Unit-I

Blood and cardio vascular system: Hematopoiesis and blood volume regulation

Objective:

In this section you will learn about how blood cells are formed and blood volume is regulated. You will also understand the basic structure of heart.

Introduction:

Cardiovascular system deals with study of heart and blood vessels. The human heart is made up 4 chambers namely; 2 atria and 2 ventricles. Atria are thin-walled chambers. Right atrium receives venous blood from peripheral parts of body. The venous blood is brought to right atrium by superior and inferior vena cavae. Left atrium receives oxygenated blood from lungs. This blood is brought to left atrium by pulmonary veins. The two atria also act as weak pumps and blood is pumped from right and left atria into right and left ventricles, respectively

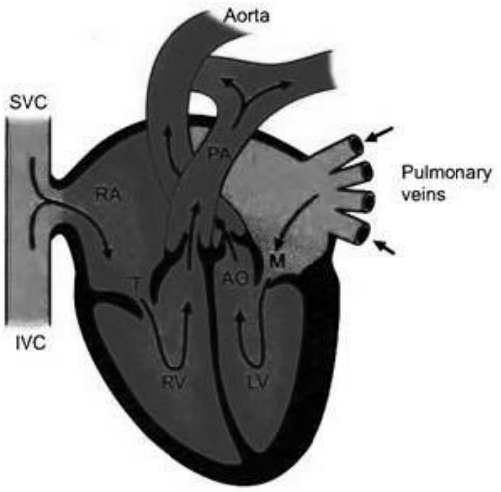


Fig. 3.1: Chambers of heart with valves and blood vessels and direction of flow of blood through chambers and blood vessels

Comparatively, ventricular walls are thicker than the atrial walls. Right ventricle is less thick-walled than the left ventricle. The ventricles act as primary pumps. From the right ventricle, blood is pumped into pulmonary circulation and from the left ventricle blood is pumped into systemic circulation (Fig. 3.2). Volume of blood pumped out by the right or the left ventricle every minute is the cardiac output.

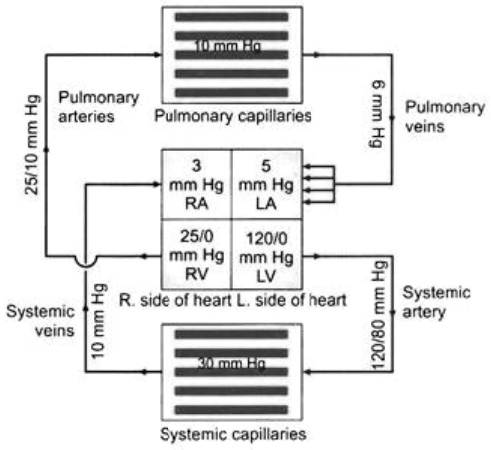


Fig. 3.2: Vessels and chambers of heart involved in systemic and pulmonary circulations and the pressure

Blood Vessels:

The systemic circulation starts with aorta. Aorta acts as a damping vessel. Aortic wall contains plenty of collagen and elastic fibers. Collagen fibers prevent rupture of the vessel. The elastic fibers are stretched when blood enters aorta from left ventricle during ventricular systole (contraction of ventricle).

Blood that enters aorta during ventricular systole is accommodated due to the stretching of elastic fibers. During ventricular diastole (relaxation of ventricle), the accommodated blood in aorta is made to flow towards periphery due to the recoiling of the elastic fibers. It is because of this, aorta acts as a secondary pump.

Blood that enters systemic circulation supplies oxygen and nutrients to the tissues and at the same time it removes carbon dioxide and metabolic wastes from tissues. Systemic circulation is a high pressure area. The pressure will be around 120/80 mm Hg.

Venous blood that has entered right ventricle is pumped into pulmonary artery. This blood takes part in exchange of gases at lungs. Because of this, oxygen from lung diffuses into blood and at the same time carbon dioxide is diffuses from blood into alveolar air. The oxygenated blood is returned to left side of heart by pulmonary vein. The pulmonary circulation is a low pressure area and the pressure will be around 25/10 mm Hg.

The Heart lies in the thoracic cavity between the two lungs in the mediastinal space and behind the sternum. It is directed more to the left than to the right side. The anterior surface of the heart faces the sternum, the posterior surface faces the vertebral column and the interior surface rests on diaphragm. The heart is composed of a specialized cardiac muscle which possesses the special property of automatic rhythmical contraction.

The heart is consisting of three layers:

1. Pericardium or outer covering layer:

The heart lies in a double membranous sac of pericardium with serous fluid between the two layers. This is known as pericardial fluid. By its lubricating action, the heart can move freely or contracts and expands without any injury. So it allows the easy movement of the heart. It keeps the heart moist and absorbs external shock.

2. The Myocardium:

This is the middle muscular layer. The thickness of the heart wall is composed of a network of heart muscle fibers which is known as myocardium. The muscular walls of the heart very in thickness. The ventricles have the thickest walls. The walls of the left ventricles are thicker then those of right ventricle because the force of contraction of the left ventricle is much greater. The walls of the auricle are composed of thinner muscles.

3. The Endocardium:

This is the inner layer of the heart. The heart is lined by endothelium layer which is known as Endocardium. The valves attached to the heart are the thickened portions of this membranes.

Structure of Heart:

Auricles and Ventricles:

The heart is divided by a septum into two sides right and left. These two parts are automatically joined together, but physiologically separate. There is normally no communication between these sides after birth.

Each side of the heart consists of two chambers:

(1) An upper thin walled atrium or auricle and

(2) The lower thick walled chamber called as ventricle.

There are two auricles or atrium right and left and two ventricles. The auricles act as receiving chambers and the ventricles the pumping chambers. The auricles and ventricles of each side communicate with one another by means of the auriculo-ventricular openings. These openings are guarded by valves which permit blood to flow from auricles to the ventricles but not in the opposite direction – that is from ventricle to auricles.

On the right side, the auriculo-ventricular opening is guarded by tricuspid valve which is composed of three flaps and on the left side by the bicuspid valve which is composed of two flaps. When the auricles are contracted, the tricuspid and bicuspid valves open and blood enters into the ventricles. But when ventricles are contracted, the flaps in the valves open like umbrella and close the auriculo ventricular openings, so that blood cannot pass back again to the auricles.

The blood in the ventricles is forced to go into the respective blood vessels which arise from them, the pulmonary artery from right ventricle and aorta from the left ventricle. The opening leading to the pulmonary artery and the aorta are guarded by different types of valves which are called semilunar valves. These valves permit the blood to enter these arteries. They prevent back flow of blood.

Two large veins named superior vena cava and inferior vena cava are attached to the right auricle which brings impure or deoxygenated blood from all parts of the body. The left auricle is attached with four pulmonary veins which carry pure or oxygenated blood from the lungs.

Hematopoiesis:

Hematopoiesis is the process by which immature precursor cells develop into mature blood cells. The currently accepted theory on how this process works is called the monophyletic theory which simply means that a single type of stem cell gives rise to all the mature blood cells in the body. This stem cell is called the pluripotential (pluripotent) stem cell.

Sites of Haematopoiesis:

Age of Human	Site of hematopoiesis
Embryo	yolk sac and liver
3rd to 7th month	spleen
4th and 5th months	marrow cavity - esp. granulocytes and platelets
7th month	marrow cavity - erythrocytes
Birth	mostly bone marrow; spleen and liver when needed
Birth to maturity	number of active sites in bone marrow decreases but retain ability for hematopoiesis
Adult	bone marrow of skull, ribs, sternum, vertebral column, pelvis, proximal ends of femurs

Structure and Function of Bone Marrow:

Bone marrow has a vascular compartment and an extra vascular compartment. The vascular compartment is supplied by a nutrient artery which branches into central longitudinal arteries which send out radial branches that eventually open into sinuses.

These sinuses converge into a central vein that carries the blood out of the bone marrow into the general circulation. Hematopoiesis takes place in the extravascular compartment. The extravascular compartment consists of a stroma of reticular connective tissue and a parenchyma of developing blood cells, plasma cell, macrophages and fat cells.

The high activity of the bone marrow is demonstrated by its daily output of mature blood cells: 2.5 billion erythrocytes, 2.5 billion platelets, 50-100 billion granulocytes. The numbers of lymphocytes and monocytes is also very high. Bone marrow is the site for other important activities in addition to hematopoiesis. These include the removal of aged and defective erythrocytes and the differentiation of B lymphocytes. It is also the site of numerous plasma cells.

Process of Hematopoiesis:

The monophyletic theory of hematopoiesis states that pluripotent stem cells multiply to produce more pluripotent stem cells, thus ensuring the steady and lasting supply of stem cells. Some of the pluripotent stem cells differentiate into precursor cells that are at least partially committed to become one type of mature blood cell. Pluripotent stem cells multiply slowly into one of five possible uni-potential stem cells which then multiply rapidly into the precursor of the specific mature blood cell for which they are destined.

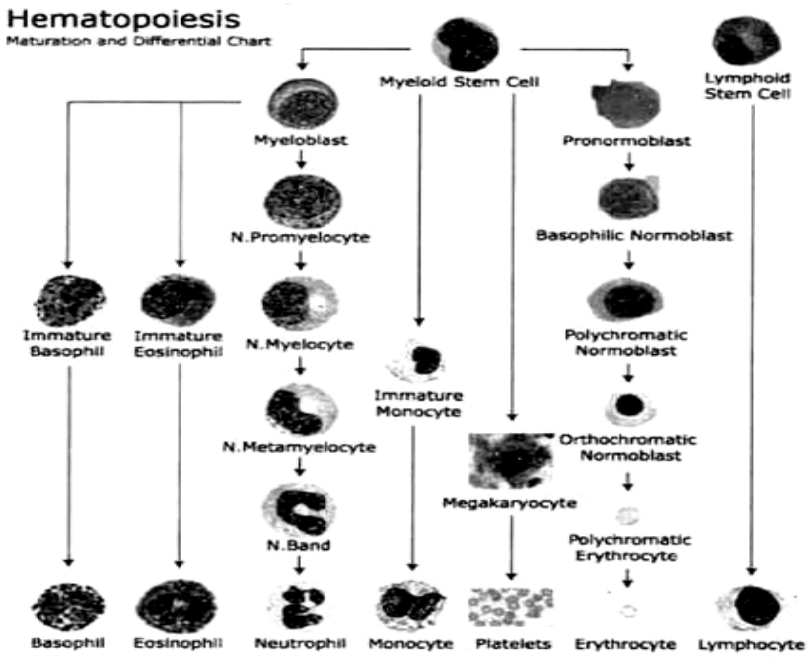


Fig. 15.1

Erythropoiesis:

The term erythropoiesis (erythro = RBC) and poiesis = to make) is used to describe the process of RBC formation or production. In humans, erythropoiesis occurs almost exclusively in the red bone marrow. (The yellow bone marrow is primarily composed of fat, but, in response to a greater need for RBC production, the yellow bone marrow can turn to red marrow.)

The red bone marrow of essentially all bones produces RBCs frc3m birth to about five years of age. Between the ages of 5 to 20, the long bones slowly lose their ability to produce RBCs. Above age 20, most RBCs are produced primarily in the marrow of the vertebrae, the sternum, the ribs, and the pelvis

RBC Production Process:

a. Kidneys respond to a lower than normal oxygen concentration in the blood by releasing the hormone erythropoietin.

b. Erythropoietin travels to the red bone marrow and stimulates an increase in the production of red blood cells (RBCs).

c. The red bone marrow manufactures RBCs from stem cells that live inside the marrow.

d. RBCs squeeze through blood vessel membranes to enter the circulation.

e. The heart and lungs work to supply continuous movement and oxygenation of RBCs.

f. Damaged or old RBCs are destroyed primarily by the spleen.

Erythroblast:

Erythroblast is a nucleated red cell. Normoblast implies normal (reaction) erythropoiesis.

Normoblastic maturation involves:

i. Reduction in cell size.

ii. Ripening of cytoplasm, i.e. hemoglobinisation.

Maturation time from pronormoblast to RBC is 7 days. Mitotic division occurs till the intermediate normoblast stage.

Pronormoblast:

12-20 μm, large nucleus surrounded by a rim of deep basophilic cytoplasm and has a perinuclear halo. Nucleus is round and has several nucleoli.

Basophilic Normoblast:

10-16 μm nucleus still large chromatin coarser and deeply staining nucleoli disappear.

Orthochromatic/Intermediate Normoblast:

8-14 μm, nucleus smaller later it becomes pyknotic and essentrically placed ultimately it is lost by extrusion.

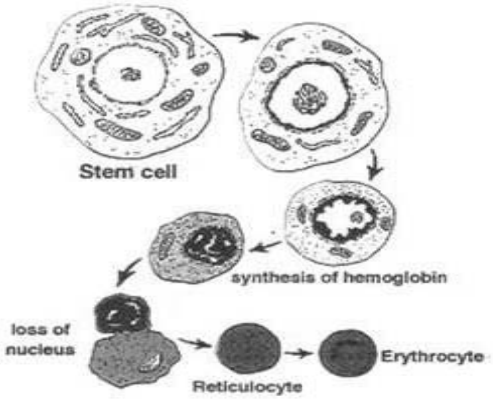


Fig. 15.2: The life cycle of a RBC

Reticulocyte:

Flat, non – nucleated, disc shaped, slightly larger than mature red blood cell. It shows diffuse pale basophilia which appears in the form of a reticulum with supra vital stains (brilliant cresy 1 blue or new ethylene blue). In 1-2 days, it loses its basophilia and become a mature erythrocyte.

Mature RBC:

They are middle sized measuring (6.6-7.7 micron). They have biconcave shape. Protein fraction of RBC is 85%. They don't have nucleus RBC is. They contain hemoglobin and are the heaviest, of all the solid component of the blood. The shape of erythrocytes is a biconcave disc.

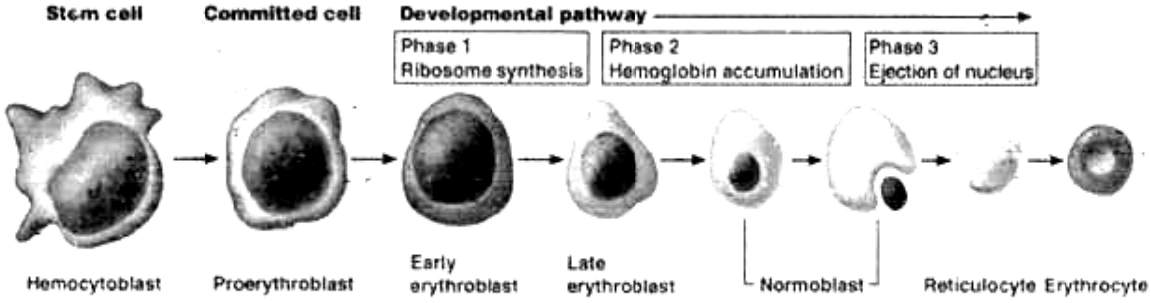


Fig. 15.3

Leucopoiesis:

The process of generating white blood cells (Leukocytes) from the pluripotent Hematopoietic Stem Cells of the, Bone Marrow. There are two significant pathways to generate various types of leukocytes -Myelopoiesis, in which leukocytes in the blood are derived from Myeloid Stem Cells, and Lymphopoiesis, in which leukocytes of the lymphatic system (Lymphocytes) are generated from lymphoid stem cells.

Myeloblast:

15-20 µm has a large round or oval nucleus, evenly stained chromatin in strands or granules with reticular appearance, 1-6 nucleoli. The cell is peroxidase negative.

Promyelocyte:

It is like myeloblast except that it contains azurophilic granules which are peroxidase positive. Nuclear chromatin becomes condenser and nucleoli are less well defined.

Metamyelocyte:

Specific neutrophilic granules appear, nucleus shows no nucleoli, (Nucleus: Cytoplasm) N: C ratio reduces; cytoplasm is pale pink, chromatin thicker and deeply stained.

Meyamyelocyte:

Nucleus is smaller and indented cytoplasm is pink with neutrophilic granules (Purplish).

Band or Stab Form:

Cell becomes still smaller, nucleus has a deep indentation, and chromatin is coarsely clumped. Cytoplasm is pink with purplish granules.

Segmented Neutrophil:

12-14 μm is size, nucleus shows 2-5 lobes, chromatin in dark purple clumps, cytoplasm has numerous, fine evenly distributed purplish granules. In female ai least 6 neutrophils/500 should show drumsticks.

The Mature Eosinophil:

16 μm in size, granules are acidophilic and larger. Nucleus is bilobed and is not masked by granule

The Mature Basophil:

It usually has a bilobed nucleus, but the nucleus is masked by about 10 large basophilic granules.

Lymphocytic and Monocytic Series:

Lymphoblast:

15-20 μm in size, resembles myeloblast, cytoplasm is agranular and moderately basophilic. Nuclear chromatin gives fine reticular appearance with up to 2 nucleoli. It is peroxidase negative.

Large Lymphocyte:

12-16 μm in size has abundant pale sky blue cytoplasm with a few purplish red granules seen in about 33% of the cells.

Small Lymphocyte:

9-12 μm in size has scanty cytoplasm. Nucleus is usually round and shows heavily clumped chromatin.

Monocytic Series:

Monoblast:

It resembles myeloblast.

Promonocyte:

Upto 20 μm in size has a large convoluted nucleus, chromatin is seen in abundant. Cytoplasm is dull grey-blue and may contain a few azurophilic granules.

Monocyte:

15-20 μm in size has abundant dull grey-blue cytoplasm with a ground glass appearance and may show vacuolation and fine azurophilic granules. It has a kidney-shaped nucleus.

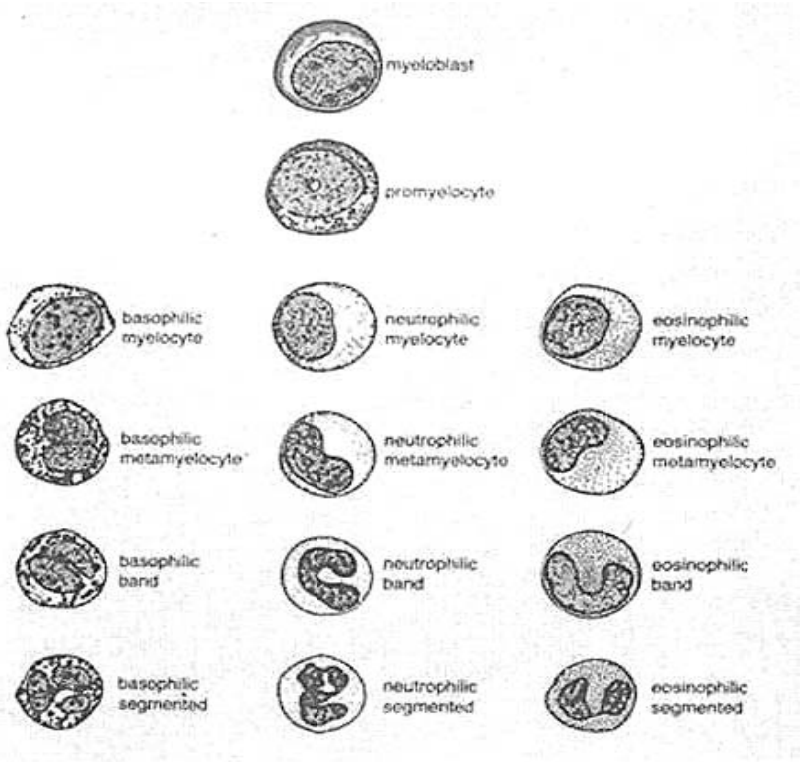


Fig. 15.5

Thrombopoiesis:

Thrombopoiesis refers to the process of thrombocyte generation. Thrombocytes are ligations of the cytoplasm from megakaryocytes. A single megakaryocyte can give rise to thousands of thrombocytes.

Thrombopoietin stimulates megakaryopoiesis, process of megakaryocyte maturation and differentiation. Thrombopoietin, upon release, binds to its receptor, found on megakaryocyte progenitor cells.

Following binding, intracellular signaling1 leads to megakaryocyte growth, maturation, membrane stability, platelet granule formation and the demarcation of the cytoplasm into regions destined to fragment into mature platelets.

These "pro-platelet processes" further fragment into platelets. This last step of pro-platelet process and platelet formation, in vitro, has been shown to be independent of thrombopoietin.

Megakaryoblast:

20-30 μm in size has a large oval or kidney-shaped nucleus with several nucleoli. It possesses relatively small amount of agranular cytoplasm.

Promegakaryocyte:

25-30 μm in diameter cytoplasm intensely basophilic with fine azurophilic granules. Nucleus may show mild lobulation and chromatin appears denser.

Megakaryocyte:

30-40 μm in diameter, it contains a single multi-lobulated or indented nucleus. Nuclear lobes may vary from 4-16 in numbers. Cytoplasm is bulky. Light blue with fine azurophilic granulation. The margin is irregular and may show fragmentation or budding. Precursor of circulating-platelets.

Mature Platelet:

1-4 μm. It is formed by fragmentation of megakaryocytic pseudopods. In circulation they acquire a discoid shaped. Cytoplasm stains light blue and contains purple reddish granules which may be clumped centrally.

Probable Questions:

1. Describe different layers of heart.
2. What is erythropoiesis. Briefly describe the process.
3. Name different types of White blood cells. How they are formed?
4. What is Hematopoiesis ? Describe different sites of Hematopoiesis.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

Unit-II

Comparative anatomy of heart structure; Cardiac cycle; neural and chemical regulation of functions of heart

Objective: In this unit you will have an idea about Comparative anatomy of heart structure; Cardiac cycle; neural and chemical regulation of functions of heart

Introduction:

Convergence is the tendency of independent species to evolve similarly when subjected to the same environmental conditions. The primitive blueprint for the circulatory system emerged around 700–600 Mya and exhibits diverse physiological adaptations across the radiations of vertebrates (Subphylum Vertebrata, Phylum Chordata). It has evolved from the early chordate circulatory system with a single layered tube in the tunicate (Subphylum Urochordata) or an amphioxus (Subphylum Cephalochordata), to a vertebrate circulatory system with a two-chambered heart made up of one atrium and one ventricle in gnathostome fish (Infraphylum Gnathostomata), to a system with a three-chambered heart made up of two atria which maybe partially divided or completely separated in amphibian tetrapods (Class Amphibia). Subsequent tetrapods, including crocodiles and alligators (Order Crocodylia, Subclass Crocodylomorpha, Class Reptilia), birds (Subclass Aves, Class Reptilia) and mammals (Class Mammalia) evolved a four-chambered heart. The structure and function of the circulatory system of each individual holds a vital role which benefits each species specifically. The special characteristics of the four-chamber mammalian heart are highlighted by the peculiar structure of the myocardial muscle.

Comparative Anatomy of Hearts

A. Heart of Fish:

a. Location:

Heart is situated ventral to the oesophagus in the pericardial section of the coelom.

b. Covering:

The heart is covered by a transparent protective covering, called pericar-dium. It is a single layer in fish. Within pericardi-um there is a pericardial fluid, protects the heart from the external injury.

c. Structure:

Heart of fishes consists of 3-chambers — a sinus venosus, a single auricle and a single ventricle. No conus arteriosus. The embryonic heart of fishes consists of 4 cham-bers which include sinus venosus, auricle, ventricle and bulbus cordis but in adult the term conus arteriosus may be used instead of bulbus cordis if it possesses cardiac muscles**.**

But according to Hildebrand (1982) , the bulbus arterious is found in the potion of cinus (Conu arteriosus) in teleosts which does not possess cardiac muscle but is highly elastic and passively evens the flow of blood into the afferent branchial arteries.

i. Sinus Venosus:

Sinus venosus is a thin walled sac. It receives deoxygenated blood by two precaval veins or ductus Cuveiri. It opens to the auricle by sinuauricular aperture, guarded by valves.

ii. Auricle:

Auricle is a thin walled single chamber of the heart. It opens into the ventricle by auriculoventricular aperture. This aperture is guarded by valves. Sinus venosus and auricle both constitute the receiving chambers of the heart.

iii. Ventricle:

Single, conical, thick- walled, forwarding chamber of the heart.

iv. Conus arteriosus:

There is no conus arteriosus. The bulbus aorta is a dilated part at the base of the ventral aorta, and not regarded as the part of the heart. It is the part of the arterial system.

B. Heart of Amphibia:

a. Location:

Heart is located mid ventrally in the anterior part of the body cavity.

b. Covering:

The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

c. Structures:

5 chambers. Sinus venosus, two auricles, single ventricle and conus arteriosus. Out of 5 chambers, the two auricles and single ventricle are regarded as permanent chambers, and sinus venosus and conus arteriosus are considered as accessory chambers.

i. Sinus Venosus:

It is a dorsally placed, thin walled triangular sac, formed by the union of two precavals and a post caval. It receives deoxygenated blood by three venacavae. It opens into right auricle through sinuauricular aperture which is guarded by sinuauricular valve. It is well developed.

ii. Auricle:

There are two unequal sized auricles. The left auricle is smaller than the right. Two auricles and a sinus venosus are the receiving parts of the heart. The two auricles are placed anterior to the ventricle. These auricles are separated internally by inter-auricular septum. Both the auricles are sharply marked off from the ventricle externally by a narrow constriction, called coronary sulcus.

The left auricle receives oxygenated blood from the lungs, through two pulmonary veins. The right auricle receives deoxygenated blood from the sinus venosus through sinuauricular aperture. Two auricles open into the ventricle by a common auriculoventricular aperture.

This aperture is guarded by membranous valves, called auriculoventricular valves. The valves remain attached with the wall of the ventricle by fine thread-like Chordae tendineae.

iii. Ventricle:

Single, thick walled highly muscular forwarding chamber, with the apex pointed towards the caudal end. The inner wall of the ventricle is thrown into muscular ridges, known as columnaecarnae.

iv. Conus Arteriosus:

From the base of the ventricle arises a stout tube-like structure called conus arteriosus (Pylangium) which proceeds forward as truncus arteriosus (Synangium). The lumen of the conus arteriosus is divided into two Channels by a spiral valve.

The left channel of the spiral valve is known as cavum pulmocutanum and the right one is called cavum aorticum. Each branch of truncus arteriosus gives three arches, known as carotid, systemic and pulmonary. The deoxygenated blood passes through the cavum pulmocutaneum to the lungs through the pulmonary arch and less oxygenated blood travels through the cavum aorticum to the different parts of the body and cephalic region. The truncus arteriosus is not to be considered as the part of the heart. It is the basal stem of the three main arteries.

e. Mechanism of Circulation:

The right auricle receives deoxygenated blood and left auricle receives oxygenated blood. The two auricles contract and the blood is driven to the ventricle. The blood mixes into the lumen of the ventricle and by contraction, reaches into the conus arteriosus.

As the conus arises from right side, a large quantity of deoxygenated blood goes to the cavum pulmocutaneoum, then goes to the pulmocutaneous arteries. The mixed blood from the middle region of the ventricle goes to the systemic arches though the cavum aorticum and lastly the oxygenated blood goes to the carotid arteries. Ultimately the spiral valve helps in the entry of blood into different arches. Therefore, it presents a transitional stage due to separation of auricle.

C. Heart of Reptiles :

a. Location:

Heart is located in the anterior part of the thoracic cavity.

b. Covering:

The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

c.Structures:

In Calotes, there are 3 permanent chambers — two auricles and an incompletely divided ventricle, but there is no conus arteriosus (Fig. 10.144D). In crocodiles, the heart is completely 4-chambered — the two auricles, and two completely divided ventricles. In crocodiles, no sinus venosus and conus arteriosus (Fig. 10.144E).

i. Sinus Venosus:

The sinus venosus of reptiles represents from larger size (turtles) to small or vestigial in other groups. It is a thin walled triangular receiving chamber placed dorsal to the auricles. It receives deoxygenated blood by three venae cavae, two anterior and one posterior. It opens into right auricle through sinuauricular aperture, guarded by sinuauricular aperture. In crocodile the sinus venosus is absent. In lizards the sinus venosus is the first chamber and contains the pace-maker.

ii. Auricle:

There are two auricles, known as right and left auricles. These are thin walled receiving chambers and placed anterior to the venricle. The auricular region is wider than the venricular portion and right auricle is larger than the left.

The inner lining of the right auricle is provided with a number of muscular ridges, known as musculipectinati. It receives the deoxygenated blood. The left auricle receives oxygenated blood through a common pulmonary vein. The pulmonary aperture is circular in outline and not provided with valves. Internally the two auricles are separated by an inter auricular septum which extends posteriorly within ventricle and possesses its tip auriculoventricular valves.

The two auricles open into the ventricle by a common auriculoventricular aperture. In the crocodiles all the structures of the auricles are same except two separate auriculoventricular apertures.

iii. Ventricle:

Incompletely divided, thick- walled, highly muscular chamber. The lumen of the ventricle is provided with an incompletely divided inter ventricular septum in most groups except crocodiles. In crocodiles this septum is complete. The two sides of the crocodilian heart are connected by an aperture, which connects between the bases of the left and right aortic trunks.

The inner cavity of the ventricle in lizards has been arbitrarily divided into three regions, namely cavum pulmonale in the right side, cavum arteriosum in the left side and cavum venosum in the middle. The right part of the inter-ventricular septum is called Cavum ventrale and the left part of the inter-ventricular septum is called cavum dorsale.

In crocodiles 4-chambered heart with only two aortic arches are seen. In crocodiles the pulmonary trunk and left aortic arch develop from the right ventricle and only right aortic arch develops from the left ventricle.

iv. Conus arteriosus: Absent

e. Mechanism of Circulation:

The heart of lizard represents a transitional stage which approaches a double circuit stage but has not reached it completely due to lacking of complete separation of the ventricle.

In crocodiles the heart is completely 4-chambered. The right part always gets deoxygenated blood and the left part gets oxygenated part. All the apertures are guarded by muscular valves that prevent the back flow of blood. The deoxygenated blood goes to the lungs through the pulmonary aorta from the right ventricle.

The oxygenated blood of the left ventricle constitutes the major circuit over the body. The blood circulation represents a double circuit stage, which attains maximum completely among reptiles.

D. Heart of Aves :

a. Location:

Heart is situated in the thoracic cavity between the two lungs and is slightly towards the left side.

b. Covering:

The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

c. Structures:

4 chambered heart. No conus arteriosus and vestigial sinus venosus. Right ventricle partly covers the left .

i. Sinus Venosus: Vestigial.

ii. Auricle:

Auricles are comparatively thick walled receiving chamber and placed anterior to ventricle. The two auricles are separated internally by inter-auricular septum. The wall of the right auricle bears sinuauricular node or pace-maker and the atrial septum bears auriculoventricular node (A V node).

The right auricle receives deoxygenated blood from three caval veins and the left auricle receives oxygenated blood through four pulmonary veins. The right auricle opens into the right ventricle by a right auriculo ventricular aperture which is guarded by a single muscular flap-like valve. The left auriculoventricular aperture is provided with 3 valve which is composed of two cusps.

iii. Ventricle:

Ventricle is a thick-walled, highly muscular forwarding chamber of the heart. It is divided into two by a complete muscular septum. The lumen of the ventricle is thrown into muscular ridges, called columnaecarnae. Only two aortic arches originate from the ventricle. The right ventricle gives rise to pulmonary arch and the left ventricle gives rise to single right aortic arch.

The opening of the arches are guarded by three cup-like semi-lumar valves. The right auriculo-ventricular aperture is guarded by a single valve but the left auriculo ventricular aperture is guarded by a membranous valve and provided with two cusps (bicuspids).

iv. Conus Arteriosus: Absent.

e. Mechanism of Circulation:

The heart of pigeon is a double circuit heart and there is no chance of mixing up of deoxygenated and oxygenated blood. The circulation represents an evolutionary advancement in birds over reptiles and its working efficiency has reached maximum.

E. Heart of Mammals:

a. Location:

Heart is situated in the thoracic cavity between the two lungs and is slightly towards the left.

b. Covering:

It is a double layered. The inner layer is called visceral layer which is highly vascularized and outer connective tissue layer, called peritoneal layer.

c. Structures:

4 chambered heart. No conus arteriosus and vestigial sinus venosus. Right ventricle partly covers the left (Fig. 10.144F). The sinus venosus is absent in adult mammals.

i. Sinus Venosus:

Absent. The embryonic sinus venosus is merged with the right atrium in the adult stage.

ii. Auricle:

Comparatively thick walled receiving chamber. The right auricle receives deoxygenated blood from the body through the anterior and posterior vena cavae. The three venae cavae open separately into the right auricle. In between the apertures of the two anterior venae cavae and to some extent guarding the posterior vena cava is the Eustachian valve.

The blood of the walls of the heart is brought to the auricle by means of an aperture, coronary sinus in the right auricle. The opening of coronary sinus is guarded by a coronary valve. The left auricle receives oxygenated blood from the lungs through four pulmonary veins. The inter-auricular septum bears an oval- shaped depression, called fossa ovalis. This depression indicates the position of foramen ovale which was the aperture during the embryonic stage.

This aperture becomes closed before the birth of the animal. The fossa ovalis is surrounded by an annular-shaped prominent ridge, called annulus ovalis. The two auricles open into the ventricles by separate apertures. The left auriculo-ventricular aperture is provided with bicuspid or mitral valve. The lumen of the left ventricle is provided with a number of muscular ridges, called trabeculae carnae. The right auriculo-ventricular aperture or ostrium is provided with a tricuspid valve which is composed of three cusps.

iii. Ventricle:

Ventricle is a thick-walled, highly muscular forwarding chamber of the heart. It is divided into two by a complete muscular septum. The lumen of the ventricle is thrown into muscular ridges, called columnaecarnae. Only two aortic arches originate from the ventricle. The right ventricle gives rise to pulmonary arch and the left ventricle gives rise to single right aortic arch. The opening of the arches are guarded by three cup-like semi-lumar valves. The right auriculo- ventricular aperture is guarded by a single valve but the left auriculo ventricular aperture is guarded by a membranous valve and provided with two cusps (bicuspids). Except the pulmonary trunk arises from the right ventricle and the left aortic arch arises from the left ventricle.

iv. Conus Arteriosus: Absent.

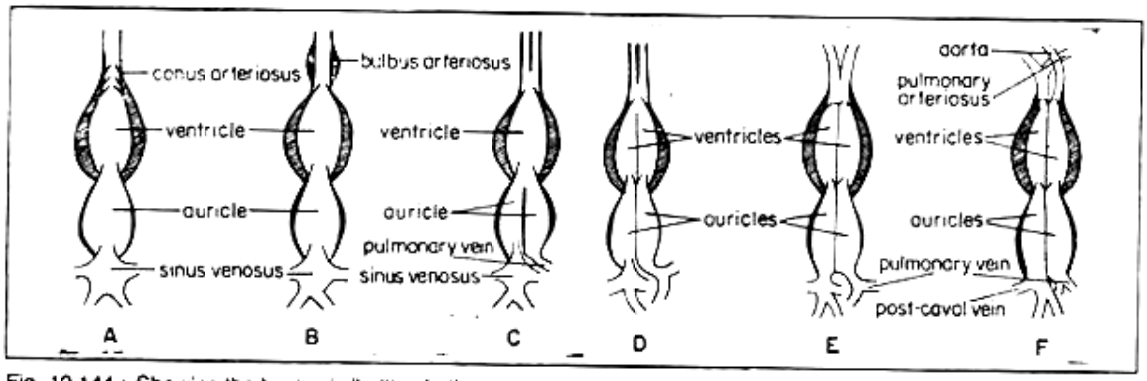


Fig. 10.144 : Showing the basic similarities in the structural plan of vertebrate hearts. A = Elasmobranchs, B = Teleost, C = Amphibia, D = Reptilia (typical), E = Crocodile and F = Birds and Mammals. Note the gradual elimination of sinus venosus and conus arteriosus in course of evolution and acquisition of two auricles and two ventricles.

e.Mechanism of Circulation:

The heart of pigeon is a double circuit heart and there is no chance of mixing up of deoxygenated and oxygenated blood. The circulation represents an evolutionary advancement in birds over reptiles and its working efficiency has reached maximum.

Cardiac Cycle:

Changes that occur in the heart during one beat are repeated in the same order in the next beat. This cyclical repetition of the various changes in heart, from beat to beat, is called cardiac cycle.

Cardiac Cycle Time:

This is the time required for one complete cardiac cycle. With the normal heart rate of 75 per minute, this time will be 60/75 = 0.8 sec. It means that every event in the cycle will be repeated at the interval of 0.8 sec. It is obvious that the cardiac cycle time will be inversely proportional to the heart rate.

In the cardiac cycle there are four main events:

i. Atrial systole.

ii. Atrial diastole.

iii. Ventricular systole.

iv. Ventricular diastole.

All the other changes are subsidiary to them.

Atrial systole initiates the cycle, because the pacemaker S.A. node is situated in it. It lasts for 0.1 sec, and is followed by atrial diastole, lasting for 0.7 sec. At the end of diastole, the atrial systole returns, and in this way, the atrial cycle goes on (total duration 0.8 sec.).

At the end of atrial systole, ventricular systole starts—duration 0.3 sec. This is immediately followed by ventricular diastole—duration 0.5 sec. At the end of diastole, ventricular systole repeats and thus the ventricular cycle goes on (total duration 0.8 sec.).

In order to follow the march of events during the cardiac cycle and their interrelations, the Fig. 7.43 should be carefully studied. In it, there are two concentric rings, divided into eight equal parts. The whole circle represents one complete cardiac cycle, so that each of its eight divisions represents 0.1 sec. The inner ring represents the atrial events, the outer ring ventricular events.

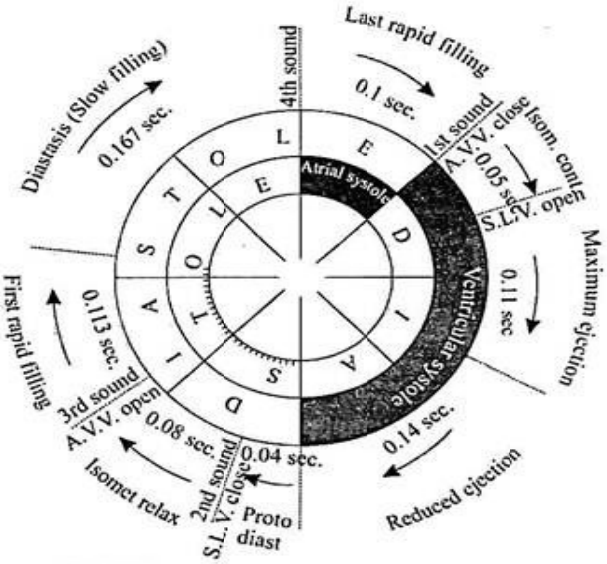


Fig. 7.43 Sequence of events during cardiac cycle.

Let us Follow Inner Ring First:

The one shaded division in it denotes atrial systole (0.1 sec.). During this period the atria contract and expel their contents into the respective ventricles. The left atrium, being further away from the S.A. node, contracts a little after the right atrium. But practically their contractions are simultaneous. The force of contraction is stronger in the first half than in the second. Because during first half or at initial stage the intra-atrial pressure remains high andduring last half the same is decreased due to expulsion of blood to the ventricle (Fig. below).

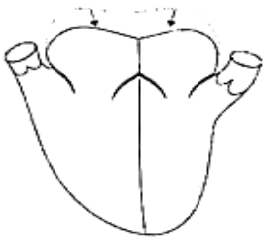


Fig. 7.44 Heart during atrial systole.
Atria shorten, A.V.V.—open.
S.L.V.—closed

After atrial systole, comes its diastole (0.7 sec.) being represented by seven un-shaded divisions in the ring. During this period the atria relax and receive blood from the great veins—the right atrium from the venae cavae, the left atrium from the pulmonary veins. At the end of this period, the atrial systole comes again and in this way, the atrial events go on.

Let us now follow the ventricular events in the outer ring. There are three shaded divisions on it, representing ventricular systole (0.3 sec.). It is followed by five un-shaded divisions, indicating ventricular diastole (0.5 sec.). On comparing the two rings, it would be found that ventricular systole commences at the end of atrial systole.

The reason for this is very clear. The impulse originating at the S. A. node will certainly overtake the atrium first, and then it will travel down the junctional tissues, enter the ventricles and stimulate their contraction. Naturally then, ventricular systole will always come after atrial systole.

From these interrelations we can deduce one fundamental rule of cardiac action that -the systoles of atrium and ventricle will never overlap. In other words, when one chamber is contracting, the other must be relaxing.

At the onset of ventricular systole, the first sound occurs. It is caused by the sudden closure of the A.V. valves due to sharp rise of intraventricular pressure. The semilunar valves open a little later, because, until the intraventricular pressure goes above that in the aorta and pulmonary artery, the semilunar valves will not open.

Thus, at the beginning of ventricular systole, there is a brief period during which both the valves are closed and the ventricles are contracting as closed cavities (Fig.below). No blood passes out and therefore, no shortening of the cardiac muscle will occur. Hence, this period is called isometric contraction period (0.05 sec.). It is marked at the onset by the closure of the A.V. valves (e.g., the first sound) and at the termination by the opening of the semilunar valves.

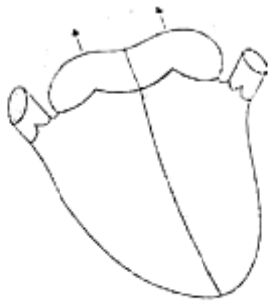


Fig. 7.45 Heart, during isometric contraction phase. Atria relaxing. Both valves closed. No shortening of ventricles.

At the end of this period, the semilunar valves open and the ejection period starts. (0.25 sec.). During this period, blood is expelled from the ventricles—from the left ventricle into the systemic aorta, from the right into the pulmonary trunk. In the first part of this period (0.11 sec.) the outflow is very rapid.

Hence, it is known as the maximum ejection period (Fig. below). In the last part (0.14 sec.) the rate of outflow slows down.

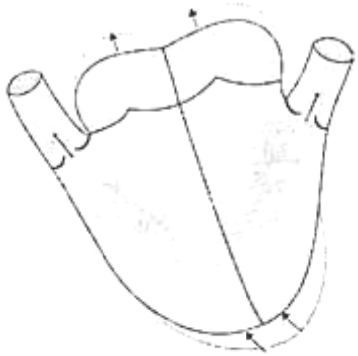


Fig. 7.46 Heart, during maximum ejection period. A.V.V.—closed. S.L.V. –open. Atria relaxing. Ventricles shortening.

Hence, it is called the reduced ejection period (Fig. 7.47). Here, the ventricular systole ends and diastole begins.

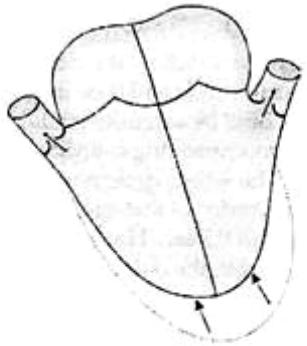


Fig. 7.47 Heart, during reduced ejection period A.V.V.—closed. S.L.V.—open. Ventricles much shortening. Atria—full

Let us Follow the Outer Ring Further:

It will be seen that after the three shaded divisions, come the five clear divisions—representing the duration of ventricular diastole (0.5 sec.). As soon as ventricles relax, the intraven-tricular pressure starts falling. The blood columns in the aorta and pulmonary trunk try to roll back towards ventricles but are stopped by the sharp closure of the semilunar valves. This produces the second sound of heart. Thus the onset of ventricular systole is marked by the first sound and its termination by the second sound (approximately).

On comparing the two rings, it will be seen that the last one division (0.1 sec.) of ventricular diastole is overlapped by atrial systole. In other words, when atria are contracting, the ventricles are still in diastole and are having the last part of their filling. It will be seen further that the first four divisions of ventricular diastole coincide with the corresponding four divisions of the atrial diastole.

From this we can come to another fundamental rule of cardiac action that—the diastole of the two chambers will always partly overlap. In the left half of the Fig. 7.43 un-shaded division will be found. In other words, both the chambers are in diastole here. This is called the diastole of the whole heart (0.4 sec.).

Let us again follow the ventricular diastole on the outer ring. As mentioned above, the second sound occurs at the end of ventricular systole. But this statement is not exact, because, till the falling of intraventricular pressure goes below the intra-aortic pressure, the semilunar valves will not close.

Consequently, there will be a short interval between the onset of diastole and the closure of the semilunar valves (i.e., the second sound). This period is called the protodiastolic period (0.04 sec.). From this it is clear that the second sound does not occur just at the end of ventricular systole but a little afterwards (i.e., after the protodiastolic period).

Although the semilunar valves have closed, yet the A.V. valves are still not open. Because, the falling intraventricular pressure takes a little time to go below that of the atria, so that the A.V. valves may open. Consequently, there will be a brief interval during which both the valves remain closed and ventricles are relaxing as closed cavities. Since no blood enters the ventricles there will be no lengthening of cardiac muscle fibres. Owing to this, it is called the isometric relaxation period (0.08 sec.—Fig. Below)

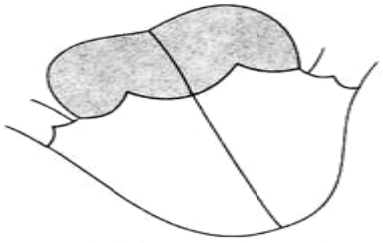


Fig. 7.48 Heart, during isometric relaxation period. Both valves closed. Ventricles relaxing. No blood entering, hence no lengthening of ventricles.

At the end of isometric relaxation period, the A.V. valves open. Blood rushes into the ventricles and ventricular filling begins. The first part of this period is known as the first rapid filling phase (0.113 sec.). Because, as soon as the A.V. valves open, blood accumulating so long in the atria, rushes in to the ventricles.

The steep fall of the intraventricular pressure during the isometric relaxation period, make the inflow all the more intense. Although the duration is brief yet the largest part of ventricular filling takes place during it. Due to rapid rush of blood a sound is produced, known as the third sound of heart (Fig. Below)

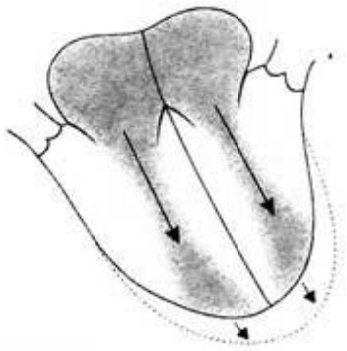


Fig. 7.49 Heart, during first rapid filling phase. A.V.V—open. Blood rushing into the ventricles Ventricles relaxing and lengthening. S.L.V.—closed.

and the next phase of diastole, the rate of filling slows down. The ventricles are already full to a large extent and ventricular pressure slowly rises. Consequently, the rate of inflow from the atria will be gradually slower. This period is called diastasis or slow inflow phase (0.167 sec.).

Although this is the longest phase of ventricular diastole, yet the amount of filling during this period is minimum. If one looks into the heart (Fig. 7.50) during this time, one will find that, the whole atrioventricular canal contains a continuous column of blood, more or less stagnant, in which the cusps of the A.V. valves are passively floating. After this period comes the last part of ventricular diastole represented by the last un-shaded division on the outer ring.

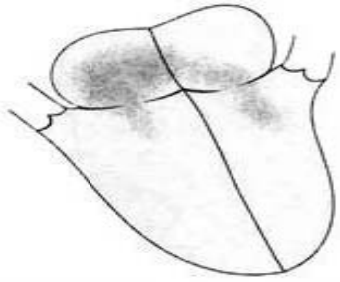


Fig. 7.50 Heart, during diastasis. A.V.V.—floating in neutral position. S.L.V.—closed. Both atira and ventricles filled up.

It is obvious that this phase corresponds with atrial systole. Due to atrial contraction, blood rushes into the ventricles and ventricular filling again becomes rapid. This phase—the last rapid filling phase (0.1 sec.) is responsible for the last part of ventricular filling. Due to rapid rush of blood, again a sound is produced—known as the fourth sound of heart.

Thus the onset of filling period is marked by the third sound and its termination by the fourth sound. Here the ventricular diastole ends. They are completely filled up, the impulse from the S.A. node arrives in the meantime and the ventricles plunge into systole again. Thus the cycle goes on.

Summary of the Sequence of Events in Cardiac Cycle:

The atrial systole is the first event (0.1 sec.). It initiates the cardiac cycle, because the pacemaker S.A. node is situated here. Due to higher atrial pressure, the first half of atrial systole is stronger than that of the last half. After systole comes the atrial diastole (0.7 sec.). These two alternately follow each other and constitute the atrial cycle (0.8 sec.).

Just after the atrial systole, the ventricular systole (0.3 sec) begins and is immediately followed by its diastole (0.5 sec.). These two events repeat alternately and make up the ventricular cycle (0.8 sec.).

At the onset of ventricular systole, the A.V. valves close producing the first sound. The semilunar valves open a little later. The interval between the closing of the A.V. valves and opening of the semilunar valves is called the isometric contraction period (0.05 sec.). During this period ventricles contract as closed cavities and intraventricular pressure steeply rises.

After this phase, comes the ejection period—when blood is pumped out of the ventricles. The first part of this period, when the outflow is very rapid, is called the maximum ejection period (0.11 sec.). The second part, when the rate of flow slows down, is known as the reduced ejection period (0.14 sec.). Here, ventricular systole ends and diastole begins.

At the beginning of ventricular diastole, the semilunar valves close producing the second sound. There is a brief interval between the beginning of diastole and the closure of the semilunar valves—known as the protodiastolic period (0.04 sec.). So that, second sound occurs actually after this period.

The A.V. valves open a little after the closing of the semilunar valves. The interval between these two is called the isometric relaxation period (0.08 sec.). During this period ventricle relax as closed cavities and intraventricular pressure steeply falls. At the end of this period, the intraventricular pressure goes below that of the atria and the A.V. valves open. Atrial blood rushes into the ventricles—producing the third sound.

Here, ventricular filling begins. The first part of filling is very rapid, being known as the first rapid filling phase (0.113 sec.). The maximum filling takes place during this brief period. The intermediate part of filling is very slow and is known as diastasis or slow inflow phase. Although this is the longest phase (0.167 sec.), yet the amount of filling is minimum.

The last part of diastole corresponds with atrial systole. Due to active contraction of the atria, filling becomes very rapid. This last rapid filling phase (0.1 sec.), is responsible for the last part of ventricular filling. Due to rapid rush of blood, another sound is produced – the so-called fourth sound of heart. Here, ventricular diastole ends and systole commences again.

Fig. 7.51 Represents the interrelations of the important events of the cardiac cycle. Condition of heart corresponding to each important phase is also shown.

Time Relations of the Various Events:

It has been noted that the cardiac cycle time is inversely proportional to heart rate. But all the phases of cardiac cycle do not proportionally vary. The duration of diastole varies much more than that of systole. For instance, with a rate of 120 per minute the cardiac cycle time will be 0.5 sec.

The systolic period will be reduced to 0.23 sec, and diastolic period to 0.27 sec. with a rate of 60 per minute the cycle time is 1 sec. Here, the systole will be 0.33 sec and diastole 0.67 sec. Thus when the rate rises from 60 to 120 per minute the systole diminishes only by 0.1 sec., whereas diastole diminishes by 0.4 sec. In other words, heart rate varies more at the expense of diastole than that of systole.

Summary of the Time Relations:

With 0.8 sec, as the cardiac cycle time (heart rate 75 per minute), the time relations of the various events are given in Table below:

Table 7.2 Cardiac events with their time relation

Cardiac Event	Duration	
▪ **Atrial Events**		
• ***Atrial Systole***		0.1 sec
- First phase (at rise of pressure)	0.05 sec	
- Second phase (at fall of pressure)	0.05 sec	
• ***Atrial Diastole***		0.7 sec
	Total	0.8 sec
▪ **Ventricular events**		
• ***Ventricular systole***		0.3 sec
- Isometric Contraction period	0.05 sec	
- Maximum Ejection period	0.11 sec	
- Reduced Ejection period	0.14 sec	
Total Ejection period	(0.25 sec)	
• ***Ventricular Diastole***		.0.5 sec
- Protodiastolic period	0.04 sec	
- Isometric Relaxation period	0.08 sec	
- First Rapid inflow	0.113 sec	
- Diastasis or slow inflow phase	0.167 sec	
- Last Rapid inflow (Atrial systole)	0.100 sec	
Total Ventricular filling time	(0.38 sec)	
	Total	0.8 sec.

Heart Rate and its Regulation:

Normal heart rate is about 60-90 beats per minute. On an average, the rate at which the heart beats is about 75 per minute. It depends on the balanced activity between the sympathetic and parasympathetic nerve influence that are acting on it. Heart rate can be increased because of either an increased activity of sympathetic nerve fibers or a decreased activity of parasympathetic nerve fibers and vice versa for a decrease in heart rate. In a newborn infant, the heart rate is about 120 beats per minute. The rate at which the heart beats is proportionate to the metabolic rate of the body. In canary birds, it can be as much as 1000 beats per minute. An increase in heart rate is known as tachycardia and a decrease is known as bradycardia.

Factors Influencing Heart Rate

Tachycardia	*Bradycardia*
Exercise	Athletes
Sympathetic stimulation	Conduction blocks
Anger, anxiety	Grief
Arrhythmias	Sleep
Thyrotoxicosis	Hypothyroidism
Fever	

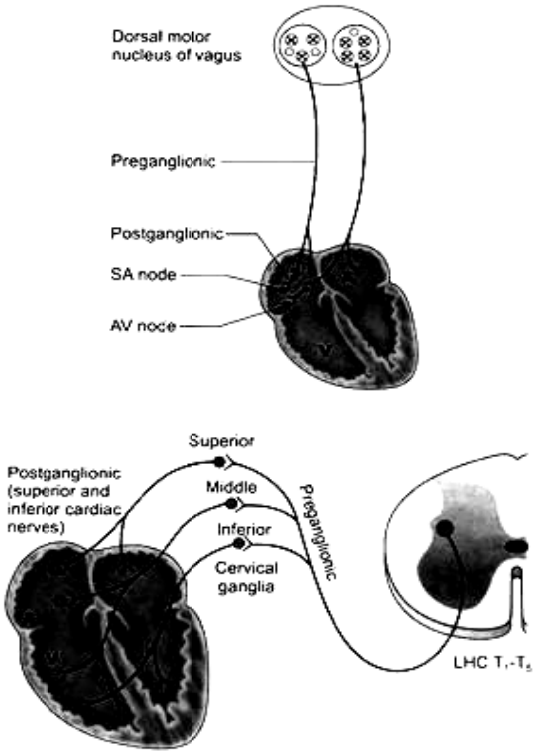


Fig. 3.27: Motor innervations to heart by parasympathetic and sympathetic nerves (above diagram is of parasympathetic and below is sympathetic)

i. The efferent nerve supply to the heart is from both sympathetic and parasympathetic nerves.

ii. The parasympathetic nerve supplying the heart comes along the vagus whereas sympathetic is from the lateral horn cells of T1-T5 segments of spinal cord. The sympathetic fibers reach heart as superior, middle and inferior cardiac nerves.

iii. Vagus nerve takes origin from the cardioinhibitory centre present in the reticular formation of the brainstem. The preganglionic fibers synapse in the ganglion cells present in the walls of the atria. From these the short postganglionic fibers supply almost all parts of heart except the apex.

iv. The neurotransmitter liberated both at the pre- and postganglionic regions will be acetylcholine. The receptors through which acetylcholine acts at the preganglionic region are termed as nicotinic receptor and at the postganglionic region are muscarinic receptor. The right vagus predominantly supplies the SA node whereas the left vagus predominantly supplies the AV node.

v. Even under normal resting conditions, there is some amount of constant activity of the vagus on heart. This is termed as vagal tone. Because of this, the normal heart rate is maintained around 75 beats per minute. If there is bilateral vagotomy (cutting of vagi on either side), even at rest the heart rate may increase to about 140-180 beats per minute.

vi. The sympathetic fibers take origin from the lateral horn cells of the upper five thoracic segments. The preganglionic fibers that have emerged out of the spinal cord ascend up and synapse in the superior, middle and inferior cervical ganglia. From these ganglia, the postganglionic fibers take origin and supply the heart.

vii. The neurotransmitter liberated by the preganglionic fibers is acetylcholine and the postganglionic fibers release noradrenaline. The influences of parasympathetic and sympathetic nerve stimulation on various activities of the heart h1ave been indicated in Table below.

Table 3.7: The effects of sympathetic and parasympathetic nerve stimulation on heart

Parasympathetic stimulation	*Cardiac activity*	*Sympathetic stimulation*	*Tropic effect*
↓	Heart rate	↑	Chronotropic
↓	Force of contraction	↑	Inotropic
↓	Conduction velocity of impulse	↑	Dromotropic
↓	Excitability	↑	Bathmotropic

Phases of Respiration and Heart Rate:

Sinus arrhythmia:

During inspiration, the heart rate is increased and during expiration, it is decreased.

Mechanism:

a. During inspiration, there will be irradiation of the impulses from the inspiratory centre to the cardioinhibitory centre which is present nearby in the reticular formation of the brainstem. These impulses from the respiratory centre will inhibit the activity of the cardioinhibitory centre and this in turn decreases the activity of vagus nerve and hence vagal tone. Consequently, heart rate gets increased.

b. During inspiration as air enters the alveoli, the stretch receptors present in the walls of the alveoli get stimulated. The impulses are carried to the brainstem through afferent vagal fibers. These afferent impulses not only inhibit the inspiratory centre but also the cardioinhibitory centre. Hence vagal tone is decreased and heart rate increases.

Regulation of Heart Rate:

Baroreceptor Mechanism:

i. There are specialized receptors namely the baroreceptors in the walls of carotid sinus and arch of aorta. The carotid sinus is located at the beginning of the internal carotid artery.

ii. The baroreceptors are stretch receptors present in the walls of the above blood vessels. Whenever there is an increase in blood pressure, the receptors get stimulated. They respond better when the blood flow in the above vessels is pulsatile.

iii. The afferent impulses from the carotid sinus are carried by sinus nerve a branch of glossopharyngeal nerve and from arch of aorta by aortic nerve a branch of vagus.

iv. The afferent impulses will stimulate the cardio inhibitory centre present in the brainstem. This will increase the number of efferent impulses along the vagus to heart. The end result will be a decrease in heart rate (Fig. 3.28). The vagal tone depends on the impulses coming from the baroreceptors. When the baroreceptors are denervated vagal tone is lost completely.

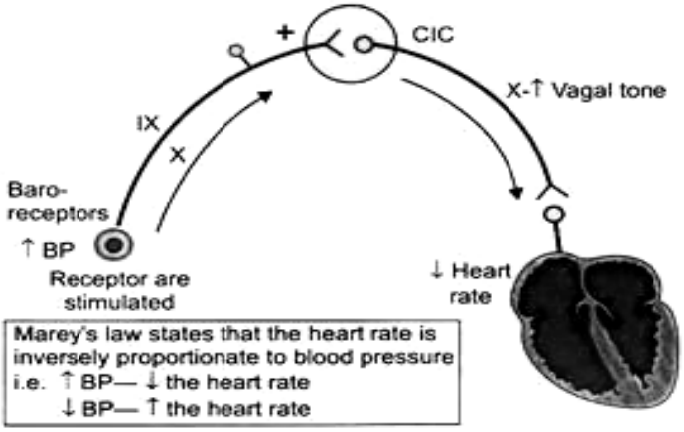


Fig. 3.28: The reflex pathway involved in Marey's reflex

v. There is an inverse relationship between blood pressure and heart rate. Heart rate is inversely proportional to blood pressure and this is termed as Marey's law. Accordingly, when blood pressure increases the heart rate is decreased. In certain conditions, like muscular exercise, anxiety, etc., there is increase of both blood pressure and heart rate (exception to Marey's law).

Nerves:

From baroreceptors (BR) to CIC—IX and X cranial nerves.

Chemoreceptor Mechanism:

i. These receptors are called as carotid and aortic bodies.

ii. The carotid body is present at the bifurcation of the common carotid artery (at the commencement of occipital artery) and aortic bodies are present at the arch of aorta.

iii. The afferent nerve that carries impulses from these receptors will be sinus nerve and aortic nerve respectively.

iv. They respond for chemical changes in blood namely, decrease in pO_2, increase of pCO_2 and increase in hydrogen ion concentration.

v. When chemoreceptor get stimulated by any of the above factors, the afferent impulses from these receptors are carried by sinus and aortic nerves.

vi. The end result will be an increase in heart rate.

Bainbridge Reflex

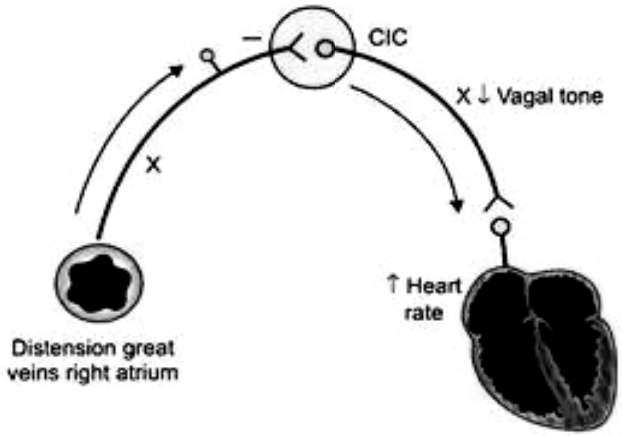


Fig. 3.29: The pathway involved in Bainbridge reflex

i. In the walls of great veins, there are stretch receptors present. They are termed as low pressure or volume receptors.

ii. Distension of the great veins leads to stimulation of these receptors.

iii. Afferent impulses from these receptors will be carried by vagus nerve.

iv. The afferent impulses inhibit the activity of cardioinhibitory centre and thereby leading to increase in heart rate.

v. The afferent impulses along the vagus will also stimulate the neurons present in the brainstem which can increase the activity of sympathetic nerves. This leads to increased sympathetic activity on heart and heart rate increases.

The other factors which can influence the heart rate are given in table below:

Table 3.8: Factors regulating heart rate

1. Higher centre activity
2. Baroreceptors
3. Bainbridge reflex
4. Chemoreceptors
5. Joint receptors
6. Pain receptors
7. Circulating chemical substance, like adrenaline

1. Pain receptor stimulation will have differential effect. When pain is from superficial parts of body (cutaneous pain), it brings about an increase in heart rate and if pain is visceral in origin, it leads to decrease in heart rate.

2. Joint receptors that are present in and around the joint will get stimulated during muscular exercise and increase the heart rate during exercise.

3. Increased intracranial tension: When there is an increased intracranial tension (e.g. in improper drainage of CSF), this will bring about a reflex bradycardia.

4. Oculocardiac reflex: When pressure is applied on the eyeball, it will bring about a decrease in heart rate.

5. Increase of body temperature will bring about an increase of heart rate.

6. Effect of adrenaline and noradrenaline: In an intact heart, adrenaline increases the heart rate while noradrenaline decreases the same. The decrease of heart rate by noradrenaline is brought about by reflex mechanism acting through baroreceptors since noradrenaline brings about an increase of mean arterial blood pressure.

7. Impulses coming from higher parts of the CNS, like limbic system, hypothalamus will also influence the heart rate, e.g. anger, fear, worry excitement, etc.

Probable Questions:

1. Describe the structural modifications of auricle from amphibia to mammals.

2. Describe the structural modifications of ventricle from amphibia to mammals.

3. How heart rate is regulated.

4. Briefly describe the Cardiac cycle.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

Unit-III

COMPARATIVE ACCOUNT OF RESPIRATORY SYSTEM IN CHORDATE

Objective: In this section you will know about Comparative account of Respiratory system in chordates

Introduction:

The steps of the evolution of terrestrial vertebrates are: change from anaerobic to aerobic life, accretion of unicells into multicellular organs, formation of a closed circulatory system, evolution of metal-based carrier pigments that improved oxygen up-take, formation of invaginated respiratory organs, physical translocation from water to land, development of a double circulation and progression from ectothermic-heterothermy to endothermic-homeothermy . In vertebrates, the blood performs the task of transporting oxygen to the cells and carbon dioxide to the external environment. The development of the respiratory organs of vertebrates is closely related to the primitive pharynx, since the gills of aquatic vertebrates and the lungs of terrestrial vertebrates and aquatic mammals have pharyngeal embryology origin.

Respiration: Respiration is the metabolic process in organisms in which organic substances are broken down to simpler products in presence (in aerobic respiration) or absence (in anaerobic respiration) of O_2 with the release of energy, water and CO_2.

a. External respiration – The exchange of oxygen and carbon dioxide between external environment and body cells is called external respiration.

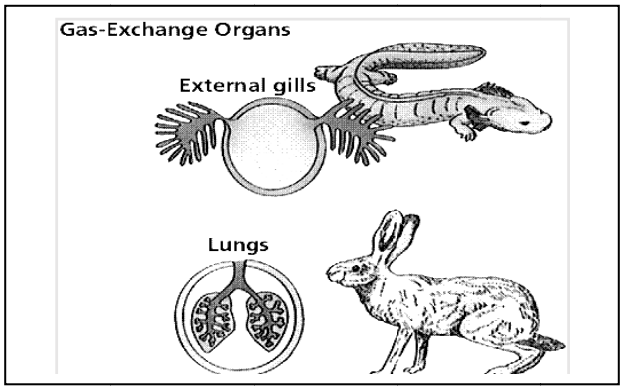
b. Internal respiration – The respiration at the cellular level for ATP production (and also CO_2) is known as internal or cellular or tissue respiration. It can be divided into two stages like **Glycolysis:** Glucose is broken down to pyruvate and **Krebs cycle:** Pyruvate is broken down by a series of cyclical reactions to water and CO_2.

Primary respiratory organs in vertebrates:

1. **Gills:** The respiratory organ used by aquatic animals to obtain oxygen from the surrounding water. The gill consists essentially of a membrane or outgrowth from the body, with a large surface area and a plentiful blood supply, through which diffusion of oxygen and carbon dioxide between the water and blood occurs.
2. **Lungs:** The respiratory organ of air–breathing vertebrates. A pair of lungs is situated in the thorax, within the ribcage. Each consists essentially of a thin moist membrane that is folded to increase its surface area. Exchange of O_2 and CO_2 takes place between blood capillaries on one side of the membrane and air on the other.

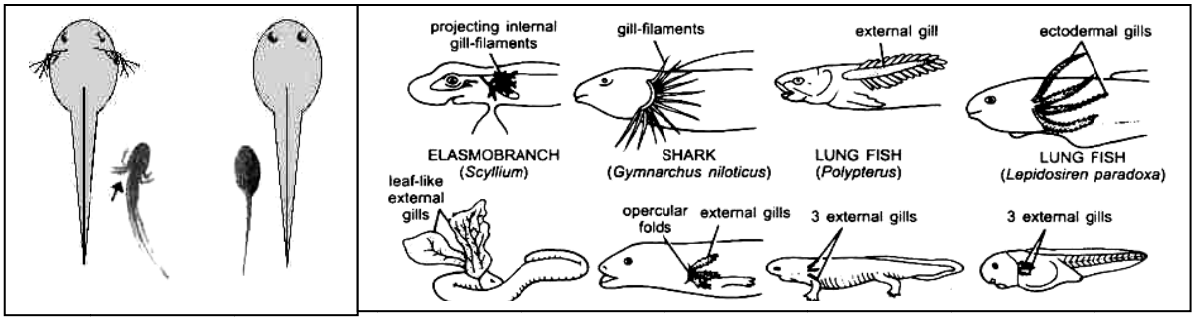
Adaptations for external respiration:

1. Primary organs in adult vertebrates are external & internal gills, swim bladders or lungs, skin, & the buccopharyngeal mucosa.
2. Less common respiratory devices include filamentous outgrowths of the posterior trunk & thigh (African hairy frog), lining of the cloaca, & lining of esophagus



I. Larval gills:

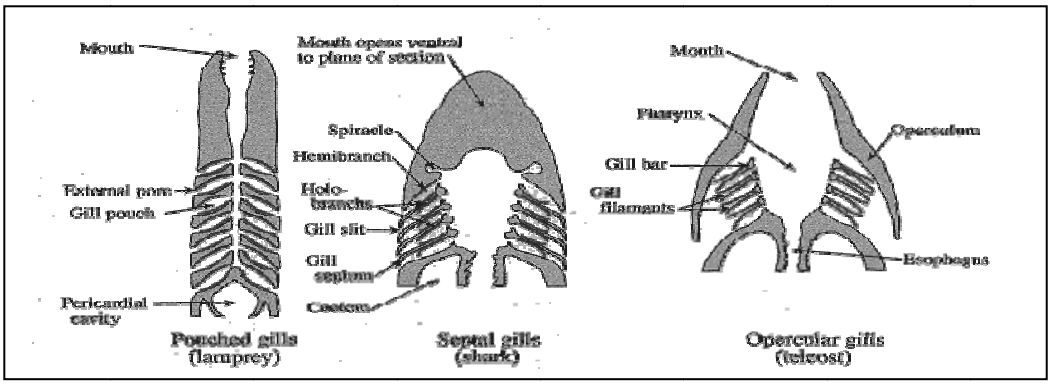
1. **External gills**
   i. Outgrowths from the external surface of 1 or more gill arches.
   ii. Found in lungfish & amphibians.
2. **Filamentous extensions of internal gills**
   i. Project through gill slits.
   ii. Occur in early stages of development of elasmobranches.
3. **Internal gills –**
   i. Hidden behind larval operculum of late anuran tadpoles.



II. Cartilaginous fishes and agnathans:

a. In cartilaginous fishes

i. 5 'naked' gill slits.
ii. Anterior & posterior walls of the 1st 4 gill chambers have a gill surface (demibranch). Posterior wall of last (5th) chamber has no demibranch.
iii. Interbranchial septum lies between 2 demibranchs of a gill arch
iv. Gill rakers protrude from gill cartilage & 'guard' entrance into gill chamber
v. Demibranchs + septum & associated cartilage, blood vessels, muscles, & nerves = holobranch

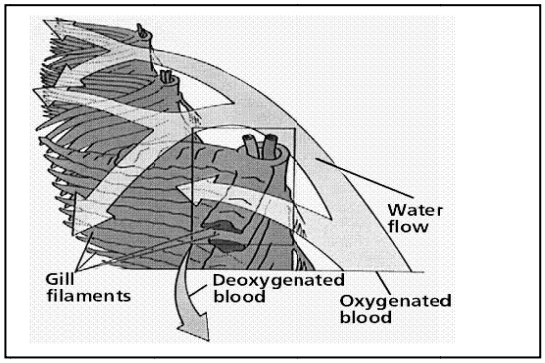


b. In agnathans:

There are 6 - 15 pairs of gill pouches connected to pharynx by afferent branchial (or gill) ducts & to exterior by efferent branchial (or gill) ducts in agnathans.

III. Gills in bony fishes (Teleost):

i. Usually have 5 gill slits.
ii. Operculum projects backward over gill chambers.
iii. Interbranchial septa are very short or absent.



IV. Lungs & associated structures:

a. Larynx

i. Tetrapods besides mammals - 2 pair of cartilages: artytenoid& cricoid

ii. Mammals - paired arytenoids + cricoid + thyroid + several other small cartilages including the epiglottis (closes glottis when swallowing)

iii. Amphibians, some lizards, & most mammals - also have vocal cords stretched across the laryngeal chamber

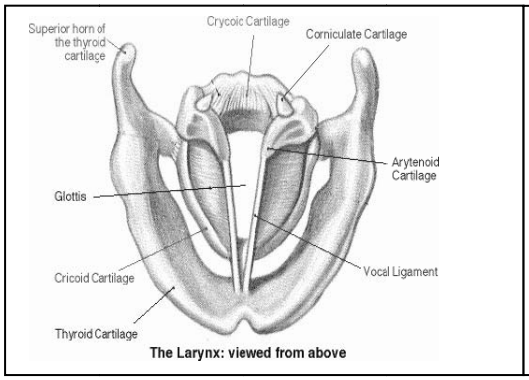
iv.

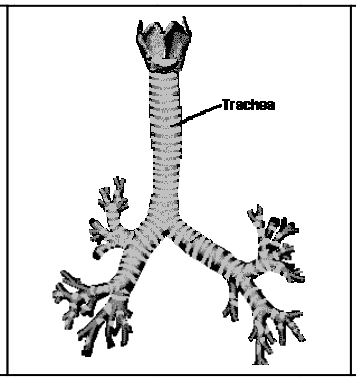
b. Trachea & syrinx:

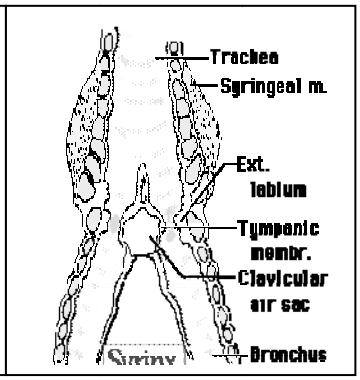
i. Usually about as long as a vertebrates neck (except in a few birds such as cranes).

ii. Reinforced by cartilaginous rings (or c-rings).

iii. Splits into 2 primary bronchi &, in birds only, forms the **syrinx** at that point.







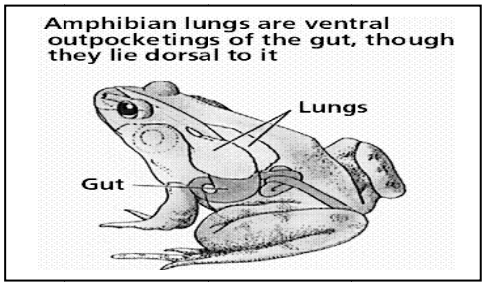
V. **Lungs**

1. Amphibian lungs

i. simple sacs

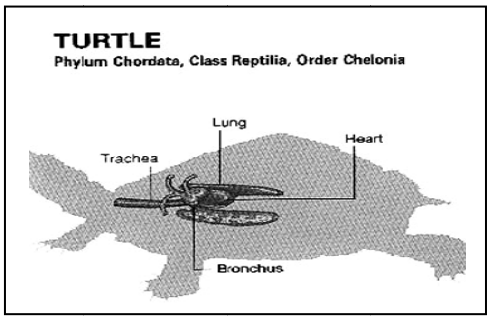
ii. internal lining may be smooth or have simple sacculations or pockets

iii. air exchanged via positive-pressure ventilation



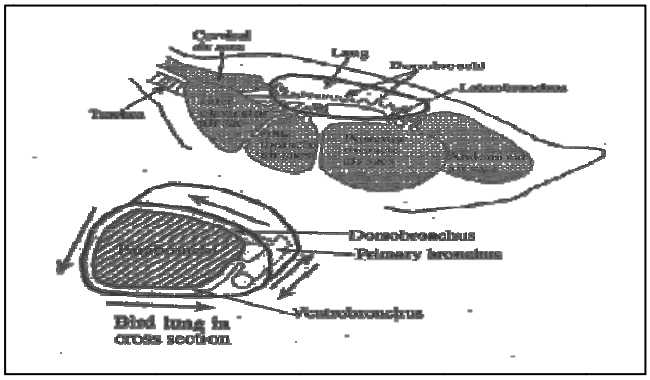
2. Reptilian lungs:

i. Simple sacs in Sphenodon& snakes
ii. Lizards, crocodilians, & turtles - lining is septate, with lots of chambers &subchambers
iii. air exchanged via positive-pressure ventilation



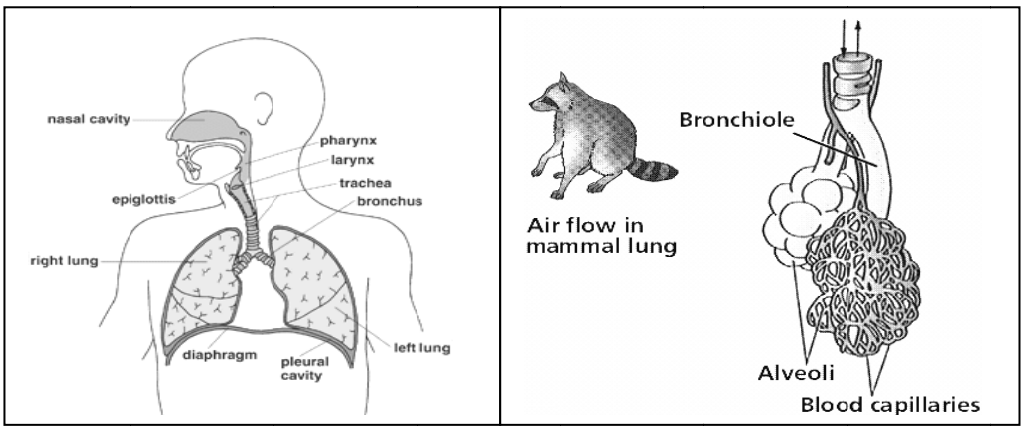
3. Avian lungs – modified from those of reptiles:

i. Air sacs (diverticula of lungs) extensively distributed throughout most of the body
ii. Arrangement of air ducts in lungs ----> no passageway is a dead-end
iii. Air flow through lungs (parabronchi) is unidirectional



4. Mammalian lungs:

i. Multichambered & usually divided into lobes
ii. Air flow is bidirectional: Trachea <--> primary bronchi <--> secondary bronchi <--> tertiary bronchi <--> bronchioles <--> alveoli.
iii. Air exchanged via negative pressure ventilation, with pressures changing due to contraction & relaxation of diaphragm & intercostal muscles.



V. Accessory respiratory organs:
Gills are the chief respiratory organs in aquatic vertebrates, like fishes and some aquatic urodeles, etc. The land vertebrates have the lungs for respiration. There are also other accessory structures for respiration, i.e., for taking oxygen directly from water or air.

1. Skin:
Some fishes are able to survive outside water. The common eel, Anguilla can travel by wriggling on damp grass though it has no special respiratory organs, but it has vascular areas in the skin by which it can breathe both in water and on land. Secondly the opening of the operculum is small and rounded so that the eel can retain water in the branchial chamber and journey on land.
In amphibians also, the moist skin is highly vascular. Lungless salamanders (plethodonts) respire only through skin. Their larvae loses gills at metamorphosis and lungs do not develop in adults. African male hairy frog, Astylosternus have vascular hairy cutaneous outgrowths which act as respiratory surface.Vascular caudal fin of Periophthalmus (mud-skipper) acts as respiratory organ during submergence. In the mud-skipper Periophthalmus of the Indian and Pacific Oceans the caudal fin is highly vascular, the head and trunk of the fish project above water when it perches on a rock, only the caudal fin remains submerged and acts as a respiratory organ.

2. Labyrinth:
(i) The Indian climbing perch *Anabas scandens* (Fig. 44.6) has special air chambers above the gills, where three concentrically folded bony laminae, called labyrinth form organs are developed from the first epibranchial bone on each side. Their covering vascular mucous membrane brings about respiration. Anabas is so dependent on air that even in water it comes to the surface to gulp air and it is asphyxiated if prevented from doing so. It can survive for long periods on land and makes excursions by means of its many long spines on the operculum and ventral fins.
(ii) In Ophiocephalus there is an accessory branchial cavity on each side above the gills.

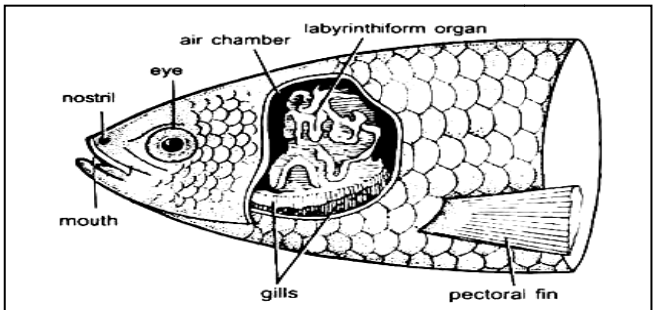


Fig. 44.6. Diagram showing the accessory respiratory organs of *Anabas*.

3. Epithelial Lining:
The loach Misgurnus swallows air which passes through the intestine and is voided by the anus, the highly vascular mucous membrane absorbs oxygen from the air, and carbon dioxide is also passed through the anus. There may be other special organs for gaseous interchange. In Calichthys rectal respiration takes place, the rectum is highly vascular into which water is alternately taken in and pumped out.

4. Pharyngeal Diverticula:
The Indian 'Cuchia eel' Amphipnous has poorly developed gills, but on each side of the body there is a vascular sac as an outgrowth of the pharynx which opens anteriorly into the first gill-cleft. These sacs are respiratory.

5. Branchial Diverticula:
In the Indian catfish Saccobranchus there is a pair of large air sacs, each arising from the branchial chamber and extending laterally backwards into the trunk muscles. They can be filled with air for respiration. The catfish *Clarias* (Fig. 44.8), found in Indian and African rivers, has a pair of supra-branchial organs, each lying on one side and divided into two parts, a highly branched arborescent

organ formed from second and fourth branchial arches, and a vascular sac of the branchial chamber which encloses the arborescent organ.
Several gill-fans formed by coalescing of gill-filaments close the entrance of the suprabranchial organ. Air is taken into the organ through the mouth continuously, and Clarias cannot only live outside water for several hours but it can move along on damp grass. Accessory respiratory organs are found generally in tropical fishes of amphibious habit, they are devices for sustaining life out of water.

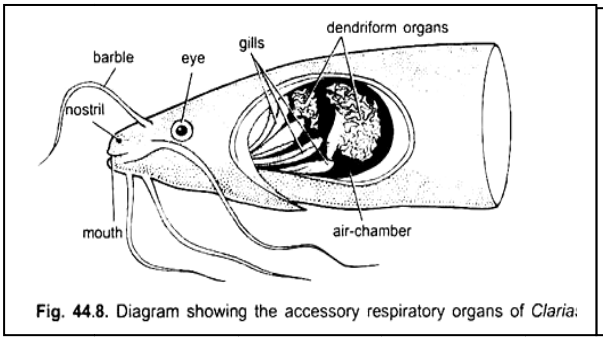
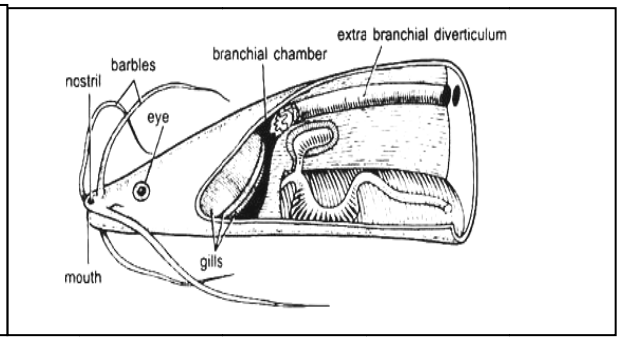
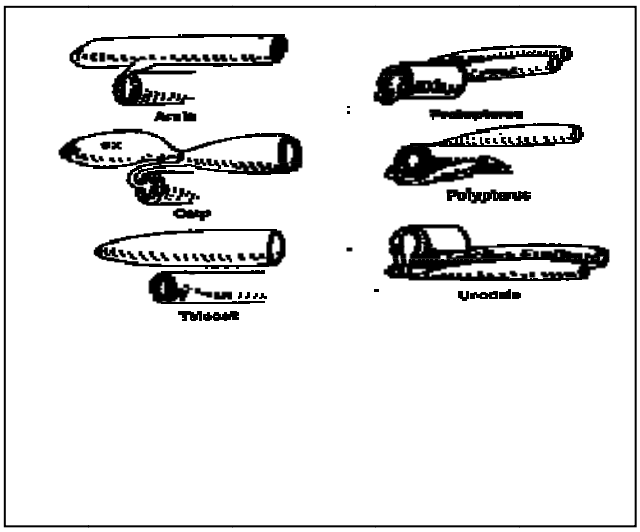
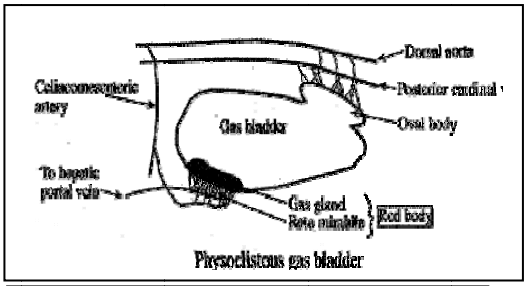


Fig. 44.8. Diagram showing the accessory respiratory organs of *Clarias*

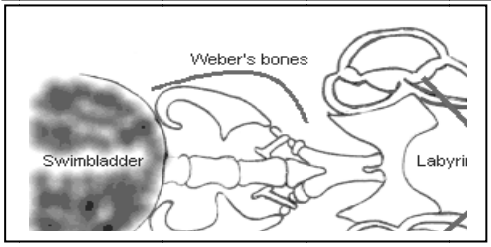


6. Swim bladder & origin of lungs –
Swim or air-bladder arises as a diverticulum from the pharynx or oesophagus in bony fishes. It is originally lateral in position but becomes dorsal. It usually lies below or lateral to the vertebral column outside the coelom. In most vertebrates swim bladder develops as an outpocketing of pharynx or esophagus that becomes one or a pair of sacs (swim bladders or lungs) filled with gases derived directly or indirectly from the atmosphere.
Similarities between swim bladders & lungs indicate they are the same organs.Vertebrates without swim bladders or lungs include cyclostomes, cartilaginous fish, and a few teleost (e.g., flounders and other bottom-dwellers).Besides respiration, swim bladder plays important role in the hearing and sound production.





Physoclistous gas bladder



a. Swim bladders-

i. May be paired or unpaired (see diagram above)
ii. Have a pneumatic duct that usually connects to the esophagus. The duct remains open (physostomous) in bowfins and lungfish, but closes off (physoclistous) in most teleosts.
iii. Serve primarily as a hydrostatic organ (regulating a fish's specific gravity)
iv. Gain gas by way of a 'red body' (or red gland); gas is resorbed via the oval body on posterior part of bladder

It may also play important roles in:

i. **Hearing** - some freshwater teleosts (e.g., catfish, goldfish, & carp) 'hear' by way of pressure waves transmitted via the swim bladder and small bones called Weberian ossicles (see diagram below)
ii. **Sound production** - muscles attached to the swim bladder contract to move air between 'sub-chambers' of the bladder. The resulting vibration creates sound in fish such as croakers, grunters, & midshipman fish.
iii. **Respiration** - the swim bladder of lungfish has number subdivisions or septa (to increase surface area) & oxygen and carbon dioxide is exchanged between the bladder & the blood.

Respiratory or Pulmonary Volumes and Capacities

The quantities of air the lungs can receive, hold or expel under different conditions are called pulmonary (= lung) volumes. Combinations of two or more pulmonary volumes are called pulmonary (= lung) capacities.

Respiratory or Pulmonary Volumes (Lung Volumes):

1. Tidal Volume (TV):

It is the volume of air inspired or expired during normal breath. This is about 500 mL, i.e., a healthy man can inspire or expire about 6000 to 8000 mL of air per minute. The lowest value is of tidal volume.

2. Inspiratory Reserve Volume (IRV):

It is the extra amount of air that can be inspired forcibly after a normal inspiration. Thus it is forced inspiration. It is about 2500 to 3000 ml. of air.

3. Expiratory Reserve Volume (ERV):

It is the extra amount of air that can be expired forcibly after a normal expiration. Thus it is forced expiration. It is about 1000 to 1100 ml.

4. Residual Volume (RV):

It is the volume of air which remains still in the lung after the most forceful expiration. It is about 1100 mL to 1200 ml.

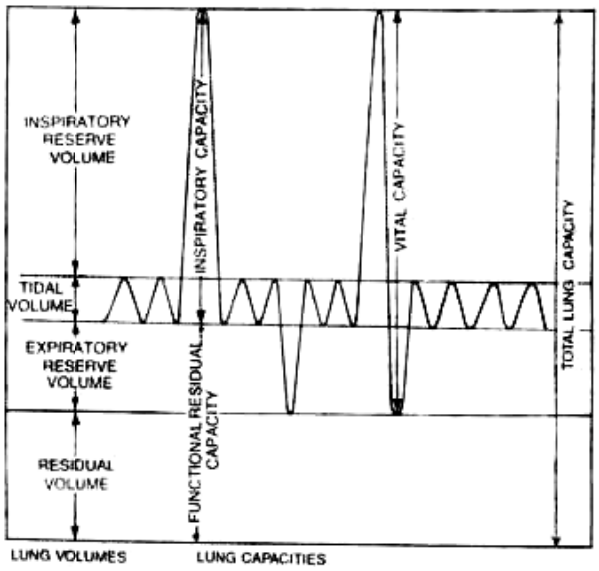


Fig. 17.8. Diagram showing Pulmonary volumes and Pulmonary capacities.

Respiratory or Pulmonary Capacities (Lung Capacities):

1. Inspiratory Capacity (IC):
It is the total volume of air a person can inspire after a normal expiration. It includes tidal volume and inspiratory reserve volume (TV + IRV).

2. Expiratory Capacity (EC):
It is the total volume of air a person can expire after a normal inspiration. This includes tidal volume and expiratory reserve volume (TV + ERV).

3. Functional Residual Capacity (FRC):

Volume of air that will remain in the lungs after a normal expiration is called functional residual capacity. This includes residual volume and the expiratory reserve volume (RV + ERV).

4. Vital Capacity (VC):

The maximum volume of air a person can breathe in after a forced expiration or the maximum volume of air a person can breathe out after a forced inspiration is called vital capacity. This includes tidal volume, inspiratory reserve volume and expiratory reserve volume (TV + IRV + ERV).

In fact total lung capacity minus residual volume is called vital capacity. VC varies from 3400 mL to 4800 ml. depending upon age, sex and height of the individual. The vital capacity is higher in athletes, mountain dwellers than in plain dwellers, in men than women and in the young ones than in the old persons.

5. Total Lung Capacity (TLC):

It is the total volume of air present in the lungs and the respiratory passage after a maximum inspiration. It includes vital capacity and the residual volume (VC + RV). All pulmonary volumes and capacities are about 20 to 25 per cent less in women than in men and they are greater in tall persons and athletes than in small and asthenic (slight build) people.

Respiratory Quotient (RQ):

Respiratory quotient is the ratio of the volume of carbon dioxide produced to the volume or oxygen consumed over a period of time in respiration.

RQ = Volume of CO_2 evolved/Volume of O_2 absorbed
Respiratory quotient varies with different foods utilized in respiration. For glucose, RQ (RQ $6CO_2/6O_2 – 1$), for fats it is about 0.7, for proteins it is about 0.9 and for organic acids it is about 1.3 or 1.4.

In anaerobic respiration, there is no consumption of oxygen. Carbon dioxide is produced in most of the cases. Therefore R.Q. is infinity. The respiratory quotient indicates the type of food oxidized in the body of the animal during respiration.

TRANSPORT AND EXCHANGE OF GASES

Exchange of Gases:

(A) Exchange of gases between alveoli and blood:

The exchange of gases (i.e., oxygen and carbon dioxide) between lung alveoli and pulmonary capillaries is called external respiration.

It occurs as follows:

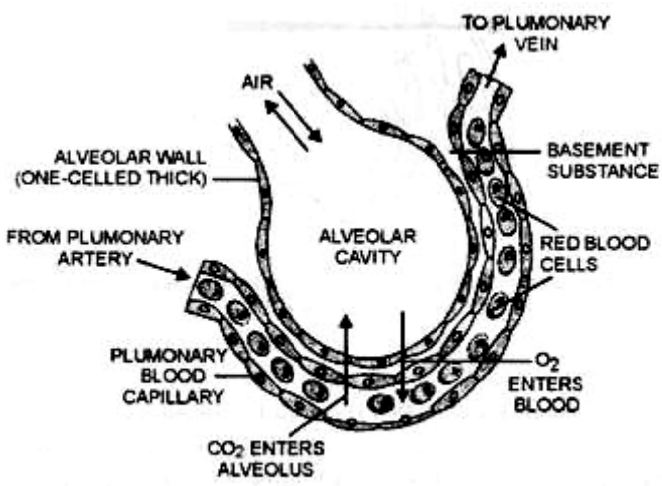


Fig. 17.9. A diagram of a section of an alveolus with a pulmonary blood capillary.

The wall of the alveoli is very thin and has rich network of blood capillaries. Due to this, the alveolar wall seems to be a sheet of flowing blood and is called respiratory membrane (= alveolar-capillary membrane).

The respiratory membrane consists mainly of:

(a) Alveolar epithelium,

(b) Epithelial basement membrane,

(c) A thin interstitial space

(d) Capillary basement membrane and

(e) Capillary endothelium.

All these layers form a membrane of 0.2 mm thickness. The respiratory membrane has a limit of gaseous exchange between alveoli and pulmonary blood. It is called diffusing capacity. The diffusing capacity is defined as the volume of gas that diffuses through the membrane per minute for a pressure difference of 1 mm Hg.It is further dependent on the solubility of the diffusing gases. In other words at the particular pressure difference, the diffusion of carbon dioxide is 20 times faster than oxygen and

that of oxygen is two times faster than nitrogen.The partial pressure of oxygen (PO_2) in the alveoli is higher (104 mm Hg) than that in the deoxygenated blood in the capillaries of the pulmonary arteries (95 mm Hg.). As the gases diffuse from a higher to a lower concentration, the movement of oxygen is from the alveoli to the blood. The reverse is the case in relation to carbon dioxide.

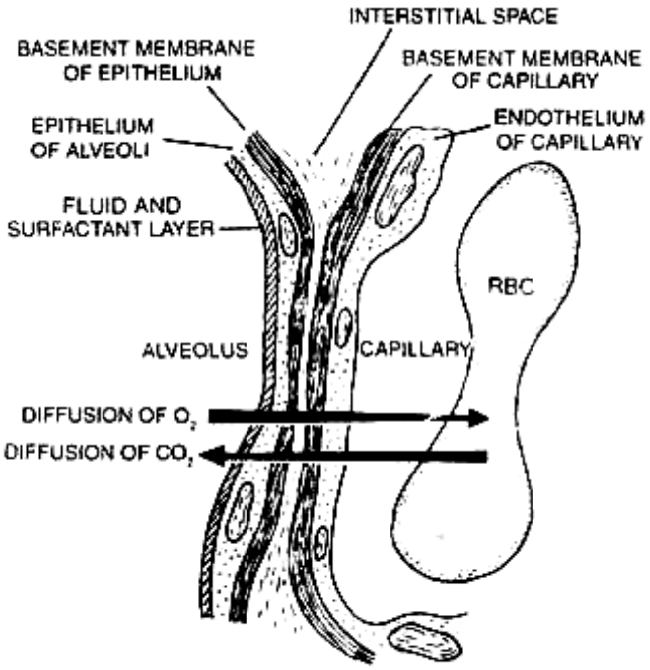


Fig. 17.10. Diagram showing Ultrastructure of Alveolar Respiratory membrane and Red blood corpuscle (RBC).

The partial pressure of carbon dioxide (PCO_2) is higher in deoxygenated blood (45 mm Hg) than in alveoli (40 mm Hg), therefore, carbon dioxide passes from the blood to the alveoli. The partial pressure of nitrogen (PN_2) is the same (537 mm Hg) in the alveoli as it is in the blood. This condition is maintained because nitrogen as a gas is not used up by the body.

(B) Exchange of gases between blood and tissue cells :

The exchange of gases (i.e., oxygen and carbon dioxide) between tissue blood capillaries and tissue cells is called internal respiration. The partial pressure of oxygen is higher (95mm Hg) than that of the body cells (40 mm Hg) and the partial pressure of carbon dioxide is lesser (40 mm Hg) than that of the body cells (45 mm Hg).

Therefore, oxygen diffuses from the capillary blood to the body cells through tissue fluid and carbon dioxide diffuses from the body cells of the capillary blood via tissue fluid. Now the blood becomes deoxygenated. The latter is carried to the heart and hence to the lungs.

Transport of Gases :

Blood transports oxygen and carbon dioxide.

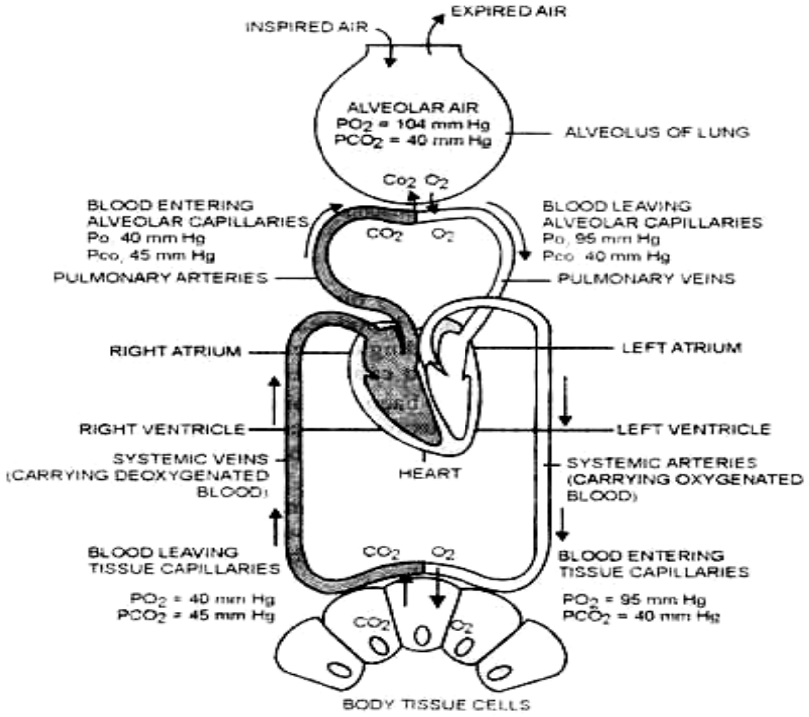


Fig. 17.11. Diagrammatic representation of exchange of gases (i) between the alveolus and pulmonary blood capillary and (ii) between the blood capillary and body tissue cells.

(A) Transport of Oxygen in the Blood:

Blood carries oxygen from the lungs to the heart and from the heart to various body parts.

Oxygen is transported in the following manners:

(i) As dissolved gas. About 3 per cent of oxygen in the blood is dissolved in the plasma which carries oxygen to the body cells.

(ii) As oxyhemoglobin. About 97% of oxygen is carried in combination with hemoglobin of the erythrocytes.

Hemoglobin (Hb) consists of a protein portion called globin and a pigment portion called heme. The heme portion contains four atoms of iron, each capable of combining with a molecule of oxygen. Four molecules of oxygen bind one molecule of hemoglobin. Oxygen and hemoglobin combine in an easily reversible reaction to form oxyhemoglobin.

$$\underset{\text{Haemoglobin}}{Hb} + \underset{\text{Oxygen}}{O_2} \underset{\text{In tissues}}{\overset{\text{In lungs}}{\rightleftharpoons}} \underset{\text{Oxyhaemoglobin}}{HbO_2}$$

Under the high partial pressure, oxygen easily binds with hemoglobin in the pulmonary (lung) blood capillaries. When this oxygenated blood reaches the different tissues, the partial pressure of oxygen declines and the bonds holding oxygen to hemoglobin become unstable. As a result, oxygen is released from the blood capillaries.

A normal person has about 15 grams of hemoglobin per 100 ml of blood. 1 gram of hemoglobin binds about 1.34 ml of O_2. Thus on an average 100 ml of blood carries about 20 ml (19.4 ml exactly) of O_2Hence under normal conditions, about 5 ml of oxygen is transported to tissues by 100 ml. of blood. During exercise or under strenuous conditions, the muscle cells consume more oxygen. The partial pressure of oxygen in the tissue falls, as a result of which, the blood at the tissue level has only 4.4 ml of oxygen/100 ml of blood. Thus about 15 ml. of oxygen is transported by hemoglobin during exercise.

Oxygen-hemoglobin Dissociation curve (=Oxygen Dissociation Curve):
The amount of oxygen that can bind with hemoglobin is determined by oxygen tension. This is expressed as a partial pressure of oxygen (PO_2). The percentage of hemoglobin that is bound with O_2 is called percentage saturation of hemoglobin.
The relationship between the partial pressure of oxygen (PO_2) and percentage saturation of the hemoglobin with oxygen (O_2) is graphically illustrated by a curve called oxygen hemoglobin dissociation curve (also called oxygen dissociation curve).

Normal Oxygen Hemoglobin Dissociation Curve:
Under normal conditions, the oxygen hemoglobin dissociation curve is sigmoid shaped or 'S' shaped. The lower part of the curve indicates dissociation of oxygen from hemoglobin. The upper part of the curve indicates the acceptance of oxygen by hemoglobin.

When the partial pressure of oxygen is 25 mm Hg the hemoglobin gets saturated to about 50%. It means the blood contains 50% oxygen. The partial pressure at which the hemoglobin saturation is 50% is called P_{50}. At 40 mm Hg of partial pressure of oxygen, the saturation is 75%. It becomes 95% when the partial pressure of oxygen is 100 mm Hg.

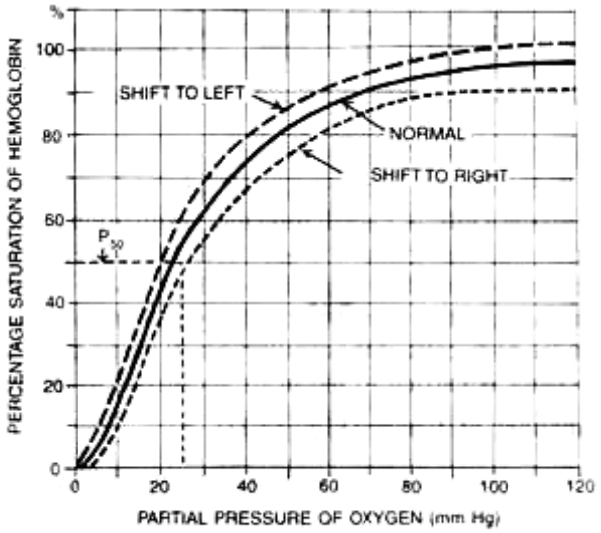


Fig. 17.12. Oxygen-haemoglobin dissociation curve .

Factors Affecting Oxygen Hemoglobin Dissociation Curve:
The oxygen hemoglobin dissociation curve is shifted either to right or left by various factors.

Shift to Right:
Shift to right indicates dissociation of oxygen from hemoglobin.

The oxygen-hemoglobin curve is shifted to right in the following conditions:

(1) Decrease in partial pressure of oxygen.

(2) Increase in partial pressure of carbon dioxide (Bohr effect).

(3) Increase in hydrogen ion concentration and decrease in pH (acidity).

(4) Increased body temperature.

(5) Excess of 2, 3 diphosphoglycerate (DPG). DPG is a by-product in glycolysis. It is present in RBCs.

Shift to Left:
Shift to left indicates acceptance (association) of oxygen by hemoglobin.

The oxygen hemoglobin dissociation curve is shifted to left in the following conditions:
(1) In the fetal blood, because, fetal hemoglobin has more affinity for oxygen than the adult hemoglobin.

(2) In the low temperature and high pH.

Bohr Effect:
An increase in carbon dioxide in the blood causes oxygen to be displaced from the hemoglobin. This is Bohr effect. This is an important factor increasing oxygen transport. It is named after the Danish physiologist Christian Bohr (1855-1911).

The presence of carbon dioxide decreases the affinity of hemoglobin for oxygen and increases release of oxygen to the tissues. The pH of the blood falls as its CO_2 content increases so that when the PCO_2rises the curve shifts to the right and the P_{50} rises. As stated in the oxygen hemoglobin dissociation curve, the partial pressure at which the hemoglobin saturation is 50% is called P_{50}.

Factors Influencing Bohr Effect:
All the factors, which shift the oxygen hemoglobin dissociation curve to the right (mentioned above) increase the Bohr effect.

(B) Transport of Carbon dioxide:
In the oxidation of food, carbon dioxide, water and energy are produced. Carbon dioxide in gaseous form diffuses out of the cells into the capillaries, where it is transported in three ways.

(i) Transport of CO_2 in Dissolved Form:
Because of its high solubility, about 7 percent carbon dioxide gets dissolved in the blood plasma and is carried in solution to the lungs. Thus as compared to O_2, a much larger volume of CO_2 is transported in dissolved form. This is about 7% of all the CO_2 transported by blood from tissues to the lungs.

(ii) Transport of CO_2 as Bio-carbonate:

The largest fraction of carbon dioxide (about 70%) is converted to bicarbonate ions (HCO_3^-) and transported in plasma. When carbon dioxide diffuses into the RBCs, it combines with water, forming carbonic acid (H_2CO_3). H_2CO_3 is unstable and quickly dissociates into bicarbonate ions and hydrogen ions:

$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \xrightleftharpoons[\text{anhydrase}]{\text{Carbonic}} \underset{\text{Carbonic acid}}{H_2CO_3} \xrightleftharpoons[\text{anhydrase}]{\text{Carbonic}} \underset{\text{Bicarbonate ion}}{HCO_3^-} + \underset{\text{Hydrogen ion}}{H^+}$$

Although this reaction also occurs in plasma, it is thousands of times faster in erythrocytes because they (and not plasma) contain carbonic anhydrase (karbon' ik an-hi' dras), an enzyme that reversibly catalyzes the conversion of carbon dioxide and water to carbonic acid.

Hydrogen ions released during the reaction bind to hemoglobin, triggering the Bohr effect; thus, no oxygen release is enhanced by carbon dioxide loading (as HCO_3). Because of the buffering effect of hemoglobin, the liberated hydrogen ions cause little change in pH under resting conditions. Hence, blood becomes only slightly more acidic (the pH declines from 7.4 to 7.34) as it passes through the tissues.

Chloride Shift (Hamburger's Phenomenon):

Since the rise in the HCO_3^- content of red cells is much greater than that in plasma as the blood passes through the capillaries, about 70% of the HCO_3 formed in the red cells enters the plasma.

The excess HCO_3 leaves the red cells in exchange for Cl^- (Fig. 17.13). This exchange is called the chloride shift. Because of it, the Cl^-content of the red cells in venous blood is, therefore, significantly greater than in arterial blood. The chloride shift occurs rapidly and is essentially complete in 1 second. Consequently, the red cells take up water and increase in size.

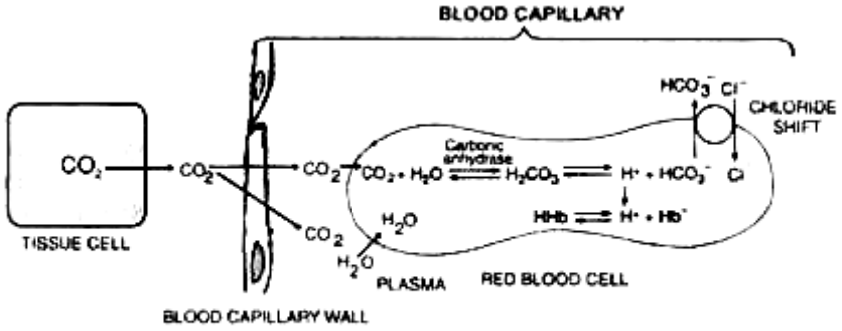


Fig. 17.13. Transport of CO_2 between tissue cell and tissue blood capillary. It is also showing chloride shift.

(iii) Transport of CO_2 as Carbaminohemoglobin. About 23 per cent CO_2 is carried by hemoglobin as carbaminohemoglobin. In addition to reacting with water, carbon dioxide also reacts directly with amine radicals (NH_2) of hemoglobin to form an unstable compound carbaminohemoglobin (Hb CO_2). This is reversible reaction.

CO_2 + Hb (Hemoglobin) ⇋ $HbCO_2$ (Carbominohemoglobin).

Every 100 mL of deoxygenated blood delivers approximately 4 mL of CO_2 to the alveoli.

Release of Carbon Dioxide in the Alveoli of Lung:

The pulmonary arteries carry deoxygenated blood to the lungs. This blood contains carbon dioxide as dissolved in blood plasma, as bicarbonate ions and as carbaminohemoglobin.

(i) CO_2 is less soluble in arterial blood than in venous blood. Therefore, some CO_2 diffuses from the blood plasma of the lung capillaries into the lung alveoli.
(ii) For the release of CO_2 from the bio-carbonate, a series of reverse reactions takes place. When the hemoglobin of the lung blood capillaries takes up O_2, the H^+ is released from it.
Then, the Cl^- and HCO_3^- ions are released from KC1 in blood, and $NaHCO_3$ in the RBC respectively. After this HCO_3^- reacts with H^+ to form $H_2CO_3^-$ As a result H_2CO_3 splits into carbon dioxide and water in the presence of carbonic anhydrase enzyme and CO_2 is released into the alveoli of the lungs.
(iii) High PO_2 in the lung blood capillaries due to oxygenation of hemoglobin favours separation of CO_2 from carbaminohemoglobin.

Haldane Effect:
It was proposed by J.S. Haldane, a Scotish physiologist, 1860-1936. Binding of oxygen with hemoglobin tends to displace carbon dioxide from the blood. This is called Haldane effect. It is far more important in promoting carbon dioxide than is the Bohr effect which promotes oxygen transport. The Haldane effect encourages CO_2 exchange in both the tissues and lungs.
It is quantitatively far more important in promoting CO_2 transport than the Bohr effect in promoting O_2 transport. Thus, Haldane effect and Bohr effect complement each other. In the tissues addition of $CO_2$to the blood facilitates unloading of O_2 by Bohr effect. In turn, O_2unloading favors uptake of CO_2 by Haldane effect.

Regulation of Respiration (Regulation of Breathing):
Respiration is under both nervous and chemical regulation.

1. Neural Regulation:
Normal quiet breathing occurs involuntarily. Adult human beings breathe 12 to 14 times per minute, but human infants breathe about 44 times per minute. In each breathe in human beings, inspiration accounts for about two and expiration for about three seconds.

The respiratory centre is composed of groups of neurons located in the medulla oblongata and pons varolii. Hence respiratory centre is divided into the medullary respiratory centres and pons respiratory centres.

Medullary Respiratory Centres:
(i) Dorsal Respiratory Group (DRG):
It is located in dorsal portion of the medulla oblongata. The dorsal respiratory group mainly causes inspiration.

(ii) Ventral Respiratory Group (VRG):
It is located in the ventrolateral part of the medulla oblongata. The ventral respiratory group can cause either inspiration or expiration, depending upon which neurons in the group are stimulated.

Pons Respiratory Centres:
(i) Pneumotaxic centre:
It is located in the dorsal part of pons varolii. The function of the pneumotaxic centre is primarily to limit inspiration.

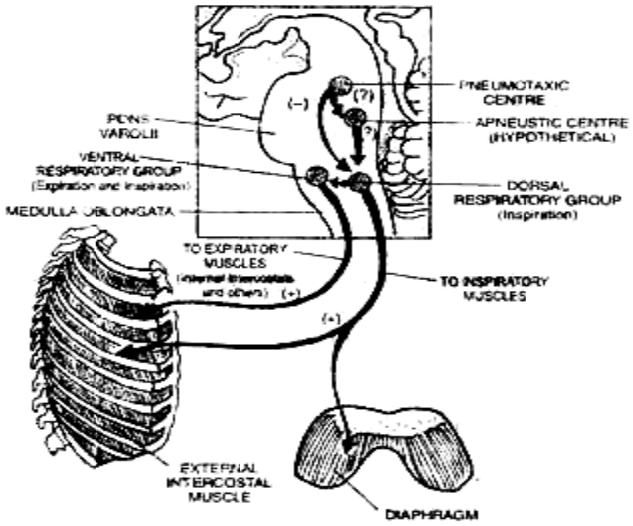


Fig. 17.15 Respiratory centre in human brain.

(ii) Apneustic Centre:

There is another strange centre called the apneustic centre, located in the lower part of the pons varolii. The function of this centre is not well understood but it is thought that it operates in association with the pneumotaxic centre to control the depth of inspiration. Apneustic centre is considered hypothetical.

2. Chemical Regulation:

The largest number of chemoreceptors is located in the carotid bodies. However, a sizeable number of chemoreceptors are in the aortic bodies.

The carotid bodies are located bilaterally in the bifurcation of the common carotid arteries and their afferent nerve fibres pass through glossopharyngeal cranial nerves and hence to the dorsal respiratory area of the medulla oblongata. The aortic bodies are located along the arch of the aorta and their afferent nerve fibres pass through the vagi (sing, vagus), cranial nerves and hence to the dorsal respiratory area.

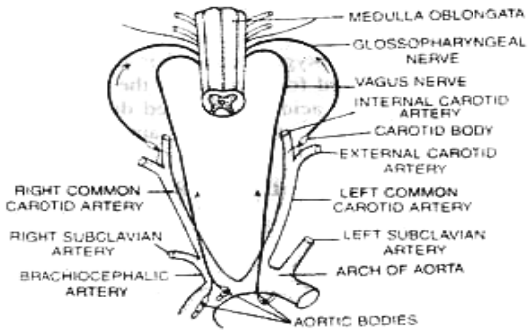


Fig. 17.16. Respiratory regulation by the carotid and aortic bodies.

Excess carbon dioxide or hydrogen ions mainly stimulate the respiratory centre of the brain and increase the inspiratory and expiratory signals to the respiratory muscles.Increased CO_2 lowers the pH resulting acidosis. However, oxygen does not have a significant direct effect on the respiratory centre of the brain in controlling respiration. Thus carotid and aortic bodies send chemical signals to the respiratory centre in the medulla oblongata.

Probable Questions:

1. What is the difference between external respiration and internal respiration.
2. Write down two characteristics of each amphibian, reptilian, avian and mammalian lung.
3. What is Swim bladders? Discuss significance of swim bladders.
4. Define tidal volume, Inspiratory Reserve Volume, Expiratory Reserve Volume ,Residual Volume Inspiratory Capacity, Expiratory Capacity, Functional Residual Capacity, Vital Capacity.
5. How oxygen is transported in the body.
6. Discuss about CO_2 transport.
7. What is Chloride shift ?
8. What is Haldane Effect ?
9. Discuss about respiration regulation.
10. Discuss about accessory respiratory organs in fishes.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

Unit-IV

Nervous system : Gross neuroanatomy of brain and spinal cord; neural control of muscle tone

Objective : In this unit you will know about Gross neuroanatomy of brain andspinal cord; neural control of muscle tone

Location and Protective Coverings of the Brain:

The brain is the anterior most part of the central neural system which is lodged in the cranial cavity (cranium) of the skull. The human brain weighs from 1220 to 1400 grams.The human neural system has about 100 billion neurons, majority of them occur in the brain. The brain is covered by three membranes or meninges (sing, meninx). The innermost membrane, the piamater is thin, very delicate and vascular and invests the brain closely.

The next is arachnoid membrane (also called arachnoid mater), which is a thin "spider webby" structure from which it gets its name. The outermost membrane, the duramater is the tough fibrous membrane adhering closely to the inside of the skull.Between the arachnoid membrane and piamater is a space known as sub-arachnoid space. The space which is present between the arachnoid and duramater is called subdural space. The sub-arachnoid space is filled with cerebrospinal fluid.

This fluid serves as a pad to cushion the central nervous system from shocks. It also provides a medium for exchange of food materials, wastes, respiratory gases and other materials. The subdural space contains a little fluid which is not cerebrospinal fluid. The membranous areas between the cranial bones of the foetal skull are called fontanels.

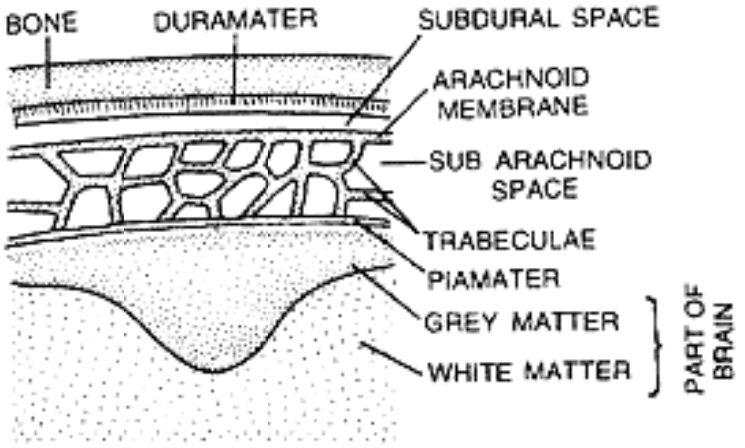


Fig. 21.3. Diagram showing meninges, grey matter and white matter of brain.

Structure and Functions of Human Brain:

The human brain is divisible into three parts:

1. Fore brain or Pros encephalon includes olfactory lobes, cerebrum and diencephalon.

2. Mid brain or Mesencephalon comprises corpora quadrigemina and crura cerebri.

3. Hind brain or Rhomb encephalon consists of cerebellum, pons varolii and medulla oblongata.

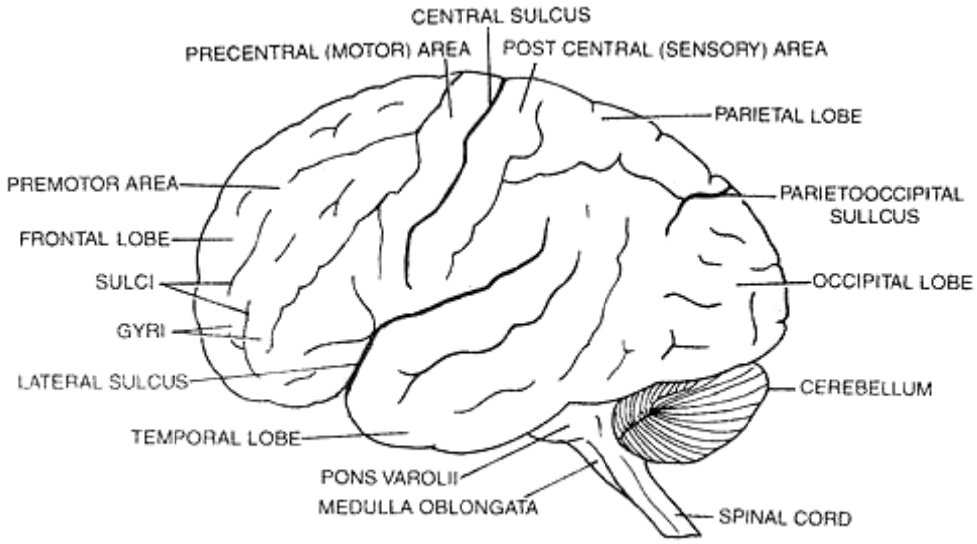


Fig. 21.4. Human brain in lateral view.

Fore Brain:

(i) Olfactory lobes:
The anterior part of the brain is formed by a pair of short club- shaped structures, the olfactory lobes. Each lobe consists of two parts, an anterior olfactory bulb and a posterior olfactory tract. They are fully covered by the cerebral hemispheres and are, therefore, only visible in the ventral view of the brain. A pair of olfactory nerves arises from the olfactory lobes.

Function:
Olfactory lobes are concerned with the sense of smell.

(ii) Cerebrum:
The cerebrum is the largest and most complex of all the parts of the human brain. It consists of left and right hemispheres connected by a large bundle of myelinated fibres, the corpus callosum and other smaller fibre bundles.

Anteriorly the corpus callosum is folded back to form the genu. Posteriorly the corpus callosum curves ventrally to form rounded splenium which joins a fibrous strip called fornix. The fornix is a paired structure, one of which is present in each hemisphere. Left cerebral hemisphere is smaller than the right.

Cerebral Cortex:
The outer portion of cerebrum is called the cerebral cortex that makes up the grey matter of the cerebrum. The surface of the cortex is greatly folded. The upward folds, or gyri (sing, gyrus), alternate with the downward grooves, or sulci (sing, sulcus).

Beneath the grey matter there are present millions of medullated nerve fibres, connecting the neurons of the cerebral cortex with those located elsewhere in the brain. The large concentration of medullated nerve fibres gives this tissue an opaque white appearance. Hence they are collectively called White matter.

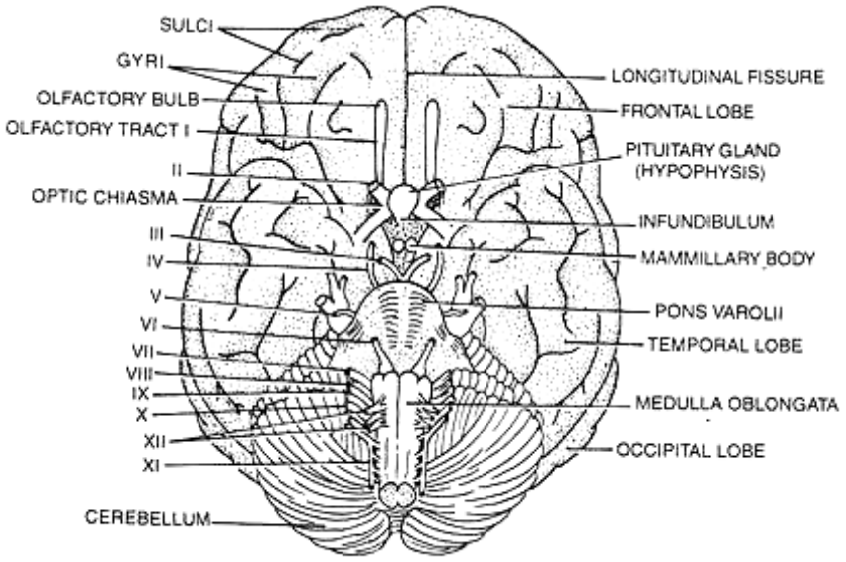


Fig. 21.5. Human brain in ventral view. The numbers I to XII indicate the cranial nerves.

Lobes:

A very deep fissure, the longitudinal fissure, separates the two cerebral hemispheres. Each cerebral hemisphere of the cerebrum is divided into four lobes: frontal, parietal, temporal and occipital lobes. The central sulcus separates the frontal lobe from the parietal lobe. The lateral sulcus separates the frontal lobe from the temporal lobe. The parieto-occipital sulcus separates the parietal lobe from the occipital lobe.

Functional Areas of the Cerebrum:

In each cerebral hemisphere, there are present three types of functional areas:

(a) Sensory areas:

They receive impulses from the receptors.

(b) Association areas:

They interpret the input, store the input and initiate a response in light of similar past experience. Thus the associated areas are involved in memory, learning and reasoning,

(c) Motor areas:

They transmit impulses to the effectors.

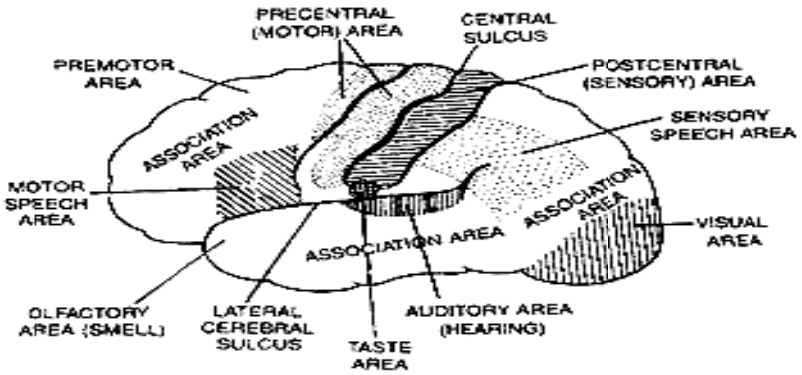


Fig. 21.6. A cerebral hemisphere (half of the cerebrum) showing the functional areas.

The pre-central (motor) area lies in the frontal lobe immediately anterior to the central sulcus. The nerve cells are called pyramidal cells which initiate the contraction of voluntary muscles. The post central (sensory) area (somaesthetic area) lies in the parietal lobe immediately posterior to the central sulcus. It perceives sensations of pain, temperature, pressure and touch.

The sensory speech area is situated in the lower part of the parietal lobe and extends into the temporal lobe. It perceives the spoken word. The auditory (hearing) area lies immediately below the lateral sulcus in the temporal lobe. It is the centre for hearing. Wernicke's area is usually located in the left temporal lobe that plays a role in understanding speech and writing words.The visual area lies in the greater part of occipital lobe. It is the centre for sight. The olfactory (smell) area lies deep within the temporal lobe. It receives the impulses from the nose via olfactory nerve and interprets them.

The taste area lies in the parietal lobe above the lateral sulcus in the post central (sensory) area. The nerve impulses from the tongue are interpreted here. The motor speech area (also called Broca's motor speech area) lies in the frontal lobe. Other functional areas of the cerebrum include the visual association area in the occipital area, parietal association area in the parietal lobe, the frontal association area in the frontal lobe and temporal association area in the temporal lobe. (An association area is a portion of the cerebral cortex that neither receives direct sensory stimuli nor directly initiates motor impulses; instead, it appears to process and interpret sensory impulses).

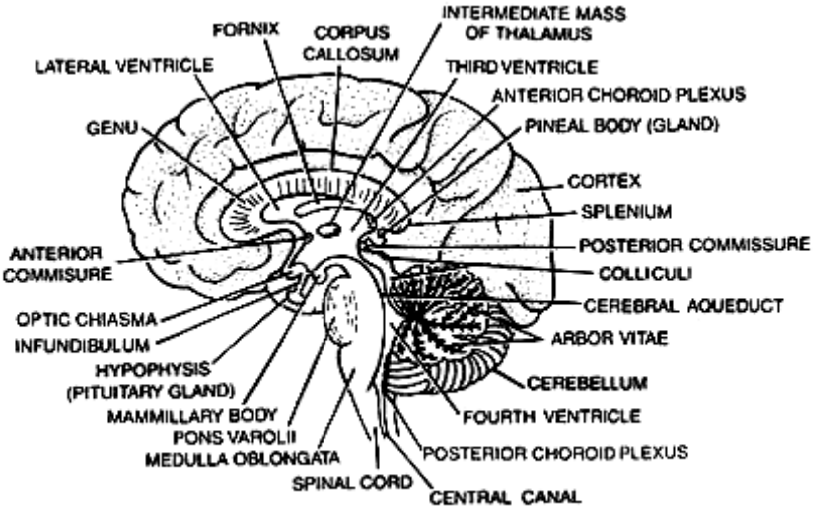


Fig. 21.7. Median section of human brain.

Basal Ganglia (Basal Nulcei):
Basal ganglia are the scattered masses of grey matter, submerged in the subcortical substance of cerebral hemispheres. The corpus striatum, the largest nucleus in the basal ganglia is a mass of grey matter situated at the base of the cerebral hemispheres in close relation to the thalamus. The lenticular nucleus is also a part of basal ganglia.

The main functions of the basal ganglia are:

(i) Control of the movements during voluntary motor activity,

(ii) Control of reflex muscular activity,

(iii) Control of muscle tone,

(iv) Control of automatic associated movements and

(v) Role in arousal mechanism. Parkinson's disease occurs due to damage of the basal ganglia. Wilson's disease is due to damage of the lenticular nucleus.

Functions:

Each lobe of cerebral hemisphere performs specific functions:

(a) In the frontal lobe creative ideas occur,

(b) In the temporal lobe sounds are interpreted so that one can understand what is being spoken,

(c) In the parietal lobe feelings about touch, hot and cold and pain are registered. It is this area that allows to accurately follow directions on map, reading a clock or dressing a person,

(d) The occipital lobe is where eyes see, and interpret what is seen.

A summary of major functions of cerebral lobes has been given below.

Cerebral Lobe	Major Functions
Frontal lobe	Inner monitoring of complex thoughts and actions, creative ideas, translation of perceptions and memories into plans of muscle movement, reality testing by judgement, *controls intellectual ability* to abstract, reasoning, decision making, expression of emotions, willpower and personality.
Parietal lobe	Registration of sensory perception of touch, pain, heat and cold, knowledge about position in space, taking in information from environment, organising it and communicating to rest of brain.
Temporal lobe	Decoding and interpretation of sound, language comprehension, smell, memory and emotion.
Occipital lobe	Decoding and interpretation of visual information; shape and colour.

(iii) Diencephalon:
Its main parts are epithalamus, thalamus and hypothalamus. Epithalamus is thin and not formed of nervous tissue. Its anterior part is vascular and folded to form the anterior choroid plexus.

Just behind the anterior choroid plexus, the epithelium forms a short stalk, the pineal stalk which has a rounded body, the pineal body, at its tip. The pineal body is an endocrine gland and, therefore, secretes a hormone, named melatonin. The thalamus, which lies superior to the mid brain is composed primarily of grey matter.

The optic nerves which come from the eyes, form a crossing, the optic chiasma in front of the hypothalamus. The hypophysis (pituitary gland) is directly attached to the hypothalamus by a stalk, the infundibulum.The pituitary gland is an endocrine gland and secretes certain hormones. Behind the infundibulum, a pair of small rounded eminences, the mammillary bodies are present. They are like nipple and hence their name.

Functions of Hypothalamus:
Although hypothalamus is relatively small (4 grams, about 1/300 of the total brain mass) yet it is highly vascular. It integrates and controls the visceral activities. It maintains homeostasis. It provides anatomical connection between the nervous and endocrine systems by its relationship to the pituitary gland.

Through connections with pituitary gland, it controls growth and sexual behaviour. Hypothalamus is thermoregulatory centre. Hence it is called "thermostat" of the body.

It keeps body temperature at roughly 37°C by means of a complex thermostat system. It is also associated with behavioural activities. Appetite, thirst and satiety (feeling of being satisfied) centres are located in the hypothalamus. It also influences respiration and heart beat.

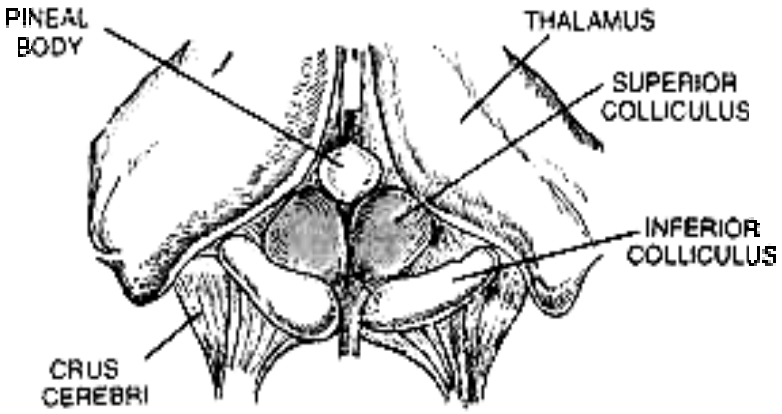


Fig. 21.8. Part of Human brain in posterior view to show some structures in detail.

Mid Brain:
(i) Corpora quadrigemina:
The upper or superior surface of the mid brain has two pairs of rounded protrusions collectively called the corpora quadrigemina; one pair is called superior colliculi and the other pair is called inferior colliculi. The superior and inferior colliculi of each side are termed the corpora bigemina.

Functions:
The superior colliculi are concerned with the sense of sight. However, the inferior colliculi are concerned with hearing.

(ii) Cerebral peduncles (Crura cerebri):
These are two bundles of fibres which lie on the lower or inferior surface of the mid brain.

Function:
They relay impulses back and forth between the cerebrum, cerebellum, pons and medulla.

Hind Brain:
(i) Cerebellum:
The second largest part of the human brain is the cerebellum (means simply "little cerebrum"). It is well developed in human brain. It consists of two lateral cerebellar hemispheres and central worm - shaped part, the vermis.

Like the cerebrum, the cerebellum has its grey matter on the outside, comprising three layers of cells and fibres. The middle layer contains characteristically large flask shaped Purkinje cells. The Purkinje's cells rank among the most complex of all neurons. The cerebellum also has Golgi cells, basket cells and granule cells.

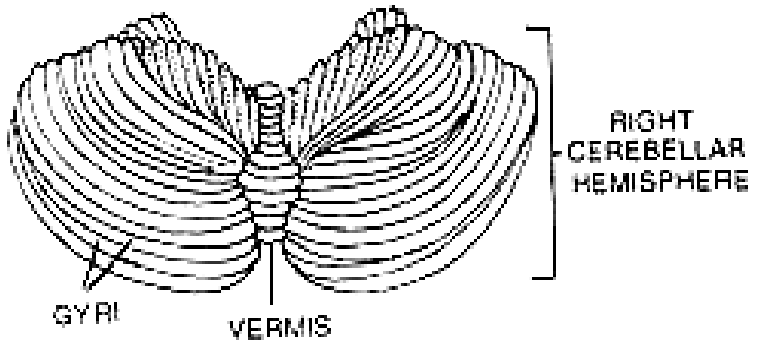


Fig. 21.9. The cerebellum viewed from below.

A cross section of the cerebellar hemispheres shows a branching tree like arrangement of grey and white matter called the arbor vitae ("tree of life").

Functions:
The cerebellum controls rapid muscular activities, such as running, typing and even talking. All activities of the cerebellum are involuntary, but may involve learning in their early stages. Alcohol affects the cerebellum. Since, alcohol is a depressant, it interferes with the functions of the cerebellum.

(ii) Pons Varolii:
It is situated in front of the cerebellum below the mid brain and above the medulla oblongata. It consists mainly of nerve fibres which form a bridge (pons— bridge) between the two hemispheres of the cerebellum and of fibres which pass between the higher levels of the brain and the spinal cord. Pneumotaxic centre is present in pons varolii.

Functions:
Pons varolii relays impulses between the medulla oblongata and more superior part of the brain, between the hemispheres of the cerebellum and between the cerebrum and cerebellum. The pneumotaxic centre limits inspiration.

(iii) Medulla oblongata:
It extends from the pons varolii above and is continuous with the spinal cord below. Its shape is like a pyramid. The medulla oblongata has a very thin, non vascular folded structure on its lower side called the posterior choroid plexus.

Functions:
Medulla oblongata receives and integrates signals from spinal cord and sends resulting signals to the cerebellum and thalamus. It contains centres that regulate heart rate, blood pressure, breathing, swallowing, salivation, sneezing, vomiting and coughing and some other involuntary movements.

Brain Stem:
The mid brain, pons varolii and medulla oblongata are collectively called the brain stem, connecting the fore brain and spinal cord.

Limbic System:
Components of Limbic System:
Certain components of the cerebrum and diencephalon constitute the limbic system (limbus L. a border or edge or fringe of a part).

Its main components are the following:
(i) Hippocampus:
Its shape roughly resembles the sea horse. It is located inside the temporal lobe

(ii) Amygdala or Amygdaloid nucleus (L., Gr. amygdale – almond):
It is almond shaped and is located in the tip of the temporal lobe,

(iii) Septal nuclei:
These are located within the septal area formed by the regions under corpus callosum and the paraterminal gyrus (a cerebral gyrus),

(iv) Mamillary bodies:
These are present behind the infundibulum.

(v) Basal ganglia:
They are scattered masses of grey matter.

Functions of limbic system:
(a) It is sometimes called the "emotional brain" because it controls emotional behaviour expressed in the form of joy, sorrow, fear, fight, friendship, liking and disliking,

(b) It controls food habits necessary for survival of the individual,

(c) It also controls sex behaviour necessary for survival of the species.

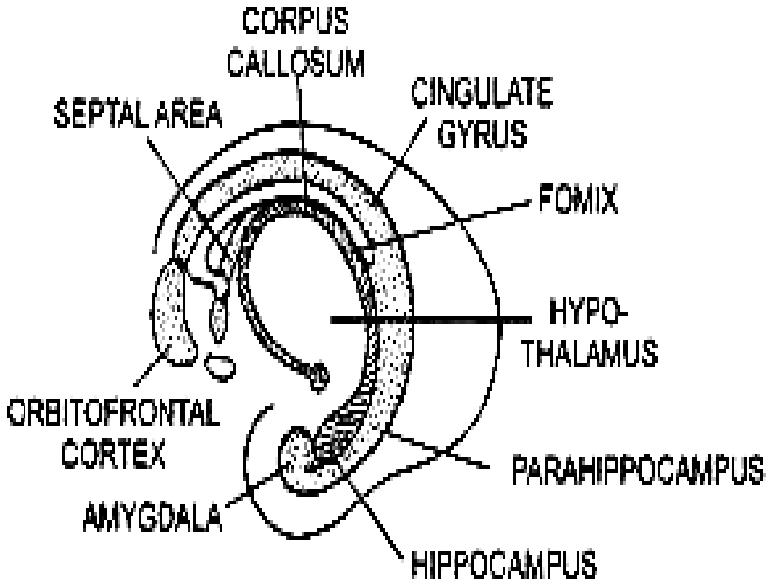


Fig. 21.10. The Limbic System

Ventricles of the Brain:

The ventricles consist of four hollow, fluid filled spaces inside the brain. A lateral ventricle lies inside each hemisphere of the cerebrum. Each lateral ventricle is connected to the third ventricle by an interventricular foramen (foramen of Monro).

The third ventricle consists of a narrow channel between the hemispheres through the area of the thalamus. It is connected by the cerebral aqueduct or aqueduct of Sylvius or iter in the midbrain portion of the brainstem to the fourth ventricle in the pons and medulla. The fourth ventricle is continuous with the central canal of the spinal cord.

Three openings in the roof of the fourth ventricle, a pair of lateral apertures (foramina of Luschka) and a median aperture (foramen of Magendie) allow cerebrospinal fluid to move upward to the subarachnoid space that surrounds the brain and spinal cord. Ventrolateral wall of each paracoel appears striated and hence called corpus striatum.

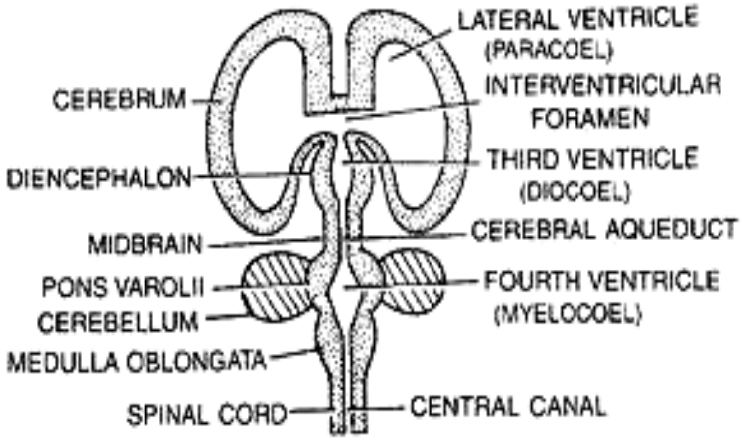


Fig. 21.11. Schematic representation of the ventricles of human brain.

Cerebrospinal Fluid (CSF):
The cerebrospinal fluid is secreted by anterior choroid plexus and posterior choroid plexus and is found inside the ventricles of the brain, the central canal of the spinal cord and in the subarachnoid space around the brain and spinal cord.

The cerebrospinal fluid performs the following functions:

(i) Protection of the Brain and Spinal cord:
CSF protects the delicate brain and spinal cord by providing shock-absorbing medium. It acts as cushion jolts to the central nervous system.

(ii) Buoyancy to the Brain:
Since the brain is immersed in the CSF, the net weight of the brain is reduced from about 1.4 kg to about 0.18 kg. Thus the pressure at the base is reduced.

(iii) Excretion:
CSF carries harmful metabolic wastes, drugs and other substances from the brain to the blood.

(iv) Endocrine Medium for the Brain:
Certain hormones are released into CSF. These hormones are carried to different parts of the brain by CSF where they may act.

Mammalian Characters in Human Brain:

(i) Olfactory lobes are small and solid.

(ii) Cerebral hemispheres are quite large in size and divided into lobes.

(iii) Corpus callosum is also found.

(iv) Optic lobes are solid and further divided into corpora quadrigemina.

(v) Pons varolii is present.

(vi) Cerebellum is very much folded and solid.

Comparative anatomy of Brain

Structure of Brain in Amphibia:

1. Forebrain:
The two olfactory lobes are fused together anteriorly. Olfactory peduncle is absent. These lobes are responsible for the sense of smell.

2. Cerebral Hemispheres:
Cerebral hemispheres are divided by a longitudinal fissure. The roof of the hemisphere is thick, smooth and nervous. Corpora striata are poorly developed and are connected by anterior commissures. Lumen of each hemisphere is reduced by the thickening of its lateral and median wall. Cerebrum is the centre for intelligence, conscience and control of voluntary muscles.

On the dorsal side of the diencephalon epiphysis is small. Pineal body of unknown function. Anterior choroid plexus is present. Ventral side of the diencephalon bears an x- shaped optic chiasma. Presence of infundibulum and pituitary body. The lateral sides of the diencephalon are thick to form optic thalami.

3. Midbrain:
Optic lobes are large and hollow ovoid bodies and two in number. Crura cerebri are longitudinally placed nerve fibres-like structures placed ventrally. Optocoels are present.

4. Hind Brain:
Cerebellum is small and does not overlap the structures in front and behind. It represents a thin transverse band-like structure.

5. Medulla Oblongata:
Medulla oblongata is thick and gradually tapers behind as a spinal cord. It gives rise 4 pairs of cranial nerves. The roof of the medulla oblongata is formed by a thin vascular membrane, called the posterior choroid plexus. The corpora restiformia are absent in medulla oblongata. It regulates the breathing, heartbeat, and metabolism.

Structure of Brain inReptiles:
1. Forebrain:
Olfactory lobes are small and connected to the brain by olfactory peduncle.

2. Cerebral Hemispheres:
Cerebral hemispheres are larger than amphibians. The two hemispheres are elongated and are separated medially by a deep fissure. The dorsal surface of the hemispheres is smooth and thin but the lateral and ventral walls are thick that constitute the corpora striata.

Due to accumulation of large amount of grey matters the roof of cerebral hemispheres is called neopallium. On the dorsal surface of the diencephalon there are tub projections, called parietal organ, situated at the anterior of the pineal body.Another projection, called paraphysis, which is present in reduced condition. On the ventral side hangs infundibulum and pituitary body. Lateral ventricles of the cerebral hemispheres are less spacious. Hippocampal lobes are differentiated. Anterior commissures and corpus albicans are absent.

3. Midbrain:
Optic lobes are more developed than those of amphibia and two in number, called corpora bigemina. They are hollow with optocoels. Crura cerebri at the floor and poorly developed.

4. Hind Brain:
Cerebellum is very small, semicircular and flap-like. It contains ventricle. Floccular lobes and pons varolii are absent. The surface of the cerebellum is smooth.

5. Medulla Oblongata:
Medulla oblongata tapers gradually and joins with the spinal cord. Its roof is thin and vascular that forms the posterior choroid plexus.

Structure of Brain in Aves:

1. Forebrain:
Olfactory lobes are reduced. Olfactory bulbs and tract are much reduced.

2. Cerebral Hemispheres:
Cerebral hemispheres exhibit considerable increase in size. These two lobes are separated by a median dorsal fissure. Corpora striata are prominent that reduces the lateral ventricles to very narrow spaces.

The corpus striatum is differentiated into hyper-striatum, meso-striatum and palaeo-striatum. The roof of cerebral hemispheres is called neopallium. The neopallium is un-convoluted. The diencephalon is inconspicuous and completely covered by cerebral hemispheres and cerebellum.

3. Midbrain:
Two large optic lobes with optocoels much reduced. Crura cerebri are well developed.

4. Hind Brain:
Cerebellum is more advanced than the reptilian cerebellum. It is divided two lateral and one median lobe. The median lobe is called vermis and lateral lobes are called flocculi. It is solid because the 4th ventricle does not extend it. Generally surface is extensively folded.

5. Medulla Oblongata:
Medulla tapers and joins with the spinal cord. Posterior choroid plexus is completely covered by cerebellum. Ventral flexure is highly developed.

Structure of Brain in Guinea-Pig:
1. Forebrain:
Olfactory lobes are small and club shaped. Olfactory peduncle is absent.

2. Cerebral Hemispheres:
Cerebral hemispheres are very large. They occupy two-thirds of the whole brain. The two cerebral hemispheres are connected by a band of nerve tracts that connect ventrally, called corpus callosum. Each hemisphere is subdivided into 4 lobes, called frontal, parietal, temporal and occipital, by grooves, called Sylvian fissure. Neopallium is highly developed. Presence of corpus albicans.

3. Midbrain:
Optic lobes are small and four in number (corpora quadrigemina) Optocoels are highly developed.

4. Hind Brain:
Cerebellum is more complex and highly advanced than the cerebellum of pigeons. It is large, elongated and solid. It is subdivided into 5 lobes — namely a median vermis, two lateral lobes and two flocculi. The surface of cerebellum is thrown into numerous folds, called gyri and grooves between the folds called sulci.

On the ventral side the two halves of the cerebellum are connected by a broad band, known as pons varolii.

5. Medulla Oblongata:
The lateral walls and floor of the medulla are highly thickened. Its roof forms highly vascularized posterior choroid plexus.

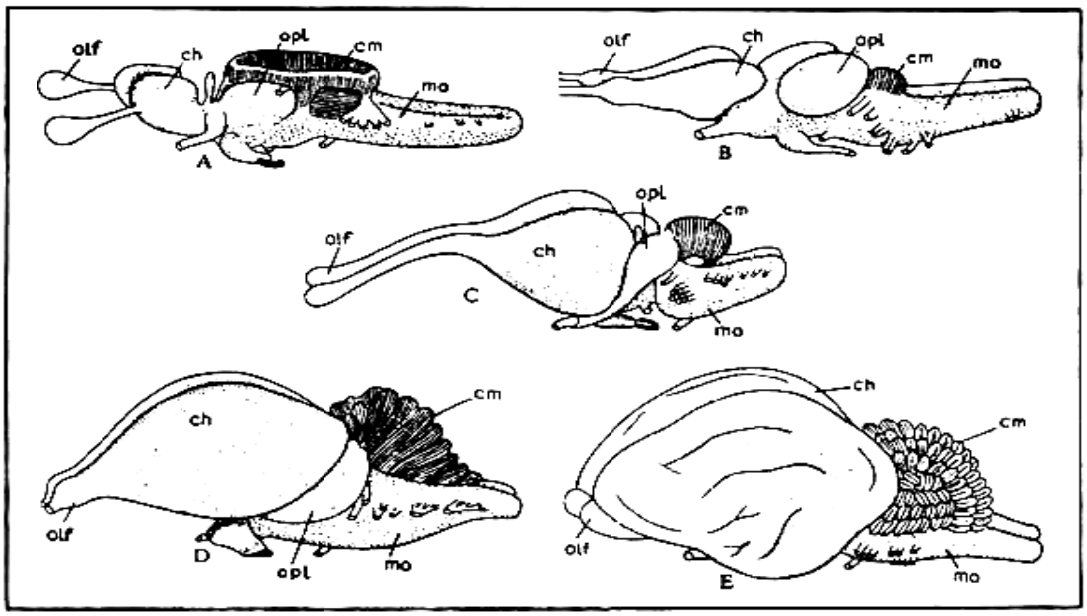


Fig. 10.154 : Showing the basic similarities in the structural organisation of brain in different vertebrates. Brain of fish (A), amphibia (B), reptile (C), bird (D) and mammal (E). Note the gradual aquisition of complexities in cerebral hemisphere, cerebellum and optic lobes. olf = olfactory lobe, ch = cerebral hemisphere, cm = cerebellum, opl = optic lobe and mo = medulla oblongata.

Structure of Human Spinal Cord:

Location and Coverings (Meninges):
It is a posterior part of central nervous system which runs mid-dorsally within the vertebral column. It lies in the neural canal of the vertebral column. The spinal cord is surrounded by the same three protective membranes (meninges) as found in the brain, viz., a thin innermost piamater, the middle webby arachnoid membrane (arachnoid mater) and the outer tough duramater.

The subarachnoid space is filled with cerebrospinal fluid. There is an additional space, the epidural space above the Dura mater. The epidural space contains fatty and connective tissues and veins.

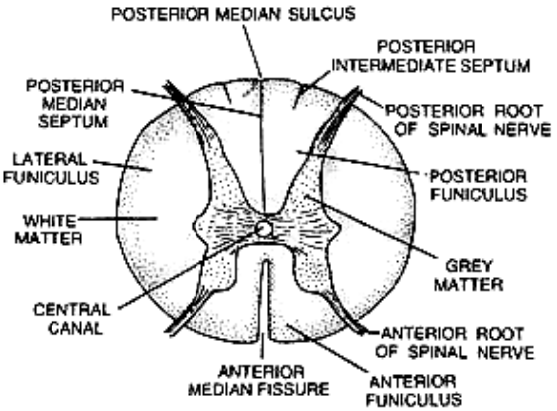


Fig. 21.12. T.S. Human spinal cord.

External Structure:
The spinal cord extends from the medulla oblongata. It is continuous, to the level of the second lumbar vertebra. In an adult the spinal cord is from 42 to 45 cm long. Its diameter varies at different levels, being enlarged in the cervical and lumbar regions.

The cord is also flattened. The cervical enlargement extends from the fourth cervical to the first thoracic vertebrae; it is the region from which nerves supplying the arms arise. It may seem strange that the lumbar enlargement should be in the thoracic region; this is the case because the spinal cord grows at a slower rate than the vertebral column.By adulthood the area within the vertebral column below the second lumbar vertebra contains spinal nerves that branch from the spinal cord at higher levels.

These spinal nerves are collectively called, the cauda equina, or "horse's tail". The spinal cord ends as the conus medullaris. The conus medullaris ends at the level of the intervertebral disc between the first and second lumbar vertebral in adults. Actually the conus medullaris is a conical portion of lower spinal cord.From the conus medullaris a fine connective tissue filament, the filum terminate, extends down to the coccygeal region. The filum terminate consists mostly of pia mater.

The spinal cord does not extend to the coccygeal region because during development the vertebral column elongates more rapidly than the spinal cord. The filum terminate anchors the spinal cord within the vertebral column. In-fact, the filum terminale is a long slender filament at the end of the spinal cord.

Internal Structure:
The internal anatomy of the spinal cord is best seen in cross section. Two indentations, the posterior median sulcus and the anterior median fissure, separate the spinal cord into left and right symmetrical halves. The inner butterfly-shaped area is the grey matter of the spinal cord.

Grey matter is so named because it lacks myelin and therefore, appears grey in an unstained preparation. Surrounding the grey matter are bundles of myelinated nerve fibres, called fasciculi or white columns, which together form the white matter of the spinal cord.In each segment of the spinal cord a spinal nerve arises from each side of the cord. Each spinal nerve connects with the cord through two nerve roots.

The dorsal nerve root consists of a bundle of sensory axons (carrying incoming signals) whose cell bodies are located in the dorsal root ganglion. These axons extend into the posterior horn of the grey matter, where they often form synapses with other neurons, some of which are called intemeurons.Interneurons, short neurons confined to the grey matter of the cord, form synapses with other intemeurons and with the motor neurons whose cell bodies are located in the anterior horn of the grey matter.

Aggregations of motor axons (carrying outgoing signals) from these cell bodies form the ventral nerve roots. The lateral horns lie between the anterior and posterior horns. The hollow central canal contains cerebrospinal fluid.

Spinal Tracts:
Along the white matter of the spinal cord there are two kinds of fasciculi, or bundles of axons, the ascending tracts, which carry sensory impulses to the brain, and the descending tracts, which carry motor impulses from the brain to the spinal nerves at various levels of the cord.

The human spinal cord is made up 31 segments. From each of these segments, a pair of spinal nerves takes origin.

There are 31 pairs of spinal nerves. Of 31 pairs:

i. 8 belong to cervical segments

ii. 12 belong to thoracic segments

iii. 5 belong to lumbar segments

iv. 5 belong to sacral segments

v. 1 belongs to coccygeal segment.

In a transverse section of spinal cord, in the central part will be H-shaped grey matter and this will be surrounded by white matter. The grey matter and white matter on either side have continuity through commissures (Fig. 9.14).

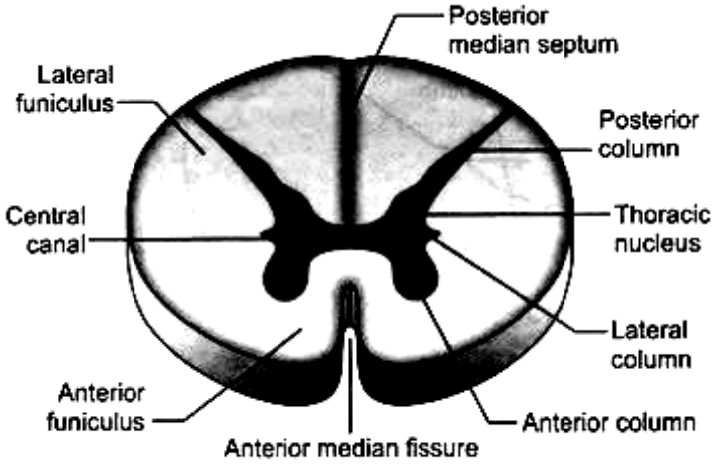


Fig. 9.14: Diagram of transverse section of spinal cord

In the grey matter, nerve cell bodies are present whereas the white matter is composed of compactly packed nerve fibers in the form of tracts. The tracts which carry impulses from peripheral parts of body towards higher parts of CNS through spinal cord are called ascending tracts. Likewise, tracts which carry information from higher parts of CNS to motor neurons present in spinal cord are called descending tract (Fig. 9.15).

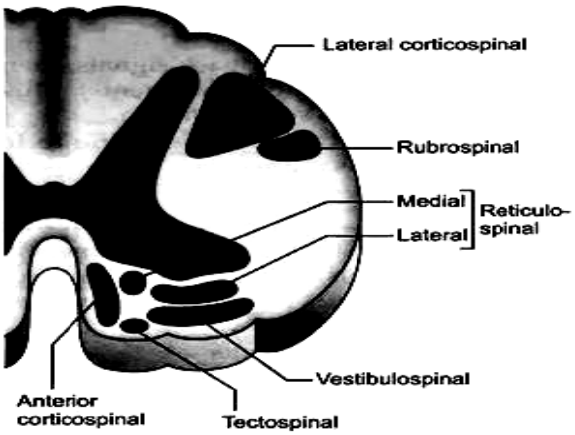


Fig. 9.15: Transverse section of spinal cord showing some of the important ascending and descending tracts

Structural and functional integrity of both grey matter and white matter areas is essential for normal functioning of nervous system. The cell bodies in grey matter area and tracts in white matter are both present in a bilaterally symmetrical way in spinal cord.

Ascending Tracts:
i. Carry information from spinal cord to higher parts of CNS.

ii. They are essential for sensory perception as impulses should reach brain from peripheral parts of body.

iii. Most of these tracts end in sensory areas of cerebral cortex.

iv. Some of the important ascending tracts are dorsal column tracts, lateral spinothalamic tract, anterior/ventral spin thalamic tract.

Dorsal Column Tract or Posterior Column Tract or Tract of Goll and Burdach or Fascicules Gracilis and Cuneatus:

Sensations carried by these tracts are:
1. Fine touch
2. Tactile localization
3. Tactile discrimination (2 point discrimination)
4. Pressure sensation
5. Vibratory sensation
6. Proprioception or sense of position and joint movement and is also known as kinesthetic sensation.
7. Stereognosis.

The above sensations from peripheral parts of body are carried by posterior column tracts to cerebral cortex. Nerve fibers carrying these sensations are A beta fibers.

Receptors involved will be:

a. Merkel's disk

b. Meissner's corpuscle

c. Pacinian corpuscle

d. Ruffini's end organ

e. Receptors in and around joints

Touch sensation has dual pathways. Fine touch sensation is carried by posterior column tracts and crude touch sensation is carried by anterior or ventral spinothalamic tract.

From the receptors, A beta fibers carry impulse. When posterior nerve fibers reach spinal cord, the nerve trunk of posterior spinal nerve separates into two divisions, namely medial and lateral divisions between dorsal root ganglion and spinal cord. The fibers going to contribute for formation of posterior column tracts enter spinal cord through medial division.

These fibers reach posterior funiculus of spinal cord and ascend up on same side of spinal cord. In the brainstem, at the level of medulla oblongata, these fibers synapse in two different nuclei, namely gracile and cuneate nuclei. First order neurons are posterior root ganglion cells.The second order fibers take origin from the nuclei of gracile and cuneate and cross midline and to reach opposite side. These fibers that cross are known as internal arcuate fibers (80%). Internal arcuate fibers will be contributing for sensory decussation. Approximately, 20% of fibers which takes origin from gracile and cuneate nuclei, do not cross midline and these are known as external arcuate fibers.

They carry impulses to cerebellum of same side by passing through inferior cerebellar peduncle. The internal arcuate fibers as they ascend up further to form medial lemniscus after crossing. Fibers of medial lemniscus synapse in ventroposterolateral nucleus present in thalamus. Neurons extending from gracile and cuneate nuclei to thalamus are called as second order neurons.Fibers which take origin from ventroposterolateral nuclei pass through posterior limb of internal capsule to end in sensory area (3, 1, 2) of cerebral cortex (primary somesthetic area) present in postcentral gyrus. These fibers which end in cerebral cortex are known as thalamic/sensory radiation fibers and constitute third order neurons (Fig. 9.16).

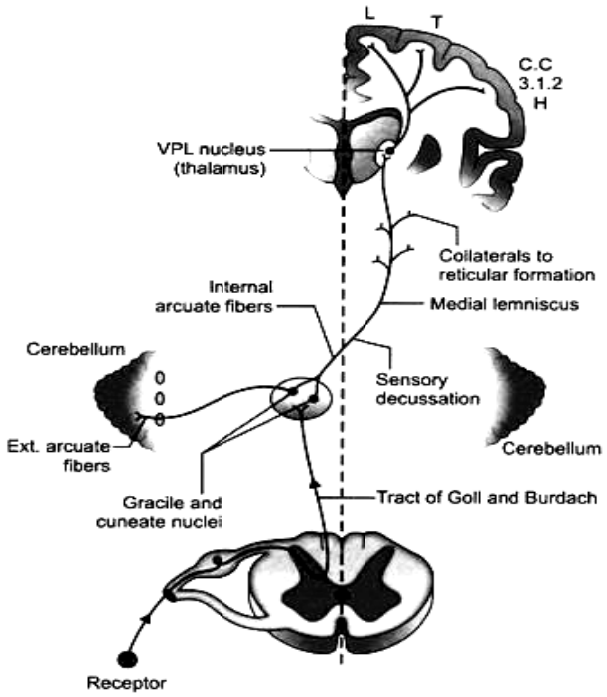


Fig. 9.16: Pathway carrying fine touch, tactile localization, etc. from peripheral parts of the body

Sensory Homunculus:

In uncrossed tract, sacral segmental fibers will be medial most and cervical fibers will be lateral most. Knowledge of topographical arrangement afferent fibers in different ascending tracts is essential in certain spinal cord lesions especially when lesion is due to degeneration of tissue around spinal canal (syringomyelia).

Stereognosis:

It is ability of person to identify some familiar/known objects even with closed eyes. The impulses for this sensation will be carried by posterior column tracts.

The person is able to identify object based on:

i. Shape

ii. Size

iii. Texture of object

Fine touch, tactile localization, and tactile discrimination, etc. from face.

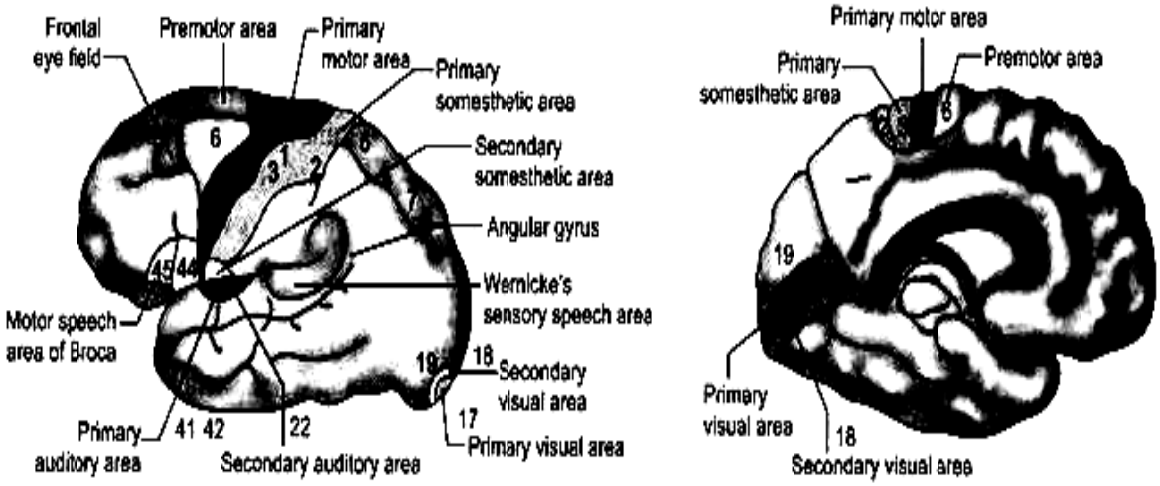


Fig. 9.17: Cerebral cortex showing some of the important areas

Fibers carrying sensations from receptor belong to 5th cranial nerve (carry sensory fibers from face). These fibers reach Gasserian ganglion. From ganglion, fibers enter pons to synapse in chief sensory nucleus. From chief sensory nucleus, 2nd order fibers take origin and cross the midline to reach the

opposite side. These fibers ascend upward as trigeminal lemniscus. They synapse in ventroposteromedial nucleus of thalamus.

From ventroposteromedial nucleus, 3rd order fibers originate. They end in lateral most part of cerebral cortex area number 3,1, and 2 present in post-central gyrus where face is represented (Fig. 9.19).

Fig. 9.18: Body representation in sensory cortex

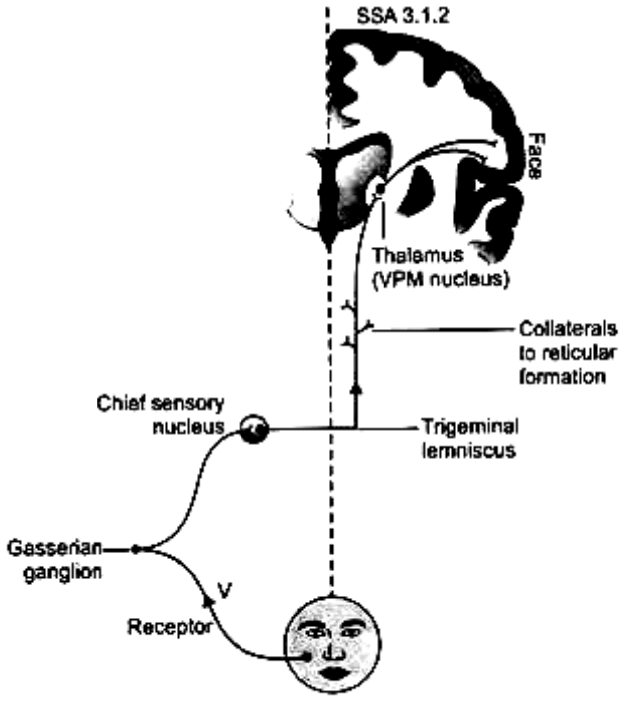


Fig. 9.19: Pathway for fine touch, tactile localization, etc. from face

Effects of Hemisection of Spinal Cord (when only one-half of spinal cord is sectioned) (Fig. 9.36):

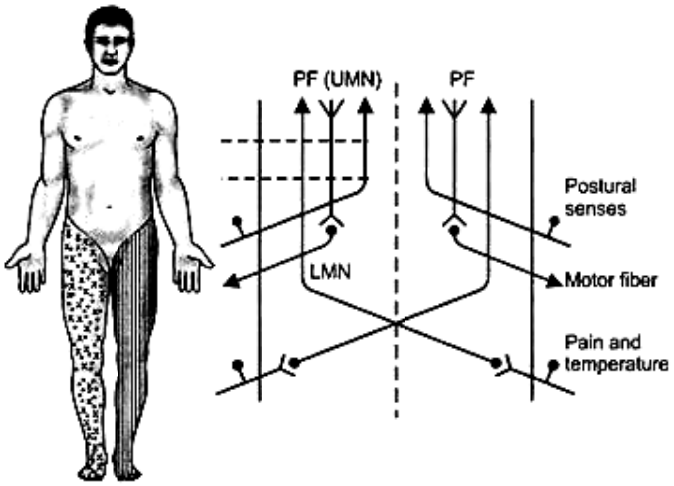


Fig. 9.36: Effect of hemisection of spinal cord

Features of effects of hemisection of spinal cord have been tabulated in Table 9.3.

Table 9.3: Effects of hemisection of spinal cord

Same side	*Opposite side*
Changes seen below the level of lesion	
Pain and temperature sensations remain intact as lateral spinothalamic tract carrying impulses cross and ascend up on contralateral half of spinal cord	Pain and temperature sensation is lost as lateral spinothalamic tract carrying from opposite half of body is damaged
Postural and other sensations are lost as posterior column tract fibers have not crossed but will cross only at level of medulla oblongata	Postural and other sensations are normal as posterior column tract which carry these impulses up ipsilaterally and has not got damaged
UMN type of paralysis because descending tracts are damaged	Normal because descending tracts are not damaged
At the level of lesion	
All sensations are lost in a dermatome, if 3 consecutive spinal nerves are damaged	Sensations will be normal
LMN lesion due to damage to anterior horn cells	Motor functions will be normal
Above the level of lesion	
Hyperesthesia due to irritation of sensory nerve fibers	No hyperesthesia

Dissociated anaesthesia:
It is a condition in which in a particular part of body, certain sensations are retained, and certain sensations are lost.

Dissociated anesthesia is seen in:
a. Hemisection of spinal cord below the level of lesion

b. Syringomyelia—pain and temperature is lost bilaterally but other sensations are retained in the affected parts of the body.

c. Tabes dorsalis—postural sensations are lost but pain and temperature is retained in the affected parts of the body.

Effect of Complete Transection of Spinal Cord:
It may occur due to accidental injury (motor vehicle, sports accidents), gunshots wound, stab injury to spinal cord. In these types of injuries, spinal cord will be cut into two different parts. Signs and symptoms are seen below level of lesion in both halves of body.

Effect on Skeletal Muscles:
i. When spinal cord is transected completely (for example at the level of T6 that is mid-thoracic level) part of body supplied below spinal segment T6 is affected. There will be complete paralysis of skeletal muscles in lower parts of body.

So no voluntary movements are possible in both lower limbs. Person suffers from paraplegia. Paraplegia results when there is transaction of spinal cord occurs anywhere above lumbosacral plexus origin but below the level of brachial plexus.

ii. If lesion is at level of C6 or C7, upper and lower limbs get paralyzed and hence result in quadriplegia (paralysis of all four limbs).

Effect on Blood Pressure [BP]:
i. If lesion is at T6 or anywhere between T6 and sacral segments, fall of blood pressure limited. Fall of blood pressure is not much since sympathetic nerves function above the level of section will be normal.

ii. If lesion is above T1, there will be severe fall of blood pressure since all lateral horn cells are separated from vasomotor centre which bring facilitatory impulses to lateral horn cells (sympathetic fibers take origin from LHC of thoracolumbar segments).

There will be complete loss of vasomotor tone.

Effect on Respiration:
i. If lesion is above C3, it leads to instantaneous death due to paralysis of all muscles of respiration.

ii. If lesion is below C5, diaphragmatic respiration continues, only intercostal muscles get paralyzed. (Intercostal muscles are supplied by nerve coming from T1 to T10 segments of spinal cord.)

Consequences of Complete Transaction of Spinal Cord:
1. Stage of spinal shock

2. Stage of recovery of reflex activity

3. Stage of reflex failure.

Functions of Spinal Cord:
(i) The stimuli are passed from and to the brain through the spinal cord.

(ii) It is the centre of spinal reflex action.

Reflex action:
What happens when you touch something hot or your finger is pricked by a needle? You immediately pull your hand away, without even thinking why you are doing so. Such sudden involuntary responses to stimuli are examples of reflex action. The response may be different when your conscious thought process is involved. For example, when a doctor pricks you with an injection needle to inject a

medicine into your arm, you do not withdraw your arm immediately.Your conscious thinking tells you that the medicine is being administered to cure your disease. In this case, a message from the spinal cord goes to the cerebrum, the thinking part of your brain, and your thinking brain directs your arm to bear the pain and not pull away.

The spinal cord is the centre of reflex action. Reflex actions are produced by reflex arcs, which may be formed anywhere along the spinal cord, nearest to the receptor and effecter. A reflex arc is formed by a sensory nerve and a motor nerve joined by a connecting nerve present in the spinal cord.As the impulses do not have to travel all the way to the brain and back, the detection of stimuli and the completion of responses are faster.

Reflex action is an extremely quick action, which does not involve any thinking by the brain. If someone hits your leg with a hammer the leg is immediately withdrawn. In this type of reflex action the impact of the hammer (stimulus) received by the receptor is sent to the spinal cord through the sensory nerve. The message is received by the connecting nerve in the spinal cord.The connecting nerve then sends a response through the motor nerve to the muscles (effectors) to pull the leg away. Thus, reflex action is a sudden, involuntary motor response to a stimulus. The flow of food in the alimentary canal, blinking in strong light or in response to a sudden movement in front of the eye, sneezing, coughing, yawning, hiccupping, shivering, etc., are also reflex actions.

Peripheral nervous system:

The peripheral nervous system includes 12 pairs of cranial nerves arising from the brain and 31 pairs of spinal nerves arising from the spinal cord. The nerves from the brain and the spinal cord connect the skeletal muscles and control their activity according to the directions and demands of the body. These nerves are, therefore, related to voluntary acts, i.e., they act according to our will.

Autonomic nervous system:

The autonomic nervous system controls and integrates the functions of internal organs like the heart, blood vessels, glands, etc., which are not under the control of our will.

The autonomic nervous system has two subdivisions: sympathetic and parasympathetic. The organs receive both sympathetic and parasympathetic nerves. The two types of nerves have opposite effects on the organs, i.e., if one is stimulatory, the other is inhibitory.

Meaning of Autonomic Nervous System:

It is the part of nervous system, which regulates functioning of visceral organs. Two different limbs of autonomic nervous system are sympathetic and parasympathetic nervous system components.

Sympathetic takes origin from lateral horn cells of thoracolumbar segments of spinal cord whereas parasympathetic takes origin from cranial (in brain) and sacral (in spinal cord) regions. In parasympathetic component, nerves included are cranial nerves 3, 7, 9 and 10 and also pelvic nerve from sacral segments of spinal cord (Fig. 9. 56).

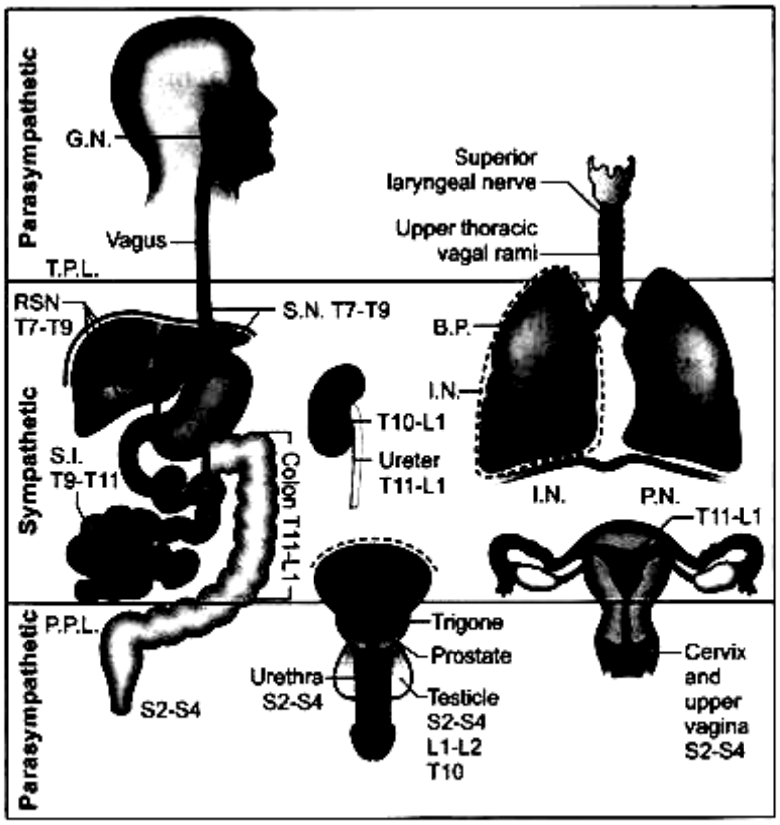


Fig. 9.56: Parts of the body that are influenced by sympathetic and parasympathetic activity

i. Centres for autonomic nervous system are present in hypothalamus.

ii. The anterior hypothalamus controls activity of parasympathetic and posterior hypothalamus that of sympathetic nerves.

iii. In any autonomic nervous system pathway, there are two neurons along efferent pathway. The preganglionic and postganglionic. The neurotransmitter at pre- and post-ganglionic regions in para-sympathetic part is acetylcholine.

In sympathetic, at preganglionic region, it is acetylcholine whereas in post-ganglionic region in most of parts of body it is noradrenaline. In some parts, at the postganglionic region of sympathetic nervous system, the neurotransmitter liberated is ACh.

iv. Acetylcholine can act through nicotinic or muscarinic receptors present in pre- and postganglionic regions respectively.

v. Noradrenaline can act through alpha or beta receptors.

vi. Alpha receptors are of two types namely alpha 1 and alpha 2 and likewise even beta receptors are of two types namely beta 1 and beta 2.

Difference between Sympathetic Nerve Fibre and Parasympathetic Nerve Fibre:

1. Sympathetic nerve arise from thoraco-lumber (T_1 to L_3) region of the spinal cord.

2. Ganglia are nearer to CNS. The ratio of pre and postganglionic fibres is generally 1: 20 or more. So, the post ganglionic fibers are longer.

3. Neurotransmitters are ACh (in ganglia) and norepinephrine (at neuro-effector junctions).

4. Sympathetic activity increases in stress and emergency.

5Parasympathetic nerves arise from the craniosacral (III, VII, IX, X, $S_2 – S_4$) region of the CNS.

6. Ganglia are away from CNS, and on or close to the organs. The ratio is generally 1: 1. So, the postganglionic fibres are shorter.

7. Neurotransmitters are ACh in both the ganglia and nouro-effector junctions.

8. Parasympathetic activity predominates during rest.

In most of the places (e.g., in smooth muscles of the gut, bladder, and heart) the sympathetic and parasympathetic systems produce opposite effects; but in some other organs only one system operates.

The sweat glands and most blood vessels, for example, have only a sympathetic innervation and the ciliary muscle of the eye has only a parasympathetic innervation. On the other hand, the two systems may produce similar, rather than opposite effects in an organ (e.g., salivary glands).

Functions of Autonomic Nervous System:

1. Regulation of functions of visceral organs, like heart, gastrointestinal tract, etc., and thereby help to regulate heart rate, blood pressure and gastrointestinal secretion and motility.

2. In lungs, bring about bronchodilation when acts through sympathetic nerve and vice versa effect, when parasympathetic nerve is acting.

3. Sweat glands are supplied by sympathetic cholinergic fibers and are involved in regulation of body temperature. In addition to this, during thermoregulation, goose pimples are formed due to piloerrection which is brought about by contraction of erector pilorum muscle.

4. Secretions from adrenal medulla are regulated by activity of sympathetic nerves (pelvic splanchnic nerve).

5. In eyes, pupillary dilation is by sympathetic nerve stimulation and constriction by parasympathetic nerve stimulation.

6. Defecation and micturition are possible due to activity of parasympathetic nerves stimulation.

7. In male reproductive system, erection of penis is due to parasympathetic stimulation and ejaculation of semen is because of sympathetic nerve activity.

How does the nervous tissue cause the muscles to act?

When an electrical signal from a nerve cell reaches a synapse it causes the axon bulb to release a chemical. This chemical, which is discharged at the junction between the nerve cell and the muscle cell, causes the cell membrane of the muscle cell to move some ions in the muscle cell. This triggers a series of changes, ultimately causing the muscle to contract or relax.

Probable Questions:

1. How does the nervous tissue cause the muscles to act?

2. What are the Functions of Autonomic Nervous System?

3. Describe Difference between Sympathetic Nerve Fibre and Parasympathetic Nerve Fibre.

4. What is autonomic nervous system?

5. Write a short note on Reflex action.

6. Describe the external and internal structure of spinal cord.

7. What are the differences between reptilian, avian and mammalian brain?

8. Write a short notes on ventricles of brain.

9. Give the structure and function of cerebellum.

10. Give the structure and function of mid brain.

11. What are the main functions of basal ganglia?

12. . Give the structure and function of fore brain.

13. write a short note on protective coverings of brain.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French

2. Textbook of Animal Physiology by Anjali Mishra.

3. Principles of Animal Physiology by Moyes and Schulte

4. Introduction To Animal Physiology by Kay Ian

Unit-V

Thermoregulation : Body temperature–Physical, chemical, neural regulation; acclimation and acclimatization

Objective: In this section you will learn Thermoregulation:Bodytemperature- Physical, chemical,neuralregulation;acclimationandacclimatization.

Introduction:

The regulation of body temperature by any means is called thermoregulation. One of the most important examples of homeostasis is the regulation of body temperature.

Not all animals can do this physiologically. Animals that maintain a fairly constant body temperature (birds and mammals) are called endotherms, while those that have a variable body temperature (all others) are called ectotherms. Endotherms normally maintain their body temperatures at around 35 - 40°C, so are sometimes called warm-blooded animals, but in fact ectothermic animals can also have very warm blood during the day by basking in the sun, or by extended muscle activity 9e.g. bumble bees, tuna). The difference between the two groups is thus that endothermic animals use internal corrective mechanisms, whilst ectotherms use behavioural mechanisms (e.g. lying in the sun when cold, moving into shade when hot). Such mechanisms can be very effective, particularly when coupled with internal mechanisms to ensure that the temperature of the blood going to vital organs (brain, heart) is kept constant.

In humans, body temperature is controlled by the thermoregulatory centerin the hypothalamus. It receives input from two sets of thermoreceptors :receptors in the hypothalamus itself monitor the temperature of the blood as it passes through the brain (the core temperature), and receptors in the skin (especially on the trunk) monitor the external temperature.

Both sets of information are needed so that the body can make appropriate adjustments. The thermoregulatory center sends impulses to several different effectors to adjust body temperature: Our first response to encountering hotter or colder condition is voluntary - if too hot, we may decide to take some clothes off, or to move into the shade; if too cold, we put extra clothes on - or turn the heating up! It is only when these responses are not enough that the thermoregulatory centreis stimulated. This is part of the autonomic nervous system, so the various responses are all involuntary.

When we get too hot, the heat loss centrein the hypothalamus is stimulated; when we get too cold, it is the heat conservation centreof the hypothalamus which is stimulated. Note that some of the responses to low temperature actually generate heat (thermogenesis), whilst others just conserve heat. Similarly some of the responses to cold actively cool the body down, while others just reduce heat production or transfer heat to the surface. The body thus has a range of responses available, depending on the internal and external temperatures. The thermoregulatory centre normally maintains a set point of 37.5 ± 0.5 °C in most mammals.

Mechanisms of thermoregulation

As a refresher, animals can be divided into endotherms and ectotherms based on their temperature regulation.

- **Endotherms**, such as birds and mammals, use metabolic heat to maintain a stable internal temperature, often one different from the environment.
- **Ectotherms**, like lizards and snakes, do not use metabolic heat to maintain their body temperature but take on the temperature of the environment.

Both endotherms and ectotherms have **adaptations**—features that arose by natural selection—that help them maintain a healthy body temperature. These adaptations can be behavioral, anatomical, or physiological. Some adaptations increase heat production in endotherms when it's cold. Others, in both endotherms and ectotherms, increase or decrease exchange of heat with the environment.

We will look at three broad categories of thermoregulatory mechanisms in this article:

- Changing behavior
- Increasing metabolic heat production
- Controlling the exchange of heat with the environment

An ectotherm (from the Greek ἐκτός (ektós) "outside" and θερμός (thermós) "hot"), is an organism in which internal physiological sources of heat are of relatively small or quite negligible importance in controlling body temperature. Such organisms (for example frogs) rely on environmental heat sources,which permit them to operate at very economical metabolic rates. Some of these animals live in environments where temperatures are practically constant, as is typical of regions of the abyssal ocean and hence can be regarded as homeothermic ectotherms. In contrast, in places where temperature varies so widely as to limit the physiological activities of other kinds of ectotherms, many species habitually seek out external sources of heat or shelter from heat; for example, many reptiles regulate their body temperature by basking in the sun, or seeking shade when necessary in addition to a whole host of other behavioral thermoregulation mechanisms. For home captivity as pet, reptile owners can use a UVB/UVA light system to assist the animals' basking behaviour.

In contrast to ectotherms, endotherms rely largely, even predominantly, on heat from internal metabolic processes, and mesotherms use an intermediate strategy.In ectotherms, fluctuating ambient temperatures may affect the body temperature. Such variation in body temperature is called poikilothermy, though the concept is not widely satisfactory and the use of the term is declining. In small aquatic creatures such as Rotifera, the poikilothermy is practically absolute, but other creatures (like crabs) have wider physiological options at their disposal, and they can move to preferred temperatures, avoid ambient temperature changes, or moderate their effects.Ectotherms can also display the features of homeothermy, especially within aquatic organisms. Normally their range of ambient environmental temperatures is relatively constant, and there are few in number that attempt to maintain a higher internal temperature due to the high associated costs

Behavioral strategies

How do *you* regulate your body temperature using behavior? On a hot day, you might go for a swim, drink some cold water, or sit in the shade. On a cold day, you might put on a coat, sit in a cozy corner, or eat a bowl of hot soup.

Nonhuman animals have similar types of behaviors. For instance, elephants spray themselves with water to cool down on a hot day, and many animals seek shade when they get too warm. On the other hand, lizards often bask on a hot rock to warm up, and penguin chicks huddle in a group to retain heat.

Some ectotherms are so good at using behavioral strategies for temperature regulation that they maintain a fairly stable body temperature, even though they don't use metabolic heat to do so.

Increasing heat production—thermogenesis

Endotherms have various ways of increasing metabolic heat production, or **thermogenesis**, in response to cold environments.

One way to produce metabolic heat is through muscle contraction for example, if you shiver uncontrollably when you're very cold. Both deliberate movements such as rubbing your hands together or going for a brisk walk and shivering increase muscle activity and thus boost heat production.

Non-shivering thermogenesis provides another mechanism for heat production. This mechanism depends on specialized fat tissue known as **brown fat**, or brown adipose tissue. Some mammals, especially hibernators and baby animals, have lots of brown fat. Brown fat contains many mitochondria with special proteins that let them release energy from fuel molecules directly as heat instead of channeling it into formation of the energy carrier ATP.

Controlling the loss and gain of heat

Animals also have body structures and physiological responses that control how much heat they exchange with the environment:

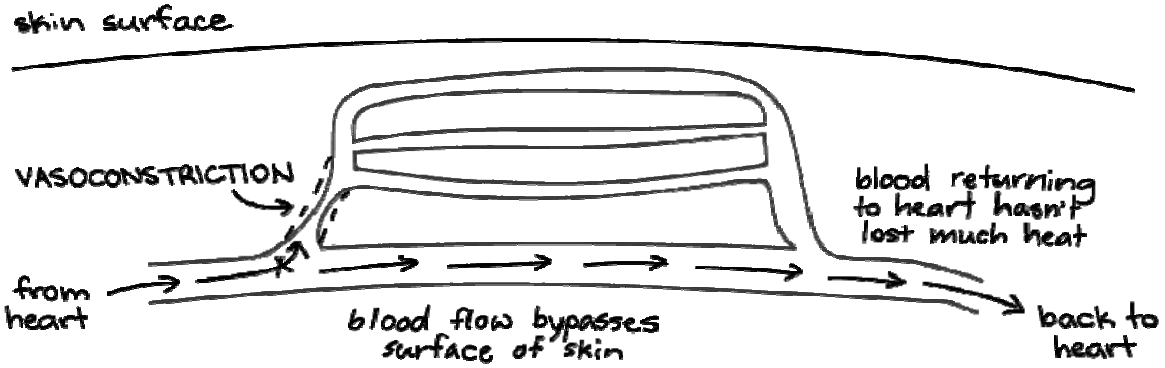
- Circulatory mechanisms, such as altering blood flow patterns
- Insulation, such as fur, fat, or feathers
- Evaporative mechanisms, such as panting and sweating

Circulatory mechanisms

The body's surface is the main site for heat exchange with the environment. Controlling the flow of blood to the skin is an important way to control the rate of heat loss to—or gain from—the surroundings.

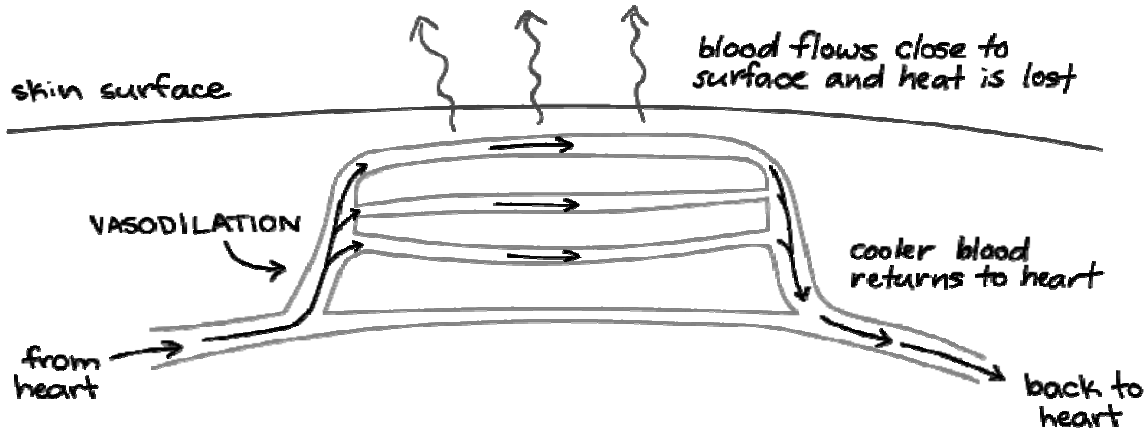
Vasoconstriction and vasodilation

In endotherms, warm blood from the body's core typically loses heat to the environment as it passes near the skin. Shrinking the diameter of blood vessels that supply the skin, a process known as **vasoconstriction**, reduces blood flow and helps retain heat.



A bed of capillaries near the surface of the skin is fed by a blood vessel that can be vasoconstricted—narrowed—or vasodilated—expanded—to control flow of blood through the capillaries. When it is cold, this blood vessel is vasoconstricted, and the blood coming from the heart does not enter the capillary bed, instead traveling through an alternative "shunt" blood vessel that lets it bypass the skin surface. Thus, the blood returning to the heart has not lost much heat.

On the other hand, when an endotherm needs to get rid of heat say, after running hard to escape a predator these blood vessels get wider, or dilate. This process is called **vasodilation**. Vasodilation increases blood flow to the skin and helps the animal lose some of its extra heat to the environment.



A bed of capillaries near the surface of the skin is fed by a blood vessel that can be vasoconstricted—narrowed—or vasodilated—expanded—to control flow of blood through the capillaries. When it is hot, this blood vessel is vasodilated, and the blood coming from the heart enters the capillary bed, avoiding an alternative "shunt" blood vessel that would let it bypass the skin surface. As it travels close to the skin, the blood loses heat to the cooler environment and is thus cooled by the time it exits the capillary bed on its way back to the heart.

Furry mammals often have special networks of blood vessels for heat exchange located in areas of bare skin. For example, jackrabbits have large ears with an extensive network of blood vessels that allow rapid heat loss. This adaptation helps them live in hot desert environments

Some ectotherms also regulate blood flow to the skin as a way to conserve heat. For instance, iguanas reduce blood flow to the skin when they go swimming in cold water to help retain the heat they soaked up while on land.

Countercurrent heat exchange

Many birds and mammals have **countercurrent heat exchangers**, circulatory adaptations that allow heat to be transferred from blood vessels containing warmer blood to those containing cooler blood. To see how this works, let's look at an example.

In the leg of a wading bird, the artery that runs down the leg carries warm blood from the body. The artery is positioned right alongside a vein that carries cold blood up from the foot. The descending, warm blood passes much of its heat to the ascending, cold blood by conduction. This means that less heat will be lost in the foot due to the reduced temperature difference between the cooled blood and the surroundings and that the blood moving back into the body's core will be relatively warm, keeping the core from getting cold.

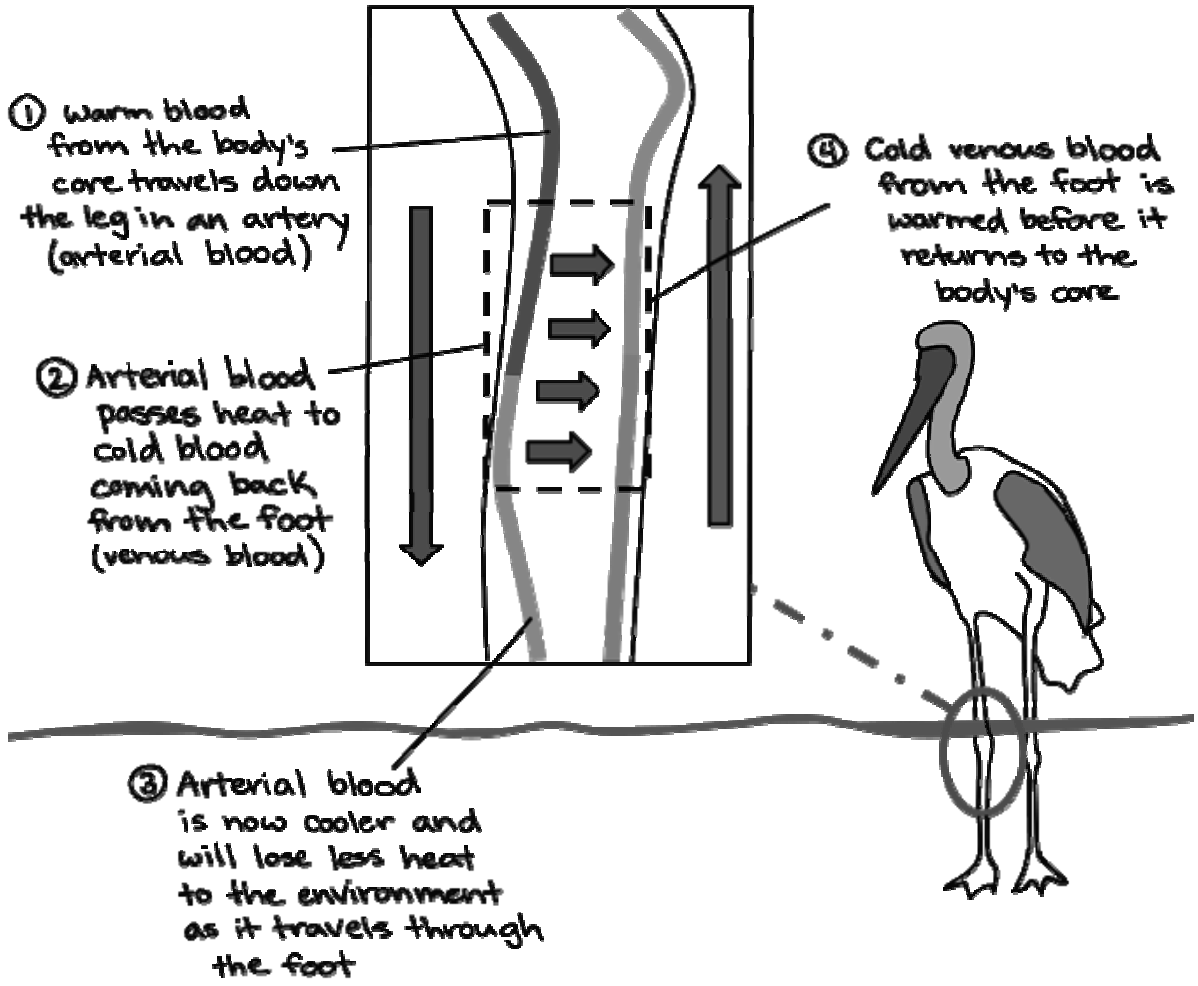


Diagram of blood vessel arrangement in the leg of a wading bird

1. Warm arterial blood from the body's core travels down the leg in an artery.
2. Arterial blood passes heat to cold venous blood coming back from the foot.
3. Arterial blood is now cooler and will lose less heat to the environment as it travels through the foot.
4. Cold venous blood ascending from the foot is warmed before it returns to the body's core.

Insulation

Another way to minimize heat loss to the environment is through **insulation**. Birds use feathers, and most mammals use hair or fur, to trap a layer of air next to the skin and reduce heat transfer to the environment. Marine mammals like whales use blubber, a thick layer of fat, as a heavy-duty form of insulation.

In cold weather, birds fluff their feathers and animals raise their fur to thicken the insulating layer. The same response in people—goosebumps—is not so effective because of our limited body hair. So, most of us wear a sweater!

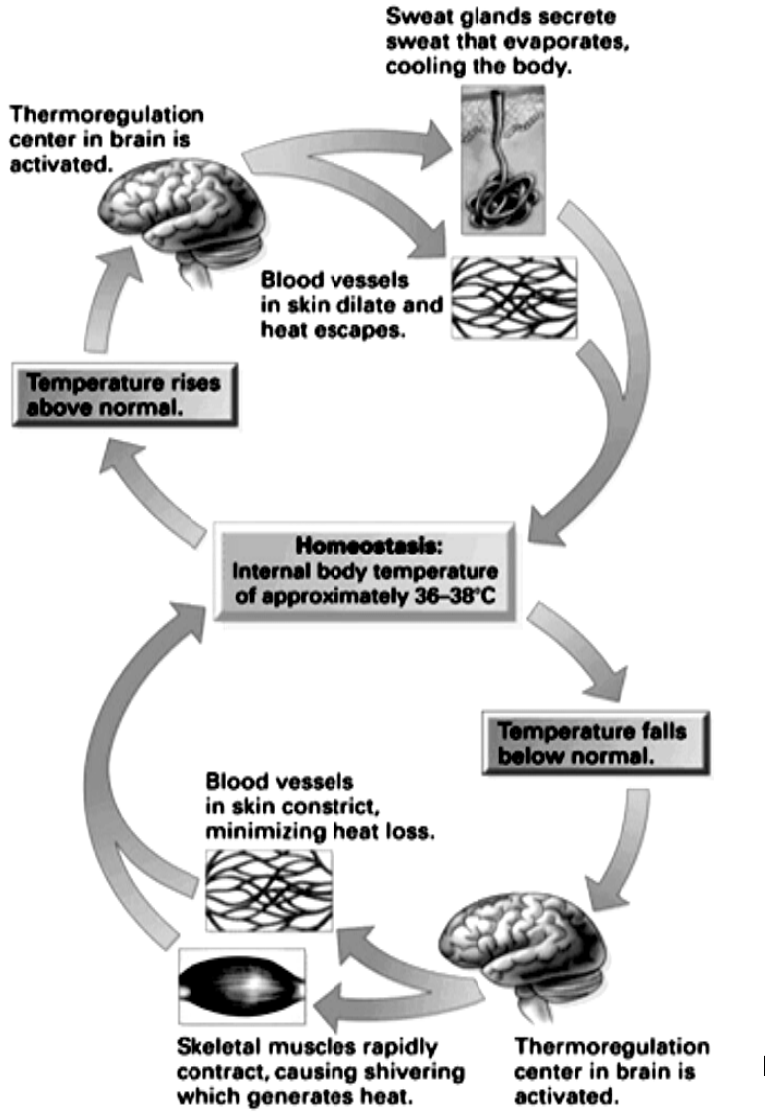
Evaporative mechanisms

Land animals often lose water from their skin, mouth, and nose by evaporation into the air. Evaporation removes heat and can act as a cooling mechanism.

For instance, many mammals can activate mechanisms like sweating and panting to increase evaporative cooling in response to high body temperature.

- In sweating, glands in the skin release water containing various ions—the "electrolytes" we replenish with sports drinks. Only mammals sweat.
- In panting, an animal breathes rapidly and shallowly with its mouth open to increase evaporation from the surfaces of the mouth. Both mammals and birds pant, or at least use similar breathing strategies to cool down.
  In some species, such as dogs, evaporative cooling from panting combined with a countercurrent heat exchanger helps keep the brain from overheating.

Thermoregulation mechanism



Effector	Response to low temperature	Response to high temperature
Smooth muscles in arterioles in the skin.	Muscles contract causing vasoconstriction. Less heats carried from the core to the surface of the body, maintaining core temperature. Extremities can turn blue and feel cold and can even be damaged (frostbite).	Muscles relax causing vasodilation. More heats carried from the core to the surface, where it is lost by convection and radiation (conduction is generally low, except when in water). Skin turns red.
Sweat glands	No sweat produced.	Glands secrete sweat onto surface of skin, where it evaporates. Since water has a high latent heat of evaporation, it takes heat from the body. High humidity, and tight clothing made of man-made fibers reduce the ability of the sweat to evaporate and so make us uncomfortable in hot weather. Transpiration from trees has a dramatic cooling effect on the surrounding air temperature.
Erector pili muscles in skin (attached to skin hairs)	Muscles contract, raising skin hairs and trapping an insulating layer of still, warm air next to the skin. Not very effective in humans, just causing "goose bumps".	Muscles relax, lowering the skin hairs and allowing air to circulate over the skin, encouraging convection and evaporation.
Skeletal muscles	Shivering: Muscles contract and relax repeatedly, generating heat by friction and from metabolic reactions (respiration is only 40% efficient: 60% of increased respiration thus generates heat).	No shivering.
Adrenal and thyroid glands	Glands secrete adrenaline and thyroxin respectively, which increases the metabolic rate in different tissues, especially the liver, so generating heat.	Glands stop secreting adrenaline and thyroxin.
Behaviour	Curling up, huddling, and finding shelter, putting on more clothes.	Stretching out, finding shade, swimming, removing clothes.

ACCLIMATION & ACCLIMATIZATION

Acclimation: When an animal is subjected to changed condition in laboratory, animal shows compensatory changes to new environment. These changes are known as **acclimation**. In other words acclimation is adjustment to laboratory conditions.

Types of acclimation: In case of temperature regulation acclimation is of two types

i) Cold Acclimation

ii) Hot or warm Acclimation

Acclimatization – Compensatory adjustment of organism to change in the environment, in nature or natural condition is known as acclimatization.

Adjustment to seasonal changes in temperature comes under acclimatization.

• Normally when an animal is exposed to a **Ta** lower than normal then initially the body temperature **Tb** drops, thereafter the animal compensates and tries to bring the **Tb** to normal.

• When exposed to hot **Ta** the **Tb** initially increases and then the animal tries to bring it to normal through compensation.

• As shown in the figure when an animal from normal temperature ta is subjected to cold ta, then there is initial drop in body temperature as shown by position 1. If the animal is exposed to hot environment there is initial rise in body temperature shown by pattern 1. If the body temperature (tb) from 1 settles to 0 then the compensation is known as "**perfect compensation**" & acclimation as **"Perfect Acclimation".**

• If the temperature settles between 1 & 0 i.e. 2 then compensation is not full proof so known as '**partial compensation**' & hence known as "**partial Acclimation**"

• If the body temperature (tb) settles to pattern 4 i.e. in cold Ta the body becomes warmer than normal & in hot Ta it become cooler than normal then the compensation is known as "**over shoot**" or "**excess compensation**".

• And if the body temperature as shown from pattern 1 changes to 3 i.e. in cold it further drops & in hot environment it further rises then the compensation is not there or it indicates "no compensation." The animals, depending upon their genetic ability show different type of compensation. However it is difficult to generalize about compensation because there are too many variations in acclamatory compensation.

• Many animals show compensatory changes in particular range of temperature.

• So also the acclimation depends on physiological state of animal such as hormonal state, health, injuries etc.

• Not only the animals show different type of acclamatory compensation. It is found that in an animal different tissues show different type of acclamatory compensations. This is of course is related to the importance of the tissue in the body.

• As shown in diagram below a fish trout when acclimated to 16 to 80^0C temperature following Precht"s pattern was seen in which brain showed perfect acclimation, liver showed over shoot compensation, whole body showed partial compensation & gills showed no compensation.

• It is generally observed that the animals which show torpidity & hibernation or are poor in compensatory acclimation. Whereas the animals that remain active in extreme environment are better in compensatory acclimation.

• The animals which live in tropics & polar environment enjoy stable environment & hence are poor in compensatory acclimation as compared to those living in temperate environment which have more temperature fluctuations. Changes that occur during Acclimation & Acclimatization.

Physiological changes that take place in Acclimation & Acclimatization:

• In cold acclimation protein & enzyme secretion is more as compared to hot acclimation. All the changes during acclimation are not understood except the following:

i) Protein & phospholipid synthesis & turnover.

ii) Synthesis of isozymes suitable for new environment.

iii) Modification of membrane lipids.

• Normally membrane lipids become unsaturated fats so that they remain fluid in cold condition.

• Likewise animals show behavioral responses (refer to Gradient selection in poikilotherms) Cold Acclimation

• When birds or mammals are kept at low temperature in laboratory, initially their body temperature falls down but then they show rise in metabolic rate, rise in O_2 consumption. This change is proportional to change in temp.

• As oxidation is faster, the food intake rises. The enzymes in liver, muscles and mitochondria rise. Glucose, pentose, fatty acid pathways are mobilized. They become faster. Adrenal & thyroid secretion rises. Most important is that the peripheral circulation rises to keep skin warm & in them normally insulation by fur coat does not change.

Cold Acclimatization

• This is a slow process & is seasonal. The change is prolonged & gradual & hence the compensatory changes are different. In nature when change occurs, the animal shows changes in thickness of fur coat. They reduce the peripheral circulation & both these prevent heat loss.

• This is because in environment with approach of winter there is scarcity of food.

• It is found that the thickness of fur rises in large animals.

• In them fat is mobilized & unsaturated fat gets deposited in joints & extremity so that the flexibility is maintained.

• Their CNS, tissue, nerves gradually gain resistance to stand the cold. Sparrow gain resistance at rate 60 drop / month. Sensitivity of tissues is increased. There is higher sensitivity of the tissues to neurotransmitter or the transmitter subs.

• Along with these the animal show behavioral changes in them e.g., Dog, rats, bees show aggregation phenomenon in cold season.

Probable Questions:

1. Define thermoregulation.

2. write down the difference between ectothermy and endothermy.

3. What is thermogenesis. Briefly describe the mechanism of thermogenesis.

4. Describe counter current heat exchange.

5. Define acclimation and acclimatization.

6. describe behavioral adaptations for heat loss.
7. Describe physiological changes during acclimation and acclimatization.
8. Describe the role of vasoconstriction and vasodilation in thermoregulation.
9. Write down the response to high and low temperature with a chart.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

HARD CORE THEORY PAPER (ZHT 205)

Structure and Function of Chordate & Biosystematics and taxonomy

Group B:Biosystemeatics and taxonomy

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 205 (Structure and Function of Chordate & Biosystematics and taxonomy)	VI	Species concept: Evolutionary and biological species concept; difficulties in application of biological species concept.	1.5	1	1	91-98
	VII	Theories of biological classification: classification and phylogeny-types of classification, hierarchic classification.		1	1	99-112
	VIII	Zoological nomenclature, basic knowledge of naming genus and species.		1	1	113-121
	IX	Phenetic method of classification, numerical phenetics and numerical taxonomy.		1	1	122-127
	X	Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidi andistance); Cluster analysis (different methods).		1	1	128-137
	XI	Cladistic method of classification, difference in the application of phenetic and cladistic classification; cladistic and cladogram, eludistic methods, application of parsimony.		1	1	138-144
	XII	Morphological taxonomy and molecular taxonomy; Construction of phylogenetic trees using molecular data.		1	1	145-160

Unit- VI

Speciesconcept: Evolutionary and biological species concept; difficulties in application of biological species concept.

Objective: In this unit you will learn Species concept: Evolutionary and biological species concept; difficulties in application of biological species concept.

Introduction:

Linnaeus (1735) conceived "species" as a unchangeable unit. This monotypic or static concept prevailed till the 19th century. The system of classification followed by Linnaeus is an artificial system. While defining a species only the morphological characters were considered by him. Later Lamarck (1809) and Darwin (1859) put forwarded their evolutionary thoughts.As a result the monotypic concept was replaced by polytypic or dynamic concept. The latter concept states that the species undergo modification in course of time, in order to adapt themselves to the ecological niches and may gradually form another species under favourable conditions.
But in recent years, the approach of biology has radically changed. Today, while ascribing characters, physiological, genetic, ecological and phylogenetic points are taken into consideration. These new insights have moulded the idea of species.

Dobzhansky (1937) has defined the species as **"a group of individuals which while passing through the ordeal of evolution has been physiologically and genetically incompatible of inbreeding with other group of individuals"**. Emerson (1941) proposed that "a species is that which has evolved by reproductive isolation and a genetically distinct group of natural population".Mayr (1963) called a species as **"groups of actually or potentially interbreeding natural populations which are morphologically distinct and reproductively isolated from the neighbouring natural groups".**
Authorities on this line, however, laid much less emphasis on morphological distinctness but have given much emphasis on reproductive isolation. Mayr's definition of the species is based upon the biological parameters, such as reproductive isolation and a common gene pool, hence it is called biological species concept.Simpson (1961) viewed species as "a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own evolutionary role and tendencies".

This is the evolutionary concept of species in which the time factor has been added to and the concept has laid much emphasis on phyletic lineage. Christoffersen (1995) proposes that **"a species is a single lineage of ancestral-descendant sexual populations genetically integrated by historically contingent events of interbreeding".** This is the ontological species concept.
The most important finding has come from the studies of genetics. It is known that all the morphological, physiological and ecological characters are based on genes which are present over the chromosomes.The difference between one species and another, rests on the genetic make-up. In certain cases, e.g., butterfly, it has been recorded that individuals having similar chromosomal make up (i.e., members of the same species), exhibit differences in morphological features. Such variations within the same species are now considered as infraspecies, which are not taxonomically recognised.

It is propounded that the expression of characters of a particular species is not due to a definite set of genes in one individual but a collective gene pool of all the inbreeding individuals. The species today is thus regarded as "the sum of all its variations".With the new concept of species in mind modern taxonomists want to establish a natural system of classification.

The goal of which is:
(1) To establish correct interrelationship amongst the existing forms (horizontal classification) and

(2) To find out the relationship of existing forms with their ancestral forms (vertical classification).

This has made the task more complicated. Two factors—difficulty in the proper assessment of characters and non-availability of suitable fossil materials, are acting as hindrances to complete the classification.

For this reason in various instances (e.g., insect) morphological features and phylogenetic speculations are still used, which has resulted into the coming of a mixed system of classification. The mixture is between natural system and artificial system.

Types of Species:
Various types of species are recognized, of which the followings are:

(i) Allopatric species (Gk. allos = other, patris = native land):
The two or more related species that have disjunct geographical ranges are called allopatric species. Examples of such species are Indian lion (*Panthera leo persica*) and African lion (*Panthera leo leo*).

(ii) Sympatric species (Gk. syn = with, together):
Two or more species are said to be sympatric when their geographical distributions overlap, though they may segregate into different ecological niche. Examples of this type are the fig-frog (*Rana grylio*) and the gopher frog (*R. areolata*). The former is extremely aquatic, while the latter species is restricted to the margins of swampy areas.

(iii) Parapatric species:

These are the species which have the geographical ranges with a very narrow region of overlap. Example of this type is the flightless Australian grasshoppers, *Moraba scurra* and *M. viatica*. Parapatric species are formed in nature mostly through chromosomal rearrangements.

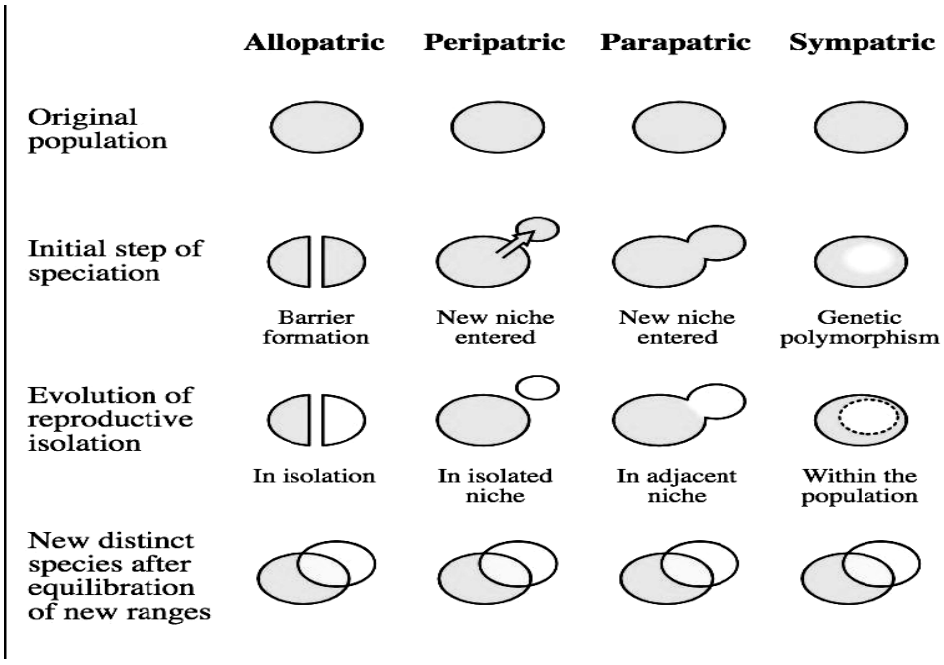


Fig: Schematic diagram of Different types of speciation

(iv) Sibling species:

Two or more than two closely related species which are morphologically alike but behaviourally or reproductively isolated from each other. Examples are *Drosophila persimilis* and *D. pseudoobscura*. The mosquito *Anopheles maculipennis* complex consists of several subspecies, of which a few are vector of malaria and the rest are harmless.

(v) Cryptic species:
The species which are alike on the basis of observed features but are genetically and sexually they are different are cryptic species. There is a confusion between the terms sibling species and cryptic species. The cryptic species are incapable of interbreeding but the sibling species can interbreed and are incapable of producing fertile hybrids.

(vi) Monotypic species:
When a genus includes a single species but does not include any subspecies, e.g., *Vampyroteuthis*, a vampire squid which is a single monotypic genus and also contains a single species, *V. infernalis* (monotypic species). Blackwelder (1967) states that the species with a single subspecies, called monotypic species.

(vii) Polytypic species:
When a species contains two or more subspecies, it is called polytypic species. Examples are tiger, *Panthera tigris* which has several subspecies; such as—(i) Indian tiger, *Panthera tigris tigris,* (ii) the Chinese tiger, *P. t. amoyensis*, (iii) the Siberian tiger, *P. t. altaica*, (iv) the Javan tiger, *P. t. sondaica,* etc.

(viii) Endemic species:
The species which are found in a particular region, called endemic species. Usually the species of oceanic islands which are found in a limited geographic area are called endemic species. The Darwinian finches are the endemic species of Galapagos Islands. The lungfish, *Neoceratodus forsteri* occurs in Mary and Burnett rivers of Queensland in Australia, is an example of endemic species.

(ix) Transient species:
Species among contemporaneous organisms, fossil or recent, called transient species. Blackwelder (1967) has defined that the species are the ones existing contemporaneously, as a cross section of the lineages of evolutionary species.

(x) Agamo species:
Species are those which consist of uniparental organisms. They may produce gametes but fertilization does not take place. They reproduce by obligatory parthenogenesis. In case of bees, wasps, rotifers the haploid eggs develop into haploid individuals and the haploid eggs are not fertilized by sperms.

(xi) Panmictic species:
Species in which a single interbreeding population occurs.

(xii) Apomictic species:
Species in which there is mixing of gametes between different individuals.

(xiii) Incipient species:
A natural population which are about genetically isolated from the rest of population of the parent species due to geographical barrier but has not accomplished all qualities for reproductive isolation from the parent population.

There are three main concepts of species.

The concepts are:

1. Morphological Species Concept

2. Genetic Species Concept

3. Biological Species Concept.

1. Morphological Species Concept:

This is the traditional concept of species. It was originally introduced by Carolus Linnaeus in his Systema Naturae in 1758. According to this concept, a population group of morphologically distinct organisms constitutes a species.

The main objections to this concept are that it does not take into consideration:

(i) The range of variation in size, colour, form & weight.

(ii) The genetic diversity

(iii) The common origin of related species and

(iv) The change of species in time.

Morphological species concept does not take into account of pronounced sexual dimorphism exhibited by different organisms. For example, the males and females of river duck, the mallard, were originally placed under separate species. The males were described as *Anas boschas* and females as *A. platyrhyechos*.In many other birds like birds of paradise, humming birds, wood warblers etc. females differ more from the males of their own species than from the females of other related species. In many deep-sea angler fishes of the family Ceratioidei, the males are much smaller in size than females, live as parasitic forms upon them. Such dwarf males, originally mistaken as separate species, attach themselves by the mouth of the female and feed up their body fluids.

Insects and lower invertebrates like rotifers, echiuroids like Bonellia exhibit more pronounced morphological differences in the two sexes. Larval stages of several vertebrates and invertebrates are so markedly different from their parents that they were very often placed in separate species.

2. Genetic Species Concept:

According to Lotsy (1918) a species is a group of genetically identical individuals. This view was supported by some geneticists. But this definition of species is incorrect because even the off-springs of same parents have different genetic constitution. Only identical twins are genetically similar.

3. Biological Species Concept:

This is the modern concept of species proposed and developed by Dobzhansky in 1937 and Mayr in 1942. According to Dobzhansky mendelian population sharing a common gene pool constitute a species. The most uptodate and convincing definition of species has been given by Mayr (1942) to his book “Systematics and the origin of species”. According to him, species are the groups of actually or potentially interbreeding natural populations that are reproductively isolated from each such groups.

The general characters of animal species may be summarized as follows:

1. Each species possesses a common gene pool.

2. Each species is in a process of continually adjusting to its environment.

3. Each species fills an ecological niche not exactly utilized by another species.

4. Each species possesses a constellation of isolating mechanisms that indirectly or directly prevent exchange of genes with related species.

5. Each species has the capacity to give rise to new species.

Shortcomings of the Biological Species Concept :

(i) Lack of information:
Due to lack of proper information systematists face some problems when applied to some cases.

(a) The morphological differences are observed due to sexual dimorphism, age differences and genetical polymorphism and individual variation can be unmasked through the study of life history and through the population analysis. The taxonomists mostly work on preserved museum specimens. So reproductive isolation is not verified in the preserved specimens. Again biological species concept is not applicable in fossil specimens.

(b) The closely related two populations live in a continuous area but show preferences for different habitats. In this case, two populations fail to interbreed due to living in different habitats. So it is difficult to apply the biological species concept on these populations because these populations are either distinct species or failure of interbreeding due to living in different habitat.An example of drongo birds is recorded in central Africa. Species A, *Dicrurus ludwigii* are found in the evergreen rainy forest areas and species B, *D. adsimilis* are found in the open grassy land areas. They live in two ecological niches with a distance of 50 m apart and do not interbreed.

(ii) Apomictic or asexual groups:
Biological species concept is not applicable in apomictic species (i.e., asexually reproducing groups) that do not fulfil interbreeding criterion which is the most important characteristic feature in biological species concept. Apomictic groups show uniparental reproduction by parthenogenesis, apomixes and budding, etc.

Uniparental reproduction is seen in lower invertebrates and lower vertebrates. The descendants of apomictic groups are termed agamospecies or binoms, paraspecies but Ghiselin (1987), Mayr (1988a) stated that these are not considered as 'species'.To solve this dilemma, Simpson (1961), Mayr (1963, 1969) and M.J.D. White (1978) discussed the problem on the basis of discussion of Dougherty (1955) and Stebbins (1966).

Attempts to define agamospecies or asexual species with or without using the word population have not been successful. There are well defined morphological discontinuity among the uniparentally reproductive organisms. These discontinuities are produced by natural selection among the various mutants which occur in asexual clones.

(iii) Sibling or Cryptic species:
Biological species concept is not applicable in sibling or cryptic species because members of sibling or cryptic species are all alike, not separated morphologically but reproductively isolated populations.

(iv) Incompleteness of speciation:
Evolution is a gradual and continuous process. To attain a new species, especially three attributes are necessary, such as reproductive isolation, ecological difference and morphological differentiation. There are many species which represents an incomplete stage during speciation. To apply the biological species concept in these cases becomes difficult.

(v) Hybridization:
According to biological species concept, two good species fail to interbreed. If the reproduction isolation breaks down, the two good species interbreed and produce fertile hybrid.

4. Evolutionary Species Concept:
Not all taxonomists specially paleontologists are not satisfied with the biological species concept. They preferred a definition of species which are related to the evolution.Simpson (1961) has proposed a definition with many modifications that is **"an evolutionary species is a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies".**

Simpson has stated that the above definition not only is consistent with biological or genetical concept of species but it helps to clarify and to remove some limitations of the biological species concept. Mayr (1982) has stated that the above definition is related to the phyletic lineage, not indicates a species concept.The evolutionary concept is applicable only to the isolated population and incipient species but not applicable to a single species. Simpson tried to solve the species definition by adding the time dimension in this species definition. Reif (1984) and Mayr (1987) have stated that there are many demerits in evolutionary species concept.Wiley (1978) has provided a revised definition of evolutionary species concept. He stated that "an evolutionary species is a single lineage of ancestral-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate". Mayr and Ashlock (1991) stated that the concept has developed on the basis of a species taxon, not of the species category.Christoffersen (1995) proposed the ontological species concept that is "a species is a single lineage of ancestral descendant sexual populations genetically integrated by historically contingent events of interbreeding". This definition of Christoffersen has given stress on the interbreeding nature of a species.

Subspecies and varieties:

Subspecies is an aggregate of local breeding populations of a given species which has become recognizably different from another population of the same species. A subspecies usually differ from other similar breeding groups of the same species both taxonomically and with respect to certain gene pool characteristics.The subspecies name is written immediately after species name. So the whole constitute a trinominal.The term variety was used to describe the non-genetic variants of phenotype caused by the climatic effects. But now the term subspecies replaces the term variety.

a. Clines:
The term 'cline' was introduced by Huxley (1939). It refers to a gradient (decrease or increase) within a continuous population. The regular or continuous variations occur for genotypes (genocline) or phenotypes (phenocline).One of the best examples of clinal gradation is exhibited by the meadow frog of North America, *Rana pipens*. It is found throughout the Paire grass land and includes a number of

temperature adapted races, which exhibit an orderly variability and adaptability. When the population of North and South extremes are compared the differences are pronounced, yet between the two extremes, there is no break in variation.

b. Demes:

The term 'Deme' was introduced by Gilmour and Gregor in 1939. A deme is a community of potentially interbreeding individuals at a given locality which share a single gene pool. The term deme is always used with a prefix which characterize the deme more precisely.

1. **Topedeme** – a group of individuals existing in a certain geographic region.

2. Ecodeme – a group of individuals associated with a specific habit

3. Phenodeme – a deme differing phenotypically from others.

4. Genodeme – a deme differing from others in genotype.

5. Plastodeme – a deme differing phenotypically from others owing to the effect of environment.

c. Sibling species:

Mayr (1952) has used the term sibling species for the sympatric populations that are morphologically similar if not identical, but are reproductively isolated.

Sibling species occur in almost all animal groups. But they are more common in insects. A best studied example is the genus Drosophila. *Drosophila pseudoobscura* and *D. persimilis* are so identical in their morphology that these were described as two races of the same species (race A and race B) by Lancefield (1924).The salivary gland chromosomes of both these differ, in the arrangement of genes and also the banding patterns. Morphological differences also exist in the sex comb, male genitalia and relative wing size. These two races of flies coexist in nature over a wide area without natural hybridization.Artificial crosses between race A and race B produce F1 hybrids, of which only females are fertile and the males are sterile. Lancefield found that sterility is due to the differences in the Y-chromosome. In race A, Y chromosome is sub-metacentric and in race B, Y is a metacentric.With these observations, these biological races are now established as two distinct species and are described as 'Sibling species'. In addition to morphological and chromosomal differences, these two species exhibit differences in ecological, physiological and sexual behaviours.

d. Monotypic and polytypic species:

Species containing only a single subspecies are called ‘monotypic’ while those containing more than one species are termed ‘polytypic’.

Probable questions:

1. Define species.

2. Describe different mode of speciation with a schematic diagram.

3. Describe morphological and biological species concept with suitable examples.

4. what are the shortcomings of Biological Species concept.

5. What do you mean by sibling species, Agamo species, Apomictic species, Panmictic species and Incipient species.

6. Describe different types of subspecies categories.

Suggested Readings:

1. Darwin, C. 1859. On the Origin of Species. London: John Murray (always seek out the first edition, facsimile version, and avoid later editions).

2. Dobzhansky, T. 1937. Genetics and the Origin of Species. New York: Columbia Univ. Press (there are several later editions, and the title changed in the last).

3. Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford: Oxford Univ. Press.

4. Mayr, E. 1942. Systematics and the Origin of Species. New York: Columbia Univ. Press.

5. Simpson, G. G. 1944. Tempo and Mode of Evolution. New York: Columbia Univ. Press .

6. Otte, D. and J. A. Endler (eds.). 1989. Speciation and its Consequences. Sunderland, MA: Sinaue

UNIT- VII
THEORIES OF BIOLOGICAL CLASSIFICATION

Objective: In this unit you will learn various theories of Biological Classification.

Introduction:

Classification is a very broad term which simply means putting things in classes. Any kind of organizational scheme is a classification: for example, sorting smarties by colour, coins by diameter or cities by population. Humans seem unable to resist the urge to classify. It's one of the most basic activities of any science, because it's easier to think about a few groups of things than about lots of separate things.

In the century after Linnaeus published his system of classification, ideas about classifying organisms began to change. In 1859, Darwin published his major work on evolution, *On the Origin of Species by Natural Selection.* After that, there was more and more interest in classifying organisms, incorporating the evolutionary history, including the genetic relationships, of the organisms.

Systematics:

The term systematics is derived from Latinized Greek word-systema-as applied to the systems of classification developed by early naturalists, notably Linnaeus.

According to Simpson, Systematics is the scientific study of kinds and diversity of organisms and of any and all relationships among them. Systematics includes taxonomy, identification, classification and nomenclature and all other aspects of dealing with different kinds of organisms and data accumulated about them is also included in systematics.

Origin and Development of Systematics:
The origin and development of systematics and human civilization started simultaneously, i.e., civilization of human being is the systematics or in other words we say that in our daily life those people who maintain their houses systematically that means they are civilized. As the knowledge developed, they started naming the plants and animals according to their own choice.

The clue for the earliest classification comes from Vedas and Upanishads (1500 BC to 600 BC). In Vedas and Upanishads, several technical terms are used in the description of plants and plant parts, both morphologically and anatomically.Plants of medicinal importance were collected and studied. Charaka and Susruta, the two eminent ancient Indian scholars and ayurvedic physicians, contributed a lot to our knowledge of diversity and utility of organisms.

One of the remotest works dealing with plant-life in a scientific manner is the Vrikshayurveda (science of plants and plant-life) compiled by Para Sara, even before the beginning of Christian era, which formed the basis of botanical teaching and medical studies in ancient India.Several early Greek scholars notably Hippocrates (460-377 B.C) and Democritus (465-370 B.C.) made observations on animals but their classification was not useful. Later on Aristotle (384-322 B.C.) also studied the living organisms, viz., plants and animals and gave statement on classification that **“animals may be classified according to their way of living, their action, their habit and their bodily parts”.**

He classified the major groups of animals as birds, fishes, insects and whales. Aristotle is called the **"Father of biological taxonomy."**Theophrastus (370- 385 B.C.) who was the student of Plato and then of Aristotle, is known as the **"Father of Botany"**, classified all plants on the basis of form and texture and divided them into trees, shrubs, under-shrubs and herbs.His classification was strictly artificial and in his **"HistoriaPlantarum"** he has classified and described 480 plants.

Albertus Magnus (1193-1280) recognised the differences with the help of crude lenses in between the monocotyledons and dicotyledons and recognised the classification of Theophrastus. Otto Brunfels (1464— 1554), a German, first recognised the Perfecti and Imperfect group of plants based on the presence or absence of flowers respectively.Jerome Bock (1498-1554), another German, classified the plants into trees, shrubs and herbs. Andrea Cesalpino (1519-1603) taxonomically classified plants on the basis of habit and subdivided them on the characters of fruits and seeds.

Gaspard Bauhin (1560-1624) classified the plants on the basis of texture and form. He was first to find out the binomial nomenclature although it is usually credited to Linnaeus. In the history of systematics, John Ray (1627-1705) has done a great job. Before him the classification was baseless and there was not any strong scientific background. John Ray travelled widely in Europe with Francis Willougby (1635-1672).They not only observed and collected plants and animals, but also planned to classify them. Unfortunately, Willougby died prematurely and later John Ray published his Historia Generalis Plantarum in three volumes between 1668 and 1704. In this book, John Ray described accurately and in meticulous detail and catalogued over 18,000 plants.

He was the first person who recognised the difference between genus and species and through valuation of both similarities and dissimilarities in animals arrived at a more natural higher classification than of the former persons.After John Ray, the most remarkable man in the field of taxonomy was Carolus Linnaeus (1707- 1778) who is often referred to as the **"Father of Taxonomy"** for his outstanding contribution in this field. Linnaeus visited many European countries and made careful observations on the plants and animals.

He published his scheme of classification in his famous book Systema Naturae in 1753. The 10th Edition of this book was published in 1758 and the 1st January of that year marks the beginning of the consistent application of what is known as the **"binomial system of nomenclature"**.
This system which introduces the principles of naming an organism by two words, was first proposed by Linnaeus and is universally followed today. This work of Linnaeus became the foundation of systematics. He also laid down the clear delimitations for species.Jean Baptiste Monet de Lamarck (1744-1829) wrote Flore Francoise in 1778 in which he laid down the principles of his concept of natural classification. Charles Darwin (1809-1882) a great evolutionist, on the basis of his extensive studies and with the help of convincing evidences gathered on the Voyage of the Beagle, explained the origin of species through natural selection.

The evolution theories of Lamarck and Darwin not only greatly influenced Linnaean classical systematics but also totally rejected the pre-Lamarckian idea of the fixity of species. Ernst Haeckel (1866) introduced the method of representing phylogeny by means of trees or branching diagrams.Sir Julian Huxley (1940) introduced the new term **"New Systematics"** which incorporates the results of recent studies in various branches of life sciences in systematics which modify some of the older ideas of classical systematics. Today the definition of species is based on population because the development of population genetics which in turn influenced the further development of population systematics.

Utility of Systematics (Taxonomy):
No scientific ecological survey can be carried out without the most painstaking identification of all the species of ecological significance. There are more than one million species of animals and more than

half million species of plants described in the biosphere. In addition, there must be a large number of plant and animal species yet to be described. Without the knowledge of systematics the discovery of new species of plants and animals is not possible and we cannot know much about the organisms of our surroundings.Therefore, there is a great necessity or utility of systematics.

Systems of Classification:
There are three different systems of classifications which have been proposed so far by different taxonomists.

They are:
(i) Artificial

(ii) Natural

(iii) Phylogenetic

1. Artificial System of Classification:
This type of classification is based upon characters of convenience without relation to phylogenetic significance; classification based upon characters erroneously presented to indicate phylogenetic relationship; also classification based on a single arbitrarily chosen criterion, instead of an evolution of the totality of characters known as artificial classification.

This system of classification was adopted by Pliny in the first century A.D. for animals on the basis of habitats, e.g., land, air and water.

Accordingly animals were classified into two categories on the basis of their flying ability:
(i) Animals that can fly and

(ii) Animals that cannot fly.

In the first group unrelated animals like butterflies, birds and bats were placed together.

The classification of plants on the basis of habit into:
(i) Herbs,

(ii) Under shrubs,

(iii) Shrubs and

(iv) Trees is also an artificial one.

The criteria used in this classification, although very simple and easy to follow are arbitrary and do not reflect any natural relationship existing among the organisms. Moreover, it leads to heterogeneous assemblage of unrelated organisms under one heading and does not do justice to the totality of characteristics of an organism.

The system adopted by Linnaeus was also artificial in which the plants were classified on the basis of number and arrangement of stamens and carpels. Closely related species of organisms are kept far apart in this system of classification.

2. Natural System of Classification:
The natural classification may be defined as **"Classification based on characters which indicate natural relationships"**. The organisms of a natural systematic category agree with one another in so many characters because they are descendants of one common ancestor. The natural system of classification is based on similarity.
Zoologists and botanists differ in their interpretation of the implication of this system of classification. According to zoologists, the natural system of classification includes the phylogenetic and evolutionary trends which are evident in the word **"natural"**.
Botanists hold the opinion that the natural system does not necessarily include phylogenetic trends of plants. Here they proposed the **"phylogenetic system"** of classification separate from the **"natural system"** to include the evolutionary trends in plants. The **"natural system"** of classification of the plant kingdom was proposed by George Bentham (1800-1844) and Joseph Dalton Hooker (1817-1911).

3. Phylogenetic System of Classification:
The phylogenetic system is based on the evolutionary and genetic relationship of the organisms. It enables us to find out the ancestors or derivatives of any taxon. Our present-day knowledge is insufficient to construct a perfect phylogenetic classification and all the present phylogenetic systems are formed by the combination of natural and phylogenetic evidences.

This system is adopted by Adolph Engler (1844-1930) and Karl A.E. Prantl and John Hutchinson (1884-1974) in classifying the plants.Even the experimental biologists have learned to appreciate the necessity for sound taxonomy. The systematists can fill many gaps in our knowledge.

Systematic Hierarchy:
Since the number of animal and plant species is very large, it is not possible to either know them individually by their names or to refer them in the literature. This necessitated arranging them into categories and taxa of different grades. Then arranging these categories and taxa in an ascending order so that a higher category includes one or more lower categories and higher taxa include one or more lower taxa.

Linnaeus was the first taxonomist to establish a definite hierarchy of taxonomic categories recognized within the animal kingdom. These are classes, order, genus, species and varieties. The varieties, used by Linnaeus as an optional category of various types of intraspecific variants, was eventually discarded or replaced by the species.

These few categories sufficed to cope with small number of animals and plants known at that time.However, as the number of known species increased and with it our knowledge of the degrees of relationship of these species, the need arose for a more precise indication of the taxonomic position of species and inserting additional ones among them.Most are formed by combining the original category names with the prefixes super or sub. Thus, there are superorder, super-families and subfamilies, etc. The most frequently used additional new category name is perhaps the term tribe for a category between genus and family.

Vertebrate paleontologists also used in routine the category cohort between order and class. Some authors used terms for additional subdivisions, such as cladus, legio, and sectio. Some used infraclass below the subclass and infra-order below the suborder.Indicated in the parenthesis are the standardized endings for the names of tribes, subfamilies, families and super-families. The systematic hierarchy or Linnaean hierarchy as it is commonly known, with its need for arbitrary ranking has often been attacked as an unscientific system of classification.

Alternate methods, such as numerical scheme have been proposed but have not found favor among taxonomists, primarily for following two reasons:

(i) Assigning definite numerical values to taxa demands a far greater knowledge of the relationships of taxa than can be inferred from available evidences.

(ii) An assignment of such values would freeze the system into a family which would preclude any further improvement.

It is the very subjectivity of the Linnaean hierarchy which gives it the flexibility required by the incompleteness of our knowledge of relationships. It permits the proposal of alternate models of relationships and gives different authors an opportunity to test which particular balance between splitting and lumping permits the presentation of maximum amount of information.

Like any other scientific theory it will forever be provisional.

Taxon and Category:

Taxon (Plural: Taxa.):
The taxa are the groups of animals generally groups of species. The words insects, fishes, birds, mammals in animals; algae, fungi, ferns, mosses, grasses, etc., in plants are the groups of organisms. These are the concrete objects of classification. Any such group of such population is called taxon.

But in ordinary usage only the so called basic categories (genus, family, order, class, phylum, kingdom), are treated as such groups. The super taxa at all levels are treated as groups of the basic taxa (a superclass as a group of classes) and the sub taxa at all levels as a subdivision of the basic taxa (a suborder as a section of the order).

According to Simpson **"A taxon is a group of real organisms recognized as a formal unit at any level of a hierarchic classification."**According to Mayr, **"A taxon is a taxonomic group of any rank that is sufficiently distinct to be worthy of being assigned to a definite category."**

Category:
The group of animals are taxa. Each taxon is placed at some level in hierarchy. A category designates rank or level in a hierarchic classification. It is a class, the members of which are all the taxa assigned a given rank. A category can be higher or lower than some other one, so we may speak of a higher category.

The categories have names, but these are terms and not names in biological nomenclature. They are kingdom, phylum, class and so on. It is an error to state **"this animal belongs to category Mammalia"**, Mammalia is the name of taxon not of category.

Taxonomic Categories:
Species:
Species is the most important category in the taxonomic hierarchy. It is the basic unit in taxonomy and also in evolution. Its definition has long been one of the major problems of taxonomy. Several definitions and aspects are discussed about the definition of species.

According to Blackweldler, the species can be defined as follows:
(i) One of the groups, the one placed in the category called the species level (a species group).

(ii) The category or level at which the species groups are placed (the species level)

Two main definitions are given for species.
These are as follows:

a. Biological species:
Biological species are usually defined as groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups. This gives theoretical groups which can seldom be distinguished in practice.

Simpson has pointed out that all the definitions of animal species give us biological species. He, therefore, prefers the name genetical species for this and cites also bio-species. (It should be noted that populations do not interbreed, only individual animals and plants interbreed).

Genetical species:
Genetical species are groups of interbreeding populations which are reproductively isolated from each other. They are, thus, the same as biological species. For example, in Homo sapiens, the sapiens is a species of Homo.

Genus:
The taxa placed in the genus category are the genera. These are groups of species brought together by the taxonomists as evidenced by the fact that generic name is a part of each name of each of the included species.

The genus is involved from naming of the first species of whose name it forms the first part. Again it is possible to say that a genus is any group of species included under the one generic name by any taxonomist. This is completely subjective, but it is approximately the working definition which is the basis of most taxonomic work.

The genus cannot properly be described as the next higher level above the species, because it is common and always possible to. use subgenera between the genus and species, and to use also sections or other informal categories.

A pragmatic definition of the genus states, **"A genus is a taxonomic category containing a single species or a monophyletic group of species, which is separated from other taxa of the same rank (other genera) by a decided gap."** The genus *Felis* includes the golden cat (*Felis temincki*) the fishing cat (*Felis viverrina*) and the leopard cat (*Felis bengalensis*).

Family:
This is a taxonomic category containing one or more related genera and which is separated from other related families by important and characteristic differences. The family Felidae which includes the lion, the leopard, the tiger and all types of cats belonging to different genera. This family is distinctly separate from the family Canidae which includes dogs and foxes.

Order:
The order is the basic category of what has been called the order group which includes also super-orders, the suborders, the infra-orders and taxa at any other levels interpolated between superfamily and infraclass. In many phyla, orders are very well known groups, but in some phyla they are less well known than the classes, whereas classes do have a fairly evident uniformity throughout the animal kingdom.The orders of vertebrates, for example, are scarcely comparable to families in insects and levels vary in other groups. For example, the order Carnivora includes families Felidae and Canidae.

Class:
The class is the basic category of what has been called the class group which included also super classes and infraclasses, as well as any others interpolated among these. In the animal kingdom as a whole, the classes are undoubtedly the best known taxa, even the phyla being subject to more differences of the opinion.

A class is generally a subdivision of a phylum. For example, the order Carnivora includes the lion, the cat, etc., are included in the class Mammalia.

Phylum:
The taxa placed in the phylum category are the phyla, subdivisions of the kingdom. They may be assembled into super-phyla or subdivided into subphyla. The phylum Porifera includes three classes such as Calcarea, Hexactinellida and Demospongia.

Kingdom:
This is the highest taxonomic category. All animals are included in the animal kingdom and all plants are included in the plant kingdom.

Species as a Category:
The importance of the term species in all fields of biology is so immense that it deserves special consideration. It has already been stated that individual organisms which have many features in common and are able to breed only amongst themselves are encompassed by the term species.

The definition of species is not restricted only to the taxonomists. Nowadays the other fields of biology also consider the species to a great extent. Cytologists, geneticists, ecologists, biochemists and others have also defined the species.

Artificial Classification:
1. The artificial classification is based on fewer or even a single arbitrary character.

2. Artificial classification is based on priory weighting of taxonomic characters.

3. The characters do not show any relationship.

4. Artificial classification is a downward classification based on dichotomous branching (e.g., the animal kingdom is divided on the presence or absence of red blood cells).

5. This classification does not find any missing link or common ancestors.

6. This system does not help in establishing evolutionary and phylogenetic relationship.

This system of classification was used by Aristotle (384-322 B.C.), Linnaeus (1707-1778), and his followers.

Natural Classification:
1. The natural classification is based on more than fewer characters, and mainly totality of characters are considered.

2. It is based on posteriori weighting of the taxonomic characters.

3. Natural Classification is based on diagnostic or hidden characters which show relationships.

4. Natural Classification is an upward classification based on the grouping of related species that resemble each other and forming a hierarchy of higher taxa by grouping similar taxa of the lower rank.

5. This classification helps to find out the missing link and their common ancestors.

6. Natural Classification can establish the relationship among the taxa, so it helps to find out the phylogeny among different taxa.

This system of classification was introduced by middle of 19th century and most prominent authors are Ray Lankester (1847-1919), Mayr (1940, '65, '69), Simpson (1961), and Mayr and Ashlock (1991), and others.

Kingdom Animalia:

This is the largest group of animal classification. It includes the entire animal population (fauna) of the world.

It is divided into two subkingdoms:
Protozoa and Metazoa.

Subkingdom A. Protozoa:
About 50,000 species. Acellular microscopic animals. Solitary or colonial. Specialized cell organelles. Single to many nuclei. Nutrition holozoic, holophytic or saprozoic or parasitic. Freshwater, marine or moist terrestrial.

Phylum 1:
Protozoa (First animals). Characters as those of the subkingdom.

Subkingdom B. Metazoa:
Multicellular animals. Body comprises many cells, usually arranged in layers or tissues.

It is divided into three branches:
Mesozoa, Parazoa, Eumetazoa.

Branch I. Mesozoa:
Cellular animals having the structure of a stereo-blastula,composed of surface layer of somatic cells and interior reproductive cells.

Phylum 2:
Mesozoa (Middle animals). About 50 species,Worm-like, small. Symmetry bilateral. An external layer of ciliated digestive cells surrounding one or several reproductive cells. Parasitic in cephalopods and other invertebrates.

Examples: Dicyema, Rhopalura, etc.

Branch II. Parazoa:
Animals of cellular grade of organization with incipient tissue formation. Interior cells of several different kinds. There is no mouth or digestive tract and no organ systems are present. The body is porous with one to many internal cavities lined by choanocytes. Sessile, marine, a few freshwater. Solitary or colonial.

Phylum 3:
Porifera (Pore-bearers).About 5,000 species. Characters as those of the branch.

Examples: Scypha or Sycon, Euplectella, Hyalonema, Euspongia, etc.
Branch III. Eumetazoa:
Animals of tissue or organ-system grade of organisation with mouth and digestive tract (except when lost by parasitic degeneration). Interior cells of several kinds. Body not porous and without cavities lined by choanocytes.

Grade I. Radiata:
Eumetazoa with primary radial symmetry. Tissues are present and organ-systems are incipient. Mesoderm, usually derived from the ectoderm, is present as an incipient tissue, without a high degree of cellular specialisation. The only body space is the digestive cavity, which has a mouth, but no anus.

Phylum 4. Cnidaria (Coelenterata):
About 10,000 species. Symmetry radial, biradial or radio-bilateral. The mouth is encircled by tentacles bearing nematocysts. No rows of ciliated plates. Body cavity as coelenteron. Sessile or free swimming. Solitary or colonial. Marine or freshwater.

Examples: Hydra, Obelia, Aurelia, Metridium, Corals, etc.

Phylum 5. Ctenophora (Comb-bearers or Comb-jellies):
About 90 species. Symmetry is biradial. Tentacles when present do not encircle the mouth. No nematocysts. Eight radial rows of ciliated swimming plates. Free-swimming and marine.

Examples: Pleurobrachia, Coeloplana, Ctenoplana, etc.

Grade II. Bilateria:
Eumetazoa with bilateral symmetry, or those with embryonic bilateral symmetry later modified into radial symmetry. Organ-system grade of organisation. Mostly with a well-developed mesoderm of endodermal origin. Mostly with body spaces other than the digestive cavity. Mouth and anus generally present.

Bilateria is divided into two divisions:
Protostomia and Deuterostomia.

Division A. Protostomia:
Bilateria in which the mouth arises from the blastopore or from the anterior margin of the blastopore.

Protostomia is subdivided into three subdivisions:
Acoelomata, Pseudocoelomata, and Coelomata.

Subdivision 1. Acoelomata:
No body cavity or coelom. Space between the body wall and digestive tract is filled with mesenchyme. Excretory system of protonephrida with flame bulbs.

Superphylum Acoelomata:
Bilateria without a coelom. With mesenchyme between the body wall and digestive tract. Excretory system of protonephridia with flame bulbs. Body un-segmented or consisting of a strobila, with youngest segment toward the head.

Phylum 6. Platyhelminthes (Flatworms):
About 12,700 species. Body dorsoventrally flattened. Anus and circulatory system absent. Free living or parasitic. Terrestrial, freshwater or marine.

Examples: *Planaria, Fasciola, Taenia,* etc.
Phylum 7. Nemertinea or Rhynchocoela- (Ribbon worms):
About 750 species. Body slender, soft, very elastic and covered with cilia. No segmentation. Mouth anterior with a long eversible proboscis. Digestive tract complete with anus. Circulatory system present. Free-living. Mostly marine, few terrestrial and freshwater.

Examples: *Cerebratulus, Stichostemma*, etc.

Subdivision 2. Pseudocoelomata:
Space present between digestive tract and body wall but this space is a pseudocoel (remnant of the blastocoel) and not a coelom. Anus present with or without protonephridia, flame bulbs present or absent.

Phylum 8. Acanthocephala (Spiny-headed worms):
About 500 species. Minute parasitic worms. Proboscis protrusible (eversible) with recurved spines. No digestive tract.

Examples: *Echinorhynchus, Gigantorhynchus,* etc.

Phylum 9. Entoprocta (Moss animals):
About 60 species. Digestive tube U-shaped. Mouth and anus close together lying within a region surrounded by ciliated tentacles. Sessile. Mostly marine, few freshwater. Solitary or colonial.

Examples: *Pedicellina, Loxosoma, Urnatella,* etc.

Super phylum Aschelminthes:
An assemblage of pseudo coelomates. All have anterior mouth, posterior anus and straight digestive tube.

Phylum 10. Rotifera (Wheel animalcule):
About 1,500 species. Microscopic. Anterior end with a ciliated corona. Pharynx with internal jaws known as trophic Protonephridial system with terminal flame bulbs. Mostly freshwater, some marine.

Examples: *Brachionus, Philodina, Rotatoria,* etc.

Phylum 11. Gastrotricha (Hairy stomach worms):
About 150 species. Microscopic. Ventral surface flattened and ciliated. Cuticle with spines, plates or scales and un-segmented. Pharynx tubular devoid of trophi. Freshwater and marine.

Examples: *Chaetonotus, Macrodasys*, etc.

Phylum 12. Kinorhyncha (Jaw-moving worms):
About 60 species. Small. More or less spiny without superficial cilia. Body un-segmented with the anterior end an introvert Marine.

Examples: *Echinoderes, Pycnophyses*, etc.

Phylum 13. Nematoda (Round worms):
About 10,000 species. Body rounded, slender, covered by a continuous cuticle, often tapered at ends. Cilia absent. Epidermis divided into four or more chords. Only longitudinal muscles in the body wall. Long muscular pharynx having triradiate lumen. Free-living or parasitic. Freshwater or marine or in soil.

Examples: *Ascaris, Rhabditis, Enterobius, Ancylostoma, Wuchereria*, etc.

Phylum 14. Nematomorpha (Horse-hair worms):
About 230 species. Body long slender, cylindrical with dorsal and ventral epidermal chords. Gonoducts joining the intestine. Larval stages parasitic and adults free-living.

Examples: *Gordius, Nectonema*, etc.

Subdivision 3. Coelomata:
Animals with a true coelom and usually well-developed ectomesoderm. Excretory organs are protonephridia with or without nephrostome. Anus present.

Super-phylum Inarticulate:
Unsegmented, coelomate protostomes.

Phylum 15. Priapulida:
About 8 species. Body surface covered with spines and tubercles. Proboscis anterior. Urinogenital pores separate from the digestive tube in both sexes. Protonephridia terminating in solenocytes joining the gonoducts. Marine.

Example: *Priapulus*

Phylum 16. Sipunculida (Peanut worms):
About 275 species. Body elongated and cylindrical with retractile anterior introvert. Short hollow tentacles around the mouth. No segmentation or setae. Anus dorsal. Marine.

Examples: *Sipunculus, Phascolosoma*, etc.

Phylum 17. Mollusca (Soft-bodied animals):
About 1, 28,000 species. Body soft covered by mantle usually with an anterior head and a ventral muscular foot. Mantle secretes shell. Shell sometimes vestigial and sometimes in several pieces. Coelom reduced. Terrestrial, freshwater and marine.

Examples: *Chiton, Pila* (Snail), *Unio* (Mussel), *Sepia, Loligo* (Squid), Nautilus, etc.

Phylum 18. Echiurida (Adder-tailed worms):
About 150 species. Body cylindrical with trough-shaped elastic proboscis (non-retractile). Mouth ventral. Trunk with setae. Coelom spacious. Marine.

Examples: *Echiurus, Urechis,* etc.

Super-phylum Articulata:
Segmented coelomate animals. Characterised by segmentation of embryonic stages, even though the adult may have secondarily lost its metameric organisation.

Phylum 19. Annelida (Ringed or Segmented worms):
About 8,700 species. Body elongated and metamerically segmented. Coelom spacious, typically divided into metameric compartments. Terrestrial, freshwater and marine.

Examples: *Nereis* (Clamworm), *Pheretima* (Earthworm), *Hirudinaria* (Leech), etc.

Phylum 20. Tardigrada (Water bears or Bear animalcules) Minute:
Body cylindrical and segmented with four pairs of unsegmented legs terminating in claws. Coelom transitory replaced by a haemocoel. No appendages associated with mouth.

Examples: *Echiniscus, Hypsibius*, etc.

Phylum 21. Onychophora (Claw-bearers):
About 73 species. Body worm-like, elongated and un-segmented. Thin un-segmented cuticle covers the body. Many pairs of short un-segmented legs. Head with three pairs of appendages, two of which are associated with mouth. Tracheal system for respiration. Moist terrestrial.

Example: *Peripatus.*

Phylum 22. Pentastomatida (Tongue worms):
About 70 species. Worm-like body with two pairs of claws or short appendages at the sides of the mouth. Respiratory system absent. Parasitic in vertebrates.

Examples: *Porocephalus, Cephalobaena*. etc.

Phylum 23. Arthropoda (Joint-footed animals):
About 9,00,000 species. Body segmented with jointed appendages usually terminating in claws. Exoskeleton chitinous. Coelom greatly reduced and replaced by a haemocoel. Terrestrial, freshwater and marine. Examples : Prawns, Scorpions, Flies, Centipedes, etc.

Division B. Deuterostomia:
Bilateria in which the mouth does not arise from the blastopore or near its anterior margin. Coelom arise from the primitive gut.

Super-phylum Tentaculata (Lophophorates):
Animals with a circular, crescentic or double spirally coiled edge bearing ciliated tentacles and known as a lophophore. Mouth and anus near together. Intestine forming a loop.

Phylum 24. Phoronida:
About 15 species. Solitary worm-like animals. Body enclosed in a chitinous tube. Closed circulatory system. One pair of metanephridia Marine.

Examples: *Phoronis, Phoronopsis*, etc.

Phylum 25. Ectoprocta or Bryozoa (Moss animals):
About 4,000 species. Sessile or colonial animals with a lophophore. Body enclosed in a gelatinous, chitinous or calcareous covering. Anus outside of the lophophore region. Mostly marine, few freshwater.

Examples: *Bugula, Plumatella*, etc.

Phylum 26. Brachiopoda (Lamp shells):
About 260 species. Solitary animals with a lophophore. Body enclosed in a bivalve shell. One or two pairs of metanephridia. Marine.

Examples: *Lingula, Crania, Hemithyris*, etc.

Phylum 27. Echinodermata (Spiny-skinned animals):
About 6,000 species. Animals with secondary radial symmetry. Water vascular system present. Locomotion by tube-feet.

Examples: Starfish, Brittle star, Sea urchins, Sea lilies, etc.

Phylum 28. Chaetognatha (Arrow worms):
About 50 species. Small, slender, elongated, transparent animals. Body divided into head, trunk and tail. Hooks or bristles near mouth, paired fins on trunk and a terminal tail fin. Digestive tube complete. Planktonic and marine. Free-living.

Examples: *Sagitta, Spadella,* etc.

Phylum 29. Pogonophora (Beard worms):
About 43 species. Body elongated in a chitinous tube. Anterior end with one to many tentacles. Endoskeleton, gill-slits, and digestive tube absent. Deep water and marine.

Examples: *Siboglinum, Polybrachia, Spirobrachia,* etc.

Phylum 30. Hemichordata (Acorn worms):
About 80 species. Animals permanently bilateral with gill-slits. Endoskeleton poor. Embryo lacking a typical notochord. Marine.

Examples: *Balanoglossus* (Acorn worm), *Cephalodiscus,* etc.

Phylum 31. Chordata:
About 45,000 species. Animals permanently bilateral. A dorsal tubular nerve cord, a notochord and paired pharyngeal gill-slits present at some stage in life- history. Terrestrial, freshwater, marine.

Examples: Ascidians, Amphioxus, Fishes, Frogs and Toads, Lizards and Snakes, Birds and Mammals, etc.

Probable Questions:

1. Write a short note on Natural Classification.

2. What is Artificial Classification.

3. Describe different types of Taxonomic categories.

4. What do you mean by systematic hierarchy.

5. Write a short note on origin and development of systematics.

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors)

Unit-VIII

Zoological nomenclature, basic knowledge of naming genus and species

Objective: In this unit you will learn Zoologicalnomenclature and basic knowledge of naming genus and species.

Introduction:

Zoological Classification:
Classification is necessary to avoid confusion in the animal kingdom. It is a part of taxonomy by which we can make an orderly arrangement on the basis of the relationships. So Zoological classification can be defined as the ordering of animals into groups or sets on the basis of their relationships (Simpson, 1961). Mayr (1957, 1969) has also given a similar type of definition.According to Mayr and Ashlock (1991), a biological classification is the**"ordered grouping of organisms according to their similarities and consistencies with their inferred descent".**This definition makes classification natural because it reflects the evolutionary pathway of the organisms.

Purpose of Classification:
(i) Identification of the animals and to arrange the different types of animals into groups on the basis of relationships;

(ii) To express the degree of genetic relationships or affinity between the different types of animals.

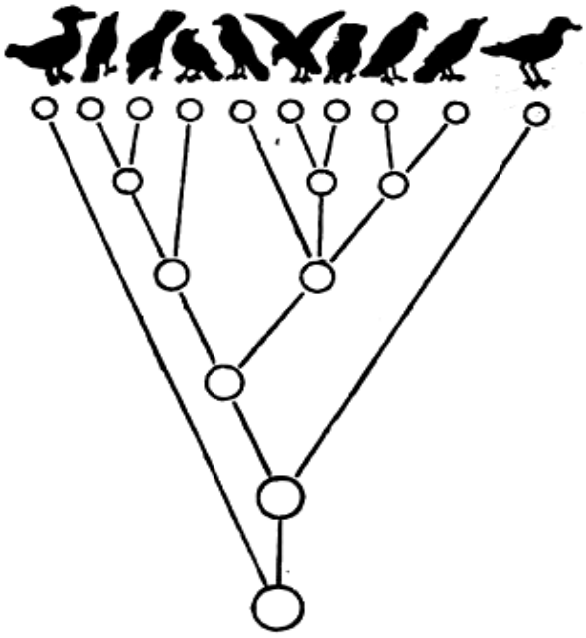


Fig. 8.1: Classification of ten different birds. Note that the classification in one hand indicates interrelationship and at the same time their origin.

Codes and Rules of Nomenclature:

There are five codes of nomenclature:
(i) International Code of Botanical Nomenclature (ICBN),

(ii) International Code of Zoological Nomenclature (ICZN),

(iii) International Code of Bacteriological Nomenclature (IC Bac N),

(iv) International Code of Viral Nomenclature (ICVN) and

(v) International Code of Nomenclature for Cultivated Plants (ICNCP).

International conferences are held from time to time to update the codes and resolve the controversies, if any.

The rules of nomenclature framed under these codes as well as the rules set by Linnaeus are as follows:

1. Each organism is given only one name consisting of two words, generic and specific.

2. Though the codes are separate for plants, animals, bacteria, etc. and the same generic name can be given to different organisms belonging to these domains, it should be avoided. However the same specific name can be given to organisms belonging to different genera. Two species belonging to the same genus cannot have similar specific names.

3. The generic name is written first. It is followed by specific name and then the name of the discoverer in full or in abbreviation.

4. The specific name can be single or compound. Usually it begins with a small letter.

5. The scientific name is printed in italics. It is underlined in handwritten description. An exception is made when the biological name is written as title of paragraph.

6. The name of the author is kept in Roman script.

7. The original names were taken from Latin and Greek languages. New names are now derived either from Latin language or are Latinized. This is because Latin language is dead and, therefore, it will not change in form or spellings with the passage of time.

8. Barring obvious error or misprint, a scientific name retains its original spellings.

9. No names are recognised prior to those used by Linnaeus in 1753 for plants in “Species Plantarum” and in 1758 for animals in the 10th edition of “Systema Naturae”.

10. The names of families and subfamilies should be based on name of type genus.

11. The names of subfamilies, families and other categories are not printed in italics. They can, however, be written in bold letters.

12. When a species is transferred or revised the name of the original worker is retained but in parenthesis, e.g., *Syzygium cumini* (L) Skeels.

13. In publishing a new name the type specimen of the material is kept.

14. A new scientific name is thought of on the basis of its characteristic, a personality or place. The selected name is such that it has no resemblance with any previously published name.

International Code of Zoological Nomenclature

Brief History of International Code of Zoological Nomenclature:

The need for a code to give a scientific name to every species was first realized by British Association for the Advancement of Science in 1842, when a set of rules were framed by it. This was also felt by American Association for the Advancement of Science in 1877. Then similar learned bodies in different countries like France, Germany and Soviet Union developed codes for their respective countries.In 1889, at the International Congress of Zoology in Paris, discussions were made to find out some common code of nomenclature. First version of the code .was adopted in the V^{th} International Congress of Zoology in Berlin in 1901. In the XV^{th} session held in London in 1958, the codes were rewritten and published on 6^{th} November, 1961 and the updated version of the code (1961) was made available in 1964 (2nd edition).

This code is concerned only up to naming of superfamily and did not satisfy the zoologists. The latest edition (4^{th} edition) of the code was published in 1999 and its effective use has started from 2000.The International Zoological Congress elects a judicial body, called International Commission of Zoological Nomenclature which interprets or recommends the provisions of the code for classification or nomenclatural problems of the animals.
Again the International Code of Zoological Nomenclature (ICZN) formed by the International Commission of Zoological Nomenclature to see the rules and principles of nomenclature and the application of these rules for both living and fossil animals.

Parts of International Code of Zoological Nomenclature:
The International Code of Zoological Nomenclature contains three main parts:
(i) The Code proper,

(ii) The Appendices and

(iii) The Official glossary.

The code proper includes a preamble followed by 90 articles which cover mandatory rules without any explanation.

There are three Appendices, of which the first two cover the status of recommendations and the third part of the Appendices is the constitution of the commission. The glossary contains the terms used in the codes with detailed definition.

Rules of Zoological Nomenclature:
At present the naming of the animal is governed by the International Code of Zoological Nomenclature. There are many rules (Articles) concerning the Zoological Nomenclature.

Of these rules, some important ones are cited below:

1. Zoological nomenclature is independent of other system of nomenclature. The scientific name of animals and plants must be different, and the generic name of a plant and an animal may be same, but this system is to be avoided. e.g., the generic name of banyan or fig tree is Ficus and the fig shell (a kind of gastropod shell) is Ficus. The scientific name of fig tree is *Ficus carica* or *F. indica*, etc., but the scientific name of the fig shell is *Ficus ficus* or *Ficus gracilis,* etc.

2. The scientific name of a species is to be binomial (Art. 5.1) and a subspecies to be trinomial (Art. 5.2).

e.g., the scientific name of Indian bull frog is *Rana tigrina*. It is binomial. The scientific name of Indian lion is *Panthera leo persica*. It is trinomial. Such a system of naming by three Latin or Latinised words is known as trinomial nomenclature. Sometimes it becomes imperative to recognise subspecies within a species and is given a third specific name.

3. The first part of a scientific name is generic (L. Genus = race) and is a single word and the first alphabet or letter must be written in Capital letter. The genus must be a noun in the nominative singular. The generic part assigns a Latin noun, a Latinized Greek or a Latinized vernacular word.

4. The second part of a name is species (L. species = particular kind) name and may be a single word or a group of words. The first alphabet or letter of the species name must be written in small letter. The species name must be adjective form in nominative singular agreeing in gender with genus name which is in noun form; e.g.:

Ending in species name	Ending in genus name	Full name of the species
Masculine ending (–i)	(–i/–us/–es)	Common mongoose (*Herpestes edwardsi)* River lapwing (*Vanellus duvaucelli*)
Feminine ending (–a/–e)	(–a/–e)	Golden cuttle fish (*Sepia esculenta*) Humpnosed viper (*Hypnale hypnale*)
Neuter ending (–um/–us, etc.)	(–um/–us, etc.)	Tusk shell (*Dentalium elephantinum*) Common crane (*Grus grus*) Lesser black-backed gull (*Larus fuscus*)

The specific name (species part) indicates distinctness while generic part shows relationship.

5. If the species names are framed after any person's name, the endings of the species are i, ii and ae, or if the species name are framed after geographical place, the endings of the species are 'ensis', 'iensis', e.g.:

Species name after person's name

Hooded cuttle fish — *Sepia prashadi* (Prasad + i)
Tree frog — *Rhacophorus jerdonii* (Jerdon + ii)
Antarctic flying squid — *Todarodes filippovae* (Filippov + ae)

Species name after place

Common Indian monitor — *Varanus bengalensis* (Bengal + ensis)
Cookiecutter shark — *Isistius brasiliensis* (Brasil + iensis)
Butterfly fish — *Chaetodon madagascariensis* (Madagascar + iensis)

6. First part of a compound species-group name is a Latin letter and denotes a character of the taxon, connected to the remaining part of the name by a hyphen (-), e.g., Sole (a kind of flat fish)—Aseraggodes sinus-arabici. L. Sinus = recess

China-rose (a kind of coloured rose)—Hibiscus rosa-sinensis. L. rosa = rose

7. If a subgenus taxon is used, it is included within parenthesis in between genus and species part and is not included in binomial and trinominal nomenclature, e.g.:

Name	Genus	Subgenus	Species	Subspecies
Fan shell (Bivalvia)	*Atrina*	*(Servatrina)*	*pectinata*	*pectinata*
Dussumieri's half beak (Osteichthyes)	*Hemirhampus*	*(Reporhampus)*	*dussumieri*	

8. The person who first publishes the scientific name of an animal, is the original author of a name, may be written after the species name along with the year of publication. The author's name may be in its abbreviated form.

Lion—*Felis leo* Linnaeus, 1758 Lion—*Felis leo* Linn., 1758 or *Felis leo* L., 1758

9. Comma is only used between author's name and the year of publication (Art. 22. A. 2.1), e.g., the scientific name of Common octopus is Octopus vulgaris Cuvier, 1797. No punctuation marks are considered one to other ends of the name, e.g., "Octopus vulgaris Cuvier, 1797" (Not considered). No diacritic mark, apostrophe (i') and hypen (-) are used in names. In German word the umlaut sign is removed from a vowel and the letter 'e' is inserted after the vowel, e.g., mulleri becomes muelleri.

10. If the original generic name given by the first author who also reported the species name, transfers the species part from one genus to the other, the name of the original author is put within parenthesis, e.g.,

Tiger:
Felis tigris Linnaeus, 1758. At first almost all the members of the cat family were placed under the genus-Felis.

Later the genus Felis was divided into two genera, the genus of the larger cats (tiger, lion, leopard, etc.) is Panthera and smaller cats such as jungle cat, fishing cat, golden cat, etc. are placed under the genus Felis, e.g.:
Lion—*Felis leo* Linnaeus, 1758 – Lion—*Panthera leo* (Linnaeus, 1758)

Jungle cat—*Felis chaus*

11. The names are not acceptable before the publication of Linnaean treatise, Systema Naturae (10^{th} edition) which was published on 1st January, 1758 except the Nomenclature of spiders which starts in 1757. The book Aranei suecici was published by C. Clerck in 1757.

12. The scientific names must be either in Latin or Latinized or so constructed that they can be treated as a Latin word.

13. The scientific names must be italicized in printed form, or underlined in hand written or in typed forms, e.g.

Indian leopard—*Panthera pardus fusca* (Meyer) [in printed form]

Indian leopard—*Panthera pardus fusca* [in handwritten or typed forms]

14. All taxa from subgenera level and above must be uninominal (Art. 4.1, 4.2) and are plural nouns for names above genus, and singular nouns for genus and subgenus. Taxon 'species' may be used as singular or plural.

15. In case of animals some rules and practices are applied on the basis of zoological codes (Art. 29.2) for the formation of suprageneric taxa from superfamily to tribe, e.g.

Taxon level	Endings of the name	Examples
Superfamily	—oidea (for vertebrates) or —acea (for invertebrates)	Hominoidea Genus *Homo* (Latin) = man Genitive *Hominis* Root *Homin*—of *Homo*
Family	—idae	Hominidae [Homin + idae]
Subfamily	—inae	Homininae [Homin + inae]
Tribe	—ini	
Subtribe	—ina	

16. A family name should be based on the basis of type-genus, e.g., Chitonidae—Chiton (type genus) + idae = Chitonidae.

17. Two species under a same genus should not have the same name.

18. Nomenclature of a hybrid/hybrids cannot be considered because the hybrids are normally individuals but not population. Thus such names have no status in nomenclature. Hybrids are typically sterile and become synaptic failure during meiosis. They are prevented from back crossing with either parental species.

19. A name published without satisfying the conditions of availability (nomen nudum = naked name) has no standing in zoological nomenclature and is best never recorded, even in synonymy.

20. A scientific valid name which is not used about 50 years in literature, then as per zoological code's provision the unused senior valid scientific name is treated as obliterated name and junior name which is used continuously in literature (atleast by 10 authors in 25 publications) becomes the accepted official name.

Remark:
The disadvantage of the binominal system is its instability and the name of a species changes every time and is transferred to a different genus (Mayr and Ashlock, 1991).

21. As per the zoological code's provision (Art. 18), the species and subspecies parts of a name may be same spelling and even the second or the third component of the name repeats the generic name (tautonomy), e.g.:

Scandinavian red fox—*Vulpes vulpes vulpes*

22. Synonyms are the different names for a same animal or a taxon (species or genus). If the several scientific names are given to a single animal by different scientists, the senior-most name is selected by law of priority. The senior-most or earliest name is called senior synonym (Art. 10.6) and is considered as valid species and the rest of the names are called junior synonyms and are treated as invalid species.

The leopard cat was named *Felis bengalensis* by Kerr and the same animal was named by Grey, *Felis chinensis*. Again this animal was named as *Prionailurus bengalensis* by Kerr. So the first name is Senior synonym and valid and the rest names are junior synonyms and are invalid.

The whale shark was named Rhiniodon typus by Smith in 1828 and the same was named *Rhinodon typicus* by Muller and Henle in 1839, *Micristodus punctatus* by Gill in 1865 and *Rhinodon pentalineatus* by Kishinouye in 1891. Here the first name is considered as senior synonym (*Rhiniodon typus*) and valid, the rest are junior synonyms and are invalid.

23. Homonyms mean when identical names are given to two or more different taxa. According to the zoological code (Art. 52.2) when two or more homonyms are found, the seniormost (oldest) homonym (Art. 52.2) is used and the junior-most homonyms are replaced with new names, e.g., Cuvier proposed the genus Echidna in 1797 for the spiny anteater.

Forster already proposed the genu Echidna in 1777 for morey eels. According to Law of Priority, Forster s genus claimed senior homonym and Cuvier's genus considered as junior homonym. Illiger replaced the Cuvier's name as Tachyglossus for spiny anteater in 1811.

24. Principle of priority:
Of all the rules of zoological nomenclature, it is the most controversial part to choose the correct name when two or more names of a single taxon are discovered. Arbitrariness in nomenclature prevails since the period from 1780-1850. The taxonomists of different countries specially in Europe were unable to consult the names of different taxa during the period of French revolution and Napoleonic wars.

A large number of synonyms appeared on these days. The continuous change of names of different taxa could be prevented when priority was adopted as a basic principle of nomenclature.

Reasons for the Changes of Name:
1. Changes dictated by scientific progress:
(i) Change of the generic part of binomial (binominal).

(ii) Change of specific name.

(iii) Synonymising of currently accepted species names.

(iv) Analysis of species complex.

2. Changes dictated by rules of nomenclature:
(i) Discovery of an earlier (senior) synonym.

(ii) Discovery of an earlier (senior) homonym.

(iii) Discovery of an earlier genotype fixation.

(iv) Discovery of inapplicable type-specimen.

Law of Priority:

The Law of Priority includes that any name given to a species or genus for the first time (from 1st January 1758 till this day) will be accepted provided:

(i) The specific name is accompanied by an indication or in descriptional figures.

(ii) The author has followed the system of Linnean binominal nomenclature.

(iii) The author has published his contention in a scientific book or journal which has been properly printed and widely circulated.

(iv) In case of a name proposed as a substitute for a name which is invalid by reason of being a homonym, with a reference to the name which is thereby replaced.

(v) In case of the generic or sub-generic name, it should accompany the genotype or sub-generic type fixation.

The Law of Priority in zoological nomenclature is a basic law of International Code and promotes stability. A zoological name and name of a taxon become valid if they belong to the category of senior synonym and senior homonym.

The Law of Priority in zoological nomenclature applies only from subspecies to family category but not to the higher categories. Priority of the zoological name and taxon are considered from the date of publication. Priority means the oldest date, month and year of the publication.

Binomial and trinomial nomenclature of animals.

Binomial Nomenclature:

The scientific method of naming plants and animals by applying two components where the first component is generic and the second part is specific, is called binomial nomenclature. It was evolved by Linnaeus and adopted by the International Code of Zoological Nomenclature.

Scientific names of a few common animals are given below:

Rohu—*Labeo rohita,*

Pigeon—*Columba livia,*

Tiger—*Panthera tigris*.

Trinomial Nomenclature:

Sometimes it becomes imperative to recognize subspecies within a species and is given a third specific name. Such system of naming is known as Trinomial nomenclature. The scientific name of the lion is *Panthera leo* (Linn.). The same species of the specimen collected in different countries shows minor differences from the original form. So a third sub-specific name becomes necessary in many cases.

The scientific name of the Indian lion is designated as *Panthera leo* persica (Linn.). A trinomen is used to recognise a subspecies. International Rules of Zoological Nomenclature recognised the trinomial nomenclature.The adoption of Latinised names for the organisms and the scheme of classification according to hierarchy are the two main themes of the classificatory secret of Linnaeus. The selection of Latin as the language of nomenclature is quite reasonable because it remains unchanged through generations and is not subjected to grammatical changes as it happens in other vernacular languages.

Probable Questions:

1. Define classification. State its purpose.
2. Briefly discuss about codes of nomenclature.
3. Briefly describe the International Code of Zoological Nomenclature.
4. Describe the parts of International Code of Zoological Nomenclature.
5. Write the rules of zoological nomenclature.
6. What is principle of priority. Give examples.
7. What is law of priority.
8. Give short notes on Binomial and trinomial nomenclature.

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors).

Unit-IX

Phenetic method of classification, numerical phenetics and numerical taxonomy

Objetive: In this unit you will learn about Phenetic method of classification, numerical phenetics and numerical taxonomy.

Introduction:

Phenetic Classification:

In biology, **phenetics** (Greek: phainein - to appear) /fɪˈnɛtɪks/, also known as **taximetrics**, is an attempt to classify organisms based on overall similarity, usually in morphology or other observable traits, regardless of their phylogeny or evolutionary relation. It is closely related to numerical taxonomy which is concerned with the use of numerical methods for taxonomic classification. Many people contributed to the development of phenetics, but the most influential were Peter Sneath and Robert R. Sokal. Their books are still primary references for this sub-discipline, although now out of print.

Phenetics has largely been superseded by cladistics for research into evolutionary relationships among species. However, certain phenetic methods, such as neighbor-joining, have found their way into phylogenetics, as a reasonable approximation of phylogeny when more advanced methods (such as Bayesian inference) are too computationally expensive.

Phenetic techniques include various forms of clustering and ordination. These are sophisticated ways of reducing the variation displayed by organisms to a manageable level. In practice this means measuring dozens of variables, and then presenting them as two or three dimensional graphs. Much of the technical challenge in phenetics revolves around balancing the loss of information in such a reduction against the ease of interpreting the resulting graphs.

The method can be traced back to 1763 and Michel Adanson (in his *Familles des plantes*) because of two shared basic principles — overall similarity and equal weighting — and modern pheneticists are sometimes called neo-Adansonians.

Phenetics versus Cladistics

- **Cladistics** can be defined as the study of the pathways of evolution. In other words, cladists are interested in such questions as: how many branches there are among a group of organisms; which branch connects to which other branch; and what is the branching sequence. A tree-like network that expresses such ancestor-descendant relationships is called a cladogram. Thus, a **cladogram** refers to the topology of a **rooted** phylogenetic tree.
- **Phenetics** is the study of relationships among a group of organisms on the basis of the **degree of similarity** between them, be that similarity molecular, phenotypic, or anatomical. A tree-like network expressing phenetic relationships is called a **phenogram**.

While a phenogram may serve as an indicator of cladistic relationships, it is not necessarily identical to the cladogram. If there is a linear relationship between the time of divergence and the degree of genetic (or morphological) divergence, the two types of trees may become identical to each other.

The **maximum parsimony** method is a typical representative of the cladistic approach, whereas the **UPGMA** method is a typical phenetic method. The other methods, however, cannot be classified

easily according to the above criteria. For example, the transformed distance method and the neighbourrelation method have often been said to be phenetic methods, but this is not an accurate description. Although these methods use similarity (or dissimilarity, i.e., distance) measures, they do not assume a direct connection between similarity and evolutionary relationship, nor are they intended to infer phenetic relationships.

The **maximum likelihood method** is a phenetic method that is statistically well founded. It has often lower variance than other methods (ie. it is frequently the estimation method least affected by sampling error) and tends to be robust to many violations of the assumptions in the evolutionary model. Even with very short sequences maximum likelihood tends to outperform alternative methods such as parsimony or distance methods. Different tree topologies are evaluated. An important disadvantage is that is very CPU intensive and thus time consuming and not appropriate for large datasets.

Which method is the better one?

It has often been argued that **character-state methods** are more powerful than **distance methods**, because the raw data is a string of character states (e.g., the nucleotide sequence) and in transforming character-state data into distance matrices some information is lost. However, while the maximum parsimony method indeed uses the raw data, it usually uses only a small fraction of the available data. For instance, in the example that we have used only three sites are informative and were used while six sites are excluded from the analysis. For this reason, this method is often less efficient than some distance-matrix methods (e.g., see Saitou and Nei 1986). Of course, if the number of informative sites is large, the maximum parsimony method is generally very effective. Distance methods only give one tree, while parsimony analyses many trees and may suggest multiple, equally likely trees, none of which is necessarily the right one. The **maximum likelihood** method does not suffer from these limitations. It uses the entire sequence information, it analyses many trees and proposes the tree with the highest likelihood. Moreover, the method seems to be robust and relatively unsensitive to violations of the evolutionary model used or to unequal rates of evolution or nucleotide bias. The method, however, is not suitable for large datasets due to its CPU-intensive nature.

Numerical Taxonomy:

Meaning of Numerical Taxonomy:
Numerical taxonomy or taximetrics, nowadays frequently and perhaps more appropriately referred to as phenetics, refers to the application of various mathematical procedures to numerically encoded character state data for organisms under study.

Thus, it is the analysis of various types of taxonomic data by mathematical or computerized methods and numerical evaluation of the similarities or affinities between taxonomic units, which are then arranged into taxa on the basis of their affinities.

According to Heywood the numerical taxonomy may be defined as the numerical evaluation of the similarity between groups of organisms and the ordering of these groups into higher ranking taxa on the basis of these similarities.The period from 1957 to 1961 saw the development of first methods and of theory of numerical taxonomy. Plants as we all know are classified based on their characters. It was Michel Adanson, a French botanist, who for the first time put forward a plan for assigning numerical

values to the similarity between organisms and proposed that equal weightage should be given to all the characters while classifying plants.

He used as many characters as possible for the classification, and such classifications came to be known as Adansonian classifications. Numerical taxonomy was however largely developed and popularized by Sneath and Sokal.The application of Adansonian principles and use of modern methods and electronic data-processing techniques, have helped in the evolution of several new classifications of plants during the past few decades.

Principles of Numerical Taxonomy:

Numerical taxonomy involves two aspects:

(a) Construction of Taxonomic Groups:
i. In numerical taxonomy, first, individuals are selected and their characters spotted out. There is no limitation to the number of characters to be considered. However, the larger the number of characters, better is the approach for generalization of the taxa.

ii. The resemblances among the individuals are then established on the basis of character analysis, which can often be worked out with the help of computers, the accuracy of which depends on the appropriateness in character. The best way to delimitate taxa is, to utilize maximum number of characters, with similar weightage given to all of them.

(b) Discrimination of the Taxonomic Groups:
When the taxonomic groups chosen for the study show overlapping of characters, discrimination should be used to select them. Discrimination analysis can be done by various techniques, specially devised for such purposes. Numerical taxonomy is thus, based on certain principles, also called neo Adansonian principles.

Following seven principles of numerical taxonomy have been enumerated by Sneath and Sokal:
(i) The greater the content of information in the taxa, and more the characters taken into consideration, the better a given classification system will be.

(ii) Every character should be given equal weightage in creating new taxa.

(iii) The overall similarity between any two entities is a function of the individual similarities in each of the many characters, which are considered for comparison.

(iv) Correlation of characters differ in the groups of organisms under study. Thus distinct taxa can be recognized.

(v) Phylogenetic conclusions can be drawn from the taxonomic structure of a group and from character correlations, assuming some evolutionary mechanisms and pathways.

(vi) The science of taxonomy is viewed and practiced as an empirical science.

(vii) Phenetic similarity is the base of classifications.

Merits of Numerical Taxonomy:

According to Sokal and Sneath, numerical taxonomy has the following advantages over conventional taxonomy:

a. The data of conventional taxonomy is improved by numerical taxonomy as it utilizes better and more number of described characters. The data are collected from a variety of sources, such as morphology, chemistry, physiology, etc.

b. As numerical methods are more sensitive in delimiting taxa, the data obtained can be efficiently used in the construction of better keys and classification systems, creation of maps, descriptions, catalogues, etc. with the help of electronic data processing systems. Numerical taxonomy has in fact suggested several fundamental changes in the conventional classification systems.

c. The number of existing biological concepts have been reinterpreted in the light of numerical taxonomy.

d. Numerical taxonomy allows more taxonomic work to be done by less highly skilled workers.

Demerits of Numerical Taxonomy:

Numerical taxonomy can however prove to be disadvantageous from the following points of view:

a. The numerical methods are useful in phenetic classifications and not phylogenetic classifications.

b. The proponents of "**biological**" species concept, may not accept the specific limits bound by these methods.

c. Character selection is the greatest disadvantage in this approach. If characters chosen for comparison are inadequate, the statistical methods may give less satisfactory solution.

d. According to Steam, different taxonometric procedures may yield different results. A major difficulty is to choose a procedure for the purpose and the number of characters needed in order to obtain satisfactory results by these mechanical aids. It is necessary to ascertain whether a large number of characters would really give satisfactory results than those using a smaller number.

Applications of Numerical Taxonomy:

Numerical taxonomy has been successfully applied in the following studies:

a. Study of similarities and differences in bacteria, other micro-organisms and several animal groups.

b. Delimitation of several angiospermic genera like Oryza, Sarcostemma Solarium, and other groups including Farinosae of Engler and a few others.

c. In the study of several other angiospermic genera including Apocynum, Chenopodium, Crotalaria, Cucurbita, Oenothera, Salix, Zinnia, wheat cultivars, Maize cultivars, etc.

d. Phytochemical data from seed protein and mitochondrial DNA RELP studies has been numerically analyzed by Mondal et al. to study the interspecific variations among eight species of cassia L .Based on the results of electrophoretic patterns, the degree of pairing affinity (PA) or similarity index was calculated by the following formula, according to the method of Sokal &Sneth and Romero Lopes et al.:

$$PA = \frac{\text{Bands common to species A and B}}{\text{Total bands in A and B}} \times 100$$

Separate dendograms expressing the average linkage were computed using the cluster method UPGMA, which showed that the eight species could be placed into two categories or clusters (Fig. 9.6) with *C. alata, C siamea, C. fistula* and *C. reginera*, all being trees or large shrubs and characterized by the absence of foliar glands on petiole or rachis and presence of dense axillary terminal racemes greater than 30 cm long, being clustered into one group, whereas the other four species, i.e., *C. occidentalis*, C. *sophera, C. mimosoides* and *C. tora*, forming the other cluster, all being herbs or undershrub's and characterized by the presence of short corymbose racemes less than 10 cm long and with foliar glands, either on petiole or rachis.

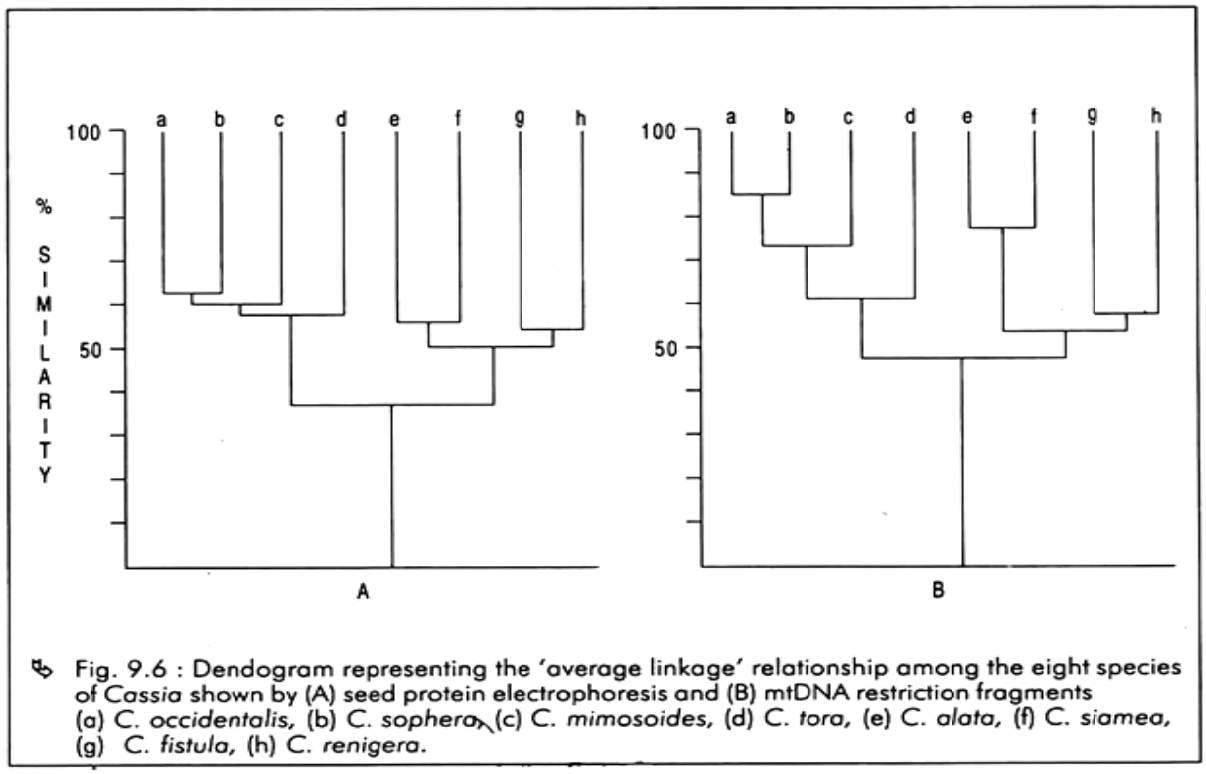


Fig. 9.6 : Dendogram representing the 'average linkage' relationship among the eight species of Cassia shown by (A) seed protein electrophoresis and (B) mtDNA restriction fragments (a) C. *occidentalis*, (b) C. *sophera*, (c) C. *mimosoides*, (d) C. *tora*, (e) C. *alata*, (f) C. *siamea*, (g) C. *fistula*, (h) C. *renigera*.

Advantage of Numerical Taxonomy over Conventional Taxonomy:

(i) It has the power to integrate data from a variety of sources-, e.g., morphology, physiology, phytochemistry, embryology, anatomy, palynology, cytology, etc.

(ii) Automation of data processing promotes efficiency.

(iii) The data can be used for creation of description key, catalogue, maps etc.

(iv) It can provide better classification.

(v) Description can improve conventional taxonomy.

(vi) A number of evolutionary concepts are interpreted by this method.

Aims of Numerical Taxonomy:

Main aim is to determine phenetic relationship between organisms or Taxa. According to Sneath and Sokal (1973) phenetic relationship means similarity or resemblance based on a set of phenotypic characters. Cladistic relationship means expression of the recency of common ancestry and it is represented by branching network of ancestor-descendent relationship.

Probable Questions:

1. Write down the advantages of Numerical Taxonomy over Conventional Taxonomy.

2. What are the applications of numerical taxonomy.

3. What are the Merits of Numerical Taxonomy?

4. What are two aspects of Numerical taxonomy.

5. Write a short notes on Phenetic classification.

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors).

Unit-X

Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidian distance); Cluster analysis (different methods)

Objective: In this section you will learn about how to prepare data matrix and similarity matrix using distance method (Manhattan distance and Euclidian distance). Different methods of cluster analysis will also be discussed in ths unit.

There are four major steps involved in the construction of taxonomic groups. The steps are:

1. Operational Taxonomic Units

2. Unit Characters

3. Estimation of Resemblances

4. Cluster Analysis.

Step - 1.

Operational Taxonomic Units (OTUs):
Operational taxonomic unit is the basic unit in numerical taxonomy. It can be an individual, species, genus, family, order or class. Since the taxonomic units employed in numerical methods are not always comparable to formal taxonomic units, they are termed as operational taxonomic units. Comparison of OTUs of equal rank is always made in numerical taxonomy.

In case of OTUs above the level of individual, adequate representation of various polymorphic forms is essential. For example, when genera are compared, they should be represented by different species. Similarly, when families are compared, they should be represented by different genera and so on.

Step - 2.

Unit Characters:
Unit characters are the characters used in numerical taxonomy. According to Sokal and Sneath (1963), unit character is defined as a taxonomic character of two or more states, which within the study at hand cannot be subdivided logically, except subdivision brought about by changes in the method of coding. Only the phenotypic characters are used as unit characters, e.g. presence or absence of an awn in a grass spikelet.

(i) Types of unit characters:
Unit characters are of two types:

i. Binary characters:
Those unit characters, which exist in two states are called binary characters, e.g. presence or absence of trichomes. They can be represented by the simplest form of coding, where characters are divided into + and – or as 1 and. 0.

In such cases, the positive characters are recorded as + or 1 and negative characters are recorded as – or 0. In case the organ possessing a given character is missing in an organism, the character is scored NC, which means "**no comparison**".

ii. Multistate characters:
Those unit characters, which exist in more than two states are called multistate characters. Such characters can be coded into number of states (1,2,3…) corresponding to their range of variation.

They may be further of two types:

Qualitative multistate characters:
These characters contain three or more contrasting forms and each form is ranked on equal footing, e.g., flower colour, which can be in any number of states – red, white, purple, yellow, etc.

Quantitative multistate characters:
These characters represent measures of the size on a continuous scale, e.g., length of the leaf, which can be 2cm, 3cm, 4cm, etc., or height of the plant, or amount of pubescence on a leaf, etc.

(ii) Admissible unit characters:

The following characters can be categorized as admissible unit characters:

a. It cannot be subdivided.

b. It must show variation among the taxa under comparison.

c. It should be inherent in the organism.

d. It should not be susceptible to environmental changes or affected by experimented conditions.

e. It must be of some diagnostic value.

(iii) Inadmissible unit characters:
As in other disciplines of taxonomy, the proper selection of unit characters is a critical point in the application of numerical taxonomy. Sokal and Sneath (1963) have listed certain characters, which cannot be utilized for numerical taxonomy and called them as inadmissible characters.

These include:
a. Attributes, which are not a reflection of the genotypes of the organisms themselves.

b. Any property, which is a logical consequence of another, either partly or wholly.

c. Characters, which do not vary within the entire sample of organisms.

(iv) Proper selection of unit characters:
Sneath and Sokal (1973) have given the following suggestions for proper selection of unit characters:
a. They should come from all parts of the organism.

b. They should belong to all the stages of the life cycle of the organism.

c. Variable characters within the group should be used.

d. Due attention should be given to characters related to morphology, physiology, ecology and distribution of the organisms.

Step - 3.
Estimation of Resemblances:
Most phenetic methods involve taxon-to-taxon distance, similarity or dissimilarity measures. Distance and dissimilarity are sometimes treated as the same thing, though a distinction can be made between them. As the name implies, distance and dissimilarity measure increase with dissimilarity between taxa, while similarity measures decrease with dissimilarity.

Thus the resemblance between two OTUs is estimated or measured either:

a. In terms of similarity i.e., percentage of characters in which they agree, or

b. In terms of dissimilarity i.e., percentage of characters in which they do not agree.

So far three methods have been devised for estimating the resemblance between the taxonomic groups, which include:

(i) Coefficients of association It is measured by the following formula:

$$S = Ns/ N_s + N_d$$

where, S is the numerical index or Simple matching index or coefficient;

N_s = the number of positive features shared by any two OTUs;

N_d = the number of positive features in one OTU and number of negative features in the other OTU.

Binary characters can be dealt with in two ways depending on whether they can be obviously polarized:

a. In terms of presence or absence, or

b. Whether the character simply has two states and it is undesirable or impossible to classify in terms of presence or absence.

Data are presented in the form of a truth table of test results between the two taxa or OTUs. Thus:

	OTU 1		
		+	—
OTU 2	+	a	b
	—	c	d

Simple matching index (S) or coefficient is defined as the number of tests giving identical results (a or d) divided by the total number of tests, thus

S= a + d / a + b + c + d (range 0 to 1)

(ii) Coefficients of correlation (r):

It is measured by the following formula:

$$r_{jk} = \frac{\sum^{n}[(X_{ij} - X_i)(X_{ik} - X_k)}{\sqrt{\{[\sum^{n}(X_{ij} - X_j)^2][\sum^{n}(X_{ik} - X_k)^2]\}}}$$

where, j and k stand for two units under comparison;

X_{ij} stands for the value of the character i in unit j;
X_{ik} stands for the value of the character i in unit k;
X_i and X_k stand for the mean of all the characters in units j and k;
n stands for number of characters.

(iii) Measurement of taxonomic distance between OTUs:
Using the convention of multidimensional space with one dimension for each character.

Taxonomic distance (d) can be measured by the following formula:

$$d_{jk} = \sqrt{\{\sum^{n}(X_{ij} - X_{jk})2 / n\}}$$

where X_{ij} is the character state of unit j for the character i;
X_{jk} is the character state of unit k; n
Σ stands for the sum over n characters;

The value of the taxonomic distance (d) is the distance in a phenetic space divided by √n.

Step - 4.

Cluster Analysis:

Cluster analysis or clustering is a type of multivariate statistical analysis. It is used to group organisms into separate clusters based on their statistical behavior. The main objective of clustering is to find similarities between organisms, and then group similar organisms together to assist in understanding relationships that might exist among them.

Thus, different OTUs are grouped together on the basis of degree of similarity and these groups of OTUs are called clusters.

(i) Cluster analysis characteristics:
Cluster analysis is based on a mathematical formulation of a measure of similarity.

There are a number of characteristics that can distinguish different approaches to cluster analysis, which include:

a. Numerical, statistical, and conceptual clusters.

b. Agglomerative vs. divisive – Agglomerative methods start with individual taxa and seek to connect them into pairs, etc. in such a way that similarities between pairs or group members are maximized at each level. In contrast, divisive clustering splits are sought in the group of taxa that will in some way maximize the collective phenotype disparity between the two groups formed by the split.

c. Overlapping vs. disjoint clusters.

d. Incremental vs. non-incremental.

e. Flat vs. hierarchical representatives.

(ii) Clustering methods:

Clustering can be achieved in two ways:

a. Monothetic system — This system employs the attributes one at a time. The monothetic method obviously leads to artificial clustering.

b. Considering all their attributes simultaneously. This method gives a natural grouping.

One cluster or a group is separated from the other by a dividing line, which indicates a distinct gap between the two. In case of intraspecific clusters, there are often few discontinuities.

c. Phenetic clustering methods — Phenetic clustering methods have largely been overtaken by cladistic methods in order to relate data to evolutionary history, and they are now rarely employed in taxonomy as they often lead to substantially different clusters when applied to real data.

The most widely used phenetic clustering methods in taxonomy include the following:

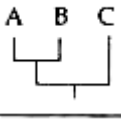
i. Nearest neighbor clustering method — It is also called single linkage clustering. In this method, phenograms are constructed by joining OTUs and groups on the basis of their most similar members, i.e., the shortest distance. Whether an OTU will join an existing cluster depends on its maximum similarity (or minimum distance from) any member of that cluster.

The phenogram is initiated by joining the two (or more) most similar OTUs.

Similarity matrix

	C	B	A
A	0.40	0.63	1.0
B	0.53	1.0	
C	1.0		

Sequence of procedures involved in the phenetic clustering of the above three taxa:

Step	Action	Result	Action	Tree
1	Find greatest similarity less than unity	0.63 (A-B)	Join A to B at level 0.63	
2	Find next highest similarity	0.53 (B-C)	Join C to A+B cluster at level 0.53	

Furthest neighbour clustering method (complete linkage):

Construction of furthest neighbor phenogram is essentially similar to that for nearest neighbor ones, with the only difference that the OTUs are joined at a level corresponding to the greatest distance between their component members instead of the shortest distance as in case of the latter. This method tends to emphasize differences between clusters.

Unweight pair-group method using arithmetic averages (UPGMA):

In this method an OTU is joined to existing clusters on the basis of their average (mean) distance to the members of that cluster (Fig. 9.1). The mean distance between two clusters of OTUs is calculated by adding together the distances between all possible pair-wise combinations of OTUs in the two clusters and dividing by the number of combinations.

The tree construction method is similar to nearest neighbour clustering. Since this method uses mean distances, it is not so subjected to the effects of aberrant OTUs, and hence preferred over nearest neighbour methods.

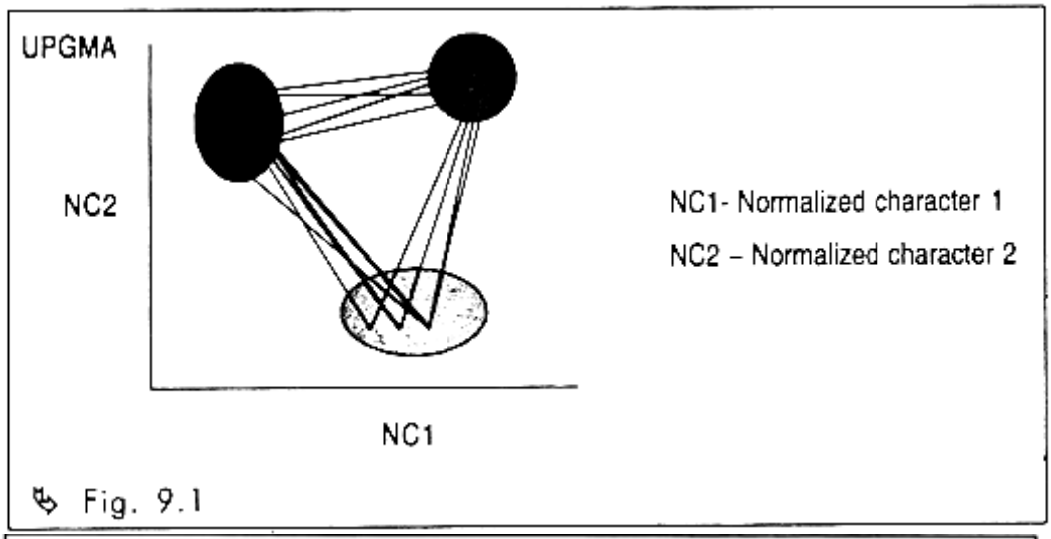


Fig. 9.1

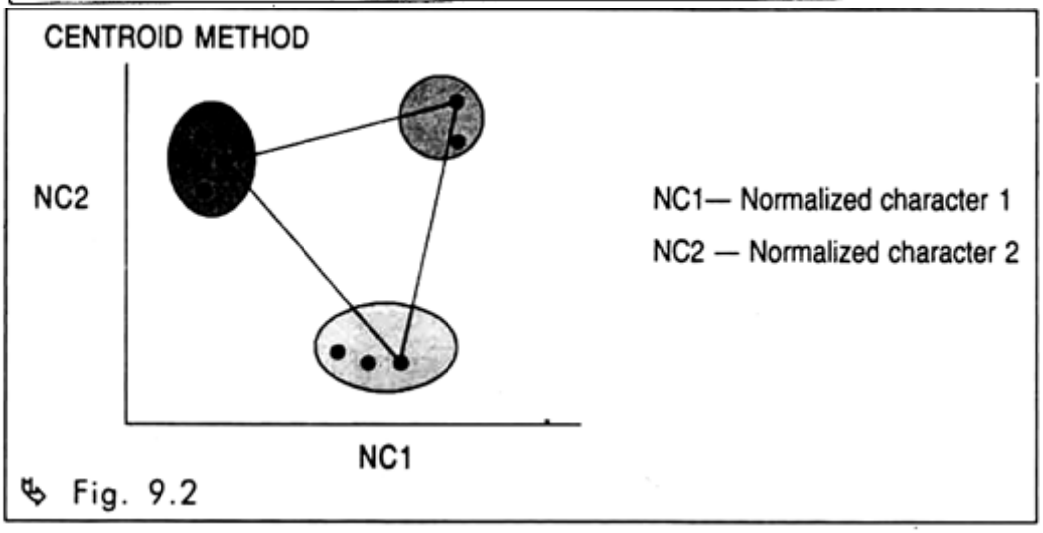


Fig. 9.2

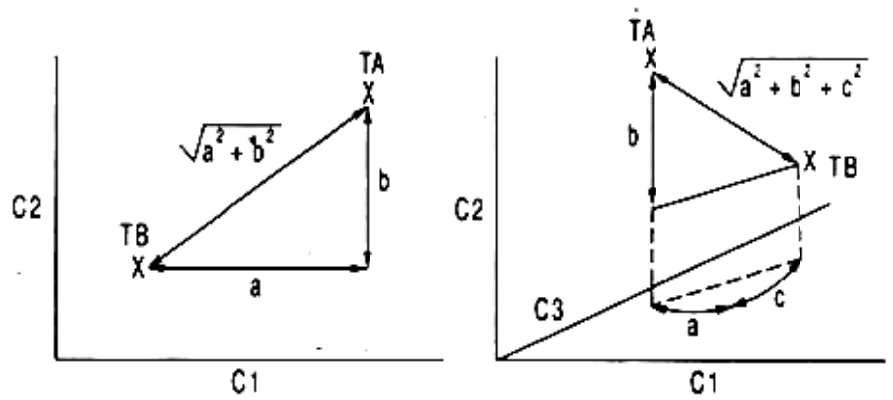


Fig. 9.3 : Illustration of the calculation of the shortest distance (Euclidean distance) between two points in 2-dimensional (left) and 3-dimensional (right) space.
[C1 - Character 1; C2- Character 2; C3- Character 3; TA – taxon A; TB – taxon B]

Weighted pair-group method using arithmetic averages (WPGMA):

This is a set of methods rather than a single one. They are essentially similar to UPGMA except that, they weight distances such that there is a tendency to emphasize separations between large clusters.

They are also therefore less subjected to the effects of single or small groups of outliers and hence preferred over nearest neighbor methods. In this method, the distances are usually weighted by some function of the number of OTUs in each cluster.

Centroid clustering:

Its basic aim is similar to UPGMA and WPGMA, but it requires the actual taxa versus character raw data, or more precisely normalized data, rather than inter-taxon distances. This method makes use of the Euclidian distances within the character hyperspace between the centroids of clusters of OTUs.

Euclidian distance is equivalent to the real physical distance by which the taxa would be separated if they were plotted in a space in which each axis represents one normalized variable and each axis is perpendicular to all others.

Calculation of Euclidian distance is shown below:

Differential shading of the similarity matrix:

This is a method to describe structure in matrices of similarity coefficients, in which similarity coefficients are grouped into five to ten evenly spaced classes, each of which are represented by different degrees of shading in the squares of half matrix.

Generally a darker shade is used for the highest value, and the lightest shade for the lowest value. Thus, the half matrix can be seen as a pattern to different shades, limited by a diagonal of squares with the darkest shade (Fig. 9.4a). Clusters can be more sharply defined by rearrangement of the sequence of OTUs.

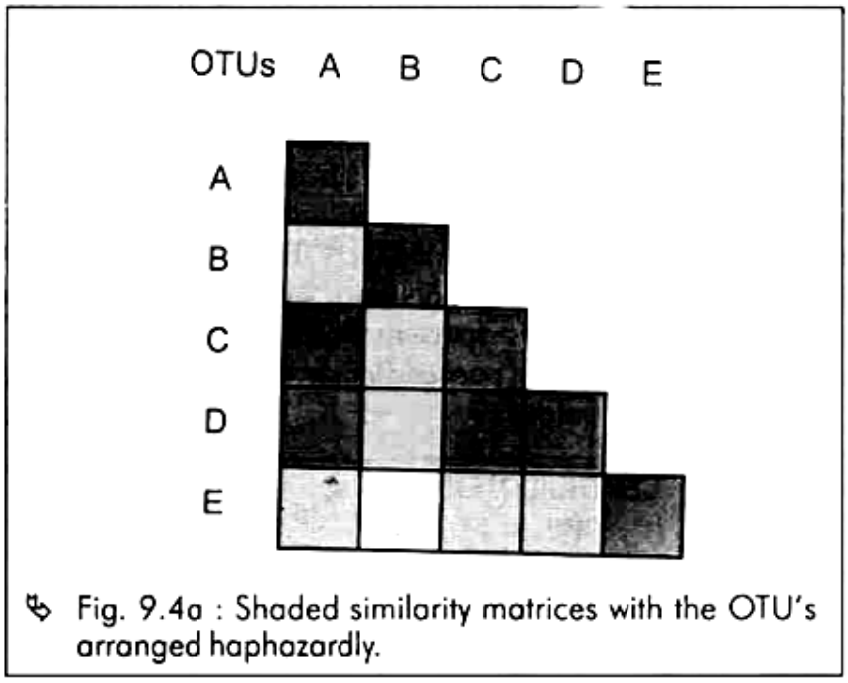


Fig. 9.4a : Shaded similarity matrices with the OTU's arranged haphazardly.

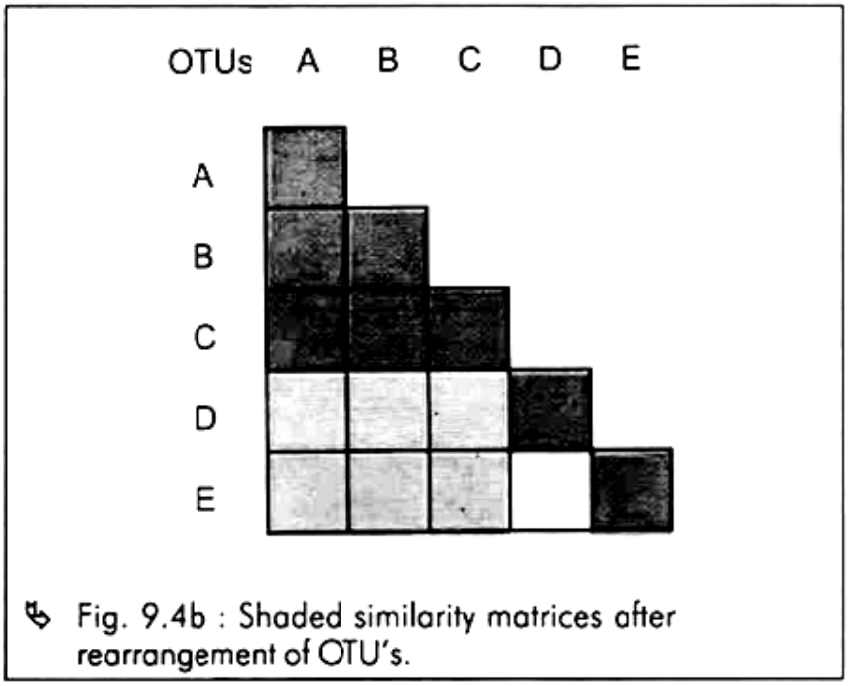


Fig. 9.4b : Shaded similarity matrices after rearrangement of OTU's.

Percentage similarity

- 100
- 90-99
- 80-89
- 70-79
- 60-69

Cluster analysis:

A large number of numerical techniques can be used to analyze the groups of related OTUs based on high similarity coefficients. These techniques include elementary cluster analysis, clustering by single, complete or average linkage, central or nodal clustering, etc. The groups of similar organisms organized in this manner are termed phenons, which are arbitrary and relative groups.

The clusters of phenons are then rearranged in a dendogram (Fig. 9.5), in which the mutually most similar taxa are paired and the pairs successively joined by the average similarity.

This finally results in a tree like dendogram when all units have been joined together, with the taxa at the tips of branches. The number of clusters is determined by drawing a horizontal (phenon line) intersecting the vertical lines of the dendogram.

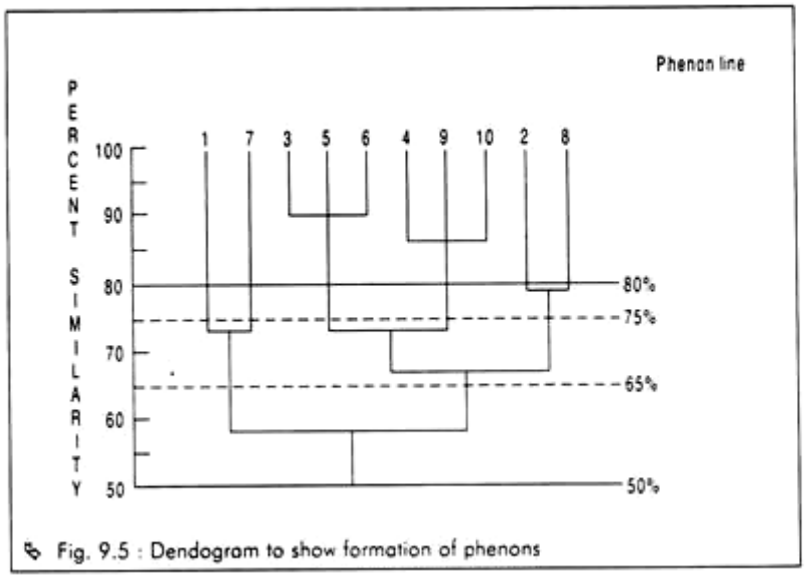


Fig. 9.5 : Dendogram to show formation of phenons

The delimitation of phenons is done by drawing a horizontal line across the dendrogram at a similarity value. For example a line at 75%, creates five 75- phenons 1; 7; 3, 5, 6; 4, 9, 10; and 2, 8., If the OTUs 1 to 10 in the above dendrogram had been species, an 80-phenon line could indicate 6 subgenera and a 65-phenon line two genera.

Probable Questions:

1. Classify Unit characters.

2. What are the types of multistate characters?

3. Define admissible and Inadmissible unit characters.

4. How Euclidian distance is calculated?

5. Describe briefly UPGMA and WUPGMA.

6. What is cluster analysis?

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors).

Unit-XI

Cladistic method of classification, difference in the application of phenetic and cladistic classification; cladistic and cladogram, eludistic methods, application of parsimony

Objective: In this section you will learn about Cladistic method of classification, difference in the application of phenetic and cladistic classification; cladistic and cladogram, eludistic methods, application of parsimony.

Introduction:

Wittering (1966) wrote a book called Phylogenetic Systematics, effectively founded the subject known as Phylogenetic systematics now called Cladistics. The term cladistics was coined by E. Mayr in 1969. Cladistics is a methodology that attempts to analyse phytogenetic data objectively, in a manner parallel to that in which taxometrics seeks to introduce objectivity into phenetics and Phenetic classification.

The methods of Wagner, Hennig etc., are known as Parsimony methods whereby they utilise the Principle of parsimony. Not only cladistics but phenetic taxonomists also used principle of Parsimony to relate extent OTUs without hypothesizing a priori. Cladistic methods differ fundamentally from taxometrics in the deductive (a priori) reasoning is used to determine routes of evolutionary change.

Hennig differentiated between Monophyletic, Paraphyletic and Polyphyletic groups and observed that taxa in a truly phylogenetic system should be only monophyletic. To identify monophyletic taxa, cladograms are constructed by considering primitive (Plesiomorphous) and advanced (apomorphous) characters.

The possession of Plesiomorphous, character-states is common by a group of taxa and is known as symplesiomorphy, and the possession of derived character-state in common is termed synapomorphy. Fig.1.shows that symplesiomorphy does not necessarily indicate monophyly.

It is equally indicative of Paraphyly, (e.g., X and Y in B) Synapomorphy indicate monophyly, (e.g., Y and Z in A), but it can be polyphyly due to parallelism, (e.g., X and Y in C) or convergence, (e.g., X and Y in D). Such cases are known as false synapomorphies.

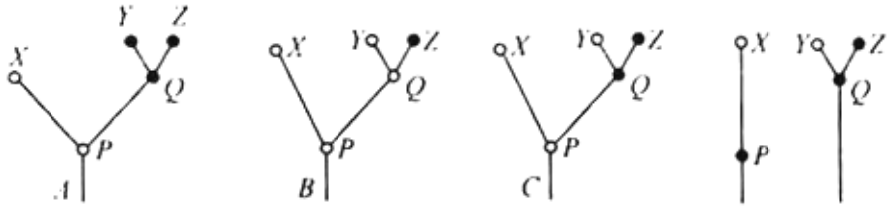


Fig. 1. Diagrams showing different origins of three species *X, Y, Z,* from the ancestral taxa *P* and *Q* in order to illustrate the concept of monophyly (A) Paraphyly (B), Polyphyly (C) and Parallelism and Convergence (D).

The basic units that are manipulated in cladistics are often known as evolutionary units (EUs), equivalent to the phenetic OTUs. Once a set of data relating to Plesimorphous versus apomorphous character-states has been accumulated for all the EUs, a data matrix can be constructed.

Cladogram is constructed based on the data. If the pairs of EUs are scored according to the number of differences in their respective character-states the table is formed which is called Manhattan distances which is in effect a dissimilarity matrix.

The dendrogram or cladograms are normally based upon the minimal or most parsimonious way in which the EUs can be connected to account for the data in the text table.

In case of fossils the cladogram are wholly hypothetical and are known together with the single hypothetical ancestor, as Hypothetical taxonomic unit (HTUs).When the hypothetical ancestral taxon is known, the evolutionary polarity is decided rooted tree or dendrogram is formed. When polarity of characters is not decided, the dendrogram obtained is not directional, this is called Unrooted tree or network. Network can become rooted by deciding a posteriori.Cladistic method basically views evolution as an ordered, divergent, step-wise transformation of characters from plesiomorphous to amorphous.

In 1977 the first symposium on cladistics for botanists was held at the AIBS meetings in EastLansing; Michigan. The proceeding of this published in 1978.

The second symposium was held in 1979 at the AIBS meeting in Stillwater, Oklahoma. As a result of disagreement between pheneticist and cladists, the cladists established the new Willi Henning society and its first meeting was held in Lawrence Kansas (Birth place of phenetics).

Methodology of Cladistics:
Depending upon the type of data and algorithms for the construction the procedure for cladistic analysis and classification varies.

Stuessy (1980) has given some conventional procedures, which are as follows:
i. Make evolutionary assumptions (select EUs, determine monophyletic grouping etc).

ii. Select characters of evolutionary interest.

iii. Describe and/or measure character states.

iv. Ascertain homologies of characters and character states.

v. Construct character state network.

vi. Determine polarity of character state network (primitive vs. derived conditions, i.e., root the character state network to form character to form character state trees. Group taxa based on synapmorphies.

vii. Construct basic data matrix.

viii. Select algorithm and generate trees (cladograms);

ix. Construct classification based upon cladograms.

x. Resolve the conflicts arisen by using some pre-defined method e.g., Parsimony.

xii. Build a tree following the rule like:

(a) All taxa go on the endpoints of the tree, never at the nodes.

(b) All nodes must have a list of Synamorphies, which are common to all the taxa above the node.

(c) All Synapmorphies appear on the tree only once, unless the character state was derived more than once through convergent evolution.

Convergence causes distortions in cladograms. This can be seen by the following phenomenon:
(a) Character-states arising by convergence are generally not logically correlated with other characters, and if a substantial number of characters is analysed those showing convergence are usually obvious.

(b) Parallelism and reversion, together termed Homoplasy; are less easily recognise. The presence of homoplasy means the correct cladogram might not be the one that appears to most parsimonious. Therefore methods depending on parsimony is less in these cases.

(c) Study of fossils led some cladists to use cladistics, i.e., Pattern or transformed cladistics merely to unravel to pattern of variation rather than detect the true geneology.

(d) Apart from parsimony methods other method is to utilize the concept of Character compatibility and is known as compatibility analysis or clique analysis. The method omits homoplasy.

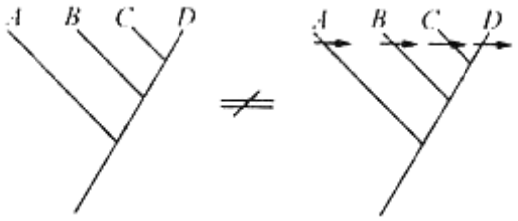
Groups of mutually compatible characters are termed cliques. This represents a group of characters in which homoplasy is absent.

For example if two characters A; B are considered with two states A_1A_2 and $B_1 B_2$ then the possible combinations are A_1B_1, A_1B_2, $A_2 B_1$ and $A_2 B_2$. The directional of evolution is A_1 to A_2 and B_1 to B_2, then if all four character state combinations are found in nature there must have been at least one reversal, i.e., A_2 to A_1 occurring twice. In this condition A and B are incompatible but if only 2 or 3 of the four combinations occur than A and B are compatible.
Goal of phylogenetic systematics is to understand the evolutionary relationships of all life on the planet and to reflect those relationships in a predictive classification.

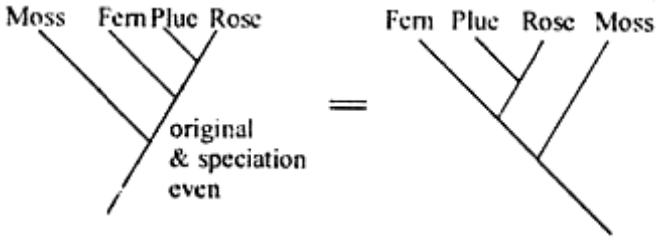
Two related components are:
(a) Discovering relationships, and

(b) Developing a classification to communicate those relationships.

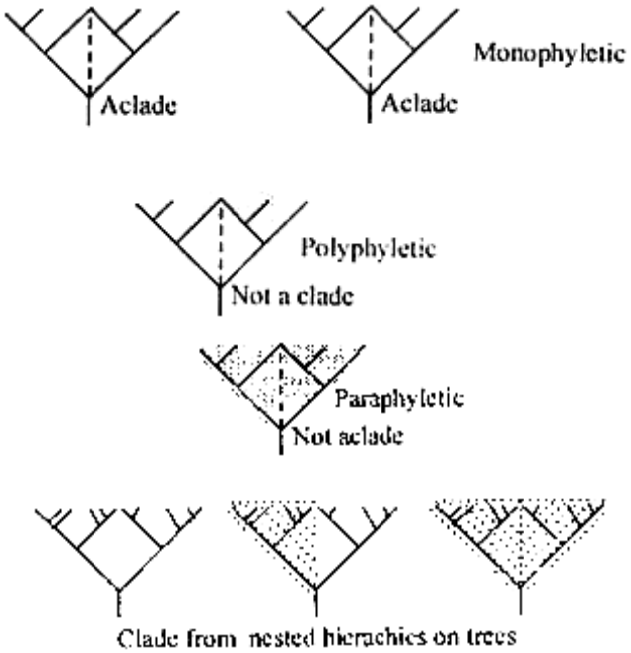
Phylogenetic tree diagrams:
Phylogenetic trees represent relative relationships between taxa. Tree diagram that seem to indicate directed character change or progress along the tips of the tree evolution produces a branching, tree like pattern of ancestor and descendent lineages-species alive today did not give rise to other species that are alive today.



Phylogenetic trees can be drawn in numerous ways, but it is always the relative position of taxa and their most recent common ancestors.



Reconstructing Phylogenetic Relationships Cladistics, methods, proposed by W. Hennin:
Based on recognition of clades: groups of organisms that descend from a common ancestor and shared derived characters.



Assumptions Characters:

(a) Heritable changes or mutations occur in lineage of reproducing organisms.

(b) Rate of mutation is greater than rate of cladogenesis or lineage splitting.

(c) Rate of mutation in characters is not so great as to swamp out evolutionary signal through multiple mutations back and forth in same character.

Vocabulary Characters:
(a) In cladistics, character can exist in two state.

(i) Derived apomorphic state

(ii) Ancestral plesiomorphic state.

(b) Shared derived character are known as Synapomorphies.

(c) Shared ancestral characters are known as Symplesiomorphies.

(d) Synapomorphy or Symplesiomorphy is relative and depends on the clade.

Difference between Organisms and their Characters:
(a) There are ancestral or primitive characters and derived or advanced characters in contrast no primitive or advanced organism.

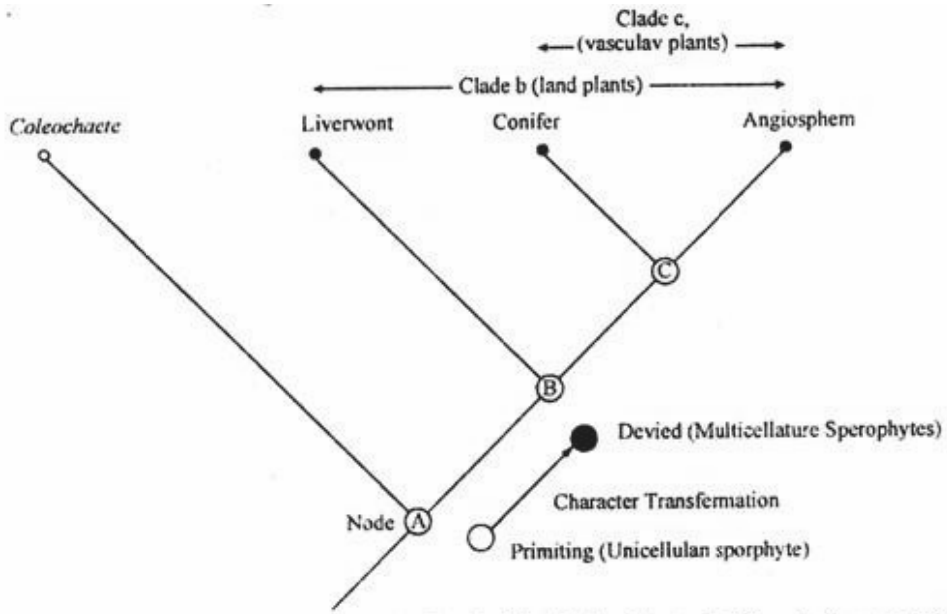


Fig. Multicellular sporophyte are Synapomorphy for clade b, the landplates but Symplesiomorphy for clade c the vascular plants.

(b) All organisms alive today have been evolving from the same length of time (from a common ancestor).

(c) Some organisms retain characters that are considered ancestral, but this does not mean that they are less evolved than others.

(d) Actually there are no "living fossil".

(e) All life is descended from a single ancestor. So we can reconstruct relationships.

(f) Evolution proceeds through a process of bifurcation even i.e., one lineage always splits into two.

Phenetics versus Cladistics:

- **Cladistics** can be defined as the study of the pathways of evolution. In other words, cladists are interested in such questions as: how many branches there are among a group of organisms; which branch connects to which other branch; and what is the branching sequence. A tree-like network that expresses such ancestor-descendant relationships is called a cladogram. Thus, a **cladogram** refers to the topology of a **rooted** phylogenetic tree.
- **Phenetics** is the study of relationships among a group of organisms on the basis of the **degree of similarity** between them, be that similarity molecular, phenotypic, or anatomical. A tree-like network expressing phenetic relationships is called a **phenogram**.

While a phenogram may serve as an indicator of cladistic relationships, it is not necessarily identical to the cladogram. If there is a linear relationship between the time of divergence and the degree of genetic (or morphological) divergence, the two types of trees may become identical to each other.

Parsimony:

Cladistic analysis depends on the principle of parsimony when selecting the best tree diagram to represent the relationships between the taxa at the tips.

(a) Parsimony assumes that the simplest solution is likely the best.

(b) In terms of tree diagrams, the most parimonious tree is the one that minimizes the number of character changes along the branches i.e., the less no. of steps the better parsimony.

Parsimony works well for morphological characters having multiple, complete character with relatively low rates of evolution.

DNA sequence data has only four (AGCT) possible characters leading to problems with tree reconstruction because it increases the likelihood of homoplasy.

Homoplasy means that the characters that look as identical through descent from a common ancestor, when in fact they are the same due to convergent evolution.

Major difference between Henning's "Phylogenetic Systematics" and "Evolutionary Systematics" is that Henning's classification only recognizes monophyletic groups. The taxa recognized in phylogenetic classification are based on their shared features and not on their differences with other taxa.

In 2000, the first draft of a purely phylogenetic code of binomial nomenclature (Phylocode) was released. Phylocode is a set of rules for naming Clades based on a phylogenetic hypothesis (tree).

(a) No ranks are associated with names.

(b) Clades are defined using "specifiers" which are reference organisms (analogous to type specimen).

(c) To define a clade, at least two specifiers are required along with their most recent common ancestors.

Probable Questions:

1. Write short notes on difference between phenetics and cladistics.

2. Describe methodology of cladistics.

3. Write short notes on Parsimony. How it is applied in taxonomical classification.

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors).

Unit-XII

Morphological taxonomy and molecular taxonomy; Construction of phylogenetic trees using molecular data

Objective: In this section you will learn about Morphological taxonomy and molecular taxonomy and also about construction of phylogenetic trees using molecular data.

Introduction:

Humans as a group are big on organizing things. Not necessarily things like closets or rooms; I personally score low on the organization front for both of those things. Instead, people often like to group and order the things they see in the world around them. Starting with the Greek philosopher Aristotle, this desire to classify has extended to the many and diverse living things of Earth.

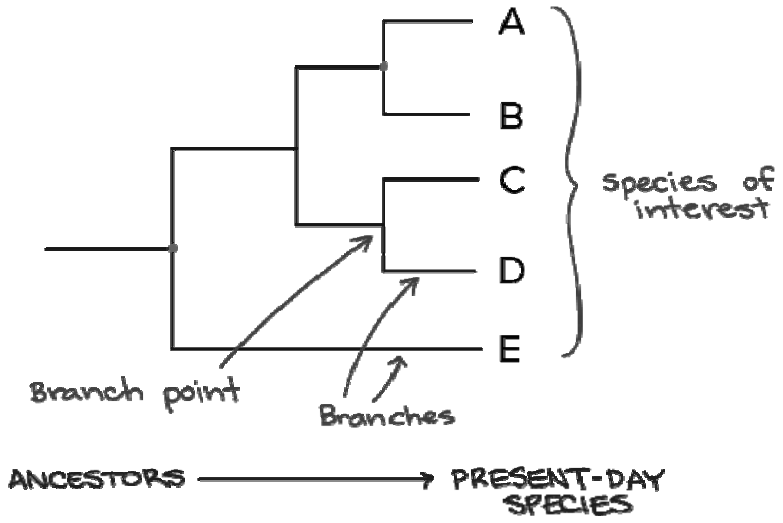
Most modern systems of classification are based on evolutionary relationships among organisms – that is, on the organisms' **phylogeny**. Classification systems based on phylogeny organize species or other groups in ways that reflect our understanding of how they evolved from their common ancestors.

In this article, we'll take a look at **phylogenetic trees**, diagrams that represent evolutionary relationships among organisms. We'll see exactly what we can (and can't!) infer from a phylogenetic tree, as well as what it means for organisms to be more or less related in the context of these trees.

Anatomy of a phylogenetic tree

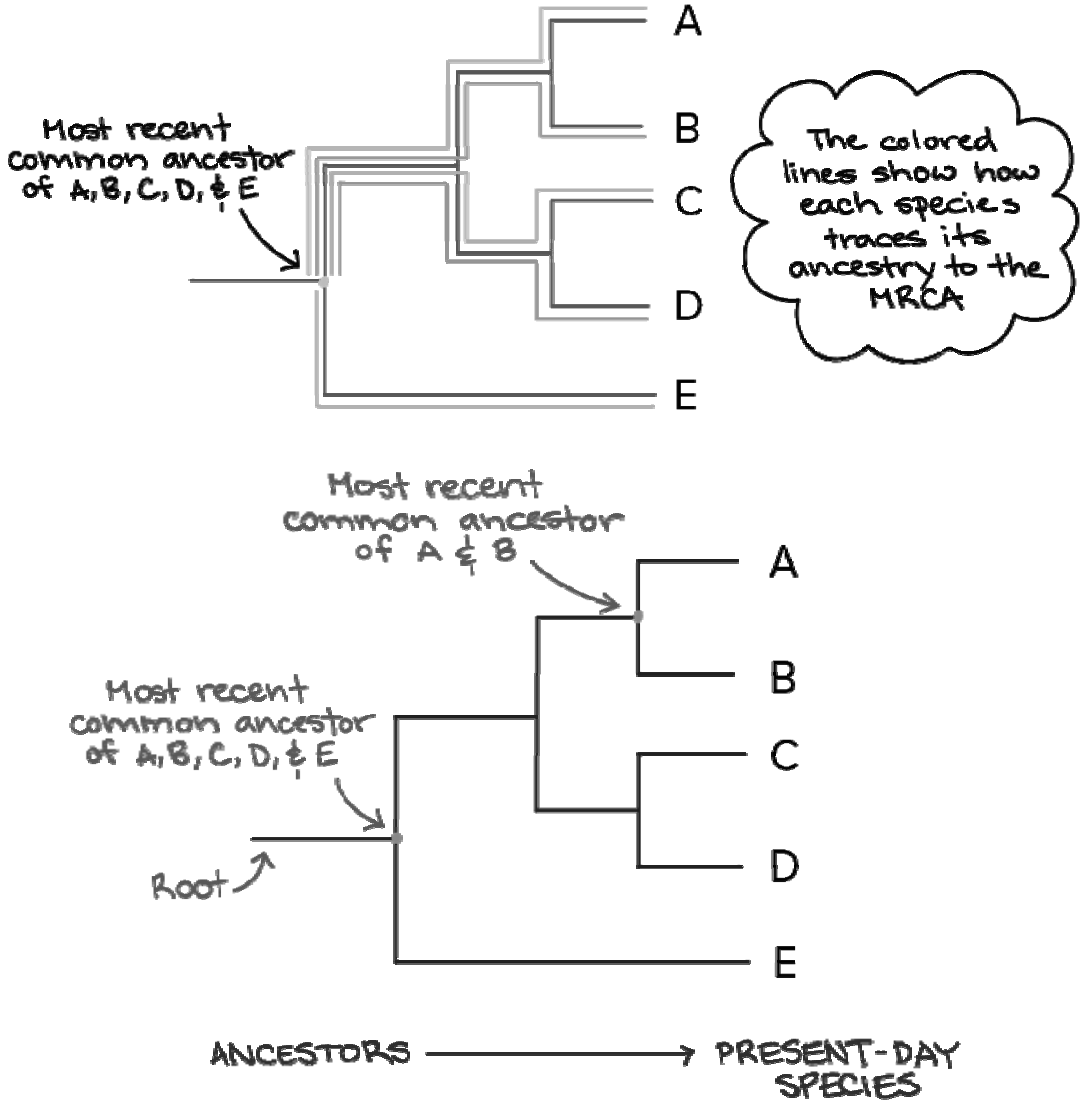
When we draw a phylogenetic tree, we are representing our best hypothesis about how a set of species (or other groups) evolved from a common ancestor^11start superscript, 1, end superscript. As we'll explore further in the article on building trees, this hypothesis is based on information we've collected about our set of species – things like their physical features and the DNA sequences of their genes.

In a phylogenetic tree, the species or groups of interest are found at the tips of lines referred to as the tree's **branches**. For example, the phylogenetic tree below represents relationships between five species, A, B, C, D, and E, which are positioned at the ends of the branches:



The pattern in which the branches connect represents our understanding of how the species in the tree evolved from a series of common ancestors. Each branch point (also called an **internal node**) represents a **divergence** event, or splitting apart of a single group into two descendant groups.

At each branch point lies the **most recent common ancestor** of all the groups descended from that branch point. For instance, at the branch point giving rise to species A and B, we would find the most recent common ancestor of those two species. At the branch point right above the **root** of the tree, we would find the most recent common ancestor of all the species in the tree (A, B, C, D, E).



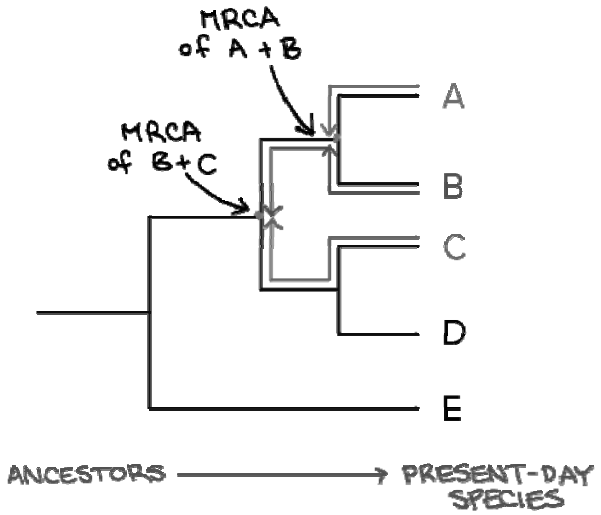
Each horizontal line in our tree represents a series of ancestors, leading up to the species at its end. For instance, the line leading up to species E represents the species' ancestors since it diverged from the other species in the tree. Similarly, the root represents a series of ancestors leading up to the most recent common ancestor of all the species in the tree.

Which species are more related?

In a phylogenetic tree, the **relatedness** of two species has a very specific meaning. Two species are *more* related if they have a *more recent* common ancestor, and *less* related if they have a *less recent* common ancestor.

We can use a pretty straightforward method to find the most recent common ancestor of any pair or group of species. In this method, we start at the branch ends carrying the two species of interest and "walk backwards" in the tree until we find the point where the species' lines converge.

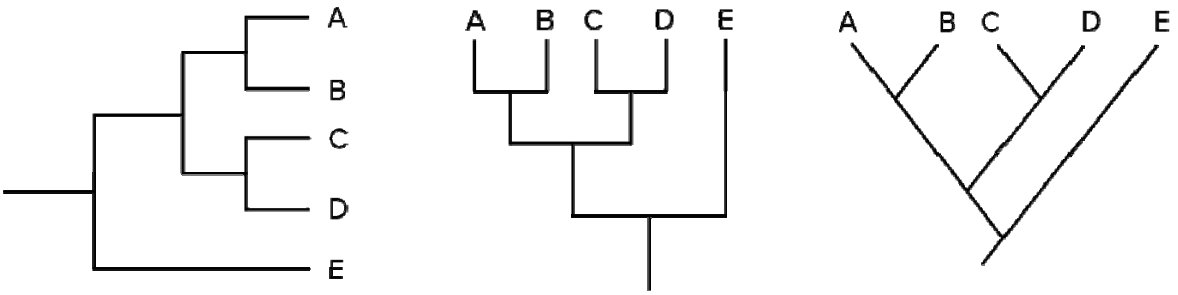
For instance, suppose that we wanted to say whether A and B or B and C are more closely related. To do so, we would follow the lines of both pairs of species backward in the tree. Since A and B converge at a common ancestor first as we move backwards, and B only converges with C after its junction point with A, we can say that A and B are more related than B and C.



Importantly, there are some species whose relatedness we can't compare using this method. For instance, we can't say whether A and B are more closely related than C and D. That's because, by default, the horizontal axis of the tree doesn't represent time in a direct way. So, we can only compare the timing of branching events that occur on the same lineage (same direct line from the root of the tree), and not those that occur on different lineages.

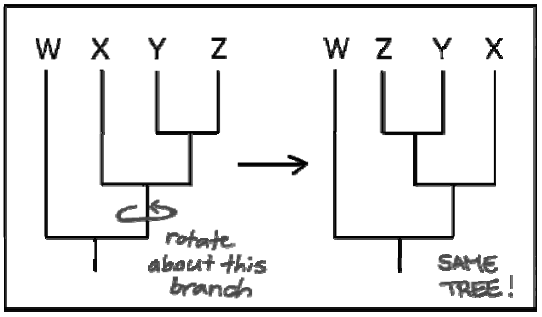
Some tips for reading phylogenetic trees

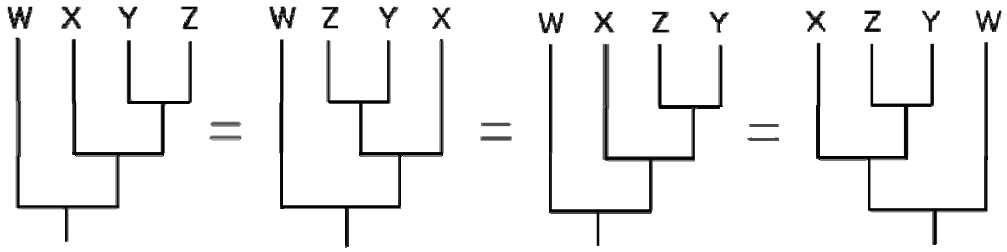
You may see phylogenetic trees drawn in many different formats. Some are blocky, like the tree at left below. Others use diagonal lines, like the tree at right below. You may also see trees of either kind oriented vertically or flipped on their sides, as shown for the blocky tree.



The three trees above represent identical relationships among species A, B, C, D, and E. You may want to take a moment to convince yourself that this is really the case – that is, that no branching patterns or recent-ness of common ancestors are different between the two trees. The identical information in these different-looking trees reminds us that it's the branching pattern (and not the lengths of branches) that's meaningful in a typical tree.

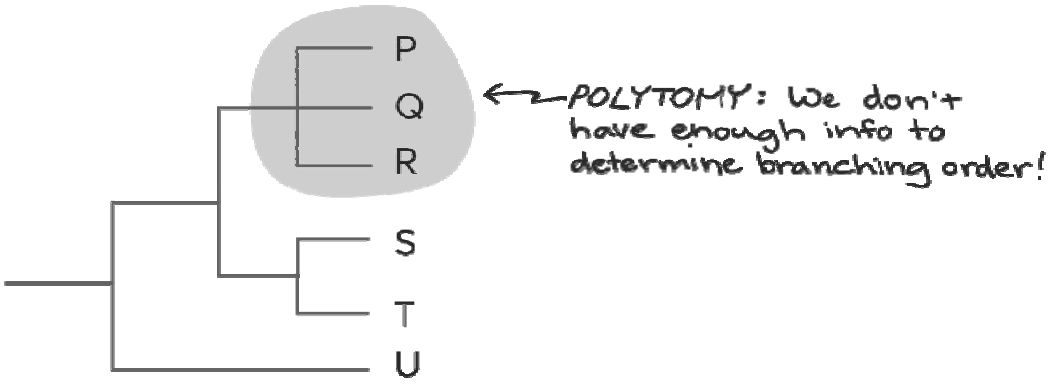
Another critical point about these trees is that if you rotate the structures, using one of the branch points as a pivot, you don't change the relationships. So just like the two trees above, which show the same relationships even though they are formatted differently, all of the trees below show the same relationships among four species:





If you don't see right away how that is true (and I didn't, on first read!), just concentrate on the relationships and the branch points rather than on the ordering of species (W, X, Y, and Z) across the tops of the diagrams. That ordering actually doesn't give us useful information. Instead, it's the branch structure of each diagram that tells us what we need to understand the tree.

So far, all the trees we've looked at have had nice, clean branching patterns, with just two lineages (lines of descent) emerging from each branch point. However, you may see trees with a **polytomy** (*poly*, many; *tomy*, cuts), meaning a branch point that has three or more different species coming off of it^22start superscript, 2, end superscript. In general, a polytomy shows where we don't have enough information to determine branching order.



If we later get more information about the species in a tree, we may be able to resolve a polytomy using the new information.

Where do these trees come from?

To generate a phylogenetic tree, scientists often compare and analyze many characteristics of the species or other groups involved. These characteristics can include external morphology (shape/appearance), internal anatomy, behaviors, biochemical pathways, DNA and protein sequences, and even the characteristics of fossils.

To build accurate, meaningful trees, biologists will often use many different characteristics (reducing the chances of any one imperfect piece of data leading to a wrong tree). Still, phylogenetic trees are hypotheses, not definitive answers, and they can only be as good as the data available when they're made. Trees are revised and updated over time as new data becomes available and can be added to the analysis. This is particularly true today, as DNA sequencing increases our ability to compare genes between species.

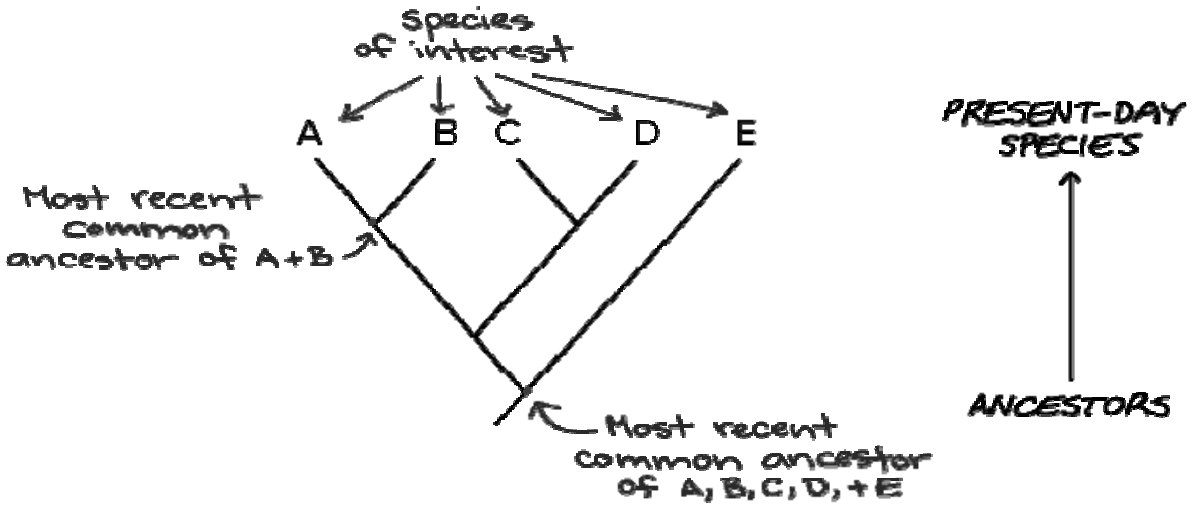
Building a phylogenetic tree

Key points:

- **Phylogenetic trees** represent hypotheses about the evolutionary relationships among a group of organisms.
- A phylogenetic tree may be built using morphological (body shape), biochemical, behavioral, or molecular features of species or other groups.
- In building a tree, we organize species into nested groups based on shared **derived traits** (traits different from those of the group's ancestor).
- The sequences of genes or proteins can be compared among species and used to build phylogenetic trees. Closely related species typically have few sequence differences, while less related species tend to have more.

Overview of phylogenetic trees

In a phylogenetic tree, the species of interest are shown at the tips of the tree's branches. The branches themselves connect up in a way that represents the evolutionary history of the species—that is, how we think they evolved from a common ancestor through a series of divergence (splitting-in-two) events. At each branch point lies the most recent common ancestor shared by all of the species descended from that branch point. The lines of the tree represent long series of ancestors that extend from one species to the next.



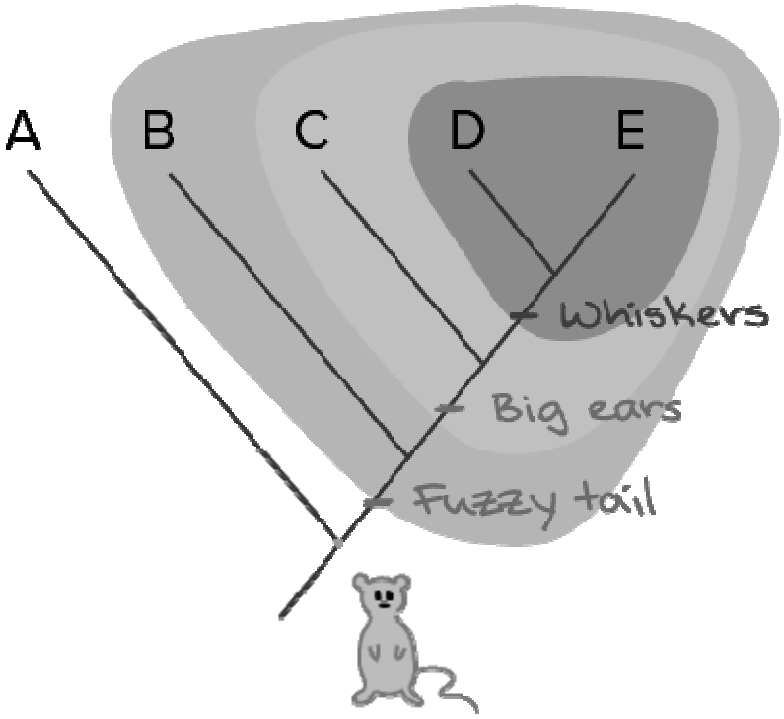
For a more detailed explanation, check out the article on phylogenetic trees.

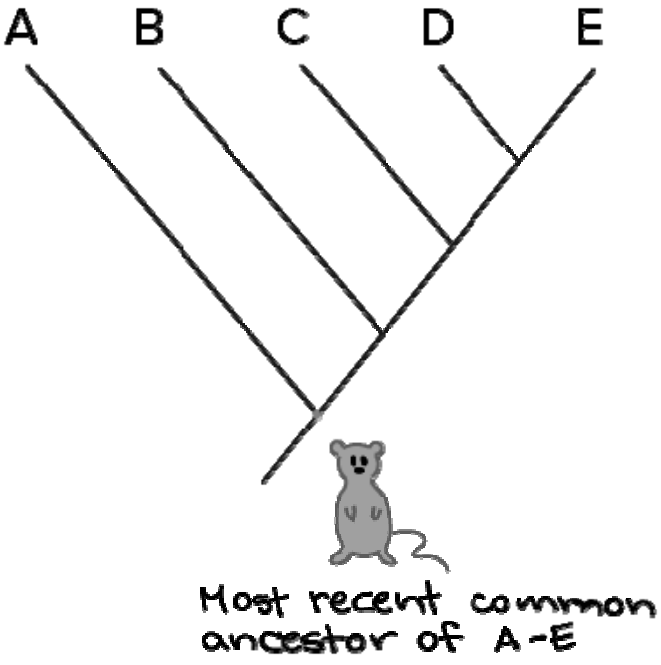
Even once you feel comfortable reading a phylogenetic tree, you may have the nagging question: *How do you build one of these things?* In this article, we'll take a closer look at how phylogenetic trees are constructed.

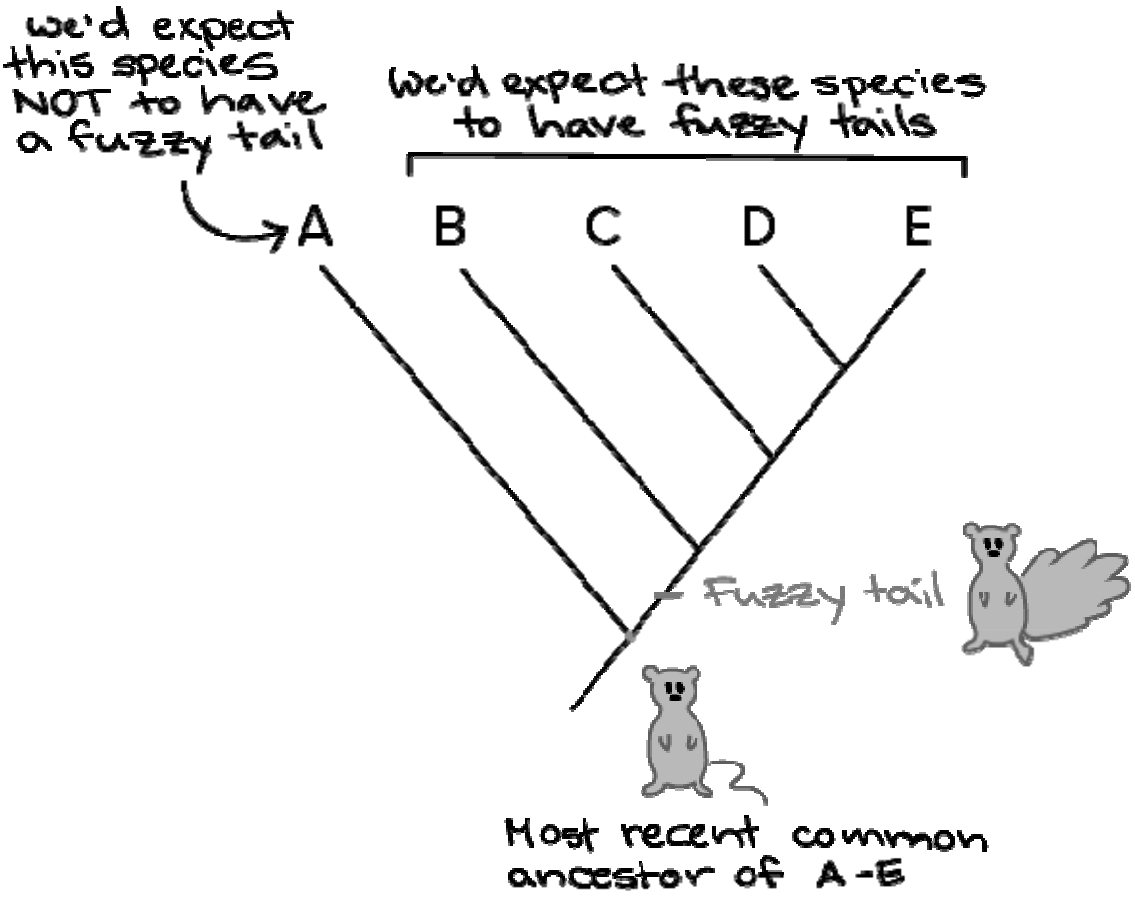
The idea behind tree construction

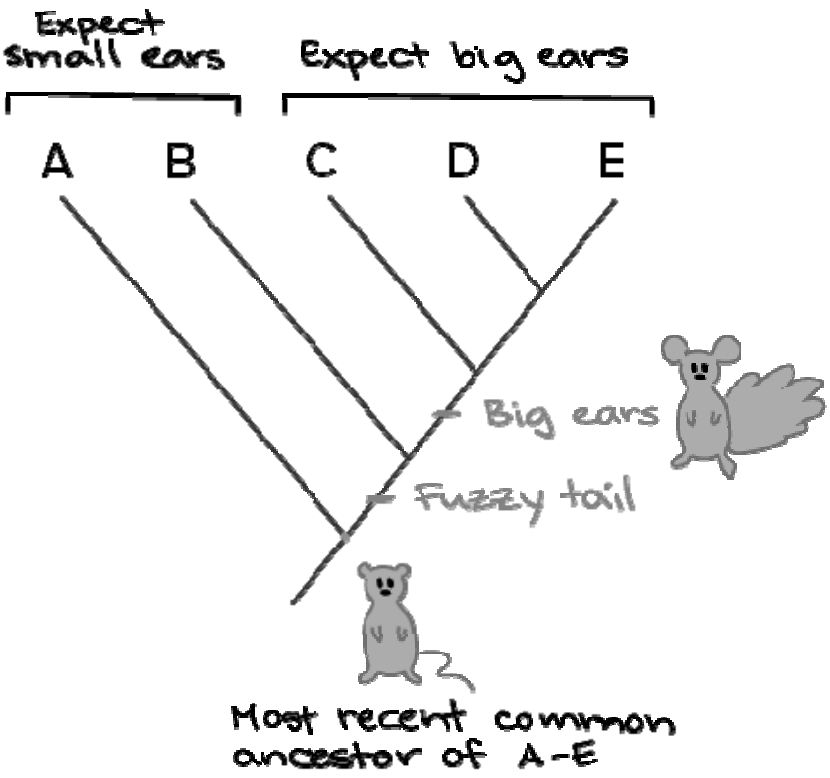
How do we build a phylogenetic tree? The underlying principle is Darwin's idea of "descent with modification." Basically, by looking at the pattern of modifications (novel traits) in present-day organisms, we can figure out—or at least, make hypotheses about—their path of descent from a common ancestor.

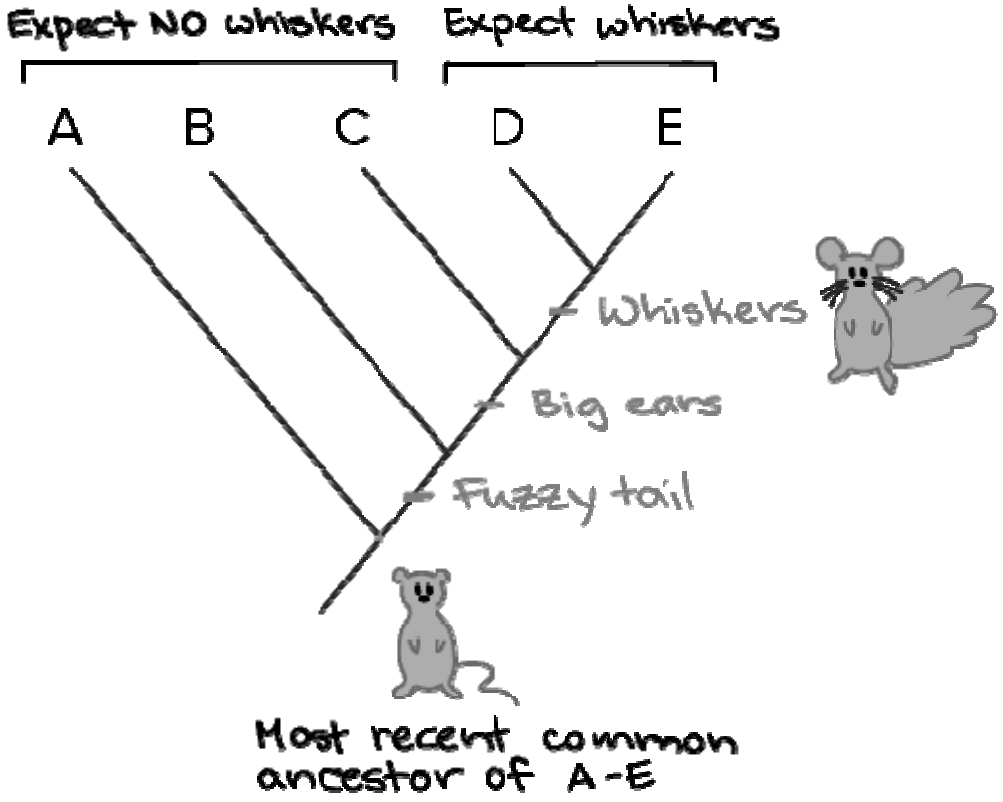
As an example, let's consider the phylogenetic tree below (which shows the evolutionary history of a made-up group of mouse-like species). We see three new traits arising at different points during the evolutionary history of the group: a fuzzy tail, big ears, and whiskers. Each new trait is shared by all of the species descended from the ancestor in which the trait arose (shown by the tick marks), but absent from the species that split off before the trait appeared.

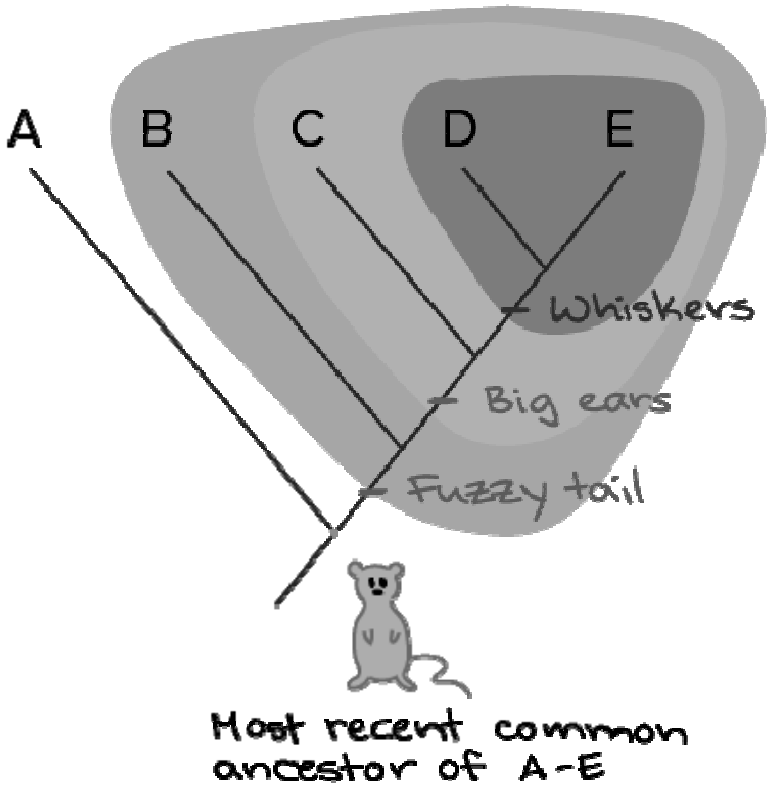

A
B
C
D
E
Whiskers
Big ears
Fuzzy tail
Most recent common ancestor of A-E


A
B
C
D
E
Most recent common ancestor of A-E


we'd expect this species NOT to have a fuzzy tail
We'd expect these species to have fuzzy tails
A
B
C
D
E
= Fuzzy tail
Most recent common ancestor of A-E


Expect small ears
Expect big ears
A
B
C
D
E
= Big ears
= Fuzzy tail
Most recent common ancestor of A-E





When we are building phylogenetic trees, traits that arise during the evolution of a group and differ from the traits of the ancestor of the group are called **derived traits**. In our example, a fuzzy tail, big

ears, and whiskers are derived traits, while a skinny tail, small ears, and lack of whiskers are **ancestral traits**. An important point is that a derived trait may appear through either loss or gain of a feature. For instance, if there were another change on the E lineage that resulted in loss of a tail, taillessness, would be considered a derived trait.

Derived traits shared among the species or other groups in a dataset are key to helping us build trees. As shown above, shared derived traits tend to form nested patterns that provide information about when branching events occurred in the evolution of the species.

When we are building a phylogenetic tree from a dataset, our goal is to use shared derived traits in present-day species to infer the branching pattern of their evolutionary history. The trick, however, is that we can't watch our species of interest evolving and see when new traits arose in each lineage.

Instead, we have to work backwards. That is, we have to look at our species of interest – such as A, B, C, D, and E – and figure out which traits are ancestral and which are derived. Then, we can use the shared derived traits to organize the species into nested groups like the ones shown above. A tree made in this way is a hypothesis about the evolutionary history of the species – typically, one with the simplest possible branching pattern that can explain their traits.

Example: Building a phylogenetic tree

If we were biologists building a phylogenetic tree as part of our research, we would have to pick which set of organisms to arrange into a tree. We'd also have to choose which characteristics of those organisms to base our tree on (out of their many different physical, behavioral, and biochemical features).

If we're instead building a phylogenetic trees for a class (which is probably more likely for readers of this article), odds are that we'll be given a set of characteristics, often in the form of a table, that we need to convert into a tree. For example, this table shows presence (+) or absence (0) of various features:

Feature	Lamprey	Antelope	Bald eagle	Alligator	Sea bass
Lungs	0	+	+	+	0
Jaws	0	+	+	+	+
Feathers	0	0	+	0	0
Gizzard	0	0	+	+	0
Fur	0	+	0	0	0

Next, we need to know which form of each characteristic is ancestral and which is derived. For example, is the presence of lungs an ancestral trait, or is it a derived trait? As a reminder, an ancestral trait is what we think was present in the common ancestor of the species of interest. A derived trait is a form that we think arose somewhere on a lineage descended from that ancestor.

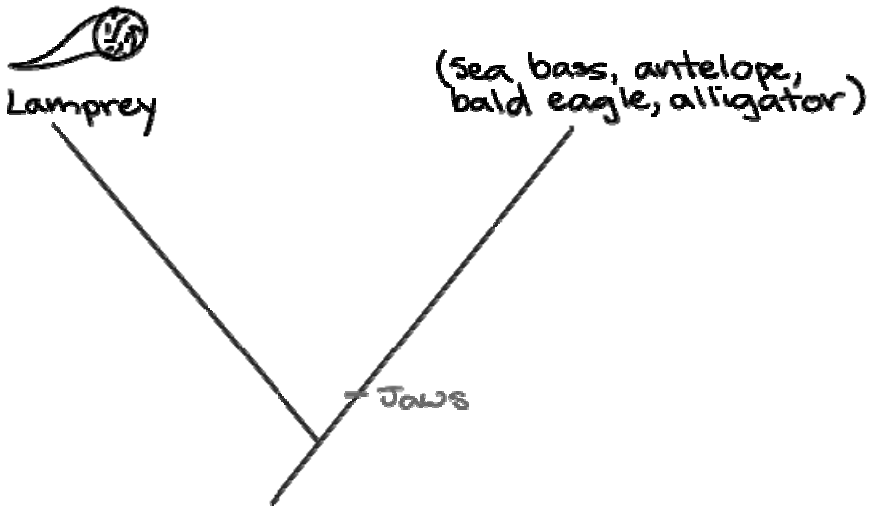
Without the ability to look into the past (which would be handy but, alas, impossible), how do we know which traits are ancestral and which derived?

- In the context of homework or a test, the question you are solving may tell you which traits are derived vs. ancestral.
- If you are doing your own research, you may have knowledge that allows you identify ancestral and derived traits (e.g., based on fossils).
- You may be given information about an **outgroup**, a species that's more distantly related to the species of interest than they are to one another.
  If we are given an outgroup, the outgroup can serve as a proxy for the ancestral species. That is, we may be able to assume that its traits represent the ancestral form of each characteristic.

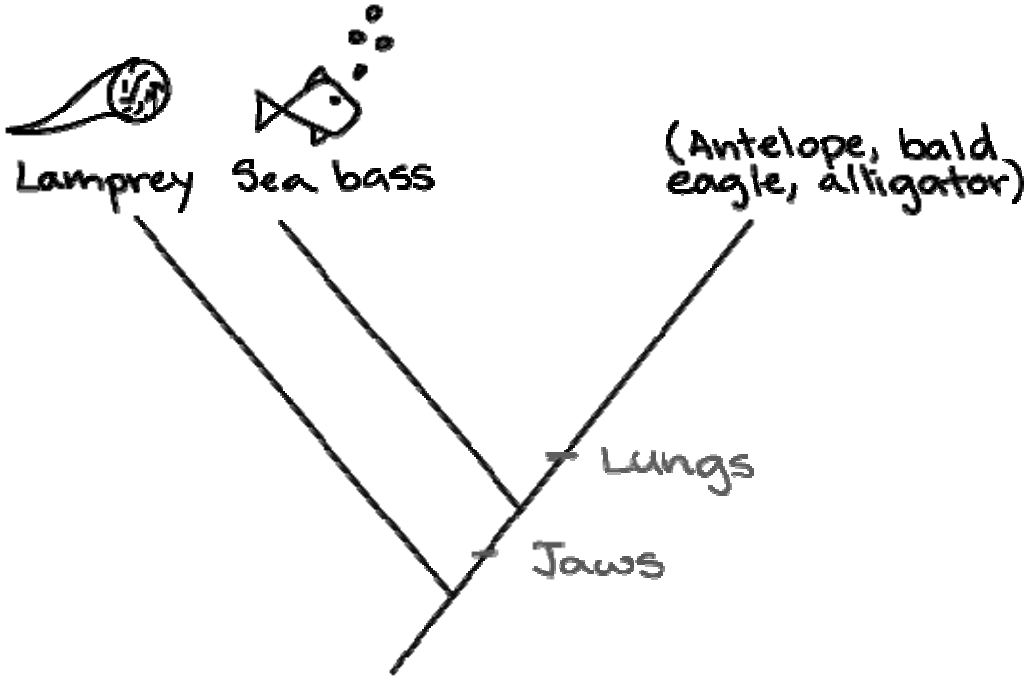
  For instance, in our example (data repeated below for convenience), the lamprey, a jawless fish that lacks a true skeleton, is our outgroup. As shown in the table, the lamprey lacks all of the listed features: it has no lungs, jaws, feathers, gizzard, or fur. Based on this information, we will assume that absence of these features is ancestral, and that presence of each feature is a derived trait.

Feature	Lamprey	Antelope	Bald eagle	Alligator	Sea bass
Lungs	**0**	+	+	+	0
Jaws	**0**	+	+	+	+
Feathers	**0**	0	+	0	0
Gizzard	**0**	0	+	+	0
Fur	**0**	+	0	0	0

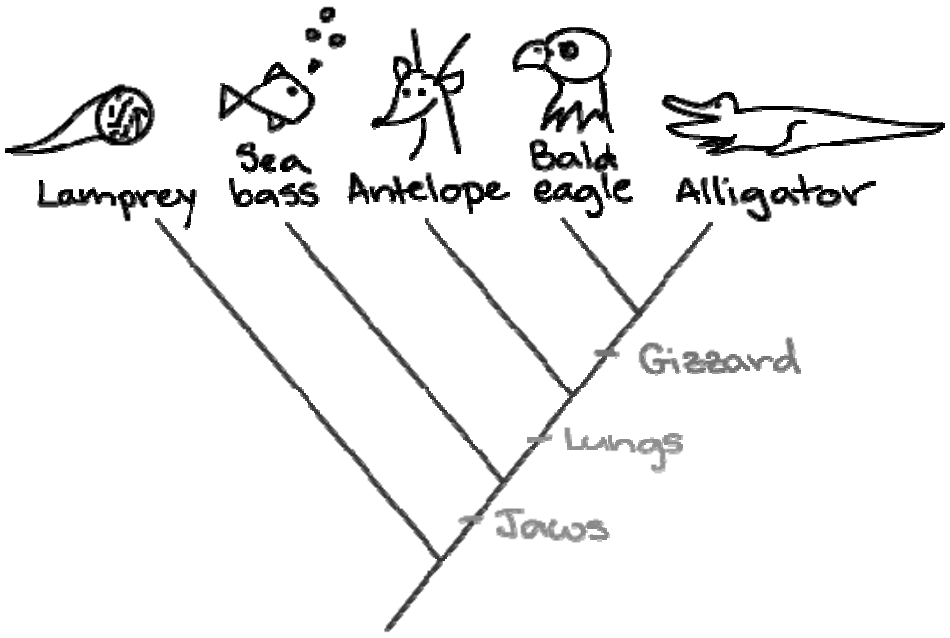
Now, we can start building our tree by grouping organisms according to their shared derived features. A good place to start is by looking for the derived trait that is shared between the largest number of organisms. In this case, that's the presence of jaws: all the organisms except the outgroup species (lamprey) have jaws. So, we can start our tree by drawing the lamprey lineage branching off from the rest of the species, and we can place the appearance of jaws on the branch carrying the non-lamprey species.



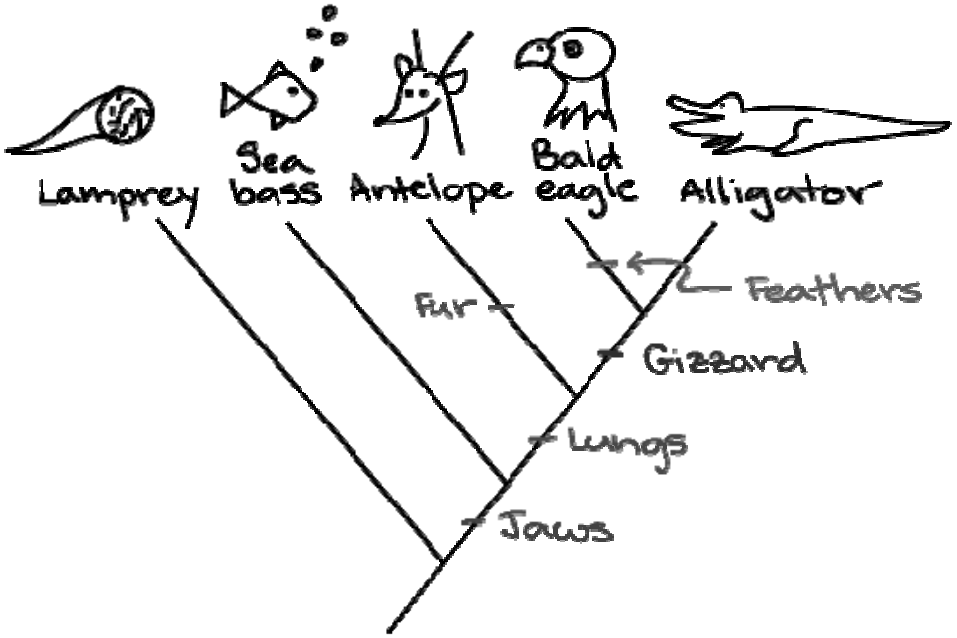
Next, we can look for the derived trait shared by the next-largest group of organisms. This would be lungs, shared by the antelope, bald eagle, and alligator, but not by the sea bass. Based on this pattern, we can draw the lineage of the sea bass branching off, and we can place the appearance of lungs on the lineage leading to the antelope, bald eagle, and alligator.



Following the same pattern, we can now look for the derived trait shared by the next-largest number of organisms. That would be the gizzard, which is shared by the alligator and the bald eagle (and absent from the antelope). Based on this data, we can draw the antelope lineage branching off from the alligator and bald eagle lineage, and place the appearance of the gizzard on the latter.



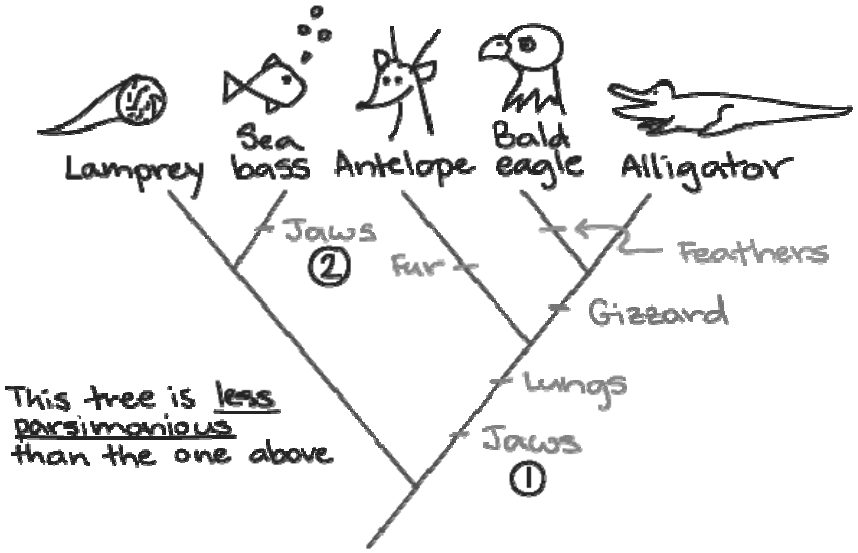
What about our remaining traits of fur and feathers? These traits are derived, but they are not shared, since each is found only in a single species. Derived traits that aren't shared don't help us build a tree, but we can still place them on the tree in their most likely location. For feathers, this is on the lineage leading to the bald eagle (after divergence from the alligator). For fur, this is on the antelope lineage, after its divergence from the alligator and bald eagle.



Parsimony and pitfalls in tree construction

When we were building the tree above, we used an approach called **parsimony**. Parsimony essentially means that we are choosing the simplest explanation that can account for our observations. In the context of making a tree, it means that we choose the tree that requires the fewest independent genetic events (appearances or disappearances of traits) to take place.

For example, we *could* have also explained the pattern of traits we saw using the following tree:

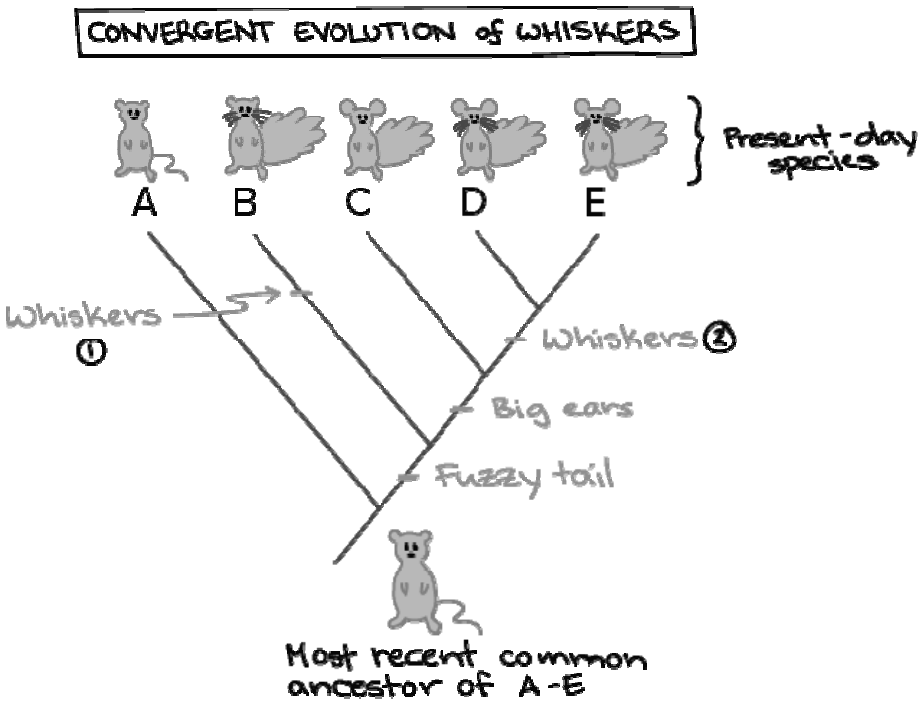


This series of events also provides an evolutionary explanation for the traits we see in the five species. However, it is *less parsimonious* because it requires more independent changes in traits to take place. Because where we've put the sea bass, we have to hypothesize that jaws independently arose two separate times (once in the sea bass lineage, and once in the lineage leading to antelopes, bald eagles, and alligators). This gives the tree a total of 666 tick marks, or trait change events, versus 555 in the more parsimonious tree above.

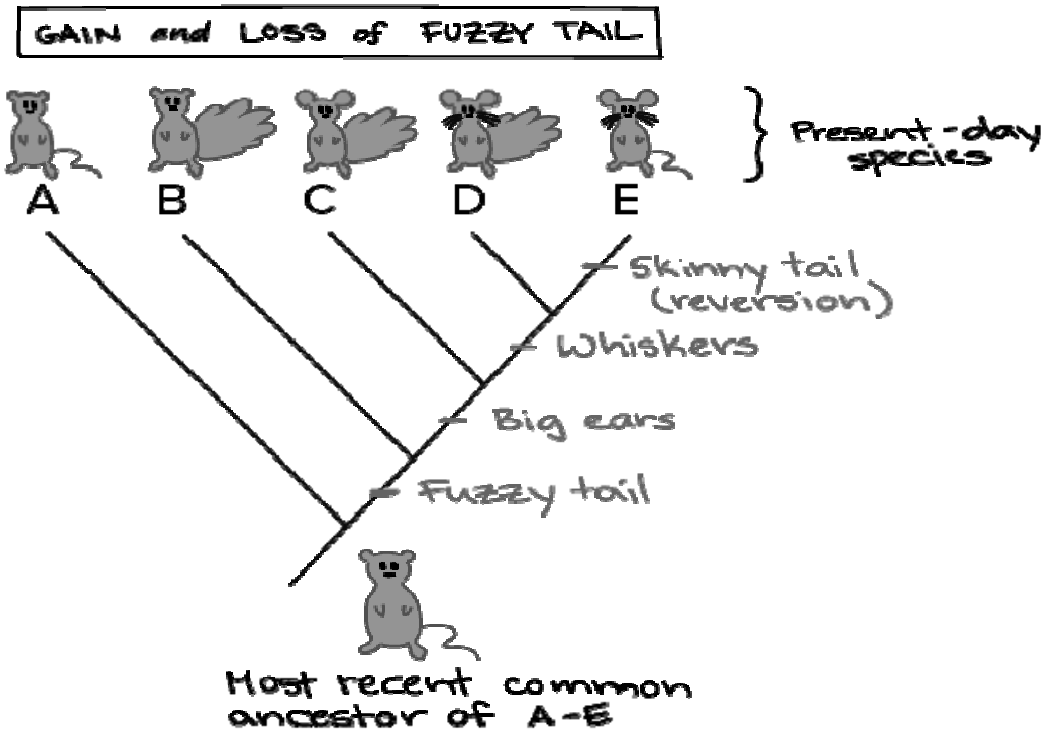
In this example, it may seem fairly obvious that there is one best tree, and counting up the tick marks may not seem very necessary. However, when researchers make phylogenies as part of their work, they often use a large number of characteristics, and the patterns of these characteristics rarely agree 100\%100%100, percent with one another. Instead, there are some conflicts, where one tree would fit better with the pattern of one trait, while another tree would fit better with the pattern of another trait. In these cases, the researcher can use parsimony to choose the one tree (hypothesis) that fits the data best.

You may be wondering: Why don't the trees all agree with one another, regardless of what characteristics they're built on? After all, the evolution of a group of species did happen in one particular way in the past. The issue is that, when we build a tree, we are reconstructing that evolutionary history from incomplete and sometimes imperfect data. For instance:

- We may not always be able to distinguish features that reflect shared ancestry (**homologous** features) from features that are similar but arose independently (**analogous** features arising by convergent evolution).



- Traits can be gained and lost multiple times over the evolutionary history of a species. A species may have a derived trait, but then lose that trait (revert back to the ancestral form) over the course of evolution.



Biologists often use many different characteristics to build phylogenetic trees because of sources of error like these. Even when all of the characteristics are carefully chosen and analyzed, there is still the potential for some of them to lead to wrong conclusions (because we don't have complete information about events that happened in the past).

Use of molecular data to build phylogenetic trees

A tool that has revolutionized, and continues to revolutionize, phylogenetic analysis is DNA sequencing. With DNA sequencing, rather than using physical or behavioral features of organisms to build trees, we can instead compare the sequences of their orthologous (evolutionarily related) genes or proteins.

The basic principle of such a comparison is similar to what we went through above: there's an ancestral form of the DNA or protein sequence, and changes may have occurred in it over evolutionary time. However, a gene or protein doesn't just correspond to a single characteristic that exists in two states.

Instead, each nucleotide of a gene or amino acid of a protein can be viewed as a separate feature, one that can flip to multiple states (e.g., A, T, C, or G for a nucleotide) via mutation. So, a gene with 300300300 nucleotides in it could represent 300300300 different features existing in 444 states!

The amount of information we get from sequence comparisons—and thus, the resolution we can expect to get in a phylogenetic tree—is much higher than when we're using physical traits.

To analyze sequence data and identify the most probable phylogenetic tree, biologists typically use computer programs and statistical algorithms. In general, though, when we compare the sequences of a gene or protein between species:

- A larger number of differences corresponds to *less* related species
- A smaller number of differences corresponds to *more* related species

For example, suppose we compare the beta chain of hemoglobin (the oxygen-carrying protein in blood) between humans and a variety of other species. If we compare the human and gorilla versions of the protein, we'll find only 111amino acid difference. If we instead compare the human and dog proteins, we'll find 151515 differences. With human versus chicken, we're up to 454545 amino acid differences, and with human versus lamprey (a jawless fish), we see 127127127 differences. These numbers reflect that, among the species considered, humans are most related to the gorilla and least related to the lamprey.

Probable Questions:

1. Describe the anatomy of a phylogenetic tree.

2. How you can determine closeness among different species.

3. How you could build a phylogenetic tree?

4. How molecular data could be used t build a phylogenetic tree?

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA: Sinauer.

2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).

3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. San Diego: Academic Press (a collection of essays by many authors).

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e sources.

Unit-I

Physiology of parasitic amoebae of man

Objective: In this unit, we will discuss about the physiology of parasitic amoeba of man.

Introducton:
The following points highlight the three important species of *Entamoeba* for which man is host. The species are:
1. *Entamoeba histolytica*
2. *Entamoeba coli*
3. *Entamoeba gingivalis*

Species 1. Entamoeba histolytica:
It was discovered by a Russian zoologist, Friedrick Losch in 1875. This is an endoparasite common in man, apes and monkeys, and may also be found in pigs, cats and rats.

E. histolytica is a microscopic endoparasite and is the causative agent of amoebic dysentery in man. It is seen inhabiting the upper part of large intestine (colon). There are two races or strains of this parasite, a smaller, common, nonpathogenic form and a much larger pathogenic form.

About 600 million people around world are infected by this parasite. Infection of this parasite is high in India, China, Philippines, Thailand, Mexico and parts of South America. A higher prevalence occurs in males, but usually the more serious and fatal complications affect women and children.

- **Morphology:**

The most active, motile and feeding form is called trophozoite, and has average about 25 um in diameter. The cytoplasm consists of a clear ectoplasm and a finely granular endoplasm. The endoplasm contains food vacuoles filled with RBC in different stages of digestion. The nucleus is single and vesicular, measures about 3 to 5 um in diameter.

Within it lies the endosome (nucleolus), which may be a single granule or a closely packed cluster of minute granules. A ring appears to surround the endosome. Spoke-like lines radiate from the endosome to the nuclear membrane.

Trophozoite produces finger-like pseudopodium and movement is irregular. As there used to be only one pseudopodium, it is typically monopodial. The type and rapidity of movement varies depending upon the consistency of surrounding medium, age of the parasite, temperature etc.

The nutrition in trophozoite is holozoic. It feeds by phagocytosis. Food usually consists of bacteria or other organic material found in the intestine. RBCs are found only in the food vacuole of pathogenic forms.

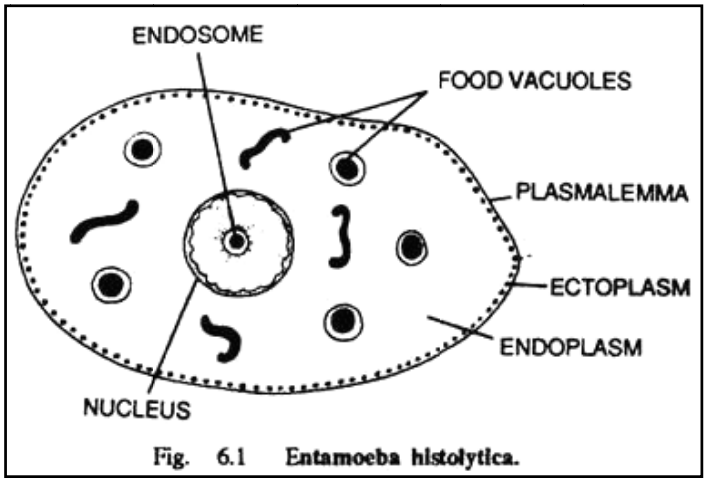


Fig. 6.1 Entamoeba histolytica.

● Pathogenesis:

The common, smaller, non-pathogeic minuta form lives in the lumen of the large intestine of man. It is harmless and feeds on bacteria. After a period of feeding and reproduction, vacuoles disappear. The *Entamoeba* becomes rounded. Soon a cyst wall begins to form, these uninucleated stages are pre-cysts.

If the resistance of gut is lowered in infected people, nonpathogenic form may change to pathogenic magna form. It can also be produced by an alternation of host's diet, increase of host cholesterol, change in the level of host sexual hormones and enzymatic action of the parasite. Magna form is invasive, and it penetrates the intestinal wall by secreting the histolytic enzymes.

They invade the submucosa by dissolving the mucosa of intestinal wall. They multiply by binary fission and produce flask-shaped ulcers containing cellular debris, RBCs, lymphocytes and bacteria. These ulcers rupture and discharge blood and mucus into the intestine that pass to outside with the stool. This results in amoebic dysentery or amoebiasis.

Other symptoms of amoebiasis are vomiting, mild fever, diarrhoea, tenderness over the sigmoidal region of the colon and hepatitis. In the stools of the patient, swarms of *Entamoeba* and RBCs are found. Stool is usually acidic. The patient is discomforted due to intense abdominal pain with the passing of blood and mucus with stools after every few minutes.

E. histolytica may not be confined to the intestine. Once a lesion is made in the intestinal wall, the amoebae can travel through blood or lymph circulation of host and can invade other tissues like liver, spleen, lungs, skin, gonads and rarely brain.

In all these soft tissues, *Entamoeba* multiplies and cause formation of abscesses. The most common extra-intestinal locus of infection is the liver, particularly the right lobe. Hence, results in hepatitis. Formation of abscesses in skin results in cutaneous amoebiasis. Abscesses in brain usually result in the death of the host.

● Treatment:

Many drugs are available for the treatment of amoebic dysentery. Alkaloid Emetine is effective in giving temporary relief. Dehydroemetine, a synthetic derivative of Emetine is equally effective. Chloroquine is used to treat amoebic hepatitis. Other medicines are iodine compounds such as. Chinioform, Diodoquin, Iroform, Humatin, Mexaform and Vioform.

Antibiotics useful in treating amoebiasis are Aureomycin, Fumagillin, Erythromycin and Terramycin. Recently both acute intestinal amoebiasis and amoebic hepatic abscess are treated with Metronidazole (1-(2- hydroxyethyl) -2 methyl 5 nitroimidazole). Selection of drugs depends on the location of infection and condition of the patient.

● Control:

1. Hands should be properly washed before handling food materials and eating the food.
2. Finger nails should be cut regularly and kept clean.
3. Uncooked vegetables like salad should be eaten only after thorough washing.
4. Avoid irrigation of vegetable by contaminated water.
5. Protection of food from contamination from housefly, cockroach etc.
6. Vegetables should be washed before cooking.

7. Avoid defaecation on vegetable fields and open grounds.

8. Quick and proper disposal of night soil.

9. Proper sanitation of surroundings.

10. Chlorination of drinking water supply.

11. Proper disposal of sewage.

12. Chemical treatment of human faeces to be used as fertilizer.

13. Covering of eatables by traders.

14. Killing of cysts with alcohol, phenol, formalin, mercuric chloride hydrochloric acid, caustic soda and potassium permanganate.

Species 2. *Entamoeba coli*:
This is found in human large intestine and is nonpathogenic. It has a worldwide distribution and the incidence of infection is more in warm countries like India, Pakistan, Burma and Sri Lanka.

Life cycle of this parasite differs from *E. histolytica. E. coli* does not invade host tissue. Trophozoite is smaller in size, ranges from 20 to 30 um in diameter. It is more sluggish than *E. histolytica*. The cytoplasm usually appears much more dense and crowded with food vaculoes. Food vacuoles contain cyst of other protozoa, bacteria, yeast cells etc., but devoid of red blood cells.

The nucleus possesses heavier peripheral chromatin and an eccentrically placed endosome. Young cysts contain a large glycogen vacuole. They may possess few nuclei. Mature cysts are slim with pointed or irregular ends. The cysts of *E. coli* are not killed by drying, this results in high incidence of infection in comparison to E. histolytica.

Cysts are excreted outside the host body through its faeces which contaminate food and water. The infection takes place when cyst contaminated water is ingested by human beings. *E. coli* occurs in the intestine of 50% of human population. Monkeys and apes also share this parasite with man.

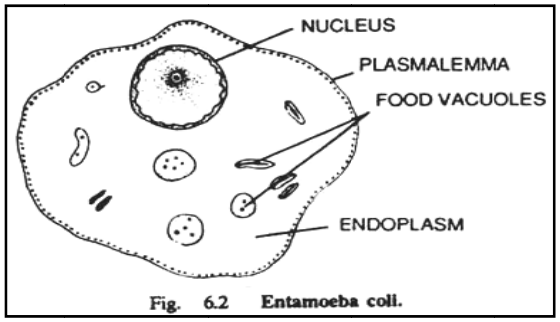


Fig. 6.2 Entamoeba coli.

● Pathogenesis:
Occasionally red blood corpuscles are reported from the food vacuoles of E. coli. Cysts are found to occur in the stools of diarrhoeic patients. Still there is no evidence to consider this parasite as a pathogenic form.

● Treatment:
Owing to nonpathogenic nature, no medical attention is required. However, the drugs available to treat amoebiasis are effective in killing this parasite.

● Control:

1. Proper disposal of sewage.
2. Quick and proper disposal of human faeces.
3. Purification of drinking water.
4. Proper washing of vegetables used in salads.
5. Cysts can be killed by Hydrochloric acid, phenol, caustic soda, cresol etc.

Species 3. *Entamoeba gingivalis*:

It inhabits in the mouth of about 70% of human population. *E. gingivalis* lives in the gingival areas around the teeth of man, other primates, dogs and cats. It is commonly found in pus-pockets of teeth of pyorrhoea infected persons and in the crypts of infected tonsils. It is the smallest species of *Entamoeba*.

There are no cysts and no intermediate hosts. It represents the simplest kind of life cycle. It is directly transmitted from mouth to mouth by contact during kissing, in feeding and cough into food at the time of dishing.

The size of *E. gingivalis* ranges from 5 to 35 um in diameter, with an average of about 15 um. It is appears similar to *E. histolytica*, but contain many more large food vacuoles, contain bacteria and white blood cells of the host. Ectoplasm is clear and central granular endoplasm is vacuolated. Many broad and blunt pseudopodia are used in locomotion.

The nucleus is lined with beaded peripheral chromatin. It contains an endosome with in a single or many closely grouped granules. Spoke-like fibrils connect the endosome with the nuclear membrane.

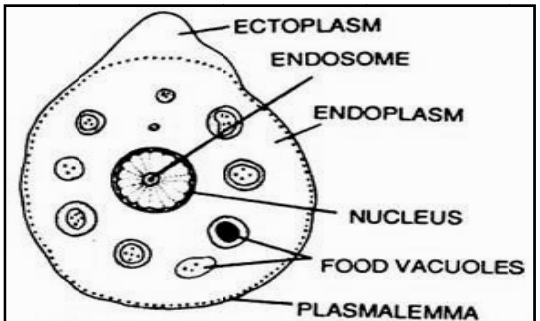


Fig. 6.3 **Entamoeba gingivalis.**

● Pathogenesis:

There is a high incidence of *E. gingivalis* in those patients of pyorrhoea. It was believed that the parasite is the causative agent of pyorrhoea. But at present, it is known that pyorrhoea is due to a bacterial infection. *E. gingivalis* increase the disease by damaging tissue in the gums. The actual pathogenecity of this parasite is still to be established.

Probable questions:

1. Mention the different species of *Entamoeba* and their respective pathogenesis.
2. What are the control measures and treatment methodology of each infection?

Suggested readings/ references:

1. http://www.biologydiscussion.com/invertebrate-zoology/amoeba/3-important-species-of-entamoeba/28196
2. http://www.biologydiscussion.com/micro-biology/species-of-entamoeba-with-diagram-microbiology/34319

Unit- II

Mode of transmission, pathogenicity and prevention of tetanus and rabies

Objective: In this unit, we will discuss about mode of transmission, pathogenicity and prevention of tetanus and rabies.

A. Tetanus:

Tetanus, also called **lockjaw**, is a serious infection caused by *Clostridium tetani*. This bacterium produces a toxin that affects the brain and nervous system, leading to stiffness in the muscles. If *Clostridium tetani* spores are deposited in a wound, the neurotoxin interferes with nerves that control muscle movement.

The infection can cause severe muscle spasms, serious breathing difficulties, and can ultimately be fatal. Although tetanus treatment exists, it is not uniformly effective. The best way to protect against tetanus is to take the vaccine.

- Tetanus is caused by the *Clostridium tetani* bacterium.
- The early symptoms of tetanus include diarrhoea, fever, and headache.
- Earlier diagnosis predicts better outcomes.

Tetanus is a serious bacterial infection.

The bacteria exist in soil, manure, and other environmental agents. A person who experiences a puncture wound with a contaminated object can develop the infection, which can affect the whole body. It can be fatal.

In the United States, there are about 30 cases a year. These are mostly people who have not been vaccinated against tetanus or who have not kept up their booster shots every 10 years.

Tetanus is a medical emergency. It will need aggressive wound treatment and antibiotics.

● Symptoms

Tetanus symptoms usually emerge about 7 to 10 days after initial infection. However, this can vary from 4 days to about 3 weeks, and may, in some cases, may take months.

In general, the further the injury site is from the central nervous system, the longer the incubation period. Patients with shorter incubation times tend to have more severe symptoms.

Muscle symptoms include spasms and stiffness. Stiffness usually starts with the chewing muscles, hence the name lockjaw.

Muscle spasms then spread to the neck and throat, causing difficulties with swallowing. Patients often have spasms in their facial muscles.

Breathing difficulties may result from neck and chest muscle stiffness. In some people, abdominal and limb muscles are also affected.

In severe cases, the spine will arch backward as the back muscles become affected. This is more common when children experience a tetanus infection.

Most individuals with tetanus will also have the following symptoms:

- ♦ bloody stools
- ♦ diarrhoea
- ♦ fever

- ♦ headache
- ♦ sensitivity to touch
- ♦ sore throat
- ♦ sweating
- ♦ rapid heartbeat

● Treatment

Wounds should be thoroughly cleaned to prevent infection.
Any cut or wound must be thoroughly cleaned to prevent infection. A tetanus-prone wound should be treated by a medical professional immediately.

A wound likely to develop tetanus is defined as:

- ♦ a wound or burn that requires surgical intervention that is delayed for over 6 hours
- ♦ a wound or burn that has a considerable amount of removed tissue
- ♦ any puncture-type injury that has been in contact with manure or soil
- ♦ serious fractures where the bone is exposed to infection, such as compound fractures
- ♦ wounds or burns in patients with systemic sepsis
- ♦ Any patient with a wound listed above should receive tetanus immunoglobulin (TIG) as soon as possible, even if they have been vaccinated. Tetanus immunoglobulin contains antibodies that kill *Clostridium tetani*. It is injected into a vein and provides immediate short-term protection against tetanus.

TIG is just short-term and does not replace the long-term effects of vaccination. Experts say that TIG injections can be safely administered to pregnant and breastfeeding mothers.

Doctors may prescribe penicillin or metronidazole for tetanus treatment. These antibiotics prevent the bacterium from multiplying and producing the neurotoxin that causes muscle spasms and stiffness.

Patients who are allergic to penicillin or metronidazole may be given tetracycline instead.

In treating muscle spasms and stiffness, patients may be prescribed:

- ♦ Anticonvulsants, such as diazepam (Valium), relax the muscles to prevent spasms, reduce anxiety, and work as a sedative.
- ♦ Muscle relaxants, such as baclofen, suppress nerve signals from the brain to the spinal cord, resulting in less muscle tension.
- ♦ Neuromuscular blocking agentsblock the signals from nerves to muscle fibers and are useful in controlling muscle spasms. They include pancuronium and vecuronium.

● Surgery

If the doctor thinks the tetanus prone wound is very large, they may surgically remove as much of the damaged and infected muscle as possible (debridement).

Debridement is the act of removing dead or contaminated tissue, or foreign material. In the case of a tetanus-prone wound, the foreign material may be dirt or manure.

● Nutrition

A patient with tetanus requires a high daily calorie intake because of increased muscle activity.

● Ventilator

Some patients may need ventilator support to help with breathing if their vocal cords or respiratory muscles are affected.

● Causes

Tetanus is caused by the *Clostridium tetani* bacterium.

Clostridium tetani spores are able to survive for a long time outside of the body. They are most commonly found in animal manure and contaminated soil, but may exist virtually anywhere.

When *Clostridium tetani* enters the body, they multiply rapidly and release tetanospasmin, a neurotoxin. When tetanospasmin enters the bloodstream, it rapidly spreads around the body, causing tetanus symptoms.

Tetanospasmin interferes with the signals travelling from the brain to the nerves in the spinal cord, and then on to the muscles, causing muscle spasms and stiffness.

Clostridium tetani enters the body mainly through skin cut or puncture wounds. Thoroughly cleaning any cut helps prevent an infection from developing.
Common ways of contracting tetanus include:

- wounds that have been contaminated with saliva or faeces
- burns
- crush injuries
- wounds that include dead tissue
- puncture wounds

Rare ways of contracting tetanus include:

- surgical procedures
- superficial wounds
- insect bites
- compound fractures
- intravenous drug use
- injections into the muscle
- dental infections

● Prevention

Most cases of tetanus occur in people who have never had the vaccine or who did not have a booster shot within the previous decade.

● Vaccination

The tetanus vaccine is routinely given to children as part of the diphtheria and tetanus toxoids and acellular pertussis (DTaP) shot.

The DTaP vaccine consists of five shots, usually given in the arm or thigh of children when they are aged:

- 2 months
- 4 months

- 6 months
- 15 to 18 months
- 4 to 6 years

A booster is normally given between the ages of 11 and 18 years, and then another booster every 10 years. If an individual is travelling to an area where tetanus is common, they should check with a doctor regarding vaccinations.

B. Rabies:

Rabies is a viral infection that mainly spreads through a bite from an infected animal. It is an RNA virus of the rhabdovirus family.

Without early treatment, it is usually fatal.

The virus can affect the body in one of two ways:

- It enters the peripheral nervous system (PNS) directly and migrates to the brain.
- It replicates within muscle tissue, where it is safe from the host's immune system. From here, it enters the nervous system through the neuromuscular junctions.

Once inside the nervous system, the virus produces acute inflammation of the brain. Coma and death soon follow.

There are two types of rabies.

- **Furious, or encephalitic rabies:** This occurs in 80 percent of human cases. The person is more likely to experience hyperactivity and hydrophobia.
- **Paralytic or "dumb" rabies:** Paralysis is a dominant symptom.
- **Transmission:**

Rabies is most common in countries where stray dogs are present in large numbers, especially in Asia and Africa.

It is passed on through saliva. Rabies can develop if a person receives a bite from an infected animal, or if saliva from an infected animal gets into an open wound or through a mucous membrane, such as the eyes or mouth. It cannot pass through unbroken skin.

In the U.S., raccoons, coyotes, bats, skunks, and foxes are the animals most likely to spread the virus. Bats carrying rabies have been found in all 48 states that border with each other.

Any mammal can harbour and transmit the virus, but smaller mammals, such as rodents, rarely become infected or transmit rabies. Rabbits are unlikely to spread rabies.

● Symptoms:

Rabies progresses in five distinct stages:

- incubation
- prodrome
- acute neurologic period
- coma
- death

● Incubation period:

This is the time before symptoms appear. It usually lasts from 3 to 12 weeks, but it can take as little as 5 days or more than 2 years.

The closer the bite is to the brain, the sooner the effects are likely to appear.

By the time symptoms appear, rabies is usually fatal. Anyone who may have been exposed to the virus should seek medical help at once, without waiting for symptoms.

● Prodrome:

Early, flu-like symptoms, include:

- a fever of 100.4 degrees Fahrenheit (38 degrees Celsius) or above
- headache
- anxiety
- feeling generally unwell
- sore throat and a cough
- nausea and vomiting
- discomfort may occur at the site of the bite

These can last from 2 to 10 days, and they worsen over time.

● Acute neurologic period:

Neurologic symptoms develop, including:

- confusion and aggression
- partial paralysis, involuntary muscle twitching, and rigid neck muscles
- convulsions
- hyperventilation and difficulty breathing
- hypersalivation or producing a lot of saliva, and possibly frothing at the mouth
- fear of water, or hydrophobia, due to difficulty swallowing
- hallucinations, nightmares, and insomnia
- priapism, or permanent erection, in males
- photophobia, or a fear of light

Toward the end of this phase, breathing becomes rapid and inconsistent.

● Coma and death:

If the person enters a coma, death will occur within a matter of hours, unless they are attached to a ventilator.

Rarely, a person may recover at this late stage.

● Why does rabies cause a fear of water?

Rabies used to be known as hydrophobia because it appears to cause a fear of water.

Intense spasms in the throat are triggered when trying to swallow. Even the thought of swallowing water can cause spasms. This is where the fear comes from.

The excess saliva that occurs is probably due to the impact of the virus on the nervous system.

If the individual could swallow saliva easily, this would reduce the risk of spreading the virus to a new host.

● Diagnosis:

At the time of a bite, there is usually no way to tell for sure whether an animal is rabid, or whether it has passed on an infection.

Lab tests may show antibodies, but these may not appear until later in the development of the disease. The virus may be isolated from saliva or through a skin biopsy. However, by the time a diagnosis is confirmed, it may be too late to take action.

For this reason, the patient will normally start a course of prophylactic treatment at once, without waiting for a confirmed diagnosis.

If a person develops symptoms of viral encephalitis following an animal bite, they should be treated as if they may have rabies.

● Treatment

If a person is bitten or scratched by an animal that may have rabies, or if the animal licks an open wound, the individual should immediately wash any bites and scratches for 15 minutes with soapy water, povidone iodine, or detergent. This might minimize the number of viral particles.
Then they must seek medical help at once.
After exposure and before symptoms begin, a series of shots can prevent the virus from thriving. This is usually effective.

Strategies include:
A fast-acting dose of rabies immunoglobulin: Delivered as soon as possible, close to the bite wound, this can prevent the virus from infecting the individual.
A series of rabies vaccines: These will be injected into the arm over the next 2 to 4 weeks. These will train the body to fight the virus whenever it finds it.
It is not usually possible to find out whether the animal has rabies or not. It is safest to assume the worst and begin the course of shots.
A small number of people have survived rabies, but most cases are fatal once the symptoms develop. There is no effective treatment at this stage.
A person with symptoms should be made as comfortable as possible. They may need breathing assistance.

- **Prevention:**

Rabies is a serious disease, but individuals and governments can do take necessary action to control and prevent, and, in some cases, wipe it out completely.
Strategies include:

- regular antirabies vaccinations for all pets and domestic animals
- bans or restrictions on the import of animals from some countries
- widespread vaccinations of humans in some areas
- educational information and awareness

In rural Canada and the U.S., agencies have dropped baits containing an oral vaccine to reduce the number of wild raccoons with rabies.
In Switzerland, the authorities distributed vaccine-laced chicken heads throughout the Swiss Alps. The foxes immunized themselves by consuming the vaccine, and the country is now almost free of rabies.
Individual precautions
Individuals should follow some safety rules to reduce the chance of contracting rabies.

- Vaccinate pets: Find out how often you need to vaccinate your cat, dog, ferret, and other domestic or farm animals, and keep up the vaccinations.
- Protect small pets: Some pets cannot be vaccinated, so they should be kept in a cage or inside the house to prevent contact with wild predators.
- Keep pets confined: Pets should be safely confined when at home, and supervised when outside.
- Report strays to the local authorities: Contact local animal control officials or police departments if you see animals roaming
- Do not approach wild animals: Animals with rabies are likely to be less cautious than usual, and they may be more likely to approach people.

- ♦ Keep bats out of the home: Seal your home to prevent bats from nesting. Call an expert to remove any bats that are already present.

In 2015, a woman died from rabies after being bitten by a bat during the night. She did not realize she had been bitten.

People are encouraged to seek medical help after an encounter with a wild animal, even if they do not have bite marks or other outward signs of injury.

The World Health Organization (WHO) calls rabies a "100-percent vaccine-preventable disease." They note that at least 70 percent of dogs in an area must be vaccinated to break the cycle of transmission.

In the U.S., vaccinations control rabies in domestic dogs. Nevertheless, between 30,000 and 60,000 people seek rabies postexposure prophylaxis every year, following contact with suspect animals. Hundreds of thousands of animals undergo tests and observation.

Between 60 and 70 dogs and around 250 cats are reported rabid each year in the U.S. Most of these have not been vaccinated, and they were exposed to the virus through wild animals, such as bats.

● Travelling:

The prevalence of rabies varies widely in different countries. In nations without a feral dog population, the rates are significantly lower.

Rabies is present in 150 countries and in all continents except Antarctica and the Arctic. Islands such as New Zealand, Australia, Mauritius and the Seychelles, are helped by their natural isolation.

Africa and Asia are the continents where rabies is most common. India has the highest number of cases.

In recent years, the prevalence of rabies in South America and the Caribbean has fallen significantly, due to rabies control programs. Official figures show that in 1990 there were 250 cases, but by 2010, there were fewer than 10.

Anyone who is travelling to an area where rabies is prevalent, or who is participating in activities where they are likely to come into contact with wild animals that may have rabies, such as caving or camping, should ask their doctor about vaccinations.

Probable questions:

1. What is lockjaw? What is the causative agent of tetanus?
2. Mention the symptoms of tetanus? How tetanus can be treated?
3. How rabies can be transmitted?
4. Explain the different stages of progression of rabies.
5. What are the preventive measures to control rabies?

Suggested readings/ references:

1. https://www.medicalnewstoday.com/articles/163063.php
2. http://www.biologydiscussion.com/human-diseases/clostridium-tetani-properties-prevention-and-treatment-with-diagram/30908
3. https://www.medicalnewstoday.com/articles/181980.php
4. https://www.cdc.gov/vaccines/hcp/vis/vis-statements/rabies.html

Unit-III

Physiology, immunopathology of *Plasmodium* sp. Immunity of *Plasmodium sp.*

Objective: In this unit, we will discuss about physiology, immunopathology of *Plasmodium* sp. and immunity of *Plasmodium* sp.

Introduction:

Malaria causes disease through a number of pathways, which depend to a certain extent on the species. Malaria is caused by a single-celled parasite of the genus *Plasmodium*; there are five species which infect humans, being *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*.

All these species are introduced into the human blood stream through the bite of an infected mosquito; the life stage of malaria at this point is called a "sporozoite", and they pass first to the liver, where they undergo an initial stage of replication (called "exo-erythrocytic replication"), before passing back into the blood and invading red blood cells (called "erythrocytes", hence this is the "erythrocytic" part of the cycle). The malaria parasites that invade red blood cells are known as merozoites, and within the cell they replicate again, bursting out once they have completed a set number of divisions. It is this periodic rupturing of the red blood cells that causes most of the symptoms associated with malaria, as the host's immune system responds to the waste products produced by the malaria parasites and the debris from the destroyed red blood cells. Different species of malaria rupture the red blood cells at different intervals, which lead to the diagnostic cycles of fever which characterise malaria; *P. vivax*, for example, tends to produce cycles of fever every two days, whereas *P. malaria* produces fever every three.

In addition, *Plasmodium falciparum* produces unique pathological effects, due to its manipulation of the host's physiology. When it infects red blood cells, it makes them stick to the walls of tiny blood vessels deep within major organs, such as the kidneys, lungs, heart and brain. This is called "sequestration", and results in reduced blood flow to these organs, causing the severe clinical symptoms associated with this infection, such as cerebral malaria.

Clinical manifestations of *Plasmodium falciparum* infection are induced by the asexual stages of the parasite that develop inside red blood cells (RBCs). Because splenic microcirculatory beds filter out altered RBCs, the spleen can innately clear subpopulations of infected or uninfected RBC modified during falciparum malaria. The spleen appears more protective against severe manifestations of malaria in naïve than in immune subjects. The spleen-specific pitting function accounts for a large fraction of parasite clearance in artemisinin-treated patients. RBC loss contributes to malarial anaemia, a clinical form associated with subacute progression, frequent spleenomegaly, and relatively low parasitemia. Stringent splenic clearance of ring-infected RBCs and uninfected, but parasite-altered, RBCs, may altogether exacerbate anaemia and reduce the risks of severe complications associated with high parasite loads, such as cerebral malaria. The age of the patient directly influences the risk of severe manifestations.

Malaria causes approximately 212 million cases and 429 thousand deaths annually. *Plasmodium falciparum* is responsible for the vast majority of deaths (99%) than others. The virulence of *P. falciparum* is mostly associated with immune response-evading ability. It has different mechanisms to evade both *Anopheles* mosquito and human host immune responses. Immune-evading mechanisms in mosquito depend mainly on the Pfs47 gene that inhibits Janus kinase-mediated activation. Host complement factor also protects human complement immune attack of extracellular gametes in Anopheles mosquito midgut. In the human host, evasion largely results from antigenic variation, polymorphism, and sequestration. They also induce Kupffer cell apoptosis at the pre-erythrocytic

stage and interfere with phagocytic functions of macrophage by hemozoin in the erythrocytic stage. Lack of major histocompatibility complex-I molecule expression on the surface red blood cells also avoids recognition by $CD8^+$ T cells. Complement proteins could allow for the entry of parasite into the red blood cell. Intracellular survival also assists the escape of malarial parasite. Invading, evading, and immune response mechanisms both in malaria vector and human host are critical to design appropriate vaccine. As a result, the receptors and ligands involved in different stages of malaria parasites should be elucidated.

Malaria is one of the most important infectious diseases in the world, and it has been recognized as the most widespread infection in tropical and subtropical areas with high rate of morbidity and mortality. An estimated 3.3 billion people are at risk of malaria in the world, of which 1.2 billion are at high risk and 97 countries had ongoing malaria transmission. It still remains a challenge in the world in general and in Africa in particular. There is an estimated 212 million cases all over the world; most of these cases (82%) were in the WHO African Regions, followed by the WHO Southeast Asia Regions (12%) and the WHO Eastern Mediterranean Regions (5%). Globally, malaria deaths were 429,000 and 90% of these deaths were in the WHO African Regions, followed by the WHO Southeast Asia Regions (7%) and the WHO Eastern Mediterranean Regions.

Plasmodium falciparum is mainly responsible for the enormous deaths (99%) than other *Plasmodium* species. Some studies associate the virulence of *P. falciparum* with its ability to escape the human and vector immune system by different mechanisms. This review is mainly focused on evading, invading, and immune response mechanisms involved in different stages of malaria parasite and on its implication for vaccine development.

In *Anopheles* mosquito vectors, the cycle begins when gametocytes are ingested by mosquitoes, but approximately 62% of mosquitoes that ingest infected blood never get infected. Inside the mosquito's gut, gametocytes are differentiated into gametes and undergo fertilization to form zygotes. The zygotes are then differentiated into ookinetes and develop into oocyst. Out of the thousand gametocytes, only 50–100 differentiated into ookinetes, and only around five develop into oocysts. The ookinetes then invade the midgut epithelium by using mosquito sugars and parasite lectins. On the other hand, oocysts developed and produce thousands of sporozoites which invade the salivary glands. During blood meal, an infected mosquito releases sporozoites in human.

Sporozoites then invade hepatocytes by using cholesterol uptake pathway. In the hepatocyte, sporozoites are enclosed in a parasitophorous vacuole to avoid lysosomal degradation. Merozoites ranging from 10,000 to 30,000 exit the schizont within 60 seconds of hepatocyte invasion by sporozoites.

Merozoite surface protein (MSP) 1 plays a main role for merozoite attachment to erythrocytes. Then, reorientation mediated by merozoite apical membrane antigen (AMA) 1 results in juxtaposition of the merozoite's apical end with the erythrocyte membrane that allows closer interaction. Tight junction between the parasite and host membrane is formed to enter the RBC. Erythrocyte-binding ligands (EBLs) and reticulocyte-binding-like protein homologs (RBLs) are involved in junction formation. EBLs are encoded by EBA175, EBA140, and EBA181genes. PfRh1, PfRh2a, PfRh2b, and PfRh4 ligands are encoded by RBL genes, but the erythrocyte's receptors to these ligands are still puzzling. Dense granules, micronemes, and rhoptries are considered as principal invasive organelles found in merozoites. In erythrocytic schizogony, a single merozoite grows and forms 16–32 daughter merozoites. The underlying cause of increased pressure and destruction of the cytoskeleton preceding rupture of erythrocytic merozoite to begin the erythrocytic cycle is not completely clear. In parallel, stress factors such as lack of nutrition and host immune pressure force merozoites to develop into

gametocytes. Finally, the cycle continues when Anopheles mosquito ingests blood containing gametocytes.

Immune Response to *P. falciparum* Malaria

Immune response against the *P. falciparum* malaria parasite is multifaceted and stage specific both in *Anopheles* mosquito vector and human host. Immunological responses could also contribute to the pathophysiology of the disease in human.

2.1. *Anopheles* Mosquito's Immune Response

Plasmodium falciparum malaria parasite is limited by several bottlenecks before establishing infection in its *Anopheles* mosquito vector. This includes the physical, microbiological, and immunological defences of the mosquito immune system. Immunological defence plays a key role when ookinete traverses the midgut and sporozoites migrate to the salivary glands. The interaction of the mosquito immune system is critical to control its vectorial capacity.

2.1.1. Physical Barriers

It is the first line of defence of Anopheles mosquito to *P. falciparum* parasites. The major physical barriers are peritrophic membrane (PM) of the midgut, cuticle of the exoskeleton, and lining of the tracheal respiratory system. Capsule formation around the parasite by mosquito melanin also has a protective role.

2.1.2. Midgut Microbiota

The microbiota found in *Anopheles* mosquitoes, such as *Asaia, Enterobacter, Pseudomonas* and *Pantoea*, induces AMPs, which stimulate a basal innate immune activity against *P. falciparum* infection.

2.1.3. Humoral Immune Response

Complement-like or thioester-containing protein (TEP) 1 that circulates in the Anopheles mosquito haemolymph is the major arm of defence in the humoral immune response of Anopheles mosquito. It forms leucine-rich repeat protein 1 (LRIM1)/anopheles plasmodium-responsive leucine-rich repeat protein (APL) 1/TEP1cut complex and gets accumulated on the ookinete surface for killing. Apolipophorin and apolipoprotein D precursors and fibrinogen-related proteins are also documented as players in midgut antiplasmodialdefence.

A study conducted in Portugal showed that hemozoin activates transcription of several key immune genes like REL2-F transcription factor that regulates TEP1, APL1, LRRD7, and FBN9 anti-Plasmodium immune factors. Anti-*Plasmodium* response of An. gambiae aga-miR-305 is also demonstrated. Antibodies also avert ookinete motility, penetration of the midgut wall, and formation of oocyst.

2.1.4. Cellular Immune Response

The primary immune cells involved in mosquito innate immune response are hemocytes. Hemocytes include granulocytes, oenocytoids, and prohemocyte subtypes that are involved in phagocytosis, melanization, and hematopoietic progenitors, respectively. Other immune effectors released by hemocytes and fat body into hemolymph are also involved in phagocytosis, secretion of antimicrobial peptides, nodule formation, agglutination, encapsulation, and melanization. Reactive oxygen species (ROS) produced by haemocyte is also involved in mosquito immunity against *P. falciparum*.

2.1.5. Signaling Pathways in Mosquito Antiplasmodial Immunity

This pathway includes Toll, immune deficiency (Imd), Janus kinase (JNK), and signal transducers and activators of transcription (STAT), which contribute to anti-Plasmodium defence. The Toll and Imd pathways target the ookinete stage of the parasite and promote activation of the mosquito TEP1

complement-like system. These pathways are activated when they recognize PAMPs, which activate NF-κB that leads activation of Rel1 and Rel2 in Toll and Imd pathways, respectively. Activation of both pathways is also important for the entry of AMPs to nucleus such as defensins, cecropins, attacin, and gambicin, which have antiplasmodial activity. Rel1 and Rel2 are negatively controlled by Cactus and Caspar, respectively. Potent anti-Plasmodium effectors such as TEP1, APL1, LRRD7, and FBN9 are also controlled by the Imd pathway. Immune-enhanced Anopheles stephensi mosquitoes by Rel2 in the midgut demonstrated better resistance to Plasmodium infection and may give clear direction to design appropriate control strategies. Currently, activation of the Imd and Toll pathways to induce the expression of AgDscam isoforms that have species-specific antiplasmodial responses is indicated. The genes that mediate these pathways and tissues in which they are produced need to be illustrated.

Janus kinase–STAT pathway is also linked with anti-Plasmodium defence but activation of these pathways is not clearly explained. The JNK pathway also regulates the expression of HPX2, NOX5, and TEP1 in haemocytes that promote TEP1-mediated lysis. The STAT pathway targets after parasites cross the midgut and change into the oocysts.
Signal transducers and activators of transcription genes (STAT1/AgSTAT-B and STAT2/AgSTAT-A) mediate immunity against the *P. falciparum* malaria parasite. AgSTAT-A is involved in transcriptional activation of NO synthase that increases reactive NO and transcription of suppressors of cytokine signaling (SOCS), which lessen the development of parasite. The exact role of AgSTAT-B is not characterized.

2.2. Immune Response to *P. falciparum* in Human

The response is complex and targets at different stages of plasmodium parasites. Immune attack involvement is high in the erythrocytic stage in contrast to preerythrocytic stage, and major immune players in the preerythrocytic and erythrocytic stages are $CD8^+$ T cells and antibodies, respectively.

2.2.1. Skin as a Physical Barrier

The skin is the first critical physical barrier and acts as first line of defence against many pathogens and is also true for *P. falciparum* malaria parasites. After inoculation, sporozoites stay in the skin for several hours and are activated into a state of readiness for the hepatic stages. Antibodies found in the skin tissues also inhibit sporozoite motility in the dermis. Approximately 50% of the sporozoites do not leave the inoculation site. As a result, this early stage could play a key role in vaccine design.

The sporozoite proteins (SPECT1 and SPECT2) were reported to be necessary to pass the skin barrier, cell traversal, and migration to the liver. This allows for sporozoites to evade destruction by phagocytes, and growth is arrested in nonphagocytic cells in the host dermis.

2.2.2. Immune Response to Pre-erythrocytic-Stage Parasites

Immune response at the pre-erythrocytic stage is targeted on free sporozoites and infected hepatocytes. Antibodies against free sporozoites and circumsporozoite protein (CSP) are important to prevent invasion of hepatocytes by neutralizing proteins required for cell traversal and invasion. It also activates complement fixation, phagocytosis, and lysis by cytotoxic NK and NKT cells. It also recognizes parasite neoantigens at the surface of infected hepatocytes and kills through an antibody-dependent cell-mediated mechanism by Kupffer cells and NK cells.

CD8+ T cells producing interferon-γ are mainly involved in killing of intrahepatic parasites. Other cells like NK, NKT, and γδT cells also kill intrahepatic parasites through secretion of type I interferons and IFN-γ.

Unlike viruses and bacteria, *P. falciparum* malaria parasites can trigger type I IFNs in the absence of Toll-like receptors (TLR3 and TLR4) and their signaling proteins (MyD88 and TRIF); rather, they use melanoma differentiation-associated gene 5 protein (MDA5) and signalling via the mitochondrial antiviral signalling protein (MAVS), which activates the transcription factors IRF3 and IRF7. Very recently, an exoerythrocyte-form (EEF) RNA was also reported to be recognized by MDA5 in hepatocytes, triggered a type I IFN response in the innate immune cells. Host iron regulatory hormone hepcidin, which impairs the growth of sporozoites, is also produced by unknown mechanisms.
Killing of infected hepatocytes and blocking of invasion by $CD8^+$ T cells and antibodies, respectively, are bottleneck phases that could be targeted by vaccine.

2.2.3. Immune Response to Erythrocytic Stage of Infection

Adaptive immunity against erythrocytic-stage *P. falciparum* is more complex than other stages. The release of merozoites from hepatocytes to invade RBCs is responsible for initiation of the erythrocytic stage. At this stage, the targets are free merozoites and intraerythrocytic parasites (schizonts). Humoral or antibody and T cell responses are important to control merozoites and intraerythrocytic parasites, respectively. Antibodies can opsonise merozoites for uptake or to inhibit invasion of RBCs. Antibody mediates cellular killing, blocks adhesion of infected RBCs to endothelium, and neutralizes parasite toxins to prevent the induction of excessive inflammation. It also marks merozoites for lysis by complement. This stage is also known by proinflammatory cytokine response that activates macrophages.

The role of $CD8^+$ T cells in the erythrocytic stage is negligible. $CD4^+$ T helper cells are also important to produce proinflammatory cytokines that activate macrophages. They also mediate an activation of specific B cell clones.

Others like NK cells and γδT cells are also involved in the immune response. IFN-γ, perforin, and granzyme produced by NK cells are responsible to kill P. falciparum-infected RBCs.

2.2.4. Immune Response against Gametocyte

Antibodies kill gametocyte through complement-mediated lysis and prevent sequestration and maturation of gametocytes in the host. Antibodies derived from host during blood meal are also highly responsible for complement-mediated killing of gametocytes and prevent gamete fusion in mosquito. Nitric oxide produced by macrophages is also important to kill gametocytes.

3. Immune Evasion

3.1. Immune Evasion Mechanisms of *P. falciparum* in *Anopheles* Mosquito

Immune evasion is a strategy used to avoid immune response attack. Similarly, *P. falciparum* parasites evade mosquito immune response to transmit to a new host. The main and critical *P. falciparum* gene used for evasion of *Anopheles* mosquito immune response is Pfs47. It inhibits JNK-mediated apoptosis by preventing activation of several caspases. Moreover, being deficient in caspase-S2 also prevents protein nitration in the mosquito midgut cells. Pfs47 gene also inhibits NOX5 and HPX2.

Host complement factors (FH) have also an influence on mosquito midgut stages. The FH receptor in *P. falciparum* gamete is glideosome-associated protein 50 (GAP50) that protects the gametes from complement attack. Blocking of this receptor may also shed light for vaccine development. Immune-

modulatory peroxidase (IMPer) is also crucial to form dityrosine network, which helps parasites to inactivate NOS.

3.2. Immune Evasion in Human

3.2.1. Immune Evasion Strategies of Liver Stage *P. falciparum* Malaria Parasites

Free sporozoites and intrahepatic parasites must pass the hurdle of host immune response in order to enter the erythrocytic stage. Research showed that sporozoites actively pass through Kupffer cells (KCs, 24%) and endothelial cells (ECs, 53%); some sporozoites can cross the gaps between an EC and a KC, but it is puzzling how sporozoites safely pass through KCs, which kill other microorganisms. To pass this barrier, the CSP binds to KC surface proteins, and this interaction produces high levels of intracellular cAMP/EPAC that prevents the formation of ROS. Sporozoites' contact with KC also downregulates the inflammatory Th1 cytokines and upregulates the anti-inflammatory Th2 cytokines. In some cases, the binding of sporozoites also induced KC apoptosis and reduced the expression of major histocompatibility complex (MHC)-I. This results in induction of T cell tolerance. The CSP antigen of sporozoites could also be responsible for the reduction of KC MHC-I expression. Sporozoites are able to manipulate the KC functions.

Once inside the hepatocyte, parasitophorous vacuole prevents lysosomal degradation. Host heme oxygenase-1 (HO-1) also enhances the development of intrahepatic parasites by modulating the host inflammatory response. Sporozoite also interferes with the mTOR pathway.

3.2.2. Intraerythrocytic Immune Evasion

The success of evasion depends on merozoites and infected red blood cell (iRBC) surface proteins. Mostly, immune evasion by intraerythrocytic parasites is the result of antigenic diversity and sequestration. Intracellular survival also assists the parasite escape by avoiding direct interaction with the immune cells. Lack of MHC-I molecule expression on the surface RBCs also helps parasite to void recognition by CD8+ T cells. They also create rosettes that help them to bind on RBC epitopes and avoid immune recognition.

Expression of variable antigenic surface proteins on iRBCs helps them to evade host immune response. Antigenic diversity is mostly developed from multicopy gene families and polymorphic alleles. PfEMP1 is one of the highly polymorphic proteins, encoded by approximately 60 copies of var genes. It has different variable domains that establish their binding to various ligands on endothelial cells. A particular var gene, known as var2csa mediates cytoadherence of iRBC to syncytiotrophoblasts of the placenta.

The variant proteins, such as RIFINs at early trophozoite stage and STEVORS at mature trophozoite stage, are possibly important in immune evasion. In general, antigenic polymorphism is the most difficult hurdle for development of effective *P. falciparum* malaria vaccines.

The second immune evasion mechanism at this stage is sequestration, mediated by PfEMP-1, RIFIN, and STEVOR multigene families. These allow iRBC adherence to vascular endothelium that protects from clearance of the parasites by spleen. Endothelium receptors such as EPCR, CSA, CD36, and ICAMs are also important for sequestration. Rosette formation and adherence are important for immune evasion through sequestration and responsible for the occurrence of cerebral malaria.

IgM, which is not specific for these parasites, also binds to PfEMP-1 molecules through their Fc portion (Fc) and promotes rosetting that may facilitate sequestration by preventing splenic elimination.

Phagocytic functions of macrophages are also hindered by P. falciparum malaria pigment or hemozoin. Macrophages that have hemozoin cannot phagocytose more iRBC and reduce the production of radical oxygen intermediates. *P. falciparum* infection also activates checkpoint inhibitor molecules.

Most *P. falciparum* parasites use sialic acid-(SA-) independent pathway to evade antibody immune response. They also divert antibody response for one antigen to another antigen. Polymorphic tandem repeats in antigens also mask the critical epitopes.

3.2.3. Immune Evasion by Merozoites

The mechanisms used for merozoite evasion include antigenic proteins. Principally, merozoite surface proteins (MSPs), PfAMA1, PfEBA, and PfRHs are involved in merozoite evasion. Among them, MSPs are highly polymorphic and play a key role to evade immune attack. Merozoite proteins show strong homology to the host protein that makes it difficult to be recognized by antibody.

3.2.4. Evading Mechanisms of Gametocytes

Antibodies are also enhancing transmission to the mosquito. Expression of Var, Rif, and Stevor proteins that provides an immune evasion is also indicated recently.

4. Conclusions

This review manly focused on the evasion, invasion, and immune response mechanisms of *P. falciparum* involved both in Anopheles mosquito vector and human host. Based on this, molecular and cellular immune molecules and cells are discussed. The receptors and ligands involved in each stage are also elucidated that gives an indication for vaccine development.

Probable question:

1. Describe the immunopathology and immunity of *Plasmodium* sp.

Suggested readings/ references:

1. https://www.hindawi.com/journals/jir/2018/6529681/
2. http://www.bloodjournal.org/content/117/2/381?sso-checked=true
3. https://www.malariasite.com/pathophysiology/
4. https://www.karger.com/Article/Pdf/402535
5. https://academic.oup.com/femsre/article/40/2/208/2570118

Unit- IV

Fish parasites and its control

Objective: In this unit, we will discuss about fish parasites and its control.

Like humans and other animals, fish suffer from diseases and parasites. Fish defences against disease are specific and non-specific. Non-specific defences include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth. If pathogens breach these defences, fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver white blood cells that attempt to destroy the pathogens.

Specific defences are specialised responses to particular pathogens recognised by the fish's body that is adaptive immune responses. In recent years, vaccines have become widely used in aquaculture and ornamental fish, for example vaccines for furunculosis in farmed salmon and koi herpes virus in koi.

● Diseases:

All fish carry pathogens and parasites. Usually this is at some cost to the fish. If the cost is sufficiently high, then the impacts can be characterised as a disease. However disease in fish is not understood well. What is known about fish disease often relates to aquaria fish and more recently, to farmed fish.
Disease is a prime agent affecting fish mortality, especially when fish are young. Fish can limit the impacts of pathogens and parasites with behavioural or biochemical means, and such fish have reproductive advantages. Interacting factors result in low grade infection becoming fatal diseases. In particular, things that cause stress, such as natural droughts or pollution or predators, can precipitate outbreak of disease.
Disease can also be particularly problematic when pathogens and parasites carried by introduced species affect native species. An introduced species may find invading easier if potential predators and competitors have been decimated by disease.

Pathogens which can cause fish diseases comprise:

- viral infections, such as esocid lymphosarcoma found in Esox species.
- bacterial infections, such as *Pseudomonas fluorescens* leading to fin rot and fish dropsy
- fungal infections
- water mould infections, such as *Saprolegnia* sp.
- metazoan parasites, such as copepods
- unicellular parasites, such as *Ichthyophthirius multifiliis* leading to ich
- Certain parasites like Helminths for example *Eustrongylides*

● Parasites:

Parasites in fish are a common natural occurrence. Parasites can provide information about host population ecology. In fisheries biology, for example, parasite communities can be used to distinguish distinct populations of the same fish species co-inhabiting a region. Additionally, parasites possess a variety of specialized traits and life-history strategies that enable them to colonize hosts. Understanding these aspects of parasite ecology, of interest in their own right, can illuminate parasite-avoidance strategies employed by hosts.
Usually parasites (and pathogens) need to avoid killing their hosts, since extinct hosts can mean extinct parasites. Evolutionary constraints may operate so parasites avoid killing their hosts, or the natural variability in host defensive strategies may suffice to keep host populations viable. Parasite

infections can impair the courtship dance of male three spine sticklebacks. When that happens, the females reject them, suggesting a strong mechanism for the selection of parasite resistance.
However, not all parasites want to keep their hosts alive, and there are parasites with multistage life cycles which go to some trouble to kill their host. For example, some tapeworms make some fish behave in such a way that a predatory bird can catch it. The predatory bird is the next host for the parasite in the next stage of its life cycle. Specifically, the tapeworm Schistocephalus solidus turns infected three spine stickleback white, and then makes them more buoyant so that they splash along at the surface of the water, becoming easy to see and easy to catch for a passing bird.
Parasites can be internal (endoparasites) or external (ectoparasites). Some internal fish parasites are spectacular, such as the philometrid nematode Philometrafasciati which is parasitic in the ovary of female Blacktip grouper; the adult female parasite is a red worm which can reach up to 40 centimetres in length, for a diameter of only 1.6 millimetre; the males are tiny. Other internal parasites are found living inside fish gills, include encysted adult didymozoid trematodes, a few trichosomoidid nematodes of the genus Huffmanela, including Huffmanelaossicola which lives within the gill bone, and the encysted parasitic turbellarianParavortex. Various protists and Myxosporea are also parasitic on gills, where they form cysts.

Fish gills are also the preferred habitat of many external parasites, attached to the gill but living out of it. The most common are monogeneans and certain groups of parasitic copepods, which can be extremely numerous. Other external parasites found on gills are leeches and, in seawater, larvae of gnathiid isopods. Isopod fish parasites are mostly external and feed on blood. The larvae of the Gnathiidae family and adult cymothoidids have piercing and sucking mouthparts and clawed limbs adapted for clinging onto their hosts. Cymothoaexigua is a parasite of various marine fish. It causes the tongue of the fish to atrophy and takes its place in what is believed to be the first instance discovered of a parasite functionally replacing a host structure in animals.
Other parasitic disorders, include Gyrodactylussalaris, *Ichthyophthirius multifiliis*, cryptocaryon, velvet disease, *Brooklynella hostilis*, Hole in the head, Glugea, Ceratomyxashasta, Kudoathyrsites, *Tetracapsuloides bryosalmonae*, Cymothoaexigua, leeches, nematode, flukes, carp lice and salmon lice.

Although parasites are generally considered to be harmful, the eradication of all parasites would not necessarily be beneficial. Parasites account for as much as or more than half of life's diversity; they perform an important ecological role (by weakening prey) that ecosystems would take some time to adapt to; and without parasites organisms may eventually tend to asexual reproduction, diminishing the diversity of sexually dimorphic traits. Parasites provide an opportunity for the transfer of genetic material between species. On rare, but significant, occasions this may facilitate evolutionary changes that would not otherwise occur, or that would otherwise take even longer.

Parasites are typically divided into two groups: ectoparasites, external parasites that live on the outside of a tropical fish host (including the gills, mouth, skin and fin surfaces), and endoparasites, which live in the tissues, blood and/or organs (including the gastrointestinal tract). Interestingly, we still don’t know exactly how intestinal parasites manage to survive without being digested along with the other contents of the intestinal tract. As you can imagine, the inside of the gastrointestinal tract is a pretty inhospitable place to live- it is full of acids and enzymes deigned to digest anything passing through it.

Some parasites are transmitted directly from fish to fish, such as some ectoparasitic protozoa and skin/gill flukes. The life cycles of these parasites may also include a free-living phase, when they can be found in the water or aquarium substrate. The life cycle of such parasites is called a direct life cycle.

Other larger parasites often have complex life cycles involving two or more hosts, including a fish. This is called an indirect life cycle. For example, the spiny-headed worm attaches to the intestinal

wall of a tropical fish (the definitive host), and after expelled by the fish defecating, the worm's eggs are eaten by freshwater shrimp (the intermediate host), which are in turn ingested by another tropical fish.
It is important to know the difference between life cycles, because this will have an effect on treatment methods and whether you will succeed when treating parasites.

● Causes and Cures:

Clinical signs of parasitic disease in tropical fish can be vague. A sick fish may be seen swimming alone, or it may swim more slowly and/or on its side. The gills may move more rapidly. The fins may be clamped or droopy, and the fish may be losing weight. There may be white spots on the skin or gills, or the tropical fish may rub against aquatic plants, ornaments or gravel. Some tropical fish appear bloated.
Most parasitic diseases occur as a result of poor water quality. Most parasitic organisms are opportunistic and may be present all the time in the aquarium or on the fish in low numbers, and only cause disease when the tropical fish is stressed. The number of tropical fish in an aquarium, water temperature, pH, lighting, type of filtration system and water chemistries all influence the health of the fish.
Another common reason for parasitic outbreak is inadequate (or a total lack of) quarantine procedures for new tropical fish prior to introducing them into your aquariums. Many diseases do not show up for two to three weeks following transport, and unfortunately this can result in serious problems being introduced into an established aquarium. A quarantine aquarium can be as simple as a bare aquarium outfitted with a heater and sponge filter. Three to six weeks of quarantine should allow owners to identify any sick tropical fish prior to introducing them into the community aquarium. During quarantine, weekly treatments with formalin (25 mg/L) are reasonable and likely to avert serious illness in many cases.
At the first sign of illness, use a test kit to make sure the values are appropriate for the types of tropical fish you are keeping. Also perform a partial water change, which is an important safety measure. If possible, move any ill fish into an isolation aquarium to more closely observe them and prevent the possible spread of any infectious organisms.
Protozoan Parasites

The majority of the fish parasites which cause disease in fish include protozoal parasites. Typically, these parasites are present in large numbers either on the surface of the fish, within the gills, or both. When they are present in the gills, they cause problems with respiration and death will commonly occur when additional stressors are present in the aquatic environment. Protozoal parasites on the skin, fins or scales only, (i.e., not affecting the gills) usually do not result in death, unless they are accompanied by a secondary bacterial infection. The more common protozoal parasites are listed below.

● ***Ichthyophthirius multifilis*:** This is probably the most common parasite of all fishes. The common name for this parasite and disease is "Ich" or "white spot". The mature parasite reaches approximately 1 mm in diameter and is commonly observed in the gills and/or skin as coalescing white spots, hence the common name. The trophont or mature stage of the parasite has a large "horseshoe" shaped nucleus, and the entire surface of the parasite is covered in cilia. The life cycle of this parasite is direct, but is spent, in part, off of the host. The trophont is within the epidermis of the host, until it leaves the fish, encysts and divides to produce many host-seeking tomites. The tomites penetrate the skin and gills of the fish to complete the life cycle. The life cycle is temperature dependent with a shorter life cycle occurring at warmer water temperatures.

Fish with a cutaneous infection will "flash", i.e., turn over and expose their white underside, whereas fish with a gill infection will "pipe", i.e., come to the surface of the water and "breathe" through their mouth. Gill lesions include epithelial hyperplasia with the presence of mature trophonts

within the gills. Cutaneous lesions also exhibit focal epidermal hyperplasia, with parasites being located beneath the hyperplastic epidermis.

● ***Trichodinia* sp**: There are three genera which form the *Trichodina* complex: *Trichodina*, Trichodonella and Tripartiella, however, all three are commonly referred to as "Trichodina". All are approximately 100 mm in diameter and have a saucer to "frisbee" shape and are ringed with cilia around its entire surface. They have a circular arrangement of tooth-like structures (denticular ring) within the body which provides them a characteristic appearance in fresh gill and skin cytology preparations. Fish with severe gill infections of trichodina will have respiratory and osmoregulatory difficulty and may "pipe" as well as "flash" if there is cutaneous involvement. Fin erosions and/or ulcerations can be observed in chronic cutaneous infections. Diagnosis of this parasitic disease is dependent upon identification of the parasite within the skin or gill cytologic preparations or histopathology.

● ***Ambiphyra* sp:** These are ciliated protozoal organisms which are thought to be free-living, but have been known to parasitize fish. They are sessile organisms with a cylindrical to conical body with oral cilia and a permanent motionless equatorial ciliary fringe. They range in size from approximately 60-100 mm and adhere to the epithelium of the skin and/or gills. Disease and death of fish have been associated with chronic infections of the gills due to mechanical blockage of respiratory epithelium. Diagnosis of this parasite is dependent upon identification of this organism within the skin or gill scrapings or histopathology.

● ***Ichthyobodo* sp:** This parasite is also known as *Costia* sp. These are obligate flagellate parasites with a direct life cycle. The free-swimming form is renniform, and approximately 10-20 mm long with two pairs of flagellae; whereas the attached form is pear-shaped and attaches to the gills and skin. Disease associated with this parasite includes increased cutaneous mucous production (hence the lay terminology of "blue slime disease"), epithelial hyperplasia of the skin and gills, ulceration and erosion of fins. This pathogen commonly causes disease in salmonid fry, resulting in high mortality. Diagnosis is dependent upon demonstration of the agent within affected fish by cytology or histopathology.

● ***Hexamita* sp:** These parasites are pyriform to oval with tapering toward the posterior end. Occasionally, rounded individuals can be identified. The organisms are 6-8 mm wide and 10-12 mm long. They have three pairs of anterior flagella which are approximately one and one-half times the length of the body. The flagella originate from the blepharoplast at the anterior end of the axostyles. These organisms can reproduce by longitudinal binary fission as well as undergo schizogony within the epithelial cells of the caeca or intestine. This parasite causes disease within the gastrointestinal tract of fish and affected fish will have clinical signs related to malnutrition and emaciation. Diagnosis is dependent upon finding the parasite from cytologic scrapings of the ceca or intestinal tract or histopathology of these organs. A poorly understood parasite, thought to be Hexamita-like is thought to be responsible for Freshwater Hole-in-the-Head and Lateral Line Erosion (FHLLE).

● ***Chilodonella* sp:** This is a motile ciliated protozoal parasite which causes disease in the skin and gills of fish. It is typically heart-shaped with the posterior end being broader and slightly notched. It measures approximately 20-40 mm in width and 30-70 mm in length and its surface is covered with cilia. There is a large macronucleus in the posterior portion of this organism and a smaller micronucleus is near or within the macronucleus. This parasite has been attributed to death of fish due to respiratory and osmoregulatory imbalances

associated with severe gill parasitism. Diagnosis is dependent upon demonstration of the organism within the affected organs by either cytology or histopathology.

● Myxozoan Parasites

This is a very large group of parasites which can cause disease in a wide variety of fishes. They are obligate parasites of tissue (histozoic forms that reside in intercellular spaces or blood vessels that reside intracellularly) and organ cavities. Key characteristics of the Myxozoa include development of a multicellular spore, presence of polar capsules in their spores and endogenous cell cleavage in both the trophozoite and sporogony stages. The method of transmission of myxozoans is unknown, but evidence suggests that at least some pathogenic myxozoans have an indirect life cycle. This life cycle may require the completion of two different life cycles involving a vertebrate (fish) and an invertebrate (annelid) host with each life cycle having its own sexual and asexual stages. Severe infestations by these parasites can result in disease and/or death of the host fish. Each parasite is somewhat species specific as well as organ specific. A few of the more common myxozoan parasites are discussed below.

● ***Myxoboluscerebralis:*** This parasite is known for causing "whirling disease" in salmonids. This is a chronic debilitating disease which is very common in the Western U. S. and is uncommon in the Midwestern and Eastern U. S., although recent "outbreaks" have been reported in New York. The parasite feeds on cartilage of the axial skeleton and clinical signs are related to this damage. This parasite produces spores within the cartilage which are oval to circular and approximately 7-9 mm in diameter and 6-7 mm long with a thick mucoid envelope on the posterior half of the spore. Histologically, myxozoan parasites within the cartilage of the axial skeleton, with morphological characteristics of *Myxoboluscerebralis*, must be observed to confirm the diagnosis.

● **Aurantiactinomyxon**: This is currently thought to be the causative agent of "proliferative gill disease" of catfish. This myxozoan parasite causes a rapid, severe, proliferation of the gill epithelium which results in impairment of respiration and osmoregulatory function. The intermediate host is thought to be a microscopic aquatic oligochaete worm, *Dero digitata*, which is found in the mud and sediment in the ponds of affected catfish. Diagnosis is dependent upon the presence of the parasite within swollen, clubbed proliferative gill lamellae, many of which are fractured due to the associated chondrodysplasia.

● ***Henneguya*** **sp:** These parasites were once thought to be responsible for the "proliferative gill disease" of catfish, but are now thought to be less pathogenic, although they can cause respiratory problems if present in large numbers. They have a very characteristic paired, long, whip-like caudal processes giving them a spermatozoan-like appearance. Size of spores range from 8-24 mm in length with the caudal processes being approximately 20-45 mm long. Diagnosis is dependent upon observing the parasites within the histopathology of the gill lamellae.

The phylum Platyhelminthes is composed of three taxonomic classes: Turbellaria, Trematoda, and Cestoda. The Turbellaria are all free-living and have no association with fish diseases. All members of the other two classes live in close relationship with host animals. The trematodes are commonly known as flukes, whereas the cestodes are known as tapeworms.

● **Monogenetic Trematodes:** This is a group of trematodes which complete their entire life cycle on the host. The adults attach to the host by a haptor or opishaptor which is a specially adapted structure on the posterior end of the parasite. This organ has hooks which allow the parasite to attach firmly to the host fish. These parasites usually cause minimal damage to fish, but will infest the skin, fin and gills of pond fishes. Severe infestations may be responsible for poor respiration and/or emaciation. The two most common monogenetic trematodes include: *Dactylogyrus* and *Gyrodactylus*.

● ***Dactylogyrus*** **sp:** This parasite is approximately 0.2 to 0.5 mm in length, reaching a maximum length of 2.0 mm. It has seven pairs of marginal hooks and usually one pair of median hooks on the opishaptor. The dactylogrids have two to four pigmented spots (known as "eyes" or "eye spots") in the anterior fourth of the body. All dactylogrids are oviparous with no uterus.

● ***Gyrodactylus*****sp:** This parasite is smaller, rarely reaching a maximum length of over 0.4 mm. All species are viviparous with one to three daughter generations being observable in the V-shaped uterus. This parasite is more commonly found on the skin and less commonly present in the gills, although severe infestation will have both organs affected.

● **Digenetic Trematodes:** This group of parasites has a complex life cycle with several successive larval generations, alternating sexual and asexual generations and changes of hosts to develop into the adult in its primary host. The life cycles of trematodes involving fishes may either use fishes as the primary hosts or as intermediate hosts. Adult trematodes may infest the intestine or gall bladder of fishes. A few of the more common digenetic trematodes are listed below.

● ***Diplostomums pathaceum:*** The life cycle of this parasite begins as an adult trematode in the intestine of gulls or other fish-eating birds. The body of the adult is 0.3-0.5 cm in length and distinctly divided into a flattened anterior forebody and a cylindrical and narrower hindbody. Eggs are shed and passed in the feces of the bird to the water. The eggs hatch in approximately 21 days into free-swimming ciliated miracidia. The miracidia infest aquatic snails as the first intermediate host by penetration of the snail's hepatopancreas. The miracidia then become a mother sporocyst, followed by one or more daughter sporocysts. Each daughter sporocyst produces many cercariae which are released into the water. These cercariae seek a second intermediate host by penetrating the fins, skin, gills or cornea of small fishes. Primary host fish which ingest the initially infected fish (second intermediate host) become infected and the life-cycle is completed when the host fish are ingested by fish-eating birds.

● ***Posthodiplostomum minimum***: This trematode has several synonyms including: *Neodiplostomum minimum*, *Neodiplostomum or chilongum* and *Postodiplostomum or chilongum*. The life cycle of this trematode is very similar to that of *D. spathaceum* above, although, infectivity of cercariae to fishes lasts no more than 24 hours after release from the snail. Each cercaria actively raises a scale and enters under the scale pocket, causing irritation to the fish. Blood, congestion and hemorrhage occur at the bases of fins or other places of cercarial penetration. The trematodes migrate from the point of entry to visceral organs of the fishes, usually within one to three hours after penetration. Metacercariae are

located in any organ of the fishes' body, but are generally more numerous in the liver, kidney, heart, spleen and other organs of abdominal viscera. With many of the digenetic trematodes, the metacercariae within the skin results in increased melanin deposition, hence the term "black spot disease". Visible white or yellow spots in the visceral organs, usually no larger than 1 mm in diameter are often referred to as "white grubs" or "yellow grubs" and could be caused by several trematode species. Diagnosis of digenetic trematode infections is dependent upon identification of the genus and species of the trematode within infected fish.

• **Cestodes:** Cestodes are a taxonomic class of organisms in which the adult stage usually lives in the intestinal tract of vertebrates. Intermediate stages live in a wide variety of body locations in both vertebrate and invertebrate hosts. The bodies of most cestodes are ribbon-shaped and divided into short segments called proglottids, hence the name "tapeworm". Diagnosis of cestodiasis is dependent upon demonstration of the parasite within the intestinal tract of the fish. Clinical signs of cestodiasis include emaciation, anaemia, discoloration of the skin, and susceptibility to secondary infections. Low numbers of pleurocercoids may be located in vital organs such as the brain, heart, spleen, kidney, or gonad and have a devastating effect on the fish.

A few of the more common cestodes are listed below.

- ***Proteocephalus ambloplitis:*** This parasite belongs to a large family of cestodes with ten recognized subfamilies. This parasite has a complicated life cycle involving a piscine second intermediate host and a piscivorous primary host fish. These parasites are observed within the intestines of bass. Eggs are expelled from gravid proglottids and pass from the host in feces. The eggs mature to an embryo which is ingested by several species of copepods as the first intermediate host. The procercoid develops from the embryo inside the copepods. The copepod is ingested by a forage-fish whereby the procercoid penetrates the intestinal wall of the second intermediate host. Some encyst in the wall of the intestine, other penetrate organs in the visceral cavity and may eventually reach the musculature. Here they develop into plerocercoids,which is then ingested by a piscivorous fish.

- ***Ligula intestinalis:*** This group of parasites is distinct for three reasons: (a) they are not highly host-specific, but can develop in a wide variety of second intermediate host fishes, (b) the pleurocercoid stage develops sexually in the second intermediate host, and (c) these cestodes are very broad in shape and for this reason have also been known as "beltworms".

- **Acanthocephala:** This group of parasites is comprised of worms with an anterior proboscis covered with many hooks. These parasites are often referred to as "thorny-headed worms". The body is composed of a presoma (proboscis and associated structures) and a cylindrical trunk. The intestinal tract of the affected fish may contain a blood-tinged fluid, and histologically, the proboscis of these parasites will be firmly attached to the intestinal mucosa. Affected fish will exhibit emaciation, lethargy, anemia, and possibly death with a marked infection. We have observed salmonids from the Great Lakes with marked infestation of this parasite with literally hundreds of these parasites embedded in the intestinal mucosa.

- **Leeches and Copepods:** Although not a common problem, occasionally, fish will be observed infected with either leeches or copepods. Leeches have long, slender flexible bodies and actively swim for an attack on their prey. Skin and underlying soft tissues are damaged and allow blood to flow into the leeches digestive tract. Leeches are not host-specific, and the damage to the skin and gills is dependent upon the number of leeches present at any time. Small fishes can be seriously injured or die due to excessive leech infestation.

Copepods include fish lice or "anchor worms". The more common fish lice include *Lepeophtheirus* and *Caligus*, and *Argulus*. The most common genus of anchor worms includes Lernae sp. All of these are external parasites which affect the fish by imbibing blood from the host fish and causing localized skin and soft tissue damage. They may also allow for secondary bacterial infection of the skin or musculature which may ultimately cause the demise of the fish.

- **Fish Lice, Gill Maggots and Anchor Worms**

Some crustaceans are parasitic to fish, including the fish louse *Argulus* and the gill maggot *Ergasilus*. Both of these parasites are usually found on newly imported fish. Because they suck blood, they can transmit certain infections between fish.

The fish louse attaches itself to the skin and fins by means of twin suckers, and feeds on the blood of the host. The intense irritation may cause fish to flash, and heavily infested tropical fish may even jump out of the water. Red lesions occur where the lice have attached, and this opens the skin up to secondary bacterial and fungal infections.

Gill maggots are usually found attached to the gills, gill covers and inside the mouth. They are several millimeters long, and the common name refers to the adult females, with their prominent, whitish, "maggotlike" egg sacs. The male does not become a parasite. Heavy infestation can result in severe gill damage, emaciation, anemia and death.

Treatment requires an organophosphate insecticide such as dimethyl phosphate (also called phosphonate), Trichlorfon and Dimilin. The drug can be applied to a backyard pond or aquarium to eradicate both adult and juvenile crustacean parasites. Baths of Trichlorfon and Dimilin are both effective. Dimilin may be used as a bath for 48 hours, at 0.01 mg/L, repeated up to three times, and Trichlorfon can be used as a bath for one hour at 0.25 to 1.0 ppm, repeated up to three times.

Anchor worms are another crustacean parasite, elongated with two egg sacs at the rear end. They usually embed in the muscle of the body wall and often penetrate as far as the internal organs. A raised ulcer usually develops at the point of attachment, and secondary infections often occur at that site. Heavy infestations may cause weight loss and death.

Anchor worms don't typically occur in aquariums. They are most often a problem with newly imported tropical fish and in garden ponds during the summer. Male anchor worms have a short life span and die after mating; females are usually seen attached to the fish host. Eggs hatch to produce free-living juvenile parasites, which eventually molt to produce adult stages. The juvenile stages can live without a host for at least five days.

As with other crustacean parasites, organophosphate insecticides are used to eliminate the free-living juvenile stages. Remove adult worms with a forceps and treat the site with a suitable antiseptic. Remove parasites weekly until no more adult worms are found.

- **Velvet Disease:** A parasitic single-celled organism known as a dinoflagellate (*Piscinoodinium*, commonly called *Oodinium*) is responsible for velvet disease. This parasite has a direct life cycle, and has free-living and parasitic stages. The obvious symptoms are a yellow-gray coating to the scales, skin and fins. Fish may flash and show increased gill movements. In advanced cases, the fish will become anorexic and float motionless in the water, and the skin may peel away in strips.

A novice may confuse this disease with ich, but fish with velvet appear as if they are sprinkled with gold dust, hence the common name of "gold dust disease." This parasite moves from fish to fish with a flagellated spore, and can live off the fish for at least 24 hours, and probably longer (up to several days).
Velvet is usually introduced with new fish and may develop into a serious problem. Heavy infestation on the gills may kill fish without causing other obvious signs of the disease. It is most often found among some anabantids, killifish and goldfish. The parasite may establish itself in the intestinal tract of fish, making treatment more difficult, and long-term control of the parasite can be quite frustrating.
Treatment with ich or velvet remedies may be used. If the fish are salt tolerant, a prolonged (see the "Ich Treatment" section for suggested durations) salt treatment using 1 teaspoon of salt per 5 gallons of water can be used to eradicate the parasite. Interestingly, because the parasites use photosynthesis, keeping the aquarium relatively dark can help during a serious outbreak.

- **Treatment:** When it comes to treatments, there are several options. Drugs or chemicals placed in the water are commonly referred to as "bath" treatments. Drugs delivered orally are generally mixed in the food, and are meant to deliver systemic effects. Injections may be utilized in cases where small numbers of fish are involved. Bath treatments are the most variable, in that a specific concentration of the chemical is placed in the water for a specific length of time. As a rule, lower concentrations are used for longer periods of time, and *vice versa*.

Salt, plain old sodium chloride, is often overlooked as the number one best chemical to manage fish health. For most purposes, simple, un-iodized table salt is adequate, although there is nothing wrong with using aquarium salt specifically sold for that purpose, such as sea salt, solar salt and salt sold for consumption by livestock.

There are three common ways to use salt in freshwater tropical fish medicine. As an aid to osmoregulation, salt may be added in a concentration of 0.5-1.0% (or 5-10 parts per million-ppm). This concentration is safe for several hours and up to several days. At this concentration, control of protozoan parasites can be achieved. Higher concentrations of salt for shorter periods of time may be used to eliminate protozoa.

Chemicals Used to Treat Protozoa

- Un-iodized salt (sodium chloride).
- Potassium permanganate.
- Copper sulfate
- Formalin-F®, Paracide-F® and Parasite-S® (37% formaldehyde gas).
- Chloroquine (nonfood fish only); freshwater dips for marine food fish
- Metronidazole (nonfood fish only)

The most common forms of visible parasites are: anchor worm (*Lernea*), fish lice (*Argulus*), gill maggots (*Ergasilus*), and fish leeches (*Psicicola*) and if left untreated these parasites can cause life threatening damage. These parasites are relatively rare in aquariums and normally introduced with new fish or plants – carefully inspect any new fish and consider quarantining them before adding them to the aquarium.
Treatments of parasites are formulated to attack parasites at the weakest part of their lifecycle so accurate treatment must be done to ensure a successful treatment and recovery.

Note: it is important to be 100% sure of a diagnosis before treatment as medicines to kill visible parasites are very strong and will affect the biological filtration so require you to manage the water quality in the aquarium during treatment.

Key areas to consider and regularly monitor to prevent future outbreaks and aid recovery:

- maintain good water quality
- test your water regularly
- consider quarantine when introducing new fish to an established aquarium

FRESHWATER FISH PARASITE TREATMENTS	
External Parasite	**Treatment**
Ich *Ichthyophthirius multifiliis & Trichodina & Ichthyobodo &Tetrahymena*	Salt, formalin, malachite green, other commercial products, inc. temp.
Gill flukes, skin flukes	Copper, malachite green, formalin, others
External protozoa, flukes	Malachite green and formalin, others
Velvet (dinoflagellates)	Antiparasite medication, salt, copper, inc. temp
Fish lice, gill maggots	formalin, potassium permanganate, metriphonate, other commercial prod.
Anchorworms	organophosphate, manual removal and then dab with antiseptic
Larval stage of digenetic fluke parasites	organophosphate, other commercial products

Internal Parasite	**Treatment**
Hexamita & Spironucleus	Metronidazole, other commercial products
Blood parasites	If flukes: praziquantel, others difficult to treat
Nematodes (roundworms)	Use of appropriate anthelmintic drug, eliminate IM host, remove dead fish, piperazine
Spiny-headed worm	Appropriate anthelmintic
Tapeworms	Praziquantel
Digenetic flukes	Difficult to treat, praziquan

Probable questions:

1. Mention the different types of fish parasites and their mode of action.
2. Describe the symptoms of different protozoan and trematode parasites of fish and their proper treatment.

Suggested readings/ references:

1. https://en.wikipedia.org/wiki/Fish_disease_and_parasites
2. http://www.fao.org/fishery/static/FAO_Training/FAO_Training/General/x6709e/x6709e15.htm
3. https://www.omicsonline.org/open-access/review-on-major-parasitic-crustacean-in-fish-2150-3508-1000175.php?aid=78430
4. https://www.jstor.org/stable/3271411?seq=1#page_scan_tab_contents
5. https://www.merckvetmanual.com/exotic-and-laboratory-animals/aquarium-fishes/parasitic-diseases-of-fish

Unit V

Parasites of edible oyster

Objective: In this unit, we will discuss about parasites of edible oyster.

Introduction:

In India substantial oyster resources exist which remain unutilized. Pioneering attempts were made by James Hornell in 1910 in developing oyster culture in the Chennai in India.

Along the Indian coast there present vast stretches of areas of brackish waters, estuaries, many of which harbour natural populations of oysters, which suggests the suitability of the habitat for oyster culture along the Indian coast.

Being filter feeders, the oysters convert primary production in the water into nutritious seafood.

The oyster resources if cultured and harvested in regulated manner may contribute to the well being of the people that depend on this resource thus helping in improving socio-economic conditions of the coastal communities and protecting the coastal as well as marine environment.

In West Bengal,the Sundarbans region is suitable for attempting oyster culture. Here the local people artificially culture of *Saccostrea cucullate* and*Crassostrea madrasensis*at four different sites of Chotomollakhali Island of Sundarbans

But there are many technical and biological problems for wide range successful oyster culture. The rate of the oyster mortality is high in this region which poses a great problem to the oyster culture in Sundarbans.

Many oysters are died before reaching the market size. This mortality of oyster may be due to the diseases, parasitic infection, harmful organisms, heavy metal deposition or climatic changes.

Recently histopathological studies have thrown some light on the possible factors responsible for many instances of large scale mortalities.

Some viruses are suspected to be responsible for the cause of diseases and mortalities.

Dermo isa dreaded disease caused by a protozoan parasite, *Perkinsusmarinus*inflicted heavy mortality among East American oysters. Gill disease and shell disease attributed to pathogenic fungus, caused heavy mortalities among Japanese and European oysters.

Oyster beds affected by the above disease have revived after a long time. Large and thick oyster beds often are prone to be affected by diseases. Predators and foulers like fishes, starfishes, gastropods, crabs, polychaetes etc. are also a menace to oyster farmers. Sudden changes in hydrological parameters like temperature, salinity cause much damage to the oyster population.

To develop the oyster resources it is necessary to ensure that the areas of oyster beds are free from pollution since oysters accumulate heavy metals and pathogens

Oysters also play a significant role as a good bioindicator and biomonitoring species

Oysters also act as "ecosystem engineers" (Lawton and Jones, 1995; Jones et al., 1994) and indirectly modulate the availability of resources to other species, by causing a physical change in biotic and abiotic materials.

The word oyster is used as a common name for a number of distinct groups of bivalve molluscs which live in marine or brackish habitats. The valves are highly calcified.

The edible oysters belong to phylum Mollusca and Class Bivalvia. There is over hundred species of edible oysters have been distributed all over the world mainly in all temperate and tropical coasts

Molluscs including bivalves and oysters form a low-cost subsistence food and pharmaceuticals of the coastal people, especially for the fishing communities.

Among shellfishes, oysters are considered to be a valuable food items as they constitute rich source of many of the elements, essential for providing a balanced diet as it provides provide highly nutritious food for human consumption as they store large quantities of glycogen and fat.

The oysters are known to show large variations in their meat quality depending on their physiological conditions and associated environmental factors.

- **Distribution of edible oysters in India**

The edible oysters occur in all tropical seas, mainly between tidal levels or in shallow waters near the estuaries. The distribution of oysters along the Indian coast shows a distinct pattern.

*Crassostrea madrasensis*is found along the east coast from Orissa to Tamil Nadu. Along the west coast it is more dominant in the south than in the north.

*C. gryphoides*is the main oyster species in the northwest region especially in the Gulf of Kutch. Mixed populations of *C. grvphoides*and *C. rivularis* are seen along the northwest coast.

Saccostrea cucullate has wider distribution and is found along with all the species of the genus *Crassostrea* occurring in India.

Apart from this, oyster populations dominated by *S. cucullata* are also seen especially in Karnataka, Maharashtra and Gujarat. This species is also widely distributed in the inshore waters of Andaman and Nicobar islands.

*Crassostrea gryphoides*and *Saccostrea cucullata*—these are the two important species of edible oysters in the Sundarbans region, West Bengal.

Phylum: Mollusca

Class: Bivalvia

Subclass: Pteriomorphia

Order: Ostreoida

Family: Ostreidae

Genus: *Crassostrea*
Dendostrea
Lopha
Ostrea
Ostreola
Saccostrea
Striostrea

- **Description of the study site**

Two sampling sites have been selected each in the western and central sectors of Indian Sundarbans, a gangetic delta at the apex of the Bay of Bengal. On this background two sampling stations (Kaikhali in central sector and Frasergunj in western sector) have been selected to analyze the concentrations of

some metals in the edible oyster species.The host oyster species have been collected from two sites of Sundarbans namely, Kaikhali and Frasergunj. *Crassostrea gryphoides* present in both the sites but *Saccostrea cucullata* has found only in Frasergunj.

- **Oyster species in Sunderban**

Saccostrea cuculata
Crassostrea madrasensis
Crassostrea gryphoides

About ***Crassostrea gryphoides*** **Schlotheim, 1813**
*Crassostrea gryphoides*shell valves are variable in shape, more elongated and more deeply cupped and hence are called cupped oysters. They contribute a great portion of animal protein in the food market. Its tender flesh forms a cheaper, nutritious and easily digestible food source.

About ***Saccostrea cucullata*** **Born, 1778**
Saccostrea cucullate commonly known as the rock oyster due to its occurrence on rocky substratum are found on both east and west coasts but are more common on the west coast. They also are consumed as food but nowhere does it form beds large enough for exploitation.

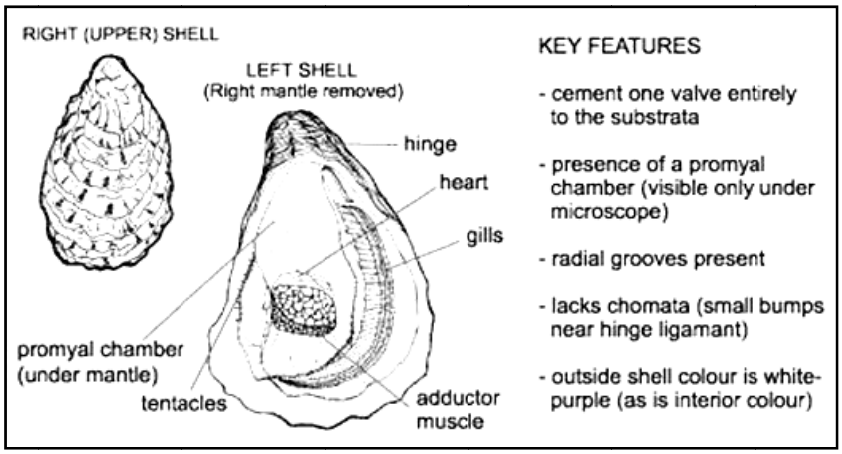
- **Utilization of oyster:** The oysters are either sold in the fresh shell on condition or as shucked meat in the domestic markets. Apart from marketing live oysters, different methods have been developed to preserve it without losing its quality. Some of the products like smoked, dried, frozen or canned oyster meat are sold in the metro cities. Oyster meat is nutritious and is relished in most nations.

Oyster meat also has nutritional and medicinal properties. The oyster meat consists of protein, glycogen and fat.

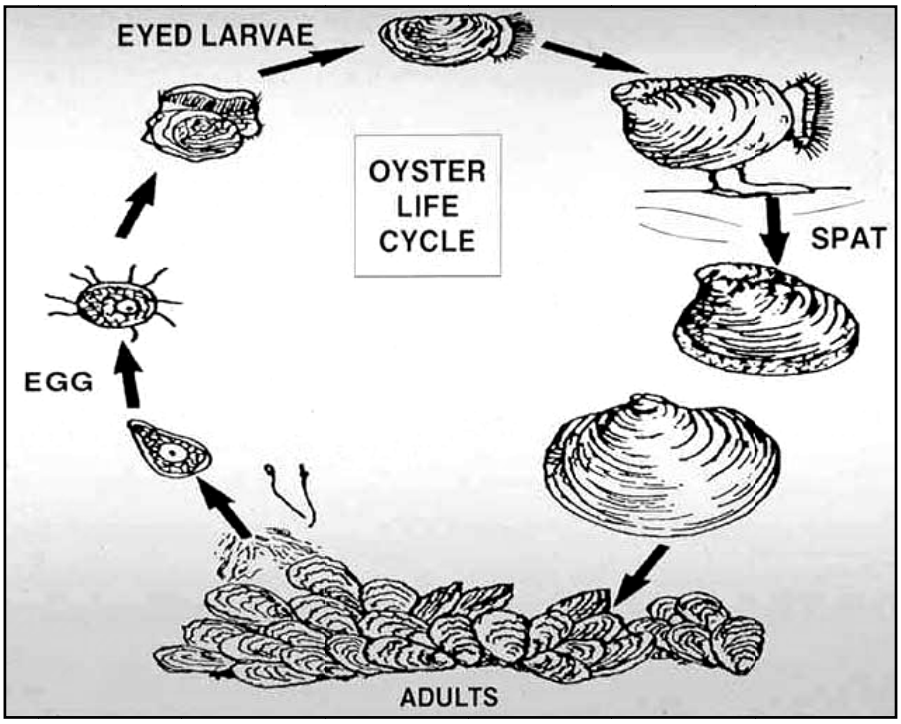
Oyster powder contains wide range of minerals and vitamins and the amino acid taurine which has complex medicinal properties. It has been found to be good for skin care, numerous heart ailments, blood pressure, liver problems, arthritis and rheumatism, diabetes, water retention and premenstrual tension.

Apart from meat, the **oyster shells** fetch a substantial return as by-product. The shell constitutes about eighty five percent of the total weight of oyster and contains fifty two to fifty five percent of calcium oxide and is used in the manufacture of calcium carbide, lime, fertilizers and cement. Further the **oyster shells** are useful as spat collectors of oyster spats. The shells are also broken to pieces of suitable size and used as poultry grit.

One of the major ecological functions of oysters is their potential to improve water quality by filtering plankton and other suspended particles from the water column.



Life History



- Reach sexual maturity at 1 year of age.
- Young oysters are male but change to female after first spawning season.
- Water temperatures above 25^{o}C trigger spawning in the.
- Salinity above 10 ppt.
- Spawn between March and November

Even though sedentary bivalves are able to withstand the wide variations in environmental conditions that commonly occur in their natural habitats, their survival are strongly influenced by fluctuations of environmental factors such as temperature, salinity, pH, food availability, nutrient levels, current speed, water depth etc.

The effect of increasing winter water-temperatures and modifications to regional salinity patterns in the spread and variation in prevalence and intensity of disease in marine bivalves.

Human-mediated climate change might also affect disease prevalence

Increased ocean temperature also caused pathogen range expansions. Climate change had been implicated in the northward expansion of Dermo and possibly MSX diseases of oysters.

Eastern oyster disease *Perkinsus marinus*) extended its range during a winter warming trend .Disease in both terrestrial and marine ecosystems could increase with future climate warming

Increased global acidification could impede calcareous shell formation, perhaps exacerbated by increased water temperature, particularly in molluscs, thereby have an impact on mollusc culture

- **Protozoan Parasites of Oyster**

Oysters are subject to various protozoan diseases.

Dermo is caused by a protozoan parasite *Perkinsus marinus.*

MSX (Multinucleated Sphere X) is caused by the protozoan Haplosporidiumnelsoni.

Disease is clearly a major factor affecting the abundance of shellfish stocks. *Haplosporidiumnelsoni* and *H. costale*had been reported to cause mortalities in the oyster, *Crassostrea virginica*

P. marinus, a water-borne pathogen most likely acquired through oyster feeding, commonly found in tissues of the digestive system

P. marinus caused mass mortalities of the oyster *Crassostrea virginica* along the east coast of America and in the Gulf of Mexico

QX disease and *Mikrocytosroughleyi* caused winter mortality

- **Dermo disease**

Dermo disease was first identified as the cause of extensive oyster mortalities in the Gulf of Mexico nearly fifty years ago.

It was originally thought to be a fungus and was named *Dermocystidiummarinum.* Even though subsequent research led to its reclassification as a protozoan parasite *Perkinsus marinus* in 1978, the disease is still commonly referred to as "Dermo".

Favourable environmental conditions are temp >20°C and salinity >15ppt

Depletes host energy resources causing decreased growth and reproduction and increased mortality

Rapidly proliferates and spreads via hemolymph (oyster blood) through tissues causing deterioration and organ failure

- **Transmission of *P. marinus***

The main method of transmission occurs when infective stages of the parasite, free in the water column, are ingested by oysters and then invade the lining of the digestive system.

Natural infections are most often caused by parasites released from the disintegration of dead oysters. P. *marinus.*

It may also be distributed by scavengers feeding on dead oysters or by *Booneaimpressa,* a parasitic snail that feeds on oyster hemolymph and acts as a vector in the transmission of the disease between live oysters.

Lifecycle of *Perkinsus marinus* has two distinct phases of the lifecycle:

1) the developmental stage

2) zoosporulation stage

The developmental stage within the host as a trophozoite in which the flagellum is lost to become a rounded immature form. This matures to form a large vacuole with a vacuoplast. Immature trophozoites free themselves through a tear in the cell wall and ultimately spread throughout the host's body. Zoosporulation occurs in which a mature trophozoite enlarges, loosing its vacuoplast. A discharge tube and pore develops in the cell wall. Palintomy occurs again, which results in numerous biflagellate zoospores moving through the discharge tube into the seawater.

- **MSX diseases**

It is often associated with a visible brown red discolouration of gill and mantle tissues.

Sporulation of *H. nelsoni*is prevalent in juvenile oysters (1-2 yrs) but sporadic in adults and occurs exclusively in the epithelial tissues of the digestive tubules. Infections appear and continue throughout the summer (mid-May to the end of October)

MSX disease was first recognized as the cause of massive oyster mortalities (90–95%) in lower Delaware Bay in 1957.

The causative agent, a single celled parasite, *Haplosporidiumnelsoni,* was originally given the acronym "MSX" because it was observed as a Multinucleated Sphere with unknown affinity ("X").

Distribution of MSX disease is fairly uniform over a large area within the higher-salinity (greater than 15 ppt – parts of salt per thousand parts of water) portion of affected estuaries

- **Transmission of *H. nelsoni***

The earliest infections of *H. nelsoni*are found in the oyster's gill.

The infective stage is water-borne.

The means by which MSX disease is transmitted is not known.

- **Probable question:**
    1. Explain the different types of protozoan parasites of edible oyster and their mode of transmission.

- **Suggested readings/ references:**

    1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4087319/
    2. https://www.ncbi.nlm.nih.gov/pubmed/28752231
    3. https://link.springer.com/article/10.1007%2Fs00436-017-5566-z
    4. http://eprints.cmfri.org.in/12069/

HARD CORE THEORY PAPER (ZHT 206)

Advanced Parasitology and Fish Biology

Group B:Fish Biology

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 206 (Advanced Parasitology and Fish Biology)	VI	Excretion and osmoregulation in fish.	1.5	1	1	45-52
	VII	Reproduction in fish : reproductive strategies, oviparity, viviparity, ovo-viviparity, Parental care, maturity stages, breeding cycle		1	1	53-66
	VIII	Structure and physiology of endocrine glands in fishes		1	1	67-78
	IX	Electroreception in fish		1	1	79-84
	X	Determination of age of fish by scale and hard parts.		1	1	85-90
	XI	Poisonous and venomous fish.		1	1	91-94
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Unit-VI

Excretion and Osmoregulation in Fish

Objective: In this Unit you will learn about Excretion and Osmoregulation in Fish

Introduction:

The process of elimination or removal of harmful substances i.e. metabolic nitrogenous wastes from the body is known as excretion and the organs associated with the removal of excretory substances known as excretory system. On the other hand, osmoregulation is the process of maintaining an internal balance of salt and water in body. Physiological systems of fishes operate in an internal fluid environment that may not match their external fluid environment.Relative concentrations of water and solutes internally must be maintained within fairly narrow limits. Since the quantity and variety of salts in both fresh and sea waters aredifferent from that in the body fluids of fish, all fish (except the myxinoids) must perform the function of osmotic regulation, i.e. fishes are homeosmotic. The physiological adjustments which are made in performing this function are truly remarkable.

Organs involved in Excretion and osmoregulation:

The functions of excretion and osmoregulation are usually closely related in animals and are performed by the same structures. In fish these structures are the gills and the kidneys.The primary excretory organ in fishes, as in other vertebrates, is the kidney. In fishes some excretion also takes place in the digestive tract, skin, and especially the gills (where ammonia is given off) but kidneys first evolved as osmoregulatory organs in fishes to remove water (freshwater) or conserve water (marine) The kidney, gills, and skin play an important role in maintaining a fish's internal environment and checking the effects of osmosis.

a. Gills:

The gills are filamentous tissues supported by a gill arch and protected by the body wall or an operculum. The blood supply consists of afferent arteries, from the ventral aorta, which divide into capillaries in the gill filaments and come together again to form the efferent arteries leading to the dorsal aorta. Although the gills function chiefly as a respiratory organ, they do very important work in excretion and osmotic regulation.

b. Kidneys:

There are great variations in the external structure of the kidney in fishes. The shape varies according to species. The kidneys occupy dorsal position in the body cavity and are placed just ventral to the vertebral column. In teleost, the kidney is distinguished into head and trunk regions. Head kidney is non-excretory and endocrine in function whereas trunk kidney (posterior kidney) is excretory in nature.

The trunk kidney or body kidney, like other vertebrates, contains renal tubules (nephrons) and interstitial lymphoid tissue. The number of renal tubules varies in different fishes. In teleosts, the

trunk kidney consists of a large number of nephrons. The functional unit of kidney is nephron. Each nephron consists of two parts, the renal corpuscles (Malpighian body) and the renal tubule (urinary tubule), the renal corpuscle or Bowman's capsule is double layer cup-like structure of uriniferous tubule which contains tuft of capillaries known as glomerulus.The remaining segment of urinary tubule (renal tubule) is divided into proximal convoluted segment the intermediate and distal segments.The distal segment is absent in marine fishes. The segment of Henle found in higher vertebrates is also absent in the fishes. The glomerulus and Bowman's capsule together constitutes the renal or Malpighian capsule. It is a filtration apparatus of kidney.The glomerular capillaries, which are the vascular part of corpuscle, is the afferent arteriole which divides and forms capillary loops. The loops reunite and leave the capsule as efferent arteriole. The renal corpuscle contains an additional group of cells known as mesangial cells. The function of mesangial cells are not known, although experimental data show that they can remove large proteins from the glomerular basal lamina. Juxtaglomerular cells are present in the wall of afferent arterioles. These cells contain secretory granules and are specialized muscular cells.

The glomeruli of freshwater teleosts are numerous and large in size. In marine teleosts, glomeruli are reduced in size and number. In extreme cases, the glomeruli disappear completely from the kidney of some marine fishes (Sea horse, pipe fish). The renal tubules are thin and short in the neck segment and consist of single layer of low epithelial cells with long cilia. The proximal convoluted are provided with cuboidal epithelial cells. The nuclei are large, round or oval. The cytoplasm contains secretary granules.The intermediate segment is well-developed in carp kidney but absent in several species of fishes. The distal convoluted segment could be distinguished because of coarse granules in the cytoplasm. The distal convoluted segments are absent in the kidney of marine fishes.

Excretion and Urine formation:

Carbon-di-oxide enters into the bicarbonate equilibrium system and most is excreted at the gills. Protein digestion yields nitrogenous compounds in addition to carbon di oxide and water. In teleost fishes these nitrogenous wastes takes the form of ammonia, a potentially toxic substance. Thus, teleports are primarily "ammoniotelic". Despite its toxicity ammonia has many advantages over urea or uric acids as the chief excretory product of nitrogen metabolism .First the small molecular size and high lipid solubility permits non ionized ammonia (NH3) to diffuse easily across the gills. Second, ionized ammonia (NH4+) is exchanged for Na+ at the gills for maintenance of relative alkalinity and internal ion balance. Third conversion of ammonia to either urea or uric acid requires energy. Thus in contrast to terrestrial forms, less energy is required to complete nitrogenous compound catabolism.

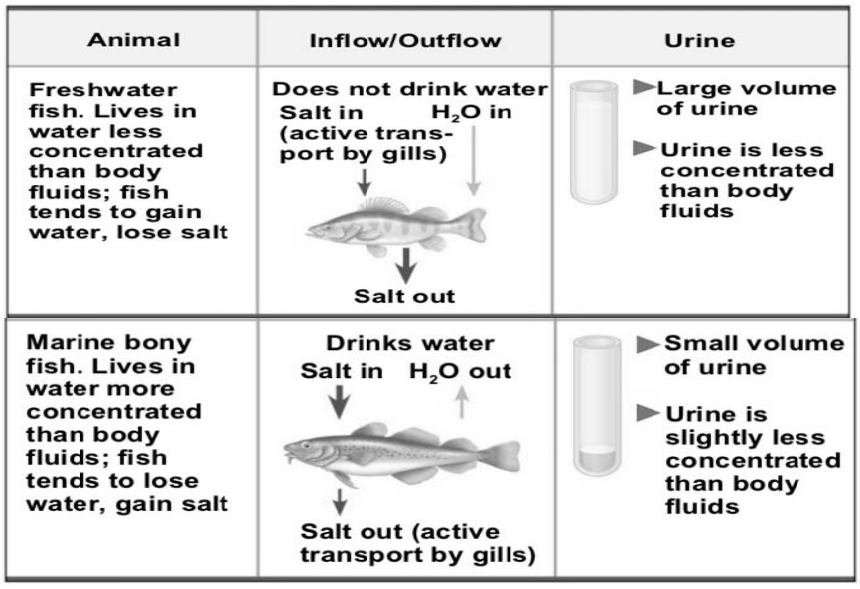

Fig: Urine concentration of Salt water and Fresh water fish

The freshwater teleosts have to excrete large amount of water which is taken through mouth. The urine of freshwater fishes contains creatine, unidentified nitrogenous compounds some of which are amino-acids, little amount of urea and ammonia.The urine is copious and has very low in concentration of electrolyte. Urine contains nitrogen amounting to 2 to 25% of total nitrogen excreted by freshwater fishes. The bulk is removed out through gills as ammonia.Marine fishes produce scanty urine, which contain Ca^{++}, Mg^{++}, SO_4^{--}, SO_4^{--} and PO_4^{--}. In addition to the creatine, creatinine and TMAO (i.e., tri-methylamine oxide) are also excreted out. Ammonia, urea and monovalent electrolytes (Na^+, Cl^-), however, are excreted mainly through gills.

Osmoregulation in Fishes

Life in an aqueous medium poses many challenges for fishes. Furthermore, the greatest challenge to the fishes is the maintenance of water and electrolyte homeostasis in the face of a broad (and sometimes rapidly changing) array of salinities. To maintain solute concentrations within limits compatible with life, fish have developed remarkable strategies for osmoregulation. According to habitat, fishes can be distinguished as marine and fresh water.The marine fishes fall into two distinct groups, (a) Those whose osmotic concentration is the same as or slightly above sea water, e.g., hagfish, elasmobranchs, Latimeria etc. This group has no major problem of water balance, because its inside and outside concentrations are equal, there is no osmotic water flow, (b) Those whose osmotic concentrations are about one third of that of sea water, e.g., lampreys, teleosts, etc. These are hyposmotic animals. They live in constant danger of losing water to the osmotically more concentrated medium.The fresh water fishes, on the other hand, have internal concentrations greater than that of their external medium. Thus, they are hyperosmotic to the medium. Therefore, the osmotic problems and the means to solve them differ drastically among fishes of different habitats.

According to habitat, fishes can be distinguished as (i) Marine, and (ii) Fresh water.

(i) The marine fishes fall into two distinct groups, (a) Those whose osmotic concentration is the same as or slightly above sea water, e.g., hagfish, elasmobranchs, Latimeria etc. This group has no major problem of water balance, because its inside and outside concentrations are equal, there is no osmotic water flow, (b) Those whose osmotic concentrations are about one third of that of sea water, e.g., lampreys, teleosts, etc. These are hypoosmotic animals. They live in constant danger of losing water to the osmotically more concentrated medium.

(ii) The fresh water fishes, on the other hand, have internal concentrations greater than that of their external medium. Thus, they are hyperosmotic to the medium. Therefore, the osmotic problems and

the means to solve them differ drastically among fishes of different habitats. A list of some fishes with habitat and osmotic characteristics is given in Table below.

Table 8.9 : Concentrations of major solutes (in millimoles per litre) in sea water and in the blood plasma of some aquatic vertebrates

	Habitat	Solute			Osmotic concentration (mOsm/litre^{-1})
		Na	K	Urea[a]	
Sea water		~450	10	0	~1000
Cyclostomes					
Hagfish (*Myxine*)	Marine	549	11		1152
Lamprey (*Petromyzon*)	Marine				317
Lamprey (*Lampetra*)	Fresh water	120	3	<1	270
Elasmobranchs					
Ray (*Raja*)	Marine	289	4	444	1050
Dogfish (*Squalus*)	Marine	287	5	354	1000
Fresh-water ray (*Potamotrygon*)	Fresh water	150	6	<1	308
Coelacanth (*Latimeria*)	Marine	197	7	350	954
Teleosts					
Goldfish (*Carassius*)	Fresh water	115	4		259
Toadfish (*Opsanus*)	Marine	160	5		392
Eel (*Anguilla*)	Fresh water	155	3		323
	Marine	177	3		371
Salmon (*Salmo*)	Fresh water	181	2		340
	Marine	212	3		400
Amphibians					
Frog (*Rana*)	Fresh water	92	3	~1	200
Crab-eating frog (*R. cancrivora*)	Marine	252	14	350	830[b]

a When no value is listed for urea, the concentration is of the order of 1 mmol per liter and osmotically insignificant. Values for ray, dogfish, and coelacanth include trimethylamine oxide

b Values for frogs kept in a medium of about 800 mOsm per liter, or four-fifths of normal sea water

A. Osmoregulation in Cyclostomes (Lampreys and Hagfishes):

The cyclostomes have two groups, Lampreys, which are anadromous, i.e. live both in sea and in fresh water, while Hag- fishes are strictly marine and stenohaline. The Lampreys, weather fresh-water or marine, have osmotic concentrations about one-quarter to one-third the concentration of sea-water. Their main problem is similar to that of teleost fish.The hagfishes are the only true vertebrates whose body fluids have salt concentrations similar to that of sea-water. In fact, the normal Na^+ concentration in hagfish blood exceeds that in their surroundings. Therefore, they have pronounced ionic regulation as an isosmotic animal.

B. Osmoregulation in Marine Elasmobranchs:

The common examples of marine elasmobranchs are sharks and rays. The salt concentration in their body fluid is roughly one- third the level of the sea-water, but they still maintain osmotic equilibrium. This is achieved by adding to the body fluids large amount of organic compounds primarily urea. Addition of different organic compounds in the body fluid/blood makes the osmotic concentration equal or slightly above the sea-water. In elasmobranchs urea is a normal component of all body fluids; this is abnormal for other vertebrates. In marine elasmobranchs, the tissue cannot function normally in the absence of such a high urea concentration. Urea is the end product of protein metabolism in vertebrates. Generally it is excreted through kidney, but the shark kidney actively reabsorbs this.The use of urea for maintaining osmotic equilibrium helps these animals to keep salt concentration much lower than those of sea-water. But urea can pose problems in the body functioning. It is known that urea destabilizes many proteins, especially enzymes. This problem is solved in elasmobranch by the presence of another organic substance trim-ethylamine (TMAO). TMAO inhibits the effect of urea on enzymes.Although the elasmobranchs have solved the osmotic problem of life in the sea by being isosmotic, they are still capable of extensive ionic regulation. They have sodium concentration much lower than that of sea-water. This means that sodium tends to diffuse from sea-water into the body.Sodium enters into the body primarily through the thin epithelium of the gill and then through the ingested food. This enhances the sodium level in the body, which must have to be excreted or

eliminated from the body. Part of the sodium excretion is undertaken in the kidney. Major excretion of Na^+ is performed by a special rectal gland. This is a small gland that opens via a duct into the posterior part of the intestine, the rectum. The gland secretes a fluid with high sodium and chloride concentrations, which is higher than the sea-water concentration.The elasmobranch blood is usually slightly more concentrated than sea-water. This higher concentration inside causes a slight osmotic inflow of water via the gills. In this way, the elasmobranchs slowly gain water osmotically, and this water is used for the formation of urine and for the secretion of rectal gland.The excess osmotic concentration is due to the presence of urea. But retention of urea solves the otherwise difficult osmotic problem of maintaining a low salt concentration while living in sea.

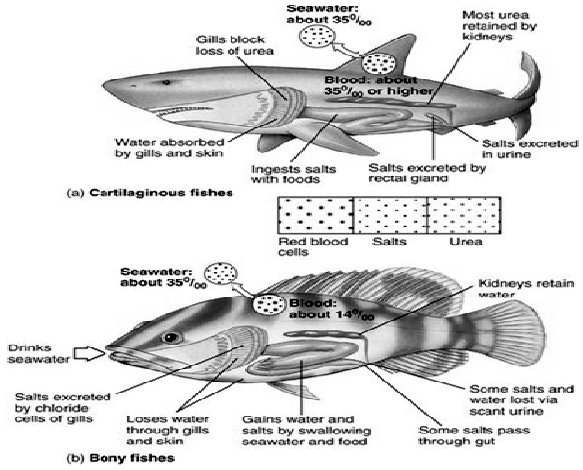

Fig:Osmoregulation in marine elasmobranch and bony fishes.

C. Osmoregulation in Freshwater Elasmobranchs:

Majority of elasmobranchs are marine, few of them are also found in freshwater. Some marine species occasionally enters rivers and lakes for various purposes. Among freshwater elasmobranchs, Carcharhinus leucas of Lake Nicaragua, four species of elasmobranchs of Perak River in Malaysia and Amazon sting ray Potamotrygon, are remarkable.Their blood concentrations are lower than those of strictly marine forms. The urea concentration is reduced to less than one third of the value of marine sharks.The problem of osmotic regulation is reduced due to the low level of solutes in the blood. The osmotic inflow of water is diminished because lower salt concentration is easier to maintain. The reduced osmotic inflow of water gives less water to be eliminated by the kidney.The urine always contains some solutes; therefore, a low urine flow reduces the urinary salt losses.

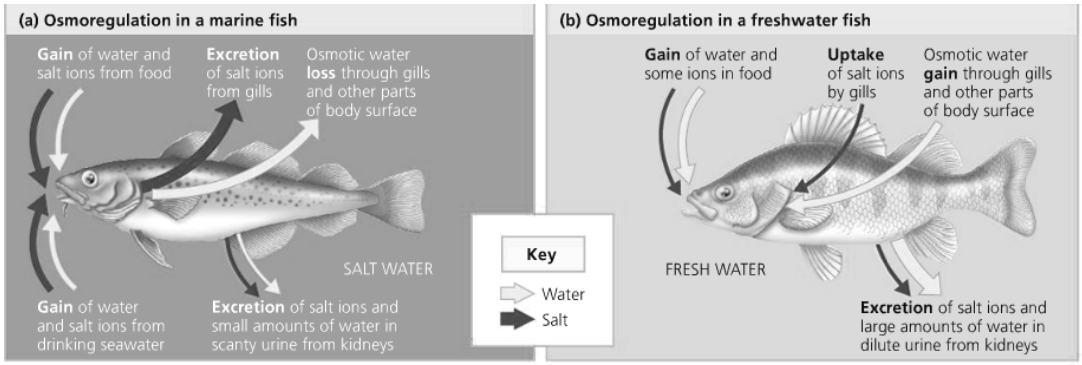

Fig: Osmoregulation in marine and freshwater fish

D. Osmoregulation in Teleosts:

Teleost fishes are living both in marine and freshwater. Both types of fishes maintain their osmotic concentration at about the quarter to one-third the level in sea-water. There is another type of fish, which roams both in sea water and fresh water. Therefore, can tolerate a wide range of salinities. These movements are often associated with the life cycle, such as breeding. But this change from one environment to the other requires profound changes in the osmoregulatory processes.

i. Osmoregulation in marine teleosts:

Marine fishes are hypoosmotic with the environment. Their main problem is losing body water to the more concentrated sea-water. The body water comes out through their body surfaces, in particular the large gill surfaces. The gill surface is more permeable to water than general body surface. These fishes compensate their inevitable water loss by drinking sea-water.Drinking of sea-water may restore the water content of the body, but impose another problem. Along with the sea-water, large amount of salts are also ingested and absorbed through the intestine. So salt concentration of the body increases.Now the problem becomes elimination of excess salt. To solve the problem, salts must be excreted in a higher concentration than in the water taken in. The teleost kidney cannot serve this purpose, because it cannot produce a urine that is more concentrated than the blood.Therefore, some other organ must participate in solving this problem of eliminating excess salts. This is done by the gills. So gills have dual function—one, participation in osmoregulation and second, gas exchange. The secretion of salt through gill is an active process, i.e., energy mediated. Because, it takes place from a lower concentration in the blood to a higher concentration in the surrounding medium.The excretion of sodium and chloride in the urine is of minor importance because teleost urine is more dilute than the body fluids.However, the kidney plays a major role in the excretion of divalent ions, magnesium and sulphate. These ions are not eliminated by the gills, which seems to transport only sodium and chloride.

Further studies show that the salt intake is happening not only through drinking sea-water, but also through the general body surface. It is also proved that fish adapted to sea-water are relatively permeable to ions and those adapted to fresh water are relatively impermeable.The ion transport is carried out, not by the general epithelial cells of the gills, but specifically by some large cells known as chloride cells. These cells are also present in the opercular cover of the fish. These cells actively transport chloride ions.

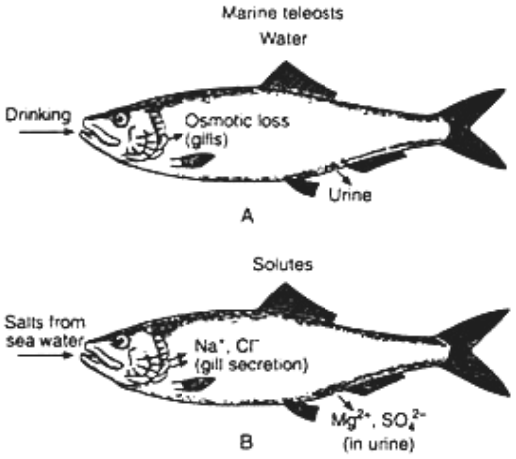

Fig. 8.38 : A marine teleost is osmotically more dilute than the water in which it lives. A. Because of the higher osmotic concentration in the medium, the fish constantly loses water, primarily across the thin gill membranes. Additional water is lost in the urine. B. To compensate for the water loss, the marine teleost drinks substantial amount of sea water. Of the ingested salts, sodium and chloride are absorbed in the intestine and eliminated via the gills by active transport (double arrow, B); magnesium and sulfate are excreted by the kidney

ii. Osmoregulation in freshwater teleosts:

The osmotic concentration of the blood of fresh water teleosts in much higher than the surrounding water (~300 mOsm/litre). Therefore, their major problem is the osmotic water inflow. Water mainly enters through the highly permeable gills. In freshwater teleosts skin is less important in transporting water inside the body, because it is less permeable.The large volume of water is excreted as urine, which is very dilute and may be produced in quantities up to one-third of the body weight per day. The urine contains 2 to 10 mmol/litre of solutes. So large urine volume also causes a substantial loss of solutes.This loss is replaced by the gills, which is also slightly permeable to ions. Some solutes are taken in with the food, but the main intake is by active transport in the gills. It is evident from the studies that skin plays only a minor, if any, role in active absorption.

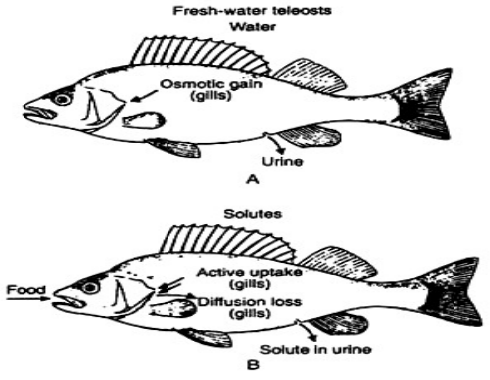

Fig. 8.39 : A fresh-water teleost is osmotically more concentrated than the medium and, therefore, suffers a steady osmotic influx of water, mainly through the gills (A). The excess water is eliminated as urine. Loss of solutes through the gills and in the urine is compensated for primarily through active uptake in the gills (double arrow, B)

Probable Questions:

1. What are the excretory organs of fishes?
2. How excretion and urine formation occur in fish?
3. Describe osmoregulation in Cyclostomes.
4. Describe osmoregulation in Marine Elasmobranchs.
5. Describe osmoregulation in fresh water Elasmobranchs.
6. Describe osmoregulation in marine Teleost.
7. Describe osmoregulation in in fresh water Teleost.

Suggested Readings:

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Unit-VII

Reproduction in Fish:Reproductive strategies, oviparity, viviparity, ovoviviparity, parental care, maturity stages, breeding cycle.

Objective: In this unit you will learn about Reproductive strategies, oviparity, viviparity, ovoviviparity, parental care, maturity stages, breeding cycle of fish.

Introduction:

The methods of reproduction in fishes are varied, but most fishes lay a large number of small eggs, fertilized and scattered outside of the body. The eggs of pelagic fishes usually remain suspended in the open water. Many shore and freshwater fishes lay eggs on the bottom or among plants. Some have adhesive eggs. The mortality of the young and especially of the eggs is very high, and often only a few individuals grow to maturity out of hundreds, thousands, and in some cases millions of eggs laid.Males produce sperm, usually as a milky white substance called milt, in two (sometimes one) testes within the body cavity. In bony fishes a sperm duct leads from each testis to a urogenital opening behind the vent or anus. In sharks and rays and in cyclostomes the duct leads to a cloaca. Sometimes the pelvic fins are modified to help transmit the milt to the eggs at the female's vent or on the substrate where the female has placed them. Sometimes accessory organs are used to fertilize females internally-for example, the claspers of many sharks and rays.In the females the eggs are formed in two ovaries (sometimes only one) and pass from the ovaries to the urogenital opening and to the outside. In some fishes the eggs are fertilized internally but shed before development takes place. Members of about a dozen families each of bony fishes (teleosts) and sharks bear live young. Many skates and rays also bear live young. In some bony fishes the eggs simply develop within the female, the young emerging when the eggs hatch (ovoviviparous).Others develop within the ovary and are nourished by ovarian tissues after hatching (viviparous). There are also other methods utilized by fishes to nourish young within the female. In all live-bearers the young are born at a relatively large size and are few in number. In one family of primarily marine fishes, the surfperches from the Pacific coast of North America, Japan, and Korea, the males of at least one species appear to be born sexually mature, although they are not fully grown.

Some fishes are hermaphroditic, an individual producing both sperm and eggs, usually at different stages of its life. Self-fertilization, however, is probably rare. Successful reproduction and in many cases defence of the eggs and young is assured by rather stereotyped but often elaborate courtship and parental behaviour, either by the male or the female or both. Some fishes prepare nests by hollowing out depressions in the sand bottom (cichlids, for example), build nests with plant materials and sticky threads excreted by the kidneys (sticklebacks), or blow a cluster of mucus-covered bubbles at the water surface (gouramis). The eggs are laid in these structures. Some varieties of cichlids and catfishes incubate eggs in their mouths.Some fishes, such as salmon, undergo long migrations from the ocean and up large rivers to spawn in gravel beds where they themselves hatched (anadromous fishes). Others undertake shorter migrations from lakes into streams or in other ways enter for spawning habitats that they do not ordinarily occupy.

Reproductive strategies in Fish:

Fish species have evolved reproductive methods and attempt physiology that allows them to be successful under a great variety of conditions. The entire combinations of habitat physiology and behaviour, the overall approaches to reproduction will be termed as reproductive strategies. Strategies may require greater number of eggs as mention or fewer eggs with greater opportunity for survival, strategies must ensure survival of a portion of the eggs through force of number, concealment, protection of nest or retention in the body; strategies must plays the earliest feeding stage of the young in the proximity of suitable and ample food and must ensure that the juvenile fish have eventual access to the living species of the adults. There are numerous ways to categorise fishes as to their reproduction, for instances, the simple breakdown into egg layers (oviparous conditions) and live bearers (ovoviviparous, viviparous).

a. Oviparity:

Oviparity or egg laying refers to the situation where the development or the fertilized of egg occurs outside the body of the female i.e. external fertilization. The young hatch when the egg envelope, shell or capsule is broken. Most fishes lay eggs that are heavier than water (Dermal eggs) but some may produce buoyant eggs that may be hydrostatically adjusted by oil inclusion, (embedded water in a large perivitelline space) or a high ratio of surface to volume to float at the surface or at some intermediate depth. Eggs of some species move freely , those of some others attached to each other or to the vegetation by means of tendrils. Demersal egg may be adhesive and deposited in clumps, sticking together through the incubation period. Some fishes engage in mass spawny with no pairing. Numerous males and females release gametes together in a suitable medium. This method of reproduction requires less energy, as the developing embryo depends completely on a concentration of nutrients within the egg called the yolk. This allows more eggs to be produced in some cases, but this lack of parental investment also reduces the offspring's likelihood of survival. Oviparous fish may be further categorized as being either ovuliparous or zygoparous.

Ovuliparity refers to the release of ova from the reproductive tract of the female followed by fertilization or activation in the external environment. Thus, all organisms that have external fertilization and this includes most teleost are said to be ovuliparous.zygoparity refer to the oviparous condition in which the zygotes (i.e. fertilized ova products of fusion between the eggs and sperm) are retained within the body of the female for a short period of time before being released into the environment. Obviously, zygoparous species display internal fertilization with their being a transfer of male sperm to the reproductive tract of the female. zygoparous reproduction characterizes all skates some sharks and a small number of teleost.Irrespective of whether the fertilization of the eggs occurs internally or the egg yolk provides externally the nutrient for the developing embryos of oviparous species.

Example: 90% Bony fishes such as Carps (*Labeorohita*, *Catlacatla*), catfishes and Horn sharkes, skates (rays) etc.

b. Viviparity:

Viviparity is a reproductive term in which female retain developing eggs inside their reproductive tract or body cavity and give birth to the off-springs capable of free living existence. Laying of the complete young adult animals, hence the mother delays to release the young individual. So long period is taken for the method of nourishment. Pseudoplacenta which found in expanded pericardial sac, internal fertilization and maternal nourishment occur. Several possible advantages are conferred by this pattern, the first of this is protection. The eggs and embryos are safe from predators.They are

protected from adverse water condition, desiccation, anoxia and injurious temperature. There is no need for large number of eggs. Usually fertilization is ensured, so that few eggs are wasted.

Example: Most selachii (themajority of sharks, stingrays, eagle rays, and giant rays) are viviparous fis h.Among bony fish, viviparity is a characteristic of eelpouts (*Zoarces viviparous)*, Bass (*Sebastes*), Baikal oil-fish (*Comephorus baicalensis*), many fish of the family Cyprinodontidae (foureyed fish, *Anableps tetrophthalmus),* and some freshwater fish of the family Hemirhamphidae.

c. Ovoviviparity:

It is intermediates between ovoparity and viviparity.Fishdonot release eggs or direct young adults. But eggs sometime remain within the body and release larvae. From the short term retention of eggs to incubating and hatching them internally is only a short step. Many species of frog fish(Family-Scorpaenidae) are ovoviviparous, releasing newly hatched larvae. The rock fishes so little by sexual morphism and have no particular specialization of the ovary where the eggs may remain in incubation. In genus-Dinematichthys eggs hatch from the ovarian follicles and develop into advance.Larvae in the lumen of ovary in Poeciliidae the youngs are retained until the juvenile stage is reached.Young remains in the follicle of ovary with sufficient yolk for development.

Parental Care In Fishes

Many species of fishes do not care for their eggs and fingerlings. They leave the spawning grounds after fertilization. For such species of fishes the lacking parental care behaviour is compensated by the production of large number of eggs. Such fishes lay over 300 million eggs at a time.

The cod fish lays over nine million eggs which are scattered at random in the open sea. Carps lay two to four million eggs at random in fresh water and adjoining aquatic vegetation. It has been estimated that about 77 percent fishes show no parental care, another 17 percent of the fish species care for the eggs only, while less than 6 percent care for eggs and newly hatched young.Fishes that produce limited number of eggs have evolved various grades of parental care behaviour:

A. Deposition of eggs in suitable places.

B. Deposition of eggs into self-made nest.

C. Concealing eggs and young's in or on their body.

D. Viviparity

E. Care of independently swimming young's.

A. Depositing Eggs in Suitable Places:
A number of fish species have developed some design of depositing their eggs in suitably protected places. They do not build nests.

(a) Deposition of eggs in sticky covering:
(i) In carps, eggs are usually laid with some special sticky covering by means of which they are attached to each other or to stones, weeds etc.

(ii) In yellow perch (*Percaflavescens*) eggs are deposited in a rope-like structure (Fig. 5.40A). The eggs are held together by a long floating membrane.

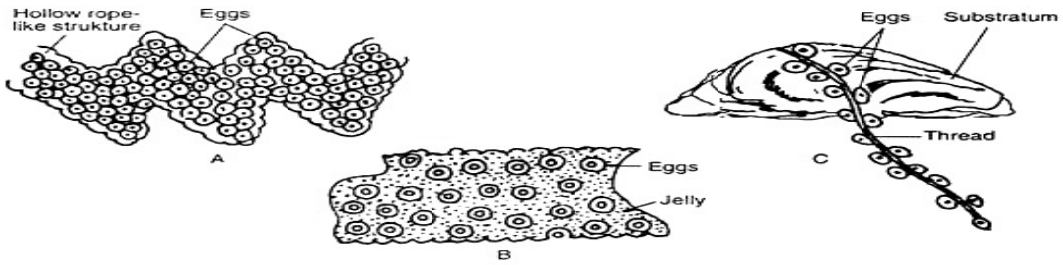


Fig. 5.40 : Deposition of eggs in sticky covering : A. Eggs deposited in a rope-like structure in yellow perch. B. Eggs deposited in a gelatinous outer coat in the case of Angler fish. C. Eggs deposited on a sticky thread secreted from the kidney of flying fishes, skippers, garfishes etc.

(iii) Angler fish (*Lophius*) lay their eggs invested by a gelatinous external coat, that remain together to form a transparent mass of enormous size (Fig. 5.40B).

(iv) Flying fishes, skippers, garfishes etc. secrete a sticky thread-like substance from their kidney, on which the eggs remain attached (Fig. 5.40C). The thread on one end remains adhered to any aquatic substratum while the other end remains free.

(b) Eggs scattered over aquatic plants:

Eggs of fishes such as pikes (*Esoxlucius*), carps (*Cyprinus carpio*), *Carrassius auratus* etc., are scattered over aquatic plants to which they remain attached.

(c) Eggs laid at suitable places:

(i) Anadromous fishes (lives in the sea but migrates to fresh water for breeding) such as Salmo solar, Acipenser, Oncorhyncus etc., lay their eggs in suitable spawning grounds. They dig excavation in gravel substrate, lay their eggs in the pits, cover them with gravel and desert them.

(ii) Sand Goby *(Pomatoschistos minutus*) lay their eggs in some protected place, where they are guarded by the male who also aerates them by his movement.

(d) Eggs deposited on dead shells of bivalves:

(i) Females of cyprinid family deposit their eggs on the dead shells of mussels.

(ii) Females of European bitterling (*Rhodeusamarus*) deposit eggs in the siphon of a fresh-water mussel by means of a long tube acting as an ovipositor (Fig. 5.41). This ovipositor is a long tube drawn out from the oviduct.

After oviposition male Rhodeus immediately sheds the sperm over the eggs and then guards them. The male Rhodeus, interestingly, are not sexually excited by the presence of the female of its own species, but by the sight of the shell of the mussel in which the eggs have been deposited.

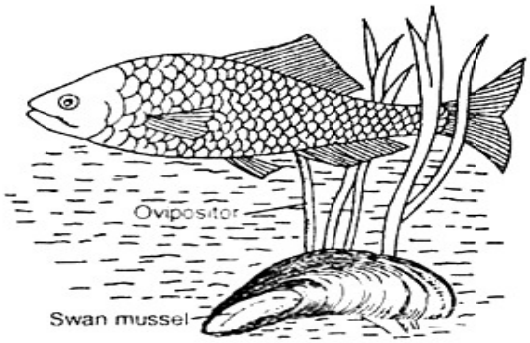


Fig. 5.41 : Oviposition by European bitterling on the shells of a dead mussel

B. Deposition of Eggs Into Self-Made Nest:

Some species of fishes prepare nests of different types for the safe deposition and protection of eggs, and for the development of their young ones. In the building of the nest, only the males or both sexes participate. Various kinds of materials such as pebbles, aquatic vegetation, secretion of their body etc., are used for nest formation.

The different types of nests built are:

(a) Basin-like nests:

(i) Male of Darter (*Etheostomacongregata*), during the spawning season, selects a suitable place called domain, which it defends repelling with vigor any attempt by rival males. Any female Darter entering its domain is allowed to remain.

The female Darter then makes a basin-like depression, sinks into it and deposits the eggs. The eggs are immediately fertilized by the male who covers the fertilized egg by a sticky secretion, secreted from its kidneys. These sticky eggs remain attached to the stone till hatching.

(ii) Fresh water sunfishes build a nest by scooping out a shallow basin-like nest at the bottom of the impoundment by carefully removing pebbles and leaving behind large stones (Fig. 5.42). A layer of fine sand remains attached with the eggs. Male sunfish guard the eggs till they hatch.

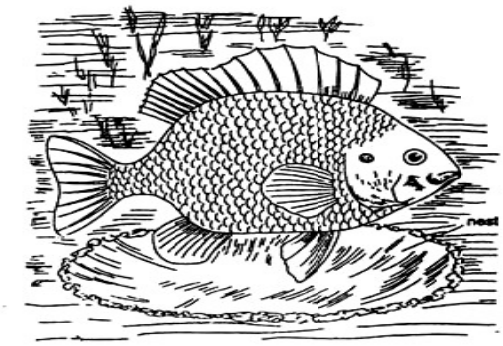


Fig. 5.42 : Basin-like nest of sunfish

(b) **Circular nest** : (i) The male Bowfin (*Amia calva*) prepares a crude pit-like circular nest (Fig. 5.43A) among aquatic vegetation. The male then invites a female. She spawns and the male fertilizes the eggs. The fertilized eggs are then protected by the male who keeps guard over the nest till the young ones are hatched. The young ones are allowed to leave the nest in a body under the protection of the father (Fig. 5.43B).

(iii) Cichlid fishes (*Haplochromisburtoni*) build basin-like nest which is guarded by both the parents.

(iv) In some North American catfishes both the male and female prepare a crude nest in the mud to lay eggs.

(b) Circular nest:

(i) The male Bowfin (*Amia calva*) prepares a crude pit-like circular nest (Fig. 5.43A) among aquatic vegetation. The male then invites a female. She spawns and the male fertilizes the eggs. The fertilized eggs are then protected by the male who keeps guard over the nest till the young ones are hatched. The young ones are allowed to leave the nest in a boa/ under the protection of the father (Fig. 5.43B).

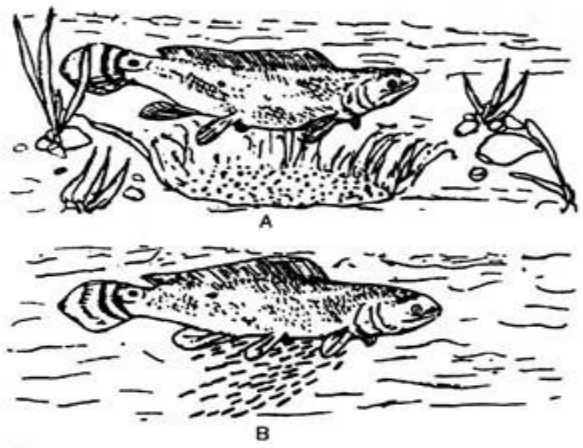


Fig. 5.43 : A. Male Bowfin prepares a circular nest. B. The hatchlings swims in a group under the protection of the father

(ii) Both the male and the female of some cat fishes (Arius) of North America prepare a crude circular nest in the mud, at the bed of the river. The diameter of the nest is about 50 cm and is sometimes provided with a protective cover of logs, stones etc. The fertilized eggs are left uncared.

(c) Hole/Burrow nest:

(i) The African lung fish (Protopterus) prepares a simple nest in the form of a deep hole in swampy places along the river bank. The male prepares the nest surrounded by long aquatic weeds and grasses and after spawning keeps guard over it, occasionally aerating the water by his slow body movement.

(ii) The South American lung fish (*Lepidosiren*) prepares a nest in the form of a burrow in swampy places, varying in length from 1 to 2 metres. The nest consists of a short vertical and a larger horizontal portion in which eggs are deposited. Males take care of the eggs. During this time they develop a long red filament from the pelvic fins which perform the function of aeration without coming out on to the surface to gulp air.

(d) Barrel-shaped nest:

Before the start of courtship the male stickleback (example: three-spined stickleback, *Gasterosteus aculeatus* and ten-spined stickleback, *Pygosteuspungitius*) builds a quite elaborate nest. The male at first selects suitable place in shallow water where the flow of water is continuous but not swift.

He then builds a nest by collecting plant fragments, rootlets, weeds and then binds them together with the help of a sticky secretion from its kidney. The various activities of males such as probing, boring, sucking and gluing result in the formation of a compact nest with tunnel to receive the eggs (Fig.5.44)

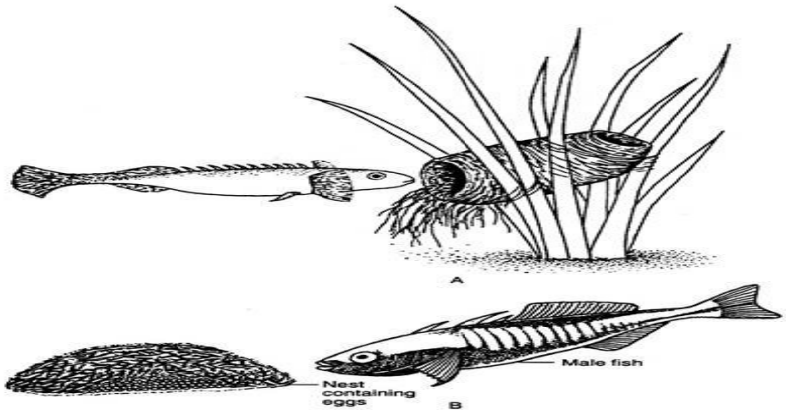


Fig. 5.44 : Barrel shaped nest of : A. Ten-spined stickleback and B. Three-spined stickleback

The male then goes out in search of a mate. The bulging abdomen of the female stimulates the male to perform a zigzag dance around her, displaying his red spot. If the female is ready to lay eggs she responds by curving her head and tail upwards. On reaching the nest the male opens the entrance of the nest with its snout. The female, after depositing two to three eggs within the nest, swims out of the nest through the opposite side of the entrance.

The male then enters the nest and fertilizes the eggs. He then comes out of the back entrance and moves out to seek another female. The same process is then repeated till sufficient eggs are deposited. The male then guards the nest and keeps the eggs aerated by fanning the nest with his fins and tail. Later, the male stops fanning and keeps close watch over the brood, not allowing any young one to go astray.

(e) Cup-shaped nest:

The male of *Apeltsquadracus* builds an elaborate cup-shaped nest, attached to rooted plants close to the bottom. After a female lays a clutch of eggs, the male builds an extension of the nest up and over the eggs, with a concave upper surface in the extension. A second female lays another clutch of eggs on the new concave floor. This process is repeated several times, until the male has several clutches of eggs stacked vertically within a multi-tiered nest.

(f) Floating nest:

The male Mormyrids (Gymnarchus) constricts a floating nest with the help of aquatic vegetation. The wall of the nest remains projected several centimeters above the water surface.

(g) Foamy nest:

(i) The nest made by American catfishes contains eggs that are suspended in a mass of bubbles and mucus produced by the male.

(ii) The male fighting fish (Beta) builds a nest by blowing bubbles of air and sticky mucus that adhere together forming a floating mass of foam on the surface of water (Fig. 5.45). The fertilized eggs are collected by the male in his mouth, who gives them a coating of mucus and sticks them to the lower surface of the foamy nest. The male then stays on guard and fights till death to defend it.

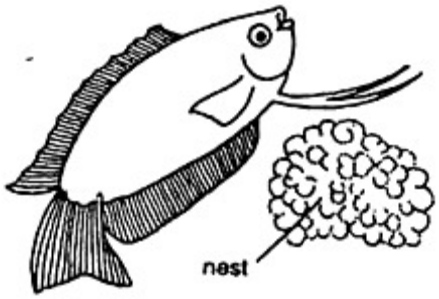


Fig. 5.45 : Male siamese fighting fish (*Beta splendens*) defending his foamy nest

(iii) The male paradise fish (*Macropodus*), also prepares a similar foamy nest. In this species the eggs are lighter and rises up to the nest without the active participation of the father.

C. Concealing Eggs and Young's in or on their Body:

Certain species of fishes have developed many structures in their body to safeguard the eggs and young ones:

(a) Eggs and young's concealed in mouth cavity:

(i) In many cichlids (Example: Tilapia), the female broods the fertilized eggs in her mouth (Fig. 5.46A). After hatching she allows the young to take refuge in her buccal cavity in times of danger.

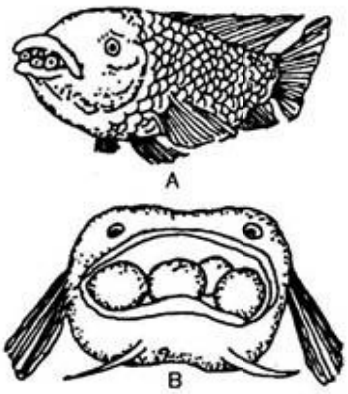


Fig. 5.46 : A. Female *Tilapia* carrying eggs in her mouth. B. The male Brazalian cat fish carrying eggs in a pouch present inside the lower lip

(ii) In most marine cat fishes (*Arius*) and cardinal fishes, the male carries the eggs and young ones in his mouth. The male does not take food during this period.

(iii) In case of the Brazilian cat-fish (*Loricariatypus*), the male has an enlarged lower lip forming a sort of pouch in which labial incubation takes place (Fig. 5.46B). This ensures safety and provides perfect aeration. During this act, the male fish do not take any food. Even after hatching the hatchings remain near the father and enter the buccal cavity of father at the slightest disturbance.

(b) Formation of egg ball: The butter fish (*Pholis*) rolls the eggs into a round ball and then one of them remains on guard by curling around it (Fig. 5.47). It is often the male that guards the egg ball till hatching of young.

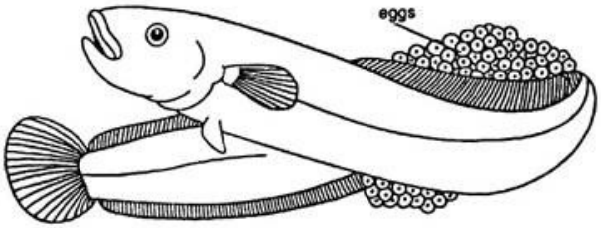


Fig. 5.47 : A butter fish (*Pholis*) curling around the egg ball

(c) Eggs attached to cephalic hook:
The male nursery fish (Kurtus) of New Guinea, carries the mass of eggs on the forehead, held in a cephalic hook (Fig. 5.48).

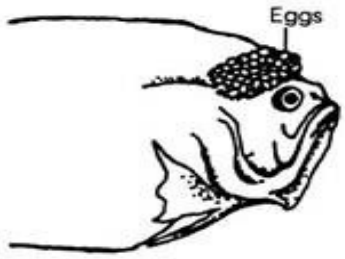


Fig. 5.48 : Male nursery fish carrying egg mass on fore-head

(d) Eggs in integumentary cups:
The cat-fish, Platystacus of Brazil, shows an interesting method of parental care. During the breeding season, the skin of the lower surface of the body of female becomes soft and spongy. Immediately after the eggs are fertilized, the female presses her body against the eggs in such a manner that each egg becomes attached to the skin by a small, stalked cup (Fig. 5.49). The eggs remain fixed in this position till hatching.

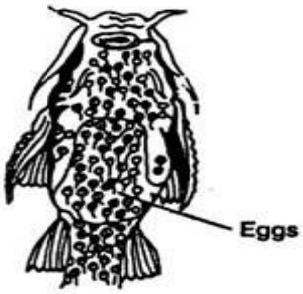


Fig. 5.49 : *Platystacus* bear eggs in integumentary cups

(e) Eggs kept in brood pouches:
(i) In sea horse (Hippocampus) fertilized eggs are transferred by the female into the brood pouch (Fig. 5.50A) of the male. The brood pouch is found on the lower surface of the abdomen. During the males so called 'pregnancy', he provides nutrients and oxygen to the fertilized eggs for several weeks.

Generally, a single large female produces enough eggs in one cycle to fill the brood pouch of nearly three large males. As the sex ratio is 1 : 1, male pouches are in short supply. Thus, males exhibit

active mate choice. They favor large ornamental ones that can fill their brood pouch with eggs quickly and sufficiently. After hatching, fry may return to the pouch when in danger,

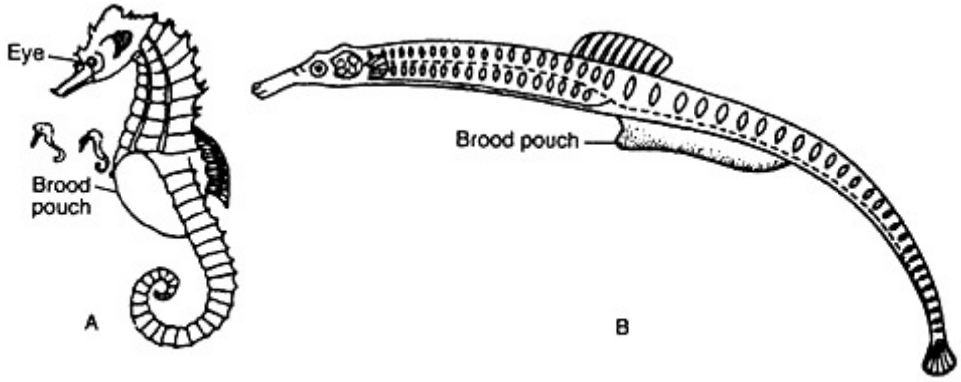


Fig. 5.50 : Brood pouch in the males of A. Sea horse and B. Pipe fish

(ii) In the male pipe-fish (*Syngnathus*), a brood pouch (Fig. 5.50B) is formed by two flaps of skin on the underside of the body on which eggs are placed by the female.

(iii) In the family solenostomidae, pelvic fins of females form the brood pouch. The eggs inside the brood pouch are kept in proper position by numerous long filaments.

(f) Egg capsules:

In oviparous elasmobranchs such as sharks and rays, the fertilized eggs are laid inside protective Horney egg capsules called mermaid purse. The shape of the purse varies in different groups (Fig. 5.51). The capsules remain attached to aquatic weeds by their tendrils. The development of the eggs is completed inside the purse.

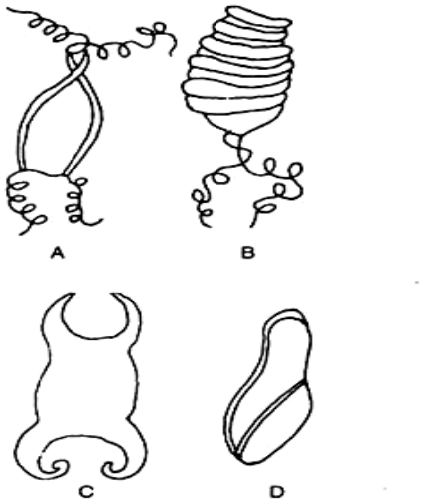


Fig. 5.51 : Different types of mermaid's purse A. shark, B. Pot Jackson shark, C. Skate, and D. Chimaera

Mermaid's Purses:

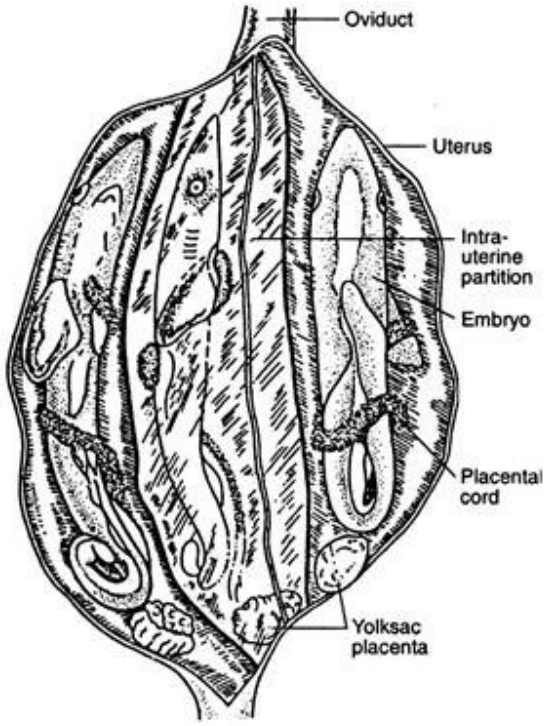
Oviparous sharks (e.g., *Scyllium*) lay fertilized eggs inside the protective horny egg capsules or mermaid's purses, which remain anchored to the sea weeds by their long tendrils. The young hatch out after rupturing the egg case.

D. Viviparity:

The highest degree of parental care is exhibited by those species which are viviparous. They have evolved internal incubation and give birth to young ones, thereby providing maximum protection.

(a) Viviparous Elasmobranchs:

Among the sharks, viviparity has been witnessed in more than a dozen families. In case of *Scoliodon, Mustelus* etc., eggs develop in the uterus. The mucous lining of the uterus forms fluid-filled protective compartments, one for each embryo. Nourishment in the form of embruotrophe or uterine milk is received by each embryo from the uterine tissue through the yolk-sac placenta (Fig. 5.52).



(b) Viviparous Bony Fishes:

(i) In teleosts, species (Example: Zoarces, Gambusia and Poicilia) belonging to the orders Cyprionodonts and Perciformes show internal fertilization and the young ones develop within the ovary but are not attached to its wall.

The embryos develop freely in a sac inside the ovary, feeding upon an "embryotrophic" material, apparently produced by the discharged ovarian follicles. The sac becomes highly vascular and remains as such during the months of pregnancy.

(ii) In the case of shiner-perch (*Cymatogasteraggregata*) the eggs also are fertilized in the ovarian follicles, but are soon released into the ovary cavity and are nourished by a secretion from the ovary (Fig. 5.53). The young are retained in the ovary until they become sexually matured.

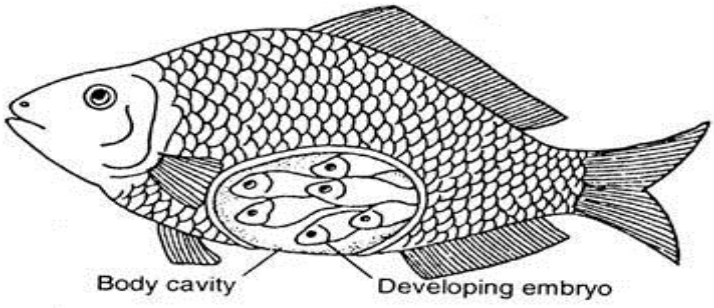


Fig. 5.53 : The body cavity of *Cymatogaster aggregata* is cut open to show fully formed youngs

(c) Ovo-viviparous:

An intermediate condition between oviparous and viviparous is observed in the case of nurse shark (*Ginglymostoma*), called ovo-viviparous. Here the eggs are covered by a horny case and the development takes place in the uterus. The fully developed young ones hatch out by breaking the shell inside the uterus.

E. Care of Independently Swimming Young's:

In the members of some families such as Gasterosteridae, Centachidae and Ictaluridae, parental care does not stop with the caring of the eggs. These fishes defend their young ones by placing them in a safe place, away from predators and enemies.

As has been dealt with earlier, the young ones of Tilapia, seahorse and pipe fish are protected by their parents either in the oral cavity of mother or in the brood pouch of the father. In the case of cichlid fish, both male and female secrete a nutritious substance from their body, which are taken up by their young ones.

Breeding cycle:

Breeding cycle means the frequency of spawning. In most cases of animals breeding cycle is cyclic or more or less regularly periodic in nature in fishes. Some fishes survive for short period of life and they breed only once.

1. Silverside (*Labidesthessicculus*): Some species survive for moderately long period but still breed only once in two or five years.
2. *Onchorhynchus*sp. (Pacific Salmon): Breed only once 2-5 years of the life span.
3. Sea lamprey (*Pteromyzon*): Breed once in 5-6
4. *Anguilla* (Freshwater eel): Breed once in 10-14 years
5. *Cyprinuscarpio*: Breed two times in a year and reproduction is very frequent.
6. *Lebistis reticulates:* They breed approx in every 4 weeks or once in a month.
7. Indian major carp: Breed once in a year.
8. Telapia*(Oreochromis mossambica):* Breed 3 times in a day.

Maturity Stages:

Determination of maturity stages record of the stage of maturity of fish examine is often required, since this bears on the condition factor and length weight relationship and may explain what otherwise might appear to be anomalous data. It is also important to know the average size or age at first maturity. One should therefore determine whether each fish is sexually mature, immature, ripe or spent.Lagler (1956) gave clear instruction on the practical procedure for fish living in temperate regions. During the breeding season the reproductive organ should be classified as immature, ripe or spent. Immature means that,there are no easily visible eggs or milt. Ripe means that the gonads contain obvious eggs or sperms and spent means that the fish has spawned. The ovaries of recently spawned fish are often flaccid and blood shots. During the remainders of the year, the fish should be classified as immature, with no egg or milts present or mature when eggs or sperms are clearly apparent.

More complex maturity classification have been suggested by Kesteven (1960) based on Buckman (1929) and by Nikolsky (1963).

From Kesteven (1960)	**From Nikolsky (1963)**
1. Virgin:Very small sexual organ closeunder the vertebral column. Testis and ovaries transparent,Colourless to grey. Eggs invisible to naked.	1. Immature: Young individuals which have not yet engaged in reproduction; gonads of very small size.
2. Maturing virgin: Testis and ovaries translucent, grey-red colour. Length half or slightly more than half of the ventral cavity. Single eggs can be seen with magnifying glass.	2. Resting stage: Sexual products have not yet begun to develop. Gonads of very small sizes. Eggs not distinguishable to the naked eye.
3. Developing: Testis and ovaries opaque, reddish with blood capillaries, occupy about half the ventral cavity. Eggs visible to the eye as whitish granules. Testis reddish white. No milt drops appear under pressure. Ovaries orange, reddish. Eggs clearly distinguishable, opaque. Testis and ovaries occupy about two third of ventral cavity.	3.Maturation: eggs distinguishable to the naked eye, a very rapid increasing weight of the gonad is in progress; testis change from transparent to a pale rose colour.
4. Gravid: Sexual organs filling ventral cavity. Testes white, drops of milt fall with pressure. Eggs completely round, some already translucent and ripe.	4. Maturity: Sexual products ripe. Gonads have achieved their maximum weight, but the sexual products are still not extruded when light pressure is applied.
5. Spawning: Roe and milt run with slight pressure. Most egg translucent with few opaque eggs left in ovary.	5. Reproduction: Sexual products are extruded in response to very light pressure on the belly; Weight of the gonad decreases rapidly on the start of spawning to its completion.

6. Spawning spent: Not yet fully empty no opaque eggs left ovary.	6. Spent condition: The sexual products have been discharged; genital aperture inflamed; gonads have the appearance of deflated sac, the ovaries usually containing a few leftover eggs and testes; some reduced sperm.
7. Spent: Testes and ovaries empty, red. A few eggs in the state of reabsorption.	7.Resting stage: Sexual products have been discharged; inflammation around the genital aperture has subsided, gonads of very small size, egg not distinguishable to the naked eye.
8. Recovering/ Spent: Testes and ovaries translucent, grey-red. Length half or slightly more than half of the length of the ventral cavity. Single eggs can be seen with magnifying glass.	

Probable questions:

1. Define viviparity, oviparity and ovoviviparity.
2. Describe different types of nests maid by fishes as an outcome of parental care.
3. Describe different types of attachment of eggs in the body of fishes.
4. Describe briefly the breeding cycle in fish.
5. Classify different stage of fish maturation according to Kesteven (1960).
6. Classify different stage of fish maturation according to Nikolsky (1963).
7. Write briefly viviparity, oviparity and ovoviviparity in fishes with examples.

Suggested Readings:

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Unit-VIII

STRUCTURE AND PHYSIOLOGY OF ENDOCRINE GLANDS OF FISH

Introduction: Endocrine glands are ductless glands that release their secretion directly into the blood or lymph and along with central nervous system regulate many kinds of body functions. Fish posses, following endocrine glands, (Fig. 19.1)

1. Pituitary Gland or Hypophysis
2. Thyroid Gland
3. Adrenal Gland
4. Corpuscles of Stannius
5. Ultimobranchial Gland
6. Urohypophysis
7. Pancreatic islets
8. Pineal Gland
9. Chromaffin Tissue
10. Gonad
11. Intestinal Tissue

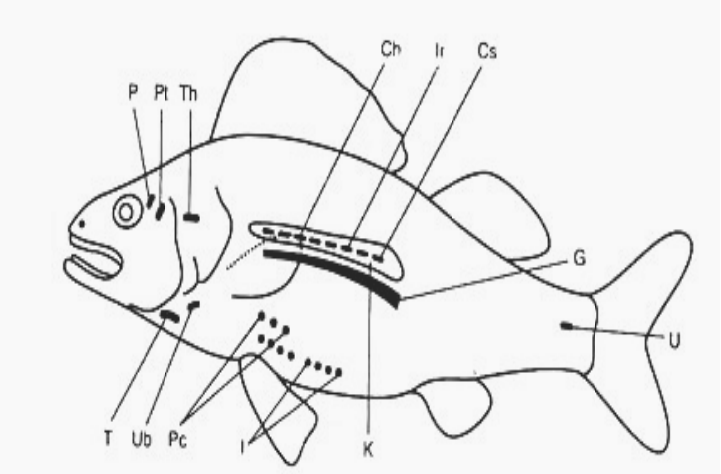

Fig. 19.1 : Schematic diagram to show position of various endocrine glands in fishes. Ch. chromaffin tissue; Cs, corpuscles of Stannius; G, gonad; I, intestinal tissue; Ir, interrenal tissue; K, kidney; P, pineal; Pc, pancreatic islets; Pt, pituitary; T, thyroid; Th, thymus; U, urohypophysis; Ub, ultimobranchial.

On the basis of organization, endocrine glands of fish, can be divided into, as follows,

A. **Discrete Endocrine Glands:** These include pituitary, thyroid and pineal glands (Fig. 19.1).
B. **Organs containing both endocrine and exocrine functions**, which include kidney,gonads and intestine. Kidney contains heterotopic thyroid follicles, interrenal, and corpuscles of Stannius.
C. **Diffused neuro-endocrines: These are scattered cells,** present in digestive tractwith endocrine function.The secretion of the cell is generally called as paracrines (e.g.,somatostatin). There aregastrointestinal peptides whose definite classification as hormone or paracrine agent has not yet been established, these are designated as putative hormones.

I. Pituitary Gland or Hypophysis

Origin: The pituitary gland occupies the same central part in the endocrine signaling system of fish that it has in mammals. This master endocrine gland originates embryologically from the two sources. One as ventral down-growth of a neural element from the diencephalon called the infundibulum to join with another, an ectodermal up-growth (extending as Rathke's pouch) from primitive buccal cavity. These two outgrowths are thus ectodermal in origin and enclose mesoderm in between them, which later on supply blood to the pituitary gland, originating from the internal carotid artery.

Location: The pituitary gland is located below the diencephalon (hypothalamus), behind the optic chiasma and anterior to saccusvasculosus, and is attached to the diencephalon by a stalk or infundibulum (Fig. 19.2). The stalked pituitary is found in *Barbus stigma* and *Xiphophorus maculatus*. The size of infundibulum varies according to the species. Usually in cyclostomes it issmaller but increases in bony fishes, with prominence in groove or depression of parasphenoid bone receiving the gland. There is no sella turcica comparable to that found inmammals in *Xiphophorus*. The short, thick- walled, hollow infundibular stalk containsa lumen, which continues with the third ventricle.

Shape and Size: The pituitary is an oval body and is compressed dorsoventrally. The size of sexually mature platy-fish has a mean anterior posterior length of 472.9 micra, with mean widthof 178 micra and mean depth of 360 micra. Male glands are smaller than those of females. On ventral aspect the gland gradually tapers caudally from rounded anterior end. The dorsal surface of the pituitary of platy-fish is concave, ventrally it is slightly convex. The pituitary gland is completely enveloped by a delicate connective tissue capsule.

Anatomy/ Histopathology: Microscopically, the pituitary gland is composed of two close associated parts:

(i) Adenohypophysis, which is a glandular part originated from the oral, ectoderm. It is further **(ii) Neurohypophysis**, which is a nervous part originated from the infundibular region of the brain.

Pituitary is broadly characterized as platybasic and leptobasic. In platybasic form (Eel), the neurohypophysis consists of flat floor of the caudal infundibulum which sends processes into disc-shaped adenohypophysis. In leptobasic, the neurohypophysis has a fairly well developed infundibulum stalk and the adenohypophysis is globular or egg shaped. There are many intermediate between the two.

i. Adenohypophysis:

It is further divided into three parts, as follows,

1. Rostral Pars Distalis or Pro-Adenohypophysis: Lying dorsal to the mesoadenohypophysis in the form of thin strip. It contains cells which secrete prolactin and corticotropin (ACTH) exclusively in addition to other hormones.

2. Proximal Pars Distalis or Meso adenohypophysis: Lying almost in between the rostral pars distalis and pars intermedia. The mesoadenohypophysis (proximal pars distalis) contains cells which produce gonadotropin (GTH) and growth hormone (GH). Thyrotropin cells may occur in either or both in rostral pars distalis and proximal pars distalis.

3. Pars Intermedia or Meta adenohypophysis: Lying at the distal tapering end of the pituitary gland (Fig. 19.2a-i). It also encompasses more neurohypophysial tissue than any other region. The metaadenohypophysis basophilic cells are PAS positive. However, the granular cells do notshow consistent staining reaction with PAS and AF stains.

The cells of pituitary store hormones in granules present in the cytoplasm. Cells of adenohypophysis classified on the basis of staining reaction to the mixture of acidic and basic dyes with secretory granules, into acidophilic and basophilic. Acidophils are rounded or oval orsometimepyrimidal shaped. They are coarsely granular, and give the cytoplasm a splotched appearance. They have round

to oval peripheral nuclei. The acidophilic cells are PAS (periodic acid Schiff) and AF (aldehyde fuschin) negative cells i.e. do not take the stains. Basophils (cyanophilic) are spherical with large, round, centrally located nuclei. Their cytoplasm is finely granular, chromophobes cells are similar in structure. The basophilic cells are AF and PAS positive i.e. take the stains.

On the basis of binding affinities with ribonucleoprotein the two classes are also classified as chromophobes and chromophils. The chromophobes have little affinity with dye while chromophils stain strongly as they have affinity with dye. Chromophilic cells which take acidic stain are called as acidophils whereas the chromophilic cells which bound basic dye are called basophilic and the cells which do not take any stain are called chromophobes. Recently on the basis of immunocytochemistry, the cells are classified according to hormones released by the pro-adenohypophysis. For example, the cells which take basophilic stain but produce adrenocorticotropic hormones, they are called ACTH cells but if secrete thyroid stimulating hormone, these cells are called thyrotrophs and if they secrete FSH hormones they are called gonadotrops although they are basophilic in nature.

The teleost hypo-thalamo-pituitary system is unique amongst vertebrates, as there is direct innervation of pars distalis by neurosecretory neurons of hypothalamus and there is loss of modification of the typical vertebrate hypothalamohypophysial portal vascular system for transport of neurohormones to pars distalis.

II. Neurohypophysis:

The neurohypophysis occupies considerable portion of the gland and possesses many interesting and distinctive features. The neurohypophysis comprises connective tissue, neuroglia cells and loosely tangled network of nerve fibres. These nerve fibres are scattered horizontally along the dorsal part of the adenohypophysis and run vertically, which are generously inter-spread with granular material, large irregularly shaped amorphous masses and large nuclei. They are located in the mid-dorsal region. The amorphous masses are called “Herring bodies”, which have an intimate relation with the di-encephalic neuro-secretory cells called nucleus preopticus by means of a fibre tract known as the preoptic neurohypophysial tract. The preoptic nucleus (nucleus preopticus, NPO) and NLT of diencephalon have their axons and nerve endings in the pituitary (Fig. 19.3). NPO are neurosecretory in nature.

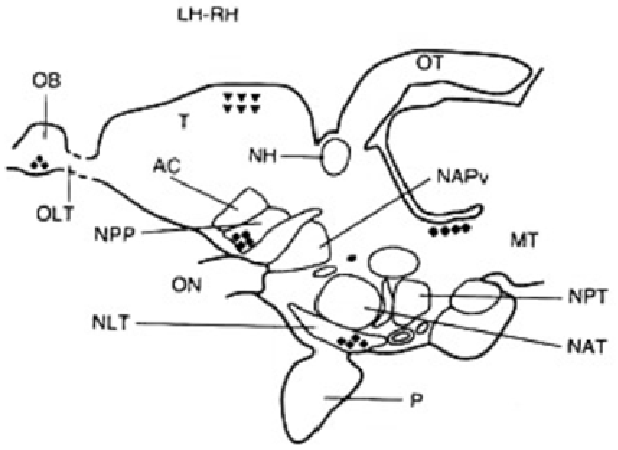

Fig. 19.3 : Diagram to show NPO and NLT in the brain of platyfish. AC, anterior commissure; MT, mid-brain tegmentum; NAT, nucleus anterioris tuberis; NAPv, nucleus anterioris periventricularis; NH, nucleus habenularis; NLT, nucleus lateral tuberis; NPO, nucleus preopticus; NPP, nucleus prepoticus periven-tricularis; NPT, nucleus posterioris tuberis; OB, olfactory bulb OLT, olfactory tract; ON, optic nerve; OT, optic tectum; P, pituitary; T, telencephalon.

Hormones and Functions of Pituitary Hormones:

There are various hormones secreted by pituitary but it is generally agreed that one cell type-one hormone concept, is correct. The different hormones secreting cell are not localized in specific region but are spread over in part of the adenohypophysis (Fig. 19.5). All hormones secreted by the pituitary are necessarily proteins or polypeptides. There exists a slight difference in the pituitary hormones of the different groups of fishes. The pituitary hormones of fishes are of two types. One which regulates the function of other endocrine glands, are called tropins or tropic hormones. These areTSH, ACTHetc.The other type directly regulates the specific enzymatic reactions in the various body cells or tissues. These hormones are melanin hormones (MH) and melanophore stimulating hormone (MSH) etc.

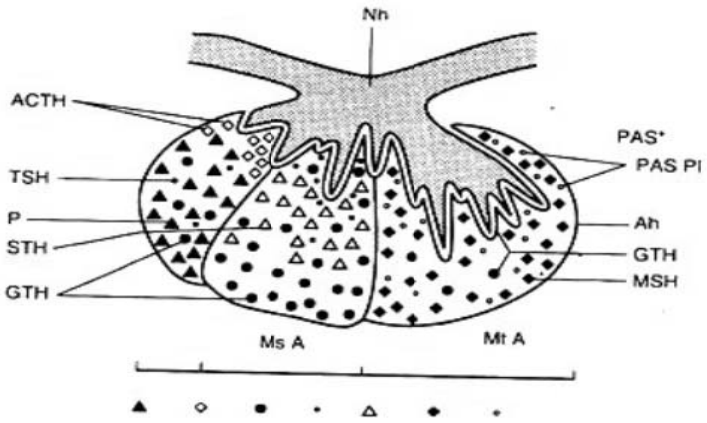

Fig. 19.5 : Section of the pituitary to show various hormone secreting cells in the adenohypophysis (Ah). ACTH, adeno corticotropic cell; GTH, gonado tropic cell; MsA, mesoadenohypophysis MtA, metaadenohypophysis; MSH melanotropic cell; Nh, neurohypophysis; PAS, periodic acid Schiff-positive cell in pars intermediate; P prolactin producing cell; STH, somatotropic cell; TSH; thyrotropic cell.

1.Thyrotropin hormone or Thyroid Stimulating Hormone (TSH) which is secreted from proadenohypophysis (rostral pars distalis) and stimulates activity of thyroid hormones. The TSH is secreted under the influence of thyroid- releasing hormones (TRH) from diencephalon in fishes.

2. Adrenocorticotropic Hormone (ACTH): It is secreted by ACTH cells located between the rostral pars distalis and the neurohypophysis. Secretion of ACTH from pituitary is stimulated by the hypothalamus through corticotrophin releasing factor (CRF) (Fig. 19.7). It activates adrenal cortex and regulates secretion of corticotrophins.

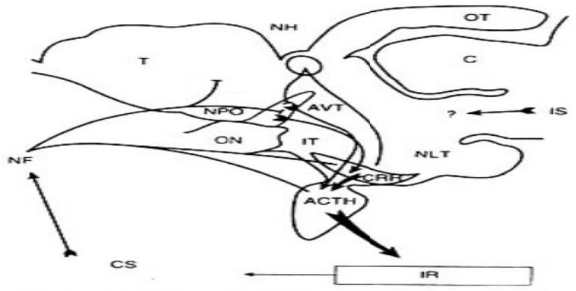

Fig. 19.7 : Diagrammatic illustration of neuroendocrine regulation of (ACTH) by a releasing hormone (RH) and (NPO)→ arrow indicates stimulatory influences; Question (?) indicates unknown pathways. AVT, arginine vasotocin; IT, isotonin; IR, inter renal; IS, input of stress; C, cerebellum; CS, cortico steroid; NF, negative feed back; NH, nucleus habenularis, NLT, nucleus lateralis tuberis; NPO, nucleus preopticus; ON, optic nerve; T, telencephalon.

3. Gonadotropin (FSH and LH): Gonadotropin (GTH) cells are richly found in the proximal pars distalis (PPD). These hormones regulates secretion of gonadal hormone and spermatogenesis and oogenesis (Fig 19.6).

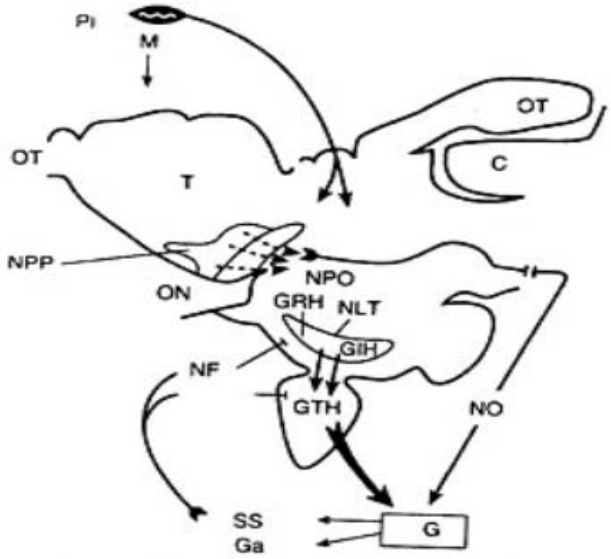

Fig. 19.6 : Diagrammatic representation of the neuroendocrine regulation of gonadotropin (GTH) secretion by releasing hormone (GRH) and one inhibitory hormone (GIH) in gold fish brain. C, cerebellum; G; gonad; Ga, gametes; M, melatonin; NF, negative feedback; NLT, nucleus lateralis tuberis; NPP, nucleus preopticus periventricularis, NPO, nucleus preopticus; NO, neural output; ON, optic nerve; OT, olfactory tract; PI, photoperiod input; SS, sex steroid; T, telencephalon. (Source; Peer and Fryer, 1983).

4.Growth hormones or Somatotropin: Mesoadenohypophysis secretes a growth hormone which accelerates increase in the body length and basal metabolic rate of fishes.

5. Prolactin: It is released from proadenohypophysis. In some fishes like mummichog (Fundulusheteroclitus), prolactin along with the intermedin enhances the laying down of melanin in the melanophores of the skin. Among the several hormones the prolactin is also involved in electrolytic regulation in teleosts but its importance in maintaining homeostasis varies according to species. The secretion of prolactin from the teleost pituitary is under an inhibitory neuroendocrine control of hypothalamic origin.

6. Melanocyte Stimulating Hormone (MSH) (Intermedin): MSH is secreted from the meta-adenohypophysis and acts antagonistically to melanin hormone (MAH). MSH stimulates melanin dispersion in the melanocytes and darkening of skin. MSH expands the pigment in the chromatophores, thus takes part in adjustment of background. It also stimulates the melanin synthesis.

7. Oxytocin and Vasopressin Hormones: In fishes the neurohypophysis secretes two hormones, i.e., oxytocin and vasopressin, which are stored in hypothalmic neurosecretory cells. These endocrine substances have effect osmoregulation, salt-water balance, mating and egg laying of fish.

Use of pituitary hormones in induced breeding:

The pituitary hormones have practical applications by injecting and implanting to force or stimulate spawning of certain fishes of great economic value, such as trouts (Salmoninae), catfishes (Ictaluridae), Mullets (Mugliliade), and sturgeons (Acipenseridae). The synthesis of sex hormones in the gonad is controlled by pituitary gonadotropin. Hence pituitary extract containing GTH are taken from sexually mature male or female fish than injected to the same species for inducing and hastening and spawning. For the preparation of pituitary extract closely related species may also be used as donor.

II. Thyroid Gland

Location: In many teleosts the thyroid gland is situated in the pharyngeal region in between the dorsal basibranchial cartilages and ventral sternohyoid muscle. The thyroid surrounds anterior and middle parts of first, second and sometimes third afferent branchial arteries of ventral aorta, as found in *Ophiocephalus* species (Fig. 19.8). In *Heteropneustes* it occupies almost the entire length of the ventral aorta and afferent arteries. In *Clariasbatrachus* the thyroid gland is concentrated around the ventral aorta, middle ends of two pairs of afferent arteries and the paired inferior jugular veins.

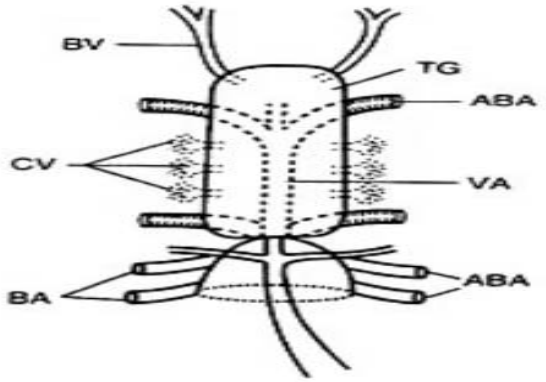

Fig. 19.8 : Ventral view of thyroid gland and their blood vessels in *Heteropneustes*. ABA, afferent branchial artery; BA, branchial vessel; BV, buccal vein; CV, commissural vessel; TG, thyroid gland; VA, ventral aorta.

Origin: The thyroid gland in fishes arises from the floor of the pharynx as a median evagination.

Shape and Size: In majority of teleosts the thyroid is un-encapsulated and thin follicles are dispersed or arranged in clusters around the base of afferent branchial arteries. It is thin-walled, sac-like, compact dark brownish and enclosed in a thin-walled capsule of connective tissue in these fishes might be correlated with the air breathing habit because thyroid gland acts here as thermoregulatory to adapt the fish to a semiterrestrial environment of low thermal capacity. In *Heteropneustes* the thyroid gland is an unpaired thin- walled brownish but cylindrical in shape. In *Clariasbatrachus* the thyroid gland is not covered by definite wall, i.e., un-capsulated and is elongated in shape.

Histology: Inteleosts, histologically the thyroid gland consists of a large number of follicles, lymph sinuses, venules and connective tissues. The follicles are round, oval and irregular in shape. Each follicle contains a central cavity surrounded by a wall composed of single layer of epithelial cells. The

central lumen of follicle is filled with colloid containing chromophilic and chromo phobic vacuoles. The follicles are supported in position by connective tissue fibres which surround them.The structure of epithelium varies according to its secretory activity. Less active follicles generally have thin epithelium. Epithelial cells are of two types:

(i) Chief cells which are columnar or cuboidal in shape, having oval nuclei and clear cytoplasm.(ii) Colloid cells or Benstay's cells possess droplets of secretory material.

The thyroid gland is highly vascularized and is generally well supplied with blood. The thyroid glands in sharks and higher teleosts are diffused in nature.

Hormones and Functions of Thyroid Hormones: Thyroid gland synthesizes thyroid hormones by combining iodine, extracted from the blood, with tyrosine. The thyroid hormones of fishes appear to be identical with those of mammals, including, monoand di-iodo-tyrosine and thyroxin. These hormones are kept stored in the thyroid follicles and are released into blood stream on metabolic demands. The release of the thyroid hormone from the follicle is controlled by the thyrotropic hormone (TSH) of pituitary. These hormones are reported to associated with various kinds of functions, such as, osmoregulation (salmons and sticklebacks), growth and nitrogen metabolism (goldfish), smolting (salmon), spawning migration (salmon), scale and bone formation and oxygen consumption in fishes.

III. Adrenal Gland or Inter-renal cells

Adrenal gland of fish is quite different from mammals. It has 3 layers of cells, cortex, inter-renal and medulla.

Location: The inter-renal cells are and situated along the post-cardinal veins as they enter the head kidney in teleosts (Fig. 19.9) while present throughout the body cavity close to the post-cardinal vein in Lamprey (Cyclostomata). In sharks (Squaliformes) they are present between the kidneys. Among the rays they lie in more or less close association with posterior kidney tissue, including some species possessing inter-renal tissue concentrated near the left and in other near the right central border of that organ.

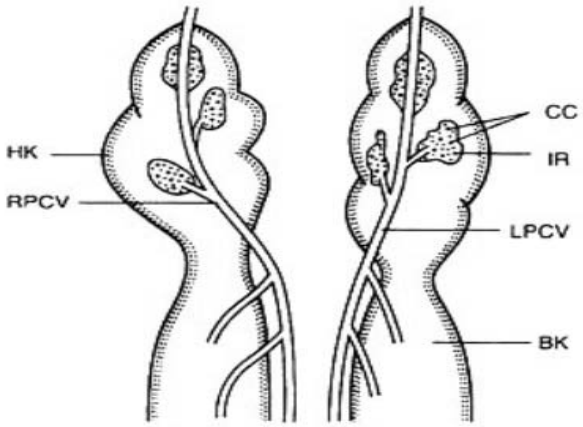

Fig. 19.9 : Digram to show location of interrenal glands in fishes. BK, body kidney, CC, chromaffin cells; HK, head kidney; IR, interrenal tissue; LPCV, left post cardinal view; RPCV, right post cardinal vein. (Source : Habia, T., 1982).

Origin: Adrenal glands originate from mesodermal layer of embryo.

Histology: The inter-renal cells are multilayered, in teleosts. In some fishes like *Puntius ticto* inter-renal cells are arranged in form of thick glandular mass while in others like *Channa punctatus* they are present in form of lobules. Each inter-renal cells, is eosinophilic and columnar with a round nucleus.

Hormones and Functions of Adrenal gland hormones: Adrenal cortical tissue or inter-renal tissue secretes two hormones, -(i) mineral corticoids, concerned with fish osmoregulation, and

(ii) glucocorticoids, which regulate carbohydrate metabolism, particularly blood sugar level.

Adrenocortical hormones secretion is under control of the adrenocorticotropic hormone (ACTH) of hypophysis. They also promote steroid fat utilization, protein catabolism, sodium retention , water and electrolyte metabolism.

IV. Corpuscles of Stannius: The corpuscles of Stannius (CS) were first described by Stannius in 1939 as discrete gland like bodies in the kidney of sturgeon.

Location: It is found attached or lodged in the kidneys of fishes particularly holostean and teleost (Fig. 19.11). In salmonids the CS is located near the middle part of mesonephros but in majority of fishes they are situated at the posterior end of kidney. In *Notopterusnotopterus* and *Heteropneustessetani*, CS found at the anterior end of kidney.

Origin: It originates as outgrowths from the pronephric or mesonephric duct of the kidney.

Shape and Size: They are asymmetrically distributed and often resembles with cysts of parasites but lie different from the latter by higher vascular supply and dull white or pink colour. The shape of the corpuscles may be flat, oval as in goldfish, trout, and salmon. There may be one CS as in *Heteropneustessetani* and *Notopterusnotopterus* (Fig. 19.11a, b), while as many as ten CS are present in some species like *Clariasbatrachus*. According to Garrett (1942), there is a gradual reduction in number of CS that has occurred during evolution of Holostei and Teleostei. The variation in number and position of CS in teleost species seems to be an embryological speciality.

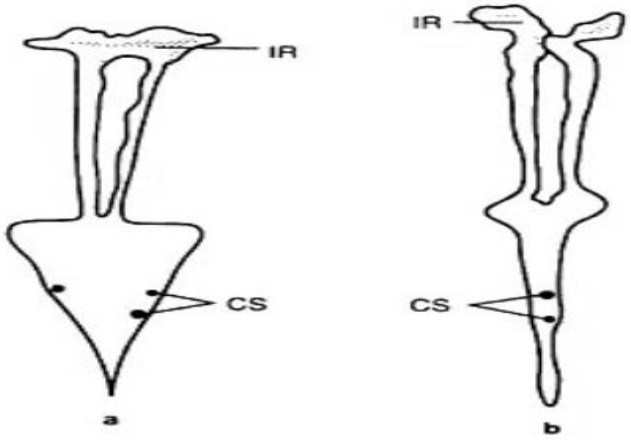

Fig. 19.11a, b : Diagram of kidney of fishes showing corpuscles of Stannius. (a) *Cirrhina mrigala*. (b) *Labeo rohita*. CS, corpuscles of Stannius; IR, interrenal corpuscles.

Histology: It is made up of columnar cells which are covered by a fibrous capsule. The columnar cells are of two types (i) AF positive and (ii) AF negative. They are filled with secretory granules. The parenchyma of CS comprises vasculo-ganglionic units consisting of a bunch of ganglion cells, blood vessels and nerve fibres. Histologically, CS are similar to the adrenal cortical cells. There is only one-cell type present in the CS of pink salmon while pacific salmon has two-cell types.

Hormones and Functions ofCorpuscles of Stannius: It secretes hypocalcin and work synergistically with pituitary gland, to exert hypercalcemic effect, in order to balance relatively constant level of serum calcium.

V. Ultimobranchial Gland: It is a small paired sac-like gland and under the control of pituitary gland.

Location: Typically, it is located between the ventral wall of oesophagus and sinus venosus or near the thyroid gland.

Origin: Embryonically the gland develops from pharyngeal epithelium near the fifth gill arch.

Histology: Histologically, it consists of parenchyma, which is solid and composed of cell cords and clumps of polygonal cells covered by capillary network. In Heteropneustes, the gland measures 0.4 x 1.5 mm in diameter in average adult of 130 to 150 mm body length.

Hormones and Functions of Ultimobranchial Gland: The gland secretes calcitonin hormone which regulates calcium metabolism. Calcitonin is said to be also related with the osmoregulation. In Japanese eels, calcitonin decreases osmolarity, sodium and chloride ions in blood. It is also known as suprapericardial bodies or post-branchial bodies.

VI. Urohypophysis

Location: It is present in the terminal part of the spinal cord (Fig. 19.13a, b, c).

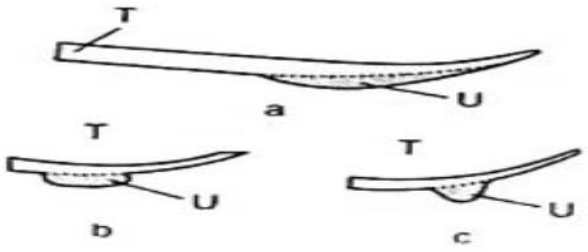

Fig. 19.13a-c : Diagram of urohypophysis of teleosts. (a) rainbow trout. (b) carp. (c) Yellow tail. T, tail; U, urohypophysis (Source : Hibiye, T., 1982)

Shape and Size: It is generally small in size. In Ayu*(P. altivelis*) the urohypophysis is extended like a bow. In carp and yellow tail it is a conspicuous oval body.

Histology: The urohypophysis is made up of spinal cord elements like neurosecretory axon, glia, and ependymal and glia fibers and meningeal derivative such as vascular reticulum and reticular fibres. Urohypophysis together with the neurosecretory cells situated in the spinal cord are called caudal neurosecretory system. The neurosecretory system is found only in elasmobranchs and teleosts. The neurosecretory cells are large nerve cells and have basophilic cytoplasm and a polymorphic nucleus.

They are diffused in the terminal part of the spinal cord. Axon terminals of these cells assemble at the ventral side of the region and form urophysis with blood capillaries.

Hormones and Functions ofUrohypophysis: The product of neurosecretory cells is deposited in urohypophysis which then releases it in circulation. Urophysis extract shows ability to contract smooth muscles of ovary and oviduct of guppy *(Poecilia reticulata*) and the sperm duct of goby (*Gillichthys mirabilis*), suggesting the possibility of involvement in reproduction and spawning. Secretion of urohypophysis may also be known as urotensins, involved in metabolic regulation. The neurosecretory system is thought to be related with osmoregulation. It corresponds to the hypo-thalamo neurosecretory system present in vertebrates.

VII. Pancreatic islets:

Location: It is found in gut walls of larval Lampreys and in hepatopancreas of most bony fishes.

Origin: It originates from mesodermal.

Histology: In some fishes like *Labeo*, *Cirrhina*, and *Channa* small islets are found near gall bladder, spleen, pyloric caeca or intestine, separated from pancreas. Such islets are often referred to as principal islets. But in some species like *Clariasbatrachus* and *Heteropneustes fossils*, large and small islets are found to be embedded in the pancreatic tissues, similar to the higher vertebrates.

In fish the islets are big and prominent and consist of three kinds of cells (Fig. 19.12a, b):

(i) The beta cells which take aldehyde fuschin stain.

(ii) Another type of cells is alpha cells, which do not take aldehyde fuschin stain. It is two types, A_1 and A_2 cells.

(iii) The function of the third type of cells is not known.

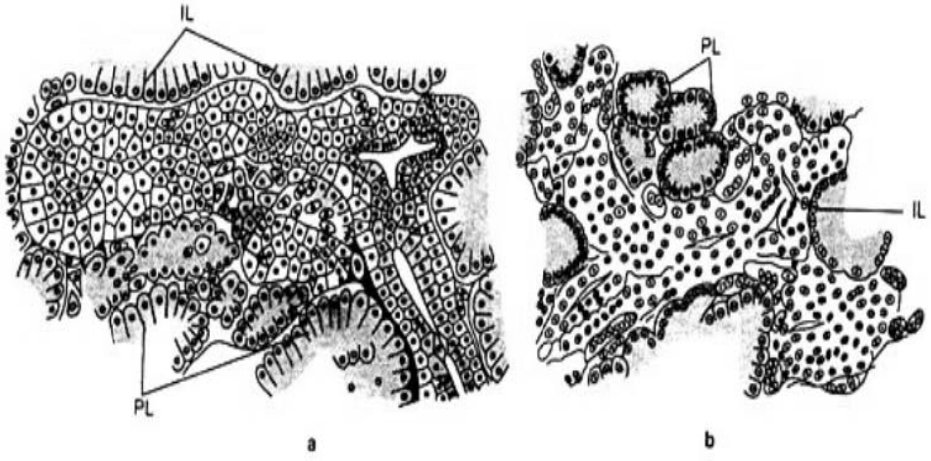

Fig. 19.12a, b : Diagram of pancreas showing endocrine components. (a) *Torpedo marmorata*. (b) *Mustelus laevis*. IL, islets of langerhans; PL, pancreatic lobule.

Hormones and Functions ofPancreatic islets: Beta cellssecrete insulin, which is involved in carbohydrate metabolism in fish and alpha cells produce glucagon.

VIII. Pineal Gland

Location: It is situated near the pituitary.

Origin: It arises as a posteromid dorsal evagination of the epithalamus.

Hormones and Functions of Pineal gland: In-spite of being a photoreceptor organ the pineal organ shows endocrine nature of doubtful function. It secretes melatonin.

Removal of pineal from Lebistes (Guppy) species causes reduced growth rate, anomalies in the skeleton, pituitary, thyroid and corpuscles of Stannius. It has been reported that thyroid and pituitary glands influence the secretion of pineal.

IX. Chromaffin Tissue or Suprarenal Bodies or Medullary Tissue:

Location: In lamprey (Cyclostomata) the chromaffin cells are present in the form of strands along the dorsal aorta as in the ventricle and the portal vein heart. In sharks and rays (Elasmobranchii) these tissues are found associated with the sympathetic chain of nerve ganglia while in bony fishes (Actinopterygii) the chromaffin cells have wide variation in their distribution. In sculpins (*Cottus*) chromaffin and adrenal cortical tissue are joined into one organ, similar to the mammalian adrenal gland (Figs. 19.10a, b, c, d).

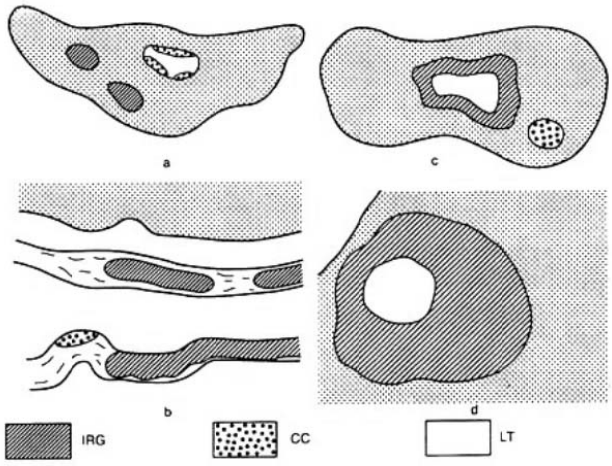

Fig. 19.10a-d : Diagram showing chromaffin tissue in fishes (a) Rainbow trout. (b) Eel. (c) Carp. (d) Yellow tail. IRG, interrenal gland; CC, chromaffin cell; LT, lymphoid tissue. (Source : Hibiya, T., 1982)

Hormone and Functions: Chromaffin tissue of fish is rich in adrenaline and noradrenaline. Injection of adrenaline and noradrenaline causes changes in blood pressure, bradycardia, branchial vasodilation, diuresis in glomerular teleosts and hyperventilation.

X. Intestinal Mucosa:

Hormones and Functions: The intestinal mucosa produces secretin and pancreozymin, which are controlled bynervous system and regulate pancreatic secretion. Secretin affects flow of enzymecarrying liquids from the pancreas, whereas pancreozymin accelerates flow ofzymogens.These hormones are usually synthesized in anterior part of the small intestine.

XI.Gonads

The sex hormones are synthesized and secreted by specialized cells of the ovaries andtestes. The release of sex hormones is under the control of mesoadenohypophysis ofpituitary.

Hormones and Functions: Male hormonetestosterone, found in elasmobranch (*Raja*) and in salmon the blood plasma. Another gonadal steroid, i.e., 11-ketotestosteron, also reported in *Oryziaslatipes* (medaka) and sockey salmon. Ovary secretes oestrogens of which estradiol-17B, estrone and estriol have been identified in many species. In some fishes progesteron is also found butwithout hormonal function. In fishes these sex hormones are necessary for maturation of gametes and secondary sex characteristics such as breeding tubercles, colouration and thematuration of gonopodia.

There is little information about the influence of gonadial hormones on thereproductive behaviour of fish. Male sex hormones are more similar to those of vertebrates than the ovarian hormones.

Probable Questions:

1. Name four major hormones secreted from Pituitary gland of fish. State their functions.

2. What is the main function of thyroid hormones in fishes?

3. Which hormone is secreted from Pineal gland. State its functions.

4. name the hormones secreted from Gonads of fishes. What is their role?

5. write short notes on Chromaffin Tissue or Suprarenal Bodies or Medullary Tissue in fish.

6. Write the names of secreted hormones from Urohypophysis in fishes and what is their functions?

Suggested Readings:

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UNIT-IX

Electroreception in Fish

Objective: In this unit we will discuss about electrolocation in fishes.

Introduction:

Electroreception or electroception is the biological ability to perceive natural electrical stimuli. It has been observed almost exclusively in aquatic or amphibious animals, because water is a much better conductor than air. The known exceptions are the monotremes (echidnas and platypuses), cockroaches and bees. Electroreception is used in electrolocation (detecting objects) and for electrocommunication. Electroreception is the biological ability to perceive natural electrical stimuli. It is observed almost exclusively in aquatic animals, particularly in fish. It is well developed in marine fishes, since salt-water is a much better conductor. Besides fish, it is also used by cockroaches, bees and monotremes (echidnas and platypuses).

Occurrence in fish:

Electroreception is found in lampreys,cartilaginous fishes (sharks, rays, chimaeras), lungfishes, bichirs,coelacanths, sturgeons, paddlefishes, catfishes, gymnotiformes,and elephantfishes. Theelectroreceptor organs in all these groups are derived embryologically froma mechanoreceptor system. In fishes they are developed from the laterallines.

Electroreceptive fishes can be divided into, as follows,

- Non-electrogenic fish: Fish those are unable to produce electric fields outside the body are called non-electrogenic fish. Most sharks, rays, eels, catfish, and lungfish are non-electrogenic fish.

- Electrogenic fish: Fish those are able to produce electric fields outside the body are called electrogenic fish. They possess a specialised electric organmade up of either disklike modified muscle cells or nerve cells called electrocytes. It is found inTorpedo rays, electric skates (Raja), electric catfish (Malapterurus), stargazers (Astroscopidae), South American knifefishes (Gymnotiformes), and African mormyrid form. Strong electric discharges form electric organs

used by fish to stun prey or repel predators while weak discharges used in electrolocation and electrocommunication.

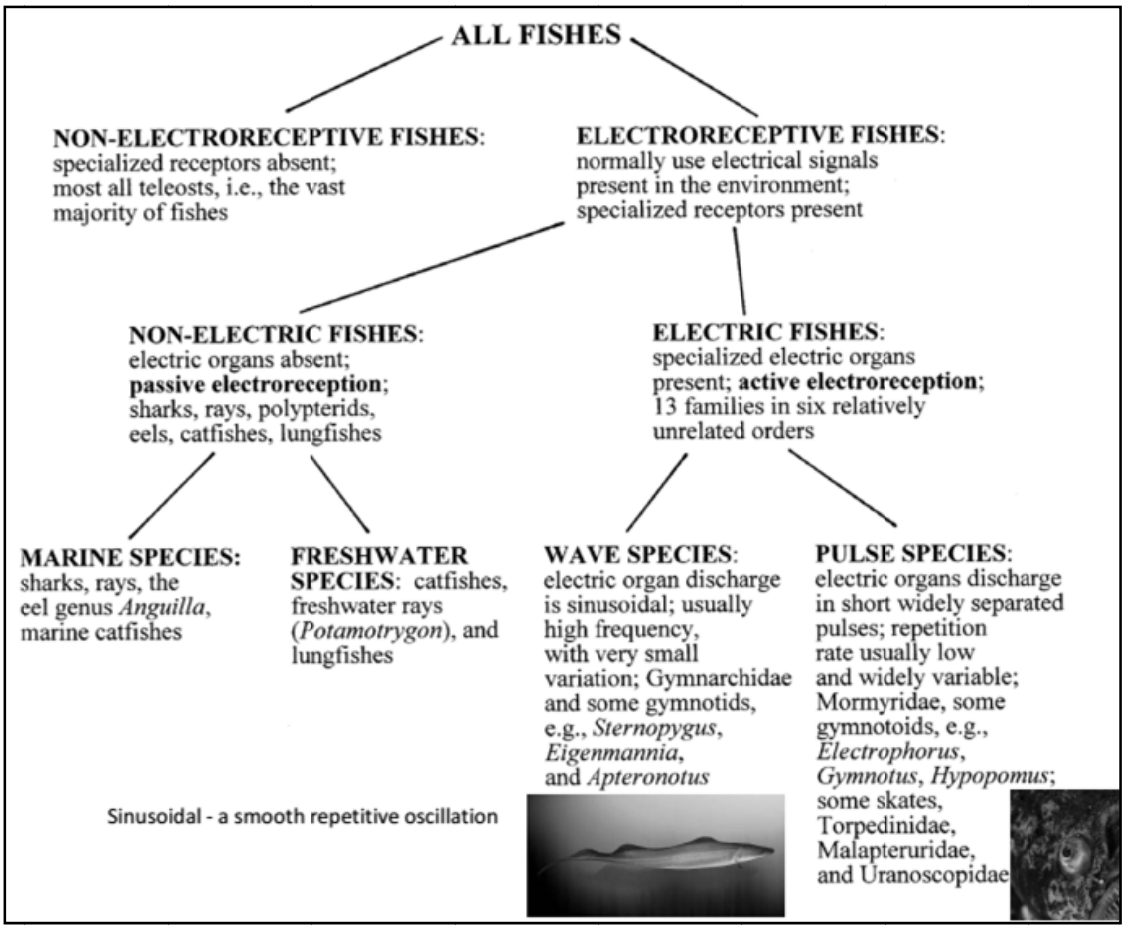

Fig 1: Distribution of electroreception among living fishes.

Function:

Electroreception is used in electrolocation (detecting objects) and for electrocommunication.

A. Electrolocation

Electroreceptive fish use this sense to locate objects around them (Fig: 2A). It is important for feeding and navigation in turbid or murky water and in dark environments, where the animal cannot depend on vision. Many fish use electric fields to detect buried prey.

Types of Electrolocation:

i. Active electrolocation

In active electrolocation, the animal senses its surrounding environment by generating electric fields and detecting distortions in these fields using electroreceptor organs. This electric field is

generated by means of a specialized electric organ consisting of modified muscle or nerves. This field may be modulated so that its frequency and wave form are unique to the species and sometimes, the individual (see Jamming avoidance response). Animals that use active electroreception include the weakly electric fish, which either generate small electrical pulses (termed "pulse-type") or produce a quasi-sinusoidal discharge from the electric organ (termed "wave-type").These fish create a potential which is usually smaller than one volt. Weakly electric fish can discriminate between objects with different resistance and capacitance values, which may help in identifying the object. Active electroreception typically has a range of about one body length, though objects with an electrical impedance similar to that of the surrounding water are nearly undetectable.

ii. Passive electrolocation

In passive electrolocation, the animal senses the weak bioelectric fields generated by other animals and uses it to locate them. These electric fields are generated by all animals due to the activity of their nerves and muscles. A second source of electric fields in fish is the ion pumps associated with osmoregulation at the gill membrane. This field is modulated by the opening and closing of the mouth and gill slits. Many fish that prey on electrogenic fish use the discharges of their prey to detect them. This has driven the prey to evolve more complex or higher frequency signals that are harder to detect. Passive electroreception is carried out solely by ampullary electroreceptors in fish. It is tuned to low frequency signals (below one up to tens of Hertz).

Fish use passive electroreception to supplement or replace their other senses when detecting prey and predators. In sharks, sensing an electric dipole alone is sufficient to cause them to try to eat it

Example:

1. Sharks and rays (Elasmobranches): Sharks heavily rely on electrolocation for hunting. Some shark embryos and pups "freeze" when they detect the characteristic electric signal of their predators. The electric field sensors of sharks and rays are called the ampullae of Lorenzini (Fig 2B). They consist of electroreceptor cells connected to the seawater by pores on their snouts and other zones of the head. Sharks may also use Earth's magnetic field to navigate the oceans using this sense.

2. Bony fish: Gymnotiformes, specifically, the electric eel (actually a knifefish, not an eel), are able to generate high voltage electric shocks as well as lower voltage pulses for navigation and prey detection in its turbid habitat. The electro-sensory organ found in polypteroids, eels, lungfish and catfish is known as tuberous organ

B. Electrocommunication

It is often species specific. Weakly electric fish communicates by modulating the electrical waveform they generate. It is used in mate attraction and territorial displays. Ampullary receptors allow fish to discriminate between own electric discharge as well as those of other fishes, and thus helps in communication with conspecifics (Fig: 2B). Mormyrid electric fish from Africa use tuberous receptors known as Knollenorgans to sense electriccommunication signals.

Example:

1. Agonistic displays: Some species of catfish use their electric discharges only in agonistic displays.

2.Mimicry: A South American riverine fish, belonging to genus *Brachyhypopomu,* mimics the pattern of low voltage electrolocative discharge of the electric eel, may be for protection purpose.

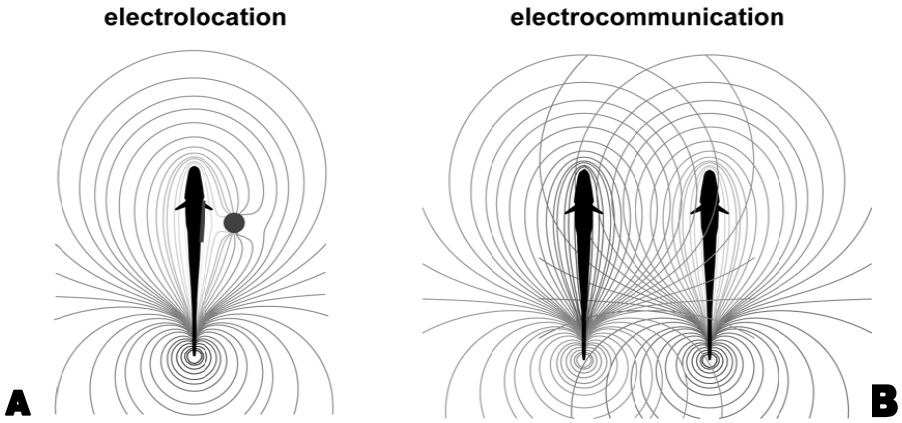

Fig 2. Electrolocation (A) and Electrocommunication (B) in Fish.

Types of Electroreception:

Electroreception can be two types – active and passive.

A. Active electroreception: It involves detection of electric fields generated by the fish itself, either by its electric organ or its motion through the earth's magnetic field. Animal use this for orientation.

It has been proposed that sharks can use their acute electric sense to detect the earth's magnetic field by detecting the weak electric currents induced by their swimming or by the flow of ocean currents.Two groups of teleost fishes, the Neotropical knifefishes(Gymnotiformes) and the African elephantfishes (Notopteroidei), are weaklyelectric and engage in *active*electroreception.

Mechanism: In active electrolocation, the animal senses its surrounding environment by generating electric fields and detecting distortions in these fields using electroreceptor organs. This electric field is generated by means of a specialised electric organ possess by some electrogenic fishes (Fig:3). This field may be modulated so that its frequency and wave form are unique to the species and sometimes, the individual. Fish can either generate small electrical pulses (termed "pulsetype") or produce a quasi-sinusoidal discharge (termed "wave-type") from the electric organ. These fish create a potential which is usually smaller than one volt. Weakly electric fish can discriminate between objects with different resistance and capacitance values, which may help in identifying the object.

Active electroreception typically has a range of about one body length, though objects with an electrical impedance similar to that of the surrounding water are nearly undetectable.

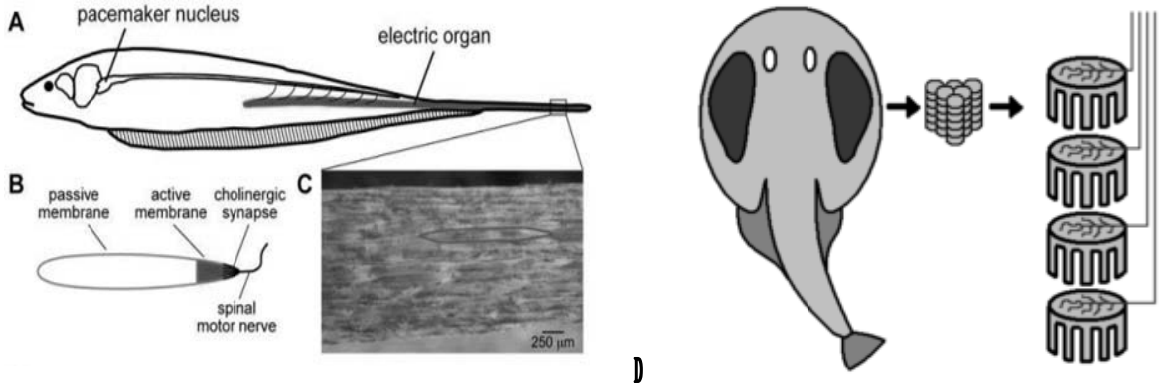

Fig. 3: A. An electric fish showing location of electric organ ; B. An electrocyte; C. A section of electric organ from the tail, with skin removed to expose the electrocytes, which are densely packed within the electric organ. A single electrocyte is outlined in red; D. An electric ray (torpedo) showing location of electric organ and electrocytes stacked within it.

B. Passive electroreception: involves detection of electric currents that originate outside the fish body. In most groups electroreception is passive, where it is usedpredominantly in predation. It is found in elasmobranches and some catfishes.

Example:

1. Predation: Sharks, particularly hammerheads, can detect a flounder that are buried under 15cm of sand.

2.Communication: Weakly electric marine skates discharge low frequency currents that are picked up by the ampullary receptors of individuals of same species.

Mechanism: In passive electrolocation, the animal senses the weak bioelectric fields generated by other animals and uses it to locate them. These electric fields are generated by all animals due to the activity of their nerves and muscles. A second source of electric fields in fish is the ion pumps associated with osmoregulation at the gill membrane. This field is modulated by the opening and closing of the mouth and gill slits. Many fish that prey on electrogenic fish use the discharges of their prey to detect them. This has driven the prey to evolve more complex or higher frequency signals that are harder to detect. Passive electroreception is carried out solely by ampullary electroreceptors (Fig. 4 B) in fish. It is tuned to low frequency signals **(**below 50 Hz)**.** These receptors have a jelly-filled canal leading from the sensory receptors to theskin surface.Fish use passive electroreception to supplement or replace their other senses when detecting prey and predators. In sharks, sensing an electric dipole alone is sufficient to cause them to try to eat it.

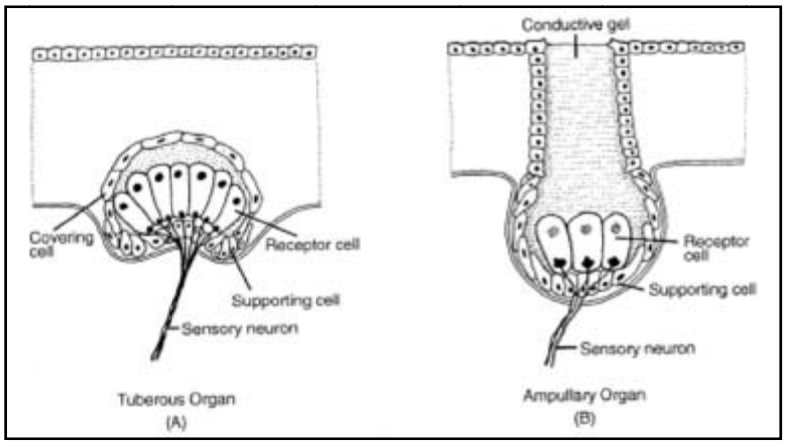

Fig 4: Electroreceptive organs in fish

Probable Questions:

1. Define electroreception in fish.

2. Briefly describe active electroreception with proper examples.

3. Briefly describe passive electroreception with proper examples.

4. What is electrolocation. Give examples.

5. What is electrocommunication. Give examples.

6. Classify fishes n the basis of their electroreceptive property.

7. Describe active and passive electrolocation with examples.

Suggested Readings:

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7.

UNIT-X

Age Determination in Fish

Objective: In this section you will learn about age determination in Fishes.

Introduction:

Determination of age and growth of fishes are one of the important aspects in the development of fisheries. Both age and growth are closely related with one other. As the fish ages, it grows, but after attaining a particular size, growth stops. Age gives an idea about sexual maturity, spawning time, catchable size, growth rate and longevity. Knowledge of all these parameters is essential in fisheries production.

Growth of fish or any organism is the change in length and weight with increase of age as a result of metabolism of nutrition. Hence growth is an index of healthy food and oxygen supply in the water-body. Proper growth of fish also indicates that the water body is devoid of any pollution.

Knowledge of age and growth of fish has many applications:
1. We can calculate the time of sexual maturity of different species.

2. Further, we can know their spawning time.

3. Growth rate of fish also indicates the suitability of particular species for a particular type of the water-body.

4. Growth rate and age of fish also indicate the size of fish at different stages, e.g., fry, fingerling, and adult of different species.

5. The study of age and growth is helpful in catching fishes by using nets of desirable mesh size.

Methods of Age and Growth Determination:

Various methods employed for determination of age of fish are:

A. Scale Method:
This method is most commonly used for determination of age of Osteichthyes (bony fish), which are provided with cycloid and ctenoid scales. The structure of scale and its development is useful in the interpretation of growth zones. The structure of scale can be seen very easily under the microscope after washing with dilute solution of caustic soda followed by staining with borax carmine.

A well-developed scale has the following structures:

1. Focus:
It is a clear area in the center, but may be shifted from the center due to irregular growth of anterior or posterior parts of scale caused by unusual overlapping of scales.

2. Circuli:
These are concentric rings present around the focus, they run parallel at regular intervals or distances. They appear as ridges.

3. Grooves:
The grooves are found between the ridges of circuli and they are responsible for maintaining the regular space between them.

4. Radii:
These are grooves found radially, viz., they run from focus to margin of scale. Radii cut the circuli present in their path.

5. Annuli:
These are wide circular troughs found in aged fish over one year. Each trough contains a few incomplete and narrow circuli different from the circuli outside it, which are complete and more widely spaced. The number of annuli represents age of a fish in years (Fig. 14.1).

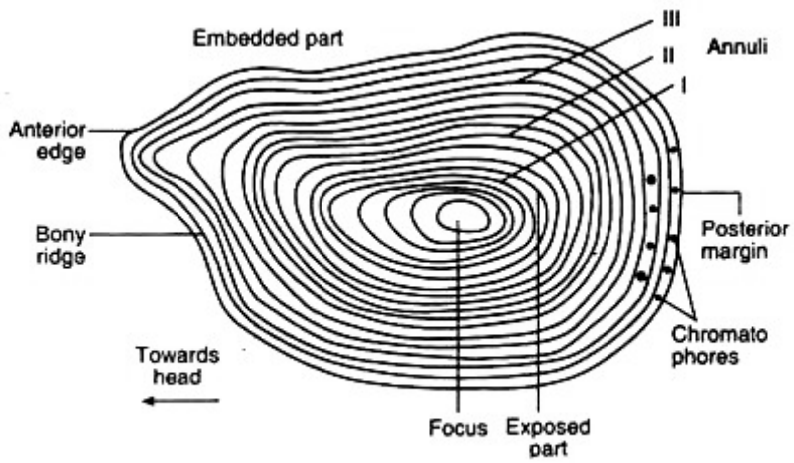


Fig. 14.1 : Scale method fish scale showing growth rings.

At the time of development of scale, focus is established first and represents the original size of the scale. As scale grows older, other structures are added and perform their functions. The grooves and circuli represent growth activity. They also indicate osteoblastic activity as a result of which secreted material is deposited around the focus. In this way every year many such circuli and grooves are formed.

A characteristic bone material, ichthylepidin, is deposited in circuli and thus their height increases which depends on the calcification. Annuli show slow growth in a year but in many fishes, during winters, annuli grow remarkably and are added yearly as fish grows.

Thus annuli are very useful in counting the age of fish and serves as year-marks on the scale for age determination. The annuli are best seen at anterior part of the scale.

Types of Annuli:
1. True Annuli:
The true annuli has following characteristic features:
(i) In cycloid scales true annuli is represented by a closely situated circuli, which is covered by widely spaced circuli.

(ii) Two complete circuli surround the trough, which is wider on the anterolateral and posterior side.

(iii) The wide part of the trough contains incomplete circuli that do not grow completely around the scale.

(iv) The trough remains narrow at the anterolateral side. In the ctenoid scales specially, the outer circulus cuts across or crosses over the incomplete circuli lying in the anterior part of the trough.

Annuli are considered as year-marks in the age determination of fish in the case of the following facts.

a. When there is a correlation between the calculated age from the scale and the size of the fish.

b. The length frequency distribution should coincide with the calculated age from the scales.

c. The calculated age should be in agreement with the age determined by other methods.

2. False Annuli:
Sometimes false annuli appear on the scales of fishes due to undesirable factors like retarded growth due to paucity of food, starvation, injury, disease, and fluctuation in temperature. These false annuli resemble the true annuli but they take position on the scale closer to the true annulus of the preceding year than the normal annulus for the next year which appear in case of normal growth (Fig. 14.2).

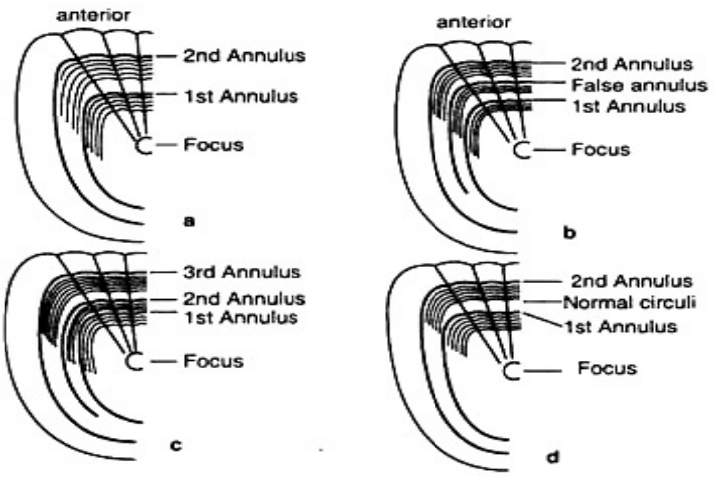


Fig. 14.2a-d : Annuli on scale in four cases of a certain species of fish. a. normal growth for a fish of a certain weight and of 2 years of age, showing annuli; b. abnormal growth for a fish of same weight and of same age, showing false annulus; c. abnormal growth in a 3-years old fish, showing skipped annulus (at 2nd annulus); d. abnormal growth for a 3-year old fish, showing overlapping annulus (at 2nd annulus)

3. Overlapping Annuli:
The position of these annuli in posterior field coincide with the annuli of the preceding year while, in anterior part, it is separated from the preceding year's annuli by 4 or 5 circuli. The overlapping of scales can occur due to a slow growth during the growing period, which is represented by an increase in the length but not in the weight of body.

4. Skipped Annulus:
This type of annulus by position coincides with the annulus of the preceding year, with no normal circuli forming in between. This abnormal function is due to the fact that the fish has not grown during one growing season (one summer) either in length or in weight.

Applications of Scale Method:
1. Fish of temperate regions shows clear rings, which are true marks. This is because there is a sharp difference between the temperatures of two seasons—summer—the period of faster growth, and winter—the period of slow growth or no growth. Therefore, the calculation of the age of fish by annuli is most reliable in temperate fish.

2. This method is more reliably applicable in case of salmons, carps, cod and herrings, established a method of estimating age of fish based on scales, which is as given on next page:

Number of Annuli on the Scale	Estimated Age	Designation of Age Group
None	Less than one year old	0+
One	One year old	1
One + a few circuli	More than one year old but less than two years old	1+
Two	Two years old	2
Two + a few circuli	More than two years old, but less than three years old	2+

Limitations of Scale Method:
1. More than one annulus are added in the extreme conditions of life, e.g., extreme cold (causes cessation of feeding), change in food quality or starvation at the time of spawning. These additional rings are called supplementary rings, which cause problem in age determination by the scale method.

2. This method cannot be applied to those fishes which live in the water with more or less uniform temperatures (tropics). This may be because in these places fishes spawn more than once and, due to fluctuations in food and chemical compositions of water due to rains and floods, formation of annulus may not be an annual feature. Thus, in the fishes of tropical regions, the growth rings do not actually represent year-marks.

B. Bone Method:
Annuli are also present on some bones. The important bones such as operculum, vertebra, supra occipital and scapula are provided with annulations. These annuli are increased in number with the age of fish. The growth rate is different in different seasons. Number of annual rings are helpful in calculating the age of fish.

Similarly, the centrum of fish is also helpful in calculating the age of fish. The centrum of fish vertebrae possess rings, which are used in age determination. For counting the rings on the centrum, it is exposed by removing the tissues attached to it using solution of 0.7% pepsin in 0.2% of hydrochloric acid. The rings on the centrum are counted under the microscope.

C. Otolith Method:

The otolith or ear-stone is present in the internal ear of the fishes and helps in balancing the body. The otolith has annulations formed by the regular deposition of calcium salts. The formation of annulations varies with the growth of fish, and the growth of fish varies with the season. The growth rate of fish is faster in the summer as compared to the winters, thus annulations formation is faster in the summer.

To get the otolith, fish is killed and dissected and otolith is taken out and annulations are counted. For this, first of all the otolith is broken and cut in transverse plane followed by polishing with liquid of high refractive index, such as immersion oils, creosote, etc. The otolith thus prepared is examined under the microscope to count the annulations for age determination and the growth rate analysis.

D. Known Age Method:

The determination of growth rate through this method requires the knowledge of age, which can be known from time of breeding. The hatchlings are kept in a tank in appropriate conditions for two or three seasons. The growth is measured periodically. The fishes are marked with tags and reintroduced in the water. The fishes are recaptured at regular intervals and idea of growth rate in relation of the time is taken.

E. Length Frequency Distribution Method:

Peterson introduced this method in the nineteenth century. In this method, in the sample of fishes of a particular fish, frequency analysis shows length of individuals of one age varying around the mean length according to normal distribution.Data of the sample is plotted and peaks are counted which represent grouping of fishes of successive lengths. Age groups are thus separated and the age of a given species is determined.

This method is suitable for determining the age of younger fishes of 2 to 4 years. This method is not reliable for calculating the age of older fishes because of overlapping of length frequencies in individuals of different ages.

F. Pectoral Spine or Fin Ray Method:

Spines of the pectoral fin is also useful in age determination. For this 3μ to 4μ thick sections of spines are cut and mounted in glycerin and then observed in the microscope. 1% to 2% of indistinct annuli or fin ray consistency are compared to 15% to 20% of annuli on the scales and, thus age is calculated.

Probable questions:

1. Describe structures of a scale.
2. What are the applications of age determination in fishes.
3. What is known age method of fish age determination?

4. What is otolith method of fish age determination?

5. What is Bone method of fish age determination?

6. What is pectoral fin method of fish age determination?

7. What are the limitations of scale method?

8. Write a short note on True Annuli.

9. Write a short note on False Annuli.

10. Write down the applications of scale method.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Sing.

UNIT-XI

Poisonous and Venomous Fish

Objective: In this unit you will know about poisonous and venomous fish.

Introduction:

Biotoxins are mainly of two types; Phytotoxins or plant poisons and Zootoxins or animal poisons. A large variety and number of marine creatures inhabiting the ocean waters pose threat to humans by virtue of their ability either to bite, lacerate or sting or contain toxic substances within their flesh, blood etc.

The information regarding the source of adventitious toxicity of fish is not only vital for development of antidotes and rational assessment of the usefulness of the fish species as food source, but it also opens an arena for the exploration of new biologically active chemical substances or biodynamic compounds of therapeutic value. Halstead and Courville (1970), Concon (1988), and Halstead (2001) have classified ichthyotoxic fish into two groups:

a) Poisonous or Phenerotoxic fish which when ingested cause a biotoxication in humans due to a toxic substance present in the fish and

b) Venomous or Acanthotoxic fish that produce poison by means of glandular structures that are equipped with a traumatogenio device to purvey their venoms.

Intermediate to the poisonous and venomous fish are the **crinotoxic fish** that produce a poison by means of glandular structures independent of true venom apparatus.

Divisions of Poisonous Fishes:

Fish poisoning is synonymous to the Icthyotoxism. Halstead has made four divisions of poisonous fishes.

a) Ichthyosarcotoxic fishes: Those fishes which contain a toxin within their musculature, viscera or skin. **Examples:** Cyclostomes, Elasmobranch, Chimaera, Tetraodon etc.

b) Ichthyootoxic fishes: Those fishes which produce a toxin generally confined particularly to female gonads. In such fishes there is a relation between gonadal activity and the production of toxin.

c) Ichthyohaemotoxic Fishes: Those fishes which have a toxin in their blood. Some freshwater eels and several marine fishes make up this group.

d) Ichthyocrinotoxic fishes: Those fishes which produce toxins by glandular secretion but lack a true venom apparatus. Example: Box fishes or trunk fishes and the lampreys. These may produce a toxic substance inn their skin and subsequently release ths toxin into the environment.

I. Poisonous Fish:

Research in the past several decades has increased our knowledge on the types of toxic fish, the nature and source of toxins and the conditions governing their toxicity. The major problem facing the marine biotoxinologist is the variability and frequent unpredictability of the toxicity of this segment of marine

life. The degree of toxicity of these fish may fluctuate periodically. The poisonous substances in fish may be concentrated in specific tissues or organs. Based on the tissue in which the toxin is present in the fish the poisonous fish are further classified into lchthyosarcotoxic (flesh, musculature, viscera or skin) eg: herrings, anchovies, tarpons and pufferfish, lchthyootoxic (roe or gonad) eg: The most dangerous forms are the genera *Barbus, Schizothorax, Tinca* (Cyprinidae) and Stichaeus (Stichaeidae). lchthyohaemotoxic (blood or serum) eg: Those fish having poisonous blood, members of the anguilliform families Anguillidae, Congridae, Muraenidae, and Ophichthidaelchthyohepatotoxic (liver) eg: sharks

List of some poisonous fish:

- Species of puffer fish are the most poisonous in the world and the second most poisonous vertebrate after the golden dart frog. The active substance, tetrodotoxin, found in the internal organs and sometimes also the skin, paralyzes the diaphragm muscles of human victims, who can die from suffocation.

- The spotted trunkfish is a coral reef fish that secretes a colorless ciguatera toxin from glands on its skin when touched. The toxin is only dangerous when ingested, and poses no immediate harm to divers. However, predators as large as nurse sharks can die as a result of eating a trunkfish.

- The giant moray is a reef fish at the top of the food chain. Like many other apex reef fish, it is likely to cause ciguatera poisoning if eaten.

II. Crinotoxic fish:

Crinotoxic fish are intermediate to poisonous and venomous fish and include filefish, pufferfish, trunkfish, boxfish, toadfish, gobyfish, catfish etc. They are able to secrete substances from their skin, known as icthyocrinotoxins, that are capable of repelling or incapacitating other marine animals. These secretions are also thought to possess antibiotic activity, protecting fish from the myriad of invading microorganisms in the marine environment, a fact that has prompted recent research on them. These have specialized cells or glands in their skin but lack a parenteral mechanism. Glandular secretions are normally released into the surrounding medium, the water. Crinotoxins are often called mucus toxins. The poison glands of ichthyocrinotoxic fish assist in the defensive mechanism of the fish by producing warning or repellent substances especially under stress conditions.

III. Venomous fish:

Fish constitute almost half the number of vertebrates on earth, and approximately 22,000 species of fish are contained in some 50 orders and 445 families. Of these, nearly 1,200 species of marine fish, including stingrays, scorpionfish, zebrafish, stonefish, weeverfish, toadfish, stargazers, and some species of shark, ratfish, catfish, surgeonfish and blenny, are known or suspected to be venomous. The vast majority of these fish is non-migratory, slow moving, and tend to live in shallow waters in protected habitats. It has been suggested that this tendency towards inactivity is closely linked with the evolution of venom apparatus.

List of someVenomous fish:

- The most venomous known fish is the reef stonefish with a remarkable ability to camouflage itself amongst rocks. It is an ambush predator that sits on the bottom waiting for prey to approach. Instead of swimming away if disturbed, it erects 13 venomous spines along its back. For defence it can shoot venom from each or all of these spines. Each spine is like a hypodermic needle, delivering the venom from two sacs attached to the spine. The stonefish has control over whether to shoot its venom, and does so when provoked or frightened. The venom results in severe pain,

paralysis and tissue death, and can be fatal if not treated. Despite its formidable defences, stonefish have predators.

- The lionfish is a venomous coral reef fish. Unlike stonefish, a lionfish can release venom only if something strikes its spines. Lionfish can aggressively dart at scuba divers and attempt to puncture their facemask with their venomous spines.
- The stargazer buries itself and can deliver electric shocks as well as venom. It is a delicacy in some cultures (cooking destroys the venom), and can be found for sale in some fish markets with the electric organ removed.
- Stingrays can sting and cause an injury with their stinger. Such envenomation can occur to people who wade in shallow water and tread on them. This can be avoided by shuffling through the sand or stamping on the bottom, as the rays detect this and swim away. The stinger usually breaks off in the wound. It is barbed, so it can easily penetrate but not so easily be removed. The stinger causes local trauma from the cut itself, pain and swelling from the venom, and possible later infection from bacteria. Occasionally severed arteries or death can result.

Different types of poisonous and venomous fish with their specific stinging apparatus:

a. Stinging apparatus in the pectoral fin:

In case of some fish the spine has been modified from outer most pectoral fin and is capable of infecting jagged food. At the base of each spine there is a sac with more or less wide opening and this is believed to contain a fluid of viscous nature which is poured out when the spine bought into action.Eg: *Ictalurus*sp., *Clariousbatrachus*

b. Stinging apparatus at operculum and dorsal spine stinger:

Long sharp spine of operculum and spine supporting the 1st dorsal fin associated with venom gland in their tip as in weaver fish has two opercular and five to eight dorsal spines with the glandular tissue surrounding each spine. There is no canal living from the gland and the venom is set free by rapture of the cell throwing down the groove and injected into the wound.Eg: Weever fish

c. Stinging apparatus at gill cover and dorsal spine:

The two spines of the 1st dorsal fin and spine on the gill cover or opercular spine constitute the venom apparatus. Each of these spines is hollowed and perforated just like the venom fang of the snake. The base of each spine is embedded in the center of the poison gland and the secretion is discharged through the spine just like as a hypodermic needle. Eg: *Thalassophryne* sp.

d. Stinger at the base of dorsal fin:

Violy and Formidable scorpion fish of tropic seas have this type of stinger. The venom gland is situated under a heavy skin beneath the bases, towards the tip of dorsal spine and each be continued into a duct situated into the deep groove on either side of the spine. The 5th spine of the dorsal fin penetrate into the skin if someone step on it and the venom is injected into the wound by pressure of the foot on the bag like venom gland. Expulsion of venom takes place when the integumentary sheath is retracted along the spine.Eg: Stone fish(*Synanceia* sp.)

e. Stringer on the each side of the caudal pedancle:

In *Acanthurus sp.* The caudal peduncle stinger is present on each side of the body with venom gland. A pair of knife sharp spine on either side of the caudal peduncle is the main poison apparatus. Venom gland located within the pine sheath is the interesting feature of the fish. The spine is directed forward but when the fish become excited it is extended from the right angles of the body and get ready for injecting.Eg: *Acanthurus sp.*

f. Shoulder stringer with venom gland:

The venom apparatus consists of two solder spines which are situated bilaterally and each of which protrudes through an integumentary sheath. The spines are sharp, conical structure with cement like material and have concentric groove lamellae. The spine is said to have double groove through which the venom flow. When the pressure is put upon the sheath of the venom containing cell as well as spine the sheath rapture and the venom is forced on the groove of the spine.Eg: *Astroscopus sp.*

g. Stinging apparatus of caudal region:

In case of elasmobranch in sting ray possess one or two spine attached to the upper middle part of the beak like tail. Glandular epitheliam lying in the ventro lateral glandular groove of the spine considered as the primary venom producing area. Each spine along their side bear dozen of bar hooks contain venom glands. Groove turns backward as pierced to the victim and the snake like duct eject poison. Besides sting ray caudal fins are also present in various other fishes.Eg: *Trigon* sp.

h. Stinger in dorsal fin with venomous glandular epithelium in groove:

Venom apparatus composed of dorsal fin spine and associated with glandular tissue that grooved along the base of the spine. The glandular cells are present in the integumentary portion and in the area of anterolateral groove. The venom secretion is holocrine type of secretion.Eg: Spiny dog fish.(*Squalusacanthias*)

i. Stinger on dorsal fin with venomous glandular epithelium:

Venom apparatus consists of dorsal spine, glandular epithelium of spine connecting membrane and the enveloping integumentary sheath. Spine has a pronounced keel area and shallow depression. Venom producing tissues lie within the depression. The secretion of the spine is discharged through the groove of the spine into the wounds.

Probable Questions:

1. What is the differences between poisonous and venomous fishes?

2. Classify poisonous fishes.

3. Write short nots on Crinotoxic fishes.

4. Write different types of stinging apparatus in poisonous fishes.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

Unit-XII

Fish Migration : Types, Theories and Significances

Objective: In this unit you will know about types, theories and significance of fish migration.

Introduction:

Migration is the movement of large number of animals from one place to another for feeding, reproduction or to escape weather extremes. When large numbers of fishes come together and move socially it is called **shoaling.** But sometimes migrating fishes exhibit high degree of coordination in their movements and carry out synchronized manoeuvres to produce different types of shapes. This is called **schooling,** as seen in tunas and sardines.

Feeding or alimental migration takes place in fishes for feeding. In high populations fishes exhaust food resources in an area quickly and therefore must migrate constantly in search of new feeding resources. Salmons, cods and sword fish constantly migrate for food from one place to another in the sea.

Spawning migration takes place in breeding season in those fishes which have spawning grounds far away from feeding places. Migratory fishes such as eels and salmons and a large number of riverine fishes spawn in tributaries of river in hills and migrate in large number for laying eggs in these oxygen rich waters.

Juvenile migration involves larval stages of fishes which hatch in spawning grounds and must migrate long distances in order to reach the feeding habitats of their parents.

Recruitment migration takes place when large number of larvae moves from nursery habitat to the habitat of adults which may sometimes be distinctly different. Adults of eels live in rivers in Europe and America but their larval stages live and grown in sea and migrate to reach rivers which may take one to two years.

Seasonal migration takes place in fishes that inhabit arctic areas where in summer climate is conducive and food abundant but as winter approaches temperatures fall below zero and food becomes scarce. Hence fishes must migrate towards subtropical and tropical areas to escape extremes of weather conditions.

Definition of Migration:
Migration of fish is defined as a class of movement which involves a long journey to a definite area for some purpose and impels the migrants to return to the region from which they have migrated. The purpose of the journey is breeding and feeding. Migration is a two-way journey. It includes emigration (outward journey) and immigration (return journey or inward journey). The fishes are notable for migration for the purpose of spawning. The inherent purpose of migration is not known.

Types of Migration:

Myers (1949) has classified the following types of fish migration.

a. Diadromous migration:
When the migrations occur in between freshwater and marine environments.

Diadromous type of migration can be divided into following three-types.

(i) Anadromous migration:
When migration occurs from sea to freshwater for spawning, called anadromous migration; e.g., Atlantic salmon (*Salmo salar*), Hilsa shad (*Tenualosailisha*), Toli shad (*Tenualosatoli*), Paradise fish (*Polynemusparadiseus*), Flat head sillago (*Sillaginopsispanijus*), Sturgeon (*Acipenser*) and Salmon (*Oncorhynchus*).

(ii) Catadromous migration:
The journey of freshwater fishes to the sea for spawning, called catadromous migration; e.g., Indian longfin eel (*Anguilla bengalensisbengalensis*), Shortfin eel (*Anguilla bicolorbicolor*), Common freshwater or European eel (*Anguilla anguilla, A. vulgaris*), American eel (*Anguilla rostrata*).

(iii) Amphidromous migration:
Migration of fishes from freshwater to sea and vice versa and is not for the purpose of breeding but for the other purposes (e.g., food). The amphidromous fishes migrate regularly at some particular stage of the life cycle.Marineamphidromy occurs in flat head mullets (*Mugil cephalus*) which spawn in the Indian seas during autumn and early winter and whose young stage spend a short period in brackish water and freshwater. They are able to survive in ponds with salinity at 87%. After spending in fresh- or brackish water they return to marine water.

b. Potamodromous migration:
Migrations of fish that occur entirely in freshwater, called potamodromous e.g., carps and trout. Trouts and carps travel long distances in large shoals in search of suitable spawning grounds and return to feeding areas after spawning.

c. Oceanodromous migration:
Migration which occurs entirely in sea, called oceanodromous migration. Horizontal and vertical distribution is considered in oceanodromous migration. Many fishes undertake short distance migrations throughout their life and some fishes like herrings, cod, tuna and plaice, cover long distance migrations.

Latitudinal Migration:
This is performed by fishes like barracudas *(Sphyraena*) and swordfish (*Xiphius*) of the warm tropical seas. They migrate to north in spring and to south in autumn.

Vertical Migration:
This is performed by many marine and freshwater fishes and is related to light, search of food, protection and also to spawning. The mackeral rises into the surface waters when there is a rich development of plankton. They eat on plankton and go to deep layers after feeding.

The swordfish, which normally lives in surface water move downwards to great depths to feed deep water fishes like scopelids. Many pelagic larvae of marine fishes perform diurnal vertical feeding migrations. They follow the vertical movements of their prey, the planktonic invertebrates which move down to great depth by day and rise to surface by night.

Many deep water fishes of the order Scopeliformes rises to spawn in the upper layers. Their eggs develop and often their larvae live feeding on the phytoplankton. Among freshwater fishes the clearest

example of vertical spawning migration is that of the Lake Baikal Comephoridae. These fishes are viviparous and rise to surface from great depth of the lake to give birth to their larvae.

Spawning Migration:
This is the migration in fishes for breeding, and so it is related to life cycle. Spawning migration is an adaptation for ensuring the most favourable conditions for the development of the eggs and the larvae. This also gives protection to early stages of fishes from predators.

There are two major types of spawning migrations. Movement from freshwater to saltwater for spawning is called catadromous migration. The reverse movement, that is, from saltwater to freshwater is termed anadromous migration.

(a) Catadromous migration:
The most famous examples of catadromous fish is the eels, *Anguilla rostrata*, the European eel and Anguilla vulgaris, the American eel. For eel, the river serves as the feeding ground while die sea serves as the spawning ground. The stimulus for the start of migration in eel is the ripening of its gonads in rivers.

Before it enters the sea, the eye of the eel becomes enlarged, sometimes becoming four times as large as the eye of freshwater eels. Its face becomes sharper and its colouration changes the back becomes darker, while the belly changes from yellow to a silvery colour. The eel starts its migration in a wellfed condition.During migration it spends enormous amount of energy so it becomes very thin. The migrating eel does not feed. Its alimentary canal degenerates considerably. Osmotic pressure of its blood rises and size of its swim-bladder decreases.

The eels migrate about 4500 km westwards from Europe or eastward from America and reach the breeding place in the Sargasso Sea off Bermuda. The adult die immediately after spawning in deep waters.The fertilized eggs hatch out into transparent, ribbon like larvae, called the leptocephali. These were erroneously called glass fishes and placed in the genus Leptocephalus. They lead a pelagic life for a year or more and undergoes metamorphosis into elvers (glass eels).

The elvers then start ascending the rivers in shoals and grow for some years to become adult eels. The adult eel, on maturity start moving towards sea, again the cycle is repeated.

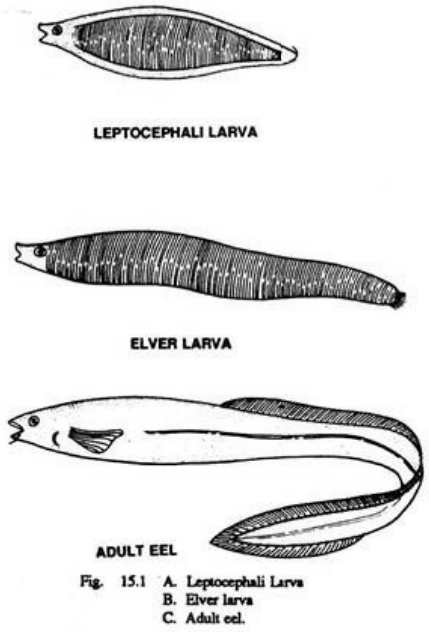


Fig. 15.1 A. Leptocephali Larva
B. Elver larva
C. Adult eel.

Catadromous migration is also performed by certain members of the families Galaxiidae and Gobiidae. But their migration is considerably shorter than those of the eel. They usually pass from the lower reaches of the rivers to the adjacent shallow parts of the sea.

(b) Anadromous migration:
Anadromous migrations are performed by lampreys, sturgeons, salmon, some shads, cyprinoids etc. The best examples are Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus nerka*).

In winter, the both sexes leave their feeding ground at sea to ascend the freshwater mountain streams, reaching the identical spot where they originally grew some years ago. The total distance travelled may be even upto 3600 km, at a speed of 30-40 km. per day.They stop feeding, alimentary tract undergoes changes into a thin thread with feebly developed pyloric caecae. Change in colour and weight too occurs. Sexual dimorphism becomes evident. The male is characterized by the possession of enlarged front teeth.

After the selection of suitable spawning grounds, the salmons segregate into pairs and produce shallow saucer-like nest where spawning takes place.Very young salmons are known as "alevins" and they remain mostly among stones. Alevins develop into next stage called "parr" and finally to adult.

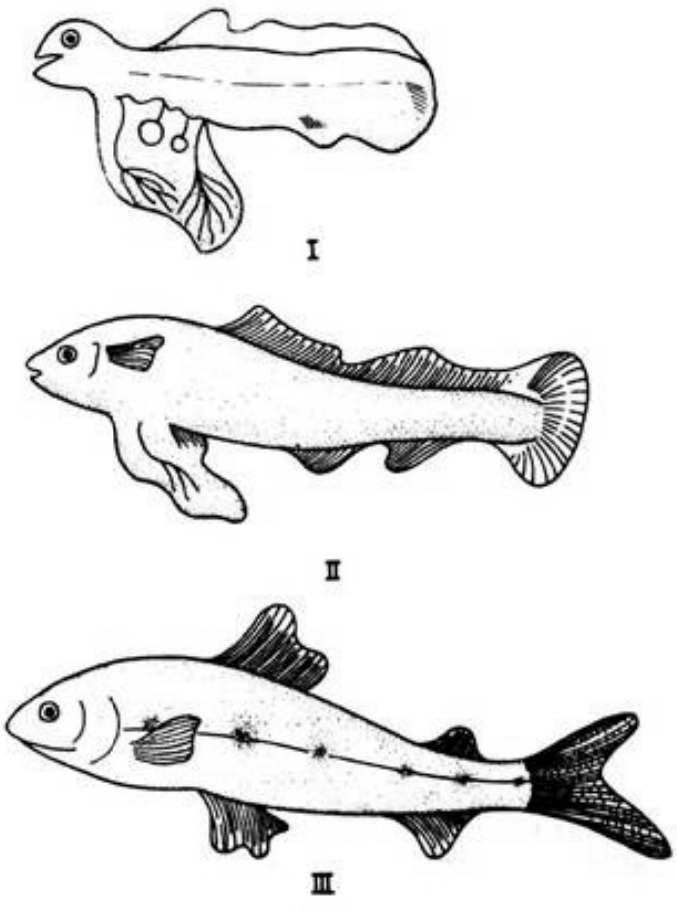


Fig. 15.2 Three stages in the development of Salmon
I & II Alevins
III 'Parr' with marks on its side.

After fertilization, salmons are very exhausted. They are called “kelts”. The males seldom return to the sea. The females recover and after a period in the sea they return to breed again. This process may be repeated several times.Some fishes do not perform significant movement like salmon. They migrate from seas to estuaries or lower reaches of river, for spawning. Such fishes are classified as fluvial anadromous (Semimigratory) fishes. Examples are many whitefishes and cyprinoids.Many freshwater fishes leave the lakes to spawn in the river. This is called as limnodromous migration. One of the common examples of this is the whitefish *Coregonuslavaretus*.

Feeding Migration:

This is the movement from spawning or overwintering grounds to the feeding grounds. Feeding migration can be passive or active. In many fishes the feeding migration even begins in the egg stage. It is a passive feeding migration of eggs and embryos from spawning to feeding ground.Active feeding migration is performed by many marine fishes like cod. Horizontal feeding migration of cod comprises regular journeys, going from one good feeding ground to another.

Overwintering Migration:

Overwintering and hibernation in fishes are a part of the life cycle of a fish. It is characterized by reduced activity, reduction or stoppage of food consumption, lack of food, poor oxygen condition, low temperature, drought etc. Overwintering do not occur in all fishes.

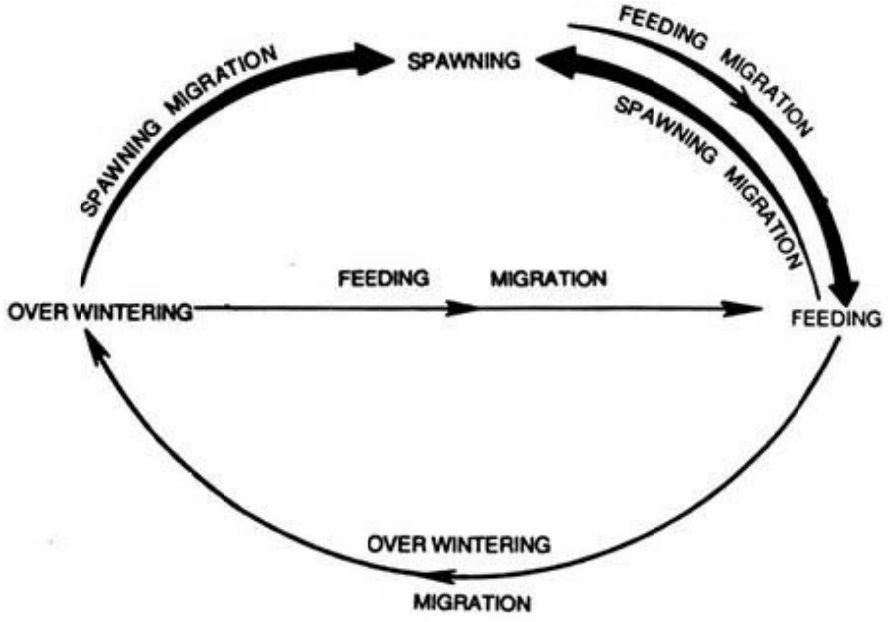


Fig. 15.3 Migration cycle of various fishes.

Overwintering migration is a movement away from feeding to wintering grounds. It occurs only in those fishes which have a wintering ground.

In the wintering ground, fish is in a state of relative inactivity and reduced metabolic rate. It requires protection against predators which are common in feeding ground. Overwintering migration is performed by marine fishes like flatfishes and freshwater fishes like grass-carp.

Shoreward Migration:

In this type of migration there is a temporary movement of fishes from water to land. For example, the common eel travel from one pond to another through moist meadow grass. The mud-skipper, *Periophthalmus* make temporary migration to land by means of modified pectoral fins. The climbing perch, Anabas migrates from water to land and even climbs trees to the height of several feet by means of the strong spines on its pelvic fins and gillcovers.

Examples of Migration:

A. Catadromous migration (Gk. kata = down, dramein = to run)

European Eel Migration:

The life-history of common river eel or the European eel (*Anguilla anguilla or A. vulgaris*), and American eel (*Anguilla rostrata*) will represent a clear idea about the catadromous migration. The common river eels or European eels are found along the shores of Europe and in inland waters of countries near the shores of Europe, some of which inhabit in Iceland, the Mediterranean countries, black sea and the red sea.

The adult eels with their sexual products are not encountered in freshwater, so their biology of reproduction was quite unknown for centuries, From the time of Aristotle the different ichthyologists tried to find out the exact spawning ground of the European river eel.

At last Danish ichthyologist Johannes Schmidt who started his investigation in 1904, ultimately succeeded in 1922 to locate the spawning place of the European river eel. It was found in the Sargasso sea of the Atlantic Ocean.

The life history of the European eel is divided into 4 phases :

a. An ordinary yellow eel representing the growing and feeding form in the river.

b. Changes of the yellow eel into the silvery eel ready for seaward migration for spawning (breeding phase).

c. A pelagic larval phase and

d. The metamorphosis of the pelagic larval phase to elver or young eels.

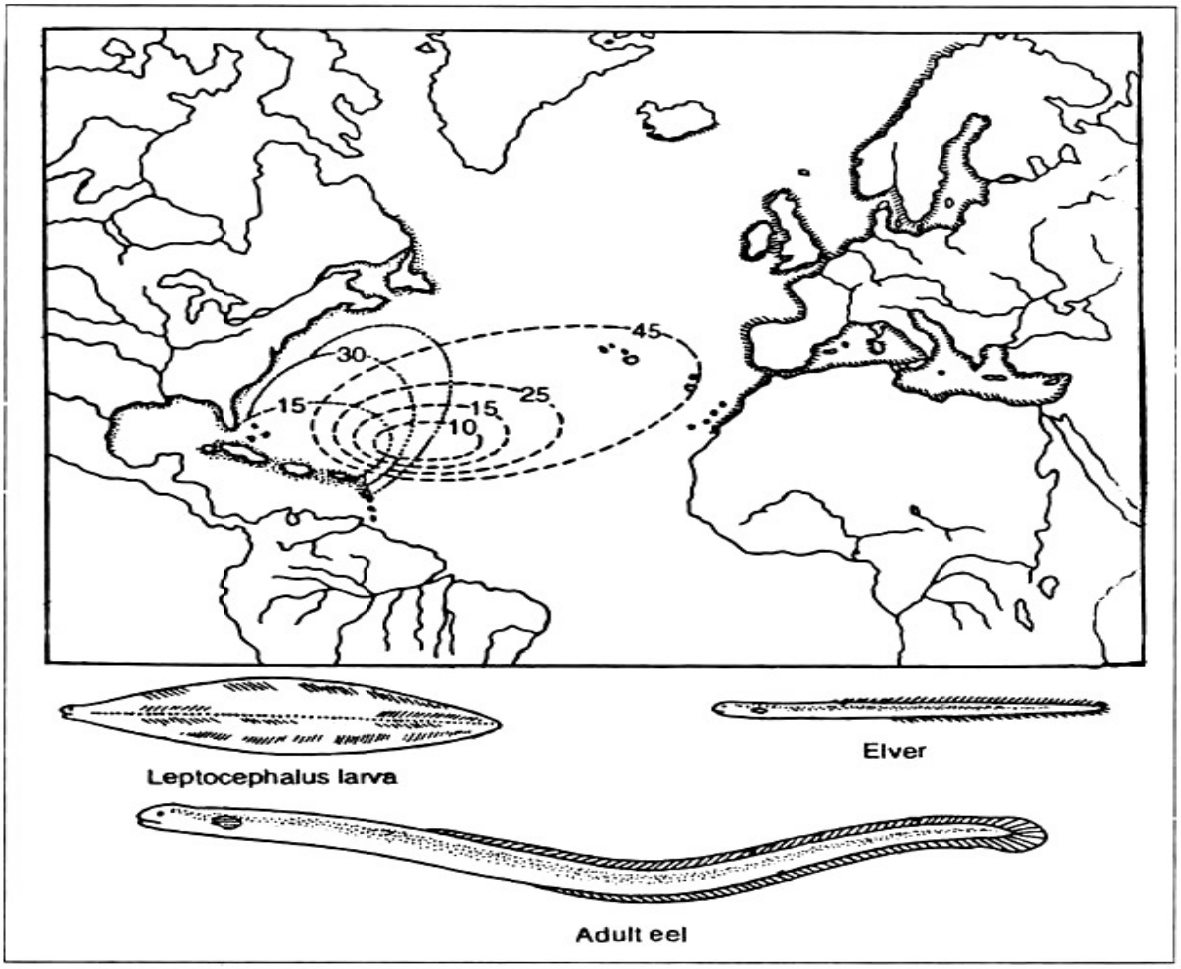


Fig. 6.109 : Life cycle of European eel (*Anguilla vulgaris*) and North American eel (*A. rostrata*). Number and curved lines in the Atlantic Ocean indicate larval distribution and length in millimeters (After Norman).

i. First phase:

The yellow colored variety living in fresh water represents the feeding and growing forms. With the advent of autumn, majority of yellow eels become silvery and prepare to undertake migration forwards the spawning ground, the Sargasso sea of Atlantic Ocean. The eels in the river spend about 10 to 12 years feeding partially on fish.

ii. Second phase:

During the transformation from yellow- colored to silvery colour, the yellow eels stop feeding, eyes become greatly enlarged, the snout becomes sharper with thinner lips and the yellow coloration is replaced by a metallic silvery color. The silver eels are recognized by having matured organs and shrunken digestive tract.

The primary development of gonads is the stimulus for the beginning of migration. The size of the eggs in the ovaries changes before migration. These silver eels first migrate down to the mouth of the rivers and then into the Atlantic Ocean. The European eels probably migrate over 6000 km between its freshwater feeding streams and its spawning ground.

The spawning ground is located in the western part of tropical waters of Atlantic Ocean between 22° and 33° N. latitude and 48° and 65° W. longitude, near Bermuda Islands. Spawning takes place from the end of winter to the middle of summer. After the completion of spawning the parents die.

iii. Third phase:
Eggs are laid in spring at depth of 500 to 700 meters with temperature ranging between 10-12°C. The fertilized eggs float for some time and the young hatch out as the pelagic larvae. The larvae are called Leptocephali.

The leptocephali are flat, glassy, leaf-like body. These tiny creatures are provided with elongated needle-like teeth for feeding. The gut has a straight tubular structure. The eyes are large and silvery. They now begin their long homeward journey. At the end of first summer when they become about 25 mm in length on average, are recorded in the Western Atlantic.

By the second summer the leptocephali reach central Atlantic and the size is about 50-52 mm in length on average. In the third summer they become about 72-75 mm in length and reach in the continental shelf of Europe. The larvae of eel are passively drifted by warm water current of the Atlantic Ocean.

iv. Fourth phase:
During autumn and winter of the 3rd year the leptocephali metamorphose to form elvers or glass eels or young eels. During metamorphosis the larvae stop feeding, their flattened body become cylindrical and the needle-like teeth is replaced by new ones. The young eels when become three years old, measure about 15-20 cm long.

Then they congregate in the mouths of rivers. Here the elvers or young eels ascend the rivers, grow in size and change their color into yellow. The males like to stay in the estuaries and the females ascend the rivers in shoals, specially at night during the spring to reach a suitable resting place. In the yellow eels teeth are lost, intestine shortens and the anal aperture moves forward.

The yellow eels spend 8-10 years on feeding and growing and on maturity, change into silver eels and start their perilous journey towards the Sargasso sea. The well fed silver eels first stop their feeding and leaving the rivers, empty into the Baltic Sea and gradually in the abyssal depths of the Atlantic Ocean.

B. Anadromous Migration:
Migration of Hilsa:

Among Indian fish, hilsa or Indian shad (*Tenualosailisha*) represents an example of anadromous migration. Its occurrence is recorded in the coastal waters of Pakistan, India, Sri Lanka, Bangladesh and Myanmar. Its presence in the estuaries, rivers and lakes (e.g., Chilka), mostly during spawning season indicates its anadromous migration.

During spawning season the hilsa population ascends the rivers of the Hooghly, the Ganges, the Mahanadi, the Godavari, the Krishna and the Kaveri and its tributaries in the eastern region of India, and the Narmada, the Tapti and the Kali in the Saurastra coast of the west India.Around the Indian coasts, the Hooghly-Matla estuarine system covers a major portion of the Ganga-Brahmaputra delta and is estimated to be 3,100 sq. miles which is the largest in India.

This estuary zone is mostly famous for hilsa migration. Before the construction of Farakka barrage on the Ganges in West Bengal the hilsa used to migrate up to Delhi during the spawning season covering a distance over 1,250 km. In Bangladesh it ascends in the rivers of Meghna, Padma, Brahmaputra and its deltaic rivers. It also migrates in the upper part of Irrawaddy river of Myanmar.

The marine distribution of hilsa in the Bay of Bengal and in the Arabian Sea is due to several factors, such as vast stretch of continental shelf, low salinity, discharge of huge amount of monsoon freshwater in the coastal region through the rivers, monsoon winds, huge abundance of planktons as food, and other favorable hydrological parameters. Oceanic properties along the Indian coasts are given below in Table 24.

Table 24 : Oceanic Propertis and Continental shelf area along the Indian Coast

Continental shelf area and Oceanic properties	*Range of Variation*
1. Continental shelf area	Along West Bengal and Orissa coast, continental shelf area, ranges from 35 to 20 km from the shore line; along Andhra and Tamil Nadu coast within 5 km; along Gujarat coast more than 100 km from the coast line; along Maharastra coast about 80–100 km.*
2. Rainfall (South-west monsoon remains active from June to September)	
(a) West Bengal, Orissa and Bangladesh	100 – more than 200 cm.*
(b) Saurastra coast	50 – 200 cm.*
3. Surface sea water temperature (°C)	23 – 30 [La Fond (1958), Miller and Jefferies (1967), Wooster, Schaefer and Robinson (1967)]
4. Surface sea water salinity (%)	17 – 36, –do–
5. Depth of euphotic layer (meter)	50 – 65, Krey and Babenerd (1976)
6. Zooplankton biomass (ml. per haul)	5 – 20, Prasad (1968)
7. Phosphate — phosphorus (µg –at/l)	0.2 – 0.6, Wyrtki (1971)
8. Nitrate — Nitrogen (µg –at/l)	0.5 – 1.0, Wyrtki (1971)
9. Primary production ($mgC/m^2/d$)	100 – 500, Krey and Babenerd (1976)
10. Chlorophyll — a (mg/m^3)	0.3 – 0.5, –do–
11. Density of Copepod (no. of per haul)	3,000 – 27,000, Kasturirangan, Saraswathi, Saraladevi, Stephen, Gopalkrishan & Kunjamma (1970).

In the sea, hilsa population is found along the east coast in the vicinity of rivers before the spawning season but in Gujarat and Maharashtra the hilsa is found about 12-16 km off the coast at a depth of about 20 fathoms.

There are two types of hilsa stocks — one estuarine and offshore stocks which are found in the lower region of the estuary and the foreshore areas of the seas, and another is riverine stock which spends throughout the year in the river, mainly in the Ganges and the Brahmaputra.The estuarine stock migrates upwardly during breeding season and after spawning they return to their natural habitat and spend throughout the year till the next breeding season to come. The riverine stock ascends in the more freshwater river zone during breeding season and after spawning they come back to the lower reaches of the river and spend the rest period until the next breeding season comes.

The life cycle of hilsa is divided into 4 phases:
(i) Egg

(ii) Larval stage or Fry,

(iii) Fingerling or Juvenile stage and

(iv) Adult stage.

The size of adults differs in sexes. The females are larger than males. The size of the sexes differs in different seasons, even in the same river. In the Ganges and in the Padma different sizes of sexes have been reported by different authors. In the Hooghly estuary the mature females are recorded about 250 mm in length.

The adult hilsa are laterally compressed, fusiform animals, having abdomen with a keel about 30-33 scutes. There are very fine numerous, closely set gill-rakes that indicate for planktonic feeding habit, predominantly zooplankton feeder. They mainly consume Cyclops, Daphnia, Moina, rotifers and protozoans. The adult hilsa in the freshwater zone of the Hooghly river are column and bottom feeders.The hilsa is probably polygamous and fertilization is external. The riverine stocks of hilsa become mature in between 1-3 years of age. Matured hilsa spawns once in a year but the time of spawning differs in different parts of India. In Hooghly estuary or freshwater region of the Hooghly river spawning takes place at the onset of evening but in the Narmada spawning takes place in the early morning.

During upstream migration schooling behaviour among hilsa is seen and males move in the upper surface layer of water and females move in the deeper water layer in river during monsoon period.Many authors reported that during spawning period the hilsa do not feed or stop feeding but Pillay (1958) has reported that the appreciable amount of food is available during spawning period, especially of hilsa of the Hooghly region. The feeding intensity is increased considerably after spawning period.In the spawning ground both the sexes discharge their garnets in freshwater. The tailed spermatozoa survive few hours in the water and ova are large, translucent at the marginal zone. The fertilized ova turn transparent after half an hour. The fertilized eggs float in water and color is about light greenish yellow. The hatched ova transform into larva or fry when they become 20-40 mm in length.

The abdomen of the larva possesses 5-7 pre-ventral scutes. At about 40-45 mm in length, the dark blotches on the lateral side are seen that indicate the fingerling or young hilsa stage. The larvae are surface feeders, predominantly zooplanktons which constitute about 70% in their food composition. When the fry attains 100 mm in size they are called finger- lings or young hilsa.

The body of young hilsa becomes laterally compressed with keeled abdomen. The abdomen possesses 30-32 scutes. The body color is silvery along the sides. A row of dark blotches is seen on the lateral sides of the body.When the fingerlings become 150 mm in size, called advanced fingerlings or youngs. At that stage they consume small shrimps and phytoplanktons. The early fingerlings (about 80 mm) are found in the lower reaches of the river and estuary zones. The youngs or advanced fingerlings (above 150 mm) occur along the foreshore areas of the seas.

Two types of anadromous migrations are seen among Indian hilsa species. Breeding or spawning migration is seen during the southwest monsoon when the Indian and Bangladesh rivers are flooded

by the monsoon rain. Another type is winter or spring migration which is influenced by the certain changes of the water temperature and rain, mainly seen in the Gangetic delta.

The correlation between temperature and movement into freshwater may be a reflection of the energetic cost of migration. In the Indus river of Pakistan and Irrawaddy river of Myanmar, the hilsa migration takes place by the molten snow that creates flood in these rivers.The adult hilsa spends most of the year in their original places except spawning season. The young and adult hilsa can tolerate certain variation of salinity. The Hooghly-Matla estuary is classified as a mixohaline range, in which salinity varies in different zones.

Mystusgulio migrates to the estuarine and freshwater zones of the Hooghly river for spawning, and *Pamapama* also migrates to the estuarine zone of the Hooghly river for both genetic and tropic reasons. Toli shad (*Tenualosatoli*) is found in the western coasts of India, ascends in rivers for spawning.

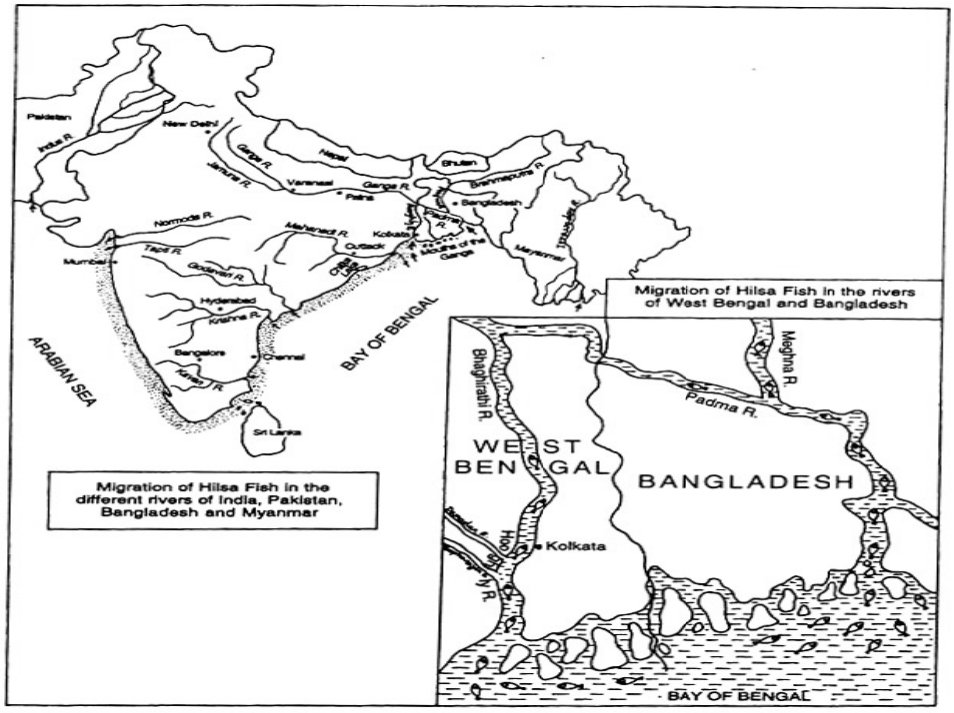


Fig. 6.110 : Migration of hilsa fish.

Causes for Fish Migration:

Fish migration is related to several factors such as physical, chemical or biological.

i. Physical factors:

The physical factors include temperature of water, rainfall, quality of water, water depths, pressure, light intensity, photoperiod, turbidity, tides and currents.

ii. Chemical factors:
The chemical factors include pH of water, salinity, dissolve of O_2 and CO_2, types of dissolved organic and inorganic substances, and taste of water.

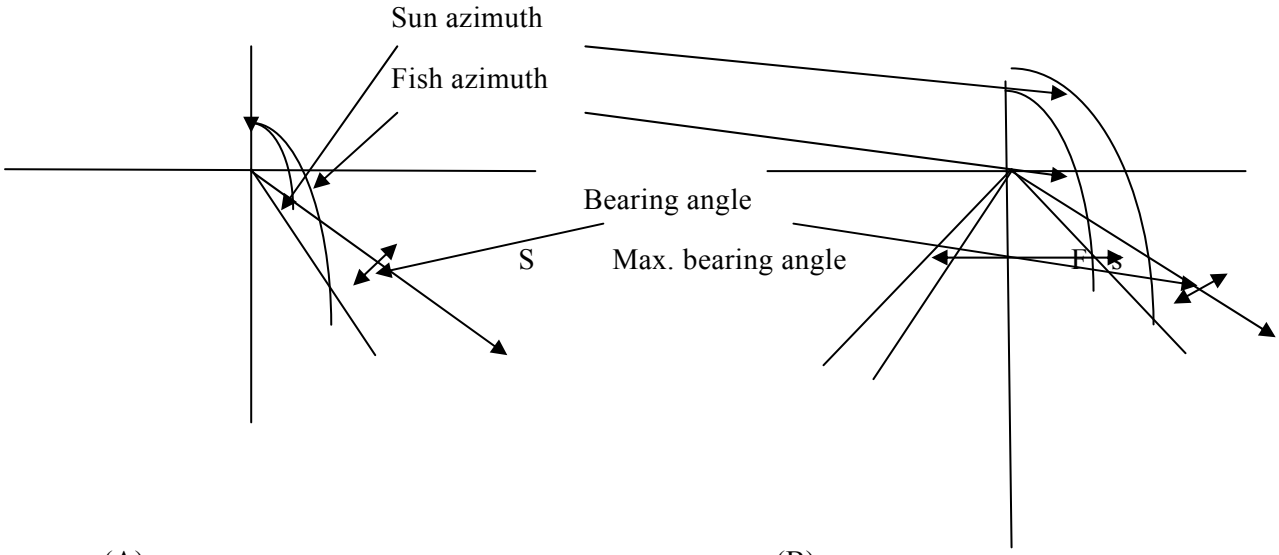
iii. Biological factors:
The biological factors are food, attainment of sexual maturity, endocrine behaviour and competitors and predators.

Theories of migration:

Odor hypothesis / Parent stream theory:
It has been proposed that each stream has a unique organic quality in terms of soil, plants etc. The specific Odor of a certain stream remain with in the brain of fish (Salmon) in their early life and helps to recognize their specific stream in the adulthood.
Mechanism of Sun compass Orientation:
Some fishes use sun as a reference point and able to correct the direction in 'Azimuth' during the day.

In this case 2 cycle period day and year are maintained.

- Azimuth: Sun azimuth can be explain as the relationship between N (North) point and horizontal Component of sun.
- When the fish starts migration, sun exposed to be on left side at noon at the and later on the side. So, adjustment direction (Correction) was done by the fish. Further work has been demonstrated that corrected route was done by the sun azimuth.
- At the fixed depth the distribution of light with azimuth angle in the horizontal place varies with the altitude of the sun. Tyler (1961) gave a clear account of the phenomenon.



(A) (B)

(B) This is a normal phenomenon when a fish is just below the depth of horizontal line, there the difference or bearing angle between sun's azimuth and fishes is short.

(C) The fish cause a correct direction with the help of azimuth. Fish cause change in direction in the angle from (F) and (S) state to F and S situation the bearing angle is maximum than the previous angle and this is advantageous in migration.

Probable Questions:

1**.** Define migration. Write down the causes for fish migration.

2. Differentiate between Anadromous migration, Catadromous migrationand

Amphidromous migration.

3. Define Feeding Migration, Overwintering Migration and Shoreward Migration.

4. With suitable example illustrate Anadromous migration in fishes.

5. With suitable example illustrate Catadromous migration in fishes.

6. Discuss various theories related to fish migration.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

SEMESTER-II

IMMUNOBIOLOGY AND HUMAN POPULATION GENETICS

ZHT-207

SELF-LEARNING MATERIAL

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

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Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

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Director
Directorate of Open and Distance Learning
University of Kalyani

HARD CORE THEORY PAPER
(ZHT–207)

Immunobiology & Human Population Genetics

Group A : Immunobiology

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 207 (Immunobiology & Human Population Genetics)	I	Basic concepts of immune system; primary and secondary lymphoid organs; Tissue, cells, molecules of immune system	1.5	1	1	7-24
	II	Innate immunity: Overview, features, neutrophils, macrophage functions, inflammation, NK cells		1	1	25-30
	III	Humoral immune system: Structure and class switching of antibodies, B cell function, maturation and development		1	1	31-40
	IV	Complement system and diseases		1	1	41-50
	V	Antigen presentation: APC, Dendritic cells, MHC genes and gene products		1	1	51-57
	VI	Vaccination and immunization: natural and artificial immunization; active immunization, vaccines.		1	1	58-63

Unit-I
Basic concepts of immune system; primary and secondary lymphoid organs; Tissue, cells, molecules of immune system

Objective: In this unit, we will discuss about the Basic concepts of immune system; primary and secondary lymphoid organs; Tissue, cells, molecules of immune system.

Immune Response:

Any foreign protein, toxin of parasites, bacteria and viruses, when enter into the body, they interfere with host physiological processes and produce harmful effects. The "chemical defense" mechanism of host organism that operates against such effects of parasites and others is called immune reaction or immune response.

Immunology: The branch of life science which deals with immune reaction is known as immunology. Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enter the body.

The immune system consists of many different organs and tissues that are found throughout the body. These organs can be classified functionally into two main groups. The primary lymphoid organs provide appropriate microenvironments for the development and maturation of lymphocytes. The secondary lymphoid organs trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen. Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole. Carried within the blood and lymph and populating the lymphoid organs are various white blood cells, or leukocytes, that participate in the immune response. Of these cells, only the lymphocytes possess the attributes of diversity, specificity, memory, and self/nonself recognition, the hallmarks of an adaptive immune response. All the other cells play accessory roles in adaptive immunity, serving to activate lymphocytes, to increase the effectiveness of antigen clearance by phagocytosis, or to secrete various immune-effector molecules. Some leukocytes, especially T lymphocytes, secrete various protein molecules called cytokines. These molecules act as immunoregulatory hormones and play important roles in the regulation of immune responses. This chapter describes the formation of blood cells, the properties of the various immune-system cells, and the functions of the lymphoid organs.

The Immune System Includes Innate and Adaptive Components:

Immunity—the state of protection from infectious disease —has both a less specific and more specific component. The less specific component, **innate immunity**, provides the first line of defense against infection. Most components of innate immunity are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a particular pathogen but that include cellular and molecular components that recognize classes of molecules peculiar to frequently encountered pathogens. Phagocytic cells, such as macrophages and neutrophils, barriers such as skin, and a variety of antimicrobial compounds synthesized by the host all play important roles in innate immunity. In contrast to the broad reactivity of the innate immune system, which is uniform in all members of a species, the specific component, adaptive immunity, does not come into play until there is an antigenic challenge to the organism.

Adaptive immunity responds to the challenge with a high degree of specificity as well as the remarkable property of "memory." Typically, there is an adaptive immune response against an antigen within five or six days after the initial exposure to that antigen. Exposure to the same

antigen sometime in the future results in a memory response: the immune response to the second challenge occurs more quickly than the first, is stronger, and is often more effective in neutralizing and clearing the pathogen. The major agents of adaptive immunity are lymphocytes and the antibodies and other molecules they produce.

Because adaptive immune responses require some time to activate, innate immunity provides the first line of defence during the critical period just after the host's exposure to a pathogen. In general, most of the microorganisms encountered by a healthy individual are readily cleared within a few days by defence mechanisms of the innate immune system before they activate the adaptive immune system.

Haematopoiesis:

All blood cells arise from a type of cell called the hematopoietic stem cell (HSC). Stem cells are cells that can differentiate into other cell types; they are self-renewing—they maintain their population level by cell division. In humans, haematopoiesis, the formation and development of red and white blood cells, begins in the embryonic yolk sac during the first weeks of development. Here, yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic haemoglobin. In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the foetal liver and then to the spleen; these two organs have major roles in haematopoiesis from the third to the seventh months of gestation. After that, the differentiation of HSCs in the bone marrow becomes the major factor in haematopoiesis, and by birth there is little or no haematopoiesis in the liver and spleen.

Cells of the Immune System:

It is remarkable that every functionally specialized, mature blood cell is derived from the same type of stem cell. In contrast to a unipotent cell, which differentiates into a single cell type, a hematopoietic stem cell is multipotent, or pluripotent, able to differentiate in various ways and thereby generate erythrocytes, granulocytes, monocytes, mast cells, lymphocytes, and megakaryocytes.

Lymphoid progenitor cells: Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. The other types of white blood cells play important important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines.

The lymphocytes can be broadly subdivided into three populations—B cells, T cells, and natural killer cells—on the basis of function and cell-membrane components. Natural killer cells (NK cells) are large, granular lymphocytes that do not express the set of surface markers typical of B or T cells. Resting B and T lymphocytes are small, motile, nonphagocytic cells, which cannot be distinguished morphologically.

- **B lymphocytes:** The B lymphocyte derived its letter designation from its site of maturation, in the bursa of Fabricius in birds; the name turned out to be apt, for bone marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. After interacting with antigen, it differentiates into antibody secreting plasma cells and memory cells.
- **T lymphocytes:** T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen

binding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane-bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC).

T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus, the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. $CD4^+$ T cells generally function as T helper (T_H) cells and are class-II restricted; $CD8^+$ T cells generally function as T cytotoxic (T_C) cells and are class-I restricted. Thus the ratio of T_H to T_C cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders.

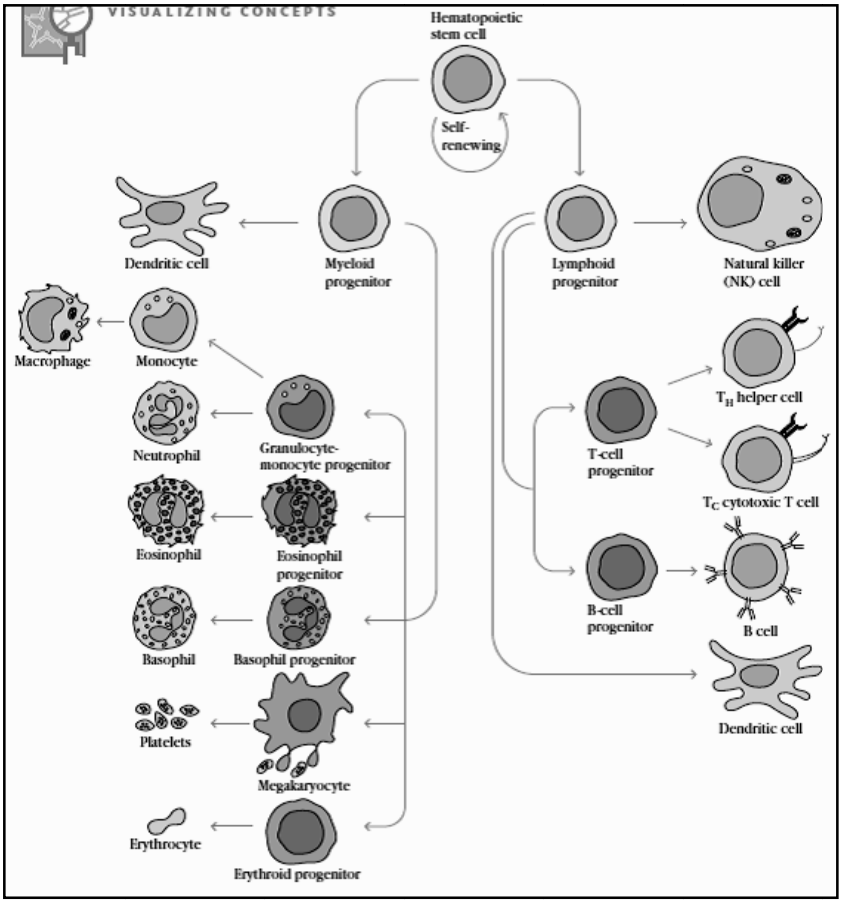


Figure: Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors.

- **Natural killer cells:** The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as **antibody-dependent cell mediated cytotoxicity (ADCC).**

TABLE 2-4 Normal adult blood-cell counts

Cell type	Cells/mm^3	%
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil		50–70
Lymphocyte		20–40
Monocyte		1–6
Eosinophil		1–3
Basophil		<1

Myeloid progenitor cells: The myeloid progenitor cells consist of mononuclear phagocytes, granular leukocytes and dendritic cells.

- **Mononuclear Phagocytes:** The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissues. During haematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 hours, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

i. Pulmonary alveolar macrophages in the lung

ii. Histiocytes in connective tissues

iii. Kupffer cells in the liver

iv. Glomerular Mesangial cells in the kidney

v. Microglial cells in the brain

vi. Osteoclasts in bone

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated T_H cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN-gamma) secreted by activated T_H cells.

Functions of macrophages:

1. Phagocytosis: Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called **chemotaxis**. The next step in phagocytosis is **adherence** of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material.

Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome**, which then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a lysosome to form a **phagolysosome**.

Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis.**

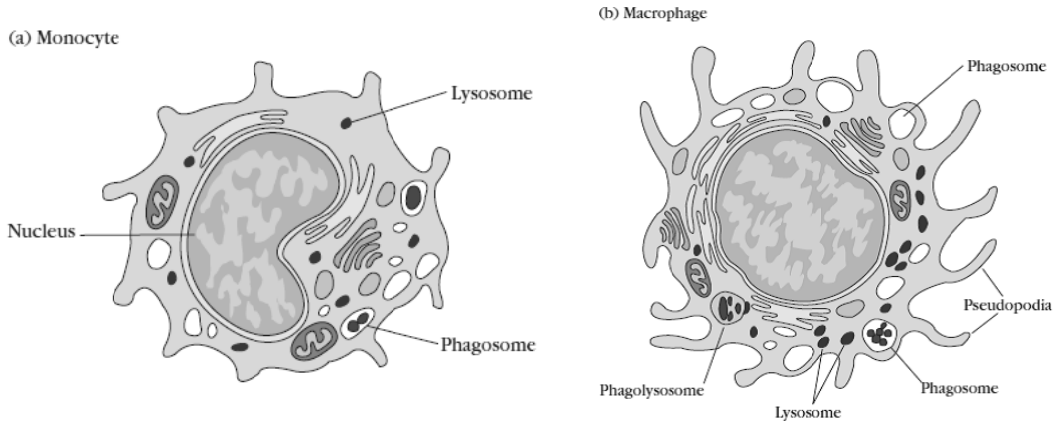


Figure: Typical morphology of a monocyte and a macrophage. Macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes.

2. Antimicrobial and cytotoxic activities: A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms. Many of the mediators of cytotoxicity are in the form of reactive forms of oxygen (ROS).

a. Oxygen dependent killing mechanisms: Activated phagocytes produce a number of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates that have potent antimicrobial activity. During phagocytosis, a metabolic process known as the **respiratory burst** occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen peroxideand chloride ions.

Hypochlorite, the active agent of household bleach, is toxic to ingested microbes. When macrophages are activated with bacterial cell-wall components such as lipopolysaccharide (LPS) or, in the case of mycobacteria, muramyl dipeptide (MDP), together with a T-cell–derived cytokine (IFN-gamma), they begin to express high levels of nitric oxide synthetase (NOS), an enzyme that oxidizes L-arginine to yield L-citrulline and nitric oxide (NO), a gas:

L-arginine + O_2 +NADPH → NO + L-citrulline + NADP

Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. Recent evidence suggests that much of the

antimicrobial activity of macrophages against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

b. Oxygen independent killing mechanisms: Activated macrophages also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins. These molecules are cysteine-rich cationic peptides containing 29–35 amino-acid residues. Each peptide, which contains six invariant cysteines, forms a circular molecule that is stabilized by intramolecular disulfide bonds. These circularized defensin peptides have been shown to form ion-permeable channels in bacterial cell membranes. Defensins can kill a variety of bacteria, including *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. Activatedmacrophages also secrete tumor necrosis factor (TNF-alpha), a cytokine that has a variety of effects and is cytotoxic for some tumor cells.

TABLE 2-6 Mediators of antimicrobial and cytotoxic activity of macrophages and neutrophils

Oxygen-dependent killing	Oxygen-independent killing
Reactive oxygen intermediates	Defensins
$O^{\cdot}{}_2^-$ (superoxide anion)	Tumor necrosis factor α
$OH^{\cdot}$ (hydroxyl radicals)	(macrophage only)
H_2O_2 (hydrogen peroxide)	Lysozyme
ClO^- (hypochlorite anion)	Hydrolytic enzymes
Reactive nitrogen intermediates	
NO (nitric oxide)	
NO_2 (nitrogen dioxide)	
HNO_2 (nitrous acid)	
Others	
NH_2CL (monochloramine)	

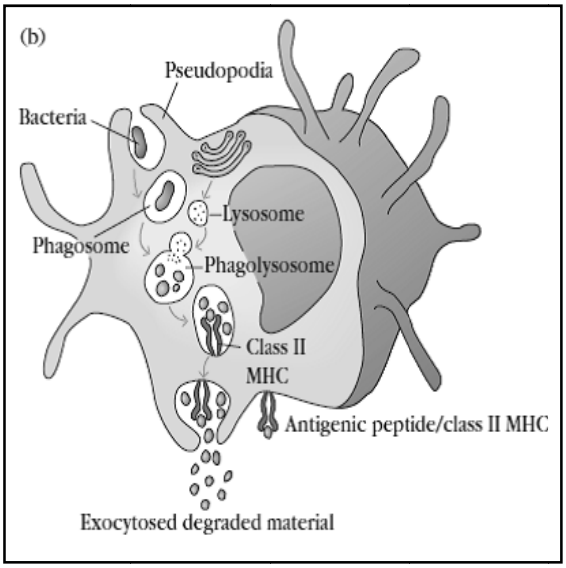


Figure: Phagocytosis and processing of exogenous antigen by macrophages.

- **Granulocytic Cells:** The granulocytes are classified as **neutrophils**, **eosinophils**, or **basophils** on the basis of cellular morphology and cytoplasmic staining characteristics. The neutrophil has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus. The eosinophil has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not.

  a. Neutrophils: Neutrophils are produced by haematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the

bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called **leucocytosis**, is used medically as an indication of infection. Movement of circulating neutrophils into tissues, called **extravasation**, takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces.

b. Eosinophils: Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defence against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.

c. Basophils: Basophils are nonphagocytic granulocytes that function by releasing pharmacologically active substances from their cytoplasmic granules. These substances play a major role in certain allergic responses.

d. Mast cells: Mast-cell precursors, which are formed in the bone marrow by haematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and enter the tissues. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances. Mast cells, together with blood basophils, play an important role in the development of allergies.

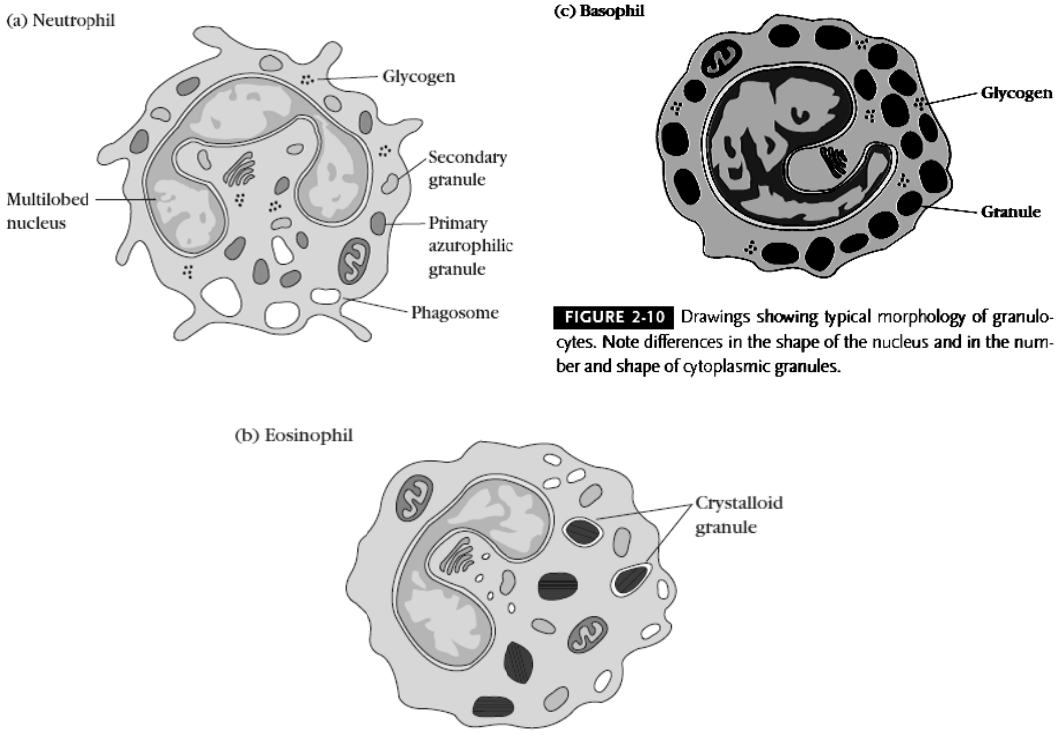


FIGURE 2-10 Drawings showing typical morphology of granulocytes. Note differences in the shape of the nucleus and in the number and shape of cytoplasmic granules.

e. Dendritic cells: The dendritic cell (DC) acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. Dendritic cells can be difficult to isolate because the conventional procedures for cell isolation tend to damage their long extensions. The development of isolation techniques that employ enzymes and gentler dispersion has facilitated isolation of these cells for study in vitro. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to T_H cells. Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Each arises from hematopoietic stem cells via different pathways and in different locations.

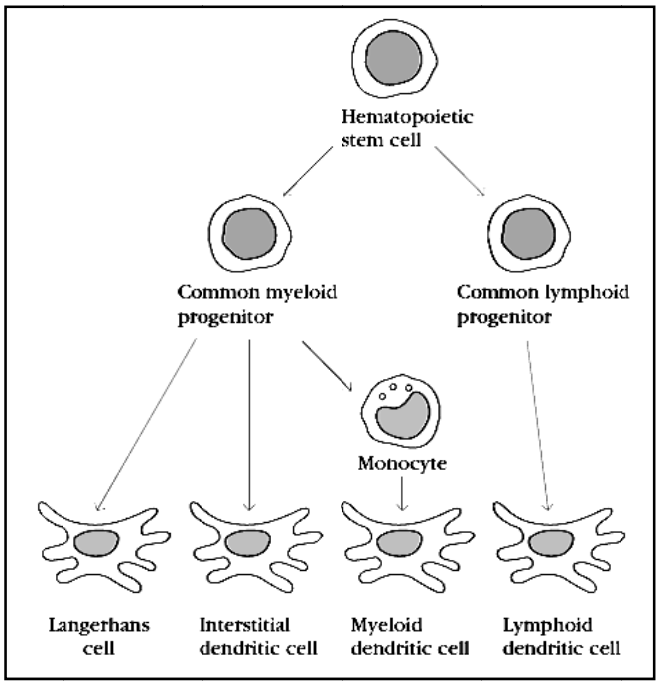


FIGURE 2-11 Dendritic cells arise from both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells. Some dendritic cells also arise from the lymphoid lineage. These considerations do not apply to follicular dendritic cells, which are not derived from bone marrow.

Organs and tissues of Immune System

The immune system consists of many different organs and tissues that are found throughout the body. These organs can be classified functionally into two main groups. The **primary lymphoid organs** provide appropriate microenvironments for the development and maturation of lymphocytes. The **secondary lymphoid organs** trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen. Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole.

Carried within the blood and lymph and populating the lymphoid organs are various white blood cells, or leukocytes, that participate in the immune response. Of these cells, only the lymphocytes possess the attributes of diversity, specificity, memory, and self/nonself recognition, the hallmarks of an adaptive immune response. All the other cells play accessory roles in adaptive immunity, serving to activate lymphocytes, to increase the effectiveness of antigen clearance by phagocytosis, or to secrete various immune-effector molecules. Some leukocytes, especially T lymphocytes, secrete various protein molecules called cytokines. These molecules act as immunoregulatory hormones and play important roles in the regulation of immune responses. This chapter describes the formation of blood cells, the properties of the various immune-system cells, and the functions of the lymphoid organs.

The immune system consists of a network of diverse organs and tissue which vary structurally as well as functionally from each other. These organs remain spreaded throughout the body. Basically, immune system is a complex network of lymphoid organs, tissues and cells.

These lymphoid organs can be categorized under two types depending upon their functional aspects: **i. Primary lymphoid organ. ii. Secondary lymphoid organ**.

- **Primary or Central Lymphoid Organs:**

Immature lymphocytes generated in haematopoiesis, the process of formation and development of blood cells, mature and become committed to a particular antigenic specificity within the primary lymphoid organs, namely, thymus, bursa of Fabricius (in birds) and bone marrow (in mammals). A lymphocyte becomes immunocompetent, i.e., capable of mounting an immune response only after it matures within a primary lymphoid organ.

1. Thymus: Thymus is a greyish, flat, bilobed lymphoid organ situated above the heart and extending into the neck on the front and sites of trachea. It develops from the epithelium of third and fourth pharyngeal pouches and, on maturity, acts as the site of development and maturation of lymphocytes named thymus-derived lymphocytes or T-lymphocytes or T-cells.

The thymus reaches peak activity in childhood and attains its largest size at puberty. Thereafter, the thymus begins to atrophy without any apparent effect on T-lymphocyte function and is extremely small in old age.

Of the T-lymphocytes produced in thymus only 5% leave the thymus as viable cells. Though the reason for this apparent wasteful process is not known, some believe that it is the elimination of T-lymphocyte clones that react against self. The function of the thymus is to generate T-lymphocytes and to confer immunological competence on to them during their stay in the organ. T-lymphocytes so educated in the thymus become capable of mounting cell-mediated immune response against appropriate antigen.

2. Bursa of Fabricius: It is a primary lymphoid organ in birds where stem cells from yolk sac, foetal liver, and bone marrow mature, proliferate, and differentiate into bursa-derived lymphocytes called B-lymphocytes or B-cells. Surgical removal of bursa (bursectomy) from newly hatched chickens destroys their subsequent ability to produce antibodies. The B-cells mature, proliferate, and differentiate into bursa and then migrate from it and reach outer or

superficial cortex of the germinal follicles and medullary cords of peripheral lymph nodes and lymphoid follicles of spleen where, following appropriate antigenic stimulation, transform into plasma cells and secrete antibodies. Like thymus, the bursal of Fabricius starts to shrink or atrophy at puberty.

3. Bone Marrow: Bone marrow is the site of origin and development of B-lymphocytes or B-cells (bone marrow derived lymphocytes) in mammals particularly in humans and mice after birth. Before birth, the yolk sac, foetal liver, and total bone marrow are the major sites of B-lymphocyte maturation. Bone marrow, therefore, is the mammalian equivalent of the bursa of Fabricius in birds.

Development of B-lymphocytes (B-cells) begins with the differentiation of lymphoid stem cells into the earliest distinctive progenitor B cells (pro-B cell), which proliferate within the bone marrow filling the extravascular spaces between large sinusoids in the shaft of a bone.

Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone marrow stromal cells.

- **Secondary or Peripheral Lymphoid Organs:** As stated earlier, the lymphocytes mature, proliferate, and differentiate in the primary or central lymphoid organs. These lymphocytes migrate from there via circulation to the secondary or peripheral lymphoid organs. Here they bind appropriate antigens and undergo further antigen-dependent differentiation.

  Once in the secondary lymphoid organs, the lymphocytes do not remain there but move from one lymphoid organ to another through the blood and lymphatic's. The passage of lymphocytes facilitates the induction of an immune response. Lymph nodes and the spleen are the most highly organized secondary or peripheral lymphoid organs, whereas mucosa-associated lymphoid tissue (MALT) is the less organized lymphoid tissue.

  1. Lymph Nodes: Lymph nodes are small, encapsulated, bean-shaped structures clustered at junctions of the lymphatic vessels which are distributed throughout the body. Lymph nodes contain a reticular network packed with lymphocytes, macrophages and dendritic cells, and filter out pathogenic microorganisms and antigens from the lymph.

  As the lymph percolates through a lymph node, any pathogen or antigen that is brought in with the lymph is trapped by the phagocytic cells and dendritic cells.

  Because of the presence of large number of T-lymphocytes in it. the Para-cortex is also referred to as a thymus-dependent area in contrast to the cortex which is a thymus-independent area. Medulla, the inner most region of lymph node, is more sparsely populated with lymphoid-lineage cells. Of the lymphoid-lineage cells present, many are plasma cells actively secreting antibody molecules.

  2. Spleen: The spleen, which is about 5 inches long and 200 g in weight in adults, is an ovoid encapsulated, and the largest secondary or peripheral lymphoid organ. Spleen is specialized for trapping blood-borne antigens and is present high in the left abdominal cavity and being

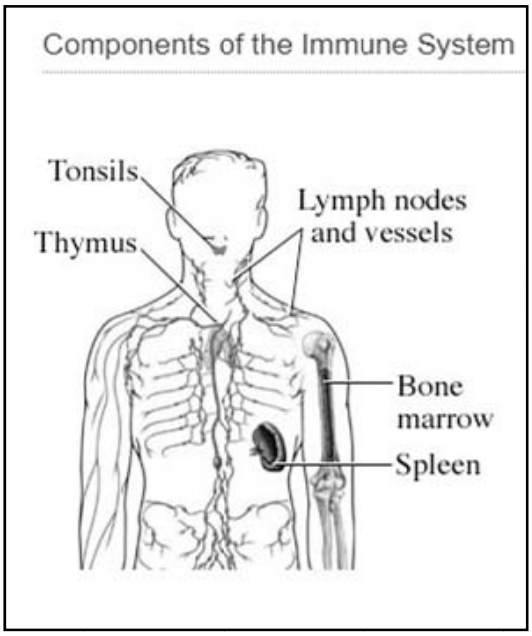
encapsulated, its capsule extends a number of projections, called trabeculae, into the interior resulting in the formation of compartments.

When the blood-borne antigens enter the spleen the B- and T-lymphocytes present in periarteriolar lymphoid sheath (PALS) are initially activated. Here, interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules (Major Histocompatibility Complex molecules) to T_H cells (T helper cells). Once activated, these T_H cells can then activate B- lymphocytes (B-cells). The activated B-lymphocytes, together with some T_H cells then migrate to primary follicles in the marginal zone. When the primary follicles are challenged by antigen, they differentiate into characteristic secondary follicles.

3. Mucosal-Associated Lymphoid Tissue (MALT): The mucous membranes lining the alimentary, respiratory, and genitourinary systems have a very large combined surface area, which is constantly exposed to numerous antigens and is the major site of entry for most pathogens.

These vulnerable membrane surfaces possess a group of organized lymphoid tissues which defend it from pathogens and antigens. The group of organized lymphoid tissues is known collectively as mucosal-associated lymphoid tissue (MALT).

There are several types of MALT; the most studied one is the gut-associated lymphoid tissue (GALT) which includes tonsils, Peyer's patch, appendix, and loosely organised clusters of lymphoid cells in the lamina propria of intestinal villi. Mucosal-associated lymphoid, tissue (MALT) is functionally very significant in immune system of the body because of the presence of large number of antibody-producing plasma cells in it. The number of plasma cells in MALT exceeds that of the total of the number of plasma cells present in spleen, lymph nodes, and bone marrow.



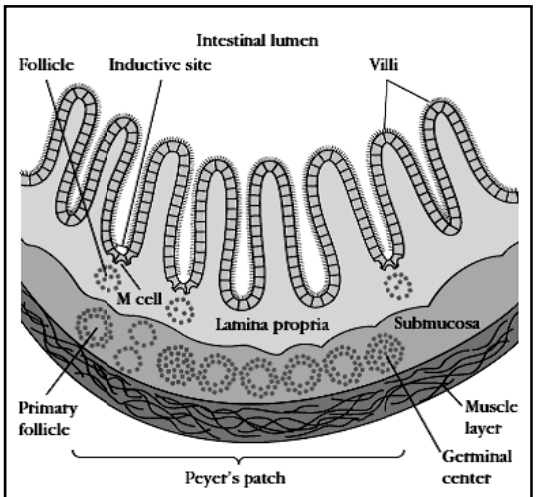


FIGURE 2-21 Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the submucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

An infection that begins in one area of the body eventually involves cells, organs, and tissues that may be distant from the site of pathogen invasion. Antigen from distant sites can arrive at lymph nodes via lymph and dendritic cells, thereby assuring activation of T cells and B cells and release

of these cells and their products to the circulation. Inflammatory processes bring lymphocytes and other leukocytes to the site of infection. Thus, although dispersed throughout the body, the components of the immune system communicate and collaborate to produce an effective response to infection.

Vertebrate orders differ greatly in the kinds of lymphoid organs, tissues, and cells they possess. The most primitive vertebrates, the jawless fishes, have only gut-associated lymphoid tissues, lack B and T cells, and cannot mount adaptive immune responses. Jawed vertebrates possess a greater variety of lymphoid tissues, have B and T cells, and display adaptive immunity.

Molecules of immune system:

1. Cytokines:

- Cytokines are a group of low-molecular- weight regulatory proteins secreted by WBC and other cells in the body.

- Cytokine secretion is very specific and self- limited event as because they are not usually stored as performed molecules. Cytokine synthesis is initiated by new gene transcription as a consequence of cellular activation. Once synthesized, cytokines are rapidly secreted, resulting in a burst of release when needed.

- After secretion, cytokines (almost 60 different types of cytokines) regulate immune and inflammatory reactions (Fig. 8.1A, B).

- Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells (Fig. 8.2). The nature of the target cell for a particular cytokine is determined by the presence of specific membrane receptors. Cytokines are so specific due to their high affinity for which Pico molar concentrations of cytokines can mediate a biological effect.

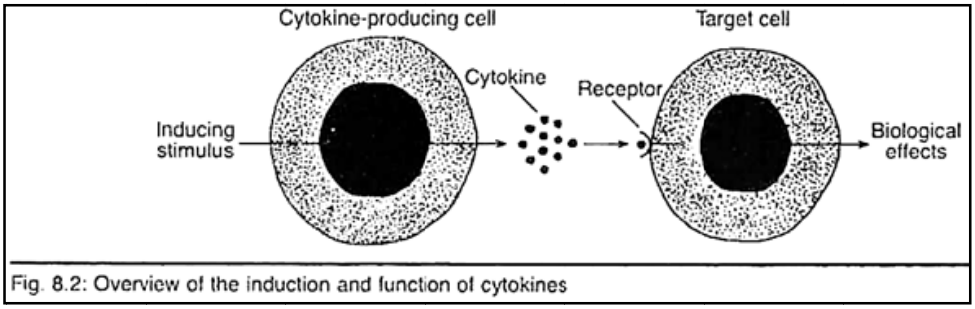


Fig. 8.2: Overview of the induction and function of cytokines

- Cytokine actions may be local or systemic.

  (i) Most of the cytokines act close to where they are produced, A particular cytokine when binds to receptors on the membrane of the same cell from where it has been secreted is called autocrine action (Fig. 8.3A).

  (ii) When secreted cytokines bind to receptors on a target cell in close proximity to the producer cells, it is called paracrine action (Fig. 8.3B).

  (iii) In most of the cases, cytokines act on cells that are in contact with the cytokine producers but when cytokines are produced in large amounts, it may enter the circulation and act at a distance from the site of production (Fig. 8.3C). This is called endocrine action.

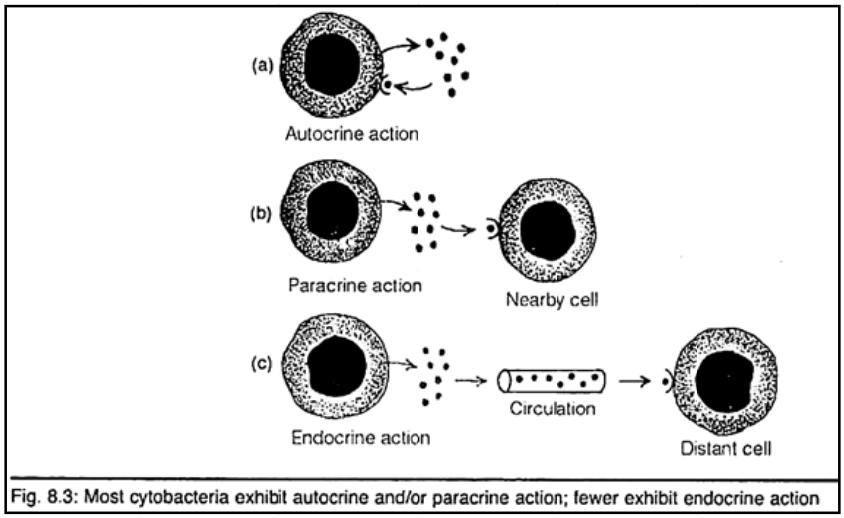


Fig. 8.3: Most cytobacteria exhibit autocrine and/or paracrine action; fewer exhibit endocrine action

- **Types of Immunity:** There are two major types of immunity: innate or natural or nonspecific and acquired or adaptive.

  (A) Innate or Natural or Nonspecific Immunity (L. innatus = inborn):

  Innate immunity is inherited by the organism from the parents and protects it from birth throughout life. For example humans have innate immunity against distemper, a fatal disease of dogs.

  As its name nonspecific suggests that it lacks specific responses to specific invaders. Innate immunity or nonspecific immunity is well done by providing different barriers to the entry of the foreign agents into our body. Innate immunity consists of four types of barriers— physical, physiological, cellular and cytokine barriers.

  1. Physical Barriers: They are mechanical barriers to many microbial pathogens. These are of two types. Skin and mucous membrane.

(a) Skin: The skin is physical barrier of body. Its outer tough layer, the stratum corneum (outermost layer of skin) prevents the entry of bacteria and viruses.

(b) Mucous Membranes: Mucus secreted by mucous membrane traps the microorganisms and immobilises them. Microorganisms and dust particles can enter the respiratory tract with air during breathing which are trapped in the mucus. The cilia sweep the mucus loaded with microorganisms and dust particles into the pharynx (throat). From the pharynx it is thrown out or swallowed for elimination with the faeces.

2. Physiological Barriers: The skin and mucous membranes secrete certain chemicals which dispose off the pathogens from the body. Body temperature, pH of the body fluids and various body secretions prevent growth of many disease causing microorganisms. Some of the important examples of physiological barriers are as follows:

(a) **Acidic pH of the stomach** kills most ingested microorganisms,

(b) **Bile** does not allow growth of microorganisms,

(c) **Cerumen** (ear wax) traps dust particles, kills bacteria and repels insects,

(d) **Lysozyme** is present in tissue fluids and in almost all secretions except in cerebrospinal fluid, sweat and urine. Lysozyme is in good quantity in tears from eyes. Lysozyme attacks bacteria and dissolves their cell walls.

(e) **Nasal Hair**. They filter out microbes and dust in nose,

(f) **Urine**. It washes microbes from urethra,

(g) **Vaginal Secretions**. It is slightly acidic which discourages bacterial growth and flush microbes out of vagina,

(h) **Sebum** (sweat). It forms a protective acid film over the skin surface that inhibits growth of many microbes.

3. Cellular Barriers: These are certain white blood corpuscles (leucocytes), macrophages, natural killer cells, complement system, inflammation, fever, antimicrobial substances, etc.

(i) Certain Leucocytes: Neutrophils and monocytes are the major phagocytic leucocytes.

(a) Polymorphonuclear Leucocytes (PMNL- neutrophils): As they have multilobed nucleus they are normally called polymorphonuclear leucocytes. Neutrophils are short lived and are highly motile phagocytic killers. Neutrophils are formed from stem cells in the bone marrow. Neutrophils are the most numerous of all leucocytes. They die after a few days and must therefore, be constantly replaced. Neutrophils constitute about 40% to 75% of the blood leucocytes in humans.

(b) Monocytes: They are the largest of all types of leucocytes and somewhat amoeboid in shape. They have clear cytoplasm (without cytoplasmic granules). The nucleus is bean-shaped. Monocytes constitute about 2-10% of the blood leucocytes. They are motile and phagocytic in nature and engulf bacteria and cellular debris. Their life span is about 10 to 20 hours. Generally they change into macrophages after entering tissue spaces.

(ii) Macrophages: Monocytes circulate in the bloodstream for about 8 hours, during which time they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages. Macrophages are long lived and are highly motile phagocytic.

Macrophages contain more cell organelles especially lysosomes. Macrophages are of two types, (a) Some take up residence in particular tissues becoming fixed macrophages (b) whereas, other remain motile and are called wandering macrophages. Wandering macrophages move by amoeboid movement throughout the tissues. Fixed macrophages serve different functions in different tissues and are named to reflect their tissue location. Some examples are given below:

(iii) Natural Killer Cells (NK Cells): Besides the phagocytes, there are natural killer cells in the body which are a type of lymphocytes and are present in the spleen, lymph nodes and bone marrow. NK cells do not have antigen receptors like T cells and B cells. NK cells cause cellular destruction in at least two ways:

(a) NK cells produce perforins which are chemicals that when inserted into the plasma membrane of a microbe make so weak that cytolysis (breakdown of cells particularly their outer membrane) occurs and creates pores in the plasma membrane of the target cells. These pores allow entry of water into the target cells, which then swell and burst. Cellular remains are eaten by phagocytes.

(b) Another function of NK cells is apoptosis which means natural cell death. It occurs naturally as part of the normal development, maintenance and renewal of cells, tissues and organs.

Thus, functions of NK cells are to destroy target cells by cytolysis and apoptosis. NK cells constitute 5%-10% of the peripheral blood lymphocytes in humans.

(iv) Complement system: Complement is a group of 20 proteins, many of which are enzyme precursors and are produced by the liver. These proteins are present in the serum of the blood (the fluid portion of the blood excluding cells and clotting factors) and on plasma membranes. They are found circulating in the blood plasma and within tissues throughout the body. They were named complement by Ehrlich because they complement the actions of other components of the immune system (e.g., action of antibody on antigen) in the fight against infection. Jules Bordet is the discoverer of complement.

Complement proteins create pores in the plasma membrane of the microbes. Water enters the microbes. The latter burst and die. The proteins of complement system destroy microbes by (i) cytolysis (ii) inflammation and (iii) phagocytosis. These proteins also prevent excessive damage of the host tissues.

(v) Inflammation: Inflammation is a defensive response of the body to tissue damage. The conditions that may produce inflammation are pathogens, abrasions (scraping off) chemical irritations, distortion or disturbances of cells, and extreme temperatures. The signs and symptoms of inflammation are redness, pain, heat and swelling.

Inflammation can also cause the loss of function in the injured area, depending on the site and extent of the injury. Inflammation is an attempt to dispose of microbes, toxins, or foreign material at the site of injury to prevent their spread to other tissues, and to prepare the site for tissue repair. Thus, it helps restore tissue homeostasis.

Broken mast cells release histamine. Histamine causes dilation of capillaries and small blood vessels. As a result more blood flows to that area making it red and warm and fluid (plasma) takes out into the tissue spaces causing its swelling. This reaction of the body is called inflammatory response.

(vi) Fever: Fever may be brought about by toxins produced by pathogens and a protein called endogenous pyrogen (fever producing substance), released by macrophages. When enough pyrogens reach the brain, the body's thermostat is reset to a higher temperature, allowing the temperature of the entire body to rise.

Mild fever strengthens the defence mechanism by activating the phagocytes and by inhibiting the growth of microbes. A very high temperature may prove dangerous. It must be quickly brought down by giving antipyretics.

4. Cytokine Barriers: Cytokines (Chemical messengers of immune cells) are low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells. They are involved in the cell to cell communication. Kinds of cytokines include interleukins produced by leucocytes, lymphocytes produced by lymphocytes, tumour necrosis factor and interferon's (IFNs). Interferon's protect against viral infection of cells.

Probable questions:

1. List the primary lymphoid organs and summarize their functions in the immune response.
2. List the secondary lymphoid organs and summarize their functions in the immune response.
3. What are the two primary characteristics that distinguish hematopoietic stem cells and progenitor cells?
4. What are the two primary roles of the thymus?
5. What effect would removal of the bursa of Fabricius (bursectomy) have on chickens?
6. Highlight the difference between innate and adaptive immunity.
7. What do you mean by hematopoiesis?
8. Classify the immune cells on the basis of origin.

9. What is antibody dependent cell mediated cytotoxicity?
10. Classify macrophages on the basis of location.
11. What is GALT and MALT?

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Unit-II

Innate immunity: Overview, features, neutrophils, macrophage functions inflammation NK cells

Objective: In this unit, we will discuss about innate immunity: overview, features, neutrophils, macrophage functions inflammation NK cells.

Innate Immunity

Innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory (Table 1-2).

The Skin and the Mucosal Surfaces Provide Protective Barriers Against Infection

Physical and anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms. The skin consists of two distinct layers: a thinner outer layer—the epidermis—and a thicker layer—the dermis. The epidermis contains several layers of tightly packed epithelial cells. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The dermis, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called sebum. Sebum consists of lactic acid and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms. A few bacteria that metabolize sebum live as commensals on the skin and sometimes cause a severe form of acne. One acne drug, isotretinoin (Accutane), is a vitamin A derivative that prevents the formation of sebum.

Breaks in the skin resulting from scratches, wounds, or abrasion are obvious routes of infection. The skin may also be penetrated by biting insects (e.g., mosquitoes, mites, ticks, fleas, and sandflies); if these harbor pathogenic organisms, they can introduce the pathogen into the body as they feed. The protozoan that causes malaria, for example, is deposited in humans by mosquitoes when they take a blood meal. Similarly, bubonic plague is spread by the bite of fleas, and Lyme disease is spread by the bite of ticks.

TABLE 1-2 Summary of nonspecific host defenses

Type	Mechanism
Anatomic barriers	
Skin	Mechanical barrier retards entry of microbes. Acidic environment (pH 3–5) retards growth of microbes.
Mucous membranes	Normal flora compete with microbes for attachment sites and nutrients. Mucus entraps foreign microorganisms. Cilia propel microorganisms out of body.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.
Low pH	Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or facilitates phagocytosis. Toll-like receptors recognize microbial molecules, signal cell to secrete immunostimulatory cytokines. Collectins disrupt cell wall of pathogen.
Phagocytic/endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.
Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

Physiological Barriers to Infection Include General Conditions and Specific Molecules

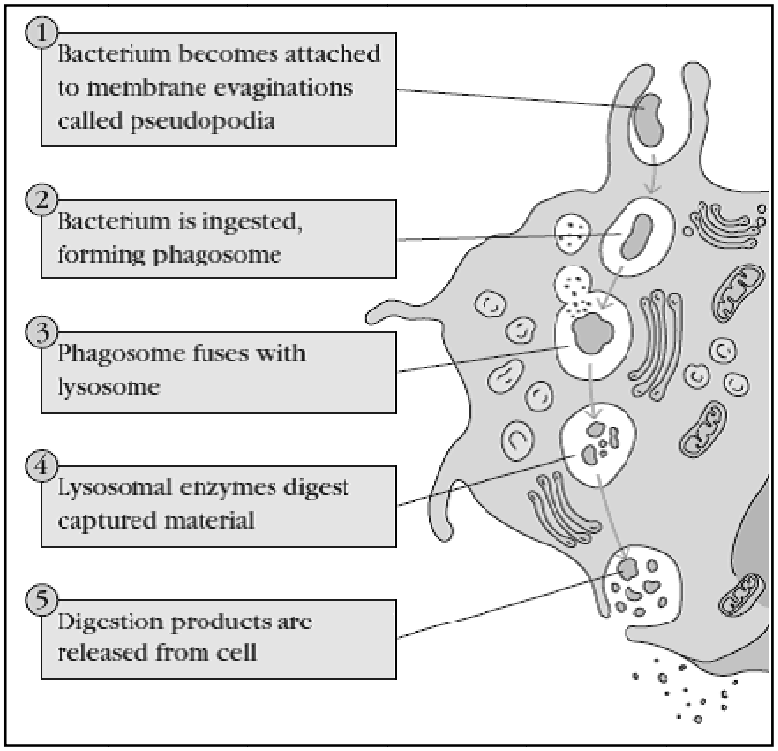
The physiological barriers that contribute to innate immunity include temperature, pH, and various soluble and cell associated molecules. Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason newborns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults.

A variety of soluble factors contribute to innate immunity, among them the soluble proteins lysozyme, interferon, and complement. Lysozyme, a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. Interferon comprises a group of proteins produced by virus-infected cells. Among the many functions of the interferons is the ability to bind to nearby cells and induce a generalized antiviral state. Complement, is a group of serum proteins that circulate in an inactive state. A variety of specific and nonspecific immunologic mechanisms can convert the inactive forms of complement proteins into an active state with the ability to damage the membranes of pathogenic organisms, either destroying the pathogens or facilitating their clearance. Complement may function as an effector system that is triggered by binding of antibodies to certain cell surfaces, or it may be activated by reactions between complement molecules and certain components of microbial cell walls. Reactions between complement molecules or fragments of complement molecules and cellular receptors trigger activation of cells of the innate or adaptive immune systems. Recent studies on collections indicate that these surfactant proteins may kill certain bacteria directly by disrupting their lipid membranes or, alternatively, by aggregating the bacteria to enhance their susceptibility to phagocytosis.

Many of the molecules involved in innate immunity have the property of pattern recognition, the ability to recognize a given class of molecules. Because there are certain types of molecules that are unique to microbes and never found in multicellular organisms, the ability to immediately recognize and combat invaders displaying such molecules is a strong feature of innate immunity. Molecules with pattern recognition ability may be soluble, like lysozyme and the complement components described above, or they may be cell-associated receptors. Among the class of receptors designated the toll-like receptors (TLRs), TLR2 recognizes the lipopolysaccharide (LPS) found on Gram-negative bacteria. It has long been recognized that systemic exposure of mammals to relatively small quantities of purified LPS leads to an acute inflammatory response. The mechanism for this response is via a TLR on macrophages that recognizes LPS and elicits a variety of molecules in the inflammatory response upon exposure.When the TLR is exposed to the LPS upon local invasion by a Gram-negative bacterium, the contained response results in elimination of the bacterial challenge.

Cells That Ingest and Destroy Pathogens Make Up a Phagocytic Barrier to Infection:
Another important innate defence mechanism is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is one type of endocytosis, the general term for the uptake by a

cell of material from its environment. In phagocytosis, a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes (Figure 1-3). Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages. Most cell types are capable of other forms of endocytosis, such as receptor-mediated endocytosis, in which extracellular molecules are internalized after binding by specific cellular receptors, and pinocytosis, the process by which cells take up fluid from the surrounding medium along with any molecules contained in it.



Inflammation Represents a Complex Sequence of Events That Stimulates Immune Responses

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. As described above, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system. Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. In the first century AD, the Roman physician Celsus described the "four cardinal signs of inflammation" as rubor (redness), tumor (swelling), calor (heat), and dolor (pain). In the second century AD, another physician, Galen, added a fifth sign: functiolaesa (loss of function). The cardinal signs of inflammation reflect the three major events of an inflammatory response (Figure 1-4):

1. **Vasodilation**—an increase in the diameter of blood vessels—of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict, resulting in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.
2. An increase in capillary permeability facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates (exudate) has a much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling (edema).
3. Influx of phagocytes from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels (margination), followed by their emigration between the capillary endothelial cells into the tissue (diapedesis or extravasation), and, finally, their migration through the tissue to the site of the invasion (chemotaxis). As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.
The events in the inflammatory response are initiated by a complex series of events involving a variety of chemical mediators whose interactions are only partly understood. Some of these

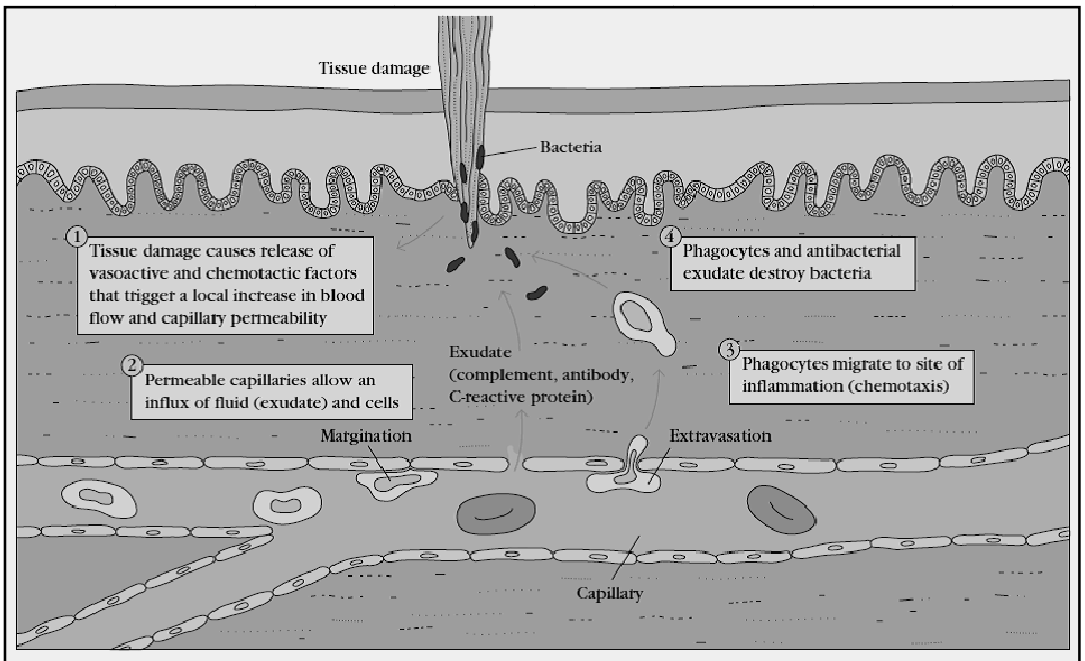


FIGURE 1-4 Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and an influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have antibacterial properties, and the phagocytes begin to engulf the bacteria, as illustrated in Figure 1-3.

mediators are derived from invading microorganisms, some are released from damaged cells in response to tissue injury, some are generated by several plasma enzyme systems, and some are

products of various white blood cells participating in the inflammatory response. Among the chemical mediators released in response to tissue damage are various serum proteins called acute-phase proteins. The concentrations of these proteins increase dramatically in tissue-damaging infections. C-reactive protein is a major acute-phase protein produced by the liver in response to tissue damage. Its name derives from its pattern recognition activity: C-reactive protein binds to the C-polysaccharide cell-wall component found on a variety of bacteria and fungi. This binding activates the complement system, resulting in increased clearance of the pathogen either by complement-mediated lysis or by a complement mediated increase in phagocytosis.

One of the principal mediators of the inflammatory response is histamine, a chemical released by a variety of cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability. Another important group of inflammatory mediators, small peptides called kinins, are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and increasedpermeability of capillaries. A particular kinin, called bradykinin, also stimulates pain receptors in the skin. This effect probably serves a protective role, because pain normally causes an individual to protect the injured area. Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of fibrin, which is the main component of a blood clot. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection. Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue forms.

Natural Killer (NK) cells:

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumour cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumour cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently

destroy the targeted cells. This is an example of a process known as **antibody-dependent cell mediated cytotoxicity (ADCC)**.

Several observations suggest that NK cells play an important role in host defence against tumours. For example, in humans the Chediak-Higashi syndrome—an autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called beige lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumour cells.

There has been growing recognition of a cell type, the NK1-T cell, that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-T cells have T cell receptors (TCRs).Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like molecules called CD1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of the cytokines needed to support antibody production by B cells as well as inflammation and the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH responses are still developing.

Probable questions:

1. Specific immunity exhibits four characteristic attributes, which are mediated by lymphocytes. List these four attributes and briefly explain how they arise.
2. Name three features of a secondary immune response that distinguish it from a primary immune response.
3. Briefly describe the three major events in the inflammatory response.
4. Innate and adaptive immunity act in cooperative and interdependent ways to protect the host. Discuss the collaboration of these two forms of immunity.
5. What are the functions of NK cell?
6. Explain the role of macrophages in elimination of foreign particulates.

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Unit-III

Humoral immune system: Structure and class switching of antibodies, B cell function, maturation and development

Objective: In this unit, we will discuss about the humoral immune system: Structure and class switching of antibodies, B cell function, maturation and development.

Humoral Immune System:

Humoral immune response is also called B-cell mediated immunity because B-lymphocytes are involved in this response. Humoral immune response is to defend the body against pathogens that may invade body fluids or humor. B cells are antigen specific.

During immune response, B cells, specific for the antigen, enlarge to become lymphoblasts that further differentiate to form plasma cells. The mature plasma cells produce gamma globulins or immunoglobulins called antibodies at a rapid rate of about 2000 molecules per second for each plasma cell. The antibodies secreted into the lymph eventually enter the blood.

The antibody molecule is the basic functional unit of this type of immune response. So this immune response is also called antibody-mediated immune response. The flow chart summarizes the various steps involved in the humoral response.

Definition of Antibody:

Antibodies are immunoglobulin's (Igs) which are produced in the body in response to the antigen or foreign bodies. Thus all antibodies are immunoglobulin's but all immunoglobulin's are not antibodies.

Location and Formation:

The antibodies may be bound to a cell membrane or they may remain free. Antibodies are produced by B lymphocytes and plasma cells. In fact B-lymphocytes get transformed into plasma cells. The mature plasma cell produces antibodies at an extremely rapid rate— about 2000 molecules per second. Antibodies direct the antibody- mediated immunity (=humoral immunity).

Types of Antibodies:

There are five types of antibodies viz:

1. IgA (Ig alpha);
2. IgD (Ig delta);
3. IgE (Ig epsilon);
4. IgG (Ig gamma) and
5. IgM (Ig mu).

Among the antibodies, IgG forms 80% of the antibodies in the body.

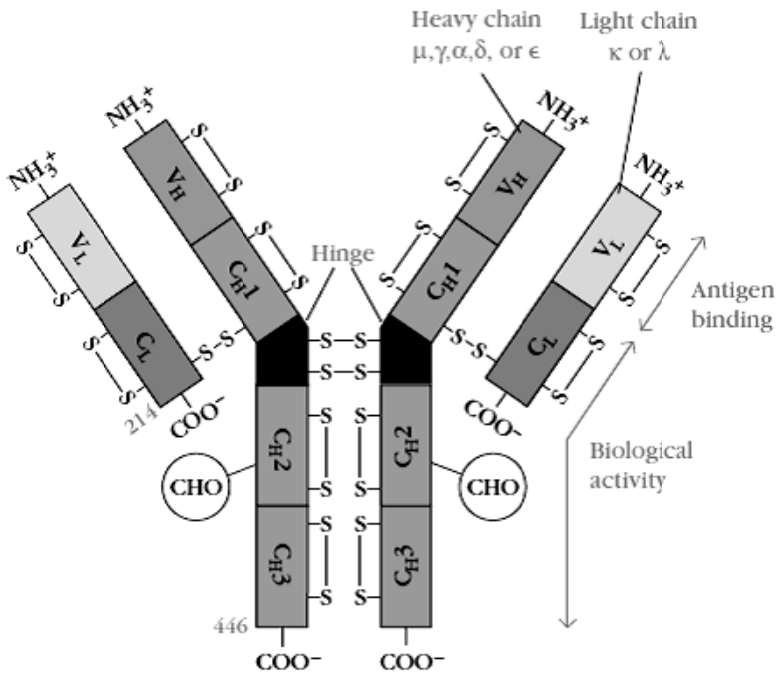

FIGURE 4-2 Schematic diagram of structure of immunoglobulins derived from amino acid sequencing studies. Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region (aqua and tan, respectively) that consists of 100–110 amino acids and differs from one antibody to the next. The remainder of each chain in the molecule—the constant (C) regions (purple and red)—exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains (γ, δ, and α) also contain a proline-rich hinge region (black). The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule.

Antibody Structure:

IgG has been studied extensively and serves as a model of basic structural unit of all Igs. An antibody molecule consists of the following parts.

(i) Heavy and Light Chains:

An antibody molecule is made up of 4 peptide chains, two small called light chains and two longer called heavy chains. Hence an antibody is represented as H_2L_2. The heavy chain has larger number of amino acids while light chain has smaller number of amino acids. Heavy and light chains may be either lambda or Kappa type.

(ii) Constant and Variable Regions:

There are two different regions the constant region and variable region in each chain of the antibody.

(iii) Disulfide Bonds and Hinge Region:

A disulfide bond joins a light chain with a heavy chain. Two disulfide bonds also link the two heavy chains. This part of the antibody displays considerable flexibility and is called the hinge region. Because the antibody "arms" can move somewhat as the hinge region bends, an antibody can assume a Y shaped molecule.

(iv) Fragment Antigen Binding (Fab) and Fragment Crystallisable (Fc):

Two identical fragments of Y-shaped molecule possess the antigen-binding sites and are thus named fragment-antigen binding (Fab). The antigen-binding sites bind to the specific antigens in a lock and key pattern, forming an antigen-antibody complex. The third fragment which lacks the ability to bind to antigen and can be crystallized, is, therefore, known as fragment crystallizable (Fc).

The stem of the Y-shaped antibody monomer is called the F_c region, so named because when antibody structure was first being identified, it was a fragment (F) that crystallized (c) in cold storage.

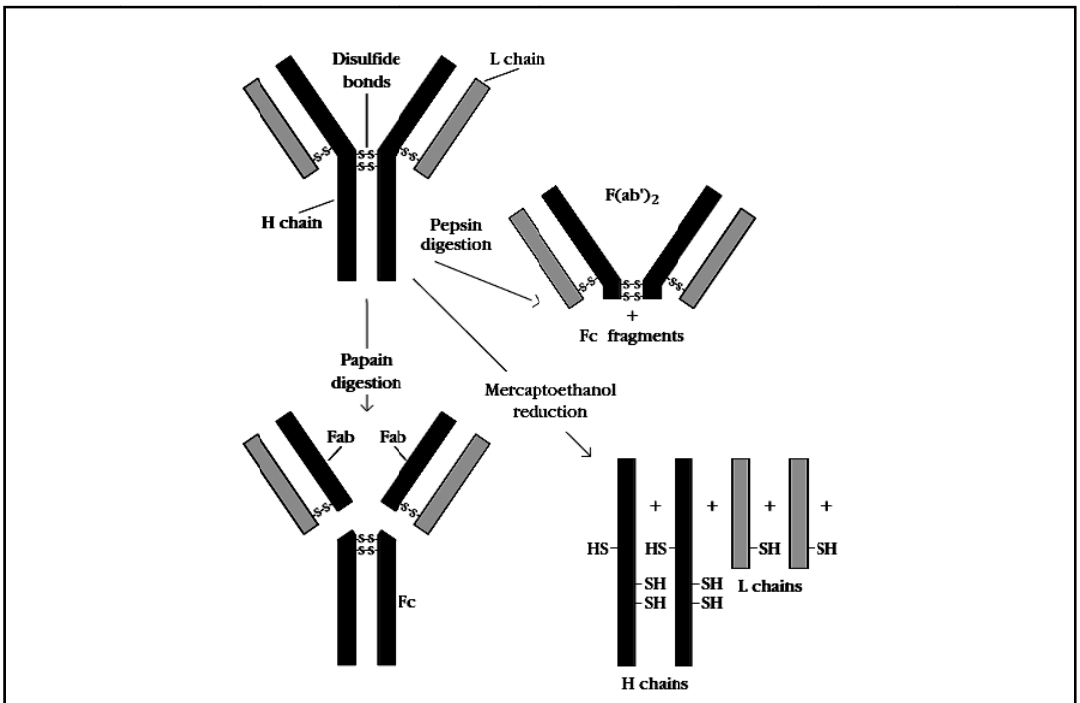

FIGURE 4-3 Prototype structure of IgG, showing chain structure and interchain disulfide bonds. The fragments produced by various treatments are also indicated. Light (L) chains are in gray and heavy (H) chains in blue.

Characteristics and Functions of Immunoglobulin's (Igs) or Antibodies:

Antibodies show the following characteristics and perform different functions.

(i) IgA:

It is the second most abundant class, constituting about 10 to 15 per cent of antibodies of serum. It is mainly found in sweat, tears, saliva, mucus, colostrum (first milk secreted by a mother) and gastrointestinal secretions.

Smaller quantities are present in blood and lymph. IgA has an extra polypeptide called a J-(joining) chain and extra protein known as secretory component. Levels decrease during stress, lowering resistance to infection. Provides localized protection in external secretions (tears, intestinal secretions, etc.) against bacteria and viruses. When IgA is excreted through faeces, it is called copro antibody.

(ii) IgD:

It is mainly found on the surfaces of B cells as antigen receptors, where it activates B cells for antigen recognition. It is about 0.2% of all antibodies in the blood.

(iii) IgE:

It is less than 0.1% of all antibodies in the blood; located on mast cells and basophils releasing histamine from mast cells and basophils. It is involved in allergic and hypersensitivity reactions; provides protection against parasitic worms. This immunoglobulin was discovered in 1966 by Ishizaka.

It exhibits unique properties such as heat lability (inactivated at 56°C in one hour). IgE mediates type I hypersensitivity (anaphylaxis). Prausnitz and Kustner in 1921 demonstrated transmission of IgE-mediated type I hypersensitivity. It is called Prausnitz-Kustner (PK) reaction. Thus IgE acts as mediator in allergic response.

(iv) IgG:

This is the most abundant class of Ig in the body constituting approximately 80% of the total Igs. It is found in the blood, lymph and intestine. It protects against bacteria and viruses by enhancing phagocytosis, neutralizing toxins and complement activation. It is the only class of antibody to cross the placenta from mother to foetus thereby conferring considerable immune protection in newborns.

(v) IgM:

IgM is about 5 to 10% of all antibodies in the blood. It is also found in lymph. It is the largest Ig which is secreted first by the plasma cells. It is so named because it is a macroglobulin at least five times larger than IgG. IgM is the oldest immunoglobulin class. It activates the B cells. It is also the earliest immunoglobin to be synthesised by the foetus, IgM has a J chain and its each dimer contains polypeptide called a secretory component.

It cannot cross the placental barrier. IgM is 500-1000 times more effective than IgG in opsonisation (to be described ahead), in bacterial action and in bacterial agglutination. But in neutralization of toxins and viruses, it is less active than IgG. It helps in complement activation.

	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ (mu)	γ (gamma)	α (alpha)	ε (epsilon)	δ (delta)
MW (Da)	900k	150k	385k	200k	180k
% of total antibody in serum	6%	80%	13%	0.002%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement. Monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B cell Receptor

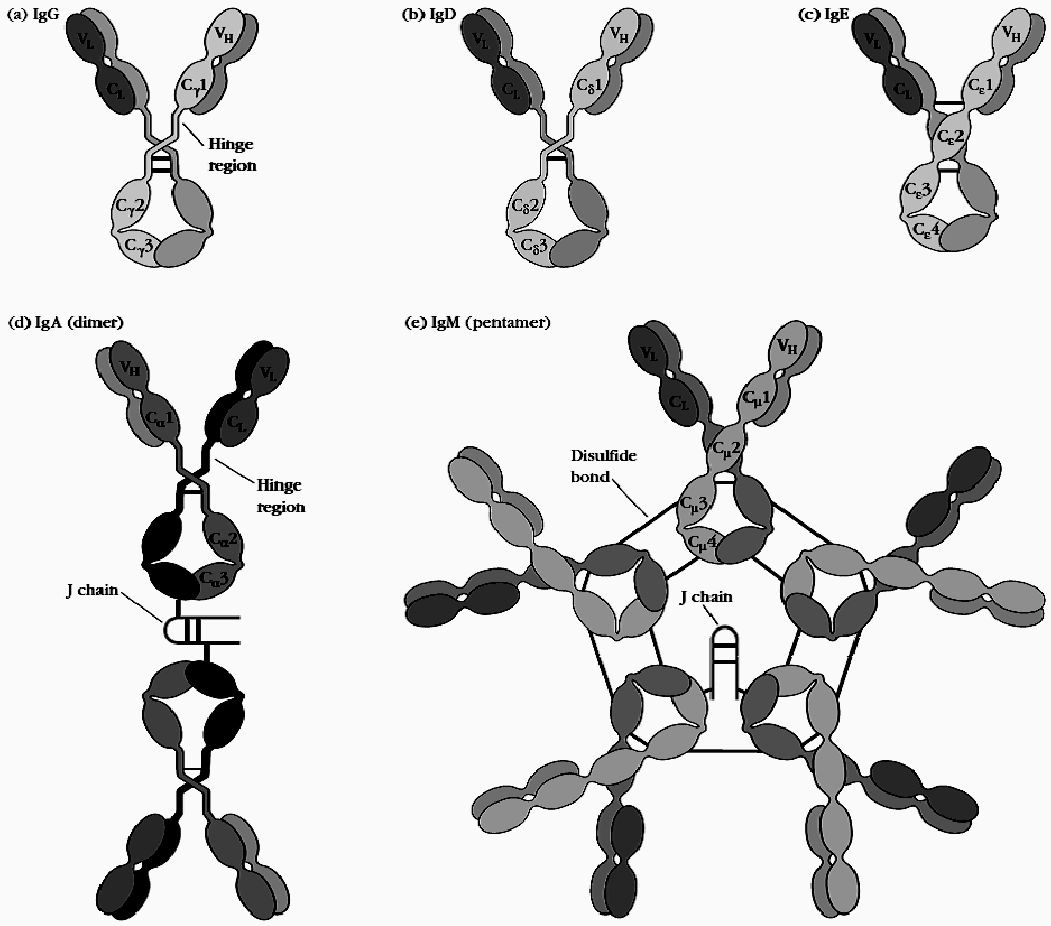

FIGURE 4-13 General structures of the five major classes of secreted antibody. Light chains are shown in shades of pink, disulfide bonds are indicated by thick black lines. Note that the IgG, IgA, and IgD heavy chains (blue, orange, and green, respectively) contain four domains and a hinge region, whereas the IgM and IgE heavy chains (purple and yellow, respectively) contain five domains but no hinge region. The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers. Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present. Not shown in these figures are intrachain disulfide bonds and disulfide bonds linking light and heavy chains (see Figure 4-2).

Class switching of antibodies:

Immunoglobulin class switching, also known as isotype switching, isotypic commutation or class-switch recombination (CSR), is a biological mechanism that changes a B cell's production of immunoglobulin (antibodies) from one type to another, such as from the isotype IgM to the isotype IgG. During this process, the constant-region portion of the antibody heavy chain is changed, but the variable region of the heavy chain stays the same (the terms "variable" and "constant" refer to changes or lack thereof between antibodies that target different epitopes). Since the variable region does not change, class switching does not affect antigen specificity. Instead, the antibody retains affinity for the same antigens, but can interact with different effector molecules.

Class switching occurs after activation of a mature B cell via its membrane-bound antibody molecule (or B cell receptor) to generate the different classes of antibody, all with the same variable domains as the original antibody generated in the immature B cell during the process of V(D)J recombination, but possessing distinct constant domains in their heavy chains.

Naïve mature B cells produce both IgM and IgD, which are the first two heavy chain segments in the immunoglobulin locus. After activation by antigen, these B cells proliferate. If these activated B cells encounter specific signaling molecules via their CD40 and cytokine receptors (both modulated by T helper cells), they undergo antibody class switching to produce IgG, IgA or IgE antibodies. During class switching, the constant region of the immunoglobulin heavy chain changes but the variable regions, and therefore antigenic specificity, stay the same. This allows different daughter cells from the same activated B cell to produce antibodies of different isotypes or subtypes (e.g. IgG1, IgG2 etc.).

The order of the heavy chain exons are as follows:

μ - IgM, δ - IgD, γ_3 - IgG3, γ_1 - IgG1, α_1 - IgA1, γ_2 - IgG2, γ_4 - IgG4, ε - IgE, α_2 - IgA2

Class switching occurs by a mechanism called class switch recombination (CSR) binding. Class switch recombination is a biological mechanism that allows the class of antibody produced by an activated B cell to change during a process known as isotype or class switching. During CSR, portions of the antibody heavy chain locus are removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces antibody of a different isotype. Double-stranded breaks are generated in DNA at conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain. DNA is nicked and broken at two selected S-regions by the activity of a series of enzymes, including Activation-Induced (Cytidine) Deaminase (AID), uracil DNA glycosylase and apyrimidic/ apurinic (AP)-endonucleases. The intervening DNA between the S-regions is subsequently deleted from the chromosome, removing unwanted μ or δ heavy chain constant region exons and allowing substitution of a γ, α or ε constant region gene segment. The free ends of the DNA are rejoined by a process called non-homologous end joining (NHEJ) to link the variable domain exon to the desired downstream constant domain exon of the antibody heavy chain. In the absence of non-homologous end

joining, free ends of DNA may be rejoined by an alternative pathway biased toward microhomology joins. With the exception of the μ and δ genes, only one antibody class is expressed by a B cell at any point in time. While class switch recombination is mostly a deletional process, rearranging a chromosome in "cis", it can also occur (in 10 to 20% of cases, depending upon the Ig class) as an inter-chromosomal translocation mixing immunoglobulin heavy chain genes from both alleles.

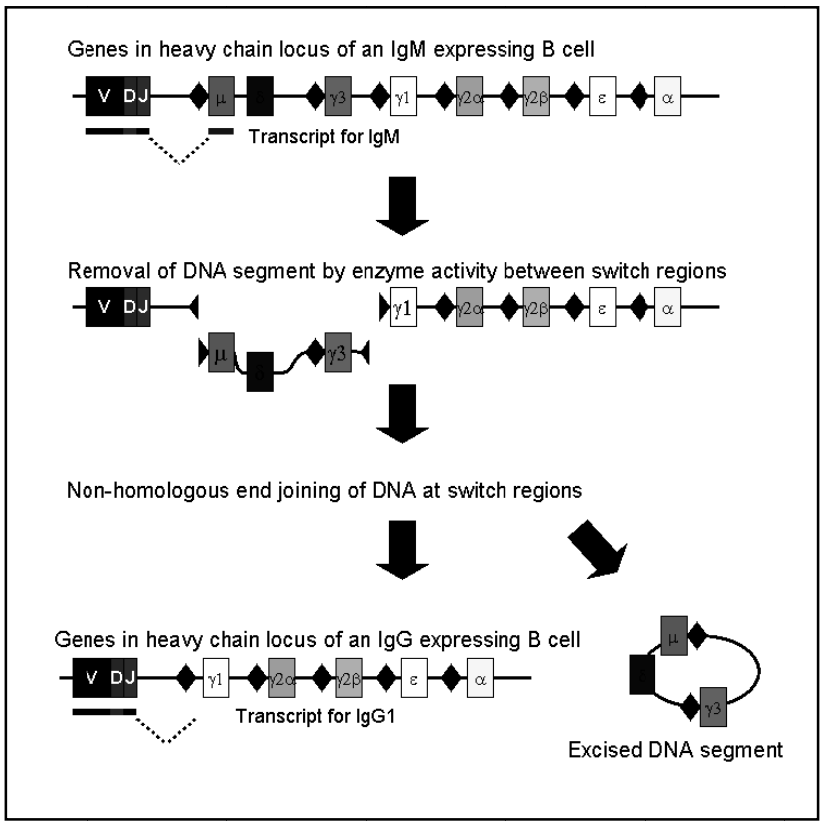

Figure: Class switching of antibody molecule

- **B-Cell Maturation and development:**

  The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac,fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

  B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the progenitor B cell (pro-B cell)—which expresses a transmembrane tyrosine phosphatase called CD45R (sometimes called B220 in mice). Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured *in vitro*, they will not progress to more mature B-cell stages unless

stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that support the developmental process.

The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation. The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell. At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness (anergy) rather than division and differentiation. Full maturation is signaled by the co-expression of IgD and IgM on the membrane.

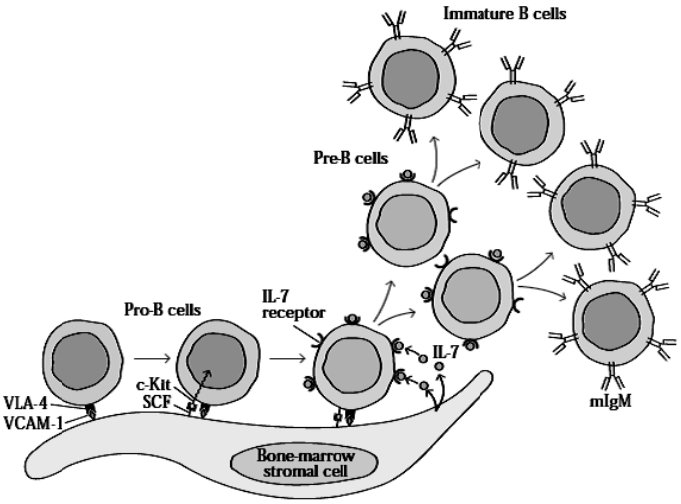

FIGURE 11-2 Bone-marrow stromal cells are required for maturation of progenitor B cells into precursor B cells. Pro-B cells bind to stromal cells by means of an interaction between VCAM-1 on the stromal cell and VLA-4 on the pro-B cell. This interaction promotes the binding of c-Kit on the pro-B cell to stem cell factor (SCF) on the stromal cell, which triggers a signal, mediated by the tyrosine kinase activity of c-Kit, that stimulates the pro-B cell to express receptors for IL-7. IL-7 released from the stromal cell then binds to the IL-7 receptors, inducing the pro-B cell to mature into a pre-B cell. Proliferation and differentiation evenutally produces immature B cells.

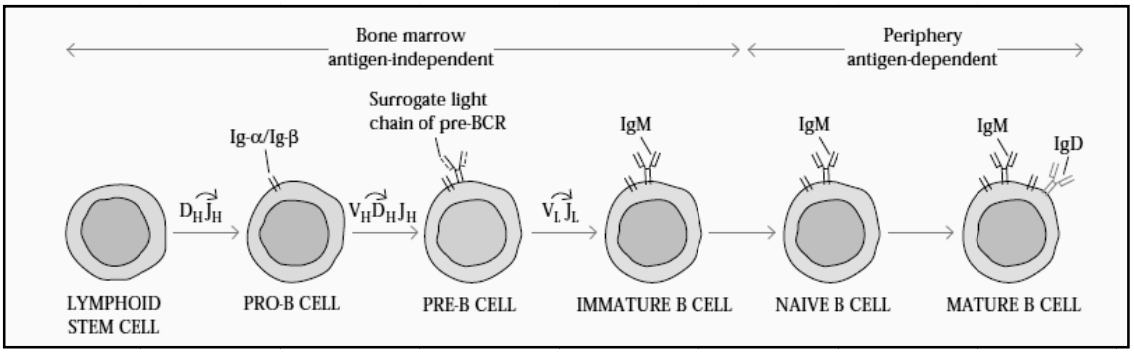

Figure: Development and maturation of B cell.

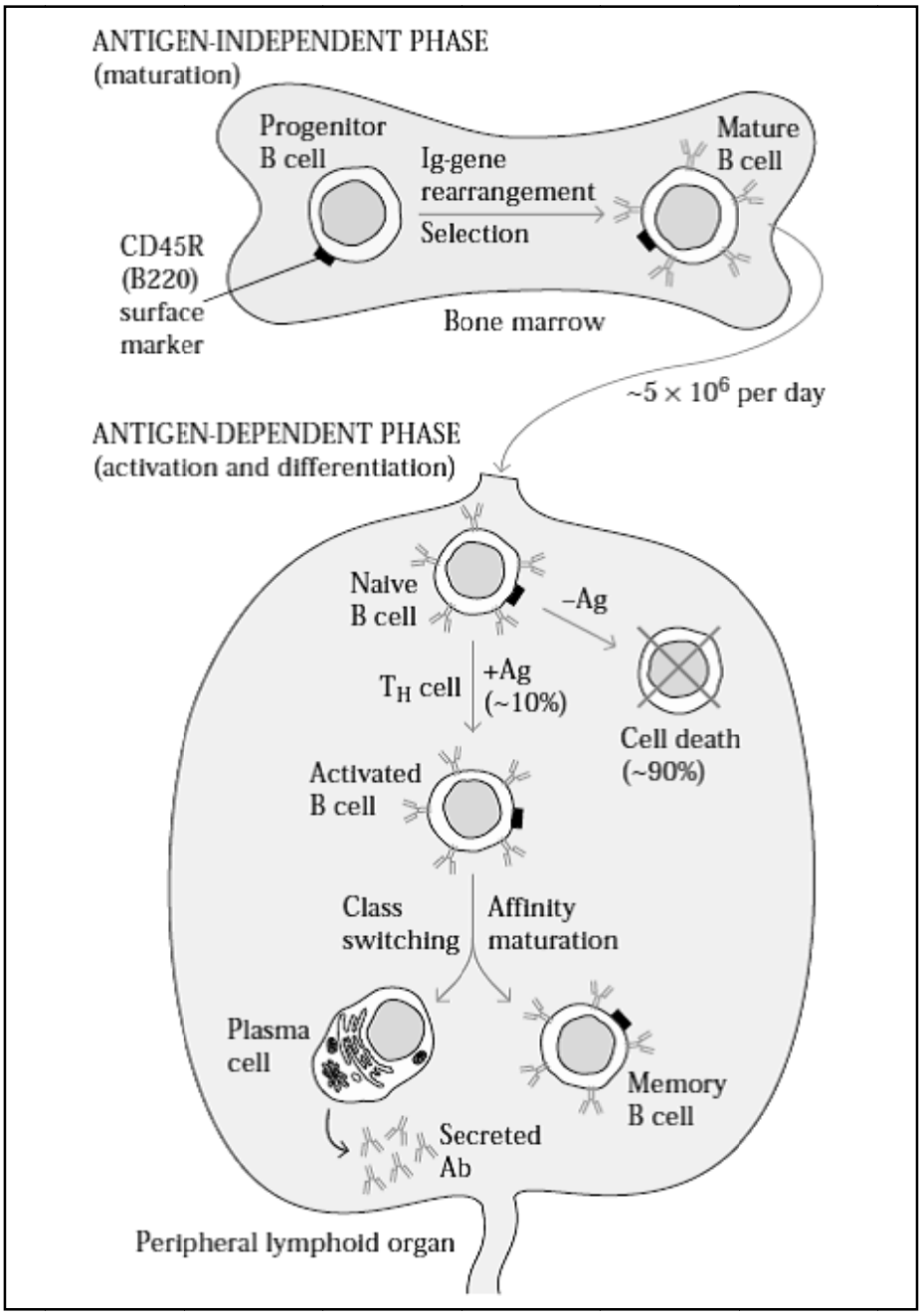

Figure: Overview of B-cell development. During the antigen- independent maturation phase, immunocompetent B cells expressing membrane IgM and IgD are generated in the bone marrow.

- **<u>Functions of B Cells</u>**
  - It regulates construction of an antigen receptor.
  - It ensures the cell specificity.
  - It helps in exporting all required cells in the periphery.
  - It helps in antibody production.
  - It helps rearrangement of their antigen receptors.

Probable questions:

1. What is humoral immune response and mention the basic components of humoral immunity.
2. Describe the basic structure of a Ig molecule.
3. Elucidate the structure and function of different Ig molecules.
4. What is class switching of antibody.
5. What are the basic functions of B cell?
6. How maturation and development occur in B cell?

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Unit-IV:
Complement system and diseases

Objective: In this unit, we will discuss about the Complement system and diseases.

Introduction:
The complement system is the major effector of the humoral branch of the immune system.
Research on complement began in the 1890s, when Jules Bordet at the Institut Pasteur in Paris showed that sheep antiserum to the bacterium *Vibrio cholera* caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity. Surprisingly, the ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibodies directed against the bacterium and was unable to kill the bacterium by itself. Bordet correctly reasoned that bacteriolytic activity requires two different substances: first, the specific antibacterial antibodies, which survive the heating process, and a second, heat-sensitive component responsible for the lytic activity. Bordet devised a simple test for the lytic activity, the easily detected lysis of antibody-coated red blood cells, called hemolysis. Paul Ehrlich in Berlin independently carried out similar experiments and coined the term complement, defining it as "the activity of blood serum that completes the action of antibody." In ensuing years, researchers discovered that the action of complement was the result of interactions of a large and complex group of proteins.

The Functions of Complement:
Research on complement now includes more than 30 soluble and cell-bound proteins. The biological activities of this system affect both innate and acquired immunity and reach far beyond the original observations of antibody mediated lysis of bacteria and red blood cells. Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms possessing the most rudimentary innate immune systems. By contrast, the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system. Thus we have a system that straddles innate and acquired immunity, contributing to each in a variety of ways. After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions (Figure 13-1) including:

- Lysis of cells, bacteria, and viruses.
- Opsonization, which promotes phagocytosis of particulate antigens.
- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules.
- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver

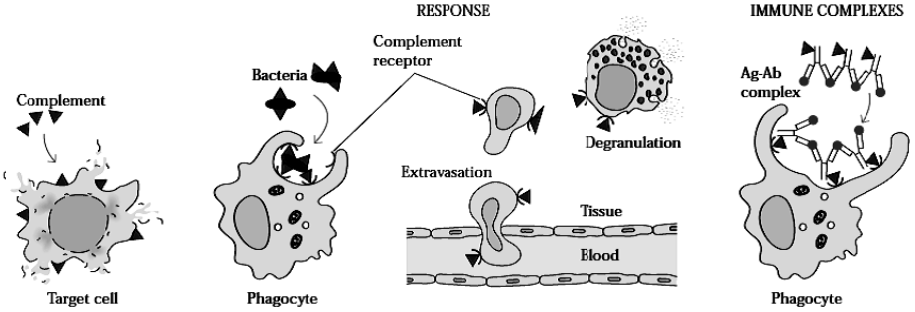
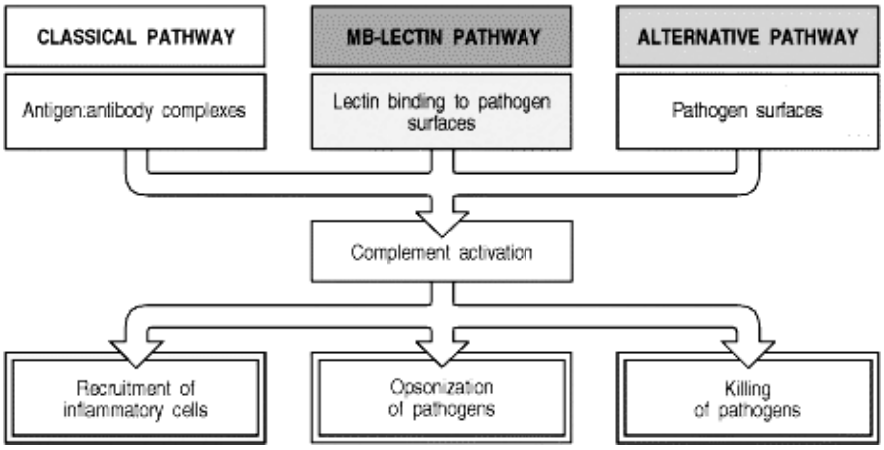


FIGURE 13-1 The multiple activities of the complement system. Serum complement proteins and membrane-bound complement receptors partake in a number of immune activities: lysis of foreign cells by antibody-dependent or antibody-independent pathways; opsonization or uptake of particulate antigens, including bacteria, by phagocytes; activation of inflammatory responses; and clearance of circulating immune complexes by cells in the liver and spleen. Soluble complement proteins are schematically indicated by a triangle and receptors by a semi-circle; no attempt is made to differentiate among individual components of the complement system here.

The Complement Components:
The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, althoughsignificant amounts are also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts. These components constitute 5% (by weight) of the serum globulin fraction. Most circulate in the serum in functionally inactive forms as proenzymes, or *zymogens,* which are inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site. The complement-reaction sequence starts with an enzyme cascade. Complement components are designated by numerals (C1–C9), by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragment resulting from cleavage of a component is designated "a" and the larger fragment designated "b" (e.g., C3a, C3b; note that C2 is an exception: C2a is the larger cleavage fragment). The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., C4b2a, C3bBb).

- **Complement Activation:**
The early steps, culminating in formation of C5b, can occur by the classical pathway, the alternative pathway, or the lectin pathway. The final steps that lead to a membrane attack are the same in all pathways.



- **The Classical Pathway Begins with Antigen-Antibody Binding:**
The classical pathway can be initiated by the binding of C1q, the first serum protein in the complement cascade, directly to the pathogen surface. It can also be activated during an adaptive immune reaction by the binding of C1q to antigen-antibody complexes, and is thus a key link between the effector mechanisms of innate and adaptive immunity. C1q is part of the C1 complex which comprises a single C1q molecule is bound to two molecules each of the zymogens C1r and C1s. C1q six globular heads, linked together by a collagen-like tail, which surround the $(C1r:C1s)_2$ complex. Binding of more than one of the C1q heads to a pathogen surface causes a conformational change in the $(C1r:C1s)_2$ complex, which leads to activation of an autocatalytic enzymatic activity in C1r; the active form of C1r then cleaves its associated C1s to generate an active serine protease.C1q, links the adaptive humoral, which leads to activation of an autocatalytic enzymatic activity in C1r; the active form of C1r then cleaves its associated C1s to generate an active serine protease.

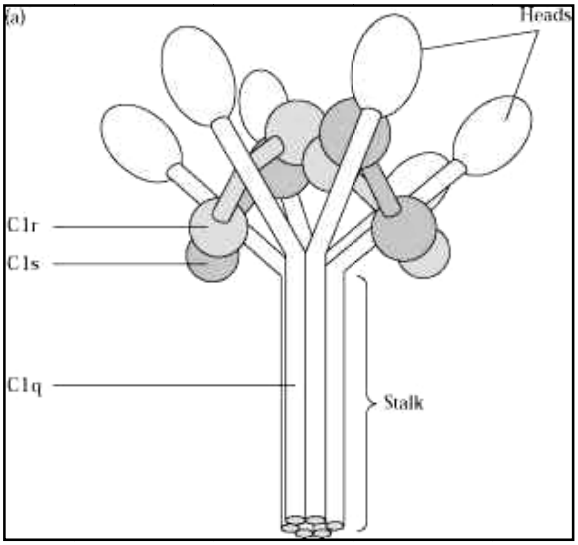


Figure: C1 complex

Once activated, the C1s enzyme acts on the next two components of the classical pathway, cleaving C4 and then C2 to generate two large fragments, C4b and C2b, which together form the C3 convertase of the classical pathway. In the first step, C1s cleaves C4 to produce C4b, which binds covalently to the surface of the pathogen. The covalently attached C4b then binds one molecule of C2, making it susceptible, in turn, to cleavage by C1s. C1s cleaves C2 to produce the large fragment C2b, which is itself a serine protease. The complex of C4b with the active serine protease C2b remains on the surface of the pathogen as the C3 convertase of the classical pathway. Its most important activity is to cleave large numbers of C3 molecules to produce C3b molecules that coat the pathogen surface. At the same time, the other cleavage product, C3a, initiates a local inflammatory response. These reactions, which comprise the classical pathway of complement activation.

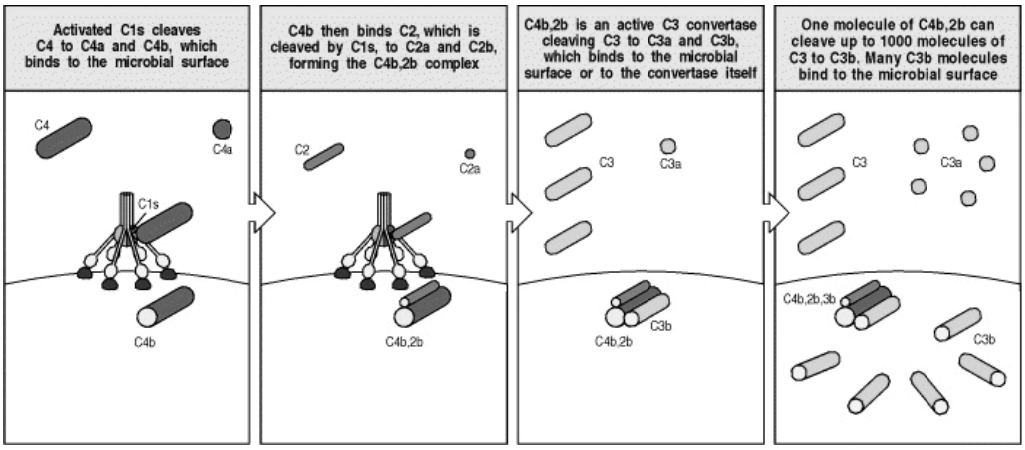


Figure: Classical Pathway Activation to form C3 Convertase

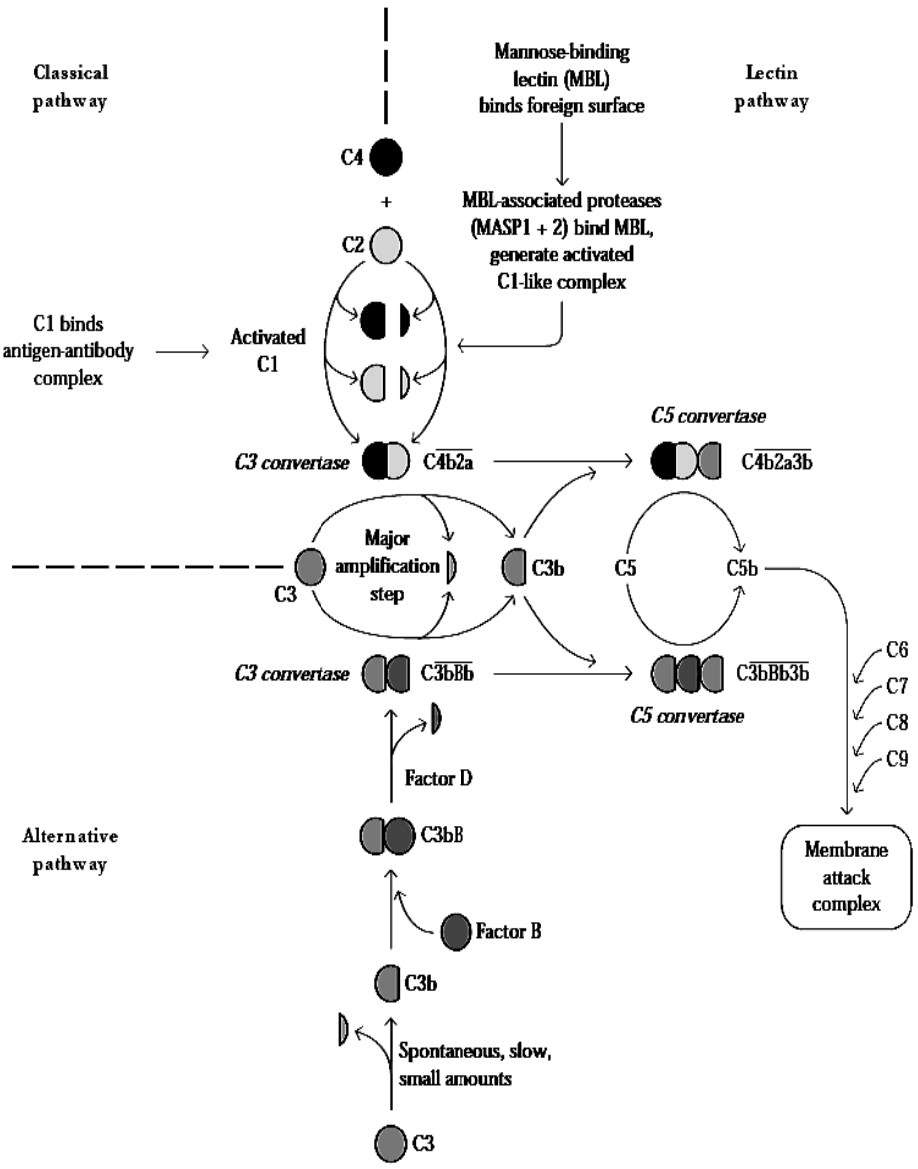


FIGURE 13-2 Overview of the complement activation pathways. The classical pathway is initiated when C1 binds to antigen-antibody complexes. The alternative pathway is initiated by binding of spontaneously generated C3b to activating surfaces such as microbial cell walls. The lectin pathway is initiated by binding of the serum protein MBL to the surface of a pathogen. All three pathways generate C3 and C5 convertases and bound C5b, which is converted into a membrane-attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in all pathways, generating large amounts of C3b, which forms part of C5 convertase. C3b also can diffuse away from the activating surface and bind to immune complexes or foreign cell surfaces, where it functions as an opsonin.

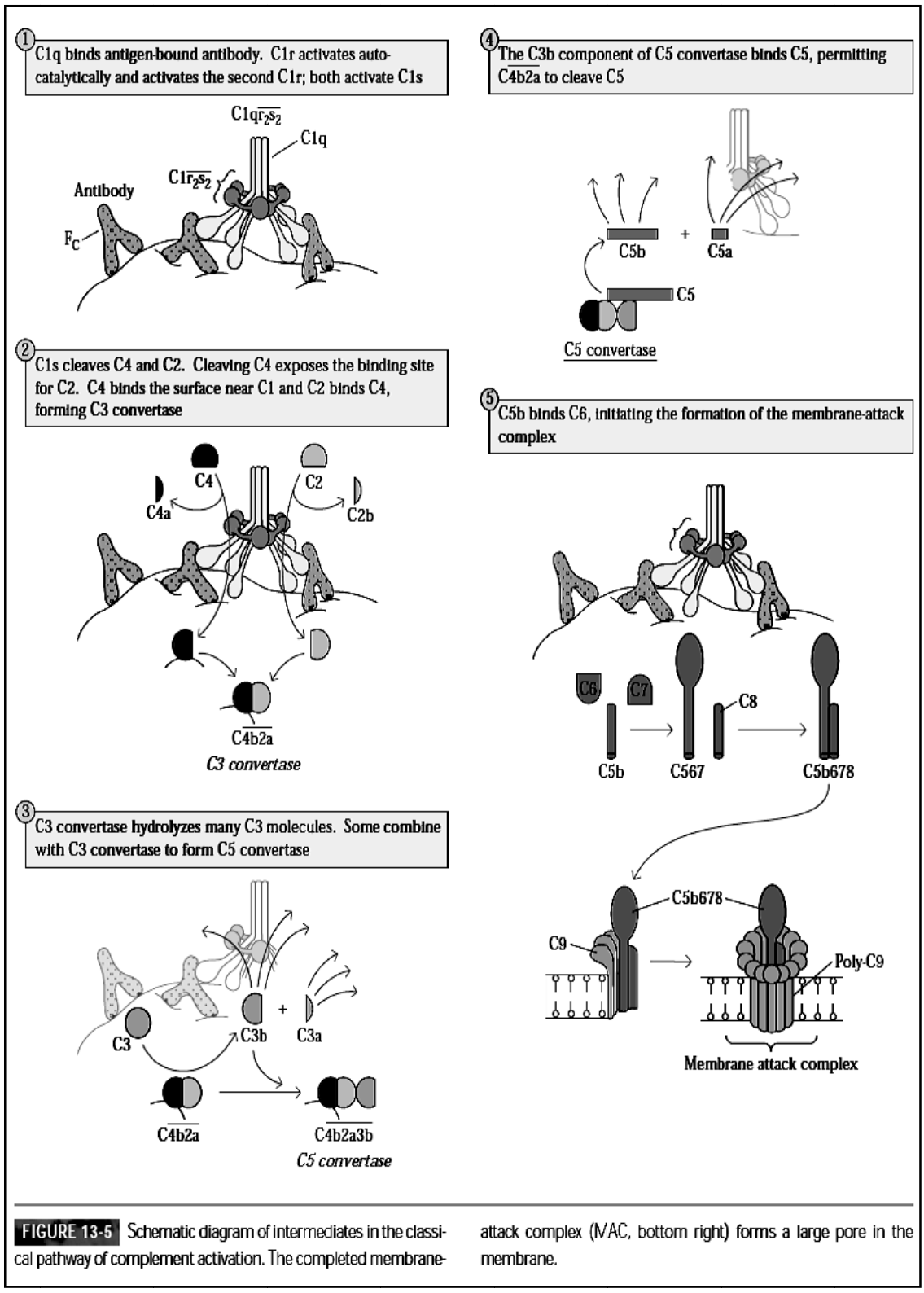


FIGURE 13-5 Schematic diagram of intermediates in the classical pathway of complement activation. The completed membrane-attack complex (MAC, bottom right) forms a large pore in the membrane.

The Alternative Pathway

This pathway is initiated upon spontaneous binding of complement components on pathogens. In the alternative pathway, a homologous C3 convertase is formed from membrane-bound C3bcomplexed with Bb.C3 is abundant in plasma, and C3b is produced at a significant rate by spontaneous cleavage (also known as 'tickover'). This occurs through the spontaneous hydrolysis of the thioester bond in C3 to form C3(H_2O) which has an altered conformation, allowing binding of the plasma protein **factor B**. The binding of B by C3(H_2O) then allows a plasma protease called **factor D** to cleave factor B to Ba and Bb, the latter remaining associated with C3(H_2O) to form the C3(H_2O)Bb complex. This complex is a fluid-phase C3 convertase, and although itis

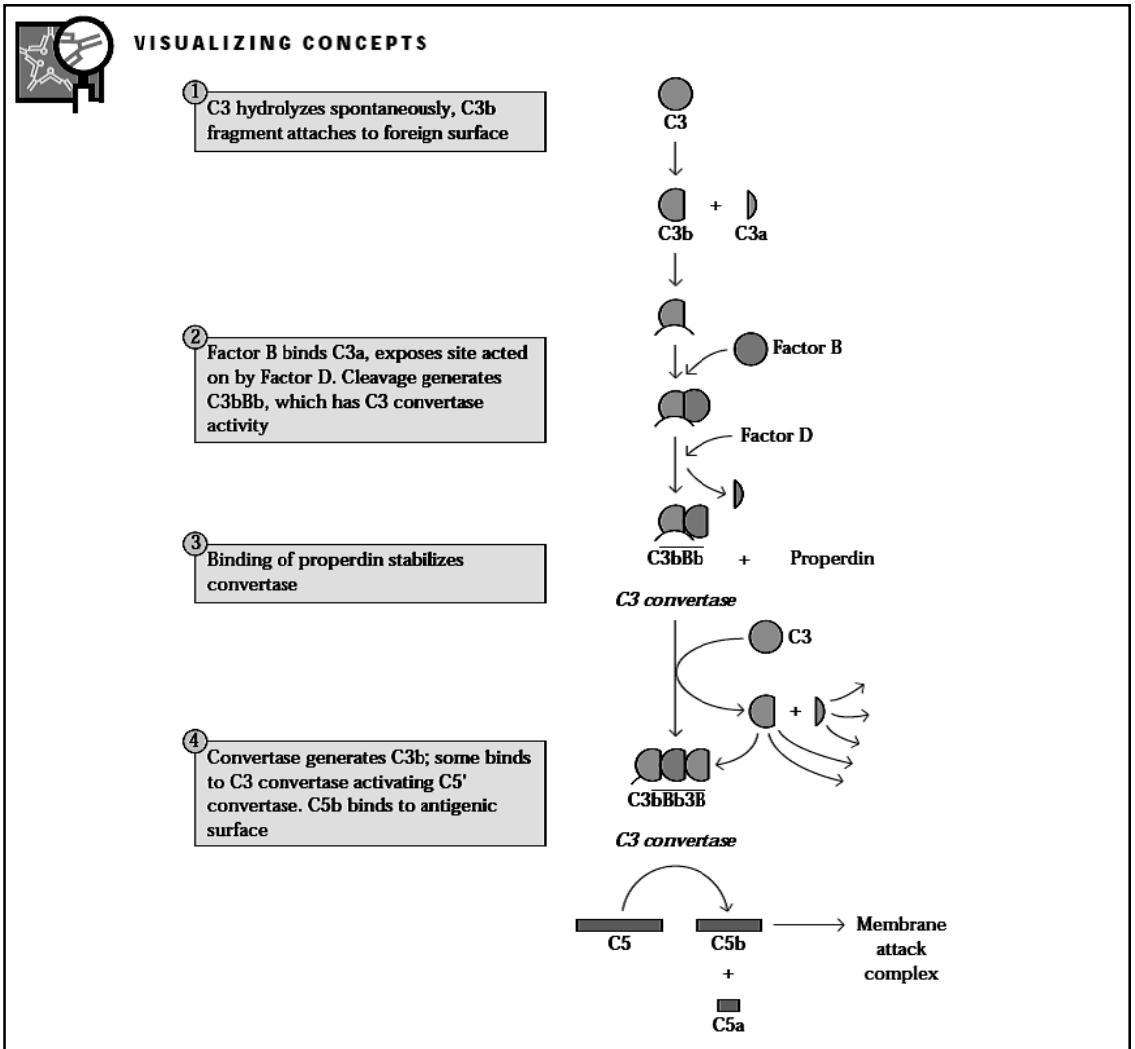


FIGURE 13-7 Schematic diagram of intermediates in the formation of bound C5b by the alternative pathway of complement activation. The C3bBb complex is stabilized by binding of properdin. Conversion of bound C5b to the membrane-attack complex occurs by the same sequence of reactions as in the classical pathway (see Figure 13-5).

only formed in small amount, it can cleave many molecules of C3 to C3a and C3b. Much of this C3b is inactivated by hydrolysis, but some attaches covalently, through its reactive thioester

group, to the surfaces of host cells or to pathogens. C3b bound in this way is able to bind factor B, allowing its cleavage by factor D to yield the small fragment Ba and the active protease Bb. This results in formation of the alternative pathway C3 convertase, C3b,Bb.

The Mannan Binding Pathway (MB-lectin pathway)is initiated by binding of the mannan-binding lectin, a serum protein, to mannose-containing carbohydrates on bacteria or viruses. The MB-lectin pathway uses a protein very similar to C1q to trigger the complementcascade. This protein, called the mannan-binding lectin (**MBL**), is a collectin, like C1q. Mannan-binding lectin binds specifically to mannose residues, and to certain other sugars, which are accessible and arranged in a pattern that allows binding on many pathogens. On vertebrate cells, however, these are covered by other sugar groups, especially sialic acid. Thus, mannan-binding lectin is able to initiate complement activation by binding to pathogen surfaces. It is present at low concentrations in normal plasma of most individuals. Mannan-binding lectin, like C1q, is a six-headed molecule that forms a complex with two protease zymogens, which in the case of the mannanbinding lectin complex (MBL complex) are MASP-1 and **MASP-2** . MASP-1 and MASP-2 are closely homologous to C1r and C1s, and all four enzymes are likely to have evolved from gene duplication of a common precursor. When the MBL complex binds to a pathogen surface, MASP-1 and MASP-2 are activated to cleave C4 and C2. Thus the MB-lectin pathway initiates complement activation in the same way as the classical pathway, forming a C3 convertase from C2b bound to C4b. People deficient in mannan-binding lectin experience a substantial increase in infections during early childhood, indicating the importance of the MB-lectin pathway for host defense.

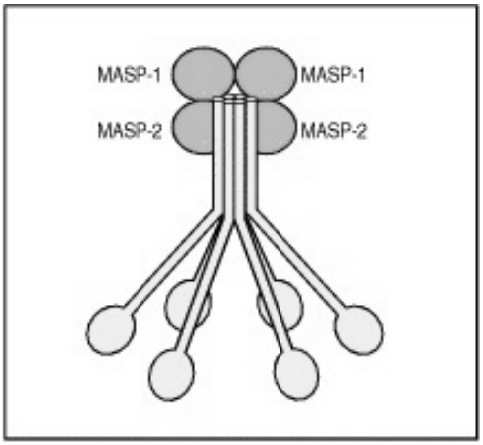


Figure: MBL complex

Generation of C5 Convertase in the Three Different Pathways

The next step in the cascade is the generation of the C5 convertases. In the classical and the MB-lectin pathways, a C5 convertase is formed by the binding of C3b to C4b,2b to yield C4b,2b,3b. By the same token, the C5 convertase of the alternative pathway is formed by the binding of C3b to the C3 convertase to form $C3b_2$,Bb. C5 is captured by these C5 convertase complexes through binding to an acceptor site on C3b, and is then rendered susceptible to cleavage by the serine

protease activity of C2b or Bb. This reaction, which generates C5b and C5a, is much more limited than cleavage of C3, as C5 can be cleaved only when it binds to C3b that is part of the C5 convertase complex. Thus, complement activation by both the alternative, MB-lectin and classical pathways leads to the binding of large numbers of C3b molecules on the surface of the pathogen, the generation of a more limited number of C5b molecules, and the release of C3a and C5a.

Formation of Membrane Attack Complex

One of the important effects of complement activation is the assembly of the terminal components of complement to form a membrane-attack complex. Theend result is a pore in the lipid bilayer membrane that destroys membrane integrity. This is thought to kill the pathogen by destroying the proton gradient across the pathogen cell membrane.

The first step in the formation of the membrane-attack complex is the cleavage of C5 by a C5 convertase to release C5b. In the next stages, C5b initiates the assembly of the later complement components and their insertion into the cell membrane. First, one molecule of C5b binds one molecule of C6, and the C5b,6 complex then binds one molecule of C7. This reaction leads to a conformational change in the constituent molecules, with the exposure of a hydrophobic site on C7, which inserts into the lipid bilayer. Similar hydrophobic sites are exposed on the later components C8 and C9 when they are bound to the complex, allowing these proteins also to insert into the lipid bilayer. C8 is a complex of two proteins, C8β and C8α-γ. The C8β protein binds to C5b, and the binding of C8β to the membrane-associated C5b,6,7 complex allows the hydrophobic domain of C8α-γ to insert into the lipid bilayer. Finally, C8α-γ induces the polymerization of 10 to 16 molecules of C9 into a pore-forming structure called the membrane-attack complex. The membrane-attack complex has a hydrophobic external face, allowing it to associate with the lipid bilayer, but a hydrophilic internal channel. The diameter of this channel is about 100 Å, allowing the free passage of solutes and water across the lipid bilayer. The disruption of the lipid bilayer leads to the loss of cellular homeostasis, the disruption of the proton gradient across the membrane, the penetration of enzymes such as lysozyme into the cell, and the eventual destruction of the pathogen.

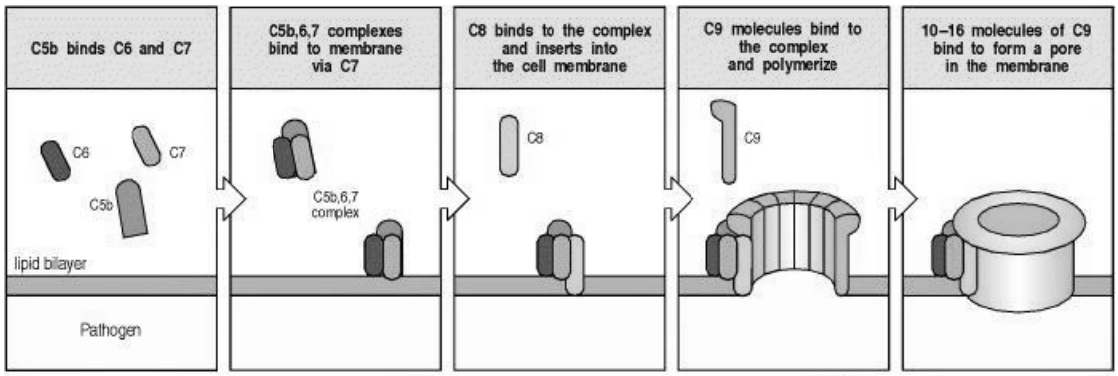


Figure: Formation of Membrane Attack Complex

TABLE 13-3 Summary of biological effects mediated by complement products

Effect	Complement product mediating*
Cell lysis	**C5b–9, the membrane-attack complex (MAC)**
Inflammatory response	
Degranulation of mast cells and basophils†	**C3a,C4a, and C5a (anaphylatoxins)**
Degranulation of eosinophils	**C3a, C5a**
Extravasation and chemotaxis of leukocytes at inflammatory site	**C3a, C5a, C5b67**
Aggregation of platelets	**C3a, C5a**
Inhibition of monocyte/macrophage migration and induction of their spreading	**Bb**
Release of neutrophils from bone marrow	**C3c**
Release of hydrolytic enzymes from neutrophils	**C5a**
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	**C5a**
Opsonization of particulate antigens, increasing their phagocytosis	**C3b, C4b, iC3b**
Viral neutralization	**C3b, C5b–9 (MAC)**
Solubilization and clearance of immune complexes	**C3b**

*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

- **Complement Deficiencies**

Classical pathway component deficiencies (C1, C4, C2)

Individuals having C1, C4 and C2 deficiencies are prone to immune complex disease, commonly systemic lupus erythematosus (SLE). The reason for this is thought to be that the complement deficiency leads to an inability to clear circulating immune complexes. This in turn leads to their deposition in tissues and an associated inflammatory response. Reduced clearance of apoptotic cells by the complement system may also lead to the development of autoantigens.

C2 deficiency is also associated with recurrent bacterial infection and an increased risk of cardiovascular disease. It is thought to influence the development of atherosclerosis.

MBL deficiency causes recurrent pyogenic infection in childhood.Deficiency of MBL increases susceptibility to *Saccharomyces cerevisiae* infection as well as pneumococcal and Neisseria infection.

Alternative pathway component deficiencies (properdin, factor B and factor D)are prone to pneumococcal and meningococcal infections. With properdin deficiency, there is a particular risk of overwhelming Neisseria infection.

C3 deficiency

C3 is required for opsonisation (the coating of pathogenic cells with opsonin to facilitate phagocytosis).A defect in the pathway that results in deficiency of C3 can lead to problems with opsonisation.

MAC deficiencies (C5-C9)

Recurrent infections are again a feature.Infection with *Neisseria meningitidis* is particularly common. More rare serotypes of this organism such as Y and W135 tend to cause the infection.

Leiner's disease

This is a paediatric condition associated with a deficiency of C5. It has also been reported with C3 andC4 deficiency.It causes wasting, chronic diarrhoea and widespread seborrhoeic dermatitis.

Probable questions:

1. What do you mean by complement system?
2. What are the basic functions of complement system?
3. Describe the classical and alternative pathway of complement activation.
4. What is membrane attack complex?
5. Summarise the biological effects mediated by complement products.
6. Name the diseases associated with deficiency in complement products.

Suggested readings/ references:

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
2. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt's Essential Immunology. Hoboken, NJ: Wiley-Blackwell.
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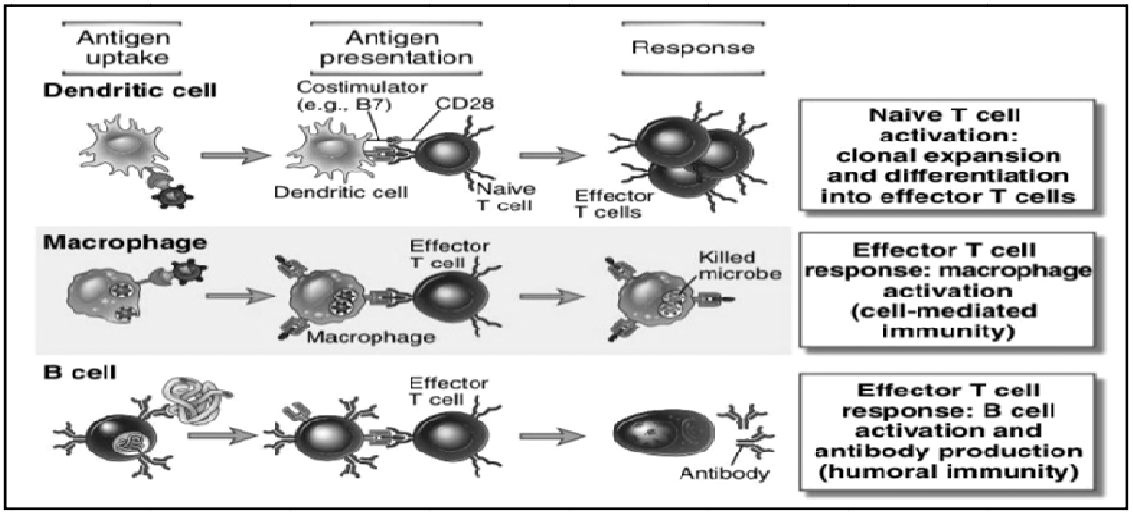
Unit-V
Antigen presentation: APC, Dendritic cells, MHC genes and gene products

Objective: In this unit, we will discuss about the Antigen presentation: APC, Dendritic cells, MHC genes and gene products.

- **Antigen Presenting Cells:**

An Antigen Presenting Cell or APC is a type of cell that displays or presents antigens complexed with major histocompatibility complex (MHC) on their surface, through a process known as antigen presentation. They are very important for the proper functioning of the Helper T cells and Cytotoxic T cells, as a result they are vital for an effective adaptive immune response.

Almost every cell of our body can serve as some form of APC due to the presence of MHC class I molecule, but there are a few cell types that are specialized for the function of antigen presentation. These specialized cells are referred to as the professional APCs. These include Macrophages, Dendritic cells and B cells. The T cells are activated by the interaction with the antigen presented on the APC, which is recognized by the T cell receptors. The T cells are able to recognize an antigen only when it is processed and presented with a MHC molecule. The Helper T cells can recognize exogenous antigens, presented with MHC class II molecule and Cytotoxic T cells can recognize endogenous antigens, presented with MHC class I molecule. The non-professional APCs that is almost every cell type of our body can present antigen to cytotoxic T cells with MHC class I molecule. But the term APC is commonly referred to the professional APCs such as dendritic cells and macrophages.



Dendritic Cells:

Dendritic cells represent the widest range of antigen presentation and are very important for the activation of the naïve T cells. These dendritic cells are able to present antigen to both the helper

T cells and cytotoxic cells. Another important phenomenon of the dendritic cells is the ability to cross-presentation. In this process they can present exogenous antigens on MHC class I molecules to the cytotoxic T cells, which was normally destined for the helper T cells. They also serve important role in peripheral tolerance that contributes to auto-immune diseases.

In normal condition, the dendritic cells exhibit very low amount of MHC class II and co-stimulatory molecules on their cell surface. These are referred to as the immature dendritic cells, which are incapable of presenting antigen to helper T cells. The activation of these immature dendritic cells require the recognition of a Pathogen Associated Molecular Pattern (PAMP) by the dendritic cell's Pattern Recognition Receptors (PRR). The activated dendritic cells then phagocytose the antigen and also upregulates the expression of MHC class II molecules and several co-stimulatory molecules, including CD40 and B7, required for T cell activation. The B7 complex can interact with CD28 on the surface of a helper T cell. Now, the fully mature and active dendritic cell moves from tissue to lymph nodes, where it encounters and activate T cells.

Macrophages:

These are a type of white blood cells that can engulf and digest cellular debris, foreign substances, microbes and even cancer cells. They are present in various forms in different parts of the body such as Kupffer cells, Histiocytes, alveolar macrophages, etc. They play important role in both innate immune response and adaptive immune response. These cells are also vital for their anti-inflammatory roles and can decrease immune reactions through the release of cytokines.

Macrophages are actually stimulated by the Interferon γ (IFNγ), secreted by the T cell. Once activated, they are capable of expressing MHC class II molecules and other co-stimulatory molecules including B7 complex. They can present phagocytosed peptide fragments to the helper T cells.

B Cells:

B cells have the ability to internalize antigens that are recognized by the B cell receptors and present it to helper T cells. B cells can also recognize soluble antigens for which their B cell receptor is specific. After they internalize the antigens, they then process the antigens and present the peptides using the MHC class II molecules.

The T Cell Receptor (TCR) of helper T cell, specific for that peptide then bind to the peptide and the B cell marker CD40 binds to CD40L on the T cell surface. Moreover, a B cell can undergo antibody isotype switching, affinity maturation, formation of memory cells when it is activated by a T cell.

Besides this professional Antigen Presenting Cells, all nucleated cells of our body can serve as non-professional APCs as they can use MHC class I molecule coupled to β2 microglubulin to present the endogenous peptides on their cell membrane. The cytotoxic T cells are able to interact

with these endogenous peptides bound to MHC class I molecule on the surface of the non-professional APCs.

MHC Molecules and MHC genes:
Immune system has elegant means of recognizing pathogens that have invaded or being taken into host cells as well as tumor antigens produced within cytosol. Proteins from within the cells are digested into short peptide fragments and they are displayed on the cell surface through binding to specialized antigen presenting molecules termed as MHC class I or Major Histocompatibility Complex class I.

In a similar fashion, peptide derived from proteins ingested from external environment by phagocytosis, are presented by MHC class II molecules. These peptide-MHC complexes serve as ligand for T cell receptors. The antigen processing and presentation pathway, upon which both activation and regulation of immune response rests, is a complex and fascinating subject.

Discovery of MHC in humans:
The proteins responsible for presenting antigen to T cells are MHC class I and II molecules, which are originally discovered as histocompatibility (transplantation) antigens. Histocompatibility refers to the ability to accept tissue graft from an unrelated donor. The MHC complex locus comprises over 100 separate genes and was discovered when it was recognized that both donor and recipient has to possess the same MHC haplotype to avoid graft rejection. The principal moieties that determine rejection were identified as MHC class I and class II but presently we know that the main purpose of MHC is not the graft rejection. The remaining genes (known as the MHC class III molecules) are sometimes very diverse. They generally encode complement system molecules, cytokines, enzymes and heat shock proteins and other essential molecules in antigen processing.

MHC class I:
The MHC class I molecule handle intrinsic antigens and present antigens to cytotoxic T cells by recognizing CD8 marker on the T cells.

The molecule have glycosylated heavy chain of 45 KD. The heavy chains are non-covalently associated with β-microglobulin of 12 KD, which is a peptide also found free in serum. The three extracellular domains designated as α_1, α_2 and α_3 are the main constituents of the heavy chain. The molecule also possesses a transmembrane region and a cytoplasmic tail. The three extracellular domain comprise 90 amino acids. α_2 and α_3 domains have interchain disulphide bonds enclosing loops of 63 and 88 amino acids respectively. The α_3 domain is structurally hmologus to the Immunoglobulin constant region and contain site that interacts with the CD8 domain of cytotoxic T cells. The extracellular portion of class I heavy chain is glycosylated and the glycosylation depends on the species and haplotype. B microglobulin domain is basically essential for expression of class I MHC molecule. The α_1 and α_2 components of the heavy chain of MHC class I forms the groove for binding antibody.

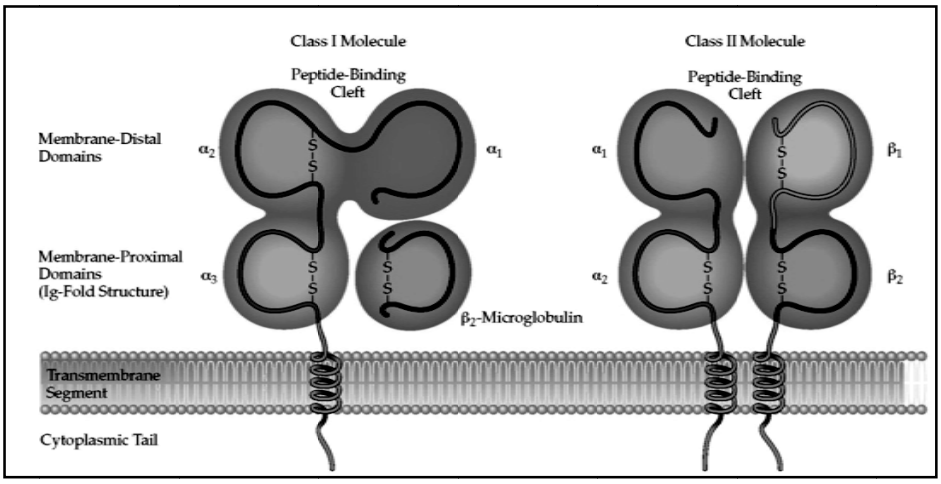


Figure: MHC Class I and MHC Class II molecule.

MHC Class II:

MHC class II binds to extrinsic antigen and present the antigen to helper T cell, which aid the B cells in generating antibody responses to extracellular protein antigens. The heavy chain of MHC class II consist of α_1 and α_2, which is a heterodimer and the light chain consist of β_1 and β_2. The α chains have molecular weight between 30-34 KD and the β chains range from 26-29 KD, depending on the locus involved. The structures of class I and class II MHC molecules are different and these differences are responsible for their functional differences. The binding groove of MHC class II molecule is more open than the binding groove of class I to accommodate longer peptide. On the other hand, MHC class I binds to short fragments of 8-10 amino acids, whereas class II binds to peptide of 13-24 amino acids.

The α and β chains of MHC class II molecules are associated by non-covalent interaction and like MHC class I molecules, the class II molecules are also membrane bound glycoprotein that contain an external domain, a transmembrane segment and the cytoplasmic anchor segment. The overall 3D structure of MHC class II molecule is more or less similar to class I molecule and the nature of the peptide binding cleft differs from the class I molecule. The peptide binding cleft of the class I molecule is closed at both ends whereas the peptide binding cleft of MHC class II molecule is open at both ends. Moreover, in class I molecules, the peptide binding domain is between α_1/α_2 whereas in case of class II MHC molecules the peptide binding domain is between the α_1/β_1.

MHC haplotype:

The loci constituting the MHC are highly polymorphic that is many alternative forms of genes or alleles exists at each locus. The MHC loci are also very closely linked. For this reason most individuals inherit alleles encoded by these closely linked loci as two sets, one from each parent. Each set of allele is referred to a haplotype and individual inherits one haplotype from the mother and one haplotype from the father. In outbred populations the offspring are generally heterozygous at many loci and will express both maternal and paternal MHC alleles as the alleles

are co-dominantly expressed. But if mice are inbred, maternal and parental haplotypes will be identical and all offspring will have that identical haplotype.

- **Antigen Processing and Presentation**

Recognition of foreign protein antigen by a T cell requires that peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell. The formation of these peptide-MHC complexes requires that a protein antigen be degraded into peptides by a sequence of events called antigen processing. The degraded peptides then associate with MHC molecules within the cell interior, and the peptide-MHC complexes are transported to the membrane, where they are displayed (antigen presentation).

Class I and class II MHC molecules associate with peptides that have been processed in different intracellular compartments. Class I MHC molecules bind peptides derived from endogenous antigens that have been processed within the cytoplasm of the cell (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells). Class II MHC molecules bind peptides derived from exogenous antigens that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway. This chapter examines in more detail the mechanism of antigen processing and the means by which processed antigen and MHC molecules are combined.

Processing of Antigen Is Required for Recognition by T Cells

The results obtained by K. Ziegler and E. R. Unanue were among those that contradicted the prevailing dogma that antigen recognition by B and T cells was basically similar. These researchers observed that TH-cell activation by bacterial protein antigens was prevented by treating the antigen presenting cells with para formaldehyde prior to antigen exposure. However, if the antigen-presenting cells were first allowed to ingest the antigen and were fixed with paraformaldehyde 1–3 h later, TH-cell activation still occurred. During that interval of 1–3 h, the antigen-presenting cells had processed the antigen and had displayed it on the membrane in a form able to activate T cells. Subsequent experiments by R. P. Shimonkevitz showed that internalization and processing could be bypassed if antigen- presenting cells were exposed to peptide digests of an antigen instead of the native antigen. In these experiments, antigen-presenting cells were treated with glutaraldehyde (this chemical, like paraformaldehyde, fixes the cell, making the membrane impermeable) and then incubated with native ovalbumin or with ovalbumin that had been subjected to partial enzymatic digestion. The digested ovalbumin was able to interact with the glutaraldehyde-fixed antigen-presenting cells, thereby activating ovalbumin specific TH cells, whereas the native ovalbumin failed to do so. These results suggest that antigen processing involves the digestion of the protein into peptides that are recognized by the ovalbumin-specific TH cells.

TABLE 8-1 Antigen-presenting cells

Professional antigen-presenting cells	Nonprofessional antigen-presenting cells	
Dendritic cells (several types)	Fibroblasts (skin)	Thymic epithelial cells
Macrophages	Glial cells (brain)	Thyroid epithelial cells
B cells	Pancreatic beta cells	Vascular endothelial cells

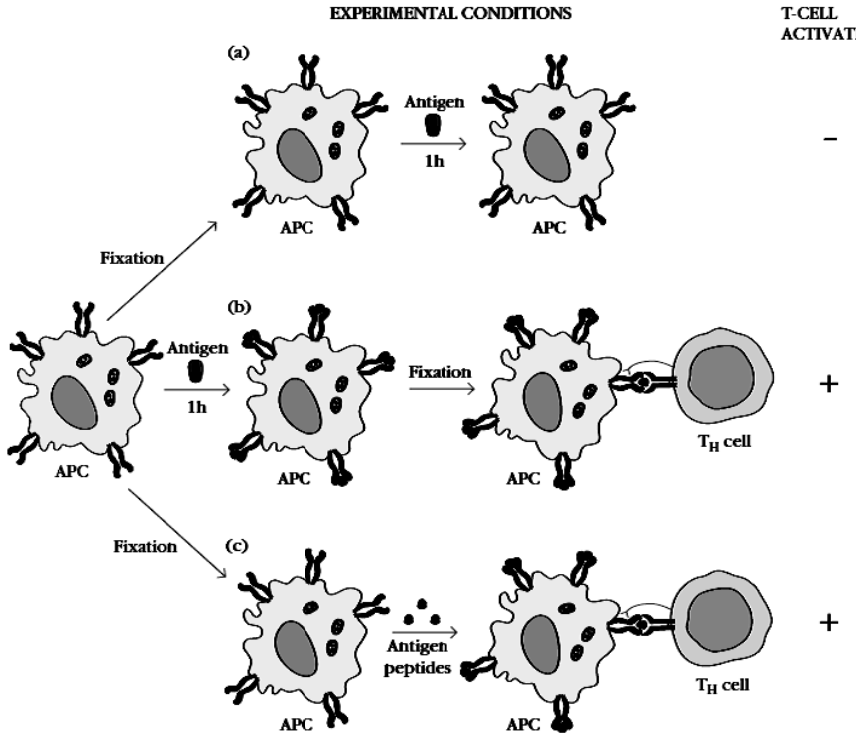


FIGURE 8-3 Experimental demonstration that antigen processing is necessary for T_H-cell activation. (a) When antigen-presenting cells (APCs) are fixed before exposure to antigen, they are unable to activate T_H cells. (b) In contrast, APCs fixed at least 1 h after antigen exposure can activate T_H cells. (c) When APCs are fixed before antigen exposure and incubated with peptide digests of the antigen (rather than native antigen), they also can activate T_H cells. T_H-cell activation is determined by measuring a specific T_H-cell response (e.g., cytokine secretion).

Most Cells Can Present Antigen with Class I MHC; Presentation with Class II MHC Is Restricted to APCs

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class I MHC molecules to CD8+ TC cells are referred to as target cells; cells that display peptides associated with class II MHC molecules to CD4+ TH cells are called antigen-presenting cells (APCs). This convention is followed throughout this text.

A variety of cells can function as antigen-presenting cells. Their distinguishing feature is their ability to express class II MHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as professional antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ from each other in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity:

Dendritic cells are the most effective of the antigen presenting cells. Because these cells constitutively express a high level of class II MHC molecules and costimulatory activity, they can

activate naive TH cells. Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or the co-stimulatory B7 membrane molecule.
B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatory B7 molecule. Several other cell types, classified as non-professional antigen-presenting cells, can be induced to express class II MHC molecules or a co-stimulatory signal (Table 8-1). Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response. Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to TC cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets.

Probable questions:

1. Describe the structure of MHC I and MHC II molecule and state their functions.
2. MHC I molecule is expressed on the surface of all nucleated cells, whereas, MHC II is restricted to APC- explain why?
3. Describe the function of APC.
4. What are the major antigen presenting cells present in the body?

Suggested readings/ references:

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
2. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt's Essential Immunology. Hoboken, NJ: Wiley-Blackwell.
3. Murphy, K., Travers, P., Walport, M., & Janeway, C. 2008. Janeway's immunobiology. New York: Garland Science.
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Unit-VI
Vaccination and immunization: natural and artificial immunization; active immunization, vaccines

Objective: In this unit, we will discuss about the vaccination and immunization: natural and artificial immunization; active immunization, vaccines.

Introduction:

A ***vaccine*** is a biological preparation that provides active acquired immunity against a particular disease. A ***vaccine*** typically contains an antigenic agent that is either the disease-causing microorganism in inactivated form or in attenuated form, its toxins, or one of its surface proteins that would generate immune response and provide protection against the disease causing agent during its future attack.

Vaccination is the process of administration of an antigenic material (a ***vaccine which might be a live pathogen in attenuated form, inactivated form or others***) to stimulate an individual's immune system to develop adaptive immunity to a pathogen. ***Vaccines*** can prevent causing of diseases or disease manifestations as vaccines help the immune system to have a memory of the pathogen. When a sufficiently large percentage of a population has been ***vaccinated***, herd immunity results..

Different Types of Vaccines

The first human vaccines against viruses were based using weaker or attenuated viruses to generate immunity. The smallpox vaccine used cowpox, a poxvirus that was similar enough to smallpox to protect against it but usually didn’t cause serious illness. Rabies was the first virus attenuated in a lab to create a vaccine for humans.

Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

Vaccine type	Vaccines of this type on U.S. Recommended Childhood (ages 0-6)
Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox) Rotavirus
Inactivated/Killed	Polio (IPV) Hepatitis A
Toxoid (inactivated toxin)	Diphtheria, tetanus (part of DTaP combined immunization)
Subunit/conjugate	*Haemophilus influenza* type b (Hib) Pertussis (part of DTaP combined immunization) Pneumococcal Meningococcal
Recombinant	Hepatitis B Vaccine
Vaccine type	**Other available vaccines**
Live, attenuated	Zoster (shingles) Yellow fever
Inactivated/Killed	Rabies
Subunit/conjugate	Human papillomavirus (HPV)

Live, attenuated vaccines currently recommended as part of the U.S. Childhood Immunization Schedule include those against measles, mumps, and rubella (via the combined MMR vaccine), varicella (chickenpox), and influenza (in the nasal spray version of the seasonal flu vaccine). In addition to live, attenuated vaccines, the immunization schedule includes vaccines of every other major type—see the table above for a breakdown of the vaccine types on the recommended childhood schedule.

The different vaccine types each require different development techniques are described as follows:

Live, Attenuated Vaccines

Attenuated vaccines can be made in several different ways. Some of the most common methods involve passing the disease-causing virus through a series of cell cultures or animal embryos (typically chick embryos). With each passage, the virus becomes better at replicating in chick cells, but loses its virulence. A virus targeted for use in a vaccine may be grown through—"passaged" through—upwards of 200 different embryos or cell cultures. Eventually, the attenuated virus will be unable to cause disease in humans but will replicate well in human cells, and can be used in a vaccine. All of the methods produce an attenuated live vaccine where the pathogen loses its ability to cause disease in the host but possess all the characteristics to be used as a vaccine.

When the resulting vaccine virus is given to a human, it will be unable to cause illness, but will still provoke an immune response that can protect against future infection.

One concern that must be considered is the potential for the vaccine virus to revert to a form capable of causing disease. Mutations that can occur when the vaccine virus replicates in the body may result in more a virulent strain. It is worth noting that mutations *are* somewhat common with the oral polio vaccine (OPV), a live vaccine that is ingested instead of injected. The vaccine virus can mutate into a virulent form and result in rare cases of paralytic polio. For this reason, OPV is no longer used in the United States, and has been replaced on the Recommended Childhood Immunization Schedule by the inactivated polio vaccine (IPV).

Protection from a live, attenuated vaccine typically outlasts that provided by a killed or inactivated vaccine.

Killed or Inactivated Vaccines

Inactivated vaccines generally termed as heat killed vaccines are created by inactivating a pathogen, typically using heat or chemicals such as formaldehyde or formalin. This destroys the pathogen's ability to replicate, but keeps it "intact" so that the immune system can still recognize it. Because killed or inactivated pathogens can't replicate at all, they can't revert to a more virulent form capable of causing disease (as discussed above with live, attenuated vaccines). However, they tend to provide a shorter length of protection than live vaccines, and are more likely to require boosters to create long-term immunity.

Toxoids

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. One example is tetanus: its symptoms are not caused by the *Clostridium tetani* bacterium, but by a neurotoxin it produces (tetanospasmin). Immunizations for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. As with organisms or viruses used in killed or inactivated vaccines, this can be done via treatment with a chemical such as formalin, or by using heat or other methods.

Immunizations created using inactivated toxins are called *toxoids*. Toxoids can actually be considered killed or inactivated vaccines, but are sometimes given their own category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria.

Subunit and Conjugate Vaccines

Both subunit and conjugate vaccines contain only pieces of the pathogens they protect against. Subunit vaccines use only part of a target pathogen to provoke a response from the immune system. This may be done by isolating a specific protein from a pathogen and presenting it as an antigen on its own. The acellular pertussis vaccine and influenza vaccine (in shot form) are examples of subunit vaccines.

Another type of subunit vaccine can be created via genetic engineering. A gene coding for a vaccine protein is inserted into another virus, or into producer cells in culture. When the carrier virus reproduces, or when the producer cell metabolizes, the vaccine protein is also created. The end result of this approach is a recombinant vaccine: the immune system will recognize the expressed protein and provide future protection against the target virus. The Hepatitis B vaccine currently used in the United States is a recombinant vaccine.

Another vaccine made using genetic engineering is the human papillomavirus (HPV) vaccine. Two types of HPV vaccine are available—one provides protection against two strains of HPV, the other four—but both are made in the same way: for each strain, a single viral protein is isolated. When these proteins are expressed, virus-like particles (VLPs) are created. These VLPs contain no genetic material from the viruses and can't cause illness, but prompt an immune response that provides future protection against HPV.

Conjugate vaccines are somewhat similar to recombinant vaccines: they're made using a combination of two different components. Conjugate vaccines, however, are made using pieces from the coats of bacteria. These coats are chemically linked to a carrier protein, and the combination is used as a vaccine. Conjugate vaccines are used to create a more powerful, combined immune response: typically the "piece" of bacteria being presented would not generate a strong immune response on its own, while the carrier protein would. The piece of bacteria can't cause illness, but combined with a carrier protein, it can generate immunity against future infection. The vaccines currently in use for children against pneumococcal bacterial infections are made using this technique.

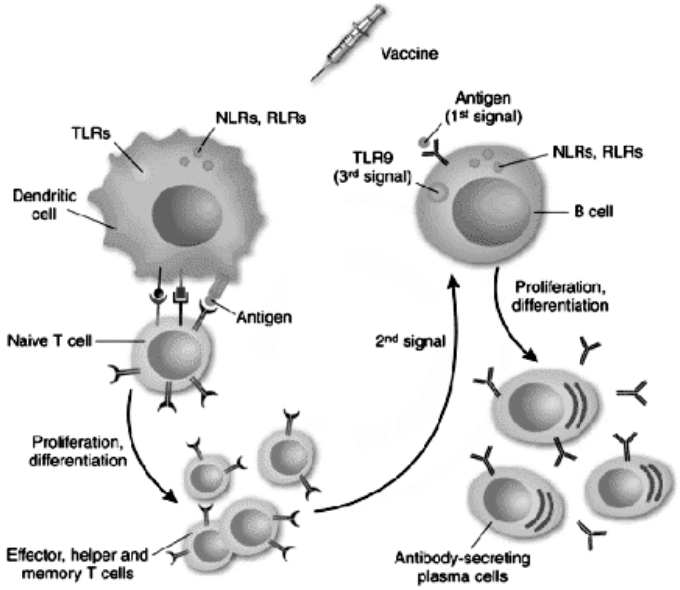
Recombinant Vaccines

Today, the rise of genetic engineering and molecular biology has had great impact on development and manufacturing process of vaccines. Specific antigenic microbes have high power to arouse the immune response against pathogens. Currently, the sequence of the

pathogenic protein antigens could be obtainable by sequencing genes of the main antigen, and producing them synthetically via recombinant DNA technology. Hepatitis B is the first and one of the most successful examples of synthetic vaccines. The surface antigen of this virus (HBsAg) is very immunogenic and effective, and able to produce high levels of antibody in the body. In the past, for providing hepatitis B vaccine, HBsAgwas purified from the plasma of infection carriers and used for vaccination; of course there were some extensive restrictions in purification, such as difficult conditions and contaminated plasma. In order to make recombinant hepatitis B vaccine, recombinant HBsAg is expressed in cells that have a powerful expression system leading to the production of virus-like particles by HBsAg which are highly immunogenic. Other kinds of common vaccines are anti-herpes simplex virus, anti-rotavirus, and anti-HPV vaccines.

How Vaccines Work?

Vaccines help develop immunity by imitating an infection. This type of infection, however, does not cause illness, but it does cause the immune system to produce T-lymphocytes and antibodies. Sometimes, after getting a vaccine, the imitation infection can cause minor symptoms, such as fever. Such minor symptoms are normal and should be expected as the body builds immunity. Once the imitation infection goes away, the body is left with a supply of "memory" T-lymphocytes, as well as B-lymphocytes that will remember how to fight that disease in the future. However, it typically takes a few weeks for the body to produce T-lymphocytes and B-lymphocytes after vaccination. Therefore, it is possible that a person who was infected with a disease just before or just after vaccination could develop symptoms and get a disease, because the vaccine has not had enough time to provide protection.



Different Generations of Vaccine

Variables	Different Types and Generations of Vaccines						
1st generation							
Live attenuated vaccines	MMR	Influenza	OPV	Chickenpox	Yellow fever	Hepatitis A	
Inactivated vaccines	Influenza	Hepatitis B	IPV	Rabies	Cholera	Plaque	Pertussis
2nd generation							
Subunit vaccines	Hepatitis B	Diphtheria	Pertussis	Anthrax	Hemophilus Influenza B		
Recombinant vaccines	Hepatitis B	HSV	Rota virus	HPV	FMD		
3rd generation							
DNA vaccines	HIV	Malaria	Cancers	Influenza	Ebola	Hepatitis	HPV

Probable questions:

1. What do you mean by immunization?

2. Describe the different types of vaccines administered in human.

3. What is toxoid? Give an example.

4. Explain the mechanism of action of a vaccine.

Suggested readings/ references:

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
2. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt's Essential Immunology. Hoboken, NJ: Wiley-Blackwell.
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HARD CORE THEORY PAPER (ZHT 207)

Immunobiology & Human Population Genetics

Group B:HumanPopulationGenetics

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 207 (Immunobiology & Human Population Genetics)	VII	Basic concept of human genetics: introduction to the structure of humangenome; human genome and mapping	1.5	1	1	67-80
	VIII	Human karyotype; karyotype and nomenclature of metaphase chromosome bands		1	1	81-90
	IX	Chromosome anomalies and diseases.		1	1	91-115
	X	Genetic analysis of complex traits - complex pattern of inheritance, quantitative traits, threshold traits.		1	1	116-130
	XI	Human genetics and society: genetic testing; human rights; gene therapy.		1	1	131-167
	XII	Quantitative genetics; variance; heritability and its measurement; inbreeding and cross breeding; QTL.		1	1	168-175

Unit-VII

Basic concep to human genetics: introduction to the structure of human genome; human genome and mapping

Objective: In this unit you will learn about Basic concept of human genetics: introduction to the structure of human genome; human genome and mapping

Introduction:

The 3.2 billion bp of our genetic blueprint is packed into 23 pairs of chromosomes, or 46 DNA molecules. Only a fraction of the genome is occupied by protein-coding exons and the majority of non-exonic sequences consists of repetitive elements. Functional exons contribute merely 2% of a genome, up to 50% of a genome is occupied by repetitive element, the remaining 48% is called unique DNA, most of which probably originated in mobile elements diverged over time beyond recognition. Different evolutionary forces shape the human genome composition and structure. It appears that different mobile elements play a significant role in this process. The human genome is a dynamic entity, new functional elements appear and old ones become extinct as genes that evolve according to birth and death rule similarly to species evolution. This confirms that the theory of evolution is truly universal and applies not only to all organisms but to all levels of life as well.Our genetic material is stored in two organelles: nucleus and mitochondria. This review is focused on the nuclear genome in which 3.2 million bp are packed in 22 pairs of autosomes and two sex chromosomes, X and Y. Human chromosomes are not of equal sizes; the smallest, chromosome 21, is 54 millionbp long; the largest, chromosome 1, is almost five times bigger with 249 million bp. Genomic sequences can be divided in several ways. From the functional point of view we can distinguish genes, pseudogenes, and non-coding DNA. Only a minute fraction of the genome — about 3% — codes for proteins. There are many pseudogenes in the human genome (0.5%) but most of the genome consists of introns and intergenic DNA. Almost half of these sequences consist of different transposons; moreover, the remaining non-coding DNA most likely originated from the single copy or in very low copies; 30% of the DNA is moderately repetitive; and 10% is considered highly repetitive.

Sequence Complexity:

The human genome contains various levels of complexity as demonstrated by reassociation kinetics. Such analyses of the human genome estimate that 60% of the DNA is either single copy or in very low copies; 30% of the DNA is moderately repetitive; and 10% is considered highly repetitive. Various staining techniques demonstrate alternative banding patterns of mitotic chromosomes referred to as karyograms. Although the three broad classes of DNA are scattered

throughout the chromosome, chromosomal banding patterns reflect levels of compartmentalization of the DNA. Using the C-banding technique yields dark-staining regions of the chromosome (or C bands), referred to as heterochromatin. These regions are highly coiled, contain highly repetitive DNA, and are typically found at the centromeres, telomeres, and on the Y chromosome. They are composed of long arrays of tandem repeats and therefore some may contain a nucleotide composition that differs significantly from the remainder of the genome (approximately 40–42% GC). That means that they can be separated from the bulk of the genome by buoyant density (caesium chloride) gradient centrifugation. Gradient centrifugation results in a major band and three minor bands referred to as satellite bands — hence the term satellite DNA. The G-banding technique yields a pattern of alternating light and dark bands reflecting variations in base composition, time of replication, chromatin conformation, and the density of genes and repetitive sequences. Therefore, the karyograms define chromosomal organization and allow for identification of the different chromosomes. The darker bands, or G bands, are comparatively more condensed, more AT-rich, less gene-rich and replicate later than the DNA within the pale bands, which correspond to the R bands by an alternative staining technique. More recently, these alternative banding patterns have been correlated to the level of compaction of scaffold-attachment regions (SARs). The human genome may also be compartmentalized into large (> 300 kb) segments of DNA that are homogeneous in base composition referred to as isochores, based on sequence analysis and compositional mapping. L1 and L2 are GC-poor (or 'light) isochore families representing about 62% of the genome. The H1, H2 and H3 (heavy) isochore classes are increasingly GC-rich. There is some correlation between isochores and chromosomal bands. G bands are almost exclusively composed of GC-poor isochores, with a minor contribution from H1. R bands can be classified further into T bands (R banding at elevated temperatures), which are composed mainly of H2 and H3 isochores, and R' (non-T R bands) which are comprised of nearly equal amounts of GC-rich (primarily H1) and GC-poor isochores. Additionally, there are five human chromosomes (13, 14, 15, 21, 22) distinguished at their terminus by a thin bridge with rounded ends referred to as chromosomal satellites. These contain repeats of genes coding for rRNA and ribosomal proteins that coalesce to form the nucleolus and are known as the nucleolar organizing regions.

Gene Distribution :

Genes may be transcribed from either the same or from the opposite strand of the genome, i.e. they may lie in the same (tail-to-head) or opposite orientation (head-to-head or tail-to-tail). Although the vast majority of the human genome accounts for non-exonic sequences, a surprisingly large number of genes occupy the same genomic space. About 6% of human genes reside in introns of other genes. For example, intron 27th of NF1 gene hosts three other genes that have small introns on their own, suggesting that they are not products of retroposition. Additionally, over 100 gene pairs are overlapping at 3 end, i.e. their 3 UTRs occupy the same region though different strands. TPR and MSF genes map to the same region of chromosome 1.

The last exon of the TPR gene is 872 nt long and overlaps completely with the last exon of the MSF gene (200 nt). Interestingly, the very end of the MSF gene overlaps with the intron of the TPR gene. Unlike in plant genomes, most of non-exonic sequences in human genome account for introns (Wong et al., 2000). However, genes are not equally distributed throughout the genome. There is a distinct association between GC-richness and gene density. This is consistent with the association of most genes with CpG islands, the 500–1000 bp GC-rich segments flanking (usually at the 5 end) most housekeeping and many tissue-specific genes. The clustering of CpG islands, as demonstrated by fluorescence in situ hybridization further depicts gene-poor and gene-rich chromosomal segments. As a consequence, more than half of human genes locate in the so-called "genomic core" (isochores H2 and H3) comprising only 12% of the human genome.

Gene Families:

Many genes can be clustered in groups of different sizes based on sequence similarity. The similarity between two genes varies from genes coding identical products to genes in which product similarity is barely detectable and/or limited to short sequence stretches called sequence motives. Genes families arose during the evolution by gene duplications over the different periods of time as reflected in sequence similarity. In general, more similar genes shared a common ancestor later (in nearer past) than genes with a weaker similarity, although gene conversion can result in very similar or identical gene copies regardless of gene duplication time. Gene duplication can occur by different mechanisms, like unequal recombination or retroposition. Not all duplicated genes remain active, some of them end up in genomic oblivion and are called pseudogenes. Some of the pseudogenes can be rescued from the genomic death by capturing a promoter and regulatory elements in the course of evolution as happened with -globin gene which was rescued by an Alu element after 200 million years of silent existence. The histone gene family is an example of very similar genes. It consists of five genes that tend to be linked, although in differing arrays of variable copy numbers dispersed in the human genome. The individual genes of a particular histone family encode essentially identical products (i.e. all H4 genes code for the identical H4 protein). Analysis of individual human genomic clones has identified isolated histone genes, e.g. H4, clusters of two or more histone genes, or clusters of all histone genes, e.g. H3-H4-H1-H3-H2A-H2B. A majority of histone genes form a large cluster on human chromosome 6 (6p21.3) and a small cluster at 1q21. Interestingly, histone genes lack introns; a rare feature for eukaryotic genes. Genes that encode ribosomal RNA (rRNA) total about 0.4% of the DNA in the human genome. The individual genes of a particular rRNA family are essentially identical. The 28S, 5.8S and 18S rRNA genes are clustered with spacer units in tandem arrays of approximately 60 copies each yielding about 2 million bp of DNA. These clusters are present on the short arms of five acrocentric chromosomes and form the nucleolar organizing regions, hence approximately 300 copies. These three rRNA genes are transcribed as a

single unit and then cleaved. 5S rRNA genes are clustered on chromosome 1q. Some genes in the human genome share highly conserved amino-acid domains with weak overall similarity. These often have developmental function. There are nine dispersed paired box (Pax) genes that contain highly conserved DNA binding domains with six -helices. The homeobox or Hox genes share a common 60 amino-acid sequence. In humans there are four Hox gene clusters, each on a different chromosome. However, the individual genes in the cluster demonstrate greater similarity to a counterpart gene in another cluster than to the other genes in the same cluster. There are pseudogenes that are the result of retroposition (retropseudogenes). The pseudogenes lack introns and the flanking DNA sequences of the functional locus and therefore are not products of gene duplication. The generation of these types of elements is dependent on the reverse transcriptase of other retroelements such as LINEs.

Repetitive Sequences:

The human genome is occupied by stretches of DNA sequences of various length that exist in variable copy number. These repetitive sequences may be in a tandem orientation or they may be dispersed throughout the genome. Repetitive sequences may be classified by function, dispersal patterns, and sequence relatedness. Satellite DNA typically refers to highly repetitive sequences with no known function and interspersed repeat sequences are typically the products of transposable element integration, including retrogenes and retropseudogenes of a functional gene.

Microsatellites, Minisatellites, andMacrosatellites:

Microsatellites are small arrays of short simple tandem repeats, primarily 4 bp or less. Different arrays are found dispersed throughout the genome, although dinucleotide CA/TG repeats are most common, yielding 0.5% of the genome. Runs of As and Ts are common as well. Microsatellites have no known functions. However, CA/TG dinucleotide pairs can form the Z-DNA conformation in vitro, which may indicate some function. Repeat unit copy number variation of microsatellites apparently occurs by replication slippage. The expansion of trinucleotide repeats within genes has been associated with genetic disorders such as Huntington disease or fragile-X syndrome. Minisatellites are tandemly repeated sequences of DNA of lengths ranging from 1 kbp to 15 kbp. For example, telomeric DNA sequences contain 10–15 kb of hexanucleotide repeats, most commonly TTAGGG in the human genome, at the termini of the chromosomes. These sequences are added by telomerase to ensure complete replication of the chromosome. Macrosatellites are very long arrays, up to hundreds of kilobases, of tandemly repeated DNA. There are three satellite bands observed by buoyant density centrifugation. However, not all satellite sequences are resolved by density gradient centrifugation, e.g. alpha satellite DNA or alphoid DNA that constitute the bulk of centromeric heterochromatin on all chromosomes. The

interchromosomal divergence of the alpha satellite families allows the different chromosomes to be distinguished by fluorescence in situ hybridization (FISH).

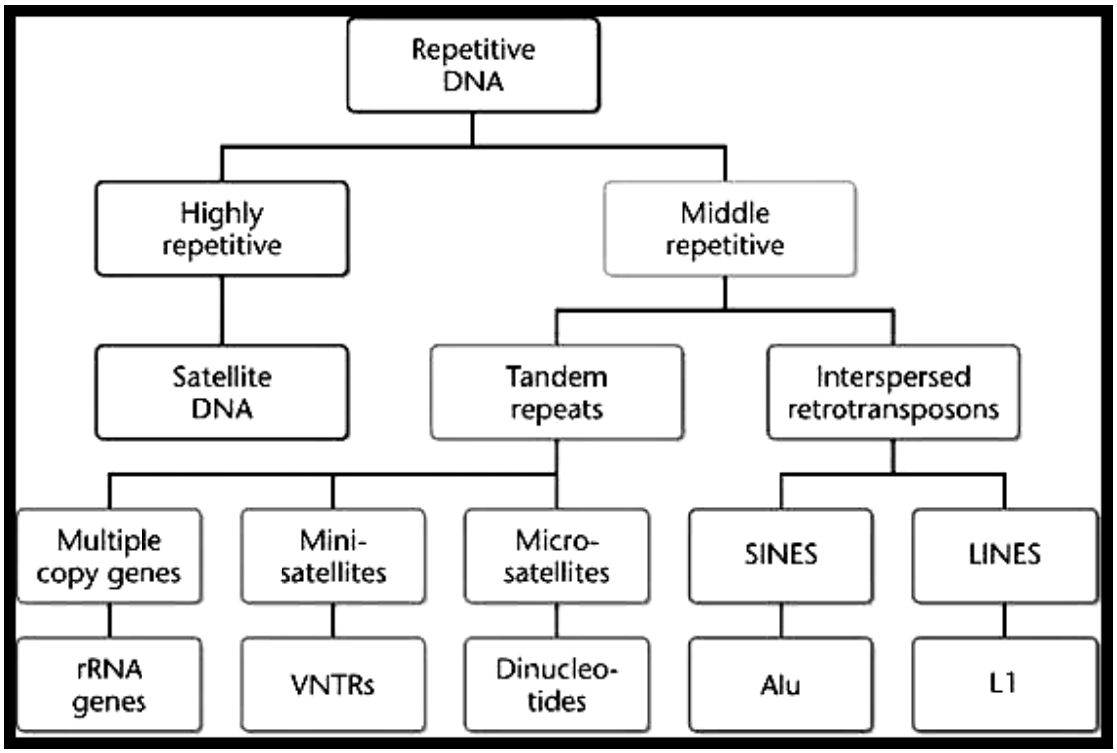


Fig: Flow charts showing various components of repetitive DNA.

Transposable Elements:

The human genome contains interspersed repeat sequences that have largely amplified in copy number by movement throughout the genome. Those sequences (transposable elements or TEs) can be divided into two classes based on the mode of transposition. The Class I elements are TEs which transpose by replication that involves an RNA intermediate which is reverse transcribed back to DNA prior to reinsertion. These are called retroelements and include LTR transposons, which are structurally similar to integrated retroviruses, non-LTR elements (LINEs and SINEs), and retrogenes. Class II elements move by a conservative cut-and-paste mechanisms, the excision of the donor element is followed by its reinsertion elsewhere in the genome. Integration of Class I and Class II transposable elements results in the duplication of a short sequence of DNA, the target site. There are about 500 families of such transposons. Most of transposition has occurred via an RNA intermediate, yielding classes of sequences referred to as retroelements (more than 400 families, e.g. Alu, L1, retrogenes, MIR). However, there is also evidence of an ancient DNA-mediated transposition (more than 60 families of class II (DNA) transposons, e.g. THE-1, Charlie, Tigger, mariner).

Retroelements:

Short interspersed repetitive elements (SINEs) and long interspersed repetitive elements (LINEs) are the two most abundant classes of repeats in human, and represent the two major classes of mammalian retrotransposons. Structural features shared by LINEs and SINEs include an A-rich 3 end and the lack of long terminal repeats (LTRs); these features distinguish them from retroviruses and related retroelements. A full-length LINE (or L1 element) is approximately 6.1 kbp although most are truncated pseudogenes with various 5 ends due to incomplete reverse transcription. There are about 100000 copies of L1 sequences in our genome. Approximately 1% of the estimated 3500 full-length LINEs have functional RNA polymerase II promoter sequences along with two intact open reading frames necessary to generate new L1 copies. Individual LINEs contain a poly-A tail and are flanked by direct repeats. LINE mobilization activity has been verified in both germinal and somatic tissues. The Alu element is estimated at 500000– 900000 copies in the human genome representing the primary SINE family, the most successful transposon in any genome. Sequence comparisons suggest that Alu repeats were derived from the 7SL RNA gene. Each Alu element is about 280 bp with a dimeric structure, contains RNA polymerase III promoter sequences, and typically has an A-rich tail and flanking direct repeats (generated during integration). Although Alu elements are present in all primate genomes, more than 2000 Alu elements have integrated within the human genome subsequent to the divergence of humans from the great apes. The human genome also contains families of retroviral-related sequences. These are characterized by sequences encoding enzymes for retroposition and contain LTRs. In addition, solitary LTRs of these elements may be located throughout the genome. There are several low abundant (10–1000 copies) human endogenous retrovirus (HERV) families, with individual elements ranging from 6 to 10 kb, collectively encompassing about 1% of the genome.

Human Genome and Mapping

Major Characteristics of human Genome:

1. The draft represents about 90% of the entire human genome. It is believed that most of the important parts have been identified.

2. The remaining 10% of the genome sequences are at the very ends of chromosomes (i.e. telomeres) and around the centromeres.

3. Human genome is composed of 3200 Mb (or 3.2 Gb) i.e. 3.2 billion base pairs (3,200,000,000).

4. Approximately 1.1 to 1.5% of the genome codes for proteins.

5. Approximately 24% of the total genome is composed of introns that split the coding regions (exons), and appear as repeating sequences with no specific functions.

6. The number of protein coding genes is in the range of 30,000-40,000.

7. An average gene consists of 3000 bases, the sizes however vary greatly. Dystrophin gene is the largest known human gene with 2.4 million bases.

8. Chromosome 1 (the target human chromosome) contains the highest number of genes (2968), while the Y chromosome has the lowest. Chromosomes also differ in their GC content and number of transposable elements.

9. Genes and DNA sequences associated with many diseases such as breast cancer, muscle diseases, deafness and blindness have been identified.

10. About 100 coding regions appear to have been copied and moved by RNA-based transposition (retro- transposons).

11. Repeated sequences constitute about 50% of the human genome.

12. A vast majority of the genome (~ 97%) has no known functions.

13. Between the humans, the DNA differs only by 0.2% or one in 500 bases.

14. More than 3 million single nucleotide polymorphisms (SNPs) have been identified.

15. Human DNA is about 98% identical to that of chimpanzees.

16. About 200 genes are close to that found in bacteria.

Meaning of Human Genome Project:

The Human Genome Project (HGP) is an International collaborative research programme which started in 1990 and completed in 2003, whose goal was the complete mapping and understanding of the three billion DNA subunits (bases), and to identify all human genes, making them accessible for further biological study.

Aims of Human Genome Project:

The project was aimed for the benefits of humankind, generation of biologists and researchers have been provided with detailed DNA information that will be key to understand the structure, organization and function of DNA in chromosomes.

Human Genome Size:

A genome is an organism's complete set of deoxyribonucleic acid (DNA), a chemical compound that contains the genetic instructions needed to develop and direct the activities of an organism. The human genome contains approx. Three billion base pairs which reside in 23 pairs of chromosomes.

Each chromosome contains hundreds and thousands of genes, and ranges in size from about 50000000 to 300000000 base pairs. The total number of genes is 30000 (approx.) and accounts for only 25% of the DNA; the rest is extra-genic DNA.

Human Genome Project Mapping:

Before beginning a sequencing project of the human genome, it was first necessary to produce a good framework map. Two general methods were developed for mapping human genome — standard method and whole genome short-gun method.

The standard method involves finding a segment of the genome and locating where it belongs. Genetic maps based on recombination frequencies between markers are useful in ordering genes. Molecular markers like RFLP, VNTRs (Microsatellites), STSs, SNPs have been used in mapping human genome.

The whole genome shotgun sequencing method involves shearing of genomic DNA followed by cloning, to produce a genomic library.

This is followed by sequencing of cloned DNA fragments at random, followed by shotgun assembly, i.e., the assembly of the fragment sequences into larger units on the basis of their overlaps. Groups of cloned DNA segments that can be aligned in an overlapping fashion to cover a region of the human genome are referred as contigs.

Yeast Artificial Chromosomes (YACs) were initially used as cloning agents when primary task was mapping. However, as the emphasis of the project shifted to sequencing. Bacterial Artificial Chromosomes (BACs) were used.

Human Genome Project had many goals some of the important goals were outlined below:

1. To identify all the approximately 20,000-25,000 genes in human DNA.

2. To determine the sequences of the 3 billion base pairs that makes up human DNA.

3. To store this information in data base.

4. To develop improvised tools for data analysis.

5. To transfer related technologies to other sectors, such as industries.

6. To address the ethical, legal and social issues (ELSI) that may arise from the project.

The methodologies involved two major approaches identifying all genes of the genome and their sequencing. For sequencing, the total DNA from a cell is isolated and converted into fragments of relatively small sizes and cloned in suitable host, this generates a genomic library of the organism. The complete sequencing of the first human chromosome, small chromosome 22, was published in December 1999. Then chromosome 21 was completely sequenced in May 2000. The first draft sequence of entire human genome was published in the famous scientific journal "Nature" on 16th February, 2001.

Individual differences in genomes:

It has to be remembered that every individual, except identical twins, have their own versions of genome sequences. The differences between individuals are largely due to single nucleotide polymorphisms (SNPs). SNPs represent positions in the genome where some individuals have one nucleotide (i.e. an A), and others have a different nucleotide (i.e. a G). The frequency of occurrence of SNPs is estimated to be one per 1000 base pairs. About 3 million SNPs are believed to be present and at least half of them have been identified.

Most of the Genome Sequence is Identified:

About 90% of the human genome has been sequenced. It is composed of 3.2 billion base pairs (3200 Mb or 3.2 Gb). If written in the format of a telephone book, the base sequence of human genome would fill about 200 telephone books of 1000 pages each.

Human Genome Project Sequence:

Sequencing means determining the exact order of the base pairs in a segment of DNA. The primary method used by the HGP to produce the finished version of the human genetic code is

map-based or BAC- based sequencing. The human DNA is fragmented into pieces that are relatively large, cloned in the bacteria, stored for replication as required.

A collection of BAG clones containing the entire human genome is called a BAC-library. In this method, each BAC clone is mapped to determine the location of that fragment in human chromosome and then the DNA letters are sequenced from each clone and their spatial relation to sequenced human DNA in other BAC clones.For sequencing, each BAC clone is cut into still smaller fragments that are about 2000 bases in length. These pieces are called **"**sub-clones". A "sequencing reaction" is carried out on these sub-clones. With the help of a computer then the short sequences are assembled into contiguous stretches of sequence of the clones.

In a short the whole process can be summarized:

i. Chromosomes, which range in size from 50 million to 250 million bases, must first be broken into much shorter pieces (sub-cloning step).

ii. Each short piece is used as a template to generate a set of fragments that differ in length from each other by a single base that will be identified in a later step (template preparation and sequencing step).

iii. The fragments in a set are separated by gel electrophoresis (separation step).

iv. The final base at the end of each fragment is identified (base-calling step). This process recreates the original sequence of As, Ts, Cs and Gs for each short piece generated in the first step.

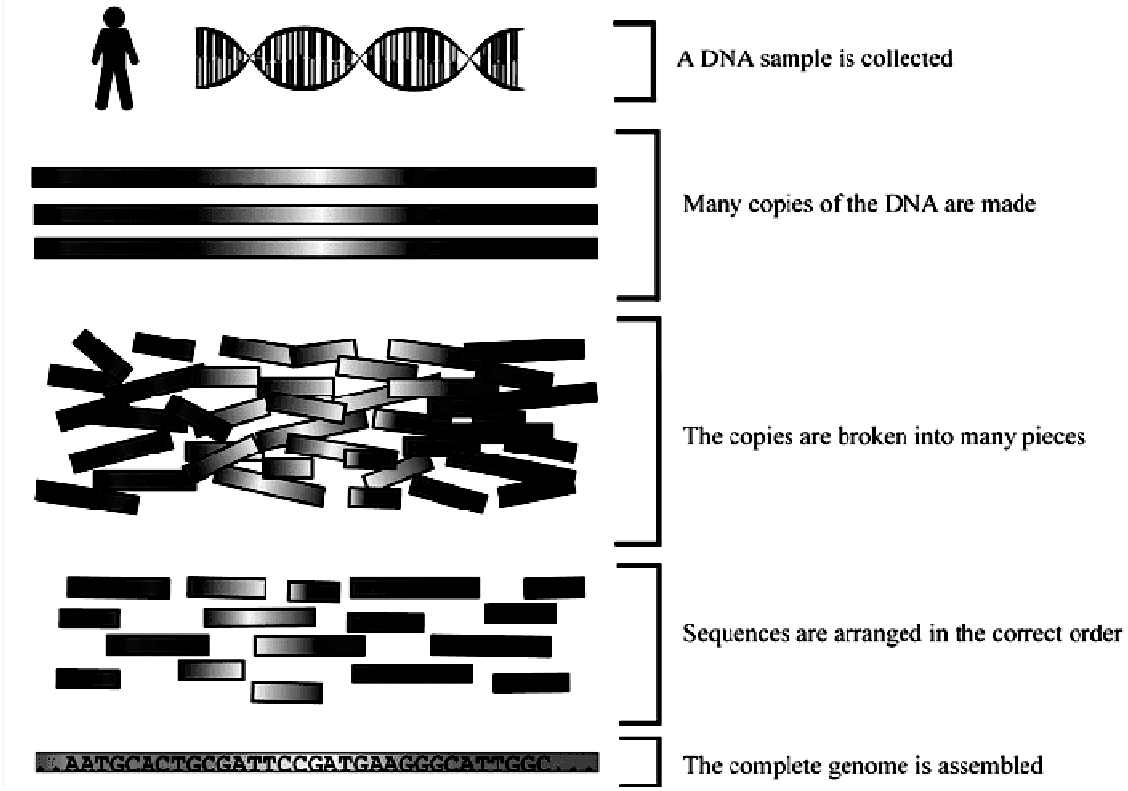
v. After the bases are 'read', computers are used to assemble the short sequences (in blocks of about 500 bases each called the read length) into long continuous stretches that are analysed for errors, gene coding regions, and other characteristics.

vi. Finished sequence is submitted to major public sequence databases, making Human Genome Project sequence data thus freely available to anyone around the world.

The human genome reference sequence do not represent any one person's genome. Rather the knowledge obtained is applicable to everyone because all humans share the same basic set of genes and genomic regulatory regions that control the development.

Researchers collected blood (female) or sperm (male) samples from different races like European, African, American (North, Central, South) and Asian ancestry and a few samples were processed as DNA resources.

Figure 2: Shotgun Whole-Genome Sequencing



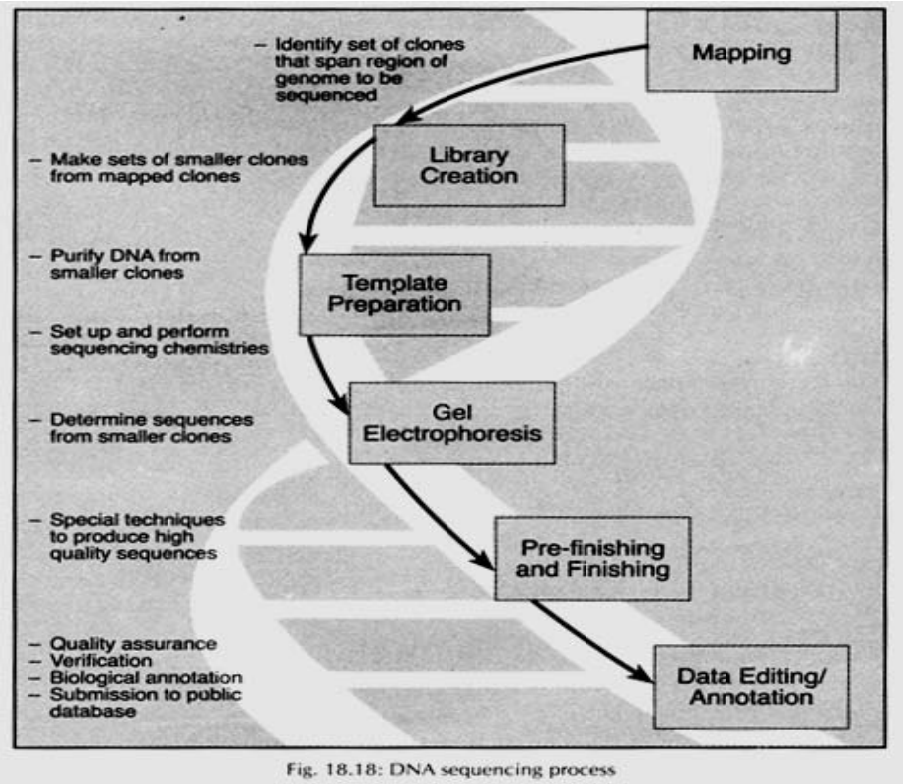


Fig. 18.18: DNA sequencing process

Outcome of Human Genome Project:

i. The human genome contains 3164.7 million chemical nucleotide bases (A, C, T and G).

ii. The average gene consists of 3000 bases, but sizes vary greatly, largest known human gene is **"dystrophin"** – 2.4 million bases.

iii. Total number of genes estimated 30000 approx.

iv. Almost all (99.9%) nucleotide bases are exactly the same in all people.

v. 50% genes are unknown for function.

vi. Less than 2% genomes code for proteins.

vii. Repeated sequences (junk DNA) is 50% of the human genome. This may contribute to create new genes, to modify and reshuffle the existing genes.

viii. A-T rich regions are gene-poor and G-C rich regions are gene-dense. Chromosome-I has the most genes (2968) and the Y chromosome has the fewest (231).

ix. Scientists have identified about 1.4 million locations where single base DNA differences (SNPs) occur in human, these findings will help to localize the disease associated sequences in the chromosomes.

x. Finding the DNA sequences underlying such common diseases as cardiovascular disease, diabetes, arthritis and cancers is being aided by human variation maps (SNPs) generated in HGP.

Benefits/Applications of Human Genome Sequencing:

It is expected that the sequencing of human genome and the genomes of other organisms will dramatically change our understanding and perceptions of biology and medicine. Some of the benefits of human genome project are given.

a. Identification of human genes and their functions:

Analysis of genomes has helped to identify the genes, and functions of some of the genes. The functions of other genes and the interaction between the gene products needs to be further elucidated.

b. Understanding of polygenic disorders:

The biochemistry and genetics of many single- gene disorders have been elucidated e.g. sickle-cell anaemia, cystic fibrosis, and retinoblastoma. A majority of the common diseases in humans, however, are polygenic in nature e.g. cancer, hypertension, diabetes. At present, we have very little knowledge about the causes of these diseases. The information on the genome sequence will certainly help to unravel the mysteries surrounding polygenic diseases.

c. Improvements in gene therapy:
At present, human gene therapy is in its infancy for various reasons. Genome sequence knowledge will certainly help for more effective treatment of genetic diseases by gene therapy.

d. Improved diagnosis of diseases:
In the near future, probes for many genetic diseases will be available for specific identification and appropriate treatment.

e. Development of pharmacogenomics:
The drugs may be tailored to treat the individual patients. This will become possible considering the variations in enzymes and other proteins involved in drug action, and the metabolism of the individuals.

f. Genetic basis of psychiatric disorders:
By studying the genes involved in behavioural patterns, the causation of psychiatric diseases can be understood. This will help for the better treatment of these disorders.

g. Understanding of complex social trait:
With the genome sequence now in hand, the complex social traits can be better understood. For instance, recently genes controlling speech have been identified.

h. Knowledge on mutations:
Many events leading to the mutations can be uncovered with the knowledge of genome.

i. Better understanding of developmental biology:
By determining the biology of human genome and its regulatory control, it will be possible to understand how humans develop from a fertilized eggs to adults.

j. Comparative genomics:
Genomes from many organisms have been sequenced, and the number will increase in the coming years. The information on the genomes of different species will throw light on the major stages in evolution.

k. Development of biotechnology:
The data on the human genome sequence will spur the development of biotechnology in various spheres.

Probable Questions:

1. How genes are distributed in human genome?

2. What do you mean by gene families?

3. Define microsatellite, macrosatellite and minisatellite.

4. Write a short note on retroelements in human genome.

5. Write the major characteristics of human genome.

6. Write the major goals of Human Genome Project.

7. What are the outcomes of Human Genome Project?

8. What are the applications of Human Genome Project?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit- VIII

Human karyo type: karyotype and nomenclature of Metaphase chromosome bands

Objective: In this unit you will learn about Human Karyotype and different banding techniques.

Introduction:

Chromosomal aberrations are abnormalities in the number or microscopically observable structure of chromosomes. The number of chromosomes in human cells is 46 with 22 autosomal pairs (one of each type contributed by the mother and one of each type from the father) and 2 sex chromosomes - 2 X chromosomes for females (one from father and one from mother) or an X and a Y chromosome for males (the X from the mother and the Y from the father). The chromosomes visible only at the metaphase stage of mitosis, 22 homologous pairs of autosomes and two sex chromosomes. Each chromosome has a characteristic size and shape in the "normal" cell. During most of the cell cycle, interphase, the chromosomes are somewhat less condensed and are not visible as individual objects under the light microscope. Mitosis, or nucleus division, is the first part of M-phase and in consists of four stages (prophase, metaphase, anaphase and telophase). However during cell division, mitosis, the chromosomes become highly condensed and are then visible as dark distinct bodies within the nuclei of cells. The chromosomes are most easily seen and identified at the metaphase stage of cell division.

The banding of chromosomes by using dyes was discovered in the late 1960's and before that cytogeneticists depended on chromosome length and position of a constriction to identify the individual chromosomes. The band width and the order of bands is characteristic of a particular chromosome - a trained cytogeneticist can identify each chromosome (1,2,3...22, X and Y) by observing its banding pattern under the microscope. chromosomes are arranged and numbered by size, from largest to smallest. This arrangement helps scientists quickly identify chromosomal alterations that may result in a genetic disorder. Identifying chromosomes has become easier in recent years by using certain staining techniques. One of the most common staining techniques involves Giemsa stain, which gives the chromosomes a banded appearance (hence Giemsa banding or G-banding) G-banding is the treatment of chromosomes in the metaphase stage with trypsin (to partially digest the protein) and stain them with Giemsa. Each homologous chromosome pair has a unique pattern of G-bands, enabling recognition of particular chromosomes. Karyotyping is the process by which doctors and geneticists take pictures of the chromosomes while the cell are undergoing mitosis. The picture is then enlarged. The picture of the chromosomes are then cut up so that each chromosome is removed. The chromosomes are matched up and attached to a paper according to size. The chromosomes pairs are numbered from largest to smallest. There are 22 pairs of chromosomes which match up exactly. Then the sex chromosomes are paired, in the female (XX) the chromosomes match and in the male (XY) the chromosomes do not match. This technique can be used to assess the "normalcy" of an individual's chromosomes and to assay for various genetic diseases such as Down's syndrome

Klinefelter’s syndrome. It is estimated that one in 156 live births have some kind of chromosomal abnormality. A chromosome is divided by its centromere into short arm (p) and long arm (q). chromosomes can be classified by the position of their centromere: - Metacentric: If its two arms are equal in length. - Submetacentric: If arms' lengths are unequal. - Acrocentric: If the p arm is so short that is hard to observe, but still present The photograph is enlarged and cut up into individual chromosomes. The homologous chromosomes can be distinguished by length and by the position of the centromere so the chromosomes can be arranged in 7 groups (A, B, C, D, E, F, G). Karyotypes are arranged with the short arm of the chromosome on top, and the long arm on the bottom. In addition, the differently stained regions and sub-regions are given numerical designations from proximal to distal on the chromosome arms. For example, Cri du chat syndrome involves a deletion on the short arm of chromosome 5. It is written as 46,XX,5p-. The critical region for this syndrome is deletion of 15.2, which is written as 46,XX,del(5)(p15.2). Peripheral Blood Karyotyping Medium With Phytohemagglutinin (PHA) is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. It is based on RPMI-1640 basal medium supplemented with L-Glutamine, fatal bovine serum and antibiotics (penicillin and streptomycin). Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing and phytohaemagglutinin supplementation. The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen (PHA), lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor (colcemid) is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

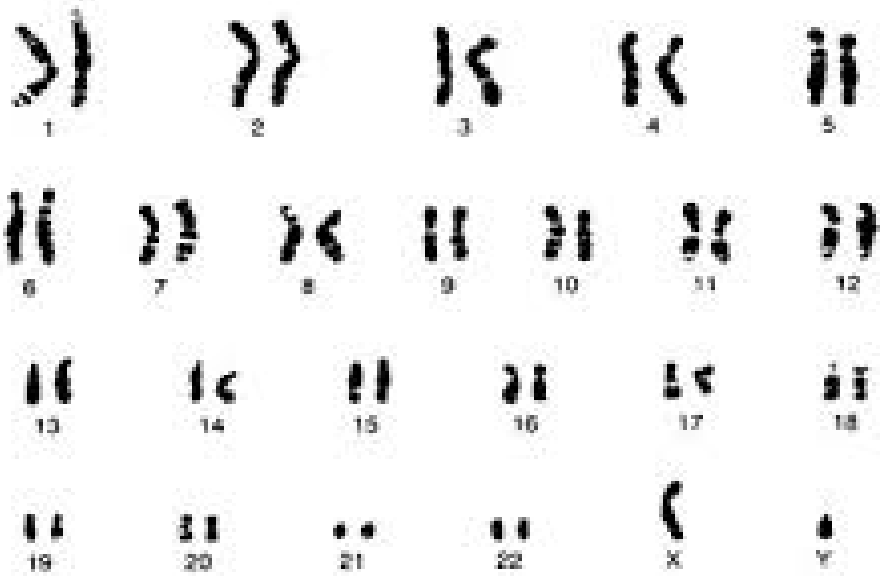


Fig. 21.1 Karyotype of a normal human male showing G-bands

Classification of Chromosomes for Karyotyping:

Chromosomes are arranged into seven groups based on size and centromere location. The centromeres can be found in the middle of the chromosome (median), near one end (acrocentric), or in between these first two (submedian)

Group A: chromosomes 1-3 are largest with median centromere.

Group B: chromosomes 4-5 are large with submedian centromere

Group C: chromosomes 6-12 are medium sized with submedian centromere

Group D: chromosomes 13-15 are medium sized with acrocentric centromere

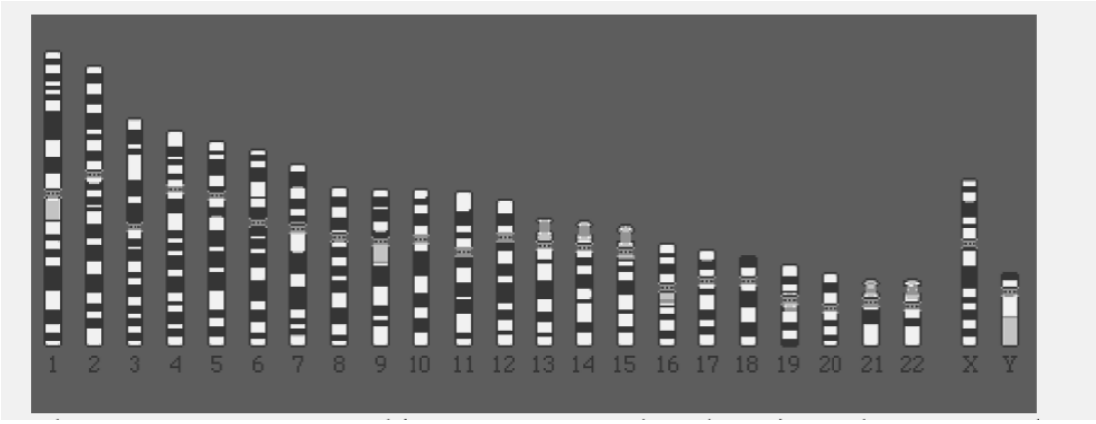
Group E: chromosomes 16-18 are short with median or submedian centromere

Group F: chromosomes 19-20 are short with median centromere

Group G: chromosomes 21-22 are very short with acrocentric centromere.

Chromosome X is similar to group C.

Chromosome Y is similar to group G



Chromosomes are arranged into seven groups based on size and centromere location. The centromeres can be found in the middle of the chromosome (median), near one end (acrocentric), or in between these first two (submedian)

Karyogram:

Photomicrographs of the chromosomes of a single representative somatic metaphase cell are clipped out and arranged in homologous pairs according to their size. If the chromosomes are small and there is difficulty in identifying the individual chromosomes, they are arranged in groups of similar chromosomes.

For example, in human, the 23 pairs of chromosomes had been divided into 7 groups represented by the letters from A to G (Denver System) and numbers (London System); the seven groups are, A (l, 2, 3), B, (4, 5), C (X, 6, 7, 8, 9, 10 11, 12), D (13, 14, 15), E (16, 17, 18), F (19, 20), and G (Y, 21, 22). Thus the X chromosome is placed in the C group, while the Y chromosome is placed in the G group. However, it is now possible to unambiguously identify each of the 23 chromosomes, and even individual chromosome arms, with the help of chromosome banding.

Idiogram or Idiotype:

It is the graphical representation of the karyotype. Generally, the idiogram is prepared to show the haploid chromosome complement of a species; it is prepared from the measurement of somatic metaphase chromosomes. Individual chromosomes must be identified for this purpose. There are techniques by which chromosomes or even specific chromosome segments can be identified. These techniques are fluorescent staining, pulse labelling, chromosome banding, and studying the tertiary constrictions and chromomeres. Chromomere pattern can be studied easily and clearly in pachytene stage in many species and in polytene giant chromosomes of several members of Diptera.

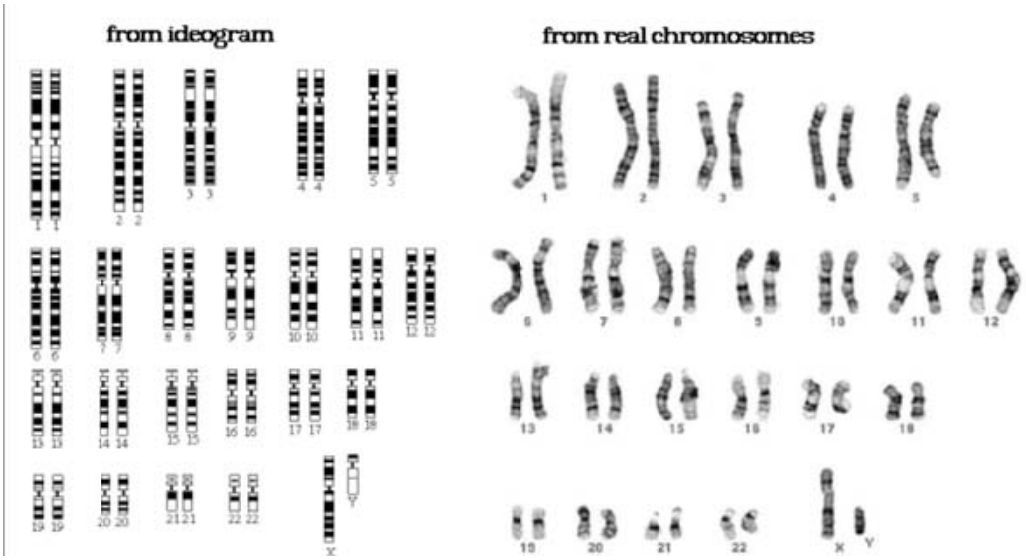
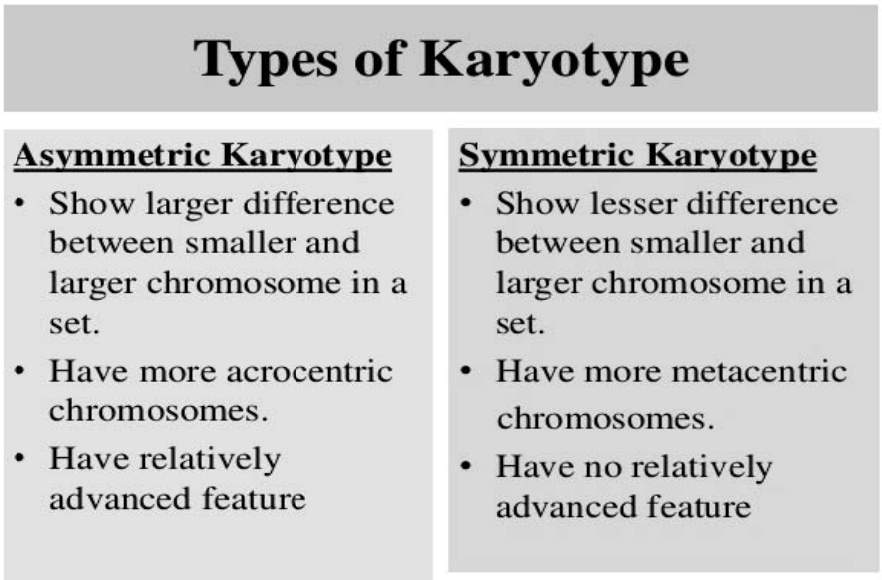


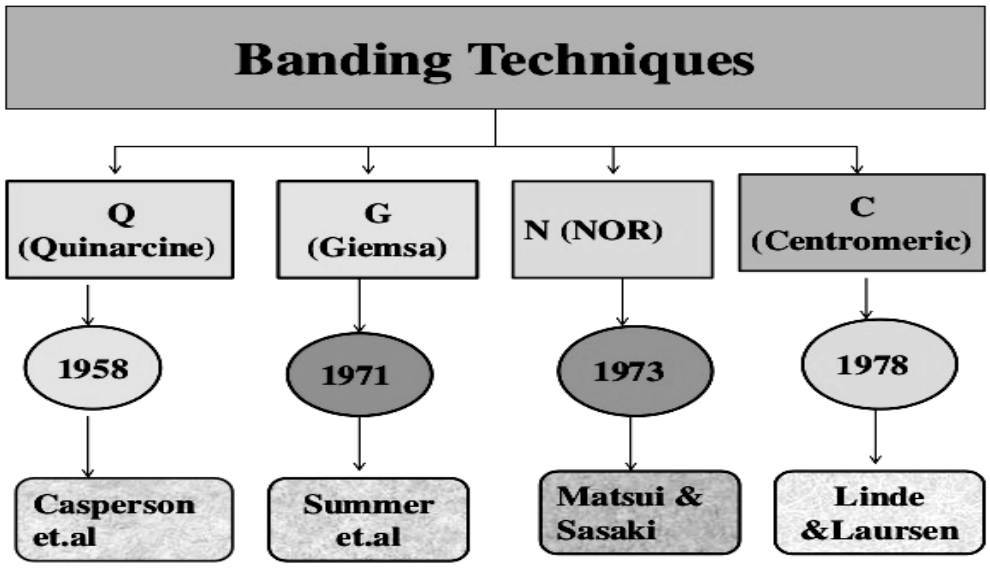
Fig: Idiogram prepared from human chromosomes



Chromosome Banding:

Chromosome banding is the "lengthwise variation in staining properties along a chromosome…normally independent of any immediately obvious structural variation," and thus excludes patterns such as those seen on polytene chromosomes of Drosophila, which have a morphological component. Although the first observations of what could be called chromosome banding were made at the end of the nineteenth century, modern chromosome banding methods date from 1968 and can be applied to chromosomes of a wide variety of species with no more than slight modifications. Following the introduction of Q-banding by Caspersson and his colleagues in 1968, Pardue and Gall inadvertently produced differential staining of heterochromatin in their pioneering in situ hybridization studies, leading directly to C-banding, and in 1971 G-banding was discovered by several authors. R-banding was also introduced in 1971. Over the next few years, many other banding techniques, too numerous to mention individually, were introduced, many of them using fluorochromes. Silver staining for nucleolus organizing regions(NORs) was introduced in 1975, methods to show chromosome replication were invented, and the use of autoimmune sera to label kinetochores immunocytochemically was discovered.

Chromosome Banding Techniques:

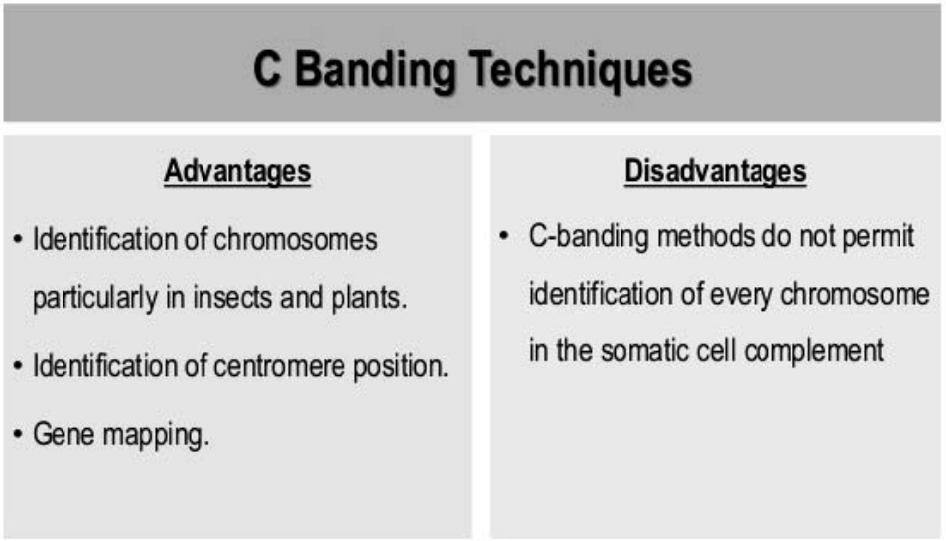


a. C-Bands:

The technique of C-banding originated after the work of Pardue and Gall who reported that constitutive heterochromatin can be stained specifically by Giemsa-solution. Each chromosome possesses a different degree of constitutive heterochromatin which enables the identification of individual chromosomes.

Constitutive heterochromatin is located near the centromere, at telomeres and in the nucleolar organizer regions; it is composed of highly repetitive DNA. C-banding represents the constitutive heterochromatin, and the banding is caused by differential staining reactions of the DNA of heterochromatin and euchromatin. The banding method is a complex technique that involves

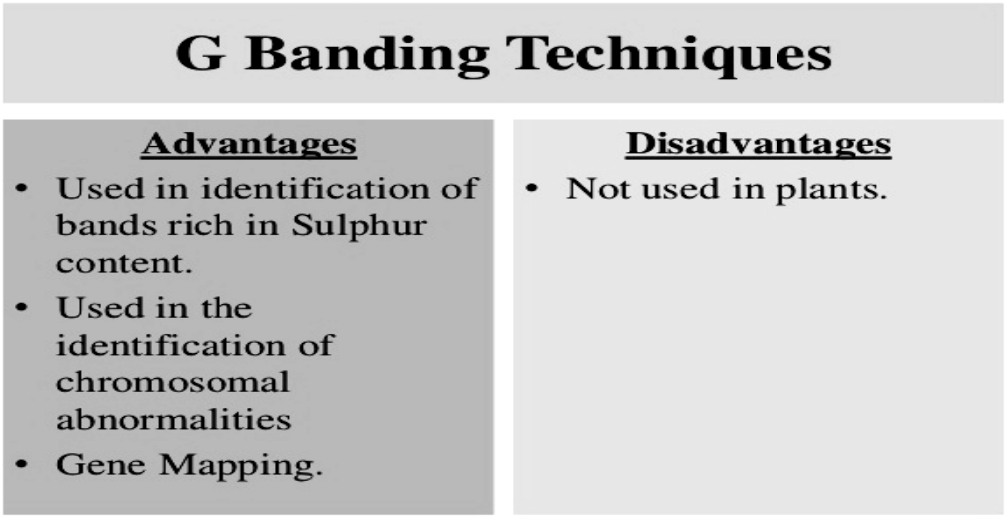
several treatments with acid, alkali or increased temperature. Denaturation of DNA is caused by these treatments. Subsequently, DNA renaturation occurs in treatments with sodium-citrate at 60°C. By these treatments, the repetitive DNA (heterochromatin) re-natures but low repetitive and unique DNAs do not re-nature. This results in differential staining of the specific chromosome regions. Giemsa-C-banding technique has been used to identify chromosomes of various plant and animal species including human. The Y chromosome of mammals is mostly heterochromatic and therefore, the technique of C-banding is quite useful for its identification.



Gupta ,P. K., 2012. Cytogenetics an advanced study, *Chapter 1*: 3-16.

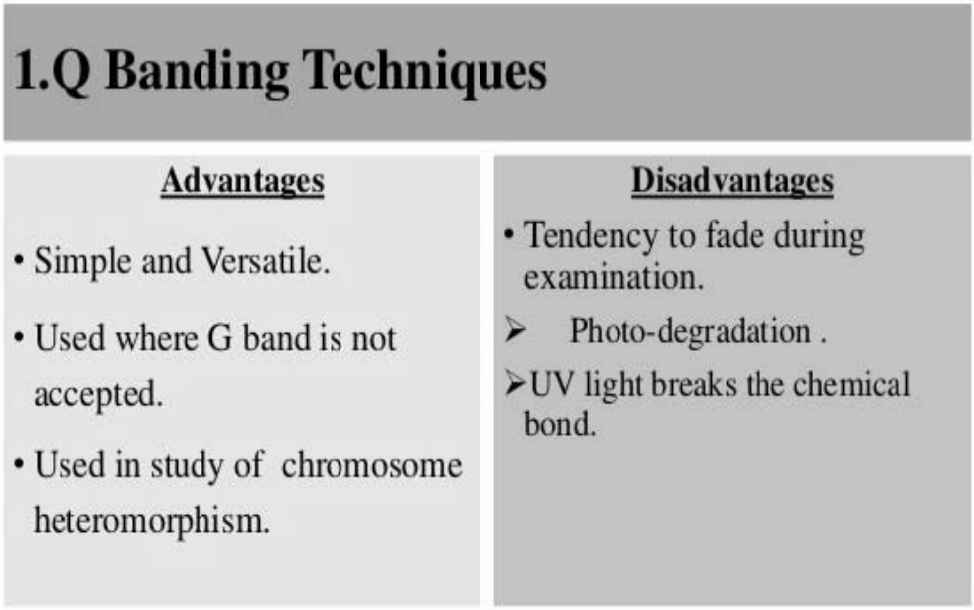
b. G-Bands:

The technique of G-banding involves Giemsa staining following pretreatment with weak trypsin solution, urea or protease. It provides greater detail than C-banding. It was first used for human chromosomes by Summer et al. in 1971. G-bands may reflect a stronger chromatin condensation. However, this technique is not suitable for plant chromosomes.



c. Q-Bands:

The method of Q-banding was developed by Caspersson et al. in 1968. The chromosomes stained with Quinacrine mustard show bright and dark zones under UV light. This technique is used to identify human and mice chromosomes.

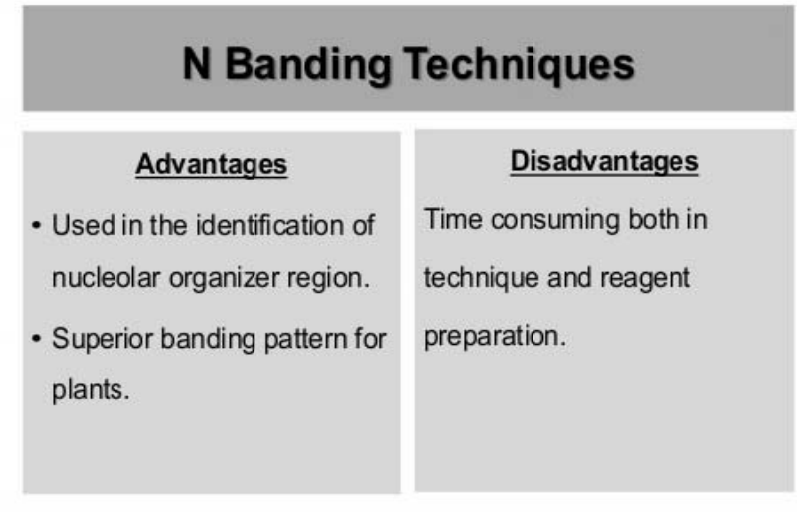


d. N-Bands:

The technique of N-banding was originally described by Matsui and Sasaki in 1973. Briefly, air-dried chromosomes slides are stained for 90 minutes with Giemsa (diluted 1 : 10 in 1/15 M phosphate buffer at pH 7.0) following extraction with 5% trichloroacetic acid at 95°C for 30 minutes and then 0.1 NHCl at 60″C for 30 minutes.The N-bands are generally located at the secondary constriction, satellites, centromeres, telomeres and heterochromatic segments. It is suggested that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar organizer region of the eukaryotic chromosomes.

The N- banding patterns have been used for the location of nucleolar regions in the different organisms, such as, mammals, birds, amphibians, fishes, insects and plants. N-banding patterns differ in the chromosomes of different species.In 1980, Islam used this method to identify the barley chromosomes from those of wheat in the reciprocal wheat-barely F, hybrids, and to detect translocations between the wheat and barley chromosomes. He also used this technique to isolate lines possessing a pair of barely chromosomes substituted for particular pair of wheat chromosomes.A modified Giemsa-N-banding technique was developed by Singh and Tsuchiya in 1982 for the identification of barley chromosomes. This method is a combination of acetocarmine staining and Giemsa-N-banding. After processing according to this method, the centromeric region looks like a **"**diamond-shaped" structure; this is not seen in other techniques.Early

metaphase or prometaphase chromosomes are more suitable for this staining as they show better banding pattern than the chromosomes at mid-metaphase in somatic cells.



Other Techniques of Chromosome Banding:

Besides the above, there are other techniques for chromosome banding, e.g., R-banding (Reverse Giemsa banding). H-banding, and T-banding (Terminal banding). Chromosome banding patterns can be used not only for the identification of individual chromosomes of an organism but also to establish evolutionary relationships between different species.

Banding patterns in human, chimpanzee, gorilla and orangutan have indicated that the evolutionary relationship between human and chimpanzee is closer than that between human and gorilla. It has further indicated that humans have a more distant evolutionary relationship with orangutans.

Uses of Chromosome Banding

G-and R-banding are the most commonly used techniquesfor chromosome identification (karyotyping) and foridentifying abnormalities of chromosome number, translocationsof material from one chromosome to another,and deletions, inversions or amplifications of chromosomesegments. This has had an invaluable impact on humangenetics and medicine and the power of this approach hasbeen augmented by combining cytogenetics with fluorescencein situ hybridization (FISH). The detection ofchromosome deletions associated with disorders, veryoften contiguous gene syndromes, provided some of the

first disease gene localizations in humans. Similarly,translocations have been important in pinpointing thelocation of disease-associated genes and the characteristictranslocationsassociated with some leukaemia is important,not only for understanding the molecular basis ofthese cancers,

but also for their diagnosis and prognosis.One of the best examples of this is the translocation between human chromosomes 9 and 22 – t(9;22)(q34:q11)– or the Philadelphia chromosome diagnostic of chronicmyelogenous leukaemia (CML).

Comparisons of chromosome banding patterns canconfirm evolutionary relationships between species andalso reveal changes in karyotype that may have beenimportant in speciation. The banding patterns of human,gorilla and chimpanzee chromosomes are almost identical,though human chromosome 2 is the result of a fusionbetween two great ape chromosomes. There are alsoextensive similarities between human chromosome bandsand those of lower primates.

Evolution of chromosome bands

Whereas Q-, G- and R-banding patterns have only beenobserved in some eukaryotes, replication banding is almostuniversal among living organisms possessing chromosomes

large enough to see by microscopy, suggesting that itis a fundamental consequence of, or requirement for, thecompartmentalization of complex genomes.Chromosomes from most mammals and birds can be GandR-banded. With amphibia, fish and plants, some species band whereasothers do not. The lowest vertebrates with reported goodG-banding are the bony fish.Evolutionary analysis of chromosome banding patternssuggests that the first cytogenetically detectable compartmentalizationthat arose in the genomes of eukaryotes wasthe temporal control of replication and differences inchromatin packaging and the segregation of some chromosomaldomains into heterochromatin. Ability to be Gbanded (and we will assume here that this is a reflection ofdifferences in chromatin structure on mitotic chromosomes) followed later. Fluorochrome banding seems tohave appeared on the scene last of all.

Probable questions:

1. Define Karyotype and Idiogram.

2. Describe grouping of chromosomes in karyotype.

3. What are the difference between symmetric and asymmetric karyotype.

4. write a note on chromosome banding. Name 4 types of chromosome banding.

5. What do you mean by C banding? state its advantages and disadvantages.

6. What do you mean by Q banding? state its advantages and disadvantages.

7. What do you mean by N banding? state its advantages and disadvantages.

8. What do you mean by G banding? state its advantages and disadvantages.

9. what are the uses of Chromosome banding ?

10. How Chromosomes bands have evolved?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit-IX

Chromosome anomalies and diseases-autosomal and sex chromosomal abnormalities; chromosomal anomalies in malignancy (chronic myeloidl eukaemia, Burkitt's lymphoma, retinoblastoma and Wilms' tumour)

Objectives: In this unit you will learn about various type of chromosomal anomalies and associated disease. You will also learn about different types of chromosomal anomalies associated with certain types of cancer such as chronicmyeloidleukaemia, Burkitt's lymphoma, retinoblastoma and Wilms' tumour.

Introduction:

Almost every cell in our body contains 23 pairs of chromosomes, for a total of 46 chromosomes. Half of the chromosomes come from our mother, and the other half come from our father. The first 22 pairs are called autosomes. The 23rd pair consists of the sex chromosomes, X and Y. Females usually have two X chromosomes, and males usually have one X and one Y chromosome in each cell. All of the information that the body needs to grow and develop comes from the chromosomes. Each chromosome contains thousands of genes, which make proteins that direct the body's development, growth, and chemical reactions.

Many types of chromosomal abnormalities exist, but they can be categorized as either numerical or structural. Numerical abnormalities are whole chromosomes either missing from or extra to the normal pair. Structural abnormalities are when part of an individual chromosome is missing, extra, switched to another chromosome, or turned upside down.

Chromosomal abnormalities can occur as an accident when the egg or the sperm is formed or during the early developmental stages of the foetus. The age of the mother and certain environmental factors may play a role in the occurrence of genetic errors. Prenatal screening and testing can be performed to examine the chromosomes of the foetus and detect some, but not all, types of chromosomal abnormalities.

Chromosomal abnormalities can have many different effects, depending on the specific abnormality. For example, an extra copy of chromosome 21 causes Down syndrome (trisomy 21). Chromosomal abnormalities can also cause miscarriage, disease, or problems in growth or development.

The most common type of chromosomal abnormality is known as aneuploidy, an abnormal chromosome number due to an extra or missing chromosome.Most people with aneuploidy have trisomy (three copies of a chromosome) instead of monosomy (single copy of a chromosome).

Down syndrome is probably the most well-known example of a chromosomal aneuploidy. Besides trisomy 21, the major chromosomal aneuploidies seen in live-born babies are: trisomy 18; trisomy 13; 45, X (Turner syndrome); 47, XXY (Klinefelter syndrome); 47, XYY; and 47, XXX.

Structural chromosomal abnormalities result from breakage and incorrect rejoining of chromosomal segments. A range of structural chromosomal abnormalities result in disease. Structural rearrangements are defined as balanced if the complete chromosomal set is still present, though rearranged, and unbalanced if information is additional or missing. Unbalanced rearrangements include deletions, duplications, or insertions of a chromosomal segment. Ring chromosomes can result when a chromosome undergoes two breaks and the broken ends fuse into a circular chromosome. An isochromosome can form when an arm of the chromosome is missing and the remaining arm duplicates.

Balanced rearrangements include inverted or translocated chromosomal regions. Since the full complement of DNA material is still present, balanced chromosomal rearrangements may go undetected because they may not result in disease. A disease can arise as a result of a balanced rearrangement if the breaks in the chromosomes occur in a gene, resulting in an absent or nonfunctional protein, or if the fusion of chromosomal segments results in a hybrid of two genes, producing a new protein product whose function is damaging to the cell.

I) Autosomal Chromosomal abnormalities:

Disorders of the autosomes are much more frequent that disorders of the sex chromosomes (Klinefelter syndrome, Turner syndrome). Typical are numeric abnormalities and we then recognize two types of disorders:

1. monosomy – the carrier lost one copy of a chromosome (45,XY);

2. trisomy – there are one more copy of a chromosome (47,XY).

1. Although the trisomies of chromosomes 18 and 13 were discovered early, there is another trisomy which occurs most – trisomy 21 (Down syndrome).

There are also structural disorders of the autosomes. The most important are deletions. Very well known are deletion of short arm of 5 chromosome (Cri du chat syndrome) and deletion of long arm of 22 chromosome (DiGeorge syndrome).

Most of carriers of autosomal mutations die during their development and usually not born. Especially monosomies in foetus are connected with abortion. Children with trisomy and

deletions who are born, suffer from physical and mental retardation and have shorter life at all. The cause of these disorders is usually meiotic nondisjunction – parents have normal karyotype.

a. Trisomy 21 (Down syndrome)

Down syndrome is very well-known trisomy. Trisomy was first described in 1866 by scientist John Down, and later elaborated upon with much greater detail through the Karyotype of Trisomy discovery in 1959..

There are three types of origin. The most common is an *extra chromosome 21* (95%). Sometimes (4% of cases) a small parts of chromosome 21 *translocate* to 14 or 22 chromosome. In 2% of cases we can find *mosaic*. Mosaic means that person has some cells with trisomy and some without. The last two examples have less severe symptoms. The extra chromosome is much worse.

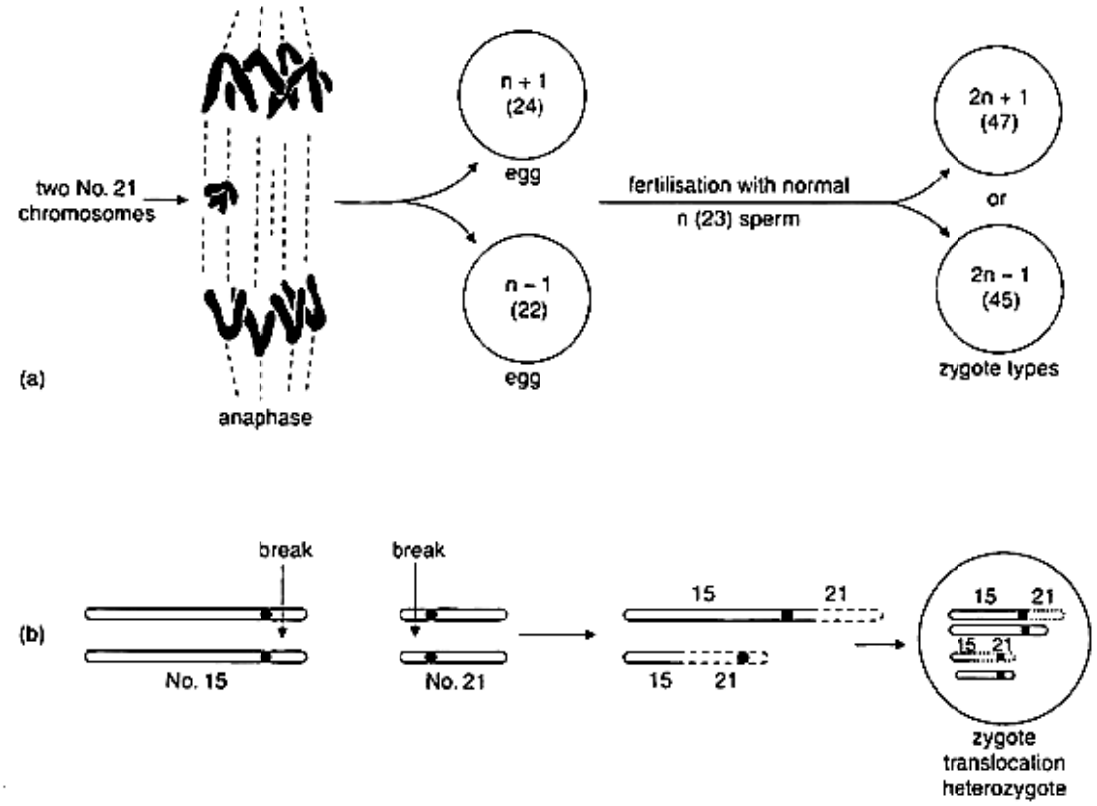


Fig. 21.2 (*a*) Origin of Down's syndrome through nondisjunction during meiosis and (*b*) through translocation.

People with Down syndrome have typical **physical appearance**. They have short body, flat faces, epicanthic folds (part of skin over the inner corner of the eye – the reason why they are sometimes called "Mongoloid"), small low-set ears and relatively large tongue. They have also

motor problems because of small muscle tone. Carriers suffer more from leukaemia, infections, cardiac malformations, epilepsy, hypothyroidism and Alzheimer disease. The mental abilities are also worse.

The IQ of people with Down syndrome is about 50. It means that they reach a level of a small child – about 6 years old. On the second side their character is very friendly, warm and loving. Congenital heart disease and leukaemia occur in many cases. Many have poor muscle tone during infancy. There is a higher incidence of Down's syndrome among children of older mothers.

The patients live for a variable number of years. A Down's male is usually sterile, females are fertile and rarely have produced offspring. The dermatoglyphic pattern (arrangement of lines on palm and fingers) shows in many cases a line called simian crease and distal axial triradius. Frequently all the ten fingers show ulnar loops.

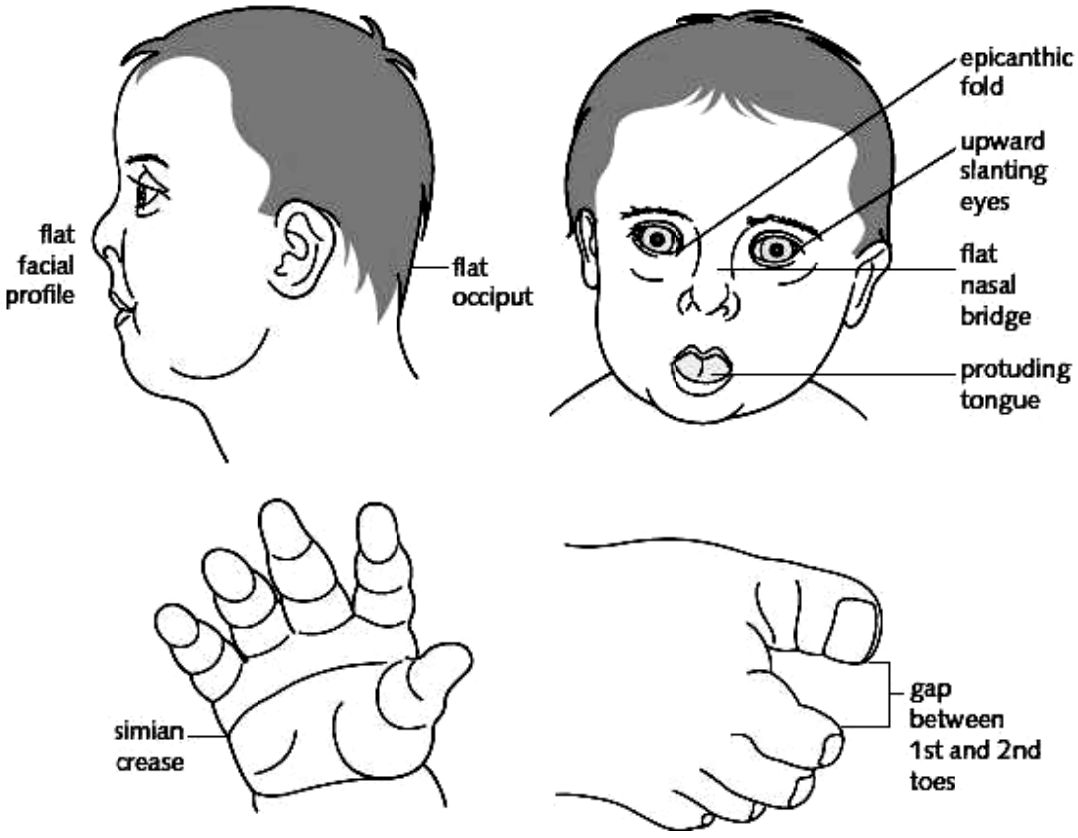


Fig: Symptoms of Down syndrome

The cause of 21 trisomymeiotic nondisjunction. Parents of the children have normal karyotype and no symptoms. Very big influence has the age of the mother. The higher age is connected with higher risks (higher means more than 35 years). The incidence is 1 in 25 live births in women older than 45 years.

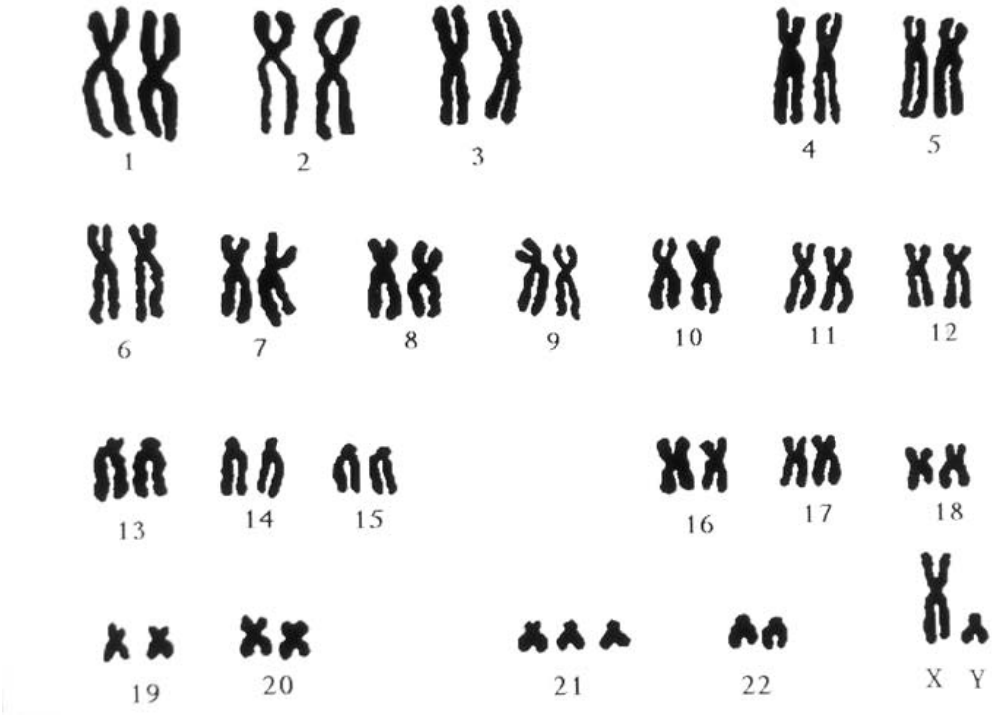


Fig: karyotype of Down Syndrome

As in most genetic diseases there is no cure for a Down's patient. Affected individuals are usually institutionalised. However, after the birth of a Mongol child it is necessary for the parents to have proper genetic counselling to prevent the birth of another child with mongolism.

An accurate diagnosis through karyotype analysis of the affected child and both parents could provide an estimate of the recurrence risk. When mongolism is due to a translocation, the abnormality can be passed on to future generations through the gametes.

A child that inherits the translocation is affected and could in turn produce victims of Down's syndrome. In contrast, Down's syndrome due to non-disjunction, which is a rare event during gametogenesis, is not familial and the condition is not inherited.

b. Trisomy 18 (Edward's syndrome)

Edward and his colleagues in 1960 described a syndrome due to trisomy of an E group chromosome (16-18) and occurring more often in females than in males. The individual may also be a mosaic having a normal cell line and an 18 trisomic line. The incidence is about 1 in 3,500 live births.

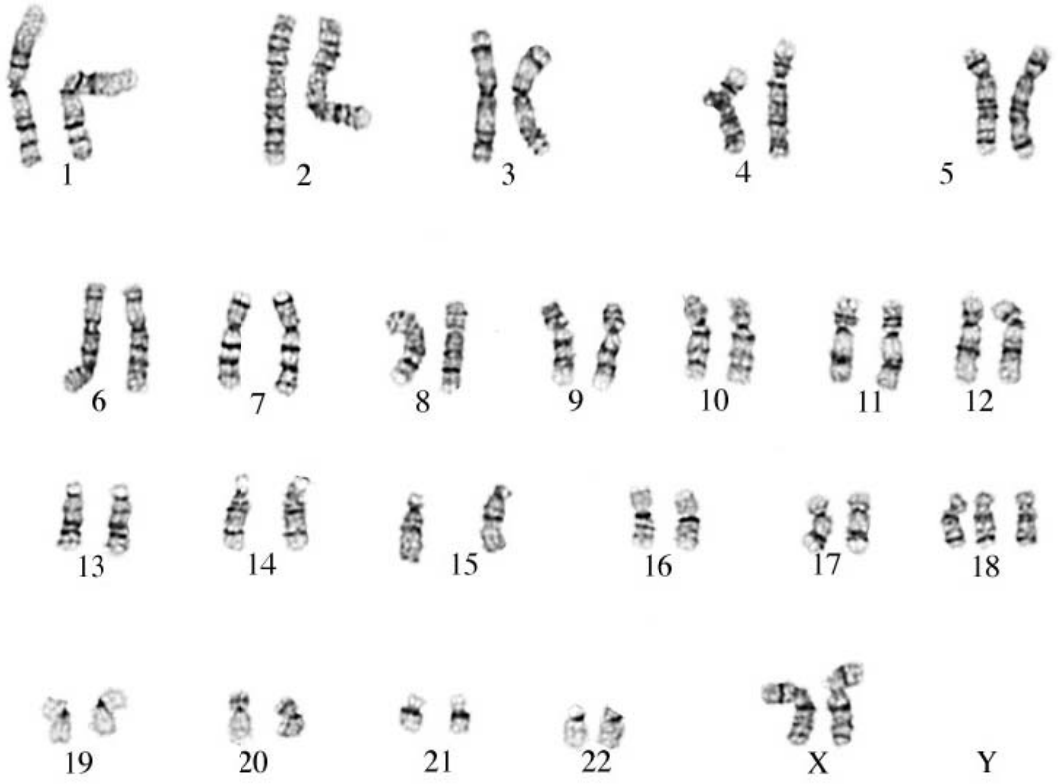


Fig: Karyotype of Edward's Syndrome

The physical and mental growth is much retarded and death usually occurs in early childhood. Patients have hyper-tonicity of skeletal muscles resulting in a peculiar characteristic by which the affected person keeps the fingers tightly clenched against the palm of the hand. The typical features also include micrognathia (small jaws), deformed ears, small sternum and pelvis, a characteristic dermatoglyphic pattern and severe retardation.

The incidence is 1 in 8000. Boys are more affected than girls. As the Down syndrome, Edwards syndrome is also influenced by the age of the mother. Nowadays there is no therapy and the cure is just palliative. Most children with trisomy 18 is not able to survive their first year and they die.

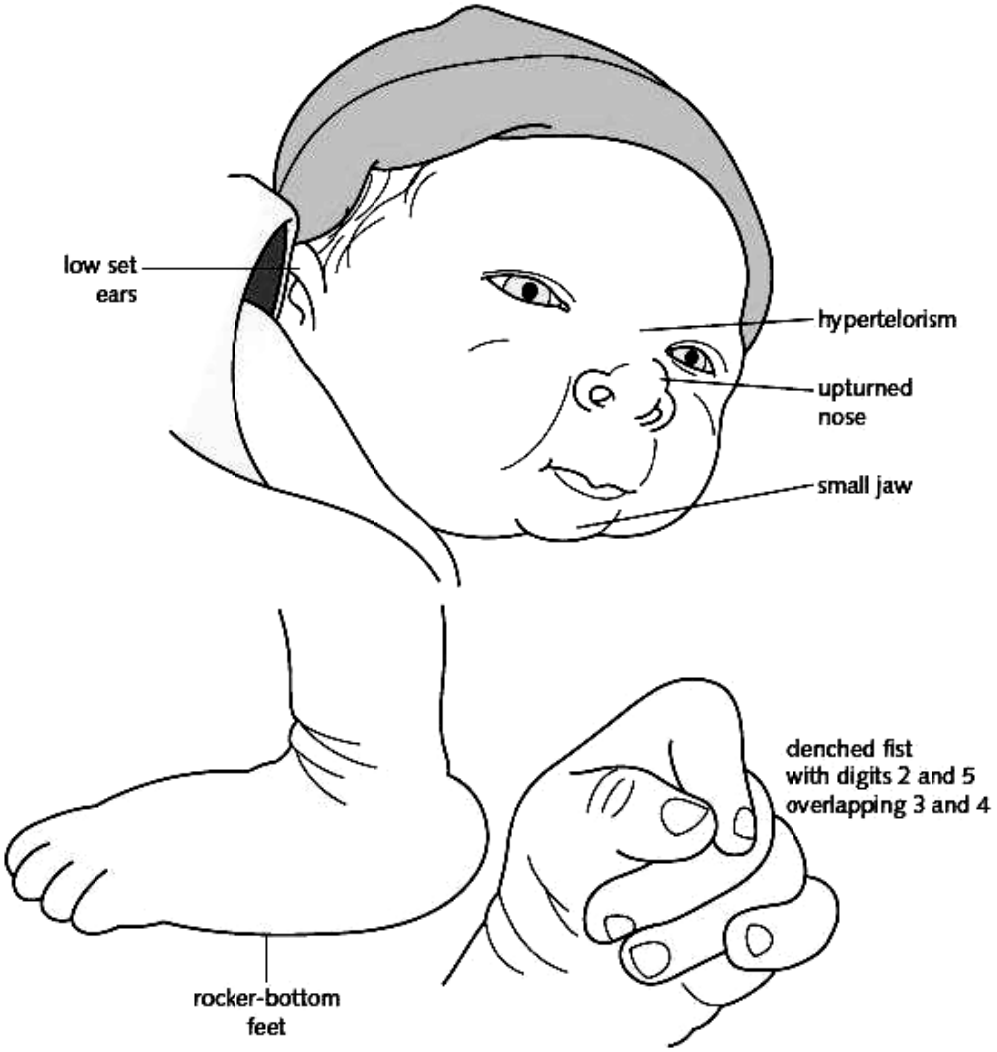


Fig: Symptoms of Edward's syndrome

c. Trisomy 13 (Patau's syndrome)

Bartholin-Patau syndrome, also called autosomal Trisomy 13, is a very severe condition first described by Dr. Klaus Patau in 1960. This genetic disorder is associated with severe intellectual disability and physical abnormalities in many parts of the body. The affected individuals often have heart defects, brain or spinal cord abnormalities, microphthalmia (very small or poorly developed eyes), cleft lip, extra fingers and/or toes, among other features.

Trisomy 13 is a chromosomal condition in which the sex ratio at birth is slightly higher in females than in males. This fact could be related to the decreased survival rate among male foetuses.Like most other trisomies (aneuploidy), Patau syndrome increasing incidence is associated with advanced maternal age, and the additional chromosome usually arises from nondisjunction in maternal meiosis resulting in three copies of 13 chromosome genetic material. This is a noninheritate case, is the result of random events during meiosis.

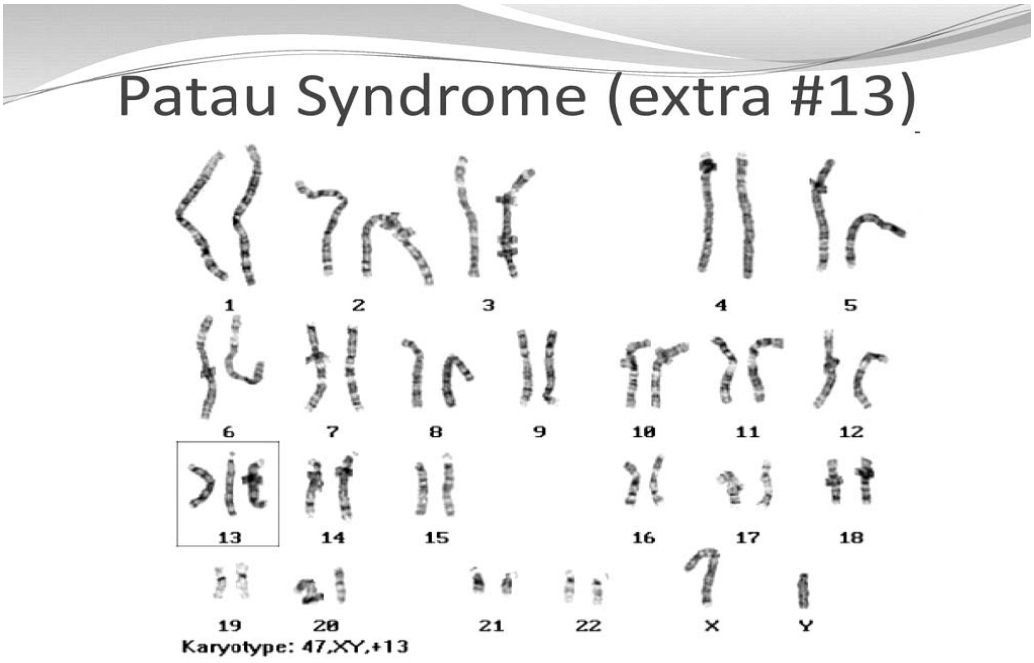


Fig : Karyotype of Patau's Syndrome

The extra genetic material disrupts the normal course of development, causing the characteristic features of trisomy 13. However, some cases derived from germinal mosaicism or by balanced chromosomal rearrangements (Robertsonian translocations), where the extra material is attached to another chromosome, are situations in which the trisomy can be inherited.The person who carries the balanced translocation involving chromosome 13 has an increased chance of passing extra material from chromosome 13 to their children.

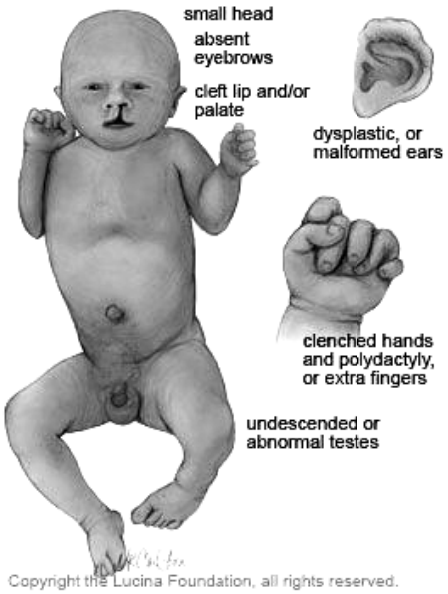
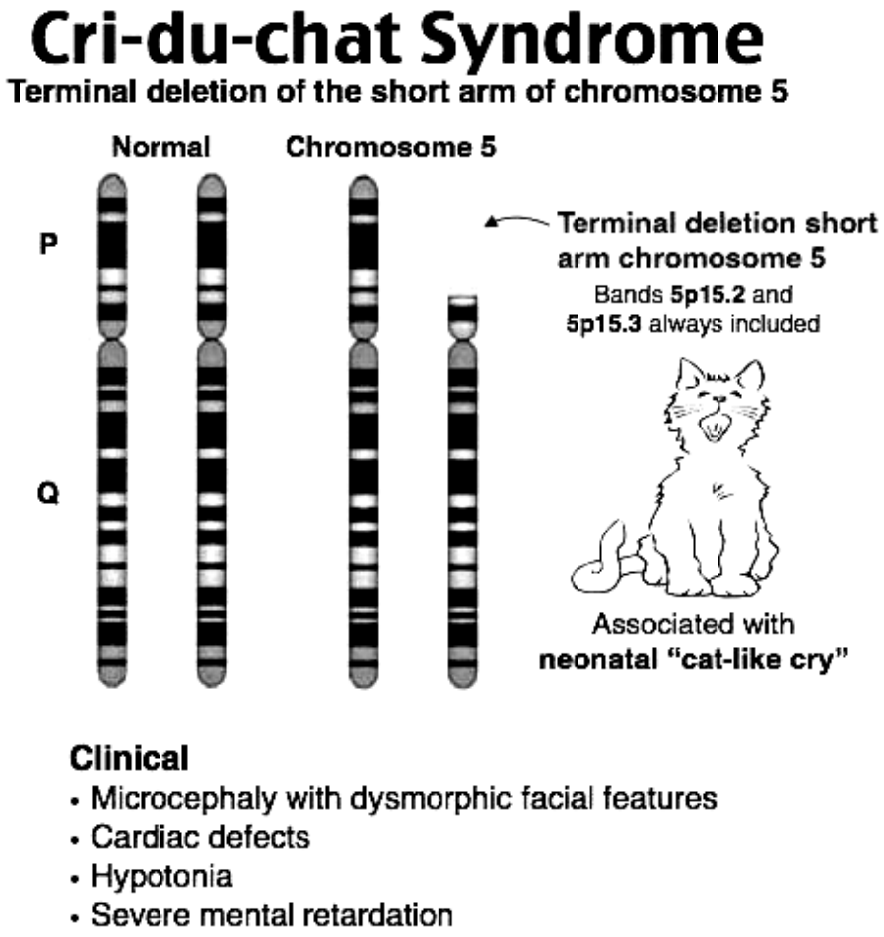


Fig : Symptoms of Patau's Syndrome

d. Cri Du Chat (Cat cry) Syndrome:

An interesting abnormality in which the affected newborn cries in a manner resembling the mewing of a cat, was first described by Lejeune in 1963 in France, hence the name cri du chat (cat cry). A small head, widely spaced eyes, receding chin and congenital heart disease are some other typical features. The condition is due to a deletion in the short arm of the B group chromosome 5, and is very rare.



e. Deletion 22q11.2 (DiGeorge syndrome)

DiGeorge syndrome is caused by the interstitial deletion on the long arm of the 22 chromosome. The deletion can be pretty variable and it is connected with different clinical manifestations. The symptoms are palatal abnormalities, congenital heart disease, facial dysmorphia, microcephaly, hypocalcaemia and thymic hypoplasia with T-cellimmunity defect. The treatment is dependent on the symptoms. It is important to start early. In serious cases of immunityinsufficiency the transplantation of bone marrow is necessary.

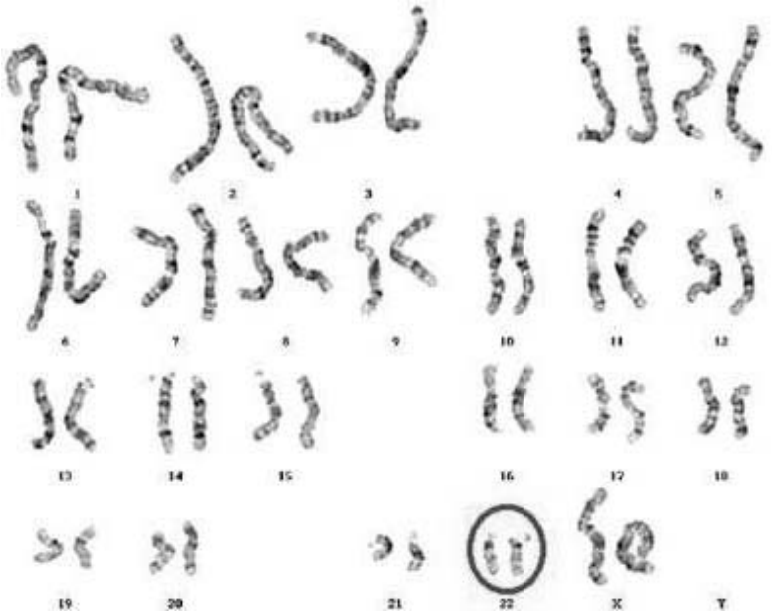


Fig: Karyotype of Di George Syndrome

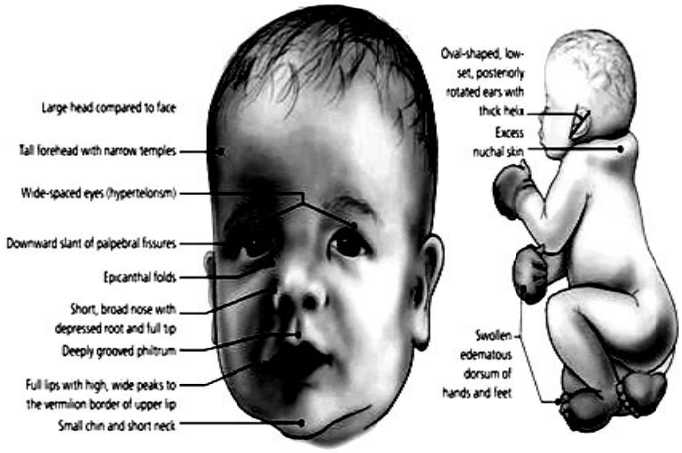


Fig : Symptoms of Di George Syndrome

B. Sex Chromosomal abnormalities:

The majority of known types of chromosomal abnormalities involve sex chromosomes. In frequency of occurrence, they are only slightly less common than autosomal abnormalities. However, they are usually much less severe in their effects. The high frequency of people with sex chromosome aberrations is partly due to the fact that they are rarely lethal

conditions. Like Down syndrome and other autosomal problems, sex chromosome gross abnormalities can be diagnosed before birth by amniocentesis and chorionic villi sampling.

Sex chromosome abnormalities are gender specific. Normal males inherit an X and a Y chromosome while females have two X's. A single Y chromosome is sufficient to produce maleness while its absence is necessary for femaleness. Female abnormalities are due to variations in the number of X chromosomes. Male abnormalities are the result of irregular numbers of either the X or the Y chromosome or both.

I. Female Sex Chromosome Abnormalities:

a. Turner syndrome :

It occurs when females inherit only one X chromosome--their genotype is X0 (i.e., monosomy X). If they survive to birth, these girls have abnormal growth patterns. They are short in stature, averaging 4 foot 7 inches as adults, and often have distinctive webbed necks (i.e., extra folds of skin), small jaws, and high arched palates. They generally lack prominent female secondary sexual characteristics. They have exceptionally small, widely spaced breasts, broad shield-shaped chests, and turned-out elbows. Their ovaries do not develop normally and they do not ovulate. The few oocytes that they produce are destroyed by the time they are two. They are in a sense postmenopausal from early childhood and are sterile. However, they can become pregnant and give birth if fertilized eggs from a donor are implanted. Women with Turner's syndrome have a higher than average incidence of thyroid disease, vision and hearing problems, heart defects, diabetes, and other autoimmune disorders. In a few individuals, there is slight mental retardation. Turner syndrome is rare. Current estimates of its frequency range from 1 in 2,000 to 1 in 5,000 female infants. If diagnosed in early childhood, regular injections of human growth hormones can increase their stature by a few inches. Beginning around the normal age of puberty, oestrogen replacement therapy can result in some breast development and menstruation. These treatments allow Turner syndrome women to appear relatively normal.

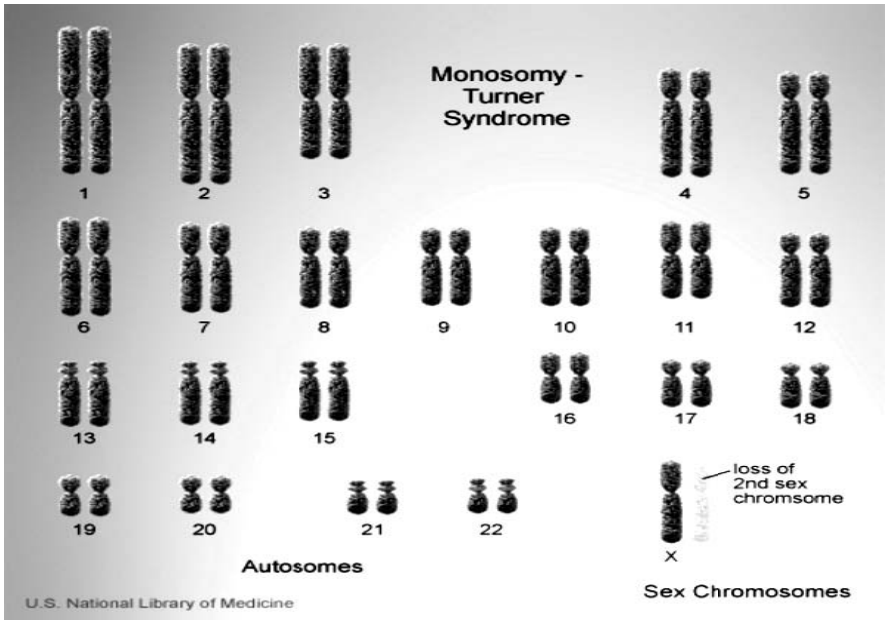


Fig : Karyotype of Turner Syndrome

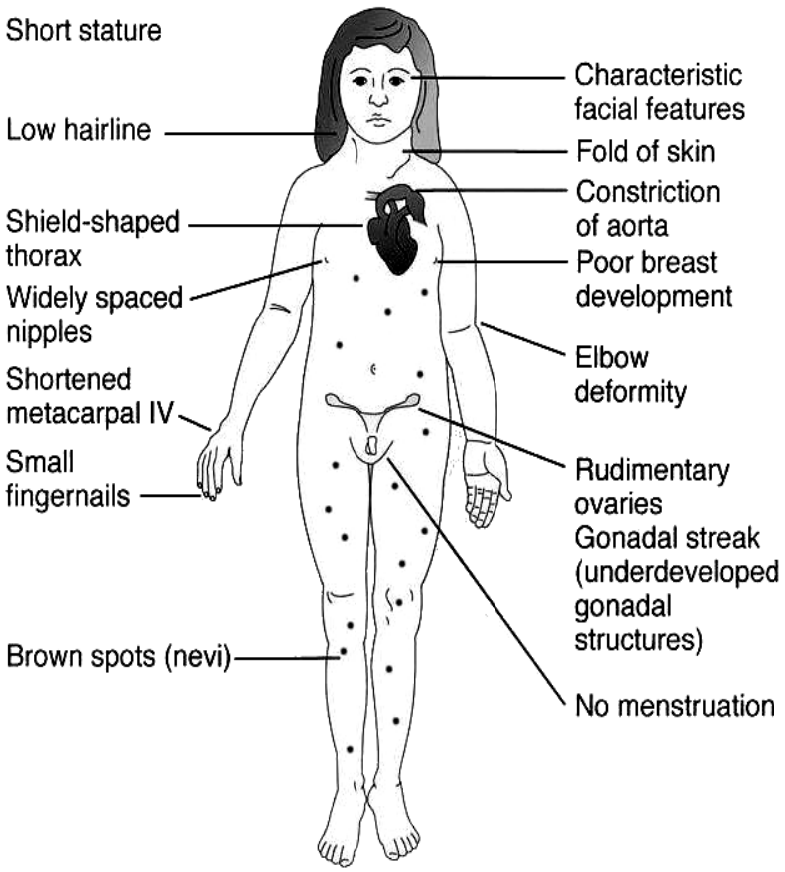


Fig: Symptoms of Turner Syndrome

b. Triple-X syndrome :

It occurs in women who inherit three X chromosomes--their genotype is XXX or more rarely XXXX or XXXXX. As adults, these "super-females" or "metafemales", as they are sometimes known, generally are an inch or so taller than average with unusually long legs and slender torsobut otherwise appear normal. They usually have normal development of sexual characteristics and are fertile but tend to have some ovary abnormalities that can lead to premature ovarian failure. They may have slight learning difficulties, especially in speech and language skills, and are usually in the low range of normal intelligence (especially the XXXX and XXXXX individuals). They frequently are very tall in childhood and tend to be emotionally immature for their size. This sometimes results in teachers and other adults labelling them as troublemakers because they expect more maturity from bigger girls. However, they are usually as emotionally mature as other girls of their age. None of these traits prevent them from being socially accepted as ordinary adult women. Individuals who are genetic mosaics (XX/XXX) have less noticeable symptoms. Triple-X syndrome is less rare than Turner syndrome, but little is known about it. The frequency is approximately 1 in 1,000 female infants and it occurs more commonly when the mother is older.

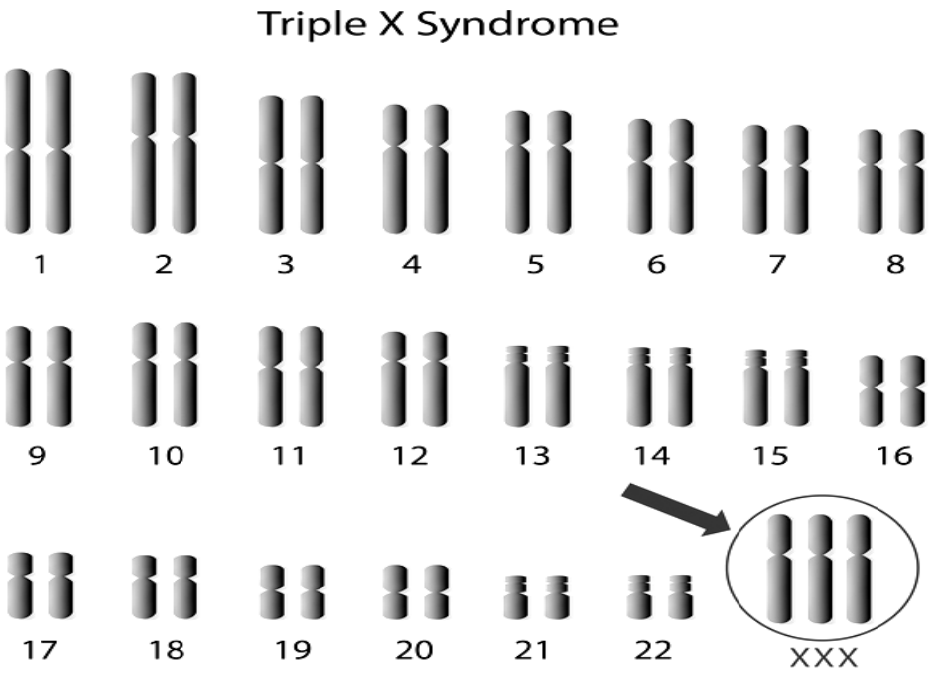


Fig : Karyotype of Triple XXX Syndrome

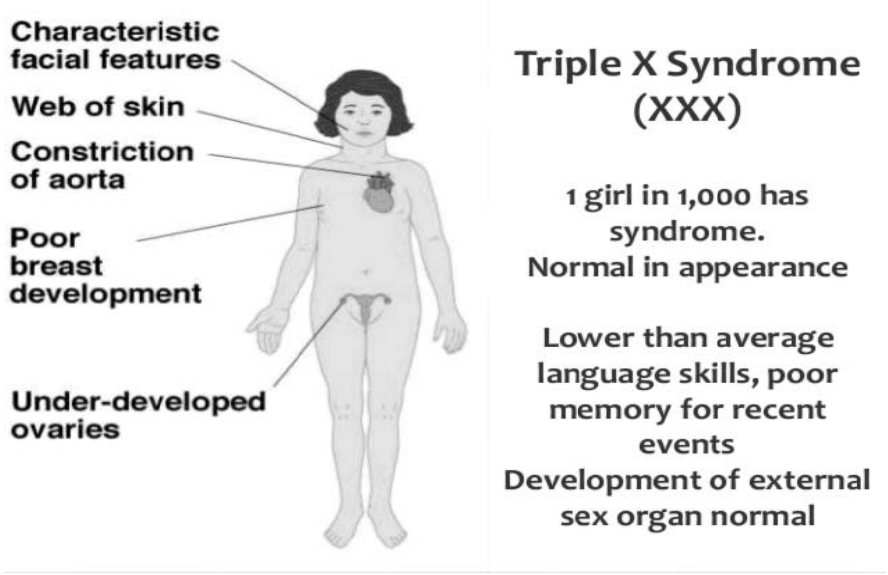


Fig: Symptoms of Triple X Syndrome

II. Male Sex Chromosome Abnormalities:

a. Klinefelter syndrome :

Males inherit one or more extra X chromosomes--their genotype is XXY or more rarely XXXY or XY/XXY mosaic. In severe cases, they have relatively high-pitched voices, asexual to

feminine body contours as well as breast enlargement, and comparatively little facial and body hair. They are sterile or nearly so, and their testes and prostate gland are small. As a result, they produce relatively small amounts of testosterone. The feminizing effects of this hormonal imbalance can be significantly diminished if Klinefelter syndrome boys are regularly given testosterone from the age of puberty on. Like triple-X females (described above), many Klinefelter syndrome men are an inch or so above average height. They also are likely to be overweight. They usually have learning difficulties as children, especially with language and short-term memory. If not given extra help in early childhood, this often leads to poor school grades and a subsequent low self esteem. However, most men who have Klinefelter syndrome are sufficiently ordinary in appearance and mental ability to live in society without notice. It is not unusual for Klinefelter syndrome adults with slight symptoms to be unaware that they have it until they are tested for infertility. They are usually capable of normal sexual function, including erection and ejaculation, but many, if not most, are unable to produce sufficient amounts of sperm for conception. Klinefelter syndrome males with more than two X chromosomes usually have extreme symptoms and are often slightly retarded mentally. Men who are mosaic (XY/XXY) generally have the least problems. There is no evidence that Klinefelter syndrome boys and men are more inclined to be homosexual, but they are more likely to be less interested in sex. They have a higher than average risk of developing osteoporosis, diabetes, and other autoimmune disorders that are more common in women. This may be connected to low testosterone production. Subsequently, regular testosterone therapy is often prescribed. The frequency of Klinefelter syndrome has been reported to be between 1 in 500 and 1 in 1000 male births. This makes it one of the most common chromosomal abnormalities. Males with Down syndrome sometimes also have Klinefelter syndrome. Both syndromes are more likely to occur in babies of older mothers.

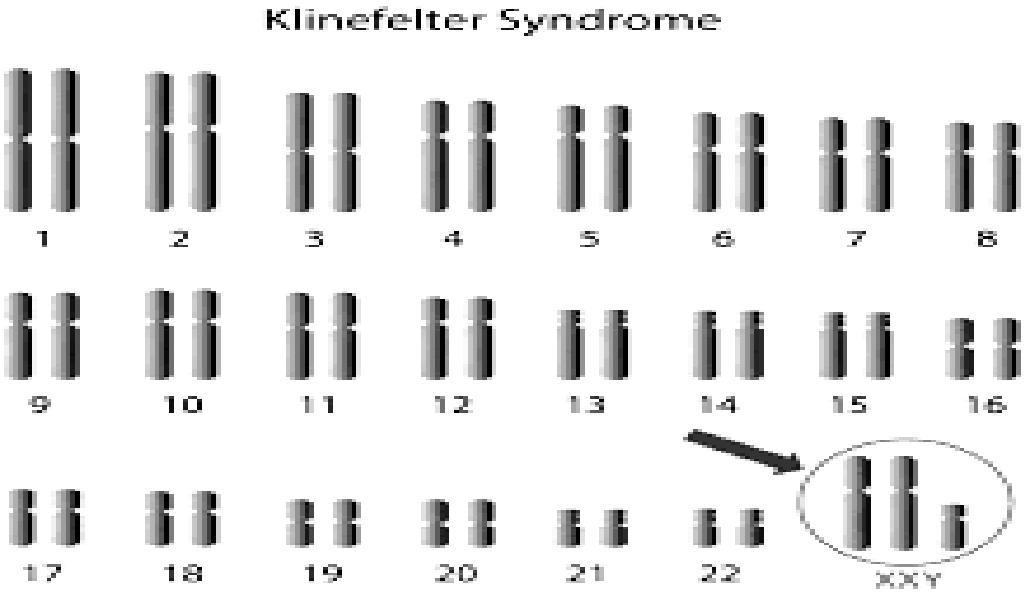


Fig : 47,XXY karyotype showing extra X Chromosome at right side

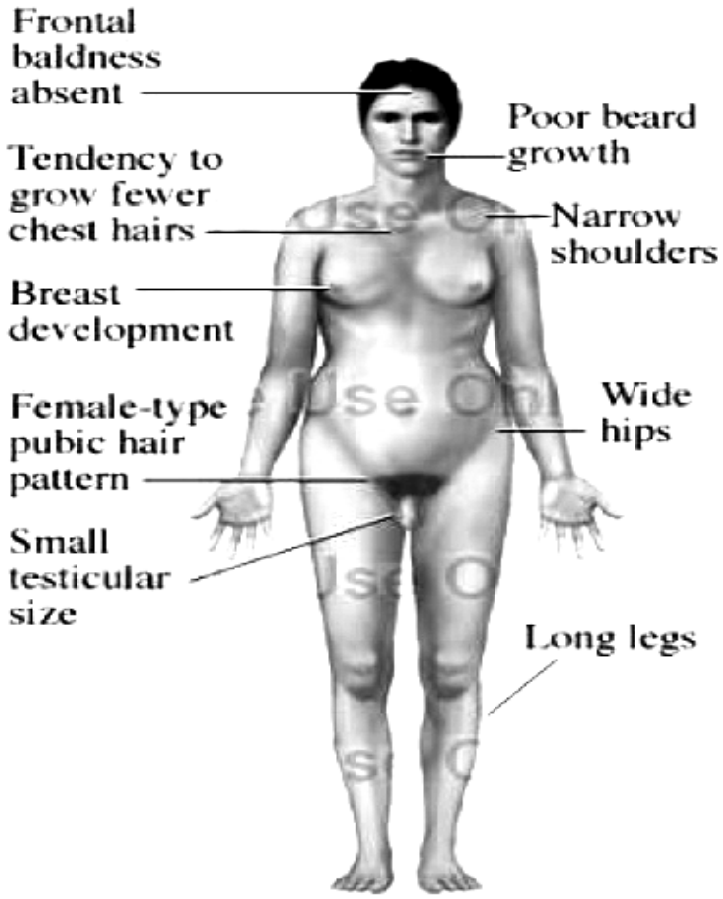


Fig: Symptoms of Klinefelter Syndrome

b. XYY syndrome (Jacobs Syndrome):

n 1965 Jacobs et al found that many of the men kept in institutions for the retarded due to aggressive and antisocial behaviour have 47 chromosomes with XYY sex chromosomes. They are usually tall but not always mentally retarded, frequently show hypogonadism and are sterile.

The presence of two Y chromosomes can be recognized as two brightly fluorescent bodies by proper staining. The discovery of this syndrome received publicity because of the possible association of a chromosome anomaly with human behaviour.Males inherit an extra Y chromosome--their genotype is XYY. As adults, these "super-males" are usually tall (above 6 feet) and generally appear and act normal. However, they produce high levels of testosterone. During adolescence, they often are slender, have severe facial acne, and are poorly coordinated. They are usually fertile and lead ordinary lives as adults. Many, if not most, are unaware that they have a chromosomal abnormality. The frequency of XYY syndrome is not certain due to statistical differences between different studies. It may be as common as 1 in 900 male births to as rare as 1 in 1500 or even 1 in 2,000. XYY syndrome is also referred to as Jacobs syndrome.

Early studies of XYY syndrome done in European prisons initially led to the erroneous conclusion that these men were genetically predisposed to antisocial, aggressive behavior, below average intelligence, and homosexuality. Contributing to the early view that XYY syndrome men have serious personality disorders was the case of Richard Speck. In 1966, he coldly murdered 8 nurses in a Chicago dormitory. At his trial, his lawyer claimed that he was innocent due to uncontrollable urges caused by his XYY genotype. This novel appeal was akin to claiming insanity or severely diminished mental competence. The jury was not convinced and found him guilty of murder. He was sentenced to life in prison where he eventually died. In fact, Richard Speck did not have an XYY genotype. However, some researchers suggest that the high testosterone levels of XYY men can make them somewhat more prone to violence and that this may cause higher rates of wife beating.

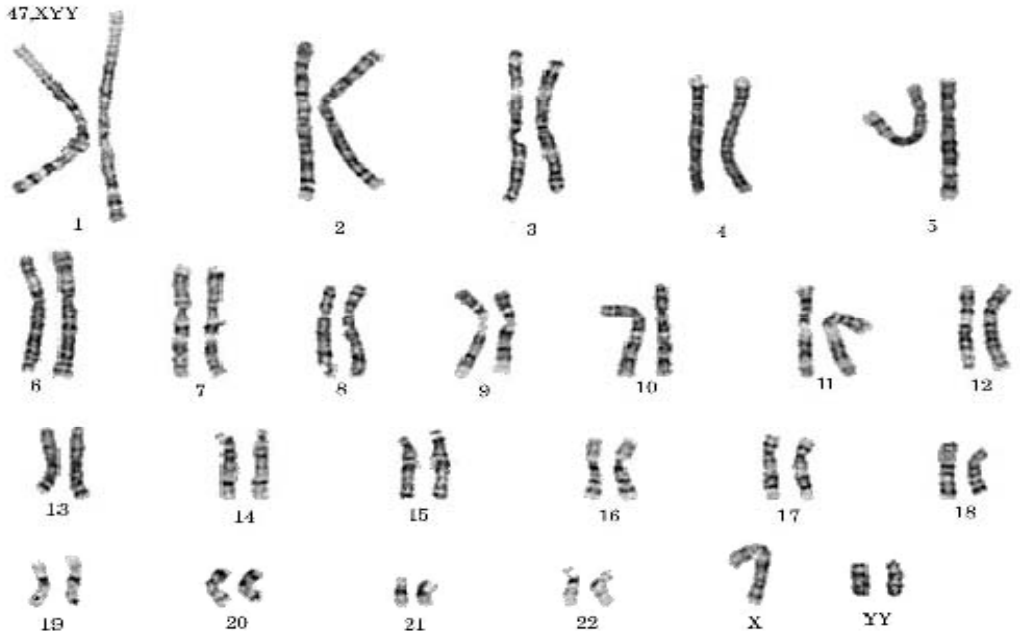


Fig : Karyotype of Jacobs Syndrome

Intersex:

Individuals with both male and female gonadal tissues are called hermaphrodites (from Greek Hermaphrodites, the son of Hermes and Aphrodite). Their karyotype analysis shows that they are mosaics having both X and Y chromosomes in their cell lines. Their buccal smears may or may not show a Barr body. Their external genitalia are often ambiguous, and they are almost always sterile.

The condition of pseudo-hermaphroditism is also included among intersexes. Such individuals are cytogenetically normal with 46, XY (male pseudo-hermaphrodites) or 46, XX (female pseudo-hermaphrodites) chromosomes and normal buccal smears for one sex only. But phenotypically they show both male and female characters.

There are two classes. Male pseudo-hermaphrodites that have testes and either ambiguous or female-like external genitalia. The female pseudo--hermaphrodites have ovaries and either ambiguous or male like external genitalia. The pseudo-hermaphrodites have some defect in the biosynthesis of testosterone in the testes or in the adrenal glands or in both.

C. Chromosomal Anomalies in Malignancy:

a. Chronic myeloid leukaemia:

Chronic myelogenous leukaemia (CML), also known as chronic myeloid leukaemia, is a cancer of the white blood cells. It is a form of leukaemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is found. It is a type of myeloproliferative neoplasm associated with a characteristic chromosomal translocation called the Philadelphia chromosome.

CML is largely treated with targeted drugs called tyrosine-kinase inhibitors (TKIs) which have led to dramatic improved long-term survival rates since 2001. These drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs. In Western countries, CML accounts for 15–25% of all adult leukaemias and 14% of leukaemias overall (including the pediatric population, where CML is less common)

Genetic cause:

CML was the first cancer to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania, USA: Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Centre.

In this translocation, parts of two chromosomes (the 9th and 22nd) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. This abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight (p210 is short for 210 kDa protein, a shorthand used for characterizing proteins based solely on size). Because abl carries a domain that can add phosphate groups to tyrosine residues (a tyrosine kinase), the bcr-abl fusion gene product is also a tyrosine kinase.

The fused BCR-ABL protein interacts with the interleukin 3beta(c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities. The action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukaemia. With

improved understanding of the nature of the BCR-ABL protein and its action as a tyrosine kinase, targeted therapies that specifically inhibit the activity of the BCR-ABL protein have been developed. These tyrosine kinase inhibitors can induce complete remissions in CML, confirming the central importance of bcr-abl as the cause of CML

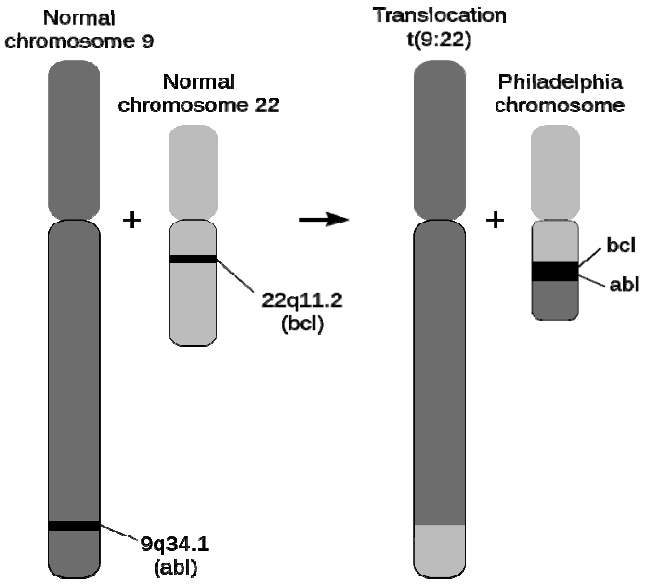


Fig: Diagram showing the translocation found in the Philadelphia chromosome

b. Burkitt's lymphoma:

Burkitt lymphoma is a cancer of the lymphatic system, particularly B lymphocytes found in the germinal centre. It is named after Denis Parsons Burkitt, a surgeon who first described the disease in 1958 while working in equatorial Africa. The overall cure rate for Burkitt's lymphoma in developed countries is about 90%, but worse in low-income countries. Burkitt's lymphoma is uncommon in adults, where it has a worse prognosis.Currently, Burkitt lymphoma can be divided into three main clinical variants: the endemic, the sporadic, and the immunodeficiency-associated variants.

The endemic variant (also called "African variant") most commonly occurs in children living in malaria endemic regions of the world (e.g., equatorial Africa, Brazil, and Papua New Guinea). Epstein-Barr virus (EBV) infection is found in nearly all patients. Chronic malaria is believed to reduce resistance to EBV, allowing it to take hold. The disease characteristically involves the jaw or other facial bone, distal ileum, cecum, ovaries, kidney, or breast.

The sporadic type of Burkitt lymphoma (also known as "non-African") is the most common variant found in places where malaria is not holoendemic. The tumour cells have a similar appearance to the cancer cells of classical endemic Burkitt lymphoma. Sporadic lymphomas are rarely associated with the Epstein–Barr virus. Non-Hodgkin lymphoma, which includes Burkitt's, accounts for 30–50% of childhood lymphoma. The jaw is less commonly involved, compared to the endemic variant. The ileocecal region is the common site of involvement.

Immunodeficiency-associated Burkitt lymphoma is usually associated with HIV infection[or occurs in the setting of post-transplant patients who are taking immunosuppressive drugs. Burkitt lymphoma can be one of the diseases associated with the initial manifestation of AIDS.By morphology (i.e., microscopic appearance) or immunophenotype, it is almost impossible to differentiate these three clinical variants. Immunodeficiency-associated Burkitt lymphoma may demonstrate more plasmocytic appearance or more pleomorphism, but these features are not specific.

Genetic cause:

While Burkitt lymphoma (BL) is associated with genetic changes involving the MYC gene and immunoglobulin genes (genes that provide instructions for antibodies), these genetic changes are acquired (not inherited), and are limited to the cancer cells . They are not passed on to offspring.

All types of Burkitt lymphoma are characterized by dysregulation of the c-myc gene by one of three chromosomal translocations. This gene is found at 8q24.

The most common variant is t(8;14)(q24;q32), which accounts for about 85% of cases. This involves c-myc and IGH@. A variant of this, a three-way translocation, t(8;14;18), has also been identified.

A rare variant is at t(2;8)(p12;q24). This involves IGK@ and c-myc.

Another rare variant is t(8;22)(q24;q11). This involves IGL@ and c-myc.

Combined, the two less-common translocations, t(2;8)(p12;q24) and t(8;22)(q24;q11), account for the remaining 15% of cases not due to the t(8;14)(q24;q32) translocation

In 2014, it was described that short non-coding RNAs named microRNAs (miRNAs) have important functions in lymphoma biology. In malignant B cells, miRNAs participate in pathways fundamental to B cell development, like B cell receptor (BCR) signalling, B cell migration/adhesion, cell-cell interactions in immune niches, and the production and class-switching of immunoglobulins. MiRNAs influence B cell maturation, generation of pre-, marginal zone, follicular, B1, plasma and memory B cells.

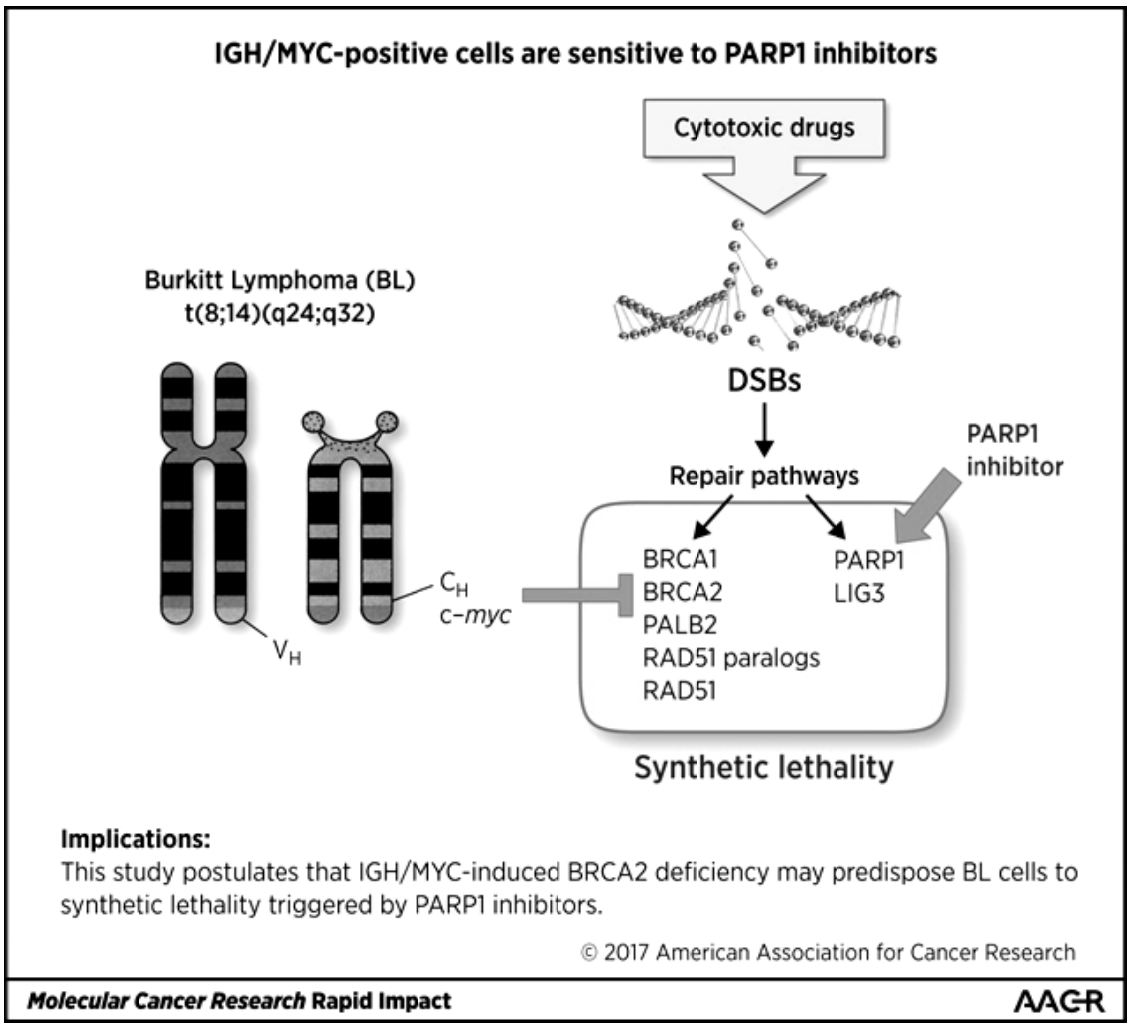


Fig: Chromosomal translocation event at Burkitt Lymphoma

c. Retinoblastoma:

Retinoblastoma (Rb) is a rare form of cancer that rapidly develops from the immature cells of a retina, the light-detecting tissue of the eye. It is the most common primary malignant intraocular cancer in children, and it is almost exclusively found in young children.

Though most children survive this cancer, they may lose their vision in the affected eye(s) or need to have the eye removed.

Almost half of children with retinoblastoma have a hereditary genetic defect associated with retinoblastoma. In other cases, it is caused by a congenital mutation in the chromosome 13 gene 13q14 (retinoblastoma protein)

The most common and obvious sign of retinoblastoma is an abnormal appearance of the retina as viewed through the pupil, the medical term for which is leukocoria, also known as amaurotic cat's eye reflex. Other signs and symptoms include deterioration of vision, a red and irritated eye with glaucoma, and faltering growth or delayed development. Some children with retinoblastoma can develop a squint, commonly referred to as "cross-eyed" or "wall-eyed" (strabismus). Retinoblastoma presents with advanced disease in developing countries and eye enlargement is a common finding.

Depending on the position of the tumours, they may be visible during a simple eye exam using an ophthalmoscope to look through the pupil. A positive diagnosis is usually made only with an examination under anaesthetic (EUA). A white eye reflection is not always a positive indication of retinoblastoma and can be caused by light being reflected badly or by other conditions such as Coats' disease. The presence of the photographic fault red eyein only one eye and not in the other may be a sign of retinoblastoma. A clearer sign is "white eye" or "cat's eye" (leukocoria)

Genetic cause:

In children with the heritable genetic form of retinoblastoma there is a mutation in the RB1 gene on chromosome 13. RB1 was the first tumour suppressor gene cloned. Although RB1 interacts with over 100 cell proteins, its negative regulator effect on the cell cycle principally arises from binding and inactivation of the transcription factorE2F, thus repressing the transcription of genes which are required for the S phase.

The defective RB1 gene can be inherited from either parent; in some children, however, the mutation occurs in the early stages of fatal development. The expression of the RB1 allele is autosomal dominant with 90% penetrance. Inherited forms of retinoblastomas are more likely to be bilateral. In addition, inherited uni- or bilateral retinoblastomas may be associated with pineoblastoma and other malignant midline supratentorialprimitive neuroectodermal tumours (PNET) with a dismal outcome; retinoblastoma concurrent with a PNET is known as trilateral retinoblastoma. A recent meta-analysis has shown that survival of trilateral retinoblastoma has increased substantially over the last decades.

The development of retinoblastoma can be explained by the two-hit model. According to the two-hit model, both alleles need to be affected, so two events are necessary for the retinal cell or cells to develop into tumours. The first mutational event can be inherited (germline or constitutional) which will then be present in all cells in the body. The second “hit” results in the loss of the remaining normal allele (gene) and occurs within a particular retinal cell.[9] In the sporadic, nonheritable form of retinoblastoma, both mutational events occur within a single retinal cell after fertilization (somatic events); sporadic retinoblastoma tends to be unilateral. Several methods

have been developed to detect the RB1 gene mutations.[10][11] Attempts to correlate gene mutations to the stage at presentation have not shown convincing evidence of a correlation.

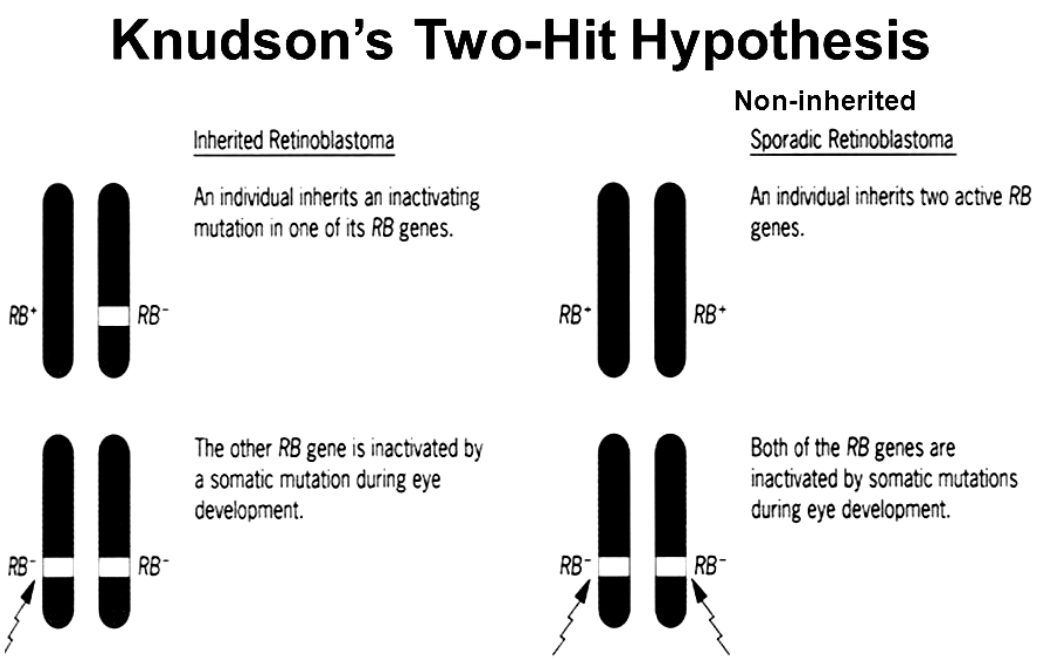


Figure 24.8 Knudson's two-hit hypothesis to explain the occurrence of inherited and sporadic cases of retinoblastoma. Two inactivating mutations are required to eliminate the function of the RB gene.

d. Wilm's tumour:

Wilms tumour is a form of kidney cancer that primarily develops in children. Nearly all cases of Wilms tumour are diagnosed before the age of 10, with two-thirds being found before age 5.

Wilms tumour is often first noticed because of abdominal swelling or a mass in the kidney that can be felt upon physical examination. Some affected children have abdominal pain, fever, a low number of red blood cells (anaemia), blood in the urine (hematuria), or high blood pressure (hypertension). Additional signs of Wilms tumour can include loss of appetite, weight loss, nausea, vomiting, and tiredness (lethargy).

Wilms tumour can develop in one or both kidneys. About 5 to 10 percent of affected individuals develop multiple tumours in one or both kidneys. Wilms tumour may spread from the kidneys to other parts of the body (metastasize). In rare cases, Wilms tumour does not involve the kidneys and occurs instead in the genital tract, bladder, abdomen, chest, or lower back. It is unclear how Wilms tumour develops in these tissues.

With proper treatment, children with Wilms tumour have a 90 percent survival rate. However, the risk that the cancer will come back (recur) is between 15 and 50 percent, depending on traits of the original tumour. Tumours usually recur in the first 2 years following treatment and develop in the kidneys or other tissues, such as the lungs. Individuals who have had Wilms tumour may experience related health problems or late effects of their treatment in adulthood, such as decreased kidney function, heart disease, and development of additional cancers.

Genetic cause:

Changes in any of several genes are involved in the formation of Wilms tumour. Wilms tumour is often associated with mutations in the *WT1* gene, *CTNNB1* gene, or *AMER1* gene. These genes provide instructions for making proteins that regulate gene activity and promote the growth and division (proliferation) of cells. *WT1*, *CTNNB1*, and *AMER1* gene mutations all lead to the unchecked proliferation of cells, allowing tumour development.

Changes on the short (p) arm of chromosome 11 are also associated with developing Wilms tumour. Two genes in this area, *IGF2* and *H19*, are either turned on or off depending on whether the copy of the gene was inherited from the mother or the father. This parent-specific difference in gene activation is a phenomenon called genomic imprinting. In some cases of Wilms tumour, abnormalities in the process of genomic imprinting on chromosome 11 lead to a loss of *H19* gene activity and increased activity of the *IGF2* gene in kidney cells. The resulting loss of *H19* gene activity, which normally restrains cell growth, and increase in *IGF2* gene activity, which promotes cell growth, together lead to uncontrolled cell growth and tumour development in people with Wilms tumour.

In most cases of Wilms tumours involving one kidney and nearly all cases involving both kidneys, the tumours are thought to arise from immature kidney tissue that never developed properly. These immature tissues are known as nephrogenic rests. It is likely that genetic changes are involved in the presence of nephrogenic rests and that additional genetic changes trigger nephrogenic rests to develop into a tumour.

Genetic conditions that share a genetic cause with Wilms tumour can also have this cancer as a feature. These conditions include WAGR syndrome, Denys-Drash syndrome, and Frasier syndrome, which are caused by mutations in the *WT1* gene. Wilms tumour has also been seen in individuals with Beckwith-Wiedemann syndrome, which can be caused by changes in the genomic imprinting of the *IGF2* and *H19* genes. Wilms tumour can be a feature of other genetic conditions caused by mutations in other genes.

Many children with Wilms tumour do not have identified mutations in any of the known genes. In these cases, the cause of the condition is unknown. It is likely that other, unknown genes are also associated with the development of Wilms tumour.

Most cases of Wilms tumour are not caused by inherited genetic factors and do not cluster in families. Approximately 90 percent of these cancers are due to somatic mutations, which means that the mutations are acquired during a person's lifetime and are present only in the tumour cells.

Mutations that are present in cells throughout the body (called germline mutations) are responsible for the remaining 10 percent of Wilms tumour cases and cause either Wilms tumour without any other signs or symptoms or syndromes in which Wilms tumour is one of multiple features. These cases follow autosomal dominant inheritance, which means one copy of the altered gene in each cell can cause a Wilms tumour-related syndrome or increase a person's chance of developing the cancer alone. Most of these cases result from new (de novo) mutations in the gene that occur during the formation of reproductive cells (eggs or sperm) or in early embryonic development.

The *AMER1* gene is located on the X chromosome (one of the two sex chromosomes), so when Wilms tumour is caused by mutations in this gene, the condition follows an X-linked dominantpattern. In females (who have two X chromosomes), a mutation in one of the two copies of the gene in each cell is sufficient to increase a person's chance of developing cancer. In males (who have only one X chromosome), a mutation in the only copy of the gene in each cell increases their cancer risk.

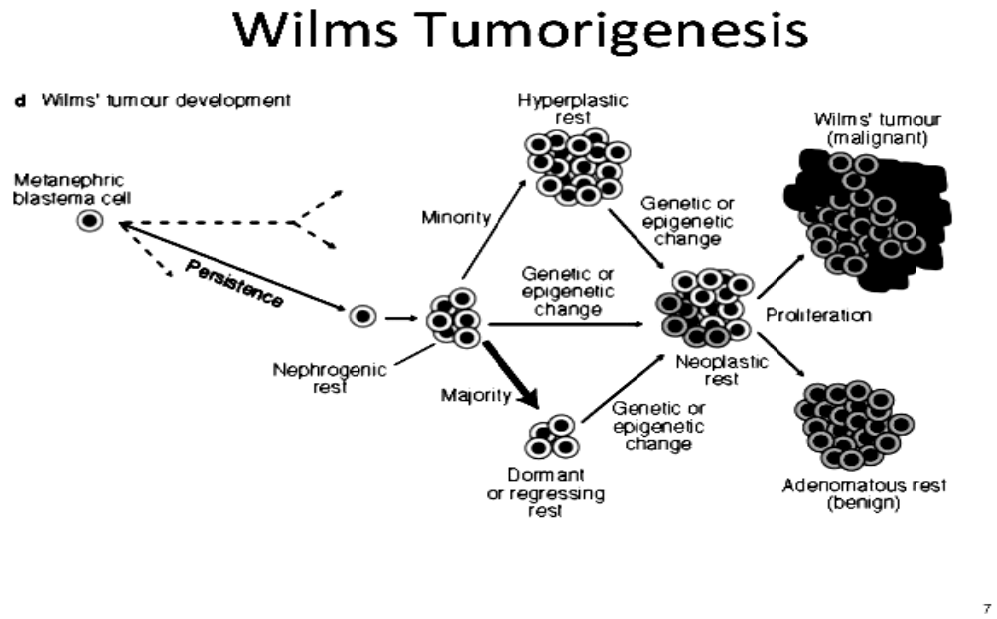


Fig: Development of Wilm's Tumour

Probable Questions:

1. What is the genetic cause of the Down Syndrome? State the symptoms of this syndrome.

2. What is the genetic cause of the Turner Syndrome? State the symptoms of this syndrome.

3. What is the genetic cause of the Klinefelter Syndrome? State the symptoms of this syndrome.

4. What is the genetic cause of the Jacob Syndrome? State the symptoms of this syndrome.

5. What is the genetic cause of the Edwards Syndrome? State the symptoms of this syndrome.

6. What is the genetic cause of the Cri du chat Syndrome? State the symptoms of this syndrome.

7. What is the genetic cause of the Patau Syndrome? State the symptoms of this syndrome.

8. What is the genetic cause of the Di George Syndrome? State the symptoms of this syndrome.

9. Define intersex.

10. What is the genetic cause of the Burkitt Lymphoma.

11. How Retinoblastoma occur in human? What is two hit hypothesis ?

12. What is the genetic cause of Wil's Tumour ?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit-X

Genetic analysis of complex traits-complex pattern of inheritance, quantitative traits, threshold traits

Objective: In this unit you will learn about Genetic analysis of complex traits-complex pattern of inheritance, quantitative traits, threshold traits

Introduction to Polygenic Traits:

Character or trait refers to any property of an individual showing heritable variation. It includes morphological, physiological, biochemical and behavioural properties. Some characters are governed by one or few genes. Such traits are referred to as qualitative characters or oligogenic characters.

On the other hand, some characters are controlled by several genes. They are known as quantitative characters or polygenic characters. The mode of inheritance of polygenic characters is termed as polygenic inheritance or quantitative inheritance. Since in polygenic inheritance several genes (factors) are involved, it is also known as multiple factor inheritance.

Features of Polygenic Traits:

The term polygene was introduced by Mather in 1941. This term has found wide usage in quantitative genetics replacing the older term multiple gene.

Main features of polygenic characters are briefly presented below:

1. Each polygenic character is controlled by several independent genes and each gene has cumulative effect.

2. Polygenic characters exhibit continuous variation rather than a discontinuous variation. Hence, they cannot be classified into clear-cut groups.

3. Effect of individual gene is not easily detectable in case of polygenic characters and, therefore, such traits are also known as minor gene characters.

4. The statistical analysis of polygenic variation is based on means, variances and co-variances, whereas the discontinuous variation is analysed with the help of frequencies and ratios. Thus, polygenic characters are studied in quantitative genetics and oligogenic characters in mendelian genetics.

5. Polygenic traits are highly sensitive to environmental changes, whereas oligogenic characters are little influenced by environmental variation.

6. Classification of polygenic characters into different clear-cut groups is not possible because of continuous variation from one extreme to the other. In case of qualitative characters, such grouping is possible because of discrete or discontinuous variation.

7. Generally the expression of polygenic characters is governed by additive gene action, but now cases are known where polygenic characters are governed by dominance and epistatic gene action. In case of oligogenic characters, the gene action is primarily of non-additive type (dominance and epistasis).

8. In case of polygenic characters, metric measurements like size, weight, duration, strength, etc. are possible, whereas in case of oligogenic characters only the counting of plants with regard to various kinds like colour and shape is possible. Thus, metric measurement is not possible in case of oligogenic characters.

9. Transgressive segregants are only possible from the crosses between two parents with mean values for a polygenic character. Such segregants are not possible in case of qualitative or oligogenic traits.

10. The transmission of polygenic characters is generally low because of high amount of environmental variation. On the other hand, oligogenic characters exhibit high transmission because there is little difference between the genotype and phenotype of such character. Thus, polygenic characters differ from oligogenic ones in several aspects (Table 12.1).

TABLE 12.1. Differences between polygenic and oligogenic traits

Polygenic Traits	*Oligogenic Traits*
1. Governed by several genes.	Governed by few genes.
2. Effect of each gene is not detectable.	Effect of each gene is detectable.
3. Usually governed by additive genes.	Governed by non-additive genes.
4. Variation is continuous.	Variation is discontinuous.
5. Separation into different classes is not possible.	Separation into different classes is possible.
6. Highly influenced by environmental factors.	Little influenced by environmental factors.
7. Statistical analysis is based on mean, variances and covariances.	Statistical analysis is based on frequencies or ratios.

In plant breeding both types of characters showing qualitative and quantitative inheritance have equal economic importance.

Similarities between Oligogenic and Polygenic Traits:

East (1916) demonstrated that polygenic characters were perfectly in agreement with Mendelian segregation and later on Fisher (1918) and Wright (1921, 1935) provided a mathematical basis for the genetic interpretation of such characters.

The quantitative characters do not differ in any essential feature from the qualitative characters, as discussed below:

1. Both quantitative and qualitative characters are governed by genes; the former is controlled by polygenes or minor genes and the latter by oligogenes or major genes.

2. Both major as well as minor genes are located on the chromosome in the nucleus.

3. The polygenic traits controlling continuous variation exhibit segregation like major genes controlling discontinuous Mendelian variation.

4. Polygenic characters show variable expression which is due to non-genetic causes i.e., environmental effects. Qualitative characters also exhibit variation in expression but to a lesser degree than polygenic traits.

5. The reciprocal crosses for both types of traits exhibit close agreement in expression of genes.

6. The phenomenon of transgression in polygenes can only be explained by Mendelian principles of inheritance.

7. Polygenes mutate like oligogenes.

8. Dominance and non-allelic interactions are common features of major genes. These features are also observed for polygenes, but are usually complete for major genes and only partial for minor genes.

9. Polygenes exhibit linkage like oligogenes. Many cases of linkage between major genes and polygenes controlling continuous variation have been reported.

Thus, quantitative genetics or biometrical genetics is an extension of Mendelian genetics firmly based on Mendelian principles of heredity.

Analysis of Polygenic Traits:

The method of analysis of quantitative inheritance differs from that of qualitative inheritance in some aspects as given below:

1. It requires various measurements of characters like weight, length, width, height, duration, etc., rather than classification of individuals into groups based on colour or shape.

2. Observations are recorded on several individuals and the mean values are used for genetical studies. Segregation into distinct classes in F_2 generation-is not obtained in the inheritance of quantitative characters. The segregants exhibit continuous range of variation from one extreme (low) to other (high) for such traits.

3. The inheritance is studied with the help of mean, variances and covariance's. These estimates can be worked out from data recorded in replicated experiment.

4. Fisher (1918) was the pioneer worker to interpret the quantitative characters in terms of Mendelian genetics. Now several biometrical techniques are available for the genetic analysis of quantitative characters. The science which deals with the genetic interpretations of quantitative characters has got separate entity as quantitative genetics or biometrical genetics.

Assumptions of Polygenic Traits:
Polygenic inheritance is based on several assumptions.

The six important assumptions are given below:
1. Each of the contributing genes involved in the expression of a character produces an equal effect.

2. Each contributing allele has either cumulative or additive effect in the expression of a character.

3. The genes involved in the expression of characters have lack of dominance. They show intermediate expression between two parents.

4. There is no epistasis among genes at different loci.

5. The linkage is in equilibrium, means there is no linkage.

6. The environmental effects are absent or may be ignored. However, last three assumptions are seldom fulfilled.

There are two types of alleles or genes in the polygenic inheritance, viz:

(1) Contributing alleles and

(2) Non-contributing alleles.

Those alleles which contribute to continuous variation are known as contributing alleles and those which do not contribute to continuous variation are referred to as non-contributing alleles. Some scientists refer to these as effective and non-effective alleles, respectively.

Examples of Polygenic Traits:
In plant genetics, examples of polygenic characters include yield per plant, days to flower, days to maturity, seed size, seed oil content, etc. Examples of qualitative characters are colour of stem, flower, pollen, etc. and their shapes.

Polygenic inheritance has been reported for various characters both in plants and animals. The most common examples include kernel colour in wheat, corolla length in tobacco, skin colour in man and ear size in maize.

These are briefly described as follows:

a. Kernel Colour in Wheat:

Nilsson Ehle (1908) studied the inheritance of kernel colour in wheat. He found that seed or kernel colour in wheat is governed by one, two and three gene pairs, because in the crosses between red and white kernel varieties, he observed that the F_1 was intermediate between the parental values and in F_2 he observed 3:1,15:1 and 63 : 1 ratios of red and white seeds in different crosses.

The last two ratios indicated that there was duplicate gene interaction; however, in depth study of coloured seeds revealed that there were different grades or shades of colour within the red coloured seeds. The red seeds of 15 : 1 ratio could be easily divided into four classes on the basis of shade of colour, viz., dark red, medium dark red, medium red and light red.

These colours were observed in the ratio of 1 : 4 : 6 : 4 : 1. This suggested that the seed colour in wheat is controlled by genes which show lack of dominance and have small cumulative effects.Here, two types of alleles are involved in the expression of character. Those which contribute to continuous variation and those which do not contribute. The first category of alleles is called effective and second as non-effective. Assume that red seed colour is controlled by two genes R_1 and R_2 and, white seed colour by r_1 and r_2.

From the cross between dark red and white seed parents, Nilsson Ehle observed the following results (Fig. 12.1):

Parents		Dark red			White
Genotypes		$R_1R_1R_2R_2$		× ↓	$r_1r_1r_2r_2$
F_1				$R_1r_1R_1r_1$	Medium Red
		R_1R_2	R_1r_2	r_1R_2	r_1r_2
F_2	R_1R_2	$R_1R_1R_2R_2$ [DR]	$R_1R_1R_2r_2$ [MDR]	$R_1r_1R_2R_2$ [MDR]	$R_1r_1R_2r_2$ [MR]
	R_1r_2	$R_1R_1R_2r_2$ [MDR]	$R_1R_1r_2r_2$ [MR]	$R_1r_1R_2r_2$ [MR]	$R_1r_1r_2r_2$ [LR]
	r_1R_2	$R_1r_1R_2R_2$ [MDR]	$R_1r_1R_2r_2$ [MR]	$r_1r_1R_2R_2$ [MR]	$r_1r_1R_2r_2$ [LR]
	r_1r_2	$R_1r_1R_2r_2$ [MR]	$R_1r_1r_2r_2$ [LR]	$r_1r_1R_2r_2$ [LR]	$r_1r_1r_2r_2$ [W]

DR = Dark Red, MDR = Medium Dark Red,
MR = Medium Red, LR = Light Red and W = White

Fig. 12.1. Inheritance of kernel colour in wheat.

Summary of Results

Effective alleles for red colour	*No. of individuals*	*Phenotype*
4	1	Dark red
3	4	Medium dark red
2	6	Medium red
1	4	Light red
0	1	White

Where 4 effective alleles were present, the seed colour was dark red, where 3 such alleles were present, the seed colour was medium dark red, with 2 effective alleles, colour was medium red and with 1 effective allele, seed colour was light red. White seed colour was produced when all the non-effective alleles were present.

2. Corolla Length in Tobacco:

Extreme differences exist in corolla length in *Nicotiana longiflora*. East (1916) studied the inheritance of corolla length in this species of tobacco. He crossed inbred lines of this species with average corolla length of 40 cm and 93 cm.

The F_1 showed intermediate expression for corolla length with 63 cm. In F_2, wide variation for corolla length was observed. The results indicated that five or more genes were involved in the expression of corolla length.

c. Skin Colour Inheritance in Man:

The inheritance of skin colour in man was studied by Davenport. The inheritance of Negro x white mattings can be explained on the basis of two gene difference. Assume that negro colour is governed by A and B genes and white colour by a and b genes.

A cross between negro and white gives birth to a child with medium skin colour called mullatoes (F_1). In F_2 generation, four distinct shades of black colour were observed besides one white (Fig. 12.2). Thus, the phenotypic ratio of 1 : 4 : 6 : 4 : 1 was observed. The individuals having 4, 3, 2, 1 and 0 effective alleles had black (negro) dark, medium, light and white colour, respectively.

The results are presented below:

Parents Negroes × White

Genotypes AABB aabb

F_1 AaBb Medium Colour Mullato

	AB	Ab	aB	ab
AB	AABB [B]	AABb [D]	AaBb [D]	AaBb [M]
Ab	AABb [D]	AAbb [M]	AaBb [M]	Aabb [L]
aB	AaBb [D]	AabB [M]	aaBB [M]	aaBb [L]
ab	AaBb [M]	Aabb [L]	aaBb [L]	aabb [W]

In bracket B = Black, D = Dark, M = Medium, L = Light and W = White

Fig. 12.2. Inheritance of skin colour in human.

Summary of Results

Effective alleles for skin colour	*Frequency*	*Phenotypes*
4	1	Black (negro)
3	4	Dark
2	6	Medium
1	4	Light
0	1	White

Subsequent studies on skin colour inheritance indicated that as many as six genes are involved in the expression of this character.

Transgressive Segregation:

Appearance of transgressive segregants in F_2 is an important feature of polygenic inheritance. Segregants which fall outside the limits of both the parents are known as transgressive segregants. Transgressive segregation results due to fixation of dominant and recessive genes in separate individuals.

Such segregation occurs when the parents are intermediate to the extreme values of the segregating population. Plant breeders use this principle to obtain superior combinations in segregating material for polygenic characters.

An example of transgressive segregation is presented as follows:

Parents	AAbb	×	aaBB
		↓	
F_1		AaBb	
F_2	AABB and aabb are transgressive segregants		

Environmental Effect:

Polygenic characters are highly sensitive to environmental changes. In other words, they are more prone to genotype x environmental interactions. The main effect of environment is to mask the small differences among different genotypes resulting in continuous variation in the character.

When the contribution of environment is 50 per cent, the distribution becomes roughly similar to normal curve and with 75 per cent contribution, it tends to reach normal distribution. For polygenic traits, generally the environmental variation ranges from 10 to 50 per cent and even more for some traits like yield. The high environmental variation results in overlapping of various classes resulting in continuous, variation.

Partitioning of Polygenic Variability:

The polygenic variation or variability present in a genetic population is measured in terms of variances.

The polygenic variation is of three types, viz:

(1) Phenotypic,

(2) Genotypic and

(3) Environmental.

These are briefly described below:

1. Phenotypic Variability:

It is the total variability which is observable. It includes both genotypic and environmental variation and hence changes under different environmental conditions. Such variation is measured in terms of phenotypic variance.

2. Genotypic Variability:

It is the inherent or genetic variability which remains unaltered by environmental conditions. This type of variability is more useful to a plant breeder for exploitation in selection or hybridization. Such variation is measured in terms of genotypic variance. The genotypic variance consists of additive, dominance and epistatic components.

3. Environmental Variability:

It refers to non-heritable variation which is entirely due to environmental effects and varies under different environmental conditions. This uncontrolled variation is measured in terms of error mean variance. The variation in true breeding parental lines and their F_1 is non-heritable. Fisher was the first to divide in 1918, the genetic variance into additive, dominance and epistatic components.

a. Additive Variance:

It refers to that portion of genetic variance which is produced by the deviations due to average effects of genes at all segregating loci. Thus, it is the component which arises from differences between two homozygotes of a gene, i.e., AA and aa. Additive genes show lack of dominance, i.e., intermediate expression.

The additive genetic variance is associated with homozygosis and, therefore, it is expected to be maximum in self-pollinating crops and minimum in cross-pollinating crops. Additive variance is fixable and, therefore, selection for traits governed by such variance is very effective.

Additive genetic variance is important for the following major reasons:

1. It is required for estimation of heritability in narrow sense and response to selection is directly proportionate to narrow sense heritability.

2. It is a pre-requisite for selection because this is the only variance which responds to selection.

3. Breeding value of an individual is measured directly by the additive gene effects. The general combining ability (gca) effect of a parent is measure of additive gene effects.

4. Additive genetic variance gets depleted proportionate to the improvement made by selection.

5. In natural plant breeding populations, additive variance is the predominant one closely followed by dominance variance.

b. Dominance Variance:

It arises due to the deviation from the additive scheme of gene action resulting from intra-allelic interaction i.e., interaction between alleles of the same gene or same locus. It is due to the deviation of heterozygote (Aa) from the average of two homozygotes (AA and aa).

Such genes show incomplete, complete or over-dominance. The dominance variance is associated with heterozygosis and, therefore, it is expected to be maximum in cross-pollinating crops and minimum in self-pollinating species.

Dominance variance is not fixable and, therefore, selection for traits controlled by such variance is not effective. Heterosis breeding may be rewarding in such situation. Dominance variance differs from additive variance in several ways. (Table 12.2).

TABLE 12.2. Differences between additive and dominance variance

Additive variance	*Dominance variance*
1. It refers to difference between homozygotes (AA/aa).	It refers to deviation of Aa from the mean of AA and aa.
2. Genes show lack of dominance.	Genes show incomplete, complete or over-dominance.
3. Associated with homozygosity and is more in inbreeders.	Associated with heterozygosity and is more in outbreeders.
4. It is fixable.	It is non-fixable.
5. Selection is very effective as it is fixable.	Selection is ineffective as it non-fixable.
6. It is the chief cause of transgressive segregation.	It is the chief cause of heterosis or hybrid vigour.

c. Epistatic Variance:

It arises due to the deviation as a consequence of inter-allelic interaction, i.e., interaction between alleles of two or more different genes or loci. The epistatic variance is of three types, viz., (i) additive x additive, (ii) additive x dominance, and (iii) dominance x dominance. They differ from each other in several aspects. (Table 12.3).

TABLE 12.3. Comparison of three types of epistatic gene action

Additive × Additive	*Additive × Dominance*	*Dominance × Dominance*
1. It refers to interaction between two loci each exhibiting lack of dominance individually.	It refers to interaction between two loci, one exhibiting lack exhibiting lack of dominance and other dominance individually.	It refers to interaction between two loci each exhibiting dominance individually.
2. This type of epistasis is fixable.	This in non-fixable.	This is also non-fixable.
3. This comes under additive type of gene action.	This comes under non-additive type of gene action.	This comes under non-additive type of gene action.
4. Selection is effective for traits governed by this type of epistasis.	Selection is not effective as this is type of epistasis is not fixable.	Selection is not effective as this is not fixable.
5. This is useful for mass selection and progeny selection.	This type of epistasis is useful for exploitation of heterosis.	This type of epistasis is also suitable for exploitation of heterosis.

(i) Additive x Additive:

In this case both the interacting loci exhibit lack of dominance individually. It is denoted as A x A and is fixable.

(ii) Additive x Dominance:

It refers to interaction between two or more loci, one exhibiting lack of dominance and the other dominance individually. It is denoted as A x D and is non-fixable.

(iii) Dominance x Dominance:
In this type of epistasis both the interacting loci exhibit dominance individually. It is represented as D x D and is non-fixable.

The first type of epistasis is fixable and, therefore, selection is effective for traits governed by such variance. The last two types of epistatic variances are unfixable and, therefore, heterosis breeding may be rewarding for traits exhibiting such variance. In natural plant breeding populations, epistatic variance has the lowest magnitude. Epistatic variance differs in many aspects from dominance variance.

Wright (1935) suggested the partitioning of genetic variance into two components, viz., additive and non-additive (dominance and epistatic components), of which only the additive component contributes to genetic advance under selection.

Mather (1949) divided the phenotypic variance into three components, namely, (1) heritable fixable (additive variance), (2) heritable non-fixable (dominance and epistatic components), and (3) non-heritable non-fixable (Environmental fraction).

In fact, the heritable fixable component of phenotypic variance will include the additive x additive fraction of the epistatic variance as well. Further, the total phenotypic variance may be partitioned as (1) fixable (additive and additive x additive components) and (2) non-fixable (dominance, additive x dominance and dominance x dominance types of epistasis and environmental fraction) components.

TABLE 12.4. Differences between dominance and epistatic variances

Dominance Variance	*Epistatic Variance*
1. It is due to interaction between genes of the same locus.	It is due to interaction between genes of two or more different loci.
2. It is of three types, *viz.*, incomplete, complete and over dominance.	It is also of three types, *viz.*, additive × additive, additive × dominance and dominance × dominance.
3. It is non-fixable.	A × A type is fixable.
4. Magnitude is higher than epistatic variance.	Magnitude is lower than dominance variance.

The above discussion may be summarized as follows:
VP = VG + VE; VG = VA + VD + VI; and VI = VAA + VAD + VDD

Where; VP = phenotypic variance, VG = genotypic variance, VA = additive variance, VD = dominance variance, VI = epistatic variance, VAA = additive x additive variance, VAD = additive x dominance variance, and VDD = dominance x dominance variance.

In homozygous genotypes, the genetic variance is of additive (A) and additive epistatic (AA) types, while in the segregating populations all the three types of genetic variances, viz., additive,

dominance and epistasis are observed. In F_2, the phenotypic variance has 1/2D (additive) and 1/4H (non-additive) components.

In a random mating populations with no epistasis and zero inbreeding, the covariance between a parent and its offspring is 1/2 VA; the covariance among half-sibs is 1/4 VA; and the covariance among full-sibs is 1/2 VA + 1/4 VD. These relationships change with the level of inbreeding in the population.

Genetic variability for important agronomic traits in almost all the crops is mainly due to the additive genetic variance. The non-additive variance also exists in nearly all crops and for many important traits, but it is generally smaller in magnitude than the additive component.

The variability present in genetic populations can be assessed in four different ways: (1) using simple measures of variability, (2) by variance component analysis, (3) by D_2 statistics, and (4) by metro glyph analysis. For details of these procedures refer Singh and Narayanan (1993).

Significance of Polygenes:

Polygenes are of prime importance to plant breeder for evolution of improved cultivars. Polygenes have great evolutionary significance. They provide variation of fine adjustment and are systems of smooth adaptive change and of speciation.

The potential genetic variability is stored in the form of linked polygenic complexes. Such stores bear mixtures of plus and minus alleles. The potential or hidden variability is released, after inter-mating of such genotypes with other genotypes, due to segregation and recombination.

Mather (1943) has nicely explained the mechanism of storage and release of polygenic variability. It is believed that in natural populations, the best adapted or the fit individuals are those that are close to the population mean for various quantitative traits.

Pleiotropy:

From our study of Mendel's laws, we have learnt that one factor or one gene is responsible for the expression of one character. However, there are also such cases where one gene influences more than one phenotypic character. Such a gene, besides producing its character (i.e., major effect), also affects other characters (i.e., secondary effect) of the body. Thus, the ability of a gene to have many effects is known as pleiotropy.

The common example of pleiotropy in man is a hereditary disease called sickle-cell anaemia or Cooley's anaemia found among certain African tribes. A recessive gene causes this disease. In homozygous condition the gene causes production of an abnormal haemoglobin. As a result the shape of the red blood cell containing it becomes sickle shaped and distorted.However, in heterozygous condition, the individuals possess both normal and abnormal haemoglobin and have mild anaemia. In Drosophila gene responsible for the size of wing also affects the eye colour, shape of spermatheca, and position of dorsal bristles.

Threshold Traits:

A threshold trait is a trait, which is inherited quantitatively, but is expressed qualitatively. Normally a lot of genes form the basis of a threshold trait, which is why it should be treated as a quantitative trait. A common characteristic of threshold traits and Mendelian traits is that they occur family wise. A threshold character is one for which the phenotypic values are discontinuous but the mode of inheritance is like that of a continuously varying character. The appearance of such characters may be connected with an environmental stimulus.

Many interesting phenotypes have a polygenic basis but are expressed as discrete character states. Examples include wing dimorphism in insects, presence or absence of enlarged horns or other structures in male insects, male size and mating behavior dimorphism in various animal taxa, life-cycle dimorphism in salamanders, trophic dimorphism in salamanders and fish, and reproductive caste dimorphism in eusocial animals. Threshold models provide a quantitative genetic framework for studying the evolution of such dimorphic phenotypes. These models assume that polymorphisms are caused by variation in an unobservable but normally distributed phenotype termed "liability". Individuals with liability above a threshold value express one phenotypic character state (induced) and those with liability below the threshold express the alternate state (uninduced). Quantitative genetic parameters, such as narrow-sense heritability and genetic correlations, can be estimated on this liability scale by considering the joint patterns of induction among relatives.

Predictive evolutionary theory requires that we understand selection and inheritance. If we are interested in the evolution of a threshold trait, then selection must be considered on the liability scale. Classical theory predicts that mass selection on liability should be frequency dependent because the threshold function shields part of the liability variation (the within-morph component) from the purifying effects of selection; the size of the cryptic fraction depends upon the proportion of the population that is induced (the population incidence). However, when populations are partitioned into groups (e.g., demes or family units), random genetic drift will lead to variations among groups in mean liability and, hence, mean incidence. Group-mean

liability can vary continuously and the function that maps fitness to group-mean liability is free to take any shape. In this way, selection can discriminate better between groups than between individuals. The threshold function causes mass selection to be inefficient, leading some to recommend that breeders apply family-level selection to more effectively change the population incidence.

Current models of selection on threshold traits consider only the genes that map directly from individuals' genotypes to phenotypes (i.e., direct genetic effects). Consequently, "family-level selection" in the classical animal breeding literature implies artificial selection on the mean phenotype of family members that results only from direct effects. In reality, the genotypes of social partners (e.g., mothers, siblings, or coresidents) often affect the phenotypes of individuals through indirect genetic effects, which are also known as associative effects. Maternal effects are the best studied and perhaps most widespread type of indirect effect and have been shown to affect the expression of threshold traits in several species, including diapause in cricket eggs sex in reptiles with temperature-dependent sex determination, the presence or absence of horns in male dung beetles, and reproductive caste in some species of ants. Indirect effects arising from both genetic and environmental effects can cause differences in group-mean fitness that lead to group-level selection that may work in concert or in conflict with individual-level selection. Family-level selection in the social evolution literature implies selection among families that have been kept intact so that social interactions contribute to the phenotypes expressed by the social members.

The evolution of traits that are affected by direct and indirect social factors can be understood more fully by partitioning selection into individual and group-level components, using the regression-based method of contextual analysis. This approach has been used to generalize Hamilton's rule, a definition of the conditions necessary for the spread of an "altruistic" trait that has opposing group and individual effects. Despite the special significance given to dichotomous traits in discussion of the evolution of altruism, such as the evolution of discrete queen and worker reproductive castes in eusocial animals, selection on threshold traits has not yet been studied using contextual analysis. Here we use this approach to explore how multilevel selection operates on a threshold trait with an underlying genetic model that includes indirect genetic effects.

Probable questions:

1. What do you mean by polygenic traits? Describe its characteristics .

2. What are the similarities between polygenic and oligogenic traits?

3. What are the differences between polygenic and oligogenic traits?

4. How polygenic traits are analysed ?

5. Describe three types of polygenic variation .

6. what is additive variance and dominance variance ? What are the differences between these two?

7. What is Pleiotropic traits ? Give examples.

8. Write a note on Threshold Traits with suitable examples .

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit-XI

Human genetics and society: genetic testing; human rights; gene therapy

Objective: In this unit you will have an idea about human genetics and society correlation. You will also learn procedure for genetic testing and also about gene therapy.

Introduction:

The betterment of human society can be achieved by following two inter-related methods:

1. By one of the method we can deal with the already existing human beings. The improvement of already existing human beings can be achieved by improving the environmental conditions, e.g., by subjecting them to better nutrition, better unpolluted ecological conditions, better education and sufficient amount of medical facilities. This type of method of improving the human race is known as euthenics.

2. By another method we can improve the future generations by improving the germplasm of existing individuals. This type of method is known as eugenics. Eugenics believes in artificial selection of physically and mentally sound individuals and discouragement of defective individuals for the inheritance of their defective germplasm to the future generations.

In other words, eugenics seeks the measures to preserve the best type of germplasm and to eliminate defective germplasm from the human society by applying the laws of inheritance to human beings. The primary aim of many of the ancient systems of eugenics was to produce a race of physically perfect human beings. The Greeks had definite ideas regarding eugenics. In Sparta, a physically perfect manhood was the chief aim, whereas the Athenians carried more for the intellectual achievements. Following the doctrine of Greeks and until the 19th century there was little interest in the eugenics. Of the particular importance was the eugenics movement in England in the last part of the nineteenth century.

The movement, spearheaded by persons of outstanding intellect such as Francis Gallon and Karl Pearson, had its objective in the application of biologically sound principles to human populations. Since the biological basis of heredity was unknown, the first objective was to establish the nature of heredity. Galton and Pearson chose to work with human beings and with what they considered important human traits such as intelligence, stature and special abilities. We now know that these characters are very complex traits and are under the control of many genes (polygenes) interacting with environmental variations. So, quite naturally the early investigators made little progress. However, Francis Galton should be credited for being the real founder of the

modem movements of eugenics. He defined the eugenics as the study of all the agencies under social control which may improve or impair the inborn qualities of fine generations of humans either physically or mentally.

Darwin also attached great importance to the eugenics and he compared it to a signpost with three directions. One of these indicates the influence of heredity on the fate of nations. Another point to the rules is that an individual should try to carry out in regard to parenthood based on the law of human heredity. The third aim indicates the regulations to be adopted by the society to encourage racial progress.

Eugenics:

Eugenics is a set of beliefs and practices that aim to improve the genetic quality of a human population by excluding (through a variety of morally criticized means) certain genetic groups judged to be inferior, and promoting other genetic groups judged to be superior. The definition of *eugenics* has been a matter of debate since the term was coined by Francis Galton in 1883.

History of Eugenics:

The concept predates the term; Plato suggested applying the principles of selective breeding to humans around 400 BCE.While eugenic principles have been practiced as early as ancient Greece, the contemporary history of eugenics began in the early 20th century, when a popular eugenics movement emerged in the United Kingdom, and then spread to many countries, including the United States, Canada, and most European countries. In this period, eugenic ideas were espoused across the political spectrum. Consequently, many countries adopted eugenic policies, intended to improve the quality of their populations' genetic stock. Such programs included both *positive* measures, such as encouraging individuals deemed particularly "fit" to reproduce, and *negative* measures, such as marriage prohibitions and forced sterilization of people deemed unfit for reproduction. Those deemed "unfit to reproduce" often included people with mental or physical disabilities, people who scored in the low ranges on different IQ tests, criminals and "deviants," and members of disfavored minority groups.

The eugenics movement became associated with Nazi Germany and the Holocaust when many of the defendants at the Nuremberg trials attempted to justify their human rights abuses by claiming there was little difference between the Nazi eugenics programs and the U.S. eugenics programs. In the decades following World War II, with the institution of human rights, many countries gradually began to abandon eugenics policies, although some Western countries, the United States, Canada, and Sweden among them, continued to carry out forced sterilizations.

Since the 1980s and 1990s, with new assisted reproductive technology procedures available, such as gestational surrogacy (available since 1985), preimplantation genetic diagnosis (available since

1989), and cytoplasmic transfer (first performed in 1996), fear has emerged about the possible revival of a more potent form of eugenics after decades of promoting human rights.

When we consider the future welfare of the human race then the following two factors alarm us greatly:

(i) The declining birth rate among the normal and superior people (those having best germplasm)

(ii) A relative rapid increase of the abnormal and defective individuals (those having defective germplasm).

For the betterment of future generation, it is necessary to increase the population of outstanding people and to decrease the population of abnormal and defective people by applying the principle of eugenics.

The eugenics can be applicable by adopting following two methods:

(A) By encouraging the marriages between desirable persons (constructive method or positive eugenics).

(B) By discouraging the marriages between undesirable persons (restrictive method or negative eugenics).

A. Positive Eugenics:

The positive eugenics attempts to increase consistently better or desirable germplasm and, thus, to preserve best germplasm of the society.

The percentage of desirable traits can be increased by adopting following measures:

a. Early Marriage of those having Desirable Traits:

It is most commonly observed fact that the highly placed persons of the society often have great ambitions for the future life. In achieving their ambitious goals, they often devote the best part of their youth and they are able to marry in their mature age (e.g., 30 to 35 years).

The biological and psychological investigation have revealed that the aged persons often lack in necessary amount of emotional warmth for the sexual activities and moreover, their germplasm also loose its vigour.

Therefore, some laws should be formulated to prevent the late marriages of highly endowed persons by applying high taxation on them and at the same time the young persons having best hereditary traits should be encouraged for early marriage.

b. Subsidizing the Fit:

Because the highly endowed persons lead a well-planned life and to avoid unnecessary difficulties in nursing the children they often prefer to have small number of children. Therefore, the selected young men and women of best eugenic value should be encouraged to increase their birth rate.

c. Eutelegenesis or Germinal Choice:

H.J. Muller has suggested that eugenically sound persons not only should increase their family size but through artificial insemination the outstanding man can serve as father to many more children than would be otherwise possible. The artificial insemination is already widely practiced to permit those women whose husbands are sterile or have some serious hereditary afflictions to bear children.

The sperms and eggs of outstanding persons can be stored for future use by quick freezing and storing them in deep freeze. These germ cells, thus, can be stored for 100 or more years. Very recently the scientists felt the urgent need of establishment of sperm and egg banks to protect these precious germ cells from the radiation. The germ cells could be collected during early adulthood and stored in lead lined containers in the deep freeze. In this state the germ cells would not be subjected to radiation exposure which might affect the donors.

In a modern technique, a woman's ovum is taken out and artificially fertilized in a test-tube. The zygote is then implanted into woman's uterus for the embryonic development. The resulting baby is called test-tube baby.

d. Education:

For the eugenically oriented reforms in the society, the people should be educated about the basic principles of human biology, human genetics, eugenics and sex. The children should be instructed about basic laws of health and they should be encouraged to develop a physically and mentally healthy body.

Moreover, sex should be free from the widespread confusion, narrow minded concepts and religious taboos and ethical bindings because that is a natural biological instinct. The children ignorant about the facts of sex may do more harm to society than otherwise.

e. By Avoiding Germinal Waste:

The wastage of best type of germplasm can be avoided by adopting following measures:

(i) The selection of marriage partners should be made with intelligence.

(ii) The social hindrance which do not allow the teachers, nuns and priests to get married, must be removed. By adopting such measures wastage of best type of germplasm due to lack of opportunities can be prevented.

(iii) The wars must be avoided because in wars the best germplasm of the society is wasted.

f. Improvement of Environmental Conditions:

Both heredity and environment have interrelated role in the development of eugenically better persons. Therefore, every person should get better food, living conditions, education and medical guidance, etc., so his or her hereditary traits can undergo their best development.

g. Promotion of Genetic Research:

Our knowledge about the genetics is not sufficient enough because we still have little information's about various human diseases and metabolic disorders which are generally related with the genes. Therefore, the research in the field of cytogenetics should be increased so that we can learn more and more about the human beings.

h. Genetic Engineering:

During the late 1970s, the science of genetics entered a new era dominated by the use of recombinant DNA technology or genetic engineering (or biotechnology) to produce novel life forms not found in nature.

Through this technology, it has been possible to transfer genes from mammals into bacteria, causing the microbes to become tiny factories for making (in relatively large quantities) proteins of great economic importance such as hormones (including growth hormone and insulin) and interferon's (lymphocyte proteins that prevent replication of a wide variety of viruses). These proteins are produced in such small quantities in humans that the cost of their extraction and purification from tissues has been very expensive, thus, limiting their medical use in prophylaxis (prevention) and therapeutics (treatment) of disease.

By genetic engineering, it has become possible to produce various blood clotting factors, complement proteins (part of immune system) and other substances for the correction of genetic deficiency diseases (euphenics). Recently, experiments have been conducted in which human cells deficient in the synthesis of purines have been obtained from the patients with Lesch- Nyhan syndrome and grown in culture; these cells have been converted to normal cells by transformation with recombinant DNA.

The exciting potential of this technique lies in the possibility of correcting genetic defects-for example, restoring the ability of a diabetic individual to make insulin or correcting immunological deficiencies. This technique is called gene therapy.

B. Negative Eugenics:

The negative eugenics attempts to eliminate the defective germplasm of the society by adopting following measures:

a. Sexual Separation of the Defective:

The defective persons may have various sex-linked diseases such as night blindness, haemophilia, colour blindness, etc., and various other defective traits which may be regulated by dominant or recessive genes.

The increase of germplasm of the persons having such defective traits in the population can be checked by keeping them away and separated from the society. Different states have wisely adopted the restricted measures in segregation of the mental defectives from the society and to place them in mental hospitals.

b. Sterilization:

The sterilization is the best means to deprive an individual from his power of reproduction without interfering with any of his normal functions. The sterilization method is based on surgical operation of sperm duct or vas deferens in males and oviducts or fallopian tubes in females.

The former is known as vasectomy and the latter is tubectomy or salpingectomy. The family planning movement in India has adopted the sterilization as the tool for controlling the rate of rapidly increasing population and in that case the sterilization is euthenical in its application than eugenical.

c. Control of Immigration:

Through immigration there are enough chances that undesirable or defective genes of different races and nationalities may intermingle with the normal germplasm of the population. Therefore, the immigration rules must be strict and the persons with undesirable hereditary traits must not be allowed to migrate from one place to another.

d. Regulation of Marriage:

Presently most human societies are money-minded and for a marriages, relationship like the wealthy or highly placed persons who, however, may have several defective genetical traits, are preferred over those who have economically weak but having eugenically sound hereditary traits. Some rule must be enacted to encourage marriages among desirable mates.

e. Birth Control:

People possessing more of undesired, dysgenic traits should be encouraged to have small families if at all allowed to reproduce. Their foetuses possessing dysgenic traits may be destroyed by abortion.

f. Statutory Ban on Marriage among Close Relatives:

Marriages among close relatives are called consanguineous marriages. The hidden recessive deleterious (e.g., lethal) traits can appear in the progeny in full view if both the parents carry

them. The chances of both the parents carrying the hidden traits are more if they are close relatives and have some common ancestors.

Therefore it is advisable not to marry a close relative. If family histories of a rare abnormality are collected and there is no dominance in them, we may generally expect that the anomaly is due to a double dose (i.e., homozygous state) of a recessive gene. If the parents are blood relatives, there will be a reasonable chance that both will be carrying at least one of the deleterious recessives possessed by one or more of their common ancestors.

Thus, by marrying a relative, one tremendously increases the chances that a recessive, which has been carried concealed down through the generations, will become expressed in the effective double dose in children. Many societies have some sort of taboo or restrictions on marriages between close relatives, which may have arisen as a result of the observation that such marriages often produced defective offspring.

In most countries, no man is allowed to legally marry his sister, mother, daughter, granddaughter, aunt or niece. Many societies have extended such restrictions to include prohibition of first cousin marriages also.

Consanguineous marriages has influenced world history:

Consanguineous marriages have been sanctioned by royalty. This is the reason for the spread of haemophilic gene to European dynasties by the offspring of Queen Victoria. The death of crown prince of Spain, Prince Alfonso, took place by profuse bleeding after a minor cut which would not have been deadly in a normal individual. This was due to X-linked recessive gene for haemophilia he inherited. This gene does not allow the blood to clot.

This condition was perpetuated in royal families of Europe because of the close intermarriages. Rasputin, “the mad monk of Russia”, obtained strong hold of Russia because he was being considered successful in treating the profuse bleeding (i.e., bleeder’s disease) attacks of Tsarevitch. This monk used his power and played a major role in the effectiveness of Russian revolution which changed the history of the world. Thus, we see how the inheritance of gene has influenced world history.

Genetic Counselling:

Negative eugenics is most widely acceptable when it is practiced through genetic counselling. A voluntary restriction of child bearing by couples with inherited genetic disorders (e.g., albinism, sickle cell anaemia, etc.) can be brought about through proper counselling by well qualified persons in the field.

The term genetic counselling is applied to service, typically available in medical settings, in which the prospective parents are provided with the estimates of the probability that they will produce children with genetically controlled defects. This vital service is intimately related to

medical and diagnostic procedures, but the counselling itself does not involve their actual performance.

Medical genetics units are often attached with certain hospitals and medical centres and they provide services of genetic counselling. A genetic counsellor may be a medical doctor or well-trained professional human geneticist.

The most common situation under which people obtain a genetic counsellor's advice is one in which phenotypically normal couple produces a first child suffering from a major defect. Understandingly they wish to know what are the chances for subsequent children to be affected. The genetic counsellor's first task, invariably, is to collect the relevant evidence by making a pedigree study of the man and the women concerned. He then proceeds to estimate the risk or probability of genetic defects among their progeny by applying established principles of inheritance, to the information already collected.

The counsellor, normally, cannot predict anything with certainty and is only in a position to explain the chances of occurrence of a trouble. The final decision is always left to the couple themselves. The role of genetic counsellor will become more important with the perfection of the technique of identification of genetic defects.

It is relatively easy for a trained clinician to identify people suffering from a genetic disease. But the identification of carriers (heterozygotes) for genetic diseases are readily identifiable when they have either a reduced level of the concerned enzyme (e.g., HGPRT enzyme in Lesch-Nyhan syndrome), or a protein/enzyme with an altered charge and, consequently, changed mobility when subjected to electrophoresis (e.g., HbS produced by persons heterozygous for the sickle cell gene). Analysis of family pedigree also provides information of the likelihood of an individual being carrier for a genetic disease.

Once genotype of both the prospective parents become known, it is a simple matter to work out the probability of their child inheriting the disease. If parents heterozygous for a genetic disease decide to produce a child, it is now possible, through appropriate tests done about 6-8 weeks after conception to advise them if their child has inherited the disease.

This is done by obtaining foetal cells from biopsies of trophoblastic villi, which form an external part of human embryo and later form a part of the placenta. Foetal cells may also be obtained by amniocentesis done usually after 18 weeks of pregnancy. The cultured foetal cells may be used for determining their karyotype, levels of critical enzymes and restriction enzyme digestion patterns of DNA.

Such an antenatal (prenatal) diagnosis is now attainable for more than 35 genetic diseases and for a variety of karyotypic defects. The sole purpose of such a diagnosis is premature termination of abnormal foetuses. This approach has helped to reduce the incidence of thalassaemia from 30 to 2

per cent a year in the Cypriot (i.e., a native or inhabitant of Cyprus) community in England. However, antenatal diagnosis may sometimes be misused, e.g., it may be used to selectively abort the foetuses of one set. Some newspaper reports show that some parents in India are using amniocentesis and ultrasound technique (another vital tool for antenatal diagnosis) to selectively abort female foetuses, obviously to save themselves from the hardships and sufferings they have to face to arrange the exceedingly high amounts of dowry for their daughters.

Some other Duties of Genetic Counsellor:

Apart from providing counselling services to high-risk individuals, a genetic counsellor must be able to provide advice to those groups or populations which have a high incidence of certain specific diseases. For example, African people have higher incidence of sickle-cell anaemia, those of Mediterranean descent are prone to thalassaemia, and East European Jews have high probability of carrying the Tay-Sach gene.

The genetic counsellor should also try to wash away the guilt feelings of the parents of the affected child by pointing out that genetic calamities can occur in any family, and the parents are not alone in their problem and no one can escape all the possible undesirable expressions of their genotype.

Genetic counselling and antenatal diagnosis provide definite relief to the possible parents and reduce the frequency of genetically defective individuals in the population. However, it is unlikely that these measures would eliminate the deleterious (lethal) alleles from a population. This is so because most genetic defects are recessive and they would persist in the population through the heterozygotes; therefore, even such an extreme selection would lead to only a slow decline in their frequency.

Further, it is not likely that all the couples in any society will willingly agree themselves, at least in foreseeable near future, to these procedures. However, genetic counselling has become a routine aspect of medical practice in most developed countries.

Euphenics:

The symptomatic treatment of genetic diseases of human beings is called euphenics. The euphenics deals with the control of several inherited human diseases, especially inborn errors of metabolism in which the missing or defective enzyme has been identified.

One example of this is the condition known as phenylketonuria or PKU, determined by an autosomal recessive gene. Babies with this defect are unable to properly metabolize an amino acid, phenylalanine, the resulting chemical imbalance causes severe mental retardation.Now it is possible to distinguish homozygotes from normal individuals by testing the urine of all new-born babies with ferric chloride. In affected children, the metabolic imbalance caused by the mutation

will turn the urine green. Once such a child is detected, a diet free of phenylalanine is prescribed and the child can develop normally.

Although a number of inherited diseases can be treated in a similar euphenic manner, but these constitute only a small fraction of known inherited disease. For the most part, biochemical geneticists could not identify the biochemical errors of many genetic diseases. In other cases, such as albinism, even though the metabolic block leading to an abnormality is known, but, it is not possible to correct it.

In future by following euphenic measures, human can get rid of certain fatal genetic diseases:

1. Intake of Missing Enzyme:

One possible euphenic measure for the future would be to supply the known missing enzyme to individuals that would allow their cells to complete the required biochemical reaction.Some attempts to do this have been made without much success. Immunological difficulties are encountered, since the enzymes being supplied are antigenic so the body produces antibodies against them.

2. Cure for Inherited Anaemia:

Scientists, studying the two anaemias in human beings, Cooley's and Lepore anaemia, resulting from abnormally low haemoglobin production in individuals homozygous for mutations at the beta-chain locus, hope that they will someday discover the factors regulating the beta gene activity and thereby increase the amount of beta chain synthesis.

Furthermore if the mechanism regulating haemoglobin synthesis in the foetus and the adult can be detected, they might be able to cure lethal conditions, such as thalassemia major and sickle cell anaemia, by suppressing synthesis of the abnormal beta chains and allowing foetal or gamma chains to be produced instead.

In fact, individuals have been discovered whose β chain locus for some unknown reason never becomes active. Such individuals function normally with foetal haemoglobin even as adults.

3. Increasing Role of Genetics to Medicine:

The increasing number of human diseases that are being discovered to have a genetic basis lend great importance to the development of such euphenic measures. Three percent of all humans have hereditary diseases which are transmitted in a Mendelian fashion.

Two diseases which account for the deaths of hundreds of thousands a year, cancer and heart disease, are thought to have some heritable component. The future works of immunogenetics can

suggest the ways by which an individual having genes for cancer may develop resistance for this disease.A study has shown that some forms of heart diseases may be inherited as an autosomal dominant trait. An understanding of the genetic basis of such heart diseases would alert persons from families with an incidence of the disease to the possibility of incurring heart conditions and perhaps cause them to alter their diets and life habits accordingly. For example, those who may inherit genes for heart trouble would then quit smoking and avoid high fat diets.

Harmful evolutionary effect of euphenics:

To restore affected individuals to normalcy by euphenic measures is the only compassionate goal for scientists to pursue but to do so is to counter the forces of natural selection that are the basis for the evolutionary strength of a species. PKU homozygotes, for example, would normally not reproduce and transmit the harmful mutations to future generations.

Selection against them would be total; however, when they do develop normally and produce progeny, every member of the next generation would be a carrier of the mutation, assuming that the wife of such person being normal. This has to add to our genetic load and to weaken the human species from the evolutionary point of view.

Genetic Testing:

Genetic testing, also known as DNA testing, allows the determination of bloodlines and the geneticdiagnosis of vulnerabilities to inherited diseases. In agriculture, a form of genetic testing known as progeny testing can be used to evaluate the quality of breeding stock. In population ecology, genetic testing can be used to track genetic strengths and vulnerabilities of species populations.

In humans, genetic testing can be used to determine a child's parentage (genetic mother and father) or in general a person's ancestry or biological relationship between people. In addition to studying chromosomes to the level of individual genes, genetic testing in a broader sense includes biochemical tests for the possible presence of genetic diseases, or mutant forms of genes associated with increased risk of developing genetic disorders.

Genetic testing identifies changes in chromosomes, genes, or proteins. The variety of genetic tests has expanded throughout the years. In the past, the main genetic tests searched for abnormal chromosome numbers and mutations that lead to rare, inherited disorders. Today, tests involve analysing multiple genes to determine the risk of developing specific diseases or disorders, with the more common diseases consisting of heart disease and cancer.The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. Several hundred genetic tests are currently in use, and more are being developed.

Because genetic mutations can directly affect the structure of the proteins they code for, testing for specific genetic diseases can also be accomplished by looking at those proteins or their metabolites, or looking at stained or fluorescent chromosomes under a microscope.

Types of Genetic Testing:

Genetic testing is "the analysis of chromosomes (DNA), proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes". It can provide information about a person's genes and chromosomes throughout life.

I. Diagnostic type testing include:

1. **Cell-free fetal DNA (cffDNA) testing**: It is a non-invasive (for the foetus) test. It is performed on a sample of venous blood from the mother, and can provide information about the foetus early in pregnancy. As of 2015 it is the most sensitive and specific screening test for Down syndrome.

2. **Newborn screening**:Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. A blood sample is collected with a heel prick from the newborn 24–48 hours after birth and sent to the lab for analysis. In the United States, newborn screening procedure varies state by state, but all states by law test for at least 21 disorders. If abnormal results are obtained, it does not necessarily mean the child has the disorder. Diagnostic tests must follow the initial screening to confirm the disease.The routine testing of infants for certain disorders is the most widespread use of genetic testing—millions of babies are tested each year in the United States. All states currently test infants for phenylketonuria (a genetic disorder that causes mental illness if left untreated) and congenitalhypothyroidism (a disorder of the thyroid gland). People with PKU do not have an enzyme needed to process the amino acid phenylalanine, which is responsible for normal growth in children and normal protein use throughout their lifetime. If there is a buildup of too much phenylalanine, brain tissue can be damaged, causing developmental delay. Newborn screening can detect the presence of PKU, allowing kids to get put on a special diet right away to avoid the effects of the disorder.

3. **Diagnostic testing**: Diagnostic testing is used to diagnose or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical mutations and symptoms. Diagnostic testing can be performed at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disease. For example, people with a family history of polycystic kidney disease (PKD) who experience pain or tenderness in their abdomen, blood in their urine, frequent urination, pain in the sides, a urinary tract infection or kidney stones may decide to have their genes tested and the result could confirm the diagnosis of PKD.

4. **Carrier testing**: Carrier testing is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing

is offered to individuals who have a family history of a genetic disorder and to people in ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition like cystic fibrosis.

5. **Preimplantation genetic diagnosis:** Genetic testing procedures that are performed on human embryos prior to the implantation as part of an in vitro fertilization procedure. Pre-implantation testing is used when individuals try to conceive a child through in vitro fertilization. Eggs from the woman and sperm from the man are removed and fertilized outside the body to create multiple embryos. The embryos are individually screened for abnormalities, and the ones without abnormalities are implanted in the uterus.

6. **Prenatal diagnosis**: Used to detect changes in a foetus's genes or chromosomes before birth. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them decide whether to abort the pregnancy. It cannot identify all possible inherited disorders and birth defects, however. One method of performing a prenatal genetic test involves an amniocentesis, which removes a sample of fluid from the mother's amniotic sac 15 to 20 or more weeks into pregnancy. The fluid is then tested for chromosomal abnormalities such as Down syndrome (Trisomy 21) and Trisomy 18, which can result in neonatal or fetal death. Test results can be retrieved within 7–14 days after the test is done. This method is 99.4% accurate at detecting and diagnosing fetal chromosome abnormalities. Although there is a risk of miscarriage associated with an amniocentesis, the miscarriage rate is only 1/400. Another method of prenatal testing is Chorionic Villus Sampling (CVS). Chorionic villi are projections from the placenta that carry the same genetic makeup as the baby. During this method of prenatal testing, a sample of chorionic villi is removed from the placenta to be tested. This test is performed 10–13 weeks into pregnancy and results are ready 7–14 days after the test was done.[12] Another test using blood taken from the fetal umbilical cord is percutaneous umbilical cord blood sampling.

7. **Predictive and presymptomatic testing:** Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that increase a person's chances of developing disorders with a genetic basis, such as certain types of cancer. For example, an individual with a mutation in *BRCA1* has a 65% cumulative risk of breast cancer. Hereditary breast cancer along with ovarian cancer syndrome are caused by gene alterations in the genes BRCA1 and BRCA2. Major cancer types related to mutations in these genes are female breast cancer, ovarian, prostate, pancreatic, and male breast

cancer. Li-Fraumeni syndrome is caused by a gene alteration on the gene TP53. Cancer types associated with a mutation on this gene include breast cancer, soft tissue sarcoma, osteosarcoma (bone cancer), leukaemia and brain tumours. In the Cowden syndrome there is a mutation on the PTEN gene, causing potential breast, thyroid or endometrial cancer. Presymptomatic testing can determine whether a person will develop a genetic disorder, such as hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder, help with making decisions about medical care and provide a better prognosis.

8. **Pharmacogenomics:** type of genetic testing that determines the influence of genetic variation on drug response. When a person has a disease or health condition, pharmacogenomics can examine an individual's genetic makeup to determine what medicine and what dosage would be the safest and most beneficial to the patient. In the human population, there are approximately 11 million single nucleotide polymorphisms (SNPs) in people's genomes, making them the most common variations in the human genome. SNPs reveal information about an individual's response to certain drugs. This type of genetic testing can be used for cancer patients undergoing chemotherapy. A sample of the cancer tissue can be sent in for genetic analysis by a specialized lab. After analysis, information retrieved can identify mutations in the tumour which can be used to determine the best treatment option.

II. Non-diagnostic testing includes:

1. **Forensic testing**: Forensic testing uses DNA sequences to identify an individual for legal purposes. Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).

2. **Paternity testing**: This type of genetic test uses special DNA markers to identify the same or similar inheritance patterns between related individuals. Based on the fact that we all inherit half of our DNA from the father, and half from the mother, DNA scientists test individuals to find the match of DNA sequences at some highly differential markers to draw the conclusion of relatedness.

3. **Genealogical DNA test:** To determine ancestry or ethnic heritage for genetic genealogy

4. **Research testing**: Research testing includes finding unknown genes, learning how genes work and advancing our understanding of genetic conditions. The results of testing done as part of a research study are usually not available to patients or their healthcare providers.

Procedure of Genetic Testing:

Genetic testing is often done as part of a genetic consultation and as of mid-2008 there were more than 1,200 clinically applicable genetic tests available.Once a person decides to proceed with genetic testing, a medical geneticist, genetic counsellor, primary care doctor, or specialist can order the test after obtaining informed consent.

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a foetus during pregnancy), or other tissue. For example, a medical procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. Alternatively, a small amount of saline mouthwash may be swished in the mouth to collect the cells. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorders, often using DNA sequencing. The laboratory reports the test results in writing to a person's doctor or genetic counsellor. Routine newborn screening tests are done on a small blood sample obtained by pricking the baby's heel with a lancet.

Risks and limitations

The physical risks associated with most genetic tests are very small, particularly for those tests that require only a blood sample or buccal smear (a procedure that samples cells from the inside surface of the cheek). The procedures used for prenatal testing carry a small but non-negligible risk of losing the pregnancy (miscarriage) because they require a sample of amniotic fluid or tissue from around the foetus.

Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. People may feel angry, depressed, anxious, or guilty about their results. The potential negative impact of genetic testing has led to an increasing recognition of a "right not to know". In some cases, genetic testing creates tension within a family because the results can reveal information about other family members in addition to the person who is tested.[The possibility of genetic discrimination in employment or insurance is also a concern. Some individuals avoid genetic testing out of fear it will affect their ability to purchase insurance or find a job.Health insurers do not currently require applicants for coverage to undergo genetic testing, and when insurers encounter genetic information, it is subject to the same confidentiality protections as any other sensitive health information. In the United States, the use of genetic information is governed by the Genetic Information Non-discrimination Act (GINA) (see discussion below in the section on government regulation).

Genetic testing can provide only limited information about an inherited condition. The test often can't determine if a person will show symptoms of a disorder, how severe the symptoms will be,

or whether the disorder will progress over time. Another major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed. Another limitation to genetic testing for a hereditary linked cancer, is the variants of unknown clinical significance. Because the human genome has over 22,000 genes, there are 3.5 million variants in the average person's genome. These variants of unknown clinical significance means there is a change in the DNA sequence, however the increase for cancer is unclear because it is unknown if the change affects the gene's function.

A genetics professional can explain in detail the benefits, risks, and limitations of a particular test. It is important that any person who is considering genetic testing understand and weigh these factors before making a decision. Other risks include accidental findings—a discovery of some possible problem found while looking for something else. In 2013 the American College of Medical Genetics and Genomics (ACMG) that certain genes always be included any time a genomic sequencing was done, and that labs should report the results.

Human Rights:

Human rights are moral principles or norms that describe certain standards of human behaviour and are regularly protected as natural and legal rights in municipal and international law. They are commonly understood as inalienable, fundamental rights "to which a person is inherently entitled simply because she or he is a human being" and which are "inherent in all human beings", regardless of their nation, location, language, religion, ethnic origin or any other status. They are applicable everywhere and at every time in the sense of being universal, and they are egalitarian in the sense of being the same for everyone. They are regarded as requiring empathy and the rule of lawand imposing an obligation on persons to respect the human rights of others, and it is generally considered that they should not be taken away except as a result of due process based on specific circumstances; for example, human rights may include freedom from unlawful imprisonment, torture and execution.

The doctrine of human rights has been highly influential within international law, global and regional institutions. Actions by states and non-governmental organisations form a basis of public policy worldwide. The idea of human rights suggests that "if the public discourse of peacetime global society can be said to have a common moral language, it is that of human rights". The strong claims made by the doctrine of human rights continue to provoke considerable scepticism and debates about the content, nature and justifications of human rights to this day. The precise meaning of the term right is controversial and is the subject of continued philosophical debate; while there is consensus that human rights encompasses a wide variety of rights such as the right to a fair trial, protection against enslavement, prohibition of genocide, free speech or a right to education (including the right to comprehensive sexuality education, among others), there is

disagreement about which of these particular rights should be included within the general framework of human rights; some thinkers suggest that human rights should be a minimum requirement to avoid the worst-case abuses, while others see it as a higher standard. In the light of emerging neurotechnologies, four new rights were identified: the right to cognitive liberty, the right to mental privacy, the right to mental integrity, and the right to psychological continuity.

Many of the basic ideas that animated the human rights movement developed in the aftermath of the Second World War and the events of the Holocaust, culminating in the adoption of the Universal Declaration of Human Rights in Paris by the United Nations General Assembly in 1948. Ancient peoples did not have the same modern-day conception of universal human rights. The true forerunner of human rights discourse was the concept of natural rights which appeared as part of the medieval natural law tradition that became prominent during the European Enlightenment with such philosophers as John Locke, Francis Hutcheson and Jean-Jacques Burlamaqui and which featured prominently in the political discourse of the American Revolution and the French Revolution. From this foundation, the modern human rights arguments emerged over the latter half of the 20th century, possibly as a reaction to slavery, torture, genocide and war crimes, as a realisation of inherent human vulnerability and as being a precondition for the possibility of a just society

Gene Therapy:

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases.

Gene therapy is the process of inserting genes into cells to treat diseases. The newly introduced genes will encode proteins and correct the deficiencies that occur in genetic diseases. Thus, gene therapy primarily involves genetic manipulations in animals or humans to correct a disease, and keep the organism in good health. The initial experiments on gene therapy are carried out in animals, and then in humans. Obviously, the goal of the researchers is to benefit the mankind and improve their health.

An overview of gene therapy strategies is depicted in Fig. 13.1. In gene augmentation therapy, a DNA is inserted into the genome to replace the missing gene product. In case of gene inhibition therapy, the antisense gene inhibits the expression of the dominant gene.

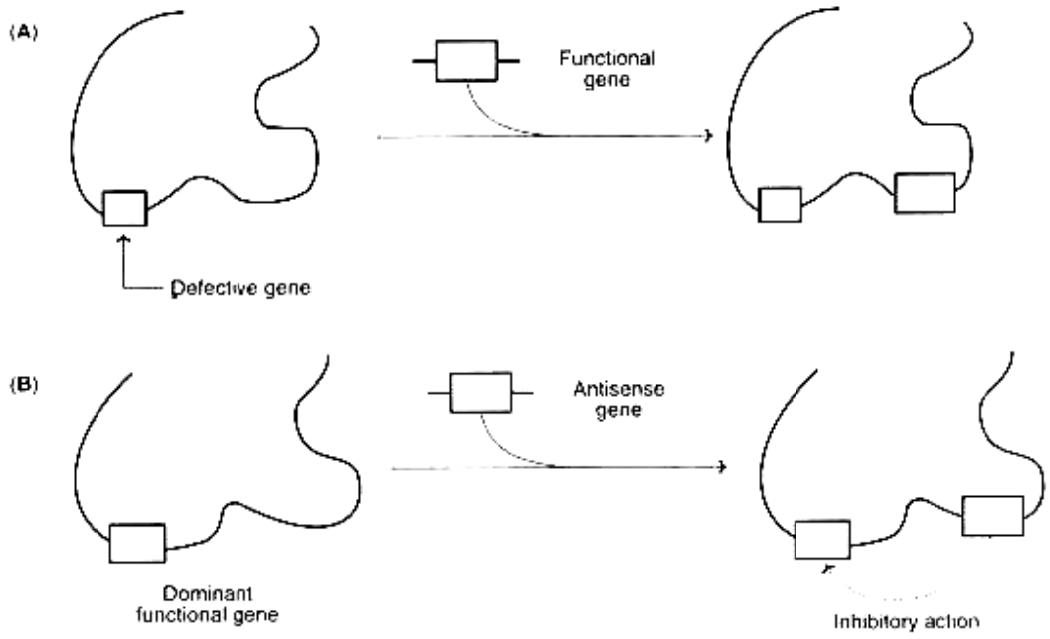


Fig. 13.1 : Overview of two major gene therapy strategies (A) Gene augmentation therapy (B) Gene inhibition therapy.

I. General gene therapy strategies

a. Gene Augmentation Therapy (GAT):

For diseases caused by loss of function of a gene, introducing extra copies of the normal gene may increase the amount of normal gene product to a level where the normal phenotype is restored (Fig. 23.1). As a result GAT is targeted at clinical disorders where the pathogenesis is reversible.

It also helps to have no precise requirement for expression levels of the introduced gene and a clinical response at low expression levels. GAT has been particularly applied to autosomal recessive disorders where even modest expression levels of an introduced gene may make a substantial difference.

Dominantly inherited disorders are much less amendable to treatment; gain-of-function mutations are not treatable by this approach and, even if there is a loss-of- function mutation, high expression efficiency of the introduced gene is required: individuals with 50% of normal gene product are normally affected, and so the challenge is to increase the amount of gene product towards normal levels.

b. Targeted Killing of Specific Cells:

This general approach is popular in cancer gene therapies. Genes are directed to the target cells and then expressed so as to cause cell killing. Direct cell killing is possible if the inserted genes are expressed to produce a lethal toxin (suicide genes), or a gene encoding a pro drug is inserted, conferring susceptibility to killing by a subsequently administered drug. Indirect cell killing uses immunostimulatory genes to provoke or enhance an immune response against the target cell.

c. Targeted Mutation Correction:

If an inherited mutation produces a dominant-negative effect, gene augmentation is unlikely to help. Instead, the resident mutation must be corrected. Because of practical difficulties, this approach has yet to be applied but, in principle, it can be done at different levels: at the gene level (e.g. by gene targeting methods based on homologous recombination); or at the RNA transcript level (e.g. by using particular types of therapeutic ribozymes — or therapeutic RNA editing).

d. Targeted Inhibition of Gene Expression:

If disease cells display a novel gene product or inappropriate expression of a gene (as in the case of many cancers, infectious diseases, etc.), a variety of different systems can be used specifically to block the expression of a single gene at the DNA, RNA or protein levels. Allele-specific inhibition of expression may be possible in some cases, permitting therapies for some disorders resulting from dominant negative effects.

II. Approaches for Gene Therapy:

There are two approaches to achieve gene therapy.

1. Somatic Cell Gene Therapy:

The non- reproductive (non-sex) cells of an organism are referred to as somatic cells. These are the cells of an organism other than sperm or eggs cells, e.g., bone marrow cells, blood cells, skin cells, intestinal cells. At present, all the research on gene therapy is directed to correct the genetic defects in somatic cells. In essence, somatic cell gene therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently.

2. Germ Cell Gene Therapy:

The reproductive (sex) cells of an organism constitute germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety, ethical and technical reasons, germ cell gene therapy is not being attempted at present.

The genetic alterations in somatic cells are not carried to the next generations. Therefore, somatic cell gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases. Development of gene therapy in humans for any specific disease involves the

following steps. In fact, this is a general format for introducing any therapeutic agent for human use.

a. In vitro experiments and research on laboratory animals (pre-clinical trials).

b. Phase I trials with a small number (5-10) of human subjects to test safety of the product.

c. Phase II trials with more human subjects to assess whether the product is helpful.

d. Phase III trials in large human samples for a final and comprehensive analysis of the safety and efficacy of the product.

As such, gene therapy involves a great risk. There are several regulatory agencies whose permission must be sought before undertaking any work related to gene therapy. Recombinant DNA Advisory Committee (RAC) is the supervisory body of the National Institute of Health, U.S.A., that clears proposals on experiments involving gene therapy. A large number of genetic disorders and other diseases are currently at various stages of gene therapy trials. A selected list of some important ones is given in Table 13.1.

Table 13.1 Human gene therapy trials

Disease	*Gene therapy*
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA).
Cystic fibrosis	Cystic fibrosis transmembrane regulator (CFTR).
Familial hypercholesterolemia	Low density lipoprotein (LDL) receptor.
Emphysema	α_1-Antitrypsin
Hemophilia B	Factor IX
Thalassemia	α- or β-Globin
Sickle-cell anemia	β-Globin
Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
Gaucher's disease	Glucocerebrosidase
Peripheral artery disease	Vascular endothelial growth factor (VEGF)
Fancon anemia	Fanconi anemia C
Melanoma	Tumor necrosis factor (TNF)
Melanoma, renal cancer	Interleukin-2 (IL-2)
Glioblastoma (brain tumor), AIDS, ovarian cancer	Thymidine kinase (herpes simplex virus)
Head and neck cancer	p^{53}
Breast cancer	Multidrug resistance I
AIDS	*rev* and *env*
Colorectal cancer, melanoma, renal cancer	Histocompatability locus antigen-B_7 (HLA-B_7)
Duchenne muscular dystrophy	Dystrophin
Short stature*	Growth hormone
Diabetes*	Glucose transporter-2, (GLUT-2), glucokinase
Phenylketonuria*	Phenylalanine hydroxylase
Citrullinemia*	Arginosuccinate synthetase

III. Methods of gene therapy:

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (ex vivo gene therapy)

2. Transfer of genes directly to cells inside the body (in vivo).

1. Ex vivo gene therapy:

The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cells can be cultured in the laboratory. The technique of ex vivo gene therapy involves the following steps (Fig. 13.2).

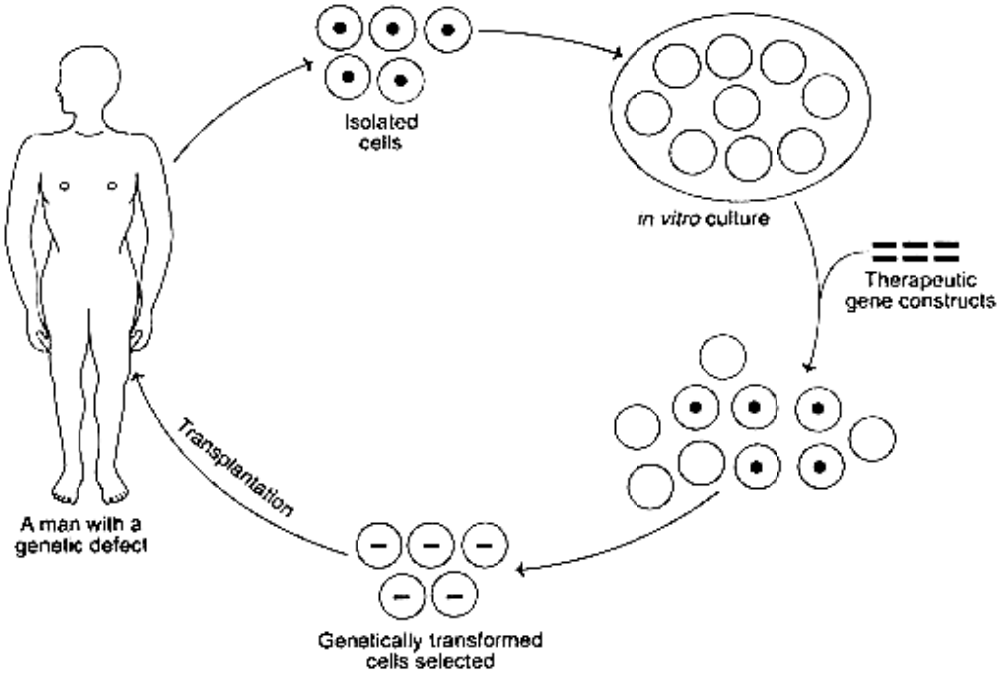


Fig. 13.2 : The procedure for ex vivo gene therapy.

1. Isolate cells with genetic defect from a patient.

2. Grow the cells in culture.

3. Introduce the therapeutic gene to correct gene defect.

4. Select the genetically corrected cells (stable trans-formants) and grow.

5. Transplant the modified cells to the patient.

The procedure basically involves the use of the patient's own cells for culture and genetic correction, and then their return back to the patient. This technique is therefore, not associated with adverse immunological responses after transplanting the cells. Ex vivo gene therapy is efficient only, if the therapeutic gene (remedial gene) is stably incorporated and continuously expressed. This can be achieved by use of vectors.

Vectors in Gene Therapy:

The carrier particles or molecules used to deliver genes to somatic cells are referred to as vectors. The important vectors employed in ex vivo gene therapy are listed below and briefly described next.

i. Viruses

ii. Human artificial chromosome

iii. Bone marrow cells.

i. Viruses:

The vectors frequently used in gene therapy are viruses, particularly retroviruses. RNA is the genetic material in retroviruses. As the retrovirus enters the host cell, it synthesizes DNA from RNA (by reverse transcription). The so formed viral DNA (referred to as provirus) gets incorporated into the DNA of the host cell.

The proviruses are normally harmless. However, there is a tremendous risk, since some of the retroviruses can convert normal cells into cancerous ones. Therefore, it is absolutely essential to ensure that such a thing does not happen.

Making retroviruses harmless:

Researchers employ certain biochemical methods to convert harmful retroviruses to harmless ones, before using them as vectors. For instance, by artificially removing a gene that encodes for the viral envelope, the retrovirus can be crippled and made harmless. This is because, without the envelope, retrovirus cannot enter the host cell. The production of a large number (billions) of viral particles can be achieved, starting from a single envelope defective retrovirus (Fig. 13.3).

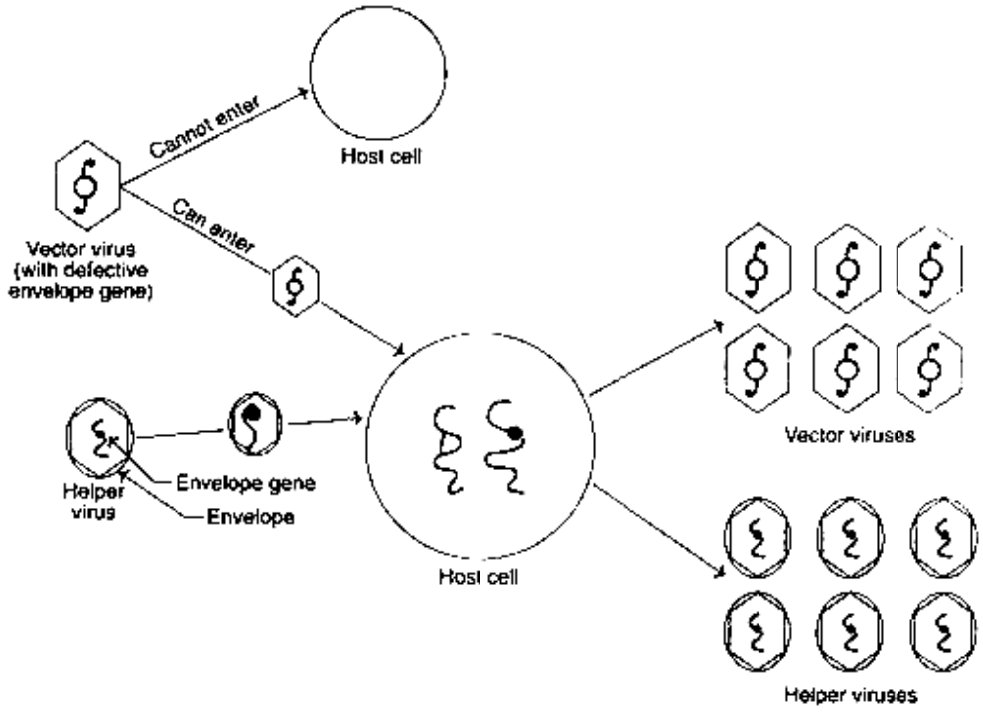


Fig. 13.3 : Large scale production of vector viruses by using helper viruses.

This is made possible by using helper viruses which contain normal gene for envelope formation. Along with the helper virus, the vector (with defective envelope gene) can enter the host cell and both of them multiply. By repeated multiplication in host cells, billions of vector and helper viruses are produced.

The vector viruses can be separated from the helper viruses and purified. Isolation of vector viruses, totally free from helper viruses, is absolutely essential. Contamination of helper viruses is a big threat to the health of the patients undergoing gene therapy.

Retroviruses in gene therapy:

The genetic map of a typical retrovirus is depicted in Fig. 13.4A. In general, the retrovirus particle has RNA as a genome organized into six regions. It has a 5′-long terminal repeat (5′-LTR), a non-coding sequence required for packaging RNA designated as psi (Ψ), a gene gag coding for structural protein, a gene pol that codes for reverse transcriptase, a gene env coding for envelope protein and a 3-LTR sequence.

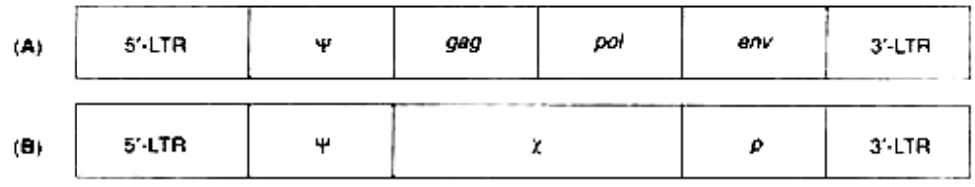


Fig. 13.4 : A retrovirus used in gene therapy. (A) General map of a typical retrovirus (B) Gene map of a modified retrovirus for use in gene therapy (LTR-Long terminal repeat; Ψ-Packaging signal sequence; gag-Coding sequence for structural protein; pol-Coding sequence for reverse transcriptase; env-Envelope protein coding sequence; χ-Therapeutic gene; p–Promoter gene).

For use of a retrovirus as a vector, the structural genes gag and pol are deleted. These genes are actually adjacent to Ψ region. In addition, a promoter gene is also included (Fig. 13.4B). This vector design allows the synthesis of cloned genes. A retroviral vector can carry a therapeutic DNA of maximum size of 8 kb.

A retroviral vector DNA can be used to transform the cells. However, the efficiency of delivery and integration of therapeutic DNA are very low. In recent years, techniques have been developed to deliver the vector RNA to host cells at a high frequency. For this purposes, packaged retroviral RNA particles are used. This technique allows a high efficiency of integration of pharmaceutical DNA into host genome.

Several modified viral vectors have been developed in recent years for gene therapy. These include oncoretrovirus, adenovirus, adenoassociated virus, herpes virus and a number of hybrid vectors combining the good characters of the parental vectors.

Murine leukaemia viruses in gene therapy:
This is a retrovirus that causes a type of leukaemia in mice. It can react with human cells as well as the mouse cells, due to a similarity in the surface receptor protein. Murine leukaemia virus (MLV) is frequently used in gene transfer.

AIDS virus in gene therapy
It is suggested that the human immunodeficiency virus (HIV) can be used as a vector in gene transfer. But this is bound to create public uproar. Some workers have been successful in creating a harmless HIV (crippled HIV) by removing all the genes related to reproduction. At the same time, the essential genes required for gene transfer are retained. There is a distinct advantage with HIV when compared with MLV. MLV is capable of bringing out gene transfer only in dividing cells. HIV can infect even non-dividing cells (e.g., brain cells) and do the job of gene transfer effectively. However, it is doubtful whether HIV can ever be used as a vector.

ii. Human Artificial Chromosome:
The details of human artificial chromosome (HAC) are described elsewhere .HAC is a synthetic chromosome that can replicate with other chromosomes, besides encoding a human protein. As already discussed above, use of retroviruses as vectors in gene therapy is associated with a heavy risk. This problem can be overcome if HAC is used. Some success has been achieved in this direction.

iii. Bone Marrow Cells:
Bone marrow contains totipotent embryonic stem (ES) cells. These cells are capable of dividing and differentiating into various cell types (e.g., red blood cells, platelets, macrophages, osteoclasts, B- and T-lymphocytes). For this reason, bone marrow transplantation is the most widely used technique for several genetic diseases.

And there is every reason to believe that the genetic disorders that respond to bone marrow transplantation are likely to respond to ex vivo gene therapy also (Table 13.2). For instance, if there is a gene mutation that interferes with the function of erythrocytes (e.g., sickle-cell anaemia), bone marrow transplantation is done. Bone marrow cells are the potential candidates for gene therapy of sickle-cell anaemia. However, this is not as simple as theoretically stated.

Table 13.2 Selected list of genetic diseases that are likely to be cured by using bone marrow cells (potential candidates for gene therapy)

Severe combined immunodeficiency (SCID)
Sickle-cell anemia
Fanconi anemia
Thalassemia
Gaucher's disease
Hunter disease
Hurler syndrome
Chronic granulomatous disease
Infantile agranulocytosis
Osteoporosis
X-linked agammaglobulinemia

Selected Examples of Ex Vivo Gene Therapy:

a. Therapy for Adenosine Deaminase Deficiency:

The first and the most publicized human gene therapy was carried out to correct the deficiency of the enzyme adenosine deaminase (ADA). This was done on September 14, 1990 by a team of workers led by Blaese and Anderson at the National Institute of Health, USA (The girl's name is Ashanti, 4 years old then).

b. Severe combined immunodeficiency (SCID):

This is rare inherited immune disorder associated with T-lymphocytes, and (to a lesser extent) B-lymphocytes dysfunction. About 50% of SCID patients have a defect in the gene (located on chromosome 20, and has 32,000 base pairs and 12 exons) that encodes for adenosine deaminase. In the deficiency of ADA, deoxyadenosine and its metabolites (primarily deoxyadenosine 5′-triphosphate) accumulate and destroy T-lymphocytes.

T-Lymphocytes are essential for body's immunity. Besides participating directly in body's defense, they promote the function of B-lymphocytes to produce antibodies. Thus, the patients of SCID (lacking ADA) suffer from infectious diseases and die at an young age. Previously, the children suffering from SCID were treated with conjugated bovine ADA, or by bone marrow transplantation.

c. Technique of therapy for ADA deficiency:

The general scheme of gene therapy adopted for introducing a defective gene in the patient has been depicted in Fig 13.2. The same procedure with suitable modifications can also be applied for other gene therapies.

A plasmid vector bearing a pro-viral DNA is selected. A part of the pro-viral DNA is replaced by the ADA gene and a gene (G 418) coding for antibiotic resistance, and then cloned. The antibiotic resistance gene will help to select the desired clones with ADA gene.

A diagrammatic representation of the treatment of ADP deficient patient is depicted in Fig. 13.5.

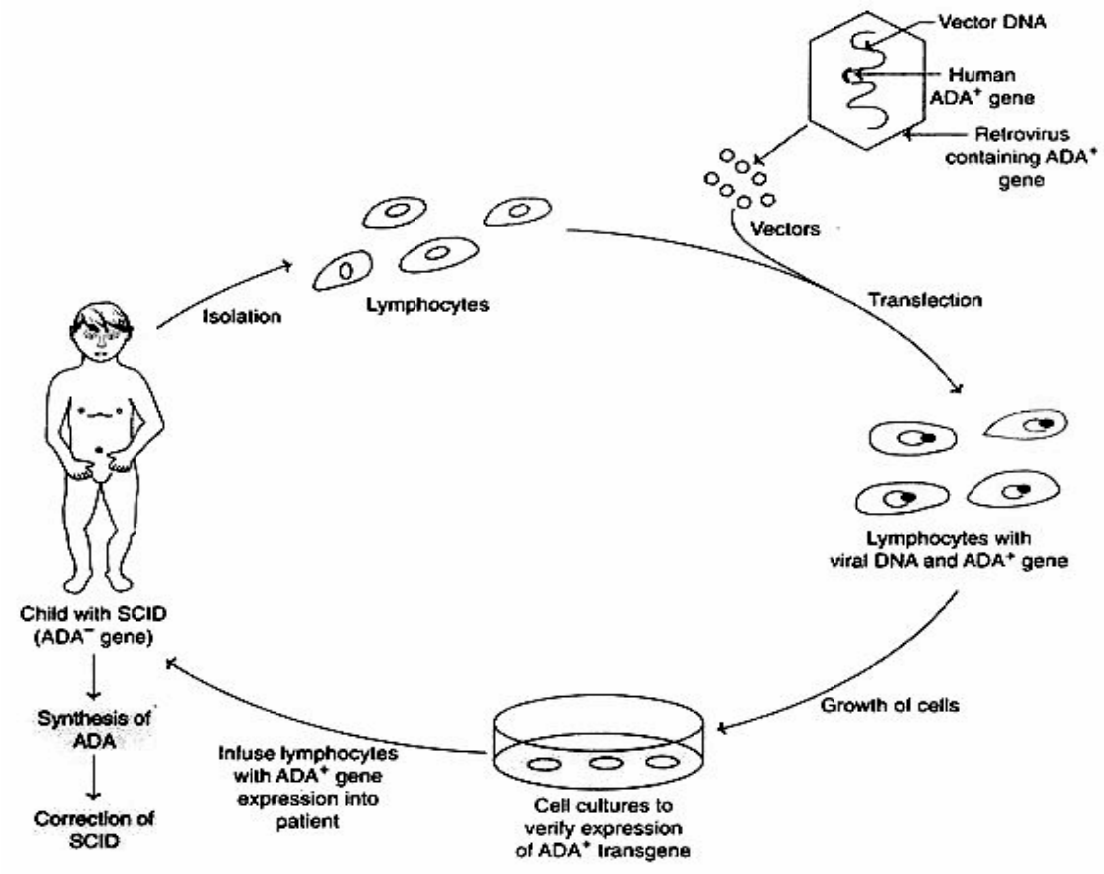


Fig. 13.5 : Treatment of adenosine deaminase (ADA) deficient patient by somatic ex vivo gene therapy (SCID-Severe combined immunodeficiency disease).

Circulating lymphocytes are removed from a patient suffering from ADA deficiency. These cells are transfected with ADA gene by exposing to billions of retroviruses carrying the said gene. The genetically-modified lymphocytes are grown in cultures to confirm the expression of ADA gene and returned to the patient. These lymphocytes persist in the circulation and synthesize ADA.

Consequently, the ability of the patient to produce antibodies is increased. However, there is a limitation. The lymphocytes have a short life span (just live for a few months), hence the transfusions have to be carried out frequently.

Transfer of ADA gene into stem cells:

In 1995, ADA gene was transferred into the stem cells, obtained from the umbilical cord blood, at the time of baby's delivery. Four days after birth, the infant received the modified cells back. By this way, a permanent population of ADA gene producing cells was established.

d. Therapy for Familial Hypercholesterolemia:

The patients of familial hypercholesterolemia lack the low density lipoprotein (LDL) receptors on their liver cells. As a result, LDL cholesterol is not metabolised in liver. The accumulated LDL-cholesterol builds up in the circulation, leading to arterial blockage and heart diseases.

Attempts are being made by gene therapists to help the victims of familial hypercholesterolemia. In fact, there is some success also. In a woman, 15% of the liver was removed. The hepatocytes were transduced with retroviruses carrying genes for LDL receptors. These genetically modified hepatocytes were infused into the patient's liver.

The hepatocytes established themselves in the liver and produced functional LDL-receptors. A significant improvement in the patient's condition, as assessed by estimating the lipid parameters in blood, was observed. Further, there were no antibodies produced against the LDL-receptor molecules, clearly showing that the genetically modified liver cells were accepted.

e. Therapy for Lesch-Nyhan Syndrome:

Lesch-Nyhan syndrome is an inborn error in purine metabolism due to a defect in a gene that encodes for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HCPRT). In the absence of HGPRT, purine metabolism is disturbed and uric acid level builds up, resulting in severe gout and kidney damage. The victims of Lesch- Nyhan syndrome exhibit symptoms of mental retardation, besides an urge to bite lips and fingers, causing self-mutilation.

By using retroviral vector system, HGPRT producing genes were successfully inserted into cultured human bone marrow cells. The major problem in humans is the involvement of brain. Experiments conducted in animals are encouraging. However, it is doubtful whether good success can be achieved by gene therapy for Lesch-Nyhan syndrome in humans, in the near future.

f. Therapy for Haemophilia:

Haemophilia is a genetic disease due lack of a gene that encodes for clotting factor IX. It is characterized by excessive bleeding. By using a retroviral vector system, genes for the synthesis of factor IX were inserted into the liver cells of dogs. These dogs no longer displayed the symptoms of haemophilia.

g. Ex Vivo Gene Therapy with Non-Autologous Cells:

The ex vivo gene therapies described above are based on the transplantation of genetically modified cells for the production of desired proteins. However, there are several limitations in using the patient's own cells (autologous cells) for gene therapy. These include lack of enough cells from target tissues, defective uptake of genes and their inadequate expression. To overcome these problems, attempts are on to develop methods to use non-autologous cells (i.e., cells from other individuals or animals). The outline of the procedure is briefly described below.

Tissue-specific cells capable of growing in culture are selected. These include fibroblasts from skin, hepatocytes from liver, and myoblasts from muscle and astrocytes from brain. These cells are cultured and genetically modified with the therapeutic gene. They are then encapsulated in artificial membrane composed of a synthetic polymer (e.g., polyether sultone, alginase-poly L-lysine-alginate).The polymeric membranes are non-immunogenic, therefore the patient can accept non-autologous encapsulated cells. Further, being semipermeable in nature, these membranes allow the nutrients to enter in, and the encoded protein (by the therapeutic gene) to pass out.

Experiments conducted in animals have shown some encouraging results for using non-autologous cells in gene therapy. The encapsulated cells were found to proliferate and produce the required protein. However, the success has been very limited in human trials.

2. In Vivo Gene Therapy:

The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue of a patient constitutes in vivo gene therapy (Fig. 13.6). Many tissues are the potential candidates for this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by viral or non- viral vector systems. The success of in vivo gene therapy mostly depends on the following parameters

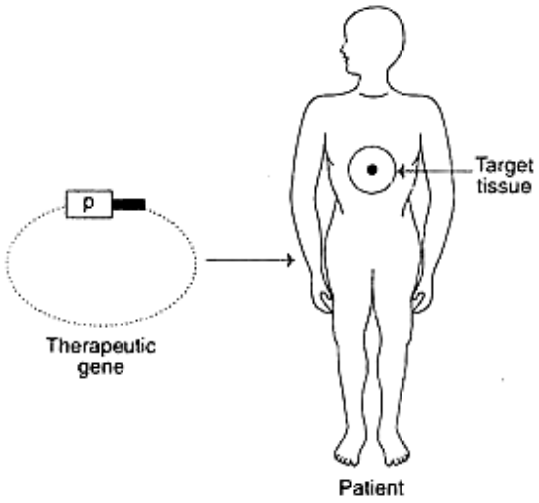


Fig. 13.6 : Diagrammatic representation of in vivo gene therapy. (p-Promoter gene specific for therapeutic gene)

i. The efficiency of the uptake of the remedial (therapeutic) gene by the target cells.

ii. Intracellular degradation of the gene and its uptake by nucleus.

iii. The expression capability of the gene.

In vivo gene therapy with special reference to gene delivery systems (viral, non-viral) with suitable examples is described.

Gene Delivery by Viruses:
Many viral vector systems have been developed for gene delivery. These include retroviruses, adenoviruses, adenoassociated viruses and herpes simplex virus.

Retrovirus vector system:
Replication defective retrovirus vectors that are harmless are being used. A plasmid in association with a retrovirus, a therapeutic gene and a promoter is referred to as plasmovirus. The plasmovirus is capable of carrying a DNA (therapeutic gene) of size less than 3.4 kb. Replication defective virus particles can be produced from the plasmovirus.

As such, for the delivery of genes by retroviral vectors, the target cells must be in a dividing stage. But majority of the body cells are quiescent. In recent years, viral vectors have been engineered to infect non-dividing cells. Further, attempts are on to include a DNA in the retroviral vectors (by engineering env gene) that encodes for cell receptor protein. If this is successfully achieved, the retroviral vector will specifically infect the target tissues.

Adenoviral vector system:
Adenoviruses (with a DNA genome) are considered to be good vectors for gene delivery because they can infect most of the non-dividing human cells. A common cold adenovirus is a frequently used vector. As the target cells are infected with a recombinant adenovirus, the therapeutic gene (DNA) enters the nucleus and expresses itself.

However, this DNA does not integrate into the host genome. Consequently, adenoviral based gene therapy required periodic administration of recombinant viruses. The efficiency of gene delivery by adenoviruses can be enhanced by developing a virus that can specifically infect target cells. This is possible by incorporating a DNA encoding a cell receptor protein.

Adeno-associated virus vector system:
Adeno-associated virus is a human virus that can integrate into chromosome 19. It is a single-stranded, non-pathogenic small DNA virus (4.7 kb). As the adeno-associated virus enters the host cell, the DNA becomes double- stranded, gets integrated into chromosome and expresses.

Adeno-associated viruses can serve as good vectors for the delivery of therapeutic genes. Recombinant viruses are created by using two plasmids and an adenovirus (i.e., helper virus) by a special technique. Some attempts were made to use therapeutic genes for the treatment of the human diseases-haemophilia (for production of blood clotting factor IX) and cystic fibrosis (for synthesis of cystic fibrosis trans membrane regulator protein) by employing adeno-associated viruses.

Therapy for cystic fibrosis:

Cystic fibrosis (CF) is one of the most common (frequency 1: 2,500) and fatal genetic diseases. It is characterized by the accumulation of sticky, dehydrated mucus in the respiratory tract and lungs. Patients of CF are highly susceptible to bacterial infections in their lungs and most of them die before reaching the age of thirty. Cystic fibrosis can be traced in European folklore, the following statement used to be said **"Woe to that child which when kissed on the forehead tastes salty. He is be witched and soon must die".**

Biochemical basis:

In the normal persons the chloride ions of the cells are pushed out through the participation of a protein called cystic fibrosis trans membrane regulator (CFTR). In the patients of cystic fibrosis, the CFTR protein is not produced due to a gene defect. Consequently, the chloride ions concentrate within the cells which draw water from the surroundings. As a result, the respiratory tract and the lungs become dehydrated with sicky mucus, an ideal environment for bacterial infections.

Gene therapy for Cystic Fibrosis:

As the defective gene for cystic fibrosis was identified in 1989, researchers immediately started working on gene therapy for this disease. Adenoviral vector systems have been used, although the success has been limited. The major drawback is that the benefits are short-lived, since the adenoviruses do not integrate themselves into host cells. Multiple administration of recombinant adenovirus caused immunological responses that destroyed the cells.

By using adeno-associated virus vector system, some encouraging results were reported in the gene therapy of CF. In the phase I clinical trials with CF patients, the vector persisted for about 70 days and some improvement was observed in the patients. Some researchers are trying to insert CF gene into the developing fetal cells (in experimental animals such as mice) to produce CFTR protein. But a major breakthrough is yet to come.

Herpes simplex virus vector system:

The retroviruses and adenoviruses employed in in vivo gene therapy are engineered to infect specific target cells. There are some viruses which have a natural tendency to infect a particular type of cells. The best example is herpes simplex virus (HSV) type I, which infects and persists in non-dividing nerve cells. HSV is a human pathogen that causes (though rarely) cold sores and encephalitis.

These are a large number of diseases (metabolic, neurodegenerative, immunological, tumours) associated with nervous system. HSV is considered as an ideal vector for in vivo gene therapy of many nervous disorders.The HSV has a double-stranded DNA of about 152 kb length as its genome. About 30 kb of HSV genome can be replaced by a cloned DNA without loss of its basic characteristics (replication, infection, packaging etc.). But there are some technical difficulties in dealing with large-sized DNAs in genetic engineering experiments. Some modified HSV vectors with reduced genomic sizes have been developed.

Most of the work on the gene therapy, related to the use of HSV as a vector, is being conducted in experimental animals. And the results are quite encouraging. HSV vectors could deliver therapeutic genes to the brain and other parts of nervous system. These genes are well expressed and maintained for long periods. More research, however, is needed before going for human trials. If successful, HSV may help to treat many neurodegenerative syndromes such as Parkinson's disease and Alzheimer's disease by gene therapy.

Gene Delivery by Non-Viral Systems:

There are certain limitations in using viral vectors in gene therapy. In addition to the prohibitive cost of maintaining the viruses, the viral proteins often induce inflammatory responses in the host. Therefore, there is a continuous search by researchers to find alternatives to viral vector systems.

a. Pure DNA constructs:

The direct introduction of pure DNA constructs into the target tissue is quite simple. However, the efficiency of DNA uptake by the cells and its expression are rather low. Consequently, large quantities of DNA have to be injected periodically. The therapeutic genes produce the proteins in the target cells which enter the circulation and often get degraded.

b. Lipoplexes:

The lipid-DNA complexes are referred to as lipoplexes or more commonly liposomes. They have a DNA construct surrounded by artificial lipid layers. A large number of lipoplexes have been prepared and used. They are non-toxic and non-immunogenic.

The major limitation with the use of lipoplexes is that as the DNA is taken up by the cells, most of it gets degraded by the lysosomes. Thus, the efficiency of gene delivery by lipoplex is very low. Some clinical trials using liposome-CFTR gene complex showed that the gene expression was very short-lived.

c. DNA-molecular conjugates:

The use of DNA-molecular conjugates avoids the lysosomal breakdown of DNA. Another advantage of using conjugates is that large-sized therapeutic DNAs (> 10 kb) can be delivered to the target tissues. The most commonly used synthetic conjugate is poly-L-lysine, bound to a specific target cell receptor. The therapeutic DNA is then made to combine with the conjugate to form a complex (Fig. 13.7).

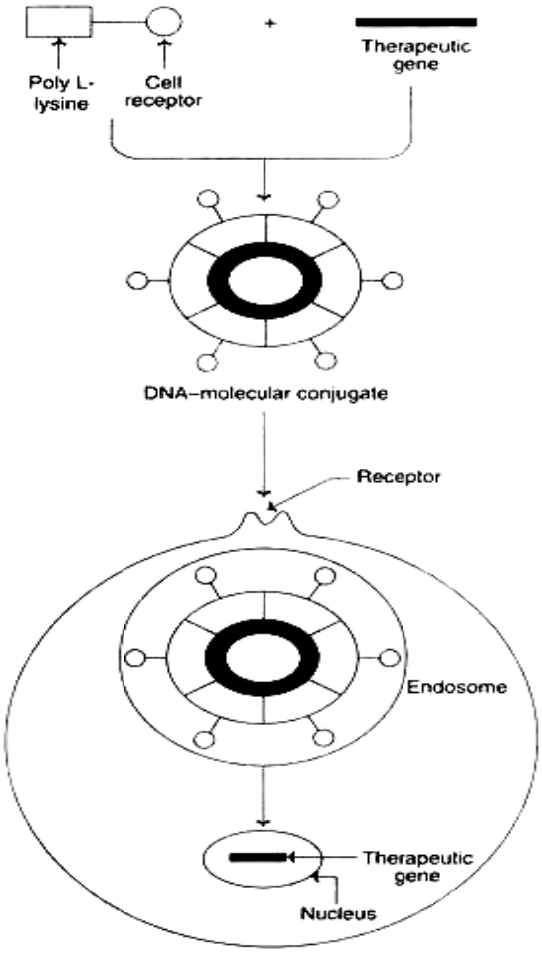


Fig. 13.7 : DNA-molecular conjugate in the delivery of therapeutic gene.

This DNA molecular conjugate binds to specific cell receptor on the target cells. It is engulfed by the cell membrane to form an endosome which protects the DNA from being degraded. The DNA released from the endosome enters the nucleus where the therapeutic gene is expressed.

d. Human artificial chromosome:

Human artificial chromosome (HAC) which can carry a large DNA one or more therapeutic genes with regulatory elements is a good and ideal vector. Studies conducted in cell cultures using HAC are encouraging. But the major problem is the delivery of the large-sized chromosome into the target cells. Researchers are working to produce cells containing genetically engineered HAC. There exists a possibility of encapsulating and implanting these cells in the target tissue.

Efficiency of gene delivery by non-viral vectors:

Although the efforts are continuously on to find suitable non-viral vectors for gene delivery, the success has been very limited. This is mainly due to the following two reasons.

1. The efficiency of transfection is very low.

2. The expression of the therapeutic gene is for a very short period, consequently there is no effective treatment of the disease.

Gene Therapy Strategies for Cancer:

Cancer is the leading cause of death throughout the world, despite the intensive treatment strategies (surgery, chemotherapy, radiation therapy). Gene therapy is the latest and a new approach for cancer treatment. Some of the developments are briefly described hereunder.

Tumour necrosis factor gene therapy:

Tumour necrosis factor (TNF) is a protein produced by human macrophages. TNF provides defence against cancer cells. This is brought out by enhancing the cancer-fighting ability of tumour- infiltrating lymphocytes (TILs), a special type of immune cells.

The tumour-infiltrating lymphocytes were transformed with a TNF gene (along with a neomycin resistant gene) and used for the treatment of malignant melanoma (a cancer of melanin producing cells usually occurs in skin). TNF as such is highly toxic, and fortunately no toxic side effects were detected in the melanoma patients injected with genetically altered TILs with TNF gene. Some improvement in the cancer patients was observed.

Suicide gene therapy:

The gene encoding the enzyme thymidine kinase is often referred to as suicide gene, and is used for the treatment of certain cancers. Thymidine kinase (TK) phosphorylates nucleosides to form nucleotides which are used for the synthesis of DNA during cell division. The drug ganciclovir (GCV) bears a close structural resemblance to certain nucleosides (thymidine). By mistake, TK phosphorylates ganciclovir to form triphosphate-GCV, a false and unsuitable nucleotide for DNA synthesis. Triphosphate-GCV inhibits DMA polymerase (Fig. 13.8).

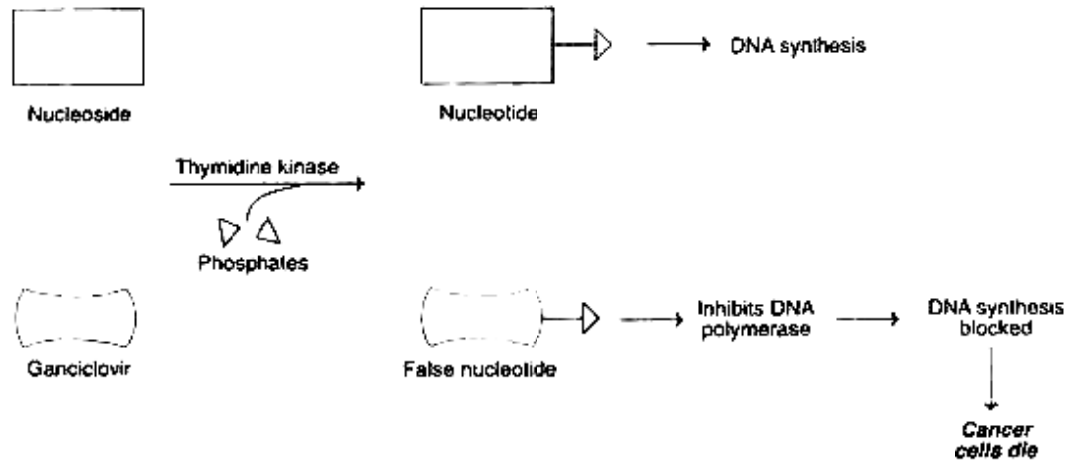


Fig. 13.8 : The action of ganciclovir mediated by thymidine kinase to inhibit the growth of cancer cells.

The result is that the elongation of the DNA molecule abruptly stops at a point containing the false nucleotide. Further, the triphosphate-GCV can enter and kill the neighbouring cancer cells, a phenomenon referred to as bystander effect. The ultimate result is that the cancer cells cannot multiply, and therefore die. Thus, the drug ganciclovir can be used to kill the cancer cells.

Ganciclovir is frequently referred to as a pro-drug and this type of approach is called pro-drug activation gene therapy. Ganciclovir has been used for treatment of brain tumours (e.g., glioblastoma, a cancer of glial cells in brain), although with a limited success.

In the suicide gene therapy, the vector used is herpes simplex virus (HSV) with a gene for thymidine kinase (TK) inserted in its genome. Normal brain cells do not divide while the brain tumour cells go on dividing unchecked.Thus, there is a continuous DNA replication in tumour cells. By using GCV-HSVTK suicide gene therapy, some reduction in proliferating tumour cells was reported. Several new strategies are being developed to increase the delivery of HSVTK gene to all the cells throughout a tumour.

Two-gene cancer therapy:

For treatment of certain cancers, two gene systems are put together and used. For instance, TK suicide gene (i.e., GCV-HSVTK) is clubbed with interleukin-2 gene (i.e. a gene promoting immunotherapy). Interleukin-2 produced mobilizes immune response. It is believed that certain proteins are released from the tumour cells on their death.

These proteins, in association with immune cells, reach the tumour and initiate immunological reactions directed against the cancer cells. Two-gene therapies have been carried out in experimental animals with colon cancer and liver cancer, and the results are encouraging.

Gene replacement therapy:

A gene named p^{53} codes for a protein with a molecular weight of 53 kilo Daltons (hence p^{53}). p^{53} is considered to be a tumour-suppressor gene, since the protein it encodes binds with DNA and inhibits replication. The tumour cells of several tissues (breast, brain, lung, skin, bladder, colon, bone) were found to have altered genes of p^{53}(mutated p^{53}), synthesizing different proteins from the original.

These altered proteins cannot inhibit DNA replication. It is believed that the damaged p^{53} gene may be a causative factor in tumour development. Some workers have tried to replace the damaged p^{53}gene by a normal gene by employing adenovirus vector systems .There are some encouraging results in the patients with liver cancer.

Gene Therapy for Aids:

AIDS is a global disease with an alarming increase in the incidence every year. It is invariably fatal, since there is no cure. Attempts are being made to relieve the effects of AIDS by gene therapy. Some of the approaches are discussed hereunder.

a. rev and env genes:
A mutant strain of human immunodeficiency virus (HIV), lacking rev and env genes has been developed. The regulatory and envelope proteins of HIV are respectively produced by rev and env genes. Due to lack of these genes, the virus cannot replicate.

Researchers have used HIV lacking rev and env genes for therapeutic purposes. T-Lymphocytes from HIV-infected patients are removed, and mutant viruses are inserted into them. The modified T-lymphocytes are cultivated and injected into the patients. Due to lack of essential genes, the viruses (HIV) cannot multiply, but they can stimulate the production of CD_8 (cluster determinant antigen 8) cells of T-lymphocytes. CD_8 cells are the killer lymphocytes. It is proved in the laboratory studies that these lymphocytes destroy the HIV-infected cells.

b. Genes of HIV proteins:
Some genes synthesizing HIV proteins are attached to DNA of mouse viruses. These genetically-modified viruses are injected to AIDS patients with clinical manifestations of the disease. It is believed that the HIV genes stimulate normal body cells to produce HIV proteins. The latter in turn stimulate the production of anti-HIV antibodies which prevent the HIV replication in AIDS patients.

c. Gene to inactivate gp120:
gp120 is a glycoprotein (molecular weight 120 kilo Daltons) present in the envelope of HIV. It is absolutely essential for binding of virus to the host cell and to bring replication. Researchers have synthesized a gene (called F105) to produce an antibody that can inactivate gp120.

In the anti- AIDS therapy, HIV-infected cells are engineered to produce anti-HIV antibodies when injected into the organism. Studies conducted in experimental animals showed a drastic reduction in the synthesis of gp120 due to anti-AIDS therapy. The production of F1IV particles was also very reduced. There are some attempts to prevent AIDS by antisense therapy.

Advantages of Gene Therapy

Gene therapy can cure genetic diseases by addition of gene or by removal of gene or by replacing a mutated gene with corrected gene.

Gene therapy can be used for cancer treatment to kill the cancerous cells.

Gene expression can be controlled.

Therapeutic protein is continuously produced inside the body which also reduces the cost of treatment in long ter

The Future of Gene Therapy:
Theoretically, gene therapy is the permanent solution for genetic diseases. But it is not as simple as it appears since gene therapy has several inbuilt complexities. Gene therapy broadly involves

isolation of a specific gene, making its copies, inserting them into target tissue cells to make the desired protein. The story does not end here.

It is absolutely essential to ensure that the gene is harmless to the patient and it is appropriately expressed (too much or too little will be no good). Another concern in gene therapy is the body's immune system which reacts to the foreign proteins produced by the new genes.The public, in general, have exaggerated expectations on gene therapy. The researchers, at least for the present, are unable to satisfy them. As per the records, by 1999 about 1000 Americans had undergone clinical trials involving various gene therapies.

Unfortunately, the gene therapists are unable to categorically claim that gene therapy has permanently cured any one of these patients. Some people in the media (leading newspapers and magazines) have openly questioned whether it is worth to continue research on gene therapy. It may be true that as of now, gene therapy due to several limitations, has not progressed the way it should, despite intensive research. But a breakthrough may come anytime, and of course, this is only possible with persistent research. And a day may come (it might take some years) when almost every disease will have a gene therapy, as one of the treatment modalities. And gene therapy will revolutionize the practice of medicine.

Probable questions:

1. Define Eugenics and Euphenics.

2. What is Positive eugenics and negative eugenics?

3. Describe the ways by which positive eugenics is achieved.

4. Describe the ways by which negative eugenics is achieved.

5. Describe genetic counselling. How it may improve human society.

6. Describe euphenic measures by which human can get rid of certain fatal genetic diseases.

7. What is genetic testing? Describe different types of diagnostic genetic testing procedures.

8. Describe different types of Non-diagnostic genetic testing methods.

9. What are the risks associated with genetic testing ?

10. Write an essay on Human Rights and its applications.

11. Define Gene therapy.

12. Describe different strategies of gene therapy ?

13. Define somatic cell gene therapy and germ cell gene therapy?

14. What is ex vivo gene therapy and in vivo gene therapy?

15. How retroviruses are used in gene therapy?

16. How gene therapy is used in treatment of Cystic fibrosis ?

17. What is suicide gene therapy?

18. Describe gene therapy treatments for AIDS?

19. What is are the advantages of gene therapy?

20. Write about the future of gene therapy?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit-XII

Quantitative genetics; variance; heritability and its measurement; in breeding and cross breeding; QTL

Objective: In this unit you will learn about Quantitative genetics, variance, heritability and its measurement. You will also have an idea about in breeding and cross breeding and QTL

Introduction:

Quantitative genetics is concerned with the inheritance of those differences between individuals that are of degree rather than of kind, quantitative rather than qualitative. These are the individual differences which, as Darwin wrote, "afford materials for natural selection to act on and accumulate, in the same manner as man accumulates in any given direction individual differences in his domestic productions." An understanding of the inheritance of these differences is thus of fundamental significance in the study of evolution and in the application of genetics to animal and plant breeding; and it is from these two fields of enquiry that the subject has received the chief impetus to its growth.

Virtually every organ and function of any species shows individual differences of this nature, the differences of size among ourselves or our domestic animals being an example familiar to all. Individuals form a continuously,graded series from one extreme to the other and do not fall naturally into sharply demarcated types. Qualitative differences, in contrast, divide individuals into distinct types with little or no connexion by intermediates. Examples are the differences between blue-eyed and brown-eyed individuals, between the blood groups, or between normally coloured and albino individuals. The distinction between quantitative and qualitative differences marks, in respect of the phenomena studied, the distinction between quantitative genetics and the parent stem of "Mendelian" genetics. In respect of the mechanism of inheritance the distinction is between differences caused by many or by few genes. The familiar Mendelian ratios, which display the fundamental mechanism of inheritance, can be seen only when a gene difference at a single locus gives rise to a readily detectable difference in some property of the organism. Quantitative differences, in so far as they are inherited, depend on gene differences at many loci, the effects of which are not individually distinguishable. Consequently the Mendelian ratios are not exhibited by quantitative differences, and the methods of Mendelian analysis are inappropriate.

The methods of study in quantitative genetics differ from those employed in Mendelian genetics in two respects. In the first place,..! since ratios cannot be observed, single progenies are uninformative, and the unit of study must be extended to "populations," that is larger groups of individuals comprising many progenies. And, in the second place, the nature of the quantitative differences to be studied requires the measurement, and not just the classification, of the

individuals. The extension of Mendelian genetics into quantitative genetics may thus be made in two stages, the first introducing new concepts connected with the genetic properties of "populations" and the second introducing concepts connected with the inheritance of measurements.

Variance:

Meaning of Variance:

It is very useful measure of dispersions which is commonly used for population data. The arithmetic mean of the squares of deviations obtained from the mean is referred to as variance. It is based on standard deviation and the square of the standard deviation is termed as variance.

$$V = \sigma^2 \text{ or } (S.D.)^2 = \frac{\Sigma(X - \bar{x})^2}{n}$$

$$\text{or } V = \frac{\Sigma d^2}{n} \text{ or } \frac{\Sigma f d^2}{n},$$

Thus, if the spread of a series is known, its standard deviation can be determined:

$$\sigma = \sqrt{variance}.$$

Co-Efficient of Variation:

Co-efficient of variation is a relative term which measures the relative magnitudes of variations present in observations and is related to the magnitude of their arithmetic mean. Since the standard deviation is independent measure of dispersion obtained from a single series of data, the comparison of variability of two different series of data is not possible directly by standard deviations.

To make it possible Karl Pearson used co-efficient of variation. Co-efficient of variation is defined as the ratio of standard deviation to arithmetic mean and is expressed in percentage. Co-efficient of variation or CV (%) = σ /x × 100, where σ = standard deviation of a series x = arithmetic mean of series.

Thus, when standard deviation of a series is compared to arithmetic mean of the series in terms of percentage, we get co-efficient of variation. It expresses the relative variability of each data series. The co-efficient of variation can be used to compare the homogeneity, consistency and stability of data of two or more series.

The data series with high value of co-efficient of variation is said to be unstable and the series with low values of co-efficient of variation are stable and they show good degree of homogeneity of data.

Example:

Compare the relative variability of two samples with the following data:

Sample A–Arithmatic mean $(\bar{x}) = 30$ and S.D. = 1.5

Sample B–Arithmatic mean $(\bar{x}) = 20$ and S.D. = 2.5

Solution. (*i*) For sample A, co-efficient of variation (CV) $= \frac{\text{S.D. or } \sigma}{\text{mean}} \times 100$

$$= \frac{1.5}{30} \times 100 = 5.0\%.$$

(*ii*) For sample B, CV $= \frac{\sigma}{\text{mean}} \times 100$

$$= \frac{2.5}{20} \times 100 = 12.50\%.$$

Example:

Calculate standard deviation, variance and co-efficient of variation form the following classified data regarding number of pods per plant recorded on 50 plants in a plot.

Number of pods/plant	1 – 3	3 – 5	5 – 7	7 – 9	9 – 11	11 – 13	13 – 15
No. of Plants	2	6	7	16	11	5	3

Solution.

Class (No. of pods per plant)	*Mid-value of class (x)*	*No. of Plants (f)*	$dx = (x - A)$	dx^2	$f.dx^2$	$f.dx$	
1 – 3	2	2	2 – 8 = – 6	36	72	– 12	
3 – 5	4	6	4 – 8 = – 4	16	96	– 24	= – 50
5 – 7	6	7	6 – 8 = – 2	4	28	– 14	
7 – 9	8 = *A*	16	8 – 8 = 0	00	00	00	
9 – 11	10	11	10 – 8 = 2	4	44	22	
11 – 13	12	5	12 – 8 = 4	16	80	20	= 60
13 – 15	14	3	14 – 8 = 6	36	108	18	
Total		$n = 50$			$\Sigma f.dx^2 = 428$	$\Sigma f.dx = 10$	

$$\text{Arithmatic mean } (\bar{x}) = A + \frac{\Sigma f.dx}{n}$$

$$\bar{x} = 8 + \frac{10}{50} = 8 + 0.2 = 8.2$$

$$\text{S.D. or } \sigma = \sqrt{\frac{\Sigma f dx^2}{n} - \left(\frac{\Sigma f dx}{n}\right)^2}$$

$$= \sqrt{\frac{428}{50} - \left(\frac{10}{50}\right)^2} = \sqrt{8.56 - 0.04}$$

$$= \sqrt{8.52} = 2.92$$

$$\text{Variance} = \sigma^2$$

$$= \left(\sqrt{8.52}\right)^2$$

$$\text{Co-efficient of variation} = \frac{\sigma}{\bar{x}} \times 100$$

$$\text{or C.V.} = \frac{2.92}{8.2} \times 100$$

$$\text{or C.V.} = \frac{292}{8.2} = 35.61\%.$$

Heritability:

Heritability**,** amount of phenotypic (observable) variation in a population that is attributable to individual genetic differences. Heritability, in a general sense, is the ratio of variation due to differences between genotypes to the total phenotypic variation for a character or trait in a population. The concept typically is applied in behaviour genetics and quantitative genetics, where heritability estimates are calculated by using either correlation and regression methods or analysis of variance (ANOVA) methods.

Heritability is expressed as $H^2 = Vg/Vp$, where H is the heritability estimate, Vg the variation in genotype, and Vp the variation in phenotype. Heritability estimates range in value from 0 to 1. If $H = 1$, then all variation in a population is due to differences or variation between genotypes (i.e., there is no environmentally caused variation). If $H = 0$, there is no genetic variation; in this case all variation in the population comes from differences in the environments experienced by individuals.Heritability is commonly used in twin studies in the field of behaviour genetics. The methodology is based on the fact that identical twins (monozygotic, or one-egg twins) share 100 percent of their genes in common and nonidentical, or fraternal, twins (dizygotic, or two-egg twins) are similar to other siblings (i.e., brothers and sisters) in that they share 50 percent of their genes in common. The correlation between identical twins is expected to be equal to 1.0 and that of fraternal twins to be 0.50. In the field of quantitative genetics, the concept of heritability is used to partition observable phenotypic variation between individuals into genetic and environmental components.

Drawbacks of Heritability Concept:

There are several drawbacks to the use of heritability estimates. First, heritability is not a measurement of how sensitive a character or trait might be to a change in environment. For example, a trait may have complete heritability ($H = 1$) yet be altered drastically by environmental change. This can be seen in certain genetic disorders of metabolism, such as phenylketonuria and Wilson disease, where heritability of phenotypic outcomes equals 1.0 but effective treatment is possible through dietary interventions. A second problem with heritability estimates is that they measure variation only within populations. In other words, a heritability estimate cannot be used to determine the causes of differences between populations, nor can it be used to determine the extent to which an individual's phenotype is determined by genes versus environment.

Furthermore, the heritability concept is subject to misuse when applied to human population differences for traits such as intelligence. For instance, studies have argued that racial differences in measures of intelligence, academic achievement, and crime rates are due to genetic rather than environmental differences. However, other studies have shown that estimates of heritability for such traits within populations do not provide information about genetic differences between populations.

Quantitative Trait Loci (QTL):

A quantitative trait locus (QTL) is a section of DNA (the locus) which correlates with variation in a phenotype (the quantitative trait). QTLs are mapped by identifying which molecular markers (such as SNPs or AFLPs) correlate with an observed trait. This is often an early step in identifying and sequencing the actual genes that cause the trait variation.

A quantitative trait locus (QTL) is a region of DNA which is associated with a particular phenotypic trait, which varies in degree and which can be attributed to polygenic effects, i.e., the product of two or more genes, and their environment. These QTLs are often found on different chromosomes. The number of QTLs which explain variation in the phenotypic trait indicates the genetic architecture of a trait. It may indicate that plant height is controlled by many genes of small effect, or by a few genes of large effect.

Typically, QTLs underlie continuous traits (those traits which vary continuously, e.g. height) as opposed to discrete traits (traits that have two or several character values, e.g. red hair in humans, a recessive trait, or smooth vs. wrinkled peas used by Mendel in his experiments).

Moreover, a single phenotypic trait is usually determined by many genes. Consequently, many QTLs are associated with a single trait. Another use of QTLs is to identify candidate genes underlying a trait. Once a region of DNA is identified as contributing to a phenotype, it can be sequenced. The DNA sequence of any genes in this region can then be compared to a database of DNA for genes whose function is already known.

Quantitative traits:

Polygenic inheritance refers to inheritance of a phenotypic characteristic (trait) that is attributable to two or more genes and can be measured quantitatively. Multifactorial inheritance refers to polygenic inheritance that also includes interactions with the environment. Unlike monogenic traits, polygenic traits do not follow patterns of Mendelian inheritance (discrete categories). Instead, their phenotypes typically vary along a continuous gradient depicted by a bell curve.

An example of a polygenic trait is human skin colour variation. Several genes factor into determining a person's natural skin colour, so modifying only one of those genes can change skin colour slightly or in some cases, such as for SLC24A5, moderately. Many disorders with genetic components are polygenic, including autism, cancer, diabetes and numerous others. Most phenotypic characteristics are the result of the interaction of multiple genes. Examples of disease processes generally considered to be results of many contributing factors.

Development of QTL Concept:

Mendelian inheritance was rediscovered at the beginning of the 20th century, and as Mendel's ideas spread geneticists began to connect Mendel's rules of inheritance of single factors to Darwinian evolution. For early geneticists, it was not immediately clear that the smooth variation in traits like body size (i.e., Incomplete Dominance) was caused by the inheritance of single genetic factors. Although Darwin himself observed that inbred features of fancy pigeons were inherited in accordance with Mendel's laws (although Darwin didn't actually know about Mendel's ideas when he made the observation), it was not obvious that these features selected by fancy pigeon breeders can similarly explain quantitative variation in nature.

An early attempt by William Ernest Castle to unify the laws of Mendelian inheritance with Darwin's theory of speciation invoked the idea that species become distinct from one another as one species or the other acquires a novel Mendelian factor. Castle's conclusion was based on the observation that novel traits that could be studied in the lab and that show Mendelian inheritance patterns reflect a large deviation from the wild type, and Castle believed that acquisition of such features is the basis of "discontinuous variation" that characterizes speciation. Darwin discussed the inheritance of similar mutant features but did not invoke them as a requirement of speciation. Instead Darwin used the emergence of such features in breeding populations as evidence that mutation can occur at random within breeding populations, which is a central premise of his model of selection in nature. Later in his career, Castle would refine his model for speciation to allow for small variation to contribute to speciation over time. He also was able to demonstrate this point by selectively breeding laboratory populations of rats to obtain a hooded phenotype over several generations. Castle's was perhaps the first attempt made in the scientific literature to direct evolution by artificial selection of a trait with continuous underlying variation, however the

practice had previously been widely employed in the development of agriculture to obtain livestock or plants with favourable features from populations that show quantitative variation in traits like body size or grain yield.

Castle's work was among the first to attempt to unify the recently rediscovered laws of Mendelian inheritance with Darwin's theory of evolution. Still, it would be almost thirty years until the theoretical framework for evolution of complex traits would be widely formalized. In an early summary of the theory of evolution of continuous variation, Sewall Wright, a graduate student who trained under Castle, summarized contemporary thinking about the genetic basis of quantitative natural variation: "As genetic studies continued, ever smaller differences were found to Mendelize, and any character, sufficiently investigated, turned out to be affected by many factors." Wright and others formalized population genetics theory that had been worked out over the preceding 30 years explaining how such traits can be inherited and create stably breeding populations with unique characteristics. Quantitative trait genetics today leverages Wright's observations about the statistical relationship between genotype and phenotype in families and populations to understand how certain genetic features can affect variation in natural and derived populations.

QTL mapping

For organisms whose genomes are known, one might now try to exclude genes in the identified region whose function is known with some certainty not to be connected with the trait in question. If the genome is not available, it may be an option to sequence the identified region and determine the putative functions of genes by their similarity to genes with known function, usually in other genomes. This can be done using BLAST, an online tool that allows users to enter a primary sequence and search for similar sequences within the BLAST database of genes from various organisms. It is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene.

Another interest of statistical geneticists using QTL mapping is to determine the complexity of the genetic architecture underlying a phenotypic trait. For example, they may be interested in knowing whether a phenotype is shaped by many independent loci, or by a few loci, and do those loci interact. This can provide information on how the phenotype may be evolving. In a recent development, classical QTL analyses were combined with gene expression profiling i.e. by DNA microarrays. Such expression QTLs (eQTLs) describe cis- and trans-controlling elements for the expression of often disease-associated genes. Observed epistatic effects have been found beneficial to identify the gene responsible by a cross-validation of genes within the interacting loci with metabolic pathway- and scientific literature databases.

Probable Questions:

1. Define variance. How it is calculated ?

2. What is coefficient of variation ?

3. What do you mean by heritability ? hat are the drawbacks of this theory ?

4. Define Quantitative trait and Quantitative trait loci.

5. How QTL mapping is done ?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

SEMESTER-II

ZOP-201
APPLIED ZOOLOGY

SELF-LEARNING MATERIAL

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

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Director
Directorate of Open and Distance Learning
University of Kalyani

OPTIONAL PAPER
(ZOP–201)

APPLIED ZOOLOGY

Group A : Immunobiology

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZOP - 201 (APPLIED ZOOLOGY)	I	Categories of wild life	4.0	1	1	7-10
	II	Wild life and wild life habitat in India: Wild life wealth of India		1	1	11-19
	III	Wild life management: Distribution, status, habitat utilization pattern, threats and survival of – Royal Bengal Tiger, Rhinoceros, Olive Ridley turtles		1	1	20-32
	IV	National and International efforts for conservation: CITES, IUCN, CBD, Protected area concept		1	1	33-47
	V	Concept of habitat and niche		1	1	48-54
	VI	Ecological principles		1	1	55-58
	VII	Community ecology: nature of communities; levels of species diversity and its Measurements		1	1	59-81
	VIII	Biogeographical zones of India		1	1	82-93
	IX	Environmental management: solid waste management with vermicomposting; Bioremediation; Bioreactors in Environment monitoring		1	1	94-117

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZOP - 201 (APPLIED ZOOLOGY)	X	Organic farming	4.0	1	1	118-124
	XI	Insect pollinators in agriculture		1	1	125-131
	XII	Integrated fish farming		1	1	132-147
	XIII	Induced breeding		1	1	148-153
	XIV	Genetics of Neurological Diseases: Pharmacogenetics and application		1	1	154-161
	XV	Preliminary knowledge on zoonotic diseases		1	1	162-167
	XVI	Immunodiagnostics: Basic of Immunology and its application		1	1	168-188

WILD LIFE AND CONSERVATION

Unit-I

Categories of wild life

Objective: In this unit you will learn about categories of wild life.

Introduction:

The International Union for Conservation of Nature (IUCN), established in 1948, is an international organization comprised of over 1,200 government and non-government members. Its objective is to promote nature conservation and sustainable use of natural resources throughout the world. This organization also focusses on issues such as poverty, gender equality, and sustainable business practices in order to address its core objective. To achieve its goal, the organization engages in field-work, data collection and analysis, lobbying, and public education outreach. IUCN has organized animals under threat on the basis of their past and present distribution, abundance, rate of decline of population, quality of natural habitats and ecological importance. Priority of conservation of a species depends on the estimation of the level of threat and its probability of its extinction over a give period of time. IUCN developed the Red Data Book to fix the priority for conservation and identify those that are in immediate need of protection. IUCN red list of threatened species is an inventory of the conservation status of the species, that are at high risk of extinction on a global scale. In 2003, IUCN developed a set of transparent , quantitative criteria to assess the conservation status of species t the regional and national level. IUCN along with species survival commission assess the status of species based on the observable decline in population, total number of living species and presence of the number of breeding pair. The current and projected trend of decline and probability of extinction in certain number of years or generation are also measured.

Reasons for species extinction

Main reasons for extinction are either natural or manmade. Through evolution, new species arise through the process of speciation and species become extinct when they are no longer able to survive in changing conditions or against superior competition. A typical species becomes extinct within 10 million years (1 crore year) of its first appearance although some species, called living fossils, survive virtually unchanged for hundreds of millions of years. Extinction, though, is usually a natural phenomenon; it is estimated that 99.9% of all species that have ever lived are now extinct.

Various anthropogenic activities causing extinction are manmade reasons. Only recently scientists have become alarmed at the high rates of recent extinctions due to various anthropogenic activities. Some of these anthropogenic activities include intentional or accidental introduction of invasive alien species, over exploitation and unscientific collection of Non-Timber Forest Produce (NTFPs) including medicinal plant, climate change, unsustainable tourism, habitat destruction, encroachment etc.

The IUCN Red List :

The IUCN is perhaps best known for its Red List of Threatened Species (also known as the Red List). The Red List, established in 1964, provides the conservation status of plant and animal species around the world. IUCN members determine the risk of a species' extinction by utilizing criteria such as population size, subpopulations, the number of mature individuals, generation, the decline in

population size, extreme fluctuations in population size, fragmented populations and habitats, habitat area size, and distribution of the population.

The Red List provides scientifically based information about species' survival, promotes public education about biodiversity, influences governmental policies, and offers advice about conservation efforts. The category assigned to each species is reassessed every 5 to 10 years by the IUCN Species Survival Commission Specialist Groups. This list is generally accepted as the most comprehensive information on the health and conservation of the world's species.

IUCN Categories: The IUCN Red List assigns a specific category to each of the evaluated species. The categories and their meanings are as follows:

a. Extinct (EX): A taxon is extinct when there is no reasonable doubt that the last individual has died.

b. Extinct in the wild (EW) : When a species only survive in cultivation, in captivity or as naturalized population (or populations) well outside from their past range.

c. Critically Endangered (CR): A taxon is called critically endangered when it is facing a high risk of extinction in the wild in immediate future. These species have 50% or higher probability of extinction within 5 years or 2 generations whichever is longer.

d. Endangered : The taxon is endangered when it is in danger of extinction and whose survival is not possible if the causal factors continue operating. These species have 20% or greater probability of extinction within 20 years or 10 generation whichever is longer.

e. Vulnerable (VU) : The taxon is vulnerable when it is not critically endangered or endangered but is facing the high risk of extinction in the wild. These species have 10% or greater chance of extinction within 100 years.

f. Near Threatened (NT) : A taxon is near threatened when it has been evaluated but does not qualify for critically endangered, endangered, vulnerable category but is close to qualifying for or is likely to qualify for a threatened category in the near future.

g. Least Concern (LC) :A taxon is least concern when it has been evaluated but does not qualify forcritically endangered, endangered, vulnerable or near threatened category. Currently these taxa are widespread and abundant.

h. Data Deficient (DD): A taxon is data deficient when there is inadequate information to make a direct or indirect assessment of its risk of extinction based on its distribution and population status.

i. Not Evaluated (NE): A taxon is not evaluated when it has not yet been assessed against the criteria.

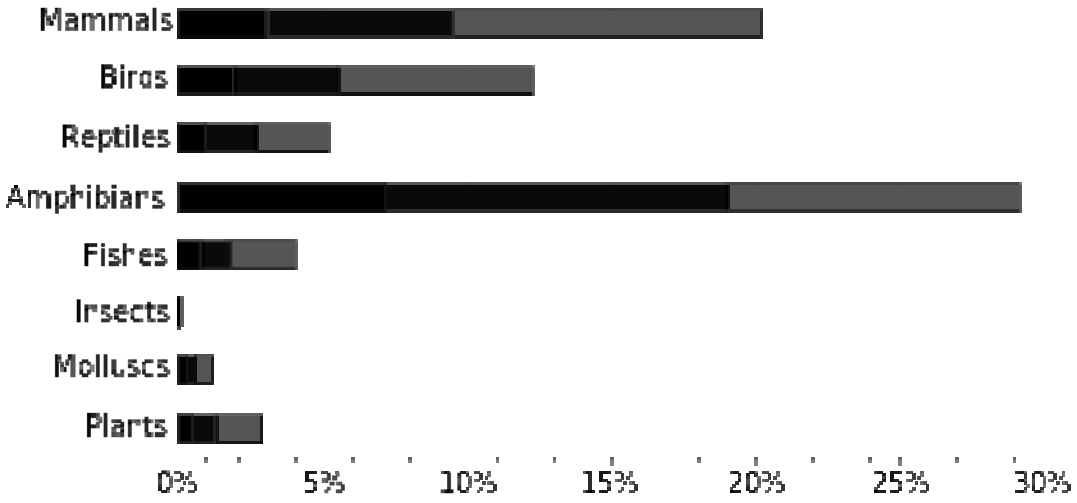

Fig: The percentage of species in several groups which are listed as critically endangered, endangered, or vulnerable on the 2007 IUCN Red List

Other categories :

a. Indeterminate: The taxon that is suspected of belonging to any of the threatened category but for which sufficient information is not available currently is called indeterminate taxon.

b. Lower risk : A taxon is in lower risk when it has been evaluated but does not qualify for any of the threatened category (critically endangered, endangered, vulnerable or near threatened) or data insufficient.

c. Rare: The taxon with small populations in the world that are not at present endangered or vulnerable are called rare taxon. These taxa are usually localized within restricted geographical areas or habitats or are thinly scattered over a more extensive range.

d. Out of danger : The taxon formerly included in any one of the extinction prone categories (critically endangered, endangered, vulnerable or near threatened) but which are now considered as relatively secured because of the effective conservation measures or the previous threat to their survival has been removed.

e. Endemic: Taxon with restricted geographical distribution are called endemic taxon. Due to such restricted distribution with small population size, they are vulnerable to both natural and anthropological threats of extinction.

Probable Questions:

1. Write a short note on IUCN.

2. Write a short note on Red list of IUCN.

3. Define nine IUCN categories.

Suggested Readings :

1. Akçakaya, H.R. and Ferson, S. 2001. RAMAS® Red List: Threatened Species Classifications under Uncertainty. Version 2.0. Applied Biomathematics, New York.

2. Akçakaya, H.R., Ferson, S., Burgman, M.A., Keith, D.A., Mace, G.M. and Todd, C.A. 2000. Making consistent IUCN classifications under uncertainty. Conservation Biology 14: 1001-1013.

3. Baillie, J. and Groombridge, B. (eds). 1996. 1996 IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland.

4. Burgman, M.A., Keith, D.A. and Walshe, T.V. 1999. Uncertainty in comparative risk analysis of threatened Australian plant species. Risk Analysis 19: 585-598.

5. Fitter, R. and Fitter, M. (eds). 1987. The Road to Extinction. IUCN, Gland, Switzerland.

6. Gärdenfors, U., Hilton-Taylor, C., Mace, G. and Rodríguez, J.P. 2001. The application of IUCN Red List Criteria at regional levels. Conservation Biology 15: 1206-1212.

Unit-II

Wild Life and Wild Life Habitat in India

Objective: In this unit you will learn about wild life and wild life habitat in India.

Introduction:

India is home to a variety of animals. Apart from a handful of domesticated animals, such as cows, buffaloes, goats, chickens,and both Bactrian and Dromedary camels, India has a wide variety of animals native to the country. It is home to Bengal and Indochinese tigers, Asiatic lions, Indian and Indochinese leopards, snow leopards, clouded leopards, various species of Deer, including Chital, Hangul, Barasingha; the Indian Elephant, the Great Indian Rhinoceros, and many others. The region's diverse is preserved in more than 120 national parks, 18 Bio-reserves and more than 500 wildlife sanctuaries across the country. India has some of the most biodiverse regions of the world and contains four of the world's 36 biodiversity hotspots – the Western Ghats, the Eastern Himalayas, Indo-Burma and Sunda Land. Wildlife management is essential to preserve the rare and endangered endemic species. India is one of the seventeen megadiverse countries. According to one study, India along with the other 16 megadiverse countries is home to about 60-70% of the world's biodiversity India, lying within the Indomalaya ecozone, is home to about 7.6% of all mammalian, 12.6% of avian (bird), 6.2% of reptilian, and 6.0% of flowering plant species.

Many Indian species are descendants of taxa originating in Gondwana, of which India originally was a part. Peninsular India's subsequent movement towards, and collision with, the Laurasian landmass set off a mass exchange of species. However, volcanism and climatic change 20 million years ago caused the extinction of many endemic Indian forms. Soon thereafter, mammals entered India from Asia through two zoogeographical passes on either side of the emerging Himalaya. As a result, among Indian species, only 12.6% of mammals and 4.5% of birds are endemic, contrasting with 45.8% of reptiles and 55.8% of amphibians. Notable endemics are the Nilgiri leaf monkey and the brown and carmine Beddome's toad of the Western Ghats. India contains 172, or 2.9%, of IUCN-designated threatened species. These include the Asian elephant, the Asiatic lion, Bengal tiger, Indian rhinoceros, mugger crocodile, and Indian white-rumped vulture, which suffered a near-extinction from ingesting the carrion of diclofenac-treated cattle.

In recent decades, human encroachment has posed a threat to India's wildlife; in response, the system of national parks and protected areas, first established in 1935, was substantially expanded. In 1972, India enacted the Wildlife Protection Act and Project Tiger to safeguard crucial habitat; further federal protections were promulgated in the 1980s. Along with over 515 wildlife sanctuaries, India now hosts 18 biosphere reserves, 10 of which are part of the World Network of Biosphere Reserves; 26 wetlands are registered under the Ramsar Convention.

The peepul tree, shown on the seals of Mohenjo-Daro, shaded Gautama Buddha as he sought enlightenment. The varied and rich wildlife of India has had a profound impact on the region's popular culture. The wildlife has also been made famous in *The Jungle Book* by Rudyard Kipling. India's wildlife has been the subject of numerous other tales and fables such as the *Panchatantra*.

Wildlife Organizations in India:

The following three organizations are dedicated for the preservation of Indian wildlife.

1. IBWL (Indian Board for Wildlife): It is an advisory body on country's wildlife constituted by Government of India in 1952.

2. BHNS (Bombay Natural- History Society): It is a non-governmental organization founded in 1881 to the cause of wildlife conservation in the country. The society conducts research and educational activities and brings out a journal on the wildlife of India.

3. WPSI (Wildlife Preservation Society of India): It is also a non-governmental body founded in 1958 at DehraDun. The society conducts tours of students and members to sanctuaries and parks, carries out research on vanishing flora and fauna, organizes a Corbett Memorial Essay competition for school students and brings forth a bilingual quarterly journal called "Cheetal"

Indian Wildlife Habitats:

A. Biosphere Reserve of India:

A biosphere reserve is a protected area in which multiple use of land is permitted by dividing it into zones, each for a particular activity.This area is meant for preserving genetic diversity in representative ecosystems (of natural biomes and biological communities) by protecting wild populations, traditional life style of tribals and domesticated plant/animal genetic resources.Under the programme MAB (Mand and Biosphere) of UNESCO creation of biosphere reserve was initiated in the year 1975.

The number of biosphere reserves established in 94 countries till May 2002 was 408. In India, 18 biosphere reserves have been set up by now (Fig. 8.1). and four of those are recognized as world heritage sites. Nanda Devi, Sunder bans, Nilgiri and Gulf of Manar.

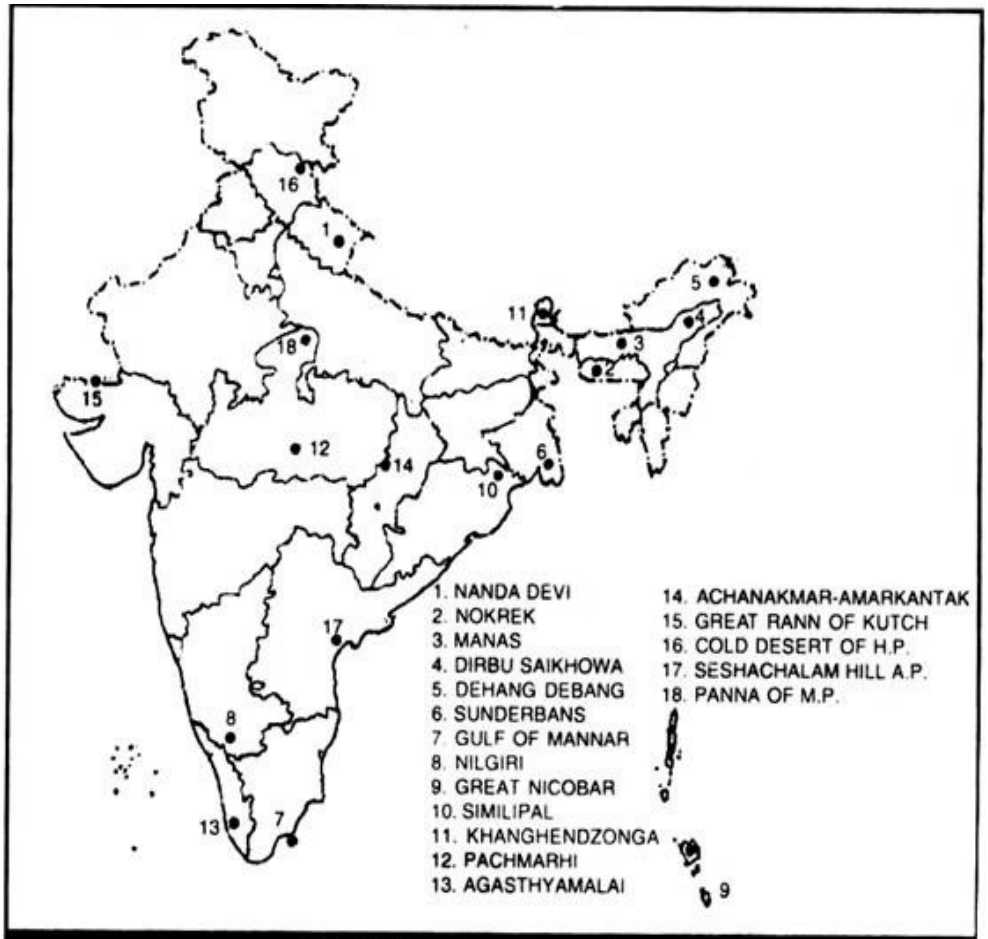

Fig 8.1. Biosphere reserves of India

List of Biosphere Reserves in India

	Name of the Biosphere Reserve	State	Area in km^2
1.	Great Rann of kutch	Gujarat	12,454
2.	Gulf of Mannar	Tamil Nadu	10,500
3.	Sunderbanas	West Bengal	9,630
4.	Cold Desert	Himachal Pradesh	7,770
5.	Nandadevi	Uttarakhand	5,860
6.	Nilgiri	T.N., Kerala, Karnatak	5,520
7.	Dehang-Dibang	Arunachal Pradesh	5,112
8.	Pachmarhi	Madhya Pradesh	4981.72
9.	Seshachalam Hills	Andhra Pradesh	4755
10.	Similipal	Odisha	4374
11.	Achanakamar-Amarkantak	Madhya Pradesh, Chattisgarh	3835
12.	Manas	Assam	2837
13.	Khangechendzonga	Sikkim	2620
14.	Agasthyamalai	Kerala, T.N.	1828
15.	Great Nicobar	Andaman & Nicobar Islands	885
16.	Nokrek	Meghalaya	820
17.	Dibru-Saikhowa	Assam	765
18.	Panna	Madhya Pradesh	

A biosphere reserve is divided into three zones: Core, buffer and manipulation.

(i) Core Zone:

In core or natural zone human activity is not allowed. This area is legally protected and undisturbed ecosystem.

(ii) Buffer zone:

The immediate surrounding area of core zone is buffer zone. Here limited human activities live like research, education and research strategy is permitted.

(iii) Transition zone:

Transition zone is the outermost or peripheral area of biosphere reserve. With the cooperation of reserve management and local people several human activities like settlements, cropping, recreation, and forestry are carried out without disturbing the environment. Buffer zone has different parts like forestry, agriculture, tourism and restoration regions. Through the restoration activities selected degraded area called restoration region is restored to natural form.

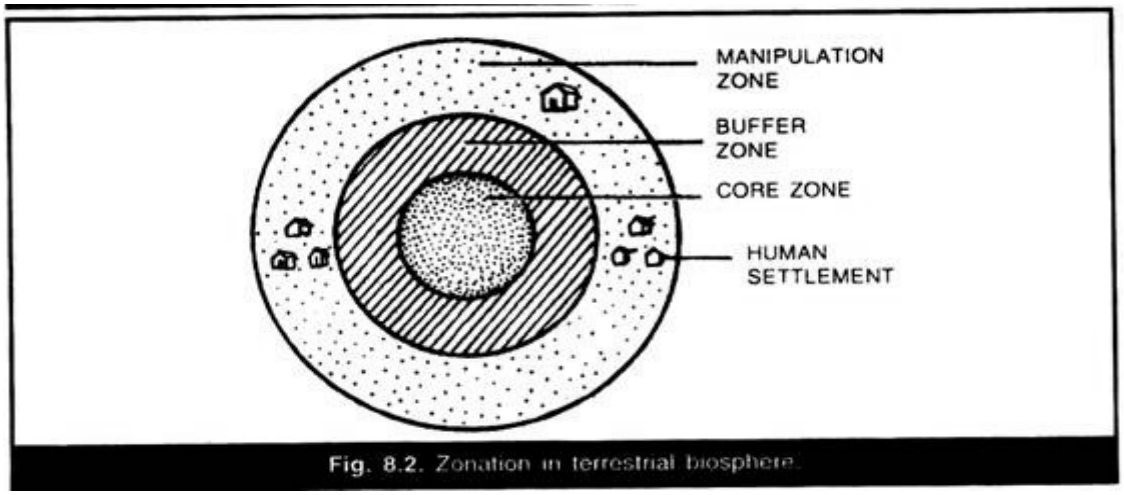

Fig. 8.2. Zonation in terrestrial biosphere.

Importance of Biosphere Reserve:

1. Conservation:

Biosphere reserves conserve genetic resources, species, ecosystems and landscapes without uprooting inhabitants. Rather the traditional life style and traditional resources of the local people are maintained.

2. Development:

A biosphere reserve is a protected area in which multiple use of land is permitted by dividing it into zones, each for a particular activity.This area is meant for preserving genetic diversity in representative ecosystems (of natural biomes and biological communities) by protecting wild populations, traditional life style of tribals and domesticated plant/animal genetic resources.

3. Restoration:

Biosphere reserve helps to rebuild any damage caused to ecosystems and habitats.

4. Education and Research:

Biosphere reserve provides a lot of scientific information for specific scientific studies and research. Exchange of information on restoration, conservation and development of biosphere can be made at national and international levels.

B. Sanctuaries of India:

A sanctuary is a protected place or area with Natural environment having optimum conditions and protection for wild animals. Shooting and hunting are prohibited in a sanctuary. But the degree of protection is lower than a national park. Manipulation up to an extent is allowed with permission of competent authority which does not harm wild life. Operations such as harvesting of timber, collection of minor forest products and private ownership rights are allowed so long as they do not affect the animals adversely. A sanctuary is established by notification of the state Forest Department and can be abolished by similar procedure. The main purpose is to provide protection to wild and indigenous animals.

Some examples of sanctuaries of India are given below:

1. Annamalai Sanctuary:
This sanctuary is situated in the southern part of Coimbatore District in Tamil Nadu and extends in a vast area of 958sq km. This sanctuary has rich fauna of animals like elephant, gaur, sambhar, spotted deer, barking deer, Nilgiritahr, lion-tailed monkeys, tiger, panther, sloth bear, langur, porcupine and pangolin.

2. Kaziranga Wildlife Sanctuary:
This sanctuary is situated on the south bank of the river Brahmaputra in Sibsagar district of Jorhat subdivision of Assam. It consists of 430sq km of forest grasslands and swamps and supports a fauna of nearly 700 rhinoceros.

Besides, it also has elephant, wild buffalo, bison, tiger, leopard, sloth bear, sambhar, swamp deer, hog deer, barking deer, wild boar, gibbon, and birds like pelican, stork and ring-tailed fishing eagle. This is, in fact, a Rhino Sanctuary.

3. Jaldapara Wildlife Sanctuary:
This sanctuary is situated in Jalpaiguri district of West Bengal and is extended in a 65 sq km stretch of grassland. It is known for rhinoceros and, hence, referred to as a Rhino Sanctuary. Besides, it also has gaur, elephant, tiger, leopard, deer, and a variety of birds and reptilian fauna.

4. Manas Wildlife Sanctuary and Tiger Reserve:
This sanctuary is situated in Kamrup district in Assam and is extended in an area of 540 sq km. River Manas passes through it. It contains the following wild animals: tiger, panther, wild dog, wild boar, rhinoceros, gaur, wild buffalo, sambhar, swamp deer and golden langur.

5. Periyar Wildlife Sanctuary:
This sanctuary is situated in Kerala and has an area of 777 sq km. It has the fauna of wild elephants, gaur, leopards, sloth bear, sambhar, barking deer, wild dogs, wild boars, black Nilgiri Langur and some birds.

6. Mudumalai Wildlife Sanctuary:
This sanctuary is situated in north-western part of Nilgiris in Tamil Nadu and extends in an area of 22 sq km. It has diverse fauna of wild elephant, gaur, sambhar, chital, barking deer, mouse deer, four-horned antelope, tiger, panther, bonnet monkey, langur, giant squirrel, flying squirrel, wild dog, jackal, wild cat, sloth bear, porcupine, pangolin, flying lizard, monitor lizard, rat snake, python and various birds.

7. Bandipur Wildlife Sanctuary:
This sanctuary is situated in south of Mysore city of Karnataka state and extends in an area of 874sq km. Its fauna includes elephant, leopard, sloth bear, wild dog, chital, panther, barking deer, porcupine and langur.

8. Sesan Gir Sanctuary:
This sanctuary is situated near Ahmedabad in Gujarat state and extends in a vast area of 1295sq km. It is the largest sanctuary of our country. It is known for Asiatic lions and other fauna includes spotted dear, blue bull, four-horned antelope, chinkara, striped hyaena, wild boar, porcupine, langur, python, crocodiles and some birds.

9. Dachigam Wildlife Sanctuary:
This sanctuary is situated in Kashmir and extends in an area of 89sq km. Its fauna includes Kashmir stag or hungal, musk deer, leopard, black bear, brown bear and baboon.

10. Bharatpur Bird Sanctuary:
It is also named as Koeladeo Ghana Sanctuary. Situated in Eastern Rajasthan, this park is known as Keoladeo Ghana National Park and is one of the world's most important heronries. It became a Sanctuary in the mid-1950's and about a third of 'its 28-sq. kms comprise a shallow, fresh-water, marsh, known as the wetlands. The dry areas are mostly scrub, thorn and mixed deciduous forest though famous for its water birds, many ", mammal species can be seen which include Sambar, blackbuck, chital, nilgai and many others, Access to Bharatpur is by a short drive from Agra, or a longer drive from Delhi or Jaipur. Should "accommodation in Bharatpur be full, Agra can be; used as a base. The sanctuary is open all year and The best times to visit are from August to March.

11. Point Calimera Wildlife Sanctuary in Tamil Nadu and includes the fauna of Chital, wild boar and black buck.

12.Sariska Sanctuary near Alwar in Rajasthan and includes tiger, leopard, spotted deer, four-horned antelope, etc.

13. Chilka Lake Sanctuary in Orissa and has variety of water birds.

14. **Shivpuri Sanctuary in Madhya Pradesh** and is known for its tiger fauna.

15. **Gautam Buddha Sanctuary in Gaya** of Bihar state and has tiger, leopard, chital, sambhar, etc.

16. **Bhimbadh Sanctuary of Monghyr** district in Bihar state and has wolf, tiger, leopard, etc.

17. Valmikinagar Sanctuary in Champaran of Bihar state and has tiger, leopard, sambhar, chital, etc.

C. National Parks of India:
A national park is a reserve of land, usually owned by a national government. It is a tract of land, which is declared public property to preserve and develop for the purpose of recreation and culture. It is protected from human development activities and pollution. National parks are protected areas of IUCN category II.

There are 10 existing national parks in India covering an area of 38,024.10 km^2, which is 1.16% of the geographical area of the country. Yellowstone National Park in California was established as the world's first protected area. The first national park in India was Hailey National Park, now known as Jim Corbett National Park, established in the year 1935.

Some examples of National Parks in India are given below:

a. Bandhavgarh National Park
Situated 'in the heart of India and the far north of Madhya Pradesh, Bandhavgarh National Park was established in 1968. Originally the private hunting preserve of the Maharaja of Rewa, it covers 453sq km of undulating terrain. The vegetation comprises mixed forest with bamboo breaks and to the south, where village land has been reclaimed, there is open grassland. The park offers varied habitats to a

large amount of \animals and excellent game viewing. Tiger sightings are common. Access to Bandhavgarh is by road from Khajuraho (reached by air from Delhi, Varanasi or Agra) or from Kanha which is a fairly long drive. The park is open from 1st November to early July.

b. **Corbett National Park**
Established in 1936, following the advice and guidelines laid down by the great hunter turned naturalist Jim Corbett, this park is located in Uttar Pradesh. Set in the undulating Siwalik Foothills of the Himalayas, the park covers 518sq km of stunning countryside. The diversity of wildlife has made it ii the most famous park in the region. Thick forest and pools of the Ramganga River are home to many animal and fish species including crocodile and Mahseer. Tiger sightings are common. Access to Corbett is by road from Delhi or a short journey through the hills from Nainital. The park is open from early November to 15 June.

c. Gir National Park
Situated in Gujarat, Gir National Park covers a core area of 259 sq. km and was established in 1965. Originally protected by Junagadh State, the area comprises mixed deciduous forest and grassland with low, rolling hills and meandering rivers. The monsoon brings little rain and much of the area is dry, making for excellent gameviewing. It remains the only home of the Asiatic Lion, slightly smaller than its African cousin and a highly protected species. There is an abundance of other wildlife to be seen and lion sightings are possible. Access to Gir is by road from Bhavnagar, Gondal, Jasdan and many other areas of Gujarat. The park is open from end October to June.

d. Kanha National Park
Situated in Madhya Pradesh and declared a national park in 1955, Kanha now covers 648sq kms and comes under the auspices of Project Tiger. The vegetation comprises forest with bamboo breaks, grassy plateaus and meadows in the valleys. The Barasingha (swamp deer) are one of the park's success stories with its population having increased seven-fold in the past 35 years. It is also home to Tara, Mark Shand's famous elephant. Tiger sightings are common. Access to Kanha is by road from Nagpur (reached by flight from Mumbai, Calcutta or Delhi) or by a longer journey from Bandhavgarh. The park is open from 1 November to 30 June.

Difference between National Parks, Sanctuaries and Biosphere Reserves:

a. Wildlife Sanctuary:

It is a consecrated place where sacred species are kept. It is not open for general public, unlike zoo. In other words, we say, it tries not to allow any activity that would place the animals in an unduly stressful situation. India has 543 wildlife sanctuaries.

Characteristics of Wildlife Sanctuary:

1. It is natural area which is reserve by a governmental or private agency for the protection of particular species.

2. Area is designated for the protection of wild animals.

3. Only animals are conserved, Could be private property also, outside activities allowed.

4. It came under the category called "**Protected Areas**". The Protected Areas are declared under **Wildlife (Protection) Act, 1972**.

5. **International Union for Conservation of Nature (IUCN)** has defined its Category IV type of protected areas

b. National Park :

It is a home to many species of birds and animals which is established by central and state government for the conservation.

Characteristics of National Park

1. Reserve area of land, owned by the government.

2. Area is protected from human exploitation, industrialization and pollution.

3. No cutting, Grazing allowed, Outside Species Allowed

4. It came under the category called "**Protected Areas**". The Protected Areas are declared under **Wildlife (Protection) Act, 1972**.

5. Conservation of 'wild nature' for posterity and as a symbol of national pride.

6. **International Union for Conservation of Nature (IUCN)**, and its **World Commission on Protected Areas**, has defined its Category II type of protected areas.

c. Biosphere Reserves:

The International Co-ordinating Council (ICC) of UNESCO designated of 'Biosphere reserve' for natural areas from November, 1971. There are 18 Biosphere Reserves in India.

Characteristics of Biosphere Reserve

1. Notified areas which cover a larger area of land which may cover multiple National Parks, Sanctuaries and reserves as well.

2. Areas are meant for conservation of biodiversity of a specific area.

3. Three areas: **Core, Buffer & Marginal**. No outside Species allowed Conservation & research purpose.

4. It is internationally recognized within the framework of **UNESCO's Man and Biosphere (MAB) programme** and nominated by national governments.

5. The Ministry of Environment and Forest provides financial assistance to the respective State governments for conservation of landscape and biological diversity and cultural heritage.

Probable Questions:

1. write a note on wildlife management organizations in India.
2. What is Biosphere reserve. Escribe different zones of an ideal biosphere reserve.
3. State the importance of Biosphere reserve.
4. What is Sanctuaries. Describe any two sanctuaries situated in India.
5. What is National park. Describe any three national parks in India.
6. State the characteristics of National Parks.
7. State the characteristics of wildlife sanctuaries.
8. State the characteristics of Biosphere reserves.
9. What are the differences between National Parks, Wildlife Sanctuaries and Biosphere reserves ?

Suggested readings:

1.https://nt.gov.au/environment/animals/classification-of-wildlife
2. Biodiversity by Maity and Maity
3. Ecology and Environmental science by Rana

Unit-III

Wild life management: Distribution, status, habitat utilization pattern, threats and survival of – Royal Bengal Tiger, Rhinoceros, Olive Ridley turtles

Objective: In this Unit you will learn distribution, habitat utilization pattern, threats and survival of Royal Bengal Tiger, Rhinoceros, Olive Ridley turtles.

Introduction:

The management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generation and to maintain its potential to meet the needs and aspirations of future generations is called the conservation. It is scientific management of wildlife to maintain it at its optimum level. The conservation of wildlife is directly related to healthy and better forests. Wildlife conservation includes protection, preservation, and perpetuation of rare species of plants and animals in their natural habitats.

A. Royal Bengal Tiger

The **Bengal tiger** is a *Panthera tigris* tigris population in the Indian subcontinent. It is listed as Endangered on the IUCN Red List since 2008, and was estimated at comprising fewer than 2,500 individuals by 2011. It is threatened by poaching, loss, and fragmentation of habitat. None of the Tiger Conservation Landscapes within its range is considered large enough to support an effective population of more than 250 adult individuals. India's tiger population was estimated at 1,706–1,909 individuals in 2010. By 2014, the population had reputedly increased to an estimated 2,226 individuals. Around 440 tigers are estimated in Bangladesh, 163–253 tigers in Nepal and 103 tigers in Bhutan. The tiger is estimated to be present in the Indian subcontinent since the Late Pleistocene, for about 12,000 to 16,500 years.

The Bengal tiger ranks among the biggest wild cats alive today. It is considered to belong to the world's charismatic megafauna. It is the national animal of both India and Bangladesh. It is also known as the Royal Bengal tiger.

Taxonomic Position:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Suborder: Feliformia

Family: Felidae

Subfamily: Pantherinae

Genus: *Panthera*

Species: *tigris*

a. Distribution and Habit:

Tigers appear to have arrived in Sri Lanka during a pluvial period, during which sea levels were depressed, evidently prior to the last glacial maximumabout 20,000 years ago. In 1929, the British taxonomist Pocock assumed that tigers arrived in southern India too late to colonize Sri Lanka, which earlier had been connected to India by a land bridge.

Results of a phylogeographic study using 134 samples from tigers across the global range suggest that the historical north-eastern distribution limit of the Bengal tiger is the region in the Chittagong Hills and Brahmaputra River basin, bordering the historical range of the Indochinese tiger.

In the Indian subcontinent, tigers inhabit tropical moist evergreen forests, tropical dry forests, tropical and subtropical moist deciduous forests, mangroves, subtropical and temperate upland forests, and alluvial grasslands. Latter habitat once covered a huge swath of grassland, riverine and moist semi-deciduous forests along the major river system of the Gangetic and Brahmaputra plains, but has now been largely converted to agricultural land or severely degraded. Today, the best examples of this habitat type are limited to a few blocks at the base of the outer foothills of the Himalayas including the *Tiger Conservation Units* (TCUs) Rajaji-Corbett, Bardia-Banke, and the transboundary TCUs Chitwan-Parsa-Valmiki, Dudhwa-Kailali and Shuklaphanta-Kishanpur. Tiger densities in these TCUs are high, in part because of the extraordinary biomass of ungulate prey.The tigers in the Sundarbans in India and Bangladesh are the only ones in the world inhabiting mangrove forests. The population in the Indian Sundarbans is estimated as 70 tigers in total.

b. Ecology and Behavior:

The basic social unit of the tiger is the elemental one of mother and offspring. Adult animals congregate only on an *ad hoc* and transitory basis when special conditions permit, such as plentiful supply of food. Otherwise they lead solitary lives, hunting individually for the dispersed forest and tall grassland animals, upon which they prey. They establish and maintain home ranges. Resident adults of either sex tend to confine their movements to a definite area of habitat within which they satisfy their needs, and in the case of tigresses, those of their growing cubs. Besides providing the requirements of an adequate food supply, sufficient water and shelter, and a modicum of peace and seclusion, this location must make it possible for the resident to maintain contact with other tigers, especially those of the opposite sex. Those sharing the same ground are well aware of each other's movements and activities.

In the Panna Tiger Reserve an adult radio-collared male tiger moved 1.7 to 10.5 km (1.1 to 6.5 mi) between locations on successive days in winter, and 1 to 13.9 km (0.62 to 8.64 mi) in summer. His home range was about 200 km^2 (77sq mi) in summer and 110 km^2 (42 sq mi) in winter. Included in his home range were the much smaller home ranges of two females, a tigress with cubs and a sub-adult tigress. They occupied home ranges of 16 to 31 km^2 (6.2 to 12.0 sq mi).

The home ranges occupied by adult male residents tend to be mutually exclusive, even though one of these residents may tolerate a transient or sub-adult male at least for a time. A male tiger keeps a large territory in order to include the home ranges of several females within its bounds, so that he may maintain mating rights with them. Spacing among females is less complete. Typically there is partial overlap with neighboring female residents. They tend to have core areas, which are more exclusive, at least for most of the time. Home ranges of both males and females are not stable. The shift or alteration of a home range by one animal is correlated with a shift of another. Shifts from less suitable habitat to better ones are made by animals that are already resident. New animals become residents only as vacancies occur when a former resident moves out or dies. There are more places for resident females than for resident males.

During seven years of camera trapping, tracking, and observational data in Chitwan National Park, 6 to 9 breeding tigers, 2 to 16 non-breeding tigers, and 6 to 20 young tigers of less than one year of age were detected in the study area of 100 km^2 (39 sq mi). One of the resident females left her territory to one of her female offspring and took over an adjoining area by displacing another female; and a displaced female managed to re-establish herself in a neighboring territory made vacant by the death of the resident. Of 11 resident females, 7 were still alive at the end of the study period, 2 disappeared after losing their territories to rivals, and 2 died. The initial loss of two resident males and subsequent take over of their home ranges by new males caused social instability for two years. Of 4 resident males, 1 was still alive and 3 were displaced by rivals. Five litters of cubs were killed by infanticide, 2 litters died because they were too young to fend for themselves when their mothers died. One juvenile tiger was presumed dead after being photographed with severe injuries from a deer snare. The remaining young lived long enough to reach dispersal age, 2 of them becoming residents in the study area.

c. Threat:

Over the past century tiger numbers have fallen dramatically, with a decreasing population trend. None of the Tiger Conservation Landscapes within the Bengal tiger range is large enough to support an effective population size of 250 individuals. Habitat losses and the extremely large-scale incidences of poaching are serious threats to the species' survival.

The challenge in the Western Ghats forest complex in western South India, an area of 14,400 square miles (37,000 km^2) stretching across several protected areas is that people live within its borders. The Save the Tiger Fund Council estimates that 7,500 landless people live illegally inside the boundaries of the 386-square-mile (1,000 km^2) Nagarhole National Park in southwestern India. A voluntary if controversial resettlement is underway with the aid of the Karnataka Tiger Conservation Project led by K. UllasKaranth of the Wildlife Conservation Society.

A 2007 report by UNESCO, "Case Studies on Climate Change and World Heritage" has stated that an anthropogenic 45-cm rise in sea level, likely by the end of the 21st century, according to the Intergovernmental Panel on Climate Change, combined with other forms of anthropogenic stress on the Sundarbans, could lead to the destruction of 75% of the Sundarbans mangroves. The Forest

Rights Act passed by the Indian government in 2006 grants some of India's most impoverished communities the right to own and live in the forests, which likely brings them into conflict with wildlife and under-resourced, under-trained, ill-equipped forest department staff. In the past, evidence showed that humans and tigers cannot co-exist.

Poaching

The most significant immediate threat to the existence of wild tiger populations is the illegal trade in poached skins and body parts between India, Nepal and China. The governments of these countries have failed to implement adequate enforcement response, and wildlife crime remained a low priority in terms of political commitment and investment for years. There are well-organised gangs of professional poachers, who move from place to place and set up camp in vulnerable areas. Skins are rough-cured in the field and handed over to dealers, who send them for further treatment to Indian tanning centres. Buyers choose the skins from dealers or tanneries and smuggle them through a complex interlinking network to markets outside India, mainly in China. Other factors contributing to their loss are urbanisation and revenge killing. Farmers blame tigers for killing cattle and shoot them. Their skins and body parts may however become a part of the illegal trade. In Bangladesh, tigers are killed by professional poachers, local hunters, trappers, pirates and villagers. Each group of people has different motives for killing tigers, ranging from profit, excitement to safety concerns. All groups have access to the commercial trade in body parts.

The illicit demand for bones and body parts from wild tigers for use in Traditional Chinese medicine is the reason for the unrelenting poaching pressure on tigers on the Indian subcontinent. For at least a thousand years, tiger bones have been an ingredient in traditional medicines that are prescribed as a muscle strengthener and treatment for rheumatism and body pain. Between 1994 and 2009, the Wildlife Protection Society of India has documented 893 cases of tigers killed in India, which is just a fraction of the actual poaching and trade in tiger parts during those years. In 2006, India's Sariska Tiger Reserve lost all of its 26 tigers, mostly to poaching. In 2007, police in Allahabad raided a meeting of suspected poachers, traders and couriers. One of the arrested persons was the biggest buyer of tiger parts in India who used to sell them off to the Chinese traditional medicinal market, using women from a nomadic tribe as couriers. In 2009, none of the 24 tigers residing in the Panna Tiger Reserve were left because of excessive poaching. In November 2011, two tigers were found dead in Maharashtra: a male tiger was trapped and killed in a wire snare; a tigress died of electrocution after chewing at an electric cable supplying power to a water pump; another tigress was found dead in Kanha Tiger Reserve landscape — poisoning is suspected to be the cause of her death.

d. Conservation:

An area of special interest lies in the "Terai Arc Landscape" in the Himalayan foothills of northern India and southern Nepal, where 11 protected areas composed of dry forest foothills and tall-grass savannas harbor tigers in a 49,000 square kilometres (19,000 sq mi) landscape. The goals are to manage tigers as a single metapopulation, the dispersal of which between core refuges can help maintain genetic, demographic, and ecological integrity, and to ensure that species and habitat conservation becomes mainstreamed into the rural development agenda. In Nepal a community-based tourism model has been developed with a strong emphasis on sharing benefits with local people and

on the regeneration of degraded forests. The approach has been successful in reducing poaching, restoring habitats, and creating a local constituency for conservation.WWF partnered with Leonardo DiCaprio to form a global campaign, "Save Tigers Now", with the ambitious goal of building political, financial and public support to double the wild tiger population by 2022. *Save Tigers Now* started its campaign in 12 different WWF Tiger priority landscapes, since May 2010.

In India

In 1973, Project Tiger was launched aiming at ensuring a viable tiger population in the country and preserving areas of biological importance as a natural heritage for the people. The project's task force visualised these tiger reserves as breeding nuclei, from which surplus animals would disperse to adjacent forests. The selection of areas for the reserves represented as close as possible the diversity of ecosystems across the tiger's distribution in the country. Funds and commitment were mustered to support the intensive program of habitat protection and rehabilitation under the project. By the late 1980s, the initial nine reserves covering an area of 9,115 square kilometres (3,519 sq mi) had been increased to 15 reserves covering an area of 24,700 square kilometres (9,500 sq mi). More than 1100 tigers were estimated to inhabit the reserves by 1984.

Through this initiative the population decline was reversed initially, but has resumed in recent years; India's tiger population decreased from 3,642 in the 1990s to just over 1,400 from 2002 to 2008.

The Indian Wildlife Protection Act of 1972 enables government agencies to take strict measures so as to ensure the conservation of the Bengal tigers. The Wildlife Institute of India estimates showed that tiger numbers had fallen in Madhya Pradesh by 61%, Maharashtra by 57%, and Rajasthan by 40%. The government's first tiger census, conducted under the Project Tiger initiative begun in 1973, counted 1,827 tigers in the country that year. Using that methodology, the government observed a steady population increase, reaching 3,700 tigers in 2002. However, the use of more reliable and independent censusing technology (including camera traps) for the 2007–2008 all-India census has shown that the numbers were in fact less than half than originally claimed by the Forest Department.[99]

Following the revelation that only 1,411 Bengal tigers existed in the wild in India, down from 3,600 in 2003, the Indian government set up eight new tiger reserves.[100] Because of dwindling tiger numbers, the Indian government has pledged US$153 million to further fund the Project Tiger initiative, set up a Tiger Protection Force to combat poachers, and fund the relocation of up to 200,000 villagers to minimize human-tiger interaction. Tiger scientists in India, such as Raghu Chundawat and UllasKaranth have called for use of technology in the conservation efforts.

In January 2008, the Government of India launched a dedicated anti-poaching force composed of experts from Indian police, forest officials and various other environmental agencies. Indian officials successfully started a project to reintroduce the tigers into the Sariska reserve. The Ranthambore National Park is often cited as a major success by Indian officials against poaching. The population increased to 1,706 in 2011 and 2,226 in 2014.[107] There are 48[108] tiger reserves in. Kuno-Palpur in Madhya Pradesh was supposed receive Asiatic lions from Gujarat. Since no lion has been transferred from Gujarat to Madhya Pradesh so far, it may be used as a sanctuary for the tiger instead.

Bengal tigers have been captive bred since 1880 and widely crossed with other tiger subspecies. Indian zoos have bred tigers for the first time at the Alipore Zoo in Kolkata. The 1997 International Tiger Studbook lists the global captive population of Bengal tigers at 210 individuals that are all kept in Indian zoos, except for one female in North America. Completion of the Indian Bengal Tiger Studbook is a necessary prerequisite to establishing a captive management program for tigers in India.

B. Rhinoceros

The **Indian rhinoceros** (*Rhinoceros unicornis*), also called the **greater one-horned rhinoceros** and **great Indian rhinoceros**, is a rhinoceros native to the Indian subcontinent. It is listed as Vulnerable on the IUCN Red List, as populations are fragmented and restricted to less than 20,000 km^2 (7,700 sq mi). Moreover, the extent and quality of the rhino's most important habitat, alluvial grassland and riverine forest, is considered to be in decline due to human and livestock encroachment. As of 2008, a total of 2,575 mature individuals were estimated to live in the wild.

The Indian rhinoceros once ranged throughout the entire stretch of the Indo-Gangetic Plain, but excessive hunting and agricultural development reduced their range drastically to 11 sites in northern India and southern Nepal. In the early 1990s, between 1,870 and 1,895 rhinos were estimated to have been alive.

Taxonomic position:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Perissodactyla

Family: Rhinocerotidae

Genus: *Rhinoceros*

Species: *unicornis*

a. Distribution and Habit:

The one-horned rhinoceros once ranged across the entire northern part of the Indian Subcontinent, along the Indus, Gangesand Brahmaputra River basins, from Pakistan to the Indian-Myanmar border, including Bangladesh and the southern parts of Nepal and Bhutan. It may have also occurred in Myanmar, southern China and Indochina. It inhabits the alluvial grasslands of the Terai and the Brahmaputra basin. As a result of habitat destruction and climatic changes its range has gradually been reduced so that by the 19th century, it only survived in the Terai grasslands of southern Nepal, northern Uttar Pradesh, northern Bihar, northern West Bengal, and in the Brahmaputra Valley of Assam.The species was present in northern Bihar and Oudh at least until 1770 as indicated in maps produced by Colonel Gentil.On the former abundance of the species, Thomas C. Jerdon wrote in 1867: This huge rhinoceros is found in the Terai at the foot of the Himalayas, from Bhutan to Nepal. It is more common in the eastern portion of the Terai than the west, and is most abundant in Assam and the Bhutan Dooars.

b. Ecology and behavior :

Adult male Indian rhinos are usually solitary. Groups consist of females with calves, or of up to six subadults. Such groups congregate at wallows and grazing areas. They are foremost active in early mornings, late afternoons and at night, but rest during hot days. They are excellent swimmers and can run at speeds of up to 55 km/h (34 mph) for short periods. They have excellent senses of hearing and smell, but relatively poor eyesight. Over 10 distinct vocalizations have been recorded. Males have home ranges of around 2 to 8 km^2 (0.77 to 3.09 sq mi) that overlap each other. Dominant males tolerate males passing through their territories except when they are in mating season, when dangerous fights break out Indian rhinos bathe regularly. The folds in their skin trap water and hold it even when they come back on land.

Indian rhinos have few natural enemies, except for tigers, which sometimes kill unguarded calves, but adult rhinos are less vulnerable due to their size. Mynahs and egrets both eat invertebrates from the rhino's skin and around its feet. *Tabanus* flies, a type of horse-fly, are known to bite rhinos. The rhinos are also vulnerable to diseases spread by parasites such as leeches, ticks, and nematodes. Anthrax and the blood-disease septicemia are known to occur. In March 2017, of a group of four tigers consisting of an adult male, female and two cubs killed a 20-year-old male rhino in the Dudhwa Tiger Reserve.

c. Threat:

Sport hunting became common in the late 1800s and early 1900s. Indian rhinos were hunted relentlessly and persistently. Reports from the middle of the 19th century claim that some British military officers in Assam individually shot more than 200 rhinos. By 1908, the population in Kaziranga had decreased to around 12 individuals.[10] In the early 1900s, the species had declined to near extinction.Poaching for rhinoceros horn became the single most important reason for the decline of the Indian rhino after conservation

measures were put in place from the beginning of the 20th century, when legal hunting ended. From 1980 to 1993, 692 rhinos were poached in India. In India's Laokhowa Wildlife Sanctuary, 41 rhinos were killed in 1983, virtually the entire population of the sanctuary. By the mid-1990s, poaching had rendered the species extinct there.
In 1950, Chitwan's forest and grasslands extended over more than 2,600 km^2(1,000 sq mi) and were home to about 800 rhinos. When poor farmers from the mid-hills moved to the Chitwan Valley in search of arable land, the area was subsequently opened for settlement, and poaching of wildlife became rampant. The Chitwan population has repeatedly been jeopardized by poaching; in 2002 alone, poachers killed 37 animals to saw off and sell their valuable horns.

Six methods of killing rhinos have been recorded:

- Shooting is by far the most common method used; rhino horn traders hire sharpshooters and often supply them with rifles and ammunition.
- Trapping in a pit depends largely on the terrain and availability of grass to cover it; pits are dug out in such a way that a fallen animal has little room to manoeuvre with its head slightly above the pit, so that it is easy to saw off the horn.
- Electrocution is used where high voltage powerlines pass through or near a protected area, to which poachers hook a long, insulated rod connected to a wire, which is suspended above a rhino path.
- Poisoning by smearing zinc phosphide rat poison or pesticides on salt licks frequently used by rhinos is sometimes used.
- Spearing has only been recorded in Chitwan National Park.
- A noose, which cuts through the rhino's skin, kills it by strangulation.

Poaching, mainly for the use of the horn in traditional Chinese medicine, has remained a constant and has led to decreases in several important populations. Apart from this, serious declines in quality of habitat have occurred in some areas, due to:

- severe invasion by alien plants into grasslands affecting some populations;
- demonstrated reductions in the extent of grasslands and wetland habitats due to woodland encroachment and silting up of beels;
- grazing by domestic livestock.

The species is inherently at risk because over 70% of its population occurs at a single site, Kaziranga National Park. Any catastrophic event such as disease, civil disorder, poaching, or habitat loss would have a devastating impact on the Indian rhino's status. However, small population of rhinos may be prone to inbreeding depression.

d. Conservation:

Rhinoceros unicornis has been listed in CITES Appendix I since 1975. The Indian and Nepalese governments have taken major steps towards Indian rhinoceros conservation, especially with the help of the World Wide Fund for Nature (WWF) and other non-governmental organizations. In the early 1980s, a rhino translocation scheme was initiated. The first pair of rhinos was reintroduced from Nepal's Terai to Pakistan's Lal Suhanra National Park in Punjab in 1982. In 1910, all rhino hunting in India became prohibited. In 1984, five rhinos were relocated to Dudhwa National Park — four from the fields outside the Pobitora Wildlife Sanctuary and one from Goalpara.

The Indian rhinoceros was initially difficult to breed in captivity. The first recorded captive birth of a rhinoceros was in Kathmandu in 1826, but another successful birth did not occur for nearly 100 years. In 1925, a rhino was born in Kolkata. No rhinoceros was successfully bred in Europe until 1956. On September 14, 1956, Rudra was born in Zoo Basel, Switzerland. In the second half of the 20th century, zoos became adept at breeding Indian rhinoceros. By 1983, nearly 40 babies had been born in captivity. As of 2012, 33 Indian rhinos were born at Zoo Basel, which means that most animals kept in a zoo are somehow related to the population in the zoo of Basel, Switzerland. Due to the success

of Zoo Basel's breeding program, the International Studbook for the species has been kept there since 1972. Since 1990, the Indian rhino European Endangered Species Programme is being coordinated there, as well, which ensures that the captive global Indian rhinoceros population stays genetically as healthy as possible. As of 2010, 174 rhinos are kept in zoos worldwide.

In June 2009, an Indian rhino was artificially inseminated using sperm collected four years previously and cryopreserved at the Cincinnati Zoo's CryoBioBank before being thawed and used. She gave birth to a male calf in October 2010. The calf died 12 hours after birth.

In June 2014, the first "successful" live-birth from an artificially inseminated rhino took place at the Buffalo Zoo in New York. As in Cincinnati, cryopreserved sperm was used to produce the female calf, Monica

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C. Olive Ridley Turtles

The **olive ridley sea turtle** (*Lepidochely solivacea*), also known as the **Pacific ridley sea turtle**, are the second smallestand most abundant of all sea turtles found in the world; this species of sea turtle is found in warm and tropical waters, primarily in the Pacific and Indian Oceans. They can also be found in the warm waters of the Atlantic Ocean.

These turtles, along with the related Kemps ridley turtle, are best known for their unique mass nesting called *arribada*, where thousands of females come together on the same beach to lay eggs.

Taxonomic Position:

Kingdom: Animalia
Phylum: Chordata
Class: Reptilia
Order: Testudines
Suborder: Cryptodira
Family: Cheloniidae
Genus: *Lepidochelys*
Species: *olivacea*

a. Distribution:

The olive ridley turtle has a circumtropical distribution, living in tropical and warm waters of the Pacific and Indian Oceans from India, Arabia, Japan, and Micronesia south to southern Africa, Australia, and New Zealand. In the Atlantic Ocean, it has been observed off the western coast of Africa and the coasts of northern Brazil, Suriname, Guyana, French Guiana, and Venezuela. Additionally, the olive ridley has been recorded in the Caribbean Sea as far north as Puerto Rico. A female individual was found alive on an Irish Sea beach on the Isle of Anglesey, British Isles in November 2016, giving this species its northernmost appearance. It was taken in by the nearby Anglesey Sea Zoo while its health was being assessed. The olive ridley is also found in the eastern Pacific Ocean from the Galapagos Islands and Chile north to the Gulf of California, and along the Pacific coast to at least Oregon Migratory movements have been studied less intensely in olive ridleys than other species of marine turtles, but they are believed to use the coastal waters of over 80 countries. Historically, this species has been widely regarded as the most abundant sea turtle in the world. More than one million olive ridleys were commercially harvested off the coasts of Mexico in 1968 alone.

The population of Pacific Mexico was estimated to be at least 10 million prior to the era of mass exploitation. More recently, the global population of annual nesting females has been reduced to about two million by 2004, and was further reduced to 852,550 by 2008. This indicated a dramatic decrease of 28 to 32% in the global population within only one generation (i.e., 20 years).

The olive ridley sea turtles are considered the most abundant, yet globally they have declined by more than 30% from historic levels. These turtles are considered endangered because of their few remaining nesting sites in the world. The eastern Pacific turtles have been found to range from Baja California, Mexico, to Chile. Pacific olive ridleys nest around Costa Rica, Mexico, Nicaragua, and the northern Indian Ocean; the breeding colony in Mexico was listed as endangered in the US on July 28, 1978.

b. Habitat and ecology:

Most observations are typically within 15 km of mainland shores in protected, relatively shallow marine waters (22–55 m deep). Olive ridleys are occasionally found in open waters. The multiple habitats and geographical localities used by this species vary throughout its life cycle. More research is needed to acquire data on and use of pelagic habitats.

Known predators of olive ridley eggs include raccoons, coyotes, feral dogs and pigs, opossums, coatimundi, caimans, ghost crabs, and the sunbeam snake. Hatchlings are preyed upon as they travel across the beach to the water by vultures, frigate birds, crabs, raccoons, coyotes, iguanas, and snakes. In the water, hatchling predators most likely include oceanic fishes, sharks, and crocodiles. Adults have relatively few known predators, other than sharks, and killer whales are responsible for occasional attacks. On land, nesting females may be attacked by jaguars. It is notable that the jaguar is the only cat with a strong enough bite to penetrate a sea turtle's shell, thought to be an evolutionary adaption from the Holocene extinction event. In observations of jaguar attacks, it was noted that the cats consumed the neck muscles of the turtle and occasionally the flippers, but left the remainder of the turtle carcass for scavengers as most likely, despite the strength of its jaws, a jaguar still cannot easily penetrate an adult turtle's shell to reach the internal organs or other muscles. In recent years, increased predation on turtles by jaguars has been noted, perhaps due to habitat loss and fewer

alternative food sources. Sea turtles are comparatively defenseless in this situation as they cannot pull their head into their shell like freshwater and terrestrial turtles. Females are often plagued by mosquitos during nesting. Humans are still listed as the leading threat to *L. olivacea*, responsible for unsustainable egg collection, slaughtering nesting females on the beach, and direct harvesting adults at sea for commercial sale of both the meat and hides.

Other major threats include mortality associated with boat collisions, and incidental takes in fisheries. Trawling, gill nets, ghost nests, longline fishing, and pot fishing, have significantly affected olive ridley populations, as well as other species of marine turtles. Between 1993 and 2003, more than 100,000 olive ridley turtles were reported dead in Odisha, India from fishery-related practices. In addition, entanglement and ingestion of marine debris is listed as a major threat for this species. Coastal development, natural disasters, climate change, and other sources of beach erosion have also been cited as potential threats to nesting grounds. Additionally, coastal development also threatens newly hatched turtles through the effects of light pollution. Hatchlings which use light cues to orient themselves to the sea are now misled into moving towards land, and die from dehydration or exhaustion, or are killed on roads.

However, the greatest single cause of olive ridley egg loss results from *arribadas*, in which the density of nesting females is so high, previously laid nests are inadvertently dug up and destroyed by other nesting females. In some cases, nests become cross-contaminated by bacteria or pathogens of rotting nests. For example, in Playa Nancite, Costa Rica, only 0.2% of the 11.5 million eggs produced in a single *arribada* successfully hatched. Although some of this loss resulted from predation and high tides, the majority was attributed to conspecifics unintentionally destroying existing nests. The extent to which *arribadas*contribute to the population status of olive ridleys has created debate among scientists. Many believe the massive reproductive output of these nesting events is critical to maintaining populations, while others maintain the traditional *arribada* beaches fall far short of their reproductive potential and are most likely not sustaining population levels.[6] In some areas, this debate eventually led to legalizing egg collection.

Threats:

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Conservation status:

The olive ridley is classified as vulnerable according to the International Union for Conservation of Nature and Natural Resources (IUCN), and is listed in Appendix I of CITES. These listings were largely responsible for halting the large-scale commercial exploitation and trade of olive ridley skins.The Convention on Migratory Species and the Inter-American Convention for the Protection and Conservation of Sea Turtles have also provided olive ridleys with protection, leading to increased conservation and management for this marine turtle. National listings for this species range from endangered to threatened, yet enforcing these sanctions on a global scale has been unsuccessful for the most part. Conservation successes for the olive ridley have relied on well-coordinated national programs in combination with local communities and nongovernment organizations, which focused primarily on public outreach and education. *Arribada* management has also played a critical role in conserving olive ridleys. Lastly, enforcing the use of turtle excluder devices in the shrimp-trawling industry has also proved effective in some areas. Globally, the olive ridley continues to receive less conservation attention than its close relative, the Kemp's ridley (*L. kempii*). Also, many schools arrange trips for students to carry out the conservation project, especially in India.Several projects worldwide seek to preserve the olive ridley sea turtle population. For example, in Nuevo Vallarta, Mexico, when the turtles come to the beach to lay their eggs, some of them are relocated to a hatchery, where they have a much better chance to survive. If the eggs were left on the beach, they would face many threats such as getting washed away with the tide or getting poached. Once the eggs hatch, the baby turtles are carried to the beach and released.Another major project, in India involved in preserving the olive ridley sea turtle population was carried out in Chennai, where the Chennai

wildlife team collected close to 10,000 eggs along the Marina coast, of which 8,834 hatchlings were successfully released into the sea in a phased manner.

Probable Questions:

1. What is the taxonomic position of Royal Bengal tiger?
2. Describe the habitat of Royal Bengal Tiger.
3. What types of threats are faced by Royal Bengal Tiger ?
4. State the conservation measures taken for Royal Bengal Tiger ?
5. What is the taxonomic position of Rhinoceros ?
6. Describe the habitat of Rhinoceros
7. What types of threats are faced by Rhinoceros ?
8. State the conservation measures taken for Rhinoceros.
9. What is the taxonomic position of olive ridley sea turtle ?
10. Describe the habitat of olive ridley sea turtle .
11. What types of threats are faced by olive ridley sea turtle.
12. State the conservation measures taken for olive ridley sea turtle.

Suggested Readings:

1.https://nt.gov.au/environment/animals/classification-of-wildlife
2. Biodiversity by Maity and Maity
3. Ecology and Environmental science by Rana

Unit- IV

National and International efforts for conservation

Objectives: In this Unit you will learn about National and International efforts for conservation: CITES, CBD, IUCN, Protected areaconcept.

Introduction:

India is one of 12 mega-diversity countries of the world. The innumerable life forms harboured by the forests, deserts, mountains, other land, air and oceans provide food, fodder, fuel, medicine, textiles, etc.There are innumerable species, the potential of which is not as yet known. It would therefore be prudent to not only conserve the species we already have information about, but also species we have not get identified and described from economic point of view.

Taxus baccata, a tree found in the sub-Himalayan regions, once believed to be of no value is now considered to be effective in the treatment of certain types of cancer. The diversity of genes, species and ecosystem is a valuable resource that can be tapped as human needs and demands change, the still more basic reasons for conservation are the moral, cultural and religious values.

Major problems with biodiversity conservation in India are:

(i) Low priority for conservation of living natural resources.

(ii) Exploitation of living natural resources for monetary gain.

(iii) Values and knowledge about the species and ecosystem inadequately known,

(iv) Unplanned urbanisation and uncontrolled industrialisation.

Biodiversity conservation in India is also impeded by a lack of knowledge of the magnitude, patterns, causes and rates of deforestation and biodiversity loss at the ecosystem and landscape level.

Poaching and trade in wildlife species are among the most important concerns in the management of protected areas today but information on poaching, trade and trade routes is sketchy and current wildlife protection and law enforcement measures are inadequate and inefficient.

Existing Policy of Biodiversity Conservation:

a. A protected area network of National Parks and Wildlife Sanctuaries have been created.

b. The Indian Council of Forestry Research and Education (ICFRE) has identified forest preservation plots of representative forest types for conservation of viable and representative areas of biodiversity.

c. A programme entitled ‘eco-development’ for in situ conservation of biological diversity involving local communities has been initiated.

d. To conserve the respective ecosystems, a Biosphere Reserve Programme is being implemented.

e. Programmes have also been launched for scientific management and wise use of fragile ecosystem,

f. Specific programmes for management and conservation of wetlands, mangroves and coral reef systems are also being implemented.

g. Six internationally significant wetlands of India have been declared as 'Ramsar Sites' under the Ramsar Convention.

h. Wildlife Protection Act is in the final stage of revision and provisions have been made for conservation reserves and community reserves to allow restrictive use to make it more people oriented. There will also be State Biodiversity Boards to control access to domestic consumers.

i. Under the World Heritage Convention, five natural sites in India, have been declared as World Heritage Sites.

j. Project Tiger and Project Elephant have been launched to protect the wildlife. Rhinos have been given special attention in selected sanctuaries and national parks.

k. The Ministry of Environment and Forests (MOEF) constituted the National Afforestation and Eco-development Board (NAEB) in August 1992, which has evolved specific schemes for promoting afforestation and management strategies and eco-development packages for promoting biomass production through a participatory planning process of Joint Forest Management (JFM) and micro-planning.

International Efforts for Conserving Biodiversity:

It is apparent that action plans and strategies, when designed appropriately and implemented, can make important contributions to conservation.

A collaborate effort of the World Resources Institute, IUCN (International Union for Conservation of Nature and Natural Resources), and UNEP (United Nations Environment Programme), working with other institutions, is leading to the preparation of a Global Strategy for the Conservation of Biodiversity, as a companion to the new version of the World Conservation Strategy now being prepared.

The aim of the Strategy is to provide a comprehensive framework to stimulate urgent, positive, innovative and coordinated action to stem the loss and degradation of the world's biological resources and enhance the contribution of these resources to human well-being.The strategy will be developed by and for national governments, NGOs (Non-government organisations), resource managers, scientists, international institutions, multilateral banks, and bilateral aid agencies.

The development of the Global Strategy will be centered around a series of regional workshops in Asia, Africa, Europe, Latin America and North America. The Global strategy will include considerations of a variety of factors influencing biological resource conservation, such as international financing, international cooperation, research, education, training, public awareness, and ecological restoration.The Earth Summit held in 1992 at Rio de Janeiro, Brazil resulted into a Convention on Biodiversity, which came into force on December, 29, 1993.

The Convention has three main objectives:
(i) Conservation of biological diversity

(ii) Sustainable use of biodiversity, and

(iii) Fair and equitable sharing of benefits that arise by the utilization of genetic resources.

The World Conservation Strategy (WTS) and the World Wide Fund for Nature (WWF) support projects worldwide to promote conservation of biological diversity.

A. Convention on International Trade in Endangered Species(CITES) :

CITES is the Convention on International Trade in Endangered Species of Wild Fauna and Flora. CITES was signed on 3rd March 1973 and entered into force on 1st July 1975. This has been in operation for almost 40 years.

a. Purpose of CITES:

The purpose of CITES is to ensure that wild fauna and flora in international trade are not exploited unsustainably.

- CITES is an international convention that combines wildlife and trade themes with a legally binding instrument for achieving conservation and sustainable use objectives
- The Convention establishes an international legal framework together with common procedural mechanisms for the strictest control of international commercial trade in species threatened with extinction, and for an effective regulation of international trade in others
- This framework and common procedural mechanism are now used by 180countries to regulate and monitor international trade in listed species

b. Basic Provisions of the Convention:

The text of the Convention outlines the basic provisions for trade and obligations of each Party such as:

- Trade procedures and requirements
- Enforcement measures
- Trade facilitation
- Exemptions and special procedures
- Marking
- Confiscations
- Reporting
- Trade with non-Parties
- Amendment of the Appendices

c. Resolutions and Decisions taken in the Conference of the Parties:

- The Conference of the Parties adopts Resolutions to guide the interpretation and implementation of the Convention, and Decisions to provide specific short-term time-bound instructions.
- Till date 89 Resolutions and 196 Decisions are in effect.

d. CITES Appendices:
Species subject to CITES regulation are divided amongst three Appendices: I, II & III.

1. Appendix I -

- ❖ Species threatened with extinction, which are or may be affected by trade.
- ❖ International (commercial) trade in wild-taken specimens is generally prohibited.
- ❖ 625 animal species and 301 plant species.

2. Appendix II -

- ❖ Species not necessarily threatened with extinction, but for which trade must be controlled to avoid their becoming so, and species that resemble species already included in Appendix II.
- ❖ International trade is permitted but regulated.
- ❖ 4685 animal species and 29105 plant species (97% of all listings).

3. Appendix III -

- ❖ Species for which a country is asking Parties to help with its protection.
- ❖ International trade is permitted but regulated (less restrictive than Appendix II).
- ❖ 147 animal species and 119 plant species.

e. Species in trade:

- ➢ Not all listed species appear in trade.
  - ❖ Of the 34,782 listed species, some 3,680 animal species and 9,577 plant species have appeared in trade during 2004-2008.
  - ❖ Of these, 11,076 species appeared in 100 or fewer shipments worldwide during this period.
  - ❖ Overall, 157 animal species and 1,878 plant species accounted for 90% of CITES transactions during this period

The Appendices require careful interpretation.

- Species listings can be annotated to specify:
  - ❖ The inclusion or exclusion of designated geographically separate populations, subspecies, species, groups of species, or higher taxa, which may include export quotas.
  - ❖ The types of specimens or export quotas.

f. CITES Permits and Certificates:

- CITES regulates the export, re-export, import and introduction from the sea of live and dead animals and plants andtheir parts and derivatives (listed species only) through a system of permits and certificates
- These permits or certificates may only be issued if certain conditions are met and which must be presented when leaving or entering a country
- For Appendix I and II species, the most important conditions are legal acquisition and that international trade must not be detrimental to their survival in the wild

g. Collaboration and cooperation:

Collaboration and cooperation at the national level are essential for CITES implementation

- CITES Authorities
- Resource sectors
- Customs

- Police
- Judiciary
- Affected stakeholders, including the private sector

h. Trade with non-Parties:

- Where export or re-export is to, or import is from, a non-Party, comparable documentation issued by the competent authorities which substantially conforms with CITES requirements for permits and certificates may be accepted.
- Parties accept documentation from States not party to the Convention only if the details of the competent authorities and scientific institutions of such States are included in the online CITES Directory.
- This also applies to specimens in transit destined for or coming from non-Parties.

Summary:

- CITES regulates international trade in wild fauna and flora listed in its Appendices on the basis of a system of permits and certificates which are issued when certain conditions are met, and which must be presented when leaving and entering a country.
- For Appendix-II and –III species, international trade is permitted but regulated, and for Appendix-I species, international (commercial) trade in wild-taken specimens is generally prohibited.
- The Conference of the Parties is the ultimate decision-making body in CITES.

B. CONVENTION ON BIOLOGICAL DIVERSITY (CBD):

Conscious of the intrinsic value of biological diversity and of the ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values of biological diversity and its components, Conscious also of the importance of biological diversity for evolution and for maintaining life sustaining systems of the biosphere, Affirming that the conservation of biological diversity is a common concern of humankind, Reaffirming that States have sovereign rights over their own biological resources, Reaffirming also that States are responsible for conserving their biological diversity and for using their biological resources in a sustainable manner, Concerned that biological diversity is being significantly reduced by certain human activities, Aware of the general lack of information and knowledge regarding biological diversity and of the urgent need to develop scientific, technical and institutional capacities to provide the basic understanding upon which to plan and implement appropriate measures, Noting that it is vital to anticipate, prevent and attack the causes of significant reduction or loss of biological diversity at source, Noting also that where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat, Noting further that the fundamental requirement for the conservation of biological diversity is the in-situ conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings, Noting further that ex-situ measures, preferably in the country of origin, also have an important role to play, Recognizing the close and traditional dependence of many indigenous and local communities embodying traditional lifestyles on biological resources, and the desire- —3 Convention On Biological Diversity 5 JUNE 1992 ability of sharing equitably benefits

arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and the sustainable use of its components, Recognizing also the vital role that women play in the conservation and sustainable use of biological diversity and affirming the need for the full participation of women at all levels of policy-making and implementation for biological diversity conservation, Stressing the importance of, and the need to promote, international, regional and global cooperation among States and intergovernmental organizations and the nongovernmental sector for the conservation of biological diversity and the sustainable use of its components, Acknowledging that the provision of new and additional financial resources and appropriate access to relevant technologies can be expected to make a substantial difference in the world's ability to address the loss of biological diversity, Acknowledging further that special provision is required to meet the needs of developing countries, including the provision of new and additional financial resources and appropriate access to relevant technologies, Noting in this regard the special conditions of the least developed countries and small island States, Acknowledging that substantial investments are required to conserve biological diversity and that there is the expectation of a broad range of environmental, economic and social benefits from those investments, Recognizing that economic and social development and poverty eradication are the first and overriding priorities of developing countries, Aware that conservation and sustainable use of biological diversity is of critical importance for meeting the food, health and other needs of the growing world population, for which purpose access to and sharing of both genetic resources and technologies are essential, Noting that, ultimately, the conservation and sustainable use of biological diversity will strengthen friendly relations among States and contribute to peace for humankind, Desiring to enhance and complement existing international arrangements for the conservation of biological diversity and sustainable use of its components, and Determined to conserve and sustainably use biological diversity for the benefit of present and future generations.

The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.

Sustainable Use of Components of Biological Diversity:

(a) Integrate consideration of the conservation and sustainable use of biological resources into national decision-making;

(b) Adopt measures relating to the use of biological resources to avoid or minimize adverse impacts on biological diversity;

(c) Protect and encourage customary use of biological resources in accordance with traditional cultural practices that are compatible with conservation or sustainable use requirements;

(d) Support local populations to develop and implement remedial action in degraded areas where biological diversity has been reduced; and

(e) Encourage cooperation between its governmental authorities and its private sector in developing methods for sustainable use of biological resources.

Access to Genetic Resources :

1. Recognizing the sovereign rights of States over their natural resources, the authority to determine access to genetic resources rests with the national governments and is subject to national legislation.

2. Each Contracting Party shall endeavor to create conditions to facilitate access to genetic resources for environmentally sound uses by other Contracting Parties and not to impose restrictions that run counter to the objectives of this Convention.

3. For the purpose of this Convention, the genetic resources being provided by a Contracting Party, as referred to in this Article and Articles 16 and 19, are only those that are provided by Contracting Parties that are countries of origin of such resources or by the Parties that have acquired the genetic resources in accordance with this Convention.

4. Access, where granted, shall be on mutually agreed terms and subject to the provisions of this Article.

5. Access to genetic resources shall be subject to prior informed consent of the Contracting Party providing such resources, unless otherwise determined by that Party.

6. Each Contracting Party shall Endeavour to develop and carry out scientific research based on genetic resources provided by other Contracting Parties with the full participation of, and where possible in, such Contracting Parties.

7. Each Contracting Party shall take legislative, administrative or policy measures, as appropriate, and in accordance with Articles 16 and 19 and, where necessary, through the financial mechanism established by Articles 20 and 21 with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms.

Entry into Force:

1. This Convention shall enter into force on the ninetieth day after the date of deposit of the thirtieth instrument of ratification, acceptance, approval or accession.

2. Any protocol shall enter into force on the ninetieth day after the date of deposit of the number of instruments of ratification, acceptance, approval or accession, specified in that protocol, has been deposited.

3. For each Contracting Party which ratifies, accepts or approves this Convention or accedes thereto after the deposit of the thirtieth instrument of ratification, acceptance, approval or accession, it shall enter into force on the ninetieth day after the date of deposit by such Contracting Party of its instrument of ratification, acceptance, approval or accession.

4. Any protocol, except as otherwise provided in such protocol, shall enter into force for a Contracting Party that ratifies, accepts or approves that protocol or accedes thereto after its entry into force pursuant to paragraph 2 above, on the ninetieth day after the date on which that Contracting Party deposits its instrument of ratification, acceptance, approval or accession, or on the date on which this Convention enters into force for that Contracting Party, whichever shall be the later.

5. For the purposes of paragraphs 1 and 2 above, any instrument deposited by a regional economic integration organization shall not be counted as additional to those deposited by member States of such organization.

CARTAGENA PROTOCOL ON BIOSAFETY TO THE CONVENTION ON BIOLOGICAL DIVERSITY:

In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

For the purposes of this Protocol:

(a) “Conference of the Parties” means the Conference of the Parties to the Convention;

(b) “Contained use” means any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment; (c) “Export” means intentional transboundary movement from one Party to another Party;

(d) “Exporter” means any legal or natural person, under the jurisdiction of the Party of export, who arranges for a living modified organism to be exported;

(e) “Import” means intentional transboundary movement into one Party from another Party;

(f) “Importer” means any legal or natural person, under the jurisdiction of the Party of import, who arranges for a living modified organism to be imported;

(g) “Living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

(h) “Living organism” means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;

(i) “Modern biotechnology” means the application of:

(a) In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or

(b) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;

(j) “Regional economic integration organization” means an organization constituted by sovereign States of a given region, to which its member States have transferred competence in respect of matters governed by this Protocol and which has been duly authorized, in accordance with its internal procedures, to sign, ratify, accept, approve or accede to it;

(k) “Transboundary movement” means the movement of a living modified organism from one Party to another Party, save that for the purposes of Articles 17 and 24 transboundary movement extends to movement between Parties and non-Parties.

This Protocol shall apply to the transboundary movement, transit, handling and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

Public Awareness and Participation:

1. The Parties shall: (a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.

2. The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21. 3. Each Party shall endeavor to inform its public about the means of public access to the Biosafety Clearing-House.

Socio-Economic Considerations :

1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.

2. The Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of living modified organisms, especially on indigenous and local communities.

Liability and Redress :

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first meeting, adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of living modified organisms, analyzing and taking due account of the ongoing processes in international law on these matters, and shall endeavor to complete this process within four years.

Financial Mechanism and Resources:

1. In considering financial resources for the implementation of this Protocol, the Parties shall take into account the provisions of Article 20 of the Convention.
2. The financial mechanism established in Article 21 of the Convention shall, through the institutional structure entrusted with its operation, be the financial mechanism for this Protocol.
3. Regarding the capacity-building referred to in Article 22 of this Protocol, the Conference of the Parties serving as the meeting of the Parties to this Protocol, in providing guidance with respect to the financial mechanism referred to in paragraph 2 above, for consideration by the

Conference of the Parties, shall take into account the need for financial resources by developing country Parties, in particular the least developed and the small island developing States among them.

4. In the context of paragraph 1 above, the Parties shall also take into account the needs of the developing country Parties, in particular the least developed and the small island developing States among them, and of the Parties with economies in transition, in their efforts to identify and implement their capacity-building requirements for the purposes of the implementation of this Protocol.
5. The guidance to the financial mechanism of the Convention in relevant decisions of the Conference of the Parties, including those agreed before the adoption of this Protocol, shall apply, mutatis mutandis, to the provisions of this Article.
6. The developed country Parties may also provide, and the developing country Parties and the Parties with economies in transition avail themselves of, financial and technological resources for the implementation of the provisions of this Protocol through bilateral, regional and multilateral channels.

Monitoring and Reporting:

Each Party shall monitor the implementation of its obligations under this Protocol, and shall, at intervals to be determined by the Conference of the Parties serving as the meeting of the Parties to this Protocol, report to the Conference of the Parties serving as the meeting of the Parties to this Protocol on measures that it has taken to implement the Protocol.

Compliance:

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first meeting, consider and approve cooperative procedures and institutional mechanisms to promote compliance with the provisions of this Protocol and to address cases of non-compliance. These procedures and mechanisms shall include provisions to offer advice or assistance, where appropriate. They shall be separate from, and without prejudice to, the dispute settlement procedures and mechanisms established by Article 27 of the Convention.

Assessment and Review:

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall undertake, five years after the entry into force of this Protocol and at least every five years thereafter, an evaluation of the effectiveness of the Protocol, including an assessment of its procedures and annexes.

Entry Into Force:

1. This Protocol shall enter into force on the ninetieth day after the date of deposit of the fiftieth instrument of ratification, acceptance, approval or accession by States or regional economic integration organizations that are Parties to the Convention.

2. This Protocol shall enter into force for a State or regional economic integration organization that ratifies, accepts or approves this Protocol or accedes thereto after its entry into force pursuant to paragraph 1 above, on the ninetieth day after the date on which that State or regional economic integration organization deposits its instrument of ratification, acceptance, approval or accession, or on the date on which the Convention enters into force for that State or regional economic integration organization, whichever shall be the later.

3. For the purposes of paragraphs 1 and 2 above, any instrument deposited by a regional economic integration organization shall not be counted as additional to those deposited by member States of such organization.

C. INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES (IUCN)

It is a membership Union uniquely composed of both government and civil society organizations in the field of nature conservation and sustainable use of natural resources. IUCN is best known for compiling and publishing red list: access the conservation status of species worldwide. Created in 1948, IUCN has evolved into the world's largest and most diverse environmental network.

Mission: Influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable.

Milestones:

- 1947-International Conference on Protection of Nature was held in Brunnen.
- 1948-IUPN (International Conference on Protection of Nature)was formed.
- 1949-IUPN and UNESCO jointly organized conference on protection of nature.
- 1956-IUPN renamed as IUCN (INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES).
- 1961-IUCN took part in setting up World Wild Life Fund (WWF).
- 1964-Red data book on the conservation status of species was first published.
- 1990-Began using World Conservation Union as official name.
- 2008-The name was revert back to IUCN.
- 2012-IUCN published the list of world's 100 most threatened species.

Present status:
Since 1948, IUCN has evolved into the world's largest and most diverse environmental network. It harnesses the experience, resources and reach of its 1,300 Member organizations and the input of some 16151 experts. IUCN is the global authority on the status of the natural world and the measures needed to safeguard it. IUCN experts are organized into six commissions dedicated to species survival, environmental law, protected areas, social and economic policy, ecosystem management, and education and communication. IUCN headquarter is now situated in Gland, Switzerland. It includes 161 countries, 217 states and Govt. agencies. There are also 1066 NGOs currently works with IUCN.

IUCN-Management and Governing body:
The IUCN Council is the principal governing body of IUCN, International Union for Conservation of Nature, in between sessions of the World Conservation Congress - the general assembly of the Union's members.Thisorganization is managed by IUCN global secretariat led by Director general Inger Andersen.
The Council is composed of:

- The President;
- Four Vice Presidents (elected by Council from among its members)
- The Treasurer;
- The Chairs of IUCN's six Commissions;
- Twenty-eight Regional Councilors;

- A Councilor from the State in which IUCN has its seat (Switzerland);
- One additional appointed Councilor.

The president of 2012-16 council is ZHANG Xinsheng (China).

IUCN council 202-16 priorities:

- Valuing and conserving nature.
- Deploying nature based solutions to climate, food and development.
- Effective and equitable governance of nature's use.

IUCN themes:

IUCN work across various themes related to conservation, environmental and ecological issues like-

- Business and biodiversity
- Climate change
- Economics
- Ecosystem management
- Environmental law
- Forests
- gender
- global policy
- marine and polar protected areas
- science and knowledge
- social policy
- species
- water
- world heritage

Regions of action:

- Asia
- Central and West Africa
- Eastern and Southern Africa
- Eastern Europe and Central Asia
- Europe
- Mediterranean
- Mexico, Central America and the Caribbean
- Oceania
- South America
- Washington D.C. Office
- West Asia

IUCN India:

India, a mega diverse country with only 2.4% of the world's land area, accounts for 7-8% of all recorded species, including over 45,000 species of plants and 91,000 species of animals. The country's diverse physical features and climatic conditions have resulted in a variety of ecosystems such as forests, wetlands, grasslands, desert, coastal and marine ecosystems which harbour and sustain high biodiversity and contribute to human well-being. Four of 34 globally identified biodiversity hotspots: The Himalayas, the Western Ghats, the North-East, and the Nicobar Islands, can be found in India.

India became a State Member of IUCN in 1969, through the Ministry of Environment, Forest and Climate Change (MoEFCC).

The IUCN India Country Office was established in 2007 in New Delhi.

IUCN Red list:

First published in 1964,The IUCN Global Species Programme working with the IUCN Species Survival Commission (SSC) has been assessing the conservation status of species, subspecies, varieties, and even selected subpopulations on a global scale for the past 50 years in order to highlight

taxa threatened with extinction, and thereby promote their conservation. This system is designed to determine the relative risk of extinction, and the main purpose of the IUCN Red List is to catalogue and highlight those plants, fungi and animals that are facing a higher risk of global extinction.

Red list categories and criteria:

- **Extinct-**
  A taxon is extinct when there is no reasonable doubt that the last individual has died.

- **Extinct in the Wild**
  A taxon is extinct in the wild when it is known only to survive incultivation, In captivity or as a naturalized population well outside the past range

- **Near Threatened(NT)**
  A taxon is NT when it has been evaluated against the criteria but does not qualify for CR, EN or VU now, but is close to qualifying for or is likely to qualify for a threatened category in the near future

- **Least Concern(LC)**
  A taxon is LC when it has been evaluated against the criteria and does not qualify for CR, EN, VU, or NT. Widespread and abundant taxa are included in this category

- **Data Deficient(DD)**
  A taxon is DD when there is inadequate information to make direct or indirect assessment of its risk of extinction based on its distribution and /or population status

- **Not Evaluated(NE)**
  A taxon is not evaluated when it has not yet been evaluated against the criteria

D. PROTECTED AREA CONCEPT:

India is one of the 17 mega diverse countries of the world. With only 2.4% of the world's land area, 16.7% of the world's human population and 18% livestock, it contributes about 8% of the known global biodiversity, however, putting enormous demands on our natural resources. India is home to world's largest wild tigers population and has got unique assemblage of globally important endangered species like Asiatic lion, Asian Elephant, One-horned Rhinoceros, Gangetic River Dolphin,Snow Leopard, Kashmir Stag, Dugong, Gharial, Great Indian Bustard, Lion Tailed Macaque etc.

Protected Area Network in India:

A National Board for Wildlife (NBWL), chaired by the Prime Minister of India provides for policy framework for wildlife conservation in the country. The National Wildlife Action Plan (2002-2016) was adopted in 2002, emphasizing the people's participation and their support for wildlife conservation. India's conservation planning is based on the philosophy of identifying and protecting representative wild habitats across all the ecosystems. The Indian Constitution entails the subject of forests and wildlife in the Concurrent list. The Federal Ministry acts as a guiding torch dealing with the policies and planning on wildlife conservation, while the provincial Forest Departments are vested with the responsibility of implementation of national policies and plans.

A network of 668 Protected Areas (PAs) has been established, extending over 1, 61,221.57 sq. kms. (4.90% of total geographic area), comprising 102 National Parks, 515 Wildlife Sanctuaries, 47 Conservation Reserves and 4 Community Reserves. 39 Tiger Reserves and 28 Elephant Reserves have been designated for species specific management of tiger and elephant habitats. UNESCO has designated 5 Protected Areas as World Heritage Sites.

As the ecosystems and species do not recognize political borders, the concept of Transboundary Protected Areas has been initiated for coordinated conservation of ecological units and corridors with bilateral and/or multilateral cooperation of the neighboring nations. There are 4 categories of the Protected Areas viz, National Parks, Sanctuaries, Conservation Reserves and Community Reserves.

1. Sanctuary:

It is an area which is of adequate ecological, faunal, floral, geomorphological, natural or zoological significance. The Sanctuary is declared for the purpose of protecting, propagating or developing wildlife or its environment. Certain rights of people living inside the Sanctuary could be permitted. Further, during the settlement of claims, before finally notifying the Sanctuary, the Collector may, in consultation with the Chief Wildlife Warden, allow the continuation of any right of any person in or over any land within the limits of the Sanctuary.

2. National Park:

It is an area having adequate ecological, faunal, floral, geomorphological, natural or zoological significance. The National Park is declared for the purpose of protecting, propagating or developing wildlife or its environment, like that of a Sanctuary. The difference between a Sanctuary and a National Park mainly lies in the vesting of rights of people living inside. Unlike a Sanctuary, where certain rights can be allowed, in a National Park, no rights are allowed. No grazing of any livestock shall also be permitted inside a National Park while in a Sanctuary, the Chief Wildlife Warden may regulate, control or prohibit it. In addition, while any removal or exploitation of wildlife or forest produce from a Sanctuary requires the recommendation of the State Board for Wildlife, removal etc., from a National Park requires recommendation of the National Board for Wildlife (However, as per orders of Hon'ble Supreme Court dated 9th May 2002 in Writ Petition (Civil) No. 337 of 1995, such removal/ exploitation from a Sanctuary also requires recommendation of the Standing Committee of National Board for Wildlife).

3. Conservation Reserves:

This can be declared by the State Governments in any area owned by the Government, particularly the areas adjacent to National Parks and Sanctuaries and those areas which link one Protected Area with another. Such declaration should be made after having consultations with the local communities. Conservation Reserves are declared for the purpose of protecting landscapes, seascapes, flora and fauna and their habitat. The rights of people living inside a Conservation Reserve are not affected.

4. Community Reserves:

It can be declared by the State Government in any private or community land, not comprised within a National Park, Sanctuary or a Conservation Reserve, where an individual or a community has volunteered to conserve wildlife and its habitat. Community Reserves are declared for the purpose of protecting fauna, flora and traditional or cultural conservation values and practices. As in the case of a Conservation Reserve, the rights of people living inside a Community Reserve are not affected.

Regulations/ laws relating to Protected Areas (PAs):

The PAs are constituted and governed under the provisions of the Wild Life (Protection) Act, 1972, which has been amended from time to time, with the changing ground realities concerning wildlife crime control and PAs management. Implementation of this Act is further complemented by other

Acts viz. Indian Forest Act, 1927, Forest (Conservation) Act, 1980, Environment (Protection) Act, 1986 and Biological Diversity Act, 2002 and the Scheduled Tribes and Other Traditional Forest Dwellers (Recognition of Forest Rights) Act, 2006. The Wildlife Crime Control Bureau of the Central Government supplements the efforts of provincial governments in wildlife crime control through enforcement of CITES and control of wildlife crimes having cross-border, interstate and international ramifications. In order to strengthen and synergize global wildlife conservation efforts, India is a party to major international conventions viz. Convention on International Trade in Endangered Species of wild fauna and flora (CITES), International Union for Conservation of Nature (IUCN), International Convention for the Regulation of Whaling, UNESCO-World Heritage Committee and Convention on Migratory Species (CMS).

Main issues concerning the management of Protected Areas:
Wildlife conservation and management in India is currently facing a myriad of complex challenges that are both ecological and social in nature. Issues such as habitat loss/fragmentation, overuse of biomass resources in the context of biotic pressures, increasing human-wildlife conflicts, livelihood dependence on forests and wildlife resources, poaching and illegal trade in wildlife parts and products, need for maintaining a broad base of public support for wildlife conservation exemplify and characterize the contemporary wildlife conservation scenario in India. The government and the civil society are taking several measures to address these issues.

Probable Questions:
1. What are the major problems in conservation of Biodiversity in India?

2. State the existing policy of Biodiversity conservation.

3. Describe the international efforts for conserving biodiversity ?

4. Write down the purpose of CITES.

5. Write a short note on CBD.

6. What is IUCN Red List?

7. Write about protected area network in India.

8. Describe the regulations/ laws relating to Protected Areas (PAs).

Suggested Readings:
1.https://nt.gov.au/environment/animals/classification-of-wildlife
2. Biodiversity by Maity and Maity
3. Ecology and Environmental science by Rana
4.http://www.envfor.nic.in/downloads/public-information/protected-area-network.pdf

Ecological Principles and Application

Unit- V

Concept of Habitat and Niche

Objective: In this unit you will learn about concept of habitat and niche.

Introduction:

Habitat refers to a specific place where a species normally lives. For example, habitat of a tiger is the forest, of a shark is the sea, and of Plasmodium are the red blood cells. More than one animal or plant may live in the same habitat. For example, tiger, deer, wolf, fox, lion, etc. may be found in the same forest.Animals exhibit habitat specificity and require specific environmental conditions to live. For example, a fish lives in an aquatic habitat, but a river fish can live only in freshwater, while a sea fish can live only in a marine habitat. Some organisms are more tolerant than the other.

A habitat can be subdivided into regions with different environmental conditions. These subdivisions are called microhabitat. For example, in a pond, some organisms are surface dwellers while some others are bottom dwellers.For a species to maintain its population, its individuals must survive and reproduce. Certain combinations of environmental conditions are necessary for individuals of each species to tolerate the physical environment, obtain energy and nutrients and avoid predators.

The total requirements of a species, i.e. resources and physical conditions determine where it can live and how abundant it can be at any one place within its range. These requirements are termed abstractly as the ecological niche. In other words, niche is a term used to indicate not only the habitat but also the role played by the organisms in the environment.G.E. Hutchinson (1958) suggested that the niche could be modelled as an imaginary space with many dimensions, in which each dimension or axis represents the range of specific environmental condition or resource that is required by the species. Thus, the niche of a plant might include the range of temperatures that it can tolerate, the intensity of light required for photosynthesis, specific humidity regimes and minimum quantities of essential soil nutrients for uptake.

A useful extension of the niche concept is the distinction between fundamental and realised niches (Fig. 2). The fundamental niche of a species includes the total range of environmental conditions that are suitable for existence without the influence of interspecific competition or predation from other

species. The realised niche describes that part of the fundamental niche was actually occupied by the species.

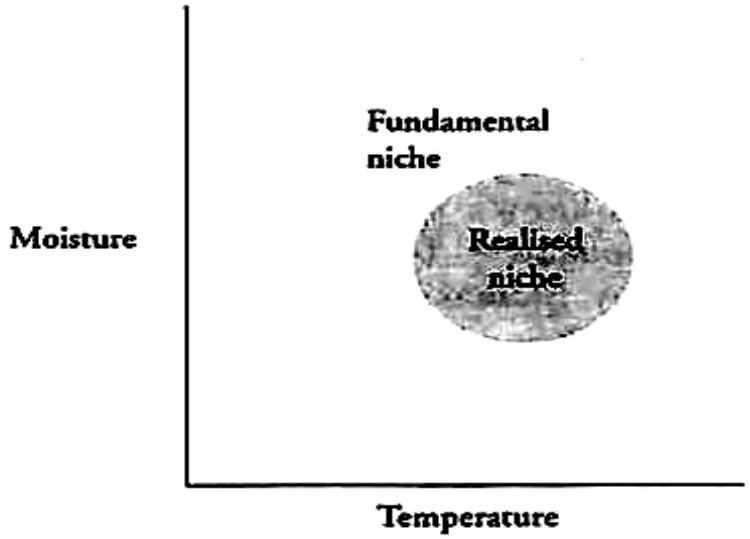


Fig. 2 A hypothetical situation where species distribution is controlled by just two environmental variables: temperature and moisture. The dotted area depicts the fundamental niche that indicates the combination of temperature and moisture condition required by the species for survival and reproduction in its habitat. The non-dotted area depicts the realised niche that is actually utilised by the organism for its survival.

A habitat possesses many niches and supports many species. An organism changes its niches as they develop. For example, the common toad, *Bufobufo* occupies the aquatic environment when it is a tadpole and feeds on algae and detritus. But after it metamorphosis's into an adult it becomes terrestrial and becomes insectivorous.According to Odum, while the habitat is the organism's 'address', its ecological niche is its 'profession'. Two organisms may be found in the same habitat, but do not occupy the same ecological niche. Each plays a different role in its habitat. Different animals that occupy similar ecological niche in different geographical regions are called 'ecological equivalents'.

Examples of Ecological Niches

a. Kirtland's Warbler

Kirtland's warbler is a rare bird that lives in small areas in Michigan's northern Lower and Upper Peninsulas. The niche of Kirtland's warbler is the jack pine forest, and the forest must have very specific conditions. Jack pine forests with areas of over 80 acres are ideal for this species. Specifically, these forests must have dense clumps of trees with small areas of grass, ferns and small shrubs in between. Kirtland's warbler nests on the ground beneath the branches when the tree is about 5 feet tall, or around 5-8 years old. When the tree reaches about 16-20 feet tall, the lower branches start to die, and the bird will no longer nest beneath the tree branches.

Jack pine forests remained virtually undisturbed during Michigan's lumber boom in the early 1800s because white pine was a much more valuable. The consistent availability of young jack pines for nesting was generated by naturally occurring wildfires in this habitat. When the lumber boom ended in the late 1800s, the wildfires continued and allowed the jack pine to spread and create more habitat for Kirtland's warbler. The species population reached its peak from 1885-1900. Humans began to alter this niche by fighting and putting out forest fires. Over time, this severely affected the Kirtland's warbler population. Large areas of jack pine forest were designated for habitat management via logging, burning, seeding and replanting in the 1970s, and the species recovered.

The image above shows a female Kirtland's Warbler, *Dendroica kirtlandii*.

Dung Beetle

As the name implies, dung beetles eat dung, both as adults and as larvae. They live on all continents except Antarctica. Dung is plentiful throughout the world, and over time, the dung beetle has learned to exploit it as a resource, and create its own niche. Dung beetles are known for the way in which they roll dung into a ball before transporting it. These balls area buried in an underground burrow to either be stored as food or used as brooding balls. The female lays eggs in the brooding ball and the larvae hatch inside. When they reach adult size, the beetles dig out of the ball and work their way to the soil surface. The actions of dung beetles serve several important functions in their habitat. Digging burrows and tunnels turns over and aerates the soil. The buried dung releases nutrients into the soil that benefits other organisms. In addition, the beetle's use of dung leaves less available for flies to breed on, thus controlling some of the fly population.

The image above shows *Khepernigroaeneus*, the Large Copper Dung Beetle, on a ball of dung.

Xerophytic Plants

Xerophytic plants have developed several adaptations to living in dry ecological niches. The adaptations evolved to help save water stored in the plant and to prevent water loss. Examples of xerophytes are cacti and aloe vera, also called succulents. These plants have thick fleshy leaves that store water, and long roots to reach water deep underground. Other adaptations that xerophytic plants use include the ability to move or fold up their leaves, dropping their leaves during dry periods, a waxy coating to prevent evaporation (called the cuticle) and thick hairy leaf coverings. The surface of plant leaves features stomata, which are tiny mouth-like structures that take in carbon dioxide and release oxygen and water. Plants usually open their stomata during the day and close them at night. Succulents do the opposite in order to reduce water loss during the heat of the day.

There are mainly three concepts about niches which are described below:

a. Grinnellian niche

The ecological meaning of niche comes from the meaning of niche as a recess in a wall for a statue, which itself is probably derived from the Middle French word *nicher*, meaning *to nest*. The term was coined by the naturalist Roswell Hill Johnson but Joseph Grinnell was probably the first to use it in a research program in 1917, in his paper "The niche relationships of the California Thrasher".

The Grinnellian niche concept embodies the idea that the niche of a species is determined by the habitat in which it lives and its accompanying behavioural adaptations. In other words, the niche is the sum of the habitat requirements and behaviours that allow a species to persist and produce offspring. For example, the behaviour of the California thrasher is consistent with the chaparral habitat it lives in—it breeds and feeds in the underbrush and escapes from its predators by shuffling from underbrush to underbrush. Its 'niche' is defined by the felicitous complementing of the thrasher's behaviour and physical traits (camouflaging color, short wings, strong legs) with this habitat.

This perspective of niche allows for the existence of both ecological equivalents and empty niches. An ecological equivalent to an organism is an organism from a different taxonomic group exhibiting similar adaptations in a similar habitat, an example being the different succulents found in American and African deserts, cactus and euphorbia, respectively. As another example, the anole lizards of the Greater Antilles are a rare example of convergent evolution, adaptive radiation, and the existence of ecological equivalents: the anole lizards evolved in similar microhabitats independently of each other and resulted in the same ecomorphs across all four islands.

b. Eltonian niche:

In 1927 Charles Sutherland Elton, a British ecologist, defined a niche as follows: "The 'niche' of an animal means its place in the biotic environment, *its relations to food and enemies*."

Elton classified niches according to foraging activities ("food habits").

For instance there is the niche that is filled by birds of prey which eat small animals such as shrews and mice. In an oak wood this niche is filled by tawny owls, while in the open grassland it is occupied by kestrels. The existence of this carnivore niche is dependent on the further fact that mice form a definite herbivore niche in many different associations, although the actual species of mice may be quite different.

Conceptually, the Eltonian niche introduces the idea of a species' response to and effect on the environment. Unlike other niche concepts, it emphasizes that a species not only grows in and responds to an environment based on available resources, predators, and climatic conditions, but also changes

the availability and behaviour of those factors as it grows. In an extreme example, beavers require certain resources in order to survive and reproduce, but also construct dams that alter water flow in the river where the beaver lives. Thus, the beaver affects the biotic and abiotic conditions of other species that live in and near the watershed. In a more subtle case, competitors that consume resources at different rates can lead to cycles in resource density that differ between species. Not only do species grow differently with respect to resource density, but their own population growth can affect resource density over time.

c. Hutchinsonian niche

The Hutchinsonian niche is an "n-dimensional hypervolume", where the dimensions are environmental conditions and resources, that define the requirements of an individual or a species to practice "its" way of life, more particularly, for its population to persist. The "hypervolume" defines the multi-dimensional space of resources (e.g., light, nutrients, structure, etc.) available to (and specifically used by) organisms, and "all species other than those under consideration are regarded as part of the coordinate system."

The niche concept was popularized by the zoologist G. Evelyn Hutchinson in 1957. Hutchinson inquired into the question of why there are so many types of organisms in any one habitat. His work inspired many others to develop models to explain how many and how similar coexisting species could be within a given community, and led to the concepts of 'niche breadth' (the variety of resources or habitats used by a given species), 'niche partitioning' (resource differentiation by coexisting species), and 'niche overlap' (overlap of resource use by different species).

Statistics were introduced into the Hutchinson niche by Robert MacArthur and Richard Levins using the 'resource-utilization' niche employing histograms to describe the 'frequency of occurrence' as a function of a Hutchinson coordinate. So, for instance, a Gaussian might describe the frequency with which a species ate prey of a certain size, giving a more detailed niche description than simply specifying some median or average prey size. For such a bell-shaped distribution, the position, width and form of the niche correspond to the mean, standard deviation and the actual distribution itself. One advantage in using statistics is illustrated in the figure, where it is clear that for the narrower distributions (top) there is no competition for prey between the extreme left and extreme right species, while for the broader distribution (bottom), niche overlap indicates competition can occur between all species. The resource-utilization approach consists in postulating that not only competition *can* occur, but also that it *does* occur, and that overlap in resource utilization directly enables the estimation of the competition coefficients. This postulate, however, can be misguided, as it ignores the impacts that the resources of each category have on the organism and the impacts that the organism has on the resources of each category. For instance, the resource in the overlap region can be non-limiting, in which case there is no competition for this resource despite niche overlap.

An organism free of interference from other species could use the full range of conditions (biotic and abiotic) and resources in which it could survive and reproduce which is called its fundamental niche. However, as a result of pressure from, and interactions with, other organisms (i.e. inter-specific competition) species are usually forced to occupy a niche that is narrower than this, and to which they are mostly highly adapted; this is termed the realized niche. Hutchinson used the idea of competition for resources as the primary mechanism driving ecology, but overemphasis upon this focus has proved to be a handicap for the niche concept. In particular, overemphasis upon a species' dependence upon resources has led to too little emphasis upon the effects of organisms on their environment, for instance, colonization and invasions.

The term "adaptive zone" was coined by the palaeontologist George Gaylord Simpson to explain how a population could jump from one niche to another that suited it, jump to an 'adaptive zone', made available by virtue of some modification, or possibly a change in the food chain, that made the

adaptive zone available to it without a discontinuity in its way of life because the group was 'pre-adapted' to the new ecological opportunity.

Hutchinson's "niche" (a description of the ecological space occupied by a species) is subtly different from the "niche" as defined by Grinnell (an ecological role, that may or may not be actually filled by a species—see vacant niches).

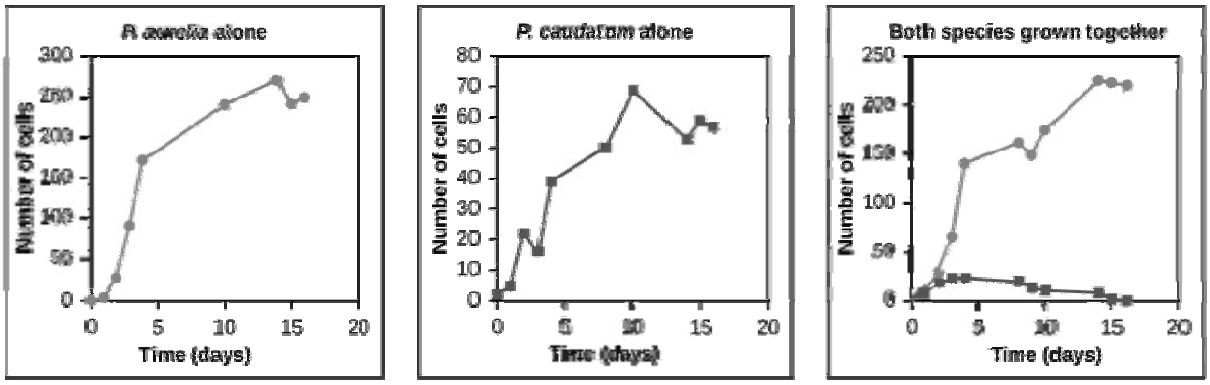
A niche is a very specific segment of ecospace occupied by a single species. On the presumption that no two species are identical in all respects (called Hardin's 'axiom of inequality') and the competitive exclusion principle, *some* resource or adaptive dimension will provide a niche specific to each species. Species can however share a 'mode of life' or 'autecological strategy' which are broader definitions of ecospace. For example, Australian grasslands species, though different from those of the Great Plains grasslands, exhibit similar modes of life.

Once a niche is left vacant, other organisms can fill that position. For example, the niche that was left vacant by the extinction of the tarpan has been filled by other animals (in particular a small horse breed, the konik). Also, when plants and animals are introduced into a new environment, they have the potential to occupy or invade the niche or niches of native organisms, often outcompeting the indigenous species. Introduction of non-indigenous species to non-native habitats by humans often results in biological pollution by the exotic or invasive species.

Competitive exclusion principle

The **competitive exclusion principle** tells us that two species can't have exactly the same niche in a habitat and stably coexist. That's because species with identical niches also have identical needs, which means they would compete for precisely the same resources.

A famous example of the competitive exclusion principle is shown in the figure below, which features two types of single-celled microorganisms, *Paramecium aurelia* and *Paramecium caudatum*. When grown individually in the lab, both species thrive. But when they are grown in the same test tube (habitat) with a fixed amount of nutrients, both grow more poorly and *P. aurelia* eventually outcompetes *P. caudatum* for food, leading to *P. caudatum*'s extinction.



Graphs a, b, and c all plot number of cells versus time in days. In Graph (a), P. aurelia is grown alone. In graph (b), *P. caudatum* is grown alone. In graph (c), both species are grown together. When grown separately, the two species both exhibit logistic growth and grow to a relatively high cell density. When the two species are grown together, *P. aurelia* shows logistic growth to nearly the same cell density as it exhibited when grown alone, but *P. caudatum* hardly grows at all, and eventually its population drops to zero.

In nature, it's rarely the case that two species occupy exactly identical niches. However, the greater the extent to which two species' niches overlap, the stronger the competition between them.

Resource partitioning

Competitive exclusion may be avoided if one or both of the competing species evolves to use a different resource, occupy a different area of the habitat, or feed during a different time of day. The result of this kind of evolution is that two similar species use largely non-overlapping resources and thus have different niches. This is called **resource partitioning**, and it helps the species coexist because there is less direct competition between them.

The anole lizards found on the island of Puerto Rico are a good example of resource partitioning. In this group, natural selection has led to the evolution of different species that make use of different resources. The figure below shows resource partitioning among 111111 species of anole lizards. Each species lives in its own preferred habitat, which is defined by type and height of vegetation (trees, shrubs, cactus, etc.), sunlight, and moisture, among other factors.

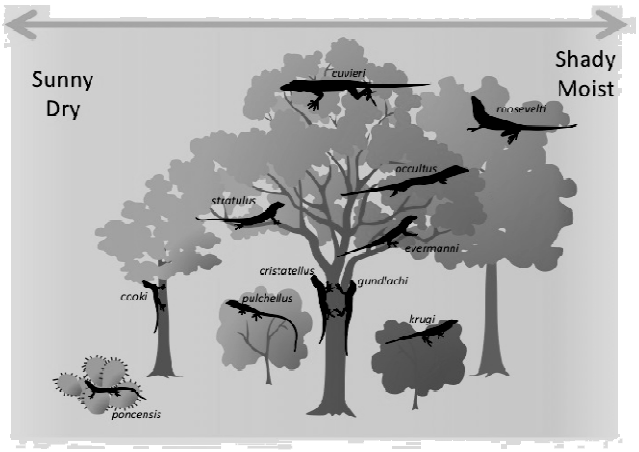


Fig: Resource partitioning

Probable Questions:

1. Define habitat and niche. What is the difference between them?
2. What is fundamental niche and realized niche ? Give examples.
3. Write notes on Grinnellian niche.
4. What is hypervolume niche.
5. Describe Eltonian niche.
6. Describe Hutchinsonian niche.
7. State competitive exclusion principle with suitable examples.
8. Describe resource partitioning with suitable examples.

Suggested readings:

1. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition*. ISBN 978-0-321-73607-9

2. Chaki, K. K., Kundu, G., and Sarker, S. (2012) Introduction to General Zoology. Vol II. ISBN: 81-7381-568-2, New Central Book Agency.

3. Ghosh, A. (2018) Notes on Habitat and Niche. Environment (http://www.yourarticlelibrary.com/ecology/notes-on-habitat-and-niche-environment /90914 2/5)

Unit-VI
Ecological Principles

Objective: In this unit you will learn about ecological principles.

Introduction:

Ecological principles are basic assumptions (or beliefs) about ecosystems and how they function.Ecological principles are built on ecological concepts to draw key conclusions that can then guide human applications aimed at conserving biodiversity. Ecological concepts include levels of biological organization(genes, populations, species, communities, ecosystems, landscapes, and regions), native species, keystonespecies, ecosystem or process, population viability/ thresholds, ecological resilience, disturbances, connectivity/ fragmentation and ecosystem management approaches.

Principles:

Principle 1. Protection of species and species' subdivisions will conserve genetic diversity

At the population level, the important processes are ultimately genetic and evolutionary because these maintain the potential for continued existence of species and their adaptation to changing conditions. The most credible surrogate for sustaining genetic variability is maintaining not only species but also the spatial structure of genetic variation within species such as sub-species and populations. This will permit the adaptability needed to sustain ecosystem productivity in changing environments and can also beget further diversity (future adaptability). Maintenance of populations distributed across a species' natural range will assist in conserving and continuation of locally adapted genetic variants. Species that are collapsing towards the edge (versus centre) of their range and disjunct populations (where a local population is disconnected from the continuous range of the species) are also particularly important to consider, given climate change, in order to conserve genetic diversity and enable adaptation.

Principle 2. Maintaining habitat is fundamental to conserving species.

A species habitat is the ecosystem conditions that support its life requirements. Habitat is the place where a species is known to occur or likely to occur, based on its ecology. Habitat can be considered at a range of spatial and temporal scales that include specific microsites (e.g., occupied by certain invertebrates, bryophytes, some lichens), large heterogeneous habitats, or occupancy of habitat during certain time periods (e.g., breeding sites, winter range areas). Therefore conserving habitat requires a multi-scale approach from regions to landscapes to ecosystems to critical habitat elements, features and structures.

Principle 3. Large areas usually contain more species than smaller areas with similar habitat.

The theory of island biogeography illustrates a basic principle that large areas usually contain more species than smaller areas with similar habitat because they can support larger and more viable populations. The theory holds that the number of species on an island is determined by two factors: the distance from the mainland and island size. These would affect the rate of extinction on the islands and the level of immigration. Other factors being similar (including distance to the mainland), on smaller islands the chance of extinction is greater than on larger ones. This is one reason why larger islands can hold more species than smaller ones. In the context of applying the theory more broadly, the "island" can be any area of habitat surrounded by areas unsuitable for the species on the island. Therefore a system of areas conserved for biodiversity that includes large areas can effectively support more viable populations.

Principle 4. All things are connected but the nature and strength of those connections vary.

Species play many different roles in communities and ecosystems and are connected by those roles to other species in different ways and with varying degrees of strength. It is important to understand key interactions. Some species (e.g., keystone species) have a more profound effect on ecosystems than others. Particular species and networks of interacting species have key, broad-scale ecosystem-level effects while others do not.

The ways in which species interact vary in addition to the strengths of those interactions. Species can be predator and/or prey, mutualist or synergist. Mutualist species provide a mutually beneficial association for each other such as fungi that colonize plant roots and aid in the uptake of soil mineral nutrients. Synergistic species create an effect greater than that predicted by the sum of effects each is able to create independently.The key issue is that it is important to determine which among the many interactions are the strong ones because those are the ones toward which attention needs to be directed.

Principle 5. Disturbances shape the characteristics of populations, communities, and ecosystems

The type, intensity, frequency and duration of disturbances shape the characteristics of populations, communities and ecosystems including their size, shape and spatial relationships.

Natural disturbances have played a key role in forming and maintaining natural ecosystems by influencing their structure including the size, shape and distribution of patches. The more regions, landscapes, ecosystems and local habitat elements resemble those that were established from natural disturbances, the greater the probability that native species and ecological processes will be maintained. This approach can be strengthened by developing an improved understanding of how ecosystems respond to both natural and human disturbances, thus creating opportunities to build resilience in the system. For example, high frequency, low intensity fires have shaped ponderosa pine ecosystems while low frequency, high intensity fires have shaped lodgepole pine ecosystems. Maintaining these ecosystems means restoring fire and/or designing management practices such as harvesting to reduce the differences between a managed landscape and a landscape pattern created by natural disturbance.

Since ecosystems can change dramatically at the site level due to natural disturbances, considering their composition and structure of habitats at the landscape-level may be more useful. For terrestrial ecosystems, this means taking into account:

- species composition;
- the amount and patch size distribution;
- the variety and proportion of seral stages of terrestrial habitat from young to old; and
- the diversity of within community structure (e.g., a variety of amounts of snags and coarse woody debris within forest stands).

It is important to recognize that for some less mobile species, distribution of habitat is potentially as influential as amount of habitat (i.e., patch size; connectivity).

Principle 6. Climate influences terrestrial, freshwater and marine ecosystems.

Climate is usually defined as all of the states of the atmosphere seen at a place over many years. Climate has a dominant effect on biodiversity as it influences meteorological variables like temperature, precipitation and wind with consequences for many ecological and physical processes, such as photosynthesis and fire behavior. For example, major temperature fluctuations in surface waters in the Pacific Ocean due to El Nino climatic events can influence weather. This in turn can (i) increase some wildlife populations (population outbreaks), (ii) impact the migration timing of some migratory bird populations, or (iii) cause loss of large native populations. Alterations to stream flow and timing of freshet resulting from climate change affects fish and waterfowl. Because of the key role of climate, rapid climate change profoundly changes ecosystems. A critical question therefore is: How should anticipated climate changes influence current conservation decisions so that ecosystems remain resilient in the future?

Application of Ecological Concepts and Principles

The applications described below provide ways of applying the ecological concepts and principles in order to conserve biodiversity. No single application will be sufficient – each approach ideally needs to be operating in conjunction with others. The applications are grouped into those that primarily relate to:

• **coarse and fine filter applications**: techniques to help conserve biodiversity; and
• **planning applications:** techniques to promote biodiversity conservation using planning tools and adaptive management that continuously improves our understanding of what needs to done and how this can be more effectively delivered over time.

Coarse and fine filter applications, includes following applications,

1. Use coarse and fine filter approaches.
2. Ensure representation in a system of protected areas.
3. Retain large contiguous or connected areas.
4. Maintain or emulate natural ecological processes.
5. Manage landscapes and communities to be responsive to environmental change.
6. Manage towards viable populations of native species.

7. Preserve rare landscape elements, critical habitats and features, and associated species.
8. Minimize the introduction and spread of invasive alien species that disrupt ecological resilience and population variability.

Planning applications, includes following applications,

1. Set objectives and targets for biodiversity in plans.
2. Manage biodiversity at multiple levels of biological organization and multiple time and spatial scales.
3. Incorporate spatial and temporal approaches to land use that are compatible with an area's natural potential.
4. Avoid land uses that convert natural ecosystems and restore damaged ecosystems.
5. Avoid, mitigate or, as a last option, compensate for the effects of human activities on biodiversity.
6. Employ adaptive management of natural resources to maximize learning.
7. Given that humans are a powerful agent of change, make science-based decisions.

Probable questions:

1. State the major principles of ecology.

2. What are the applications of ecological concepts and principles.

3. How climate influences terrestrial, freshwater and marine ecosystems?

Suggested Readings:

1. Vold, T. and D.A. Buffett (eds.). 2008. Ecological Concepts, Principles and Applications to Conservation, BC. 36 pp. Available at: www.biodiversitybc.org
2. Bunnell, F.L. 1998. Setting goals for biodiversity in managed forests. Pp. 117-153 *in* F.L. Bunnell, and J.F. Johnson. The Living Dance: Policy and practices for biodiversity in managed forests. University of British Columbia Press, Vancouver, BC. 203pp.
3. Intergovernmental Panel on Climate Change. Climate Change 2001: Synthesis Report. Available at: www.ipcc.ch/
4. MacArthur, R.H. and E.O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press. But see also: Bunnell, F.L. 1999. What habitat is an island? Pp. 1-31 *in* J. A. Rochelle, L. A. Lehmann and J. Wisniewski *(eds.).* Forest Fragmentation: Wildlife and Management Implications. Brill, Leiden, Netherlands. 340pp.

Unit-VII

Community ecology: nature of communities; levels of species diversity and its Measurements

Objectives: In this unit you will earn about Community ecology and nature of communities, levels of species diversity and its Measurements.

Introduction:

By definition, community represents the population of all species living and interacting in an area at a particular time. Population can, within limits, adapt to changes in environmental conditions. The major driving force of adaptation to environmental changes is believed by most biologists to be biological evolution, the change in a population's genetic makeup through successive generation.

Concept of Community:

A group of organisms constitute population. Each population has characteristics like natality, mor-tality, age structure, growth dynamics and so on. But when several populations share a common habitat and its resources, they interact among them-selves and develop into a biotic community or sim-ply, a community.

Microorganisms, plants and animals populations sharing a common habitat and interacting among themselves develop into biotic communities. The composition of a biotic community in any habitat is dependent upon the prevalence of environmental conditions in that habitat and the ecological amplitude of species populations.Thus the climate and other abiotic as well as biotic conditions of a habitat determine the type of community which survives and develops. The organisms of a community usually exhibit trophic (feeding) relationships among themselves. They also interact in sharing the space and there may be interactions at a reproductive and behavioural level.

Each biotic community exhibits a number of characteristics, such as diversity, density, dominance, composition and stratification. Each community has its special limit. Sometimes the boundary between two communities may be very sharp or gradual. The transitional zone or junction between two or more diverse communities is called "eco-tone". The eco-tone harbours a community termed eco-tonal community with organisms of overlapping communities and some of unique types.

Origin of Biotic Community:

All the living things make the biotic system of the earth. Neither organisms nor species populations exist by themselves in nature, but are always part of an assemblage of different species populations living together in the same area. Any assemblage of populations of living organisms in the prescribed area or habitat is termed biotic community.

For example, the different species of organisms occurring in a pond constitute the pond community. Some other communities such as swamps, deserts, large lakes, grass lands and many others of different dimensions.Similarly, the interdependent species in any environment, such as a forest, a lawn or a desert track, constitute biotic communities. A community may be composed primarily of animals, or primarily of plants, but most communities consist of both animals and plants.

The factors that influence the living organisms are known as biotic factors. In the natural world there is interdependence of one form of life on another. A population of single species is not a viable entity by itself. It will not be able to survive for long.For example, the sparrows require worms, insects or seeds or some other kind of food. Thus the most appropriate definition of biotic community— **"a biotic community is a naturally occurring assemblage of plants and animals that live in the same environment, are mutually sustaining and interdependent, and are constantly fixing and dissipating energy."**

However, the different species in a biotic community share a common environment and their relationships are based on direct or indirect functional interactions. The nature of relationship is determined by the requirements of the members of the community.Biotic community organisation results from interdependence and interaction among populations of different species in a habitat. Large number of biotic communities found in nature due to two specific reasons.

They are as follows:

(i) Existence of diverse habitats with characteristic environmental conditions and

(ii) Co-occurrence of different species whose tolerance ranges overlap with the environmental condition obtained in that habitat. When similar habitat conditions are repeated at another location, the same biotic community gets established there.

Structure of Community:

Communities may be small, consisting of few species populations in a small space, or large, comprising several species populations in a large area. The community structures, composition and other characteristics can be readily described by visual observation without actual measurement.This is a qualitative approach which is easier than the quantitative population analysis where measurements are actually made. Communities usually categories by the ecologists in various ways primarily based of habitat features like water availability, high exposure, or other habitat features.

For instance, depending on the amount of water availability, plant communities may be hydrophytic (aquatic habitats), mesophytic (moderately moist soil habitat) and xerophytic (dry or arid habitat).Similarly communities growing on conditions of abundant light are called heliophytic and those growing in shade sciophytic. Identically communities growing on various habitats designated as desert communities, mountain communities and estuarine communities and so on.

In general, a community is dynamic since it changes over time. This dynamic nature is reflected in the succession of organisms in a habitat. A series of changes results in the development of a relatively stable community, which maintains its structure and influences the climate of the area.Such a stable and mature community is called a climax community, while communities of successional stages are called seral communities. The plant community structures, composition and other characterizes can be described in both qualitative or quantitative means.

Community Dynamics:

Communities are dynamic systems constantly interacting with another system, the environment, which is equally dynamic. The community charges are gradual and imperceptible at any time but easily recognisable if observed at regular intervals over a long period of time. Seasonal changes in plant communities always occur at every place, particularly in areas where temperature variation is significant. However, in course of very long period of time at many places the communities have reached a peak stage and attained a dynamic balance with the environmental changes. The process of change in communities and their environment at one place in the course of time is called "ecological succession".

Characteristics of Biotic Community:

Each biotic community consists of very diverse organisms belonging to different kingdoms of living things. The number of species and abundance of population in communities also vary greatly. The organisms in a community depend upon each other as well as upon the non-living environment for food, shelter and reproduction.

A stable and self-sufficient community has certain specific characteristics:

(a) Dominance of Species:

Each community has one or more dominant species. These could be Pine, Oak, Sal or Teak depending on the area and climatic conditions in that biotic community. The dominant species are the most numerous plants. They are generally large in size and have the greatest biomass. They influence the local environment. The species may be fewer in number in temperate forests 90% of trees may be pines and oaks of a biotic community. The species may be more in tropical rain forests. Andaman group of islands where there are about 12 dominant species.

(b) Habitat:

Biotic community occupies particular area with specific physical environment like temperature, humidity, soil, availability of water etc., which acts as a limiting factor and regulate the population size of various species within the community.

(c) Self-Sufficiency:

On the bases of nutrition, each community comprises autotrophic and heterotrophic as perfectly balanced. Plants are autotrophic and referred as producer; animals are heterotrophic organisms and referred as consumers. The remains and dead bodies of these producers and consumers are decomposed by the decomposers (bacteria and fungi) and help in the recycling of materials.

(d) Structure and Stratification:

A stable community comprises of various strata, each community consists of the population of particular kind of species. Their growth forms help to establishment the structure of a community and on their arrangement; community shows either horizontal layering called zonation or vertical layering called stratification.

(e) Species Diversity:

Community is formed of a number of different populations. The number of species and population abundance in a community also vary greatly. Species diversity depends upon size of the area, diversity of habitats in that area, temperature, humidity, availability of water soil type, altitude etc.

(f) Dominance and Succession:

Each community has different types of population only few species especially one of them is expressed in majority and called dominants. Each community develops as a result of a directional change called succession.

Community Stratification:

Every biotic community has a vertical layering or stratification of organisms or environmental conditions. A number of examples can be cited to support the concept of community stratification from different habitats.

In grassland community three strata, namely:

(a) Subterranean,

(b) Floor and

(c) Herbaceous

may be recognized.

The subterranean stratum contains the roots of the principal vegetation and provides permanent shelter to bacteria, fungi, protozoans, nematodes, earthworms, ringworms and several other invertebrates. The floor stratum consists of basal parts of the vegetation, including the rhizomes of grass plants.

In this stratum, generally the insects, spiders, reptiles and rodents are present. The herbaceous stratum of grassland community is represented by upper parts of grasses and herbs. Several types of insects, birds and grazing mammals are included in this stratum.

In a forest community, five vertical strata are present. They are:

(a) Overstorey stratum,

(b) Understorey stratum,

(c) Transgressive stratum,

(d) Seedling stratum, and

(e) Subterranean stratum.

A typical forest may have an overstorey stratum comprised of trees that are more than forty feet in height. These trees make a canopy. Just beneath this canopy there is an understory stratum that extends from twenty feet in height to a short distance below the overstorey. A transgressive stratum extends from four feet to twenty feet or more and comprises of shorter shade loving species.

The seedling stratum begins at the soil level and extends to the lower limit of the transgressive stratum. The subterranean stratum in the forest community is moist and contains a large amount of humus. It is very prominent up-to a depth of two to three meters. Each stratum has its own group of animals. However, most animals can move from one stratum to another in search of food or in response to many biotic factors, such as insects, snails, birds and squirrels.The animals found above the soil include turtles, snakes, some birds, insects and a variety of mammals, such as rabbits, deer, wolves and foxes. The animals living in humus soil are beetles, fly larvae, spiders, annelids, antipodes, mites, protozoans, nematodes and some springtails.

In pond community, vertical stratification is very little. However, in deep ponds and lakes three strata:

(a) Littoral zone,

(b) Limnetic zone and

(c) Profundal zone

can be recognized. The littoral zone comprises shallow water region and is occupied by rooted plants. The limnetic zone occupies the depth up-to which the light penetrates and inhabited by planktons, nektons andneustons. In profundal zone, there is no penetration of light, and therefore, it has no photosynthetic organisms.

Ecotone and Edge Effect:

The zone where two or more different communities meet and integrate, is called transition zone or ecotone. This zone of integration may be narrow or wide, local (e.g., a zone between field and a forest) or regional (e.g., the transition between forest and grass land). Ecotone contains few species from both communities. The total number of species is often greater in the ecotone than in the adjoining communities.

The ecotone or transition zone exhibits a shift in dominance of the conspicuous species of both sides. It may also include a number of highly adaptable species that tend to colonize such transitional areas. Because of this, the variety (i.e., species diversity) and density of life is often greatest in such areas.This potential for the ecotone to act as a habitat for species found in neither major community is called edge effect. Thus the tendency of increased variety and density of some organisms at the community border is known as edge effect.

The organisms that occur primarily, or most abundantly, or spend the greatest amount of their time in junctions between communities are called edge species. A common example of the edge effect in action can be seen in those species of owl that live in or near ecotones between forests and grasslands. They depend on forest trees for nesting and do their hunting in the grassland, where they depend on field rodents for food.

In man-made communities such as agricultural fields, the ecotone between the field and the forest act as refuge for species formerly found in the ploughed area, as well as for other plants such as weeds. Ecotones of this type are also the prime habitat of may species of insects, game birds, and mammals.

Keystone Species:

The species, which have much greater influence on community characteristics, relative to their low abundance or biomass, are known as keystone species. Such species play a vital role in controlling the relative abundance of other species. When keystone species is removed, it causes serious disruption in the functioning of the community. For example, in the tropical rain forests, the different species of figs are the keystone species as they produce large quantity of fruits. During the time of food scarcity, these fruits are consumed by monkeys, bats, birds, etc. Thus, by protecting the fig trees, the animals dependent on them are also conserved.

Link Species:

However, only a few species act as keystone species, while others act as link species. For example, mycorrhizal fungi in soil are critical link species as they establish essential links in the absorption of nutrients from the soil and other organic substances.

Some critical link species are responsible for providing food to the network species, while others act as pollinators of flowers, and some act as agents for dispersal of seeds and fruits. Tropical rain forests are rich in critical link species due to high degree of animal dependent pollination and dispersal.

Composition of Biotic Community:

Biotic communities consist of living as well as non-living component. Non-living organisms are called as abiotic and include water, sunlight, air etc. while living organisms are referred as biotic.

There are three types of living organisms inhabit a biotic community:

The classification of biotic organisms is as follows:

(i) Producers:

Producers or autotrophs include plants, algae and bacteria that survive via converting solar energy into food. These are mainly green plants. They synthesize organic food from simple inorganic compounds, namely carbon dioxide and water, with the help of chlorophyll and sunlight, hence the name as producer. The process is called photosynthesis.

It may be briefly represented as under:

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

The producers are very important for biotic community because they:

(i) Provide food and oxygen to the animals

(ii) Reduce CO_2 and H_2O contents from the environment.
Thus, they influence animals as well the environments.

(ii) Consumers:
Consumers or heterotrophs eat plants and animals to survive. These are mainly the animals. They are unable to synthesize food for themselves. Therefore, they take other organisms or their parts, hence their name. The consumers are called herbivores when they feed on plants and carnivores if they take other animals. Grasshopper, rat, rabbit, goat and cattle are common herbivores.

Frog, wolves, tiger and lions are familiar carnivores. Cat, dog, bear and man take both plant and animal food. They are termed omnivores. The consumers also use O_2 of the environment and add to it CO_2, nitrogenous waste matter and faeces. They, thus; also influence the environment as well as the organisms. Consumers include herbivores (known as primary consumers), which eat only plants, carnivores (known as secondary consumers), which eat other animals, and omnivores, which eat both plants and animals.

Subcategories of consumers are detritivores, or detritus feeders. These creatures eat plants and animals that are already dead. Their diet consists of dead organisms, as well as organic waste. Crabs, vultures and termites are well known example of detritivores, or detritus feeders.

(iii) Decomposers:

The final type of living organism is decomposers. They change plants that have died into nutrients that allow them to survive. Animals that live in the water or soil often feed off dead decomposers. These are mainly bacteria and fungi of decay. They are also named as reducers. They obtain food from the dead producers (plants) and consumers (animals) and latter's waste products.

They decompose these materials into:

(i) Small organic molecules which they utilize themselves.

(ii) Inorganic compounds that are released into the environment for reuse as raw materials by the producers.

The decomposers not only return chemical nutrients to the environment, but also make space available for new producers. Without this, all life will ultimately cease to exit. Thus, the decomposers have a role in the environment. They are found in the soil, and the bottom of ponds, lakes and oceans.

Biotic Stability:

A biotic community is a naturally occurring group of plants and animals living in the same environment. They all interact to make the community stable. It has been seen that more the number of species, more stable is the community. A biotic community containing a large population of Eucalyptus or any other plant may be totally wiped out by a fungal disease or insect infection.

But if a biotic community contains many species and different kinds of plants, only one would wipe out at a time and the rest would survive. Since a biotic community is formed of a large number of diverse type of populations of plants and animals and microbes, all these populations are essential for biotic stability. Each species eats on a different kind of grass or shrubs. Some that feed on the same species while few feed at different stages of grass. This makes the biotic community rich and stable.

Interaction among Biotic Community:

When various species (community) live together in a biotic community a numbers of interactions take place according to specific needs of food, shelter and habits.

Following interactions have been observed:

(i) Predation:
Predation is a direct and often complex interaction of two species in a biotic community. The stronger animal called predator that captures and feeds on the weak animal called prey. The decrease in number of predators leads to an increase in number of the prey.

An increase in the predator population leads to decrease in the prey population in a biotic community. These fluctuations play an important part in regulating natural population. Tiger feeds on deer, owls on rats etc. In a common biotic community an animal could be a predator as well as a preyat different times.

(ii) Scavenging:

Scavenging is a direct food relationship where animals feed on other dead animals called scavengers where as another animal in a biotic community, which either died naturally or have been killed. They play an important role in a biotic community as food is not wasted and also is disposed off. Vulture feeds on dead bodies. Hyenas and jackals feed on left over killed animals by lion. They may feed on big animals like zebra and giraffe killed by other animals.

(iii) Parasitism:

Parasitism is a negative interaction in a biotic community where one not only derives nourishment but also lives a part or the whole of life on another organism. The parasite is an organism that lives on another organism, the host from which it obtains food as well as shelter in a biotic community. It may be between animals between plants or between animals and plants in a biotic community.

In the host parasite relationship the weak attacks on the stronger. It is beneficial to parasite and harmful to host. Generally a parasite may cause an illness or disease but not kill the host except in few specific cases. There may be number of parasites on one host.

(iv) Commensalism:

In Commensalism one organism or a population is benefited while the other is neither benefited nor harmed. In some cases the host may be able to derive some minor benefit. There is no physiological exchange of any kind. The association may be temporary or permanent in a biotic community. Remora is a small fish attaches itself to the lower side of a shark. Remora feeds on scraps of shark's food and is not harmed.

(v) Symbiosis:

Symbiosis is an association of two populations in a biotic community where both the populations are benefited. There is often a close or permanent association. In some cases both are so inter-dependent that neither can live alone in that biotic community. Lichens are the best example of symbiotic association.

(vi) Competition:

The two species in a biotic community interact in such a way that it affects their growth and survival. Both the species share the same resources like water, nutrients, space, sunlight, food, etc. of a biotic community. Members of both species compete to survive in that respective biotic community. There is a direct inhibition of one by another. There is no set pattern as anyone can succeed. Carnivorous animals like tiger and lion compete for the prey. Trees, herbs and shrubs compete for sunlight, water and nutrients in a biotic community.

(vii) Some Passive Interactions:

There are many interactions that exist in nature of a biotic community in between the animals, or between animals and the surrounding, which also help an organism to survive in that biotic community. The blending of an animal with its surrounding is called camouflaging. It is also known as protective coloration. They camouflage their body shape and colour to suit the environment. Stick insect *Carausiusmorosus* resembles a thin dry branch. Dead Leaf butterfly *Kalimaparolecta* resembles a dry leaf. Praying mantis *Mantis religiosa* resembles the green foliage. Mimicry is also a type of protective resemblance in a biotic community. In this an animal mimics another animal so as to avoid predation.

(viii) Other Interactions:

There may be many other interactions have been observed. The birds/animals are associated to bringing seed and fruit dispersal and pollination. The birds feed on ticks; they are parasites on the body of cattle. The Rufus woodpecker makes a nest in a hole in the ball shaped nest of the ants. The ferocious ants do not harm the eggs or young ones but the birds feed on these ants and keep a check on their population to continue their generation in a biotic community.

Ecological Succession or Changes in a Biotic Community:

Ecological succession is the process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and develops through increasing complexity until it becomes stable community. It is a phenomenon or process by which an ecological community undergoes more or less orderly and predictable changes following disturbance or initial colonization of new habitat.

A community is built up over a period of time. As the time passes, communities change. In a biotic community there are interactions among the organisms and between the biotic and abiotic factors like climate, light, soil, etc. All these bring about changes in a biotic community.A biotic community is a dynamic unit where tropic levels exist; there is a flow of energy and cycling of nutrients. It is a living part of an ecosystem. The wind, fire, volcanic activity or any other event in nature or man may destroy the organisms living in a biotic community.

Now if this area is left alone, a succession would start and ultimately a permanent biotic community would take shape. This process from the beginning to the climax may take thousands of years.During this time there will be an orderly and progressive replacement of one biotic community by another till a relatively stable biotic community is established. This is called as ecological succession. A complete succession is called as Sere. A Sere is made up of a number of seral stages. A climax community is die final or the seral stage.

Ecological succession may be expressed as follows:

1. The species living in a particular place gradually change over time as does the physical and chemical environment within that area.

2. Succession takes place because through the processes of living, growing and reproducing, organisms interact with and affect the environment within an area, gradually changing it.

3. Each species is adapted to thrive and compete best against other species under a very specific set of environmental conditions. If these conditions change, then the existing species will be outcompeted by a different set of species which are better adapted to the new conditions.

4. The most often quoted examples of succession deal with plant succession. It is worth remembering that as plant community's change, so will the associated micro-organism, and fungus and animal species. Succession involves the whole community, not just the plants.

5. Change in the plant species present in an area is one of the driving forces behind changes in animal species. This is because each plant species will have associated animal species which feed on it. The presence of these herbivore species will then dictate which particular carnivores are present.

6. The structure or 'architecture' of the plant communities will also influence the animal species which can live in the microhabitats provided by the plants.

7. Changes in plant species also alter the fungal species present because many fungi are associated with particular plants.

8. Succession is directional. Different stages in a particular habitat succession can usually be accurately predicted.

9. These stages, characterized by the presence of different communities, are known as 'seres'.

10. Communities change gradually from one sere to another. The seres are not totally distinct from each other and one will tend to merge gradually into another, finally ending up with a 'climax' community.

11. Succession will not go any further than the climax community. This is the final stage.

"The developmental study of vegetation necessarily rests upon the assumption that the unit or climax formation is an organic entity. As an organism the formation arises, grows, matures, and dies… Furthermore, each climax formation is able to reproduce itself, repeating with essential fidelity its development."

Causes of Plant Succession:

Climatic factors may be very important, but on a much longer time-scale than any other. Changes in temperature and rainfall patterns will promote changes in communities. As the climate warmed great successional changes took place. The tundra vegetation and bare glacial till deposits underwent succession to mixed deciduous forest.

The greenhouse effect resulting in increase in temperature is likely to bring community changes. Geological and climatic catastrophes such as volcanic eruptions, earthquakes, avalanches, meteors,

floods, fires, and high wind also bring allogenic changes. Allogenic succession is caused by external environmental influences and not by the vegetation.

Soil changes due to erosion, leaching or the deposition of silt and clays can alter the nutrient content and water relationships in the ecosystems. Autogenic succession can be brought by changes in the soil caused by the organisms there. These changes include accumulation of organic matter in litter or humic layer, alteration of soil nutrients, change in pH of soil by plants growing there.The structure of the plants themselves can also alter the community. When larger species like trees mature, they produce shade on to the developing forest floor that tends to exclude light-requiring species. Animals also play an important role in allogenic changes as they are pollinators, seed dispersers and herbivores. They can also increase nutrient content of the soil in certain areas creating patches in the habitat. This may create regeneration sites that favour certain species.

Mechanisms of Succession:

Frederic Clements in 1916, proposed the theory of ecological succession. According to this theory succession had a powerful influence on ecological community. Clements' concept is usually termed classical ecological theory.

According to Clements, succession is a process involving several phases:

(i) Nudation:

It is the development of a bare site uninhabited by any organisms. The process is usually caused by disturbances Succession begins with the development of a bare site, called Nudation. These factors can be either topographic like soil erosion, wind action etc., climatic like hails, storm, glaciations, fire etc.; or biotic. The area thus formed can sustain only autotrophic organisms which can utilize inorganic substrates. The environmental conditions are set up for the inhabitation of new species.

(ii) Migration:

The process of migration helps the arrival of seeds, spores or other reproductive propagules for establishment of species. The other species are non-native organisms which can spread widely in a community. These are usually threatening the normal ecosystem and causative agents for community disturbance.

However, in succession process, they help to alter the soil texture and function. R-selected species are often the first colonizers due to their high reproductive rates and better dispersal mechanisms.

(iii) Ecesis:

This is the initial establishment of plant community. It involves establishment and initial growth of vegetation. This is dependent on the soil structure. The stage is also called as 'colonization'. In this stage, the early colonizing species proliferate abundantly through germination, growth, and reproduction.

Ecesis is due to allogenic mechanisms. This is the stage at which the pioneer species survive through the dispersal mechanisms. The different pioneer species can have different maturation rates which allow this process to be longer and gradually allowing replacement of some species by others. The process also makes the soil structure suitable for those species.

(iv) Competition:

Once the few initial species have become established the intra as well as interspecific competition among the species starts. As vegetation became well established, grew, and spread; various species began to compete for space, light and nutrients.

This stage is called competition. The competition is usually for resources such as food, water etc. Competition is found in both plants as well as animal species. The process leads to sharing of resources (resource partitioning) or competitive exclusion.

(v) Reaction:

The environmental conditions get modified by the action of species occupying the habitat. These changes subsequently trigger the displacement and replacement of one species by another. During this phase autogenic changes affect the habitat resulting in replacement of one plant community by another.

The existing community will be unable to support itself due to the harsh conditions. The major underlying mechanism is autogenic succession in which the plants themselves alter the environmental conditions.

(vi) Stabilization:

Stabilization is the process by which the climax community gets established. A climax community is mature, self-sustaining, and stable and is the final stage of succession. The physical and chemical conditions are altered and stabilized to such levels that it supports the entire community.

The climax communities are best adapted to the regions of succession and the community structure is likely to continue until another disturbance steps in. This represents a steady state of ecological equilibrium with specific composition, structure and energy flow. Reaction phase leads to development of a climax community.

(vii) Aggregation:

Aggregation is the increase in population of the species which has become established in the area. The shrubs replace the small herbs in most successions. This also proves as a source of food for future inhabitants

a. Hydrosere:

Hydrosere is the primary succession sequence which develops in aquatic environments such as lakes and ponds. It results in conversion of water body and its community into a land community. A

hydrosere is a plant succession which occurs in an area of fresh water. Hydrosere is simply a succession which starts in water. Hydrosere, also called hydrarch involves the ecological succession in the newly formed pond or lake.

A wetland is a transitional area. Freshwater provides a good and an excellent place to observe several stages of a hydrosere at the same time. In time, an area of open freshwater such as a lake will naturally dry out, ultimately becoming woodland.

During this process, a range of different habitats such as swamp and marsh will succeed each other. This succession from open water to climax woodland is likely to take at least two hundred or more years. Some intermediate stages will last a shorter time than others.

Fresh Water:

Fresh water lack soil, minerals etc., which support the growth of vegetation. Deep freshwater will not support rooted, submerged plants because there is not enough light for photosynthesis in the depths. There will be micro-organisms and plankton floating in the water.

Ecological succession is of great importance as:

(i) It provides information, which help to have control on the growth rate of one or more species in a given geographical area.

(ii) It helps in reforestation and forest management programs.

b. Xerosere/ Lithosere:

A xerosere may include lithoseres (on rock) and psammoseres (on sand). A lithosere is a sere (succession) on rock. Lithosere is a plant succession starts on a newly exposed rock surface. Xerosere is a plant succession which is limited by water availability. It includes the different stages in a xerarch succession. Xerarch succession of ecological communities originated in extremely dry situation such as sand deserts, sand dunes, salt deserts, rock deserts etc.

Pioneer species are the first organisms that colonies an area, of which lithoseres are an example. They will typically be very hard i.e., they will be xerophytes, wind-resistant or cold-resistant. In the case of a lithosere the pioneer species will be lichens, cyanobacteria and algae, which create their own food and water.They are autotrophic and so do not require any external nutrition except sunlight. Other examples of lithoseres include communities of mosses and lichens, as they are extremely resilient and are capable of surviving in areas without soil. Xerosere or lithosere is a gradual change in community due to change in climate, nutrition etc.

Key points of Lithosere /Xerosere:

(i) Bare rock colonized, by pioneer community, for example, lichens, mosses, bacteria, that can survive in hardy conditions, and need few nutrients.

(ii) Rock slowly weathered creating thin soil.

(iii) Plants die, creating humus, leading to a more fertile soil; grasses replace the mosses and lichens as the dominant species.

(iv) Grasses decrease in number; quick-growing shrubs become dominant.

(v) Fast growing trees dominate.

(vi) Over time slower growing trees such as oak become dominant and form the climatic climax community.

Varieties of Ecological Diversity:

a. Species Diversity:

The number of species that occurs in a particular area is called its species richness (Donovan and Welden, 2002). It means the species richness in any habitat and is common currency of the study of biodiversity. Species richness index is essentially a measure of the number of species in a defined sampling unit.

The biotic community is a natural assemblage of a large number of plant and animal species in an area. However, in any particular habitat there is no considerable variation in environmental condition, the plants growing together in a community show unique uniformity in their behaviour.Vegetation, therefore, is reflection of a climate and, in general, widely separated areas having similar climate have similar aspects of landscape.Some communities, for example, tropical rain forest and coral reef community, show high species diversity with many different kinds of species living at each trophic level.Some community areas have limits but more often the community boundaries are hard to define. A clearly distinguished area or a type of area with uniform habitat conditions and supporting characteristic type of vegetation is termed biotype.

Species richness is a function of sample size. However, it should not be confused with species abundance. Each natural habitat has a variety of species, which differ in their relative abundance. No community consists of species of equal abundance; some species are rare, others are common and still others may be abundant.Species diversity measures are often more informative than species counts alone. According to Harper, (1977), there is “importance of taking an organism’s eye view of community diversity”. This comment is relevant to structural diversity as it is to species composition.

b. Resource Diversity:

It means the diversity of resources that an organism (species) utilizes. For example, some fish species in the hill-streams have a wide trophic niche and depend on zooplankton, insects, and algae and diatoms for their food (Singh and Bahuguna 1983). In many cases food resources consumed by an organism differ during different stages of the life cycle, such as fry, fingerling and adult stages in case of fish. Thus, niche width is the measure of the diversity of resources utilized by a species.

The usual approach is to use the Simpson index or the Shannon index to calculate the niche width. The number of resource types observed (e.g., types of food items eaten, varieties of habitat utilized, kinds of behaviour employed) replace number of species in the equation. A separate value must be calculated for each type of resource and measures of abundance will depend on the way in which the index is being used.

If the niche width of a particular species is under consideration then abundance may be measured as the number of individuals either eating each type of food, living in each sort of habitat, or adopting each kind of behaviour. However, if we wish to measure the niche width of an individual, then abundance can be taken as the amount of each food type eaten, the time spent in each habitat or the frequency with which each behaviour is performed.

c. Habitat Diversity:

It is the number of habitat types in a defined geographical area. This is an index, which measures the structural complexity of the habitat. This structural complexity of environment, in turn, is responsible for the presence of a wide variety of spatial and trophic niches. This means that if any habitat supports more microhabitats its biological diversity will be more as compared to a habitat which has less number of microhabitats. More studies on habitat diversity have been made for terrestrial environments.

The number of substrate types has been related to species diversity for aquatic insects, molluscs and benthic macro-invertebrates. Gorman and Karr (1978) have taken bottom type, depth and current into account to investigate the link between habitat diversity in streams and fish species diversity. The author found that habitat diversity was more in small hill streams and some tributaries than large, snow-fed rivers in the Garhwal Himalayas.

d. Differentiation Diversity:

It is also called beta diversity. It means degree of change in species composition between sites or communities or along gradients. A number of studies on faunal diversity of fish and insects have clearly indicated that their distribution and abundance is governed by gradient and altitude, among other factors (Singh et al, 1994, Singh and Nautiyal, 1990). For example, stoneflies and rheophilic fish species in the rhithron parts of hill-streams are characteristic and are absent from their potamon parts.

One study indicated that there is zonation of animals within a river; *Simuliummonticole* occurs from source to 12 km, *Simuliumvariegatum* from 12 to 35 km., and S*imuliumequinum* from 20 to 50 km. All these studies indicate that greater diversity of species and habitats means greater ecological quality.

Measurement of Species Diversity:

Any measure of species diversity, by itself, does not convey much information; we appreciate its significance only when we compare with any other measure.Measures of species diversity can be divided into three categories.

These are:

(i) Species richness indices,

(ii) Species abundance models,

(iii) Species proportional abundance based indices

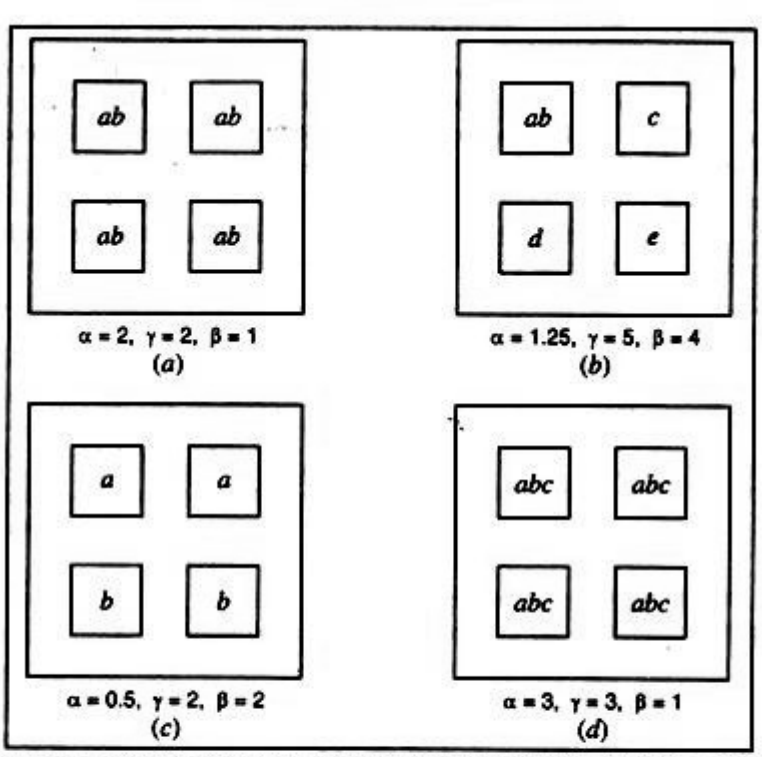


Fig. 7.1. Relationship between alpha, gamma and beta diversity (after Ricklefs and Miller, 2002). Each large box (region) has four small boxes (habitats). In (*a*), the diversity of each habitat (alpha diversity) is the smae for all 4 habitats, each contains species a and b, species richness of 2. The regional diversity (gamma) is 2. The beta diversity is gamma/alpha, 2/2 = 1. In (*b*), alpha diversity is 2 for one habitat (species a and b) and 1 for the other three (species c, d and e occur alone in a habitat), yielding an average a diversity of 1.25; gamma diversity is 5 (No. of habitats × average alpha diversity), so beta diversity is gamma/alpha, 5/1.25 = 4. In (*c*) average alpha diversity is 2/4 = 0.5, gamma diversity 2, beta diversity 2/1 = 2. In (*d*) alpha diversity is 12/4 = 3, gamma diversity 3, beta diversity is gamma/alpha, 3/3 = 1. Regions (*a*) and (*d*) have different gamma diversities but the same beta diversity, indicating little species turnover in those areas.

a. Species Richness Indices:

Species richness, as measure of diversity, has been used by ecologists. Species density or the number of species per m^2 is most commonly used to measure species richness. However, species richness increases with sample size. The smallest sample size may be 1 km^ and the largest may be the entire region or country.

Rarefaction:

As the sample sizes are always unequal, Sanders technique called Rarefaction is used to cope with this difficulty

Sanders's formula, as modified by Hurlbert (1971) is as follows:

$$E(S) = \sum\left\{1-\left[\binom{N-N_i}{n}\Big/\binom{N}{n}\right]\right\}$$

where $E(S)$ = expected number of species in the rarefied sample
n = standardized sample size
N = the total number of individuals recorded in the sample to be rarefied
N_i = the number of individuals in the *ith* species in the sample to be rarefied

The simplest approach is to take the number of individuals in the smallest sample as the standardized sample size.

This may be explained with the help of the following example:
If in one catch of fish we obtain 9 species with 23 individuals, and in another catch from the same area made for the same duration we obtained only 13 individuals belonging to 6 species, Hurlberts' formula may be used to find out the number of species we would have expected in the first catch if it too had only 13 individuals. Thus, expected number of species for the first catch x is 6.6 species (Table 7.4).

Table 7.4. Rarefaction with the help of Hurlbert's formula (see text for details)

Species	*Catch X*	*Catch Y*
A	9	1
B	3	0
C	0	1
D	4	0
E	2	0
F	1	0
G	1	1
H	0	2
I	1	0
J	0	5
K	1	3
L	1	0
Total No. of species (S)	9	6
Total No. individuals (N)	23	13

1. The term $\binom{x}{y}$ is a 'combination' which is calculated as follows.

$$\binom{x}{y} = \frac{x!}{y!(x-y)!}$$

x ! is a factorial. For example $5! = 5 \times 4 \times 3 \times 2 \times 1 = 120$

With these points in mind the computations can proceed

2.The first step is to take each species abundance from catch X and insert it in the formula

$$\left\{1-\left[\binom{N-N_i}{n}\Big/\binom{N}{n}\right]\right\}$$

$$\left\{1-\left[\left(\frac{\overline{n}}{N}\right)\Big/\left(\frac{n}{N}\right)\right]\right\}$$

Thus, for the species A which was represented by 9 individuals, the calculations are

$$\left\{1-\left[\left(\frac{14!}{13!\times 1!}\right)\Big/\left(\frac{23!}{13!\times 10!}\right)\right]\right\} = \{1-[14/1144066]\}$$

$$= 1 - 0.00 = 1.00$$

The result for each species is listed and summed to give the expected species number for catch X

Ni	
9	1.00
3	0.93
4	0.98
2	0.82
1	0.57
1	0.57
1	0.57
1	0.57
1	0.57
Expected No. of Species for catch X	E(S) = 6.58 or 6.6

Menhinick's Index (I M_n):

This index is based on the ratio of number of species (S) and the square root of the total number of individuals (N).

$I_{Mn}=S/\sqrt{N}$ or $D_{Mn} = S/\sqrt{N}$

It is claimed that this index may be used to compare samples of different sizes and that the effect of the number of individuals is reduced. However, some authors have shown that this index is not independent of sample size.

Using the data given in Table 7.4, the value of IM_n for catch x and catch y will be 1.88 and 1.66 respectively.

Margalefs index (I_{Mg}):

This index also relates the number of species to the number of individuals.

$I_{Mg} = S - 1/\log_e N$ or $D_{Mg}= (S - 1)/\ln N$

The index is influenced by sample size. However, some authors have demonstrated that both this and Manhinick's index are insensitive to changes in community structure.

Using the data given in Table 7.4, the value of for sample x and sample y will be 2.55 and 1.95 respectively.

b. Species Abundance Models:

No community has species of equal abundance. Some species are very abundant, others may have medium abundance and still others may be rare or represented by only a few individuals. This observation led to the development of species abundance models.

Species diversity data is frequently described by one or more patterns of distribution (Piclou, 1975), diversity is usually examined in relation to the following four models:

(a) The geometric series

(b) The log normal distribution

(c) The logarithmic series

(d) The broken stick model (the random niche boundary hypothesis)

When plotted on a rank abundance graph, the four models represent a progression ranging from the geometric series where a few species are dominant with the remaining fairly uncommon, through the log series and log normal distributions where species of intermediate abundance become more common and ending in the conditions represented by the broken stick model in which species are equally abundant as may be hardly observed.

c. Species Proportional Abundance Based Indices:

These indices provide an alternative approach to the measurement of diversity. These indices are called heterogeneity indices (Peet 1974) as they take both species richness and evenness into consideration. South wood (1978) called them nonparametric indices in view of the fact that no assumptions are made about the shape of the underlying species abundance distribution. The following indices are used.

Simpson's Index:

This index relates the contribution made by each species to the total number of individuals present.

$I = \Sigma p_i^2$

Where pi is the proportion of individuals in the ith species. The equation given by Wilhm (1967) is the following:

$$I = \Sigma (ni(ni-1)/N(N-1)$$

Where pi = the number of individuals in the ith species and N= the total number of individuals. The values of Simpson's index range from zero to 1 (unity) and are inversely proportional to the wealth of species (As I increases, diversity decreases). Pielou (1969) has given the following form of equation.

$$I =1- \Sigma (ni(ni-1)/N(N-1)$$

Therefore, index is usually expressed as 1 – I or l/I. The reciprocal form of Simpson's index ensures that the value of the index increases with diversity.

Shannon Index:

The index independently derived by Shannon and Wiener from the application of information theory is known as the Sharmon index of diversity. It is sometimes incorrectly referred to as the Shannon – weaver index (Krebs, 1985).

The index assumes that:

(a) All species are represented in the sample, and

(b) Individuals are randomly sampled from an 'indefinitely large' population (Pielou, 1975).

It is calculated from the equation:

$H' = -\Sigma pi \ In \ pi$

Where pi is the proportion of individuals found in the ith species. It is estimated as (ni/N). N is total number of individuals in S species. The value of Shannon index usually varies between 1.5 and 3.5 and rarely exceeds 4.5. The value of H' is related to species richness but is also influenced by the underlying species abundance distribution. May (1975) has shown that if the underlying distribution is log normal, 10 species will be required to give a value of $H' < 5.0$. Log_2 is often used to calculate Shannon index. Usually the index is obtained from the series.

$$H' = -\Sigma pi \ In \ pi - S-1/N+1-\Sigma pi^{-1}/12N^{2} + \Sigma(pi^{-1} - pi^{-2})/12N^{3}$$

Types of Diversity Indices of Biodiversity

The two types are: (1) Dominance Indices, and (2) Information-Statistic Indices.

1. Dominance Indices:

Dominance indices are weighted toward the abundance of the commonest species. A widely used dominance index is Simpson's diversity index. It takes into account both richness and evenness.

Simpson's Diversity Indices:

The term "Simpson's diversity index" can actually refer to any one of 3 closely related indices.

Simpson's Index (D):

Simpson's index measures the probability that any two individuals drawn at random from an infinitely large community will belong to same species. There are two versions of the formula for calculating D.

Either is Acceptable but is to be Consistent:

$$D = \sum (n/N)^2 \qquad D = \frac{\sum n(n-1)}{N(N-1)}$$

where, n = the total number of individuals of each species, N = the total number of organisms of all species.

The value of D ranges between 0 and 1.

With this index, 0 represents infinite diversity and 1, no diversity. That is, the bigger the value of D, the lower the diversity. This does not sound logical, so to get over this problem, D is often subtracted from 1 or the reciprocal of the index is taken.

Simpson's Index of Diversity 1-D:

This index represents the probability that two individuals randomly selected from a community will belong to different species. The value of this index also ranges between 0 and 1, but here, the greater the value, the greater the diversity.

Simpson's Reciprocal Index 1/D:

The value of this index starts with 1 as the lowest possible figure. This figure would represent a community containing only one species. The higher the value, the greater would be the diversity. The maximum value is the number of species in the sample. For example, if there are five species in the sample, then maximum value is 5.

The name Simpson's diversity index is often very loosely applied and all three related indices described above (Simpson's index, Simpson's index of diversity and Simpson's reciprocal index) have been quoted under term, depending on authors.

As an example, let us consider the following table:

Species	Number(n)	n(n – 1)
A	2	2
B	8	56
C	1	0
D	1	0
E	3	6
Total (N)	15	64

Putting the values into the formula for Simpson's index:

$$D = \frac{\sum n(n-1)}{N(N-1)} = \frac{64}{15 \times 14} = 0.3 \text{ (Simpson's index)}$$

Then, Simpson's index of diversity 1 – D = 0.7 and Simpson's reciprocal index 1/D = 3.3.

All these three values represent the same biodiversity. It is, therefore, important to ascertain which index has actually been used in any comparative studies of biodiversity. The disadvantage of Simpson's index is that it is heavily weighed toward the most abundant species, as are in all dominance indices.

The addition of rare species with one individual will fail to change the index. As a result, Simpson's index is of limited value in conservation biology if an area has many rare species with just one individual.

2. Information-Statistic Indices:

Information-statistic indices can take into account rare species in a community. Information- statistic indices are based on the rationale that diversity in a natural system can be measured in a way that is similar to the way information contained in a code or message is measured.

By analogy, if we know how to calculate the uncertainty of the next letter in a coded message, then we can use the same technique to calculate the uncertainty of the next species to be found in a community.

Shannon Index:

A widely used diversity index is Shannon index.

The Index is given by:

$$H_s = \sum_{i=1}^{s} p_i \ln p_i$$

where, p_i is the proportion of individuals found in the i^{th} species and In denotes natural logarithm.

The following table gives an example:

	Species	Abundance	p_i	$p_i \ln p_i$
	A	50	0.5	– 0.347
	B	30	0.3	– 0.361
	C	10	0.1	– 0.230
	D	9	0.09	– 0.217
	E	1	0.01	– 0.046
Total	5	100	1.00	– 1.201

Putting the values into the formula for Shannon index, $H_s = 1.201$

Even the rare species with one individual (species E) contributes some value to the Shannon index, so if an area has many rare species, their contributions would accommodate. Shannon index has a minus sign in the calculation, so the index actually becomes 1.201, not-1.201. Values of Shannon index for real communities are often found to fall between 1.5 and 3.5. The value obtained from a sample is in itself of no significance. The index becomes useful only while comparing two or more sites.

Brillouin Index:

A second information-statistic index, designed to reflect species abundance.

The Brillouin index and is given by:

$$H_B = \frac{\ln(N!) - \sum \ln(ni!)}{N}$$

where, N is the total number of individuals in the community, ni is the number of individuals in the i^{th} species.

The following table gives an example:

Species	No. of individuals	$\ln(n_i!)$
A	5	4.79
B	5	4.79
C	5	4.79
D	5	4.79
E	5	4.79
	N = 25	Σ ln(n!) = 23.95

Putting the values into the formula for Brillouin index, we get

$$H_B = \frac{\ln(25!) - 23.95}{25} = \frac{58 - 23.95}{25} = 1.362$$

This index describes a known population. There is no room for uncertainty while using this index. It places more emphasis on species richness and is moderately sensitive to sample size.

Probable questions:

1. Define ecological community.
2. Describe the structure of a community.
3. Describe the components of a community.
4. Describe stratification of a grassland.
5. describe stratification of a forest.
6. Describe stratification of a pond.
7. What is ecotone and edge effect.
8. What is keystone species and link species? Give suitable examples of each.
9. Describe different types of biotic interaction with suitable examples.
10. What do you mean by ecological succession. What do you mean by climax community ?
11. What are species diversity, resource diversity and habitat diversity ?
12. How species diversity is measured ?

Suggested Readings:

1. Robert E. Ricklefs and Gary L. Miller. ECOLOGY. *Fourth Edition*.
2. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition*. ISBN 978-0-321-73607-9

Unit-VIII

Biogeographical Zones of India

Objective: In this unit you will know about different Biogeographical zones of India

Introduction:

Biogeography is the study of the distribution of species and ecosystems in geographic space and through geological time. Organisms and biological communities often vary in a regular fashion along geographic gradients of latitude, elevation, isolation and habitat area.Phytogeography is the branch of biogeography that studies the distribution of plants. Zoogeography is the branch that studies distribution of animals.

India has only 2.4% of the world's land area, but contributes about 8.1% to global species diversity. This makes India one of the 12 mega biodiversity countries in the world. The country is divided into 10 biogeographic regions. The diverse ecological habitats such as forests, grasslands, wetlands, coastal and marine ecosystems and desert ecosystems have helped to harbor and sustain immense biodiversity in the country. The Indian subcontinent is rich in biodiversity with a good percentage of endemic flora and. fauna. This richness in biodiversity is due to variety of climate, topography and varied ecological habitats. These vary from the humid tropical Western Ghats to the hot deserts of Rajasthan, from the cold desert of Ladakh and snowcapped mountains of Himalayas to the warm coasts of peninsular India.

There are over 45,000 species of plants and 75,000 species of animals identified. There are more than 100,000 plant species and more than 300,000 animal species yet to be discovered. About 4,900 species of flowering plants are endemic to the country.These are concentrated in the floristically rich areas of North-East India, the Western Ghats, Northwest Himalayas and the Andaman and Nicobar Islands. It is estimated that 62% of the known amphibian species are endemic to India, of which a majority is found in Western Ghats. The Western Ghats in peninsular India and the North-East region are the treasure house of species diversity.The coastline of India is about 7,000 kms long where there is an abundance of seaweeds, fish, crustaceans, molluscs, corals, reptiles and mammals.

The conservation and sustainable use of biological resources in India is based on local knowledge systems and practices ingrained in Indian ethos. The country has a number .of alternative medicines, like Ayurveda, Unani, Siddha and Homeopathic systems, which are predominantly based on plant-based raw materials. Plant-based raw materials are also used in various herbal preparations in. the pharmaceutical and cosmetic industry.However, India as a country has several challenges such as overpopulation, growing demand for land, energy and water supply. Due to overexploitation there is shortage of various materials and many animal species face the danger of extinction.

India has different climate and topography in different parts and hence is termed as a mega diversity country. India occupies 10th place among plant rich countries of the world. It is essential to acquire knowledge about the distribution and environmental interaction of flora and fauna of India. With only 2.4 per cent of the total land area of the world, the known biological diversity of India contributes 8 per cent to the known global biological diversity. Biogeographic classification of India is the division of India according to biogeographic characteristics. There are ten biogeographic zones in India:

1. **Trans Himalayan zone.**
2. **Himalayan zone**
3. **Desert zone.**
4. **Semiarid zone.**
5. **Western Ghat zone.**

6. **Deccan plateau zone.**
7. **Gangetic plain zone.**
8. **North east zone.**
9. **Coastal zone**
10. **Island present near the shoreline**

Biogeographic Region	%*
Andaman & Nicobar Island	0.3
Coastal region	2.5
North East Region	5.2
Gangetic Plains	10.8
Deccan Plateau	42
Western Ghats	4
Semi Arid Region	16.6
Indian Desert Zone	6.6
Himalayan Zone	6.4
Transhimalayan Region	5.6
Total	100
*Of total geographic area	

Table 1: Total Geographical areas of biogeographical zones

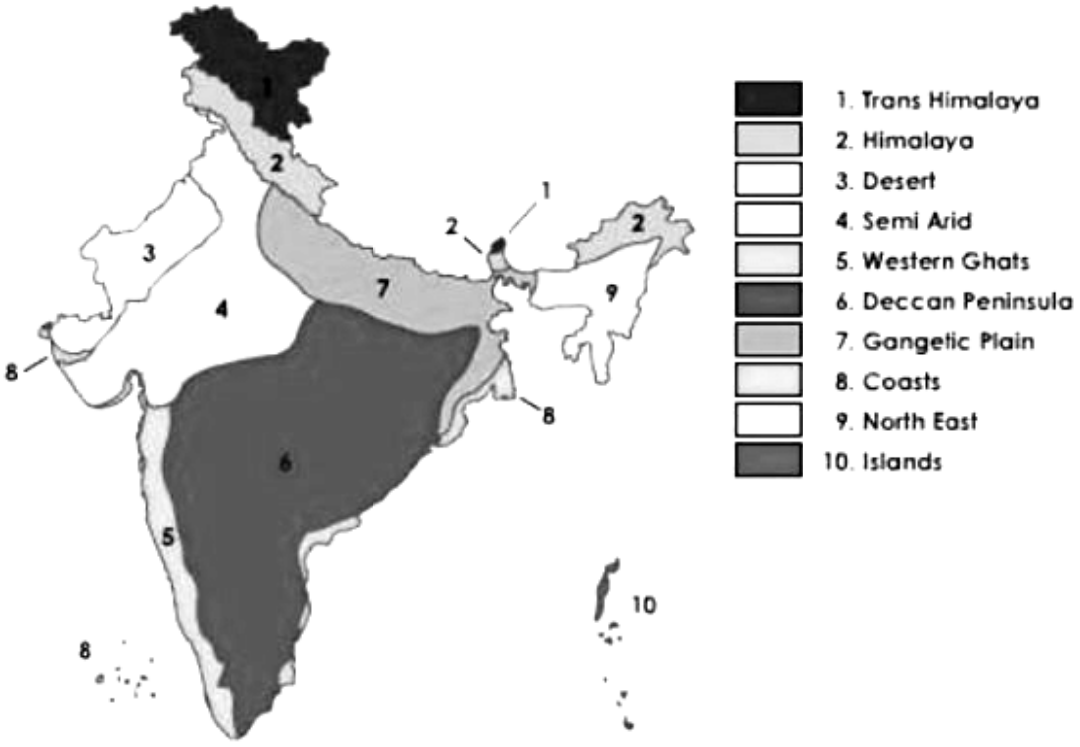


Figure-1. Map Indicating locations of various Biogeographical zones of India

1. Trans Himalayan zone:

Trans-Himalayas, eastward continuation of the most northerly ranges of the Himalayas in the southern part of the Tibet Autonomous Region of China. It consists of an ill-defined mountain area about 600 miles (1,000 km) long and 140 miles (225 km) wide in the centre, narrowing to a 20-mile (32-km) width at the eastern and western ends. The Trans-Himalayas, mainly composed of granites and volcanic rocks of Neogene and Paleogene age (i.e., about 2.6 to 65 million years old), are bounded by the Kailas (southwest), Nganglong Kangri (north), and Nyainqêntanglha (southeast) mountain ranges and by the Brahmaputra (YarlungZangbo) River (south). Trans Himalayas are divided into following ranges:

1**. Ladakh Range**

2. Zaskar Range

3. Karakoram Range

4. Kailash Range

Unlike the main Himalayas, the mountains are not divided by deep river gorges and lack a definite alignment. Passes average 17,500 feet (5,330 meters) in height, with the highest being Chargoding Pass (19,308 feet ,885 meters). The first recorded European sighting of the mountains was that of the Swedish explorer Sven Anders Hedin in 1906.

Fig 2: Kailash Range

It constitutes 5.6 per cent of the total geographical area, includes the high altitude, cold and arid mountain areas of Ladakh, Jammu & Kashmir, North Sikkim, Lahaul and Spiti areas of Himachal Pradesh. This zone has sparse alpine steppe vegetation that harbours several endemic species and is a favourable habitat for the biggest populations of wild sheep and goat in the world and other rare fauna that includes Snow Leopard and the migratory Black necked Crane *(Grus nigricollis).* The cold dry desert of this zone represents an extremely fragile ecosystem.

Fig 3: Wild goat (left) and sheep (right)

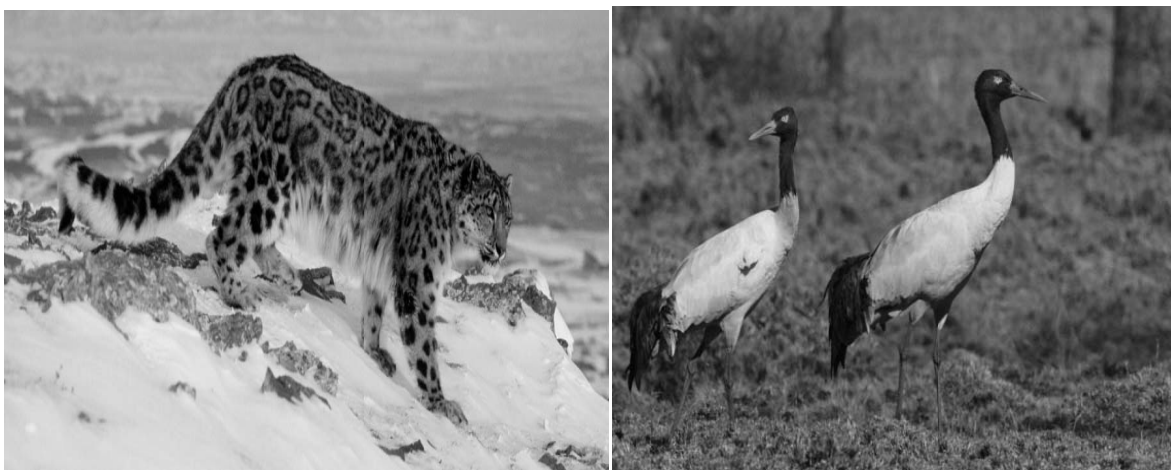


Fig 4: Snow leopardFig 5: Black necked Crane

2. Himalayan zone

The Himalayas consist of the youngest and loftiest mountain chains in the world. The Himalayas have attained a unique personality owing to their high altitude, steep gradient and rich temperate flora. The Himalayas are the northern boundaries of India. The entire mountain chain is running from Kashmir in the North-west to Assam in the north-east. The Himalayas comprise of a diverse range of biotic provinces and biomes.

The alpine and sub-alpine forests, grassy meadows and moist mixed deciduous forests . Oak, chestnut, conifer, ash, pine, deodar are abundant in Himalayas. There is no vegetation above the snowline. It provides diverse habitat for endangered species of bovids such as Bharal (*Pseudoisnayaur*), Ibex (*Capra ibex*), Markhor (*Capra falconeri*), Himalayan Tahr (*Hemitragusjemlabicus*), and Takin (*Budoreastaxicolor*). Other rare and endangered species restricted to this zone include Hangul (*Cervus eldieldi*) and Musk Deer (*Moschus moschiferus*). Panda and snow leopard are also found here.

Fig 6: Alpine Ibex

Fig 7: Musk deer

3. Desert zone

Indian desert zone, an extremely arid area west of the Aravalli hill range constituting 6.6 per cent of the total geographical area, includes the Thar and the Kutch deserts (both the salty desert of Gujarat and the sand desert of Rajasthan). Climate is characterized by very hot in dry summer and cold in winter. It has large expanses of grassland that supports several endangered species of mammals such as Wolf (*Canis lupus*), Caracal (*Felis caracal*), Desert Cat (*Felislibyca*) and birds of conservation interest viz., Houbara Bustard (*Chamydotis undulate*) and the Great Indian Bustard (*Ardeotisnigriceps*). Camel, wild asses, foxes and snakes are also found in hot arid deserts. The plants are mostly xerophytic, babul, kikar, wild palm grows in areas of moderate rainfall.

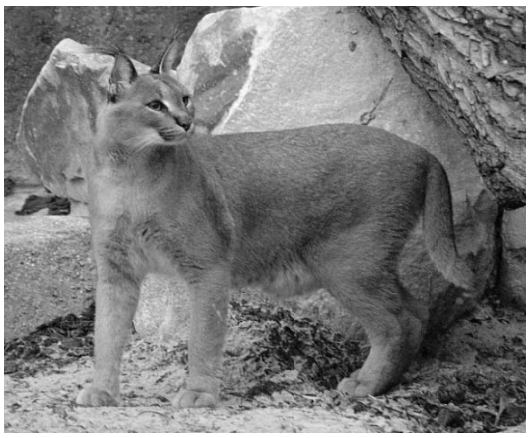


Fig 8: Caracal

Fig 9: Great Indian bustard

4. Semiarid zone

Semi-arid Region, constituting 16.6 per cent of the total geographical area, is a transition zone between the desert and the dense forests of Western Ghats. Peninsular India has two large regions, which are climatically semi-arid. This region is characterized by discontinuous vegetation cover with open areas of bare soil and soil-water deficit throughout the year. This semi-arid region also has several artificial and natural lakes and marshy lands.

The dominant grass and palatable shrub layer in this zone supports the highest wildlife biomass. Thorny shrubs, grasses, and some bamboos are present in some region. The cervid species of Sambar (*Cervus unicolor*) and Chital (*Axis axis*) are restricted to the better wooded hills and moister valley areas respectively. The Lion (*Leo persica*), an endangered carnivore species (restricted to a small area in Gujarat, specifically Gir National Park), Caracal (*Felis caraca*l), Jackal (*Canis aureus*) and Wolf (*Canis lupus*) are some of the endangered species that are characteristic of this region.

Fig 10: Sambar deer

Fig 11: Cheetal deer

Fig 12: Lion of Gir national park

5. Western Ghats zone:

The mountains along the west coast of peninsular India are the Western Ghats, which constitute one of the unique biological regions of the world. The Western Ghats extend from the southern tip of the peninsula (8°N) northwards (Kanyakumari) about 1600 km to the mouth of the river Tapti (21°N). They constitutes 4.0 per cent of the total geographical area. It is one of the major tropical evergreen forest regions in India and represents one of the two biodiversity 'hot spots'. Western Ghats are home to viable populations of most of the vertebrate species found in peninsular India, besides an endemic faunal element of its own

The mountains rise to average altitudes between 900 and 1500 m above sea level, intercepting monsoon winds from the southwest and creating a rain shadow in the region to their East. The varied climate and diverse topography create a wide array of habitats that support unique sets of plant and animal species. Apart from biological diversity, the region boasts of high levels of cultural diversity, as many indigenous people inhabit its forests.

The Western Ghats are amongst the 25 biodiversity hot-spots recognized globally. These hills are known for their high levels of endemism expressed at both higher and lower taxonomic levels. Most of the Western Ghat endemic plants are associated with evergreen forests.

The region also shares several plant species with Sri Lanka. The higher altitude forests were, if at all, sparsely populated with tribal people. Rice cultivation in the fertile valley proceeded gardens of early commercial crops like areca nut and pepper. The original vegetation of the ill-drained valley bottoms with sluggish streams in elevations below 100m would be often a special formation, the Myristica swamp.

Expansion of traditional agriculture and the spread of particularly rubber, tea, coffee and forest tree plantations would have wiped out large pockets of primary forests in valleys. The Western Ghats are well known for harboring 14 endemic species of caecilians (i.e., legless amphibians) out of 15 recorded from the region so far. This zone have large population of Nilgiri langurs, tiger, leopard and Indian elephants. Other species which are endemic in this region are Lion Tailed Macaque *(Macacasilenus),* Grizzled Giant Squirrel *(Ratufa macroura),* Malabar Civet *(Viverriculamegaspila),* NilgiriTahr *(Hemitragusbylocrius)* and Malabar Grey Hornbill *(Ocycerous griseus).*

Fig 13: Legless caecilians

Fig 14: Nilgiri langurs

Fig 15: Malabar grey hornbill

6. Deccan Plateau

Deccan Plateau is India's largest biogeographic region making 42 per cent of the total geographical area. It's a semi-arid region that falls in the rain shadow area of the Western Ghats. This bio-geographic zone of peninsular India is by far the most extensive zone, covering India's finest forests, particularly in the States of Madhya Pradesh, Maharashtra and Odisha. Majority of the forests are

deciduous in nature but there are regions of greater biological diversity in the hill ranges. The zone comprising of deciduous forests, thorn forests and degraded scrubland support diverse wildlife species.

The Deccan plateau includes the region lying south of the Satpura range. It extends up to the southern tip of peninsular India. Anaimudi is the highest peak of this region. The Deccan plateau is surrounded by the western and the eastern ghats. These ghats meet each other at the Nilgiri hills. The western ghats includes the Sahyadri, Nilgiris, Anamalai, and cardamom hills. Many rivers such as Mahanadi, Godavari, Krishna, and Kaveri originates from western ghats and flow toward the east. The eastern ghats are broken into small hill ranges by river coming from the western ghats. Most of these rivers fall into the bay of Bengal. The Godavari is the longest river in the Deccan plateau .the Narmada and the Tapi flow westwards and fall into the Arabian seaSpecies found in this region are Chital *(Axis axis),* Sambar *(Cervus unicolor),* Nilgai *(Boselapbustragocamelus)* and Chousingha *(Tetracerusquadricornis)*, Barking deer (*Muntiacusmuntjak*) and Gaur (*Antilope cervicapra*), Elephant (*Elephas maximus*) in Bihar-Orissa and Karnataka-Tamil Nadu belts, Wild Buffalo (*Bubalusbubalis*) in a small area at the junction of Orissa, Madhya Pradesh and Maharashtra and the hard ground Swamp Deer (*Cervus duvauceli*), now restricted to a single locality in Madhya Pradesh.

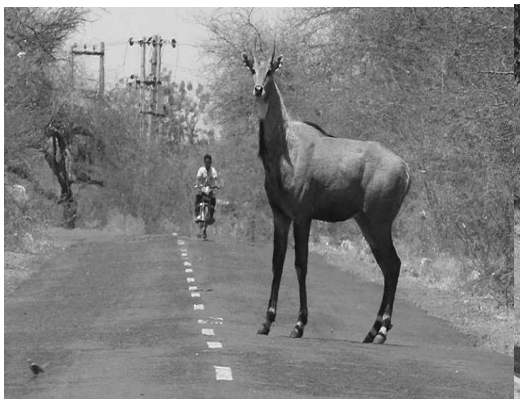


Fig 16: Nilgai

Fig 17: Indian elephants

7. Gangetic plain zone

In the North is the Gangetic plain extending up to the Himalayan foothills. This is the largest unit of the Great Plain of India. Ganga is the main river after whose name this plain is named. Gangetic plain constitutes around 10.8 per cent of the total geographical area. The Gangetic plain is topographically homogenous for hundreds of kilometers. This one of the most fertile area. Three zone can be divided Upper plain of ganga (from Delhi to Allahabad), Middle plain of ganga (from Allahabad to Bihar) and lower plain of ganga (in West Bengal).

The thickness in the alluvial sediments varies considerably with its maximum in the Ganga plains. The physio geographic scenery varies greatly from arid and semi-arid landscapes of the Rajasthan Plains to the humid and per-humid landscapes of the Delta and Assam valley in the east. Topographic uniformity, except in the arid Western Rajasthan is a common feature throughout these plains. The plain supports some of the highest population densities depending upon purely agro-based economy in some of these areas. The trees belonging to these forests are teak, sal, shisham, mahua, khair etc. The characteristic fauna of this region include Rhino (*Rhinoceros unicornis*), Elephant (*Elephas maximus*), Buffalo (*Bubalusbubalis*), Swamp Deer (*Cervus duvauceli*), Hog-Deer (*Axis porcinus*) and Hispid Hare (*Carprolagusbispidus*).

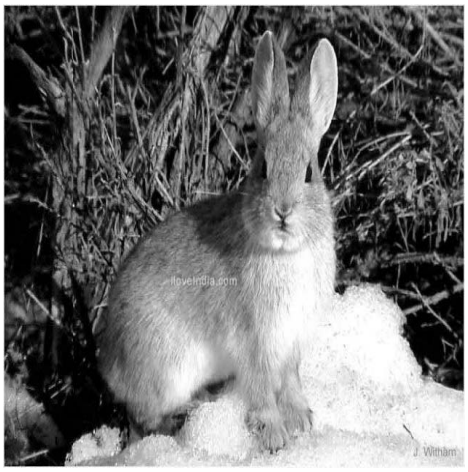


Fig 18: Indian hog deer **Fig 19: Hispid hare**

8. North East zone

North East Region constitutes 5.2 per cent of the total geographical area. This region represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese bio-geographical regions as well as being a meeting point of the Himalayan mountains and peninsular India. The North-East is thus the biogeographical 'gateway' for much of India's fauna and flora and also a biodiversity hotspot (Eastern Himalaya). Many of the species contributing to this biological diversity are either restricted to the region itself, or to the smaller localized areas of the Khasi Hills. It has several species of orchids, bamboos, ferns and other plants. Here the wild relatives of cultivated plants such as banana, mango, citrus and pepper can be grown.

9. Coastal zone

India has coastline of 7, 516.5 km. It extends from Rann of Kutch to Bangladesh. The west coast is narrow and extends from gulf of Cambay to south. Coastal region constitutes 2.5 per cent of the total geographical area with sandy beaches, mangroves, mud flats, coral reefs and marine angiosperm pastures make them the wealth and health zones of India. The coastline from Gujarat to Sunderban is estimated to be 5,423 km long. A total of 25 islets constitute the Lakshadweep, which are of coral origin, and have a typical reef lagoon system, rich in biodiversity. However, the densely populated Lakshadweep islands virtually have no natural vegetation.

The backwaters are the characteristic features of this coast. The east coast plains, in contrast are broader due to depositional activities of the east-flowing rivers owing to the change in their base levels. Extensive deltas of the Godavari, Krishna and Kaveri are the characteristic features of this coast. Mangrove vegetation is characteristic of estuarine tracts along the coast for instance, at Ratnagiri in Maharashtra. Larger parts of the coastal plains are covered by fertile soils on which different crops are grown. Rice is the main crop of these areas. Coconut trees grow all along the coast.

10. Islands

This constitutes 0.3 per cent of the total geographical area are one of the three tropical moist evergreen forests zones in India. The two groups of islands, i.e., the Arabian Sea islands and Bay Islands differ significantly in origin and physical characteristics. The Arabian Sea Islands (Laccadive, Minicoy, etc.) are the foundered remnants of the old land mass and subsequent coral formations. On the other hand, the Bay Islands lay only about 220 km. Away from the nearest point on the main land

mass and extend about 590 km. With a maximum width of 58 km the island forests of Lakshadweep in the Arabian Sea have some of the best-preserved evergreen forests of India. Some of the islands are fringed with coral reefs. Many of them are covered with thick forests and some are highly dissected. The islands house an array of flora and fauna not found elsewhere.These islands are centres of high endemism and contain some of India's finest evergreen forests and support a wide diversity of corals. In India, endemic island biodiversity is found only in the Andaman and Nicobar Islands. Some of the endemic fauna of Andaman & Nicobar islands include Narcondam hornbill, South Andaman krait etc.

Fig 20: ***Narcondam hornbill***

Fig 21: South Andaman krait

Biodiversity Hotspots in India

Of the twenty-five hotspots of biodiversity, recognized in the world, two are found in India, which extend into the neighbouring countries:

(i) The Indo-Burma region covering the Eastern Himalayas and

(ii) the Western Ghats – Sri Lankan region.

The hotspots are rich in floral wealth, reptiles, amphibians, mammals and also in their endemism.

The botanical hotspots of India include:

(1) Western Ghats,

(2) North-East India,

(3) Himalayas and

(4) Andaman and Nicobar Islands.

Eastern Himalayas:

As compared to the Western Himalayas that are colder and drier, the Eastern Himalayan ranges are much wetter with suitable climatic conditions. This supports endemism and biodiversity. The Eastern Himalayas comprise of .parts of Nepal, Bhutan, Sikkim, Arunanchal Pradesh and extends up to

Burma. The forest vegetation ranges from tropical rain forests to temperate alpine forests. Rhododendron is the dominant tree of this region.

The topography in the Eastern Himalayan region is quite varied that helps biodiversity and endemism. The rugged mountains and valleys support the virgin forests which are rich in many endemic plant species. In the Indian part of the Eastern Himalayan hotspot, about 5800 plant species are found of which around 2000 are endemic. Sikkim is one of the most blessed Indian states so far as endemism is concede; as of the 4250 plant species found there, 60% are endemic to the region.Palaeobotanists consider the Indo-Burma region to be one of the centres of origin for the flowering plants (angiosperms). Many primitive angiosperm families occurring there, such as Magnoliaceae, Winteraceae, etc. support this contention. The Eastern Himalayas are home to around 8000 species of angiosperms of which nearly 40% are endemic. The characteristic floras are Rhododendron, Alnus, Betula, Magnolia, etc.

The animals include members of the goat family, antelopes, musk deer, snow leopard, brown bear, black bear and the red panda. Due to continuous habitat destruction and hunting, many of these species are highly endangered. Andaman and Nicobar Islands are rich in littoral and Island type floras. About 2500 species of flowering plants have been recorded from there, of which 250 species are endemic. The important hotspots of Andaman & Nicobar Islands are North Andaman, spike Island, Table Island. South reef Island, Little and Great Nicobars.Western Himalayas are well-known for Alpine flora which abounds in Gymnosperms. Some 5000 species of flowering plants are known to occur in this region of which 800 species are endemic. The valley of flowers, Pithoragarh, Gori Valley, Mandal Chopta Valley, Karakoram and Laddakh are some of the major hotspots of this region.

Western Ghats:

The Western Ghats run parallel to the west coast of India and constitute more than 16,000 km strip of forests in the states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala. Locally they are also known as the Sahyadris. The Western Ghats are characterised by low hills; however, it achieves the highest elevation of 2675 m at Annamalai up to an elevation of 500 m the forests are evergreen in nature, while the forests occurring between 500-1500 m altitude are of semi-evergreen type.

The Western Ghats by virtue of having a humid tropical climate and geological stability supports one of the most biodiversity rich areas of the country. According to an estimate, of the 17,000 flowering plants species reported from India, more than 4,500 occur in the Western Ghats region. The Botanical Survey of India has listed 518 endangered species endemic to Peninsular India, the majority of which occur in the Western Ghats.The dominating plant families are Acanthaceae, Graminae, Orchidaceae, Rubiaceae, Labiatae, Compositeae and Leguminoseae. More than 200 species of rare orchids, many of them are endemic, are found in Western Ghats. Many economically important plants such as banana, rice, black pepper, ginger, etc. have spread to other parts of the country from here. The Western Ghats are home to a rich variety of fauna which are unique and many are endemic to this region. Nilgiri Langur, Lion-tailed Macaque, tiger, leopard, elephant, rare species of tortoise and other amphibians represent the faunal wealth. Many varieties of birds and fishes are also found here.

Sacred Forests:

These real hotspots of biodiversity are protected and worshiped by tribals due to religious sanctity. These are known as Devaskadu in Karnataka Devarahati in Maharashtra and Lakyntok in Meghalaya. These forests contain many rare endangered and endemic species.

Mangroves:

Mangroves are salt tolerant forest ecosystems found in Saline habitat, tropical and subtropical intertidal zones near estuaries. The total area under mangrove vegetation in India is estimated to be 6740 km and of this 4200 km^2 is covered by mangrove forest of sundarban alone and the second being in the Andaman & Nicobar Islands.

Probable Questions:

1. Define biogeography. How many biogeographic zones are there in India?

2. State the characteristics and faunal diversity in Himalayan region.

3. State the characteristics and faunal diversity in desert zone.

4. State the characteristics and faunal diversity in Gangetic plain zone.

5. State the characteristics and faunal diversity in semi arid zone of India

6. State the characteristics and faunal diversity in coastal zone of India

7 Write about biodiversity hotspots in India with special emphasis on Western Ghat region

and Eastern Himalayan region.

8. Write a short note on mangrove islands of India.

Suggested Readings:

1. Animal Ecology by Veerbala Rastogi

2. Biodiversity by Maity and Maity

3. Ecology and Environmental science by Rana

Unit-IX

Environmental management: solid waste management with vermicomposting; Bioremediation; Bioreactors in Environment monitoring

Objective: In this unit you will learn about Environmental management: solid waste management with vermicomposting; Bioremediation; Bioreactors in Environment monitoring

Introduction:

The modern day agriculture is based on intensive cropping system , which is mainly dependent on the use of agrochemicals in the form of chemical fertilizers and pesticides. In general , the production of chemical fertilizers is loading to depletion of fossil fuel , causing environmental and ground water pollution . The constant use of chemicals increases there leading because of depletion in organic carbon , change in soil pH , due to accumulation of higher amounts of salts and makes soil unproductive . The cost formed in soil affects the porosity of the soil .

To restore soil health , farmers are now showing inclination towards organic farming . For which it is necessary to produce organic manure in large quantities from the available organic wastes to maintain high level of production in the available agricultural land. Rapid utilization and industrialization in the recent years has resulted enormous amount of waste polluting the environment . City garbage in damaging the aesthetic as well as ecological quality of our surrounding . To get over this problem , in many countries and some parts of India , garbage is being used to produce manure . The multifarious being of earthworms has drafted the attention of scientists to vermicomposting which employs earth worms as bioreactor . Earth worms are above to convert organic part of municipal solid waste to rich manure , while if comply deodorizes the composting process. Thus , the rapid production of organic manure at farmer's site from locally available biodegradable organic wastes vermicompost has a bright future.

Earthworms form a major component of soil system and these organisms have been efficiently ploughing the land for millions of years assisting in the recycling or organic nutrients for the efficient growth of plants . Human interference in natural ecosystems throughout modern practices of agricultural , horticulture and silviculture involving large scale application of chemicals and pesticides has been responsible for the destruction of endemic soil fauna.

Vermicompost:

Vermicompost (also called worm compost , vermicompost worm humus vermicompost) is the conversion of biodegradable organic waste materials into granular compost through earthworms consisting of worm casts rich is nutrients and micro flora than conventional compost. Sources of the secretions of worms and associated microbes act as growth parameter along with other nutrients.

Live earthworms feed organic wastes such as farm yard wastes partially decomposed cowdung , fodder , leaf litter , gross , water hyacinth , have garbage etc . They can consume 2-5 times their body weight after using 5-10 % of feedstock for their growth . It excretes the mucons-coated undigested matter as worm costs . Wormcost consists of organic waste that has undergo physical and chemical breakdown through the activity of muscular gizzard , which grinds the material to a particle size of 1-2 micron . Species of earthworms capable for this activity are *Eixeniafoetide, Edyrilus Eugenia* and*Perionixexacavator* .

Vermicomposting:

Vermicomposting is a method by which compost or mixed manure of organic origin is prepared by the use of earthworms. It is a controlled degradation of the organic wastes for the consumption of earthworms, helps in the recycling of food wastes, reduces the waste bulk density and the final product may contain hormone-like substance which accelerates the plant growth.Vermicomposting and vermiculture are two interlinked and interdependent processes, and vermiculture can be done in the presence of decomposable waste organic matter.

Participating Organisms of the Vermicomposting:
Bacteria, micro-organisms and earthworms participate in the vermicomposting process, of which bacteria and micro-organisms are used as food for worms.

Throughout the world, 3 or 4 species are used in vermiculture but two species are used extensively in India. *Eisenia foetida* and *Eudriluseugeniae* are used throughout India for vermiculture. A list of some earthworm species is given below with some features which are used in different parts of the country.

Family Lumbricidae:
1.*Eisenia foetida*:
a. Distribution:
They are found throughout the country in muddy areas, sewage damps and tanks. The species is used in vermiculture throughout the world.

b. Features:
The colour of the body is red or brown or purple. The dorsal surface bears coloured bands often two per segment and the ventral surface is paler in colour.The clitellum occurs over 7-9 segments which include 24, 25 or 26-32 body segments. The body weight is about 1.5 gm/matured worm. Maturity attains in about 50-55 days and during adult stage the body wall becomes ridged. The species is generally called red worm or tiger worm.

Earthworm, *Eisenia* is involved in vemicomposting.

2.*Bimastos parvus*:

a. Distribution:

They occur in Kashmir, Himachal Pradesh, Punjab, Rajasthan and Uttar Pradesh

b. Features:

The body colour is brownish red. The clitellum is saddle-shaped and occurs over 6 or more segments which include 24 or 25-30. The body segments are usually 90.

Family Eudrilidae:

3. *Eudriluse ugeniae*:

a. Distribution:

Though the species was first recorded in South-West India but at present it is widely used in South India in vermiculture. The species was originally distributed in equatorial West Africa but recently it is found in most areas of the world. It is called 'night crawler' in U.S.A.

b. Features:

The colour of the body is brown or red dark violet. The clitellum occurs over 5-6 segments and include 13 or 14-18. Female gonopores are on the 14th segment and male gonopore, a few segments behind them.

The body segments usually range 145 to 196, and length of the body varies from 32 to 140 mm and diameter is about 5 to 8 mm. Maturity is attained within 40 days. The weight of the mature worms is about 4.3 mg per individual. The higher temperature tolerance is more higher than *E. foetida*.

Family Megascolecidae:

4. *Lampito mauritii* (*Megascoles mauritii*):

a. Distribution:

They are found in Punjab, South Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Kerala, Lakshadweep Islands and Andaman Island.

b. Features:

The body colour is dark yellowish except the anterior end which bears purplish tinge. The clitellum is ring-shaped and spreads over 4 segments that include 14- 17 body segments. The length of the body ranges from 80-210 mm, with diameter 3.5 to 5 mm. The total body segments vary 166-190.

**5.*Metaphire anomala (Pheretima anomala*)

a. Distribution:

The species has recorded in Kolkata (W. Bengal), Odisha, Bihar, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala.

b. Features:

Total length of the body ranges from 80 to 90 mm, with diameter varies from 5 to 5.5 mm. The clitellum is ring-shaped which spreads over 3 body segments that include 13 to 16.

6.*Pheretima posthuma*:

a. Distribution:

It is the most common species in India and South-East Asia including Japan and Australia. They prefer to live the damp soil in the gardens and also in the dead organic matter.

b. Features:

Colouration of the dorsal side is brown and the ventral side is brightly coloured. The whole length of the full grown worm measures about 200 mm and 3 to 5 mm in diameter. The body segments about 100-120. The clitellum covers 3 segments on XIV to XVI. The use of the species in vermitechnology is not well studied.

Family Octochaetidae

7. *Octochaetus surensis*:

a. Distribution:

The species was recorded in Burkul and Sur Lake in Odisha, and has also been recorded across other parts of India.

b. Features:

Colouration of the body is greyish with dark tinge at the dorsal part of the anterior region. The total length of the body measures 75 mm and diameter is about 2 to 2.5 mm. The clitellum is ring-shaped and occurs over 5 segments which include 13^{th} to 18^{th}.

8. *Dichogaster affinis*:

a. Distribution:

The species is found in Gujarat, Maharastra and South India. Outside India it is recorded in Sri Lanka, East Africa, and Madagascar etc.

b. Features:

The colour of the body is pinkish brown. The worm measures 30-32 mm in length with diameter is about 1.2 to 1.5 mm. The total body bears 140 segments. The clitellum is saddle-shaped and 8-10 segments, namely 13^{th} or 14^{th} to 21^{th} or 22^{th}.

9. *Dichogaster curgensis*:

a. Distribution:

South India.

b. Features:

The colour of the body is greyish. The length of the body measures 65-75 mm and diameter is 2 mm. The whole body contains 90-110 segments. The clitellum is ring-shaped and contains 8 segments over 13^{th} to 20^{th}.

10. *Oenerodrilus occidentalis*:

a. Distribution:

They are found in Rajasthan, Maharashtra, Karnataka and Andaman Islands.

b.. Features:

The total body length measures 15 to 30 mm and diameter is about 1 mm. The body segments are 70. The clitellum is ring-shaped and contains 6-8 segments, namely 13^{th} or 14^{th} to 19^{th} or 20^{th}.

Trophic Classification of Earthworms:

Based on the feeding habits, the earthworms are generally classified into detrivores and geophages. The detrivore earthworms feed mainly on plant litter, plan debris or mammalian dung. They collect the feed from the soil surface. These worms compose the epizoic forms.

The detrivorous earthworms are *Eisenia foetida, Eudriluseugeniae, Lampitomauritii, Polypheretimaelongata, Octochaetona serrata, Octochaetonasurensis,* etc.

The geophagous earthworms live in underground burrows and eat large quantities of deeper organic rich soil which remains beneath the surface layer. These worms are called humus feeders. *Pheretimaposthuma* and *Octochaetonathurstoni* are the examples of geophagous earthworms.

Process of Vermicomposting:

It deals with the various aspects like:

(i) Types of container,

(ii) Filling it with moist bedding

(iii) Introducing worms and

(iv) Feeding the worms on a regular basis.

These types of aspects for vermicomposting are practised in small scale vermicomposting but in larger scale the basic processes are same excepting the composting containers.

1. Composting container:

The shape or size of the containers depends upon the amount of waste to be composted and the number of earthworms which we want to culture

In 1m × 1m × 0.5 m high container 2000 adult earthworms can be maintained which can convert 200 kg wastes into composting material per month. In 2.23 sq. metre container 10 kg earthworms can be maintained which can convert about 1 ton waste per month. The selected container should be cleaned before use.

The followings are considered as composting materials which are being listed below:

(i) The animal dungs such as cattle dung, goat dung, sheep dung and poultry dropping are used as the composting material. The preliminary testing and precautions are necessary for the pathogens which can be harmful to earthworms excepting cowdung.

(ii) The agricultural waste materials which include the discarded part after harvesting and threshing of the paddy, wheat, etc. The vegetable wastes, leaf litter and sugarcane trash are considered as the composting material. The various kinds of forestry wastes, such as peels, saw dust are also considered. The leaf litters, such as mango, guava and grasses are used as composting materials.

(iii) The garbage includes various kinds of biodegradable and non-degradable materials. The biodegradable materials, such as kitchen vegetable waste, paper, should be sorted, recycled or composted.

2. Bedding material:

At the bottom of the container, a 2-3 inches thick layer of biodegradable matter, e.g., husk, grasses, stem of crops and sugar cane trash, etc. which are used as the feed of earthworms, is laid. Next to this layer, another layer of 2″-3″ thick of powdered cowdung is put.

The whole material is maintained moistened and required number, of live earthworms are released. Above this a 7″-9″ thick layer of earthworm feed matter is put and watered. The whole material is covered with a moist gunny bag.

The earthworms would multiply by consuming the food and compost the matter from upper layers. The upper layer of vermicompost becomes ready within 60-70 days. Gradually the whole matter will be converted into vermicompost within 30-40 days with the bacterial decomposition.

Feed Materials of Earthworms:

Earthworms eat decomposed organic matters. So various kinds of food are prepared used in vermiculture.

A list of feeds is given below:

1. Old cow dung which is used after 7 days.
2. Cow dung is mixed with kitchen wastes in the ratio of 10:3.
3. Cow dung is mixed with agricultural wastes in the ratio of 10:3.
4. Cow dung is mixed with rice polish or wheat bran in the ratio of 10: 3.
5. Cow dung is mixed with sewage sludge in the ratio of 10: 3.

Only cow dung or mixed dung, such as goat dung, sheep dung or poultry dropping are used. Mixed dung is prepared in the ratio of 50: 50. Any of the above-mentioned mixed materials are mixed thoroughly. Heaps should be kept in sheds places for 2-3 weeks and watered. Then the material is dried and beaten to make the material powdery which is used as feed for earthworms.

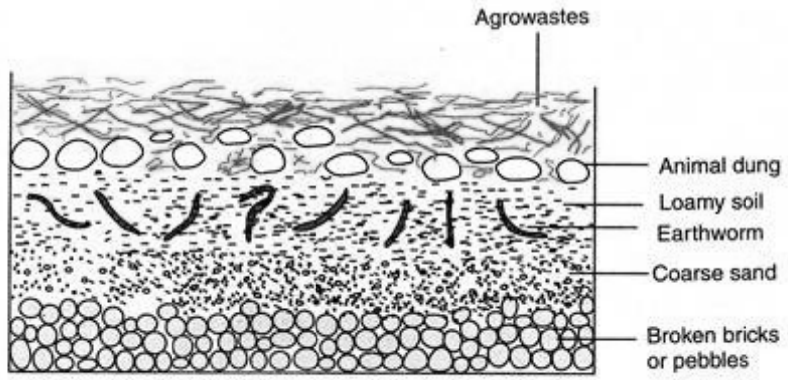


Fig. 33.1 : Diagram of a pit for operation of vermicomposting process.

Required Physical Conditions for Vermicomposting:
During vermicomposting the following conditions, such as:
(i) Moisture content,

(ii) Oxygen,

(iii) Temperature and

(iv) pH of the substrate must be noticed for the vermiculture.

(i) Moisture content: The moisture content must be maintained between 30-40%.

(ii) Oxygen : The concentration of oxygen should be optimum.

(iii) Temperature: For better results, the growth or multiplication of worms takes place between 20-30 °C.

(iv) pH of the substrate: pH of the substrate would be between 6.8-7.5.

Precautions:

1. Vermicompost pit should be completely protected from the direct sun-light.
2. To maintain moisture level, spray water on the pit as and when required.
3. Protect earthworms from birds eat insects, ant, rat.
4. Composting Mixture should be maintained at 35-40% moisture.

NutrientStatus of Vermicompost: Vermicompost is a rich source of macro and micronutrients and several hormones. The chemical and physical composition of V.V are given below-

Constituent	**range**
Organic carbon(%)	14-17.98
pH	5.63-6.7
B.D	0.984 g/cc
Total nitrogen(%)	2.6-3.0
Phosphorus	1.2-1.5
Potassium(%)	1.6-1.733
Iron(mg/l)	2.8-9.3
Zinc(mg/l)	5.7-11.7
copper(mg/l)	2.0-9.5

Application of Vermicompost:

It is a very good alternatives source of organic manure for the plant growth and aquaculture. In case of aquaculture , it does not produce higher levels of H_2S, HNO_3 and free CO_2 On decomposition in pond water and they does not cause harm to the fish culture. It can also be used in horticulture and alternate source of animal protein. Therefore , it is recommended to use of Vermicompost instead of other organic manure.

Earthworms and Plant Growth:

The effect by earthworms on plant growth maybe due to several reasons apart from the presence of macro and micronutrients in their secretions and in Vermicompost in considerable quantities. Certain metabolites produced by earthworms may also be responsible to stimulate the plant growth . It is considered that earthworms released in to the soil certain vitamins and similar substances. Though the NPK value of Vermicompost is always lower than any standard chemical fertilizer, several experiments have proved that worm cast can promote lust growth of plants.

Advantages of Vermicomposting:

1. Vermicompost is an eco-friendly natural fertilizer prepared from different raw, materials, biodegradable organic waste materials and is free from chemical inputs.
2. It does not have any adverse effect on soil , plant and environment.
3. It improves soil accretion , texture and reduce the soil erosion and compaction.
4. It improves water retention capacity of soil because of its high organic matter content.
5. Vermicompost promotes better root growth and nutrient absorption.
6. It improves soil biological, chemical and physical properties.

Bioremediation:

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration.It is rather difficult to show any distinction between biodegradation and bioremediation. Further, in biotechnology, most of the reactions of biodegradation/bioremediation involve xenobiotic.

Key Features of Bioremediation:

1. Most bioremediation treatment technologies destroy the contaminants in the soil matrix.

2. These treatment technologies are generally designed to reduce toxicity either by destruction or by transforming toxic organic compounds into less toxic compounds.

3. Indigenous micro-organisms, including bacteria and fungi, are most commonly used. In some cases, wastes may be inoculated with specific bacteria or fungi known to biodegrade the contaminants in question. Higher plants may also be used to enhance biodegradation and stabilise the soil.

4. The addition of nutrients or electron acceptors (such as hydrogen peroxide or ozone) to enhance growth and reproduction of indigenous organisms may be required.

Xenobiotic:

Xenobiotic (xenos-foregin) broadly refer to the unnatural, foreign and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents and other organic compounds. Microbial degradation of xenobiotic assumes significance, since it provides an effective and economic means of disposing of toxic chemicals, particularly the environmental pollutants.

Pseudomonas — The Predominant Microorganism For Bioremediation:
Members of the genus Pseudomonas (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic. Different strains of Pseudomonas, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycyclic aromatics and naphthalene.

About 40-50 microbial strains of microorganisms, capable of degrading xenobiotics have been isolated. Besides Pseudomonas, other good examples are Mycobacterium, Alcaligenes, and Nocardia. A selected list of microorganisms and the xenobiotics degraded is given in Table 59.1.

TABLE 59.1 A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation

Microorganism	*Pollutant chemicals*
Pseudomonas sp	Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates.
Mycobacterium sp	Benzene, branched hydrocarbons, cycloparaffins
Alcaligenes sp	Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons.
Nocardia sp	Naphthalene, alkylbenzenes, phenoxyacetate.
Arthrobacter sp	Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol.
Corynebacterium sp	Halogenated hydrocarbons, phenoxyacetate.
Bacillus sp	Long chain alkanes, phenylurea.
Candida sp	Polychlorinated biphenyls
Aspergillus sp	Phenols
Xanthomonas sp	Polycyclic hydrocarbons
Streptomyces sp	Halogenated hydrocarbons, phenoxyacetate.
Fusarium sp	Propanil
Cunninghamella sp	Polycyclic aromatics, polychlorinated biphenyls.

Consortia of microorganisms for biodegradation:
A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or

cocktail of microbes) may be more efficient. For instance, the insecticide parathion is more efficiently degraded by the combined action of *Pseudomonas aeruginosa* and *Pseudomonas stulzeri*.

Co-metabolism in biodegradation:

In general, the metabolism (breakdown) of xenobiotics is not associated with any advantage to the microorganism. That is the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term co-metabolism is often used to indicate the non-beneficial (to the microorganism) biochemical pathways concerned with the biodegradation of xenobiotics. However, co- metabolism depends on the presence of a suitable substrate for the microorganism. Such compounds are referred to co-substrates.

Factors Affecting Biodegradation:

Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O_2 supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important.

Some of the relevant features are given hereunder:

i. In general, aliphatic compounds are more easily degraded than aromatic ones.

ii. Presence of cyclic ring structures and length chains or branches decrease the efficiency of biodegradation.

iii. Water soluble compounds are more easily degraded.

iv. Molecular orientation of aromatic compounds influences biodegradation i.e. ortho > para > meta.

v. The presence of halogens (in aromatic compounds) inhibits biodegradation.

Besides the factors listed above, there are two recent developments to enhance the biodegradation by microorganisms.

Bio-stimulation:

This is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents (electron acceptors, surfactants).

Bio-augmentation:

It is possible to increase biodegradation through manipulation of genes. More details on this genetic manipulation i.e. genetically engineered microorganisms (GEMs), are described later. Bio-augmentation can also be achieved by employing a consortium of microorganisms.

Enzyme Systems for Biodegradation:

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics. The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation. The microorganism Pseudomonas occupies a special place in biodegradation.

A selected list of xenobiotics and the plasmids containing the genes for their degradation is given in Table 59.2.

TABLE 59.2 A selected list of xenobiotics and the plasmids containing genes (in *Plasmodium*) for biodegradation

Xenobiotic	*Name of plasmid in* Pseudomonas
Naphthalene	NAH
Xylene	XYL
Xylene and toluene	TOL, pWWO, XYL-K
Salicylate	SAL
Camphor	CAM
3-Chlorobenzene	pAC25

Recalcitrant Xenobiotics:

There are certain compounds that do not easily undergo biodegradation and therefore persist in the environment for a long period (sometimes in years). They are labelled as recalcitrant.

There may be several reasons for the resistance of xenobiotics to microbial degradation:

i. They may chemically and biologically inert (highly stable).

ii. Lack of enzyme system in the microorganisms for biodegradation.

iii. They cannot enter the microorganisms being large molecules or lack of transport systems.

iv. The compounds may be highly toxic or result in the formation highly toxic products that kill microorganisms.

There are a large number of recalcitrant xenobiotic compounds e.g. chloroform, freons, insecticides (DDT, lindane), herbicides (dalapon) and synthetic polymers (plastics e.g. polystyrene, polyethylene, polyvinyl chlorine).

It takes about 4-5 years for the degradation of DDT (75-100%) in the soil. A group of microorganisms (*Aspergillus flavus, Mucor aternans, Fusarium oxysporum*and *Trichoderma viride*) are associated with the slow biodegradation of DDT.

Bio-magnification:

The phenomenon of progressive increase in the concentration of a xenobiotic compound, as the substance is passed through the food chain is referred to as bio-magnification or bioaccumulation. For instance, the insecticide DDT is absorbed repeatedly by plants and microorganism.

When they are eaten by fish and birds, this pesticide being recalcitrant, accumulates, and enters the food chain. Thus, DDT may find its entry into various animals, including man. DDT affects the nervous systems, and it has been banned in some countries.

Types of Bioremediation:

The most important aspect of environmental biotechnology is the effective management of hazardous and toxic pollutants (xenobiotics) by bioremediation. The environmental clean-up process through bioremediation can be achieved in two ways—in situ and ex situ bioremediation.

a. In Situ Bioremediation:

In situ bioremediation involves a direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water). Addition of adequate quantities of nutrients at the sites promotes microbial growth. When these microorganisms are exposed to xenobiotics (pollutants), they develop metabolic ability to degrade them.

The growth of the microorganisms and their ability to bring out biodegradation are dependent on the supply of essential nutrients (nitrogen, phosphorus etc.). In situ bioremediation has been successfully applied for clean-up of oil spillages, beaches etc. There are two types of in situ bioremediation-intrinsic and engineered.

Intrinsic bioremediation:

The inherent metabolic ability of the microorganisms to degrade certain pollutants is the intrinsic bioremediation. In fact, the microorganisms can be tested in the laboratory for their natural capability of biodegradation and appropriately utilized.

Engineered in situ bioremediation:

The inherent ability of the microorganisms for bioremediation is generally slow and limited. However, by using suitable physicochemical means (good nutrient and O_2 supply, addition of electron acceptors, optimal temperature), the bioremediation process can be engineered for more efficient degradation of pollutants.

Advantages of in situ bioremediation:

1. Cost-effective, with minimal exposure to public or site personnel.

2. Sites of bioremediation remain minimally disrupted.

Disadvantages of in situ bioremediation:

1. Very time consuming process.

2. Sites are directly exposed to environmental factors (temperature, O_2 supply etc.).

3. Microbial degrading ability varies seasonally.

b. Ex Situ Bioremediation:

The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms (frequently a consortium of organisms) can be carried out at designed places. This process is certainly an improvement over in situ bioremediation, and has been successfully used at some places.

Advantages of ex situ bioremediation:

1. Better controlled and more efficient process.

2. Process can be improved by enrichment with desired microorganisms.

3. Time required in short.

Disadvantages of ex situ bioremediation:

1. Very costly process.

2. Sites of pollution are highly disturbed.

3. There may be disposal problem after the process is complete.

Metabolic Effects of Microorganisms on Xenobiotics:
Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

Detoxification:
This process involves the microbial conversion of toxic compound to a nontoxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

Activation:
Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

Degradation:
The complex compounds are degraded to simpler products which are generally harmless.

Conjugation:
The process of conjugation may involve the conversion of xenobiotics to more complex compounds. This is however, not very common.

Types of Reactions in Bioremediation:
Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

a. Aerobic bioremediation:
Aerobic biodegradation involves the utilization of O_2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes namely mono-oxygenases and- di-oxygenases are involved in aerobic biodegradation. Mono-oxygenases can act on both aliphatic and aromatic compounds while di-oxygenases oxidize aliphatic compounds.

b. Anaerobic bioremediation:
Anaerobic biodegradation does not require O_2 supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow. However, anaerobic biodegradation is cost- effective, since the need for continuous O_2 supply is not

there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehalogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de-chlorination is frequently used for dehalogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

Sequential Bioremediation*:*
In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

Biodegradation of Hydrocarbons:
Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g. Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium and Nocardia.

Biodegradation of Aliphatic Hydrocarbons:
The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are recalcitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

Biodegradation of Aromatic Hydrocarbons:
Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

The biodegradation of aromatic compounds basically involves the following sequence of reactions:
1. Removal of the side chains.

2. Opening of the benzene ring.

Most of the non-halogenated aromatic compounds undergo a series of reactions to produce catechol or protocatechuate. The bioremediation of toluene, L-mandelate, benzoate, benzene, phenol, anthracene, naphthalene, phenanthrene and salicylate to produce catechol is shown in Fig. 59.1. Likewise, Fig. 59.2, depicts the bioremediation of quinate, p-hydroxymandelate, p-hydroxybenzoylformate, p-toluate, benzoate and vanillate to produce protocatechuate.

Catechol and protocatechuate can undergo oxidative cleavage pathways. In ortho-cleavage pathway, catechol and protocatechuate form acetyl CoA, while in meta-cleavage pathway (Fig. 59.4), they are

converted to pyruvate and acetaldehyde. The degraded products of catechol and protocatechuate are readily metabolised by almost all the organisms.

Biodegradation of Pesticides and Herbicides:

Pesticides and herbicides are regularly used to contain various plant diseases and improve the crop yield. In fact, they are a part of the modern agriculture, and have significantly contributed to green revolution. The common herbicides and pesticides are propanil (anilide), propham (carbamate), atrazine (triazine), picloram (pyridine), dichlorodiphenyl trichloroethane (DDT) monochloroacetate (MCA), monochloropropionate (MCPA) and glyphosate (organophosphate). Most of the pesticides and herbicides are toxic and are recalcitrant (resistant to biodegradation). Some of them are surfactants (active on the surface) and retained on the surface of leaves.

Biodegradation of Halogenated Aromatic Compounds:

Most commonly used herbicides and pesticides are aromatic halogenated (predominantly chlorinated) compounds. The bio-degradative pathways of halogenated compounds are comparable with that described for the degradation of non-halogenated aromatic compounds . The rate of degradation of halogenated compounds is inversely related to the number of halogen atoms that are originally present on the target molecule i.e. compounds with higher number of halogens are less readily degraded.

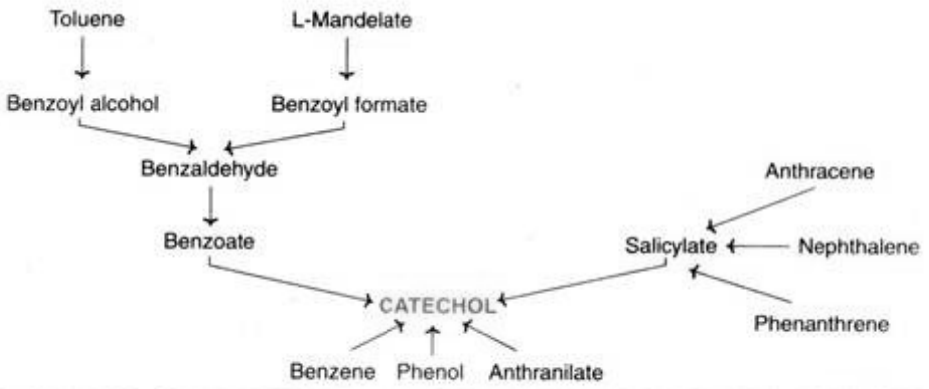


Fig. 59.1 : Bioremediation of certain aromatic compounds by bacteria to produce catechol.

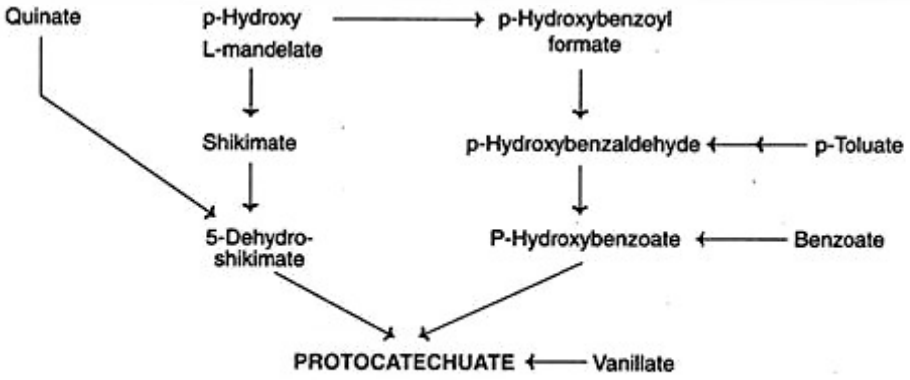


Fig. 59.2 : Bioremediation of certain organic compounds by bacteria to produce protocatechuate.

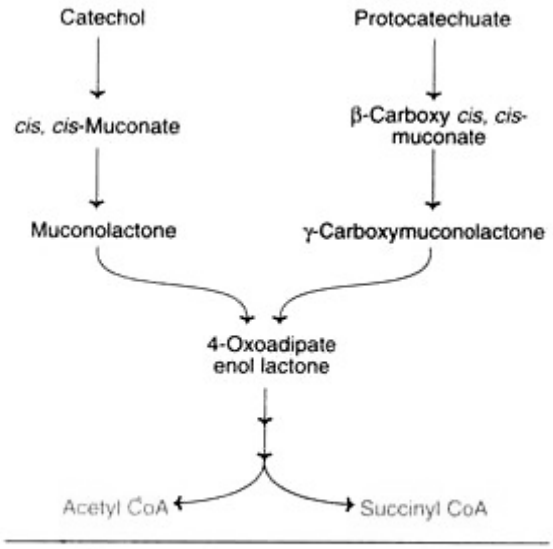


***Fig. 59.3** : Conversion of catechol and protocatechuate to acetyl CoA and succinate by **ortho–cleavage pathway**.*

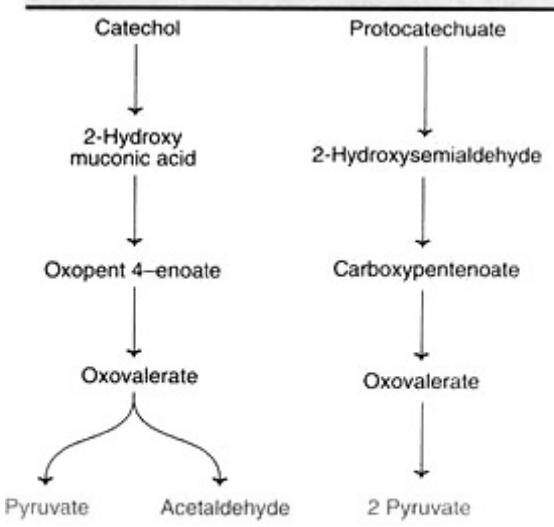


***Fig. 59.4** : Conversion of catechol and protocatechuate to pyruvate and acetaldehyde by **meta–cleavage pathway**.*

Dehalogenation (i.e. removal of a halogen substituent from an organic compound) of halogenated compounds is an essential step for their detoxification. Dehalogenation is frequently catalysed by the enzyme di-oxygenase. In this reaction, there is a replacement of halogen on benzene with a hydroxyl group.

Most of the halogenated compounds are also converted to catechol and protocatechuate which can be metabolised (Fig. 59.4). Besides Pseudomonas, other microorganisms such as *Azotobacter, Bacilluefs* and *E. coli* are also involved in the microbial degradation of halogenated aromatic compounds.

Biodegradation of Polychlorinated Biphenyls (PCBs):
The aromatic chlorinated compounds possessing biphenyl ring (substituted with chlorine) are the PCBs e.g. pentachlorobiphenyl. PCBs are commercially synthesized, as they are useful for various

purposes — as pesticides, in electrical conductivity (in transformers), in paints and adhesives. They are inert, very stable and resistant to corrosion.

However, PCBs have been implicated in cancer, damage to various organs and impaired reproductive function. Their commercial use has been restricted in recent years, and are now used mostly in electrical transformers.

PCBs accumulate in soil sediments due to hydrophobic nature and high bioaccumulation potential. Although they are resistant to biodegradation, some methods have been recently developed for anaerobic and aerobic oxidation by employing a consortium of microorganisms. Pseudomonas, Alkali genes, Corynebacterium and Acinetobacter. For more efficient degradation of PCBs, the microorganisms are grown on biphenyls, so that the enzymes of biodegradation of PCBs are induced.

Biodegradation of Some Other Important Compounds:

Organo-nitro Compounds:
Some of the toxic organo-nitro compounds can be degraded by microorganisms for their detoxification.

2, 4, 6-Trinitrotoluene (TNT):
Certain bacterial and fungal species belonging to Pseudomonas and Clostrium can detoxify TNT.

Nitrocellulose:
Hydrolysis, followed by anaerobic nitrification by certain bacteria, degrades nitrocellulose.

Synthetic detergents:
They contain some surfactants (surface active agents) which are not readily biodegradable. Certain bacterial plasmid can degrade surfactants.

Genetic Engineering for More Efficient Bioremediation:

Although several microorganisms that can degrade a large number of xenobiotics have been identified, there are many limitations in bioremediation:

i. Microbial degradation of organic compounds is a very slow process.

ii. No single microorganism can degrade all the xenobiotics present in the environmental pollution.

iii. The growth of the microorganisms may be inhibited by the xenobiotics.

iv. Certain xenobiotics get adsorbed on to the particulate matter of soil and become unavailable for microbial degradation.

It is never possible to address all the above limitations and carry out an ideal process of bioremediation. Some attempts have been made in recent years to create genetically engineered microorganisms (CEMs) to enhance bioremediation, besides degrading xenobiotics which are highly resistant (recalcitrant) for breakdown. Some of these aspects are briefly described.

Genetic Manipulation by Transfer of Plasmids:

The majority of the genes responsible for the synthesis of bio-degradative enzymes are located on the plasmids. It is therefore logical to think of genetic manipulations of plasmids. New strains of bacteria can be created by transfer of plasmids (by conjugation) carrying genes for different degradative pathways.

If the two plasmids contain homologous regions of DNA, recombination occurs between them, resulting in the formation of a larger fused plasmid (with the combined functions of both plasmids). In case of plasmids which do not possess homologous regions of DNA, they can coexist in the bacterium (to which plasmid transfer was done).

The first successful development of a new strain of bacterium (Pseudomonas) by manipulations of plasmid transfer was done by Chakrabarty and his co-workers in 1970s. They used different plasmids and constructed a new bacterium called as superbug that can degrade a number of hydrocarbons of petroleum simultaneously.

United States granted patent to this superbug in 1981 (as per the directive of American Supreme Court). Thus, superbug became the first genetically engineered microorganism to be patented. Superbug has played a significant role in the development of biotechnology industry, although it has not been used for large scale degradation of oil spills.

Creation of Superbug by Transfer of Plasmids:
Superbug is a bacterial strain of Pseudomonas that can degrade camphor, octane, xylene and naphthalene. Its creation is depicted in Fig. 59.5.

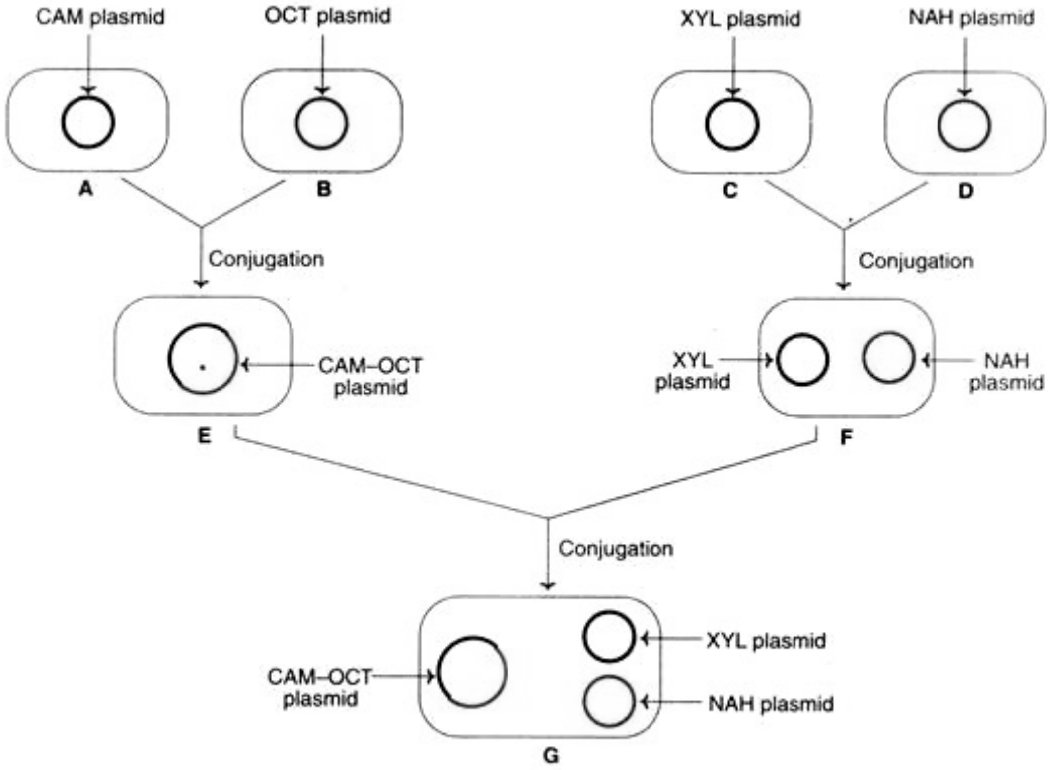


Fig. 59.5 : Creation of the superbug by transfer of plasmids (A, B, C, D, E, F and G are the different strains of bacteria containing the plasmids shown. Strain G is the superbug.)

The bacterium containing CAM (camphor- degrading) plasmid was conjugated with another bacterium with OCT (octane-degrading) plasmid. These plasmids are not compatible and therefore, cannot coexist in the same bacterium. However, due to the presence of homologous regions of DNA, recombination occurs between these two plasmids resulting in a single CAM-OCT plasmid. This new bacterium possesses the degradative genes for both camphor and octane.

Another bacterium with XYL (xylene-degrading) plasmid is conjugated with NAH (naphthalene-degrading) plasmid containing bacterium. XYL and NAH plasmids are compatible and therefore can coexist in the same bacterium. This newly, produced bacterium contains genes for the degradation of xylene and naphthalene.

The next and final step is the conjugation of bacterium containing CAM-OCT plasmid with the other bacterium containing XYL and NAH plasmids. The newly created strain is the superbug that carries CAM-OCT plasmid (to degrade camphor and octane), XYL (xylene-degrading) plasmid and NAH (naphthalene-degrading) plasmid.

Development of Salicylate—Toluene Degrading Bacteria by Plasmid Transfer:
Some attempts have been made for the creation of a new strain of the bacterium Pseudomonas putida to simultaneously degrade toluene and salicylate. Toluene-degrading (TOL) plasmid was transferred by conjugation to another bacterium that is capable of degrading salicylate (due to the presence of SAL plasmid).

The newly developed strain of Pseudomonas can simultaneously degrade both toluene and salicylate. And this occurs even at a low temperature (0-5°C). However, the new bacterium is not in regular use, as more research is being conducted on its merits and demerits.

Genetic Manipulation by Gene Alteration:
Work is in progress to manipulate the genes for more efficient biodegradation. The plasmid pWWO of Pseudomonas codes for 12 different enzymes responsible for the meta-cleavage pathway (for the conversion of catechol and protocatechuate to pyruvate and acetaldehyde, for degradation of certain aromatic compounds. Some success has been reported to alter the genes of plasmid pWWO for more efficient degradation of toluene and xylene.

Genetically Engineered Microorganisms (GEMs) in Bioremediation:
Superbug is the first genetically engineered microorganism. Several workers world over have been working for the creation of GEMs, specifically designed for the detoxification of xenobiotics. A selected list of GEMs with a potential for the degradation of xenobiotics is given in Table 59.3. Almost all these CFMs have been created by transferring plasmids.

Table 59.3 A selected list of genetically engineered microorganisms (GEMs) with the potential xenobiotics that can be degraded

Genetically engineered microorganism (GEMs)	*Xenobiotic*
Pseudomonas diminuta	Parathion
P. oleovorans	Alkane
P. cepacia	2, 4, 5-Trichlorophenol
P. putida	Mono- and dichloro-aromatic compounds
Alcaligenes sp	2, 4-Dichlorophenoxy acetic acid
Acinetobacter sp	4-Chlorobenzene

Bio-surfactant Producing GEM:

A genetically engineered *Pseudomonas aeruginosa* has been created (by Chakarabarty and his group). This new strain can produce a glycolipid emulsifier (a bio-surfactant) which can reduce the surface tension of an oil water interface. The reduced interfacial tension promotes biodegradation of oils.

GEM for Degradation of Vanillate and SDS:

A new strain of *Pseudomonas* sp (strain ATCC 1915) has been developed for the degradation of vanillate (waste product from paper industry) and sodium dodecyl sulphate (SDS, a compound used in detergents).

GEMs and Environmental Safety:

The genetically engineered microorganisms (GEMs) have now become handy tools of biotechnologists. The risks and health hazards associated with the use of GEMs are highly controversial and debatable issues. The fear of the biotechnologists and even the general public is that the new organism (GEM), once it enters the environment, may disturb the ecological balance and cause harm to the habitat. Some of the GEMs may turn virulent and become genetic bombs, causing great harm to humankind.

Because of the risks involved in the use of GEMs, so far no GEM has been allowed to enter the environmental fields. Thus, the use of GEMs has been confined to the laboratories, and fully controlled processes of biodegradation (usually employing bioreactors). Further, several precautionary measures are taken while creating GEMs, so that the risks associated with their use are minimal.Some researchers are of the opinion that GEMs will create biotechnological wonders for the environmental management of xenobiotics, in the next few decades. This may be possible only if the associated risks of each GEM is thoroughly evaluated, and fully assured of its biosafety.

Bioremediation of Contaminated Soils and Waste Lands:

Due to industrialization and extensive use of insecticides, herbicides and pesticides, the solids and waste lands world over are getting polluted. The most common pollutants are hydrocarbons, chlorinated solvents, polychlorobiphenyls and metals.Bioremediation of soils and waste lands by the use of microorganisms is gaining importance in the recent years. In fact, some success has been reported for the detoxification of certain pollutants (e.g. hydrocarbons) in the soil by microorganisms. Bioremediation of soils can be done by involving two principles-bio-stimulation and bio-augmentation.

Bio-stimulation in Soil Bioremediation:

Bio-stimulation basically involves the stimulation of microorganisms already present in the soil, by various means.

This can be done by many ways:

i. Addition of nutrients such as nitrogen and phosphorus.

ii. Supplementation with co-substrates e.g. methane added to degrade trichloroethylene.

iii. Addition of surfactants to disperse the hydrophobic compounds in water.

Addition of nutrient and co-substrates promote microbial growth while surfactants expose the hydrophobic molecules. In all these situations, the result is that there occurs bio-stimulation by effective bioremediation of polluted soil or waste land.

Bio-augmentation in Soil Bioremediation:

Addition of specific microorganisms to the polluted soil constitutes bio-augmentation. The pollutants are very complex molecules and the native soil microorganisms alone may not be capable of degrading them effectively. The examples of such pollutants include polychlorobiphenyls (PCBs), trinitrotoluene (TNT), polyaromatic hydrocarbons (PAHs) and certain pesticides.

Based on the research findings at the laboratory level (with regard to biodegradation), it is now possible to add a combination of microorganisms referred to as consortium or cocktail of microorganisms, to achieve bio-augmentation.With the development of genetically engineered microorganisms (GEMs), they can be also used to bio-augment soils for very efficient bioremediation. But the direct use of GEMs in the soils is associated with several risks and health hazards.

Techniques of Soil Bioremediation:

The most commonly used methods for the bioremediation are soils are in situ bioremediation, land farming and slurry phase bioreactors.

In Situ Bioremediation of Soils:

In situ bioremediation broadly involves the biological clean-up of soils without excavation. This technique is used for the bioremediation of sub-surfaces of soils, buildings and road ways that are polluted. Sometimes, water (oxygenated) is cycled through the sub-surfaces for increasing the efficiency of microbial degradation. There are two types of in situ soil bioremediation techniques-bioventing and phytoremediation.

Bioventing:

This is very efficient and cost- effective technique for the bioremediation of petroleum contaminated soils. Bioventing involves aerobic biodegradation of pollutants by circulating air through sub-surfaces of soil. Although, it takes some years, bioventing can be used for the degradation of soluble paraffin's and polyaromatic hydrocarbons. The major limitation of this technique is air circulation which is not always practicable.

Phytoremediation:

Bioremediation by use of plants constitutes phytoremediation. Specific plants are cultivated at the sites of polluted soil. These plants are capable of stimulating the biodegradation of pollutants in the soil adjacent to roots (rhizosphere) although phytoremediation is a cheap and environmental friendly clean-up process for the biodegradation of soil pollutants, it takes several years.

Land farming in Soil Bioremediation:

Land farming is a technique for the bioremediation of hydrocarbon contaminated soils. A diagrammatic representation of land farming system (also referred to as solid phase soil reactor) is depicted in Fig. 59.6.

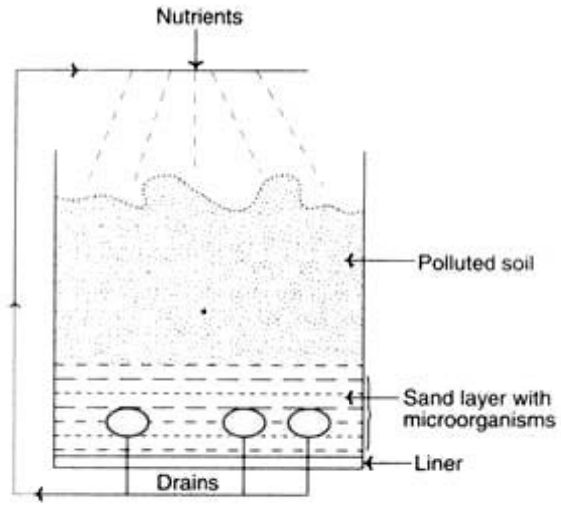


Fig. 59.6 : A diagrammatic representation of landfarming system.

The soil is excavated, mixed with microorganisms and nutrients and spread out on a liner, just below the polluted soil. The soil has to be regularly ploughed for good mixing and aeration. If the soil is mixed with compost and/or temperature is increased the efficiency of biodegradation increases.

Addition of co-substrates, and anaerobic pre-treatment of polluted soils also increases the degradation process. Land farming has been successfully used for the bioremediation of soils polluted with chloroethane benzene, toluene and xylene. The last three compounds are often referred to as BTX aromatics.

Bioreactors:

Bioremediation may also be conducted in a bioreactor, in which the contaminated soil or sludge is slurred with water in a mixing tank or a lagoon. Bioremediation systems require that the contaminated soil or sludge be sufficiently and homogeneously mixed to ensure optimum contact with the seed organisms.Bioreactors function in a manner that is similar to sewage treatment plants. There are many ways in which a bioreactor can be designed; but most are a modification of one of two systems. In the first system, which is often referred to as a trickling filter or fixed media system.

The second common bioreactor design uses a sealed vessel to mix the contaminants, amendments and micro-organisms. Recent research has expanded the capabilities of this technology, which along with its generally lower cost, has led to bioremediation becoming an increasingly attractive clean-up technology.

Slurry-phase Bioreactors in Soil Bioremediation:

Slurry-phase bioreactors are improved land farming systems. In these cases, the excavated polluted soil is subjected to bioremediation under optimally controlled conditions in specifically designed bioreactors. Due to a close contact between the xenobiotics and the microorganisms, and the optimal conditions (nutrient supply, temperature, aeration etc.), the degradation is very rapid and efficient. Slurry-phase bioreactors, however, are not suitable for widespread use due to high cost.

Bioremediation Application:

Bioremediation is the biological clean up alternatives. During past few decades this process opened up a new option in pollution abatement and cleans up of various contaminated environment. Usually variety of micro-organisms viz., bacteria and fungi were known to be the major players in the whole technology. In contrary the term phytoremediation involves the use of higher plants in clean process so that environment is harmless.

The objective of bioremediation is to exploit naturally occurring bio-degradative process to clean up contaminated sites. Firstly large number of microorganisms were characterised for use in bioremediation of diverse toxicants viz., metals, radio nuclides, phenolic compounds, pesticides and polyaromatic compounds. The process of microbial biodegradation may be of aerobic or anaerobic categories.

Limitations of Bioremediation:

The problems of onsite bioremediation by microbes are often seen for two major reasons:

First, the introduced microbe often cannot establish a niche in the environment. In fact, these introduced organisms often do not survive in a new environment beyond a few weeks.

Second, there are difficulties in delivering the introduced organisms to the site of contamination, because microorganisms like contaminants, can be strongly absorbed by solid surfaces.

Probable Questions:

1. Define vermicompost and vermicomposting.

2. state distribution and features of any four earthworm which are used in vermicomposting.

3. Describe the process of vermicomposting.

4. What are the physical conditions required for vermicomposting.

5. State the nutrient status of vermicompost.

6. Write about the nutrient status of vermicompost.

7. What are the advantages of vermicompost.

8. Define bioremediation. What is called xenobiotics?

9. Describe in situ and ex situ bioremediation.

10. What is bio augmentation and biomagnification. Give examples.

11. Describe superbug formation with suitable diagram.

12. Describe applications of Bioremediation.

13. Describe limitations of Bioremediation.

14. what is Bioreactor?

Suggested Readings:

Dr. Narendra Kumar .2005. Vermicompost and its importance for soil quality and sustainable crop production,Dept. Soil Science,G.B.PantUniv.ofAgriculture&Tech.Pantnagar,Uttarakhand.Punjab State Council for Science and Technology, Chandigarh.2008.

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Deo Kumar.2008Analyzing impact of Vermicompost in various combinations on physio-chemical properties of soil and wheat crop(Ph.D Thesis)

Richard Tom,NancyTroutmann C/M Ration corwell composting. Composting in school.Corwell University College of Agriculture and life Sciences.2007

Unit-X

Organic Farming

Objectives: In this Unit you will know about various aspects of Organic Farming.

Introduction:

Organic farming is an alternative agricultural system which originated early in the 20th century in reaction to rapidly changing farming practices. Organic farming continues to be developed by various organic agriculture organizations today. It relies on fertilizers of organic origin such as compost manure, green manure, and bone meal and places emphasis on techniques such as crop rotation and companion planting.

Organic agriculture can be defined as:

an integrated farming system that strives for sustainability, the enhancement of soil fertility and biological diversity whilst, with rare exceptions, prohibiting synthetic pesticides, antibiotics, synthetic fertilizers, genetically modified organisms, and growth hormones.

Since 1990 the market for organic food and other products has grown rapidly, reaching $63 billion worldwide in 2012. This demand has driven a similar increase in organically managed farmland that grew from 2001 to 2011 at a compounding rate of 8.9% per annum. As of 2011, approximately 37,000,000 hectares worldwide were farmed organically, representing approximately 0.9 percent of total world farmland.

History:

Agriculture was practiced for thousands of years without the use of artificial chemicals. Artificial fertilizers were first created during the mid-19th century. These early fertilizers were cheap, powerful, and easy to transport in bulk. Similar advances occurred in chemical pesticides in the 1940s, leading to the decade being referred to as the 'pesticide era'. These new agricultural techniques, while beneficial in the short term, had serious longer term side effects such as soil compaction, erosion, and declines in overall soil fertility, along with health concerns about toxic chemicals entering the food supply. In the late 1800s and early 1900s, soil biology scientists began to seek ways to remedy these side effects while still maintaining higher production & introduced biodynamic agriculture or organic farming, they used organic matter derived from plant compost & animal manures to improve the humus contents of soils, grounded in the work of early soil scientists who developed what was then called **humus farming .**

Differences between Organic and Conventional Farming Methods

In conventional farming method, before seeds are sown, the farmer will have to treat or fumigate his farm using harsh chemicals to exterminate any naturally existing fungicides. He will fertilize the soil using petroleum based fertilizers. On the flip side, the organic farmer will prepare and enrich his land before sowing by sprinkling natural based fertilizers such as manure, bone meal or shellfish fertilizer.

Before planting seeds, the organic farmer will soak the seeds in fungicides and pesticides to keep insects and pests at bay. Chemical are also incorporated in the irrigation water to prevent insects from stealing the planted seeds. On the other hand, the organic farmer will not soak his seeds in any chemical solution nor irrigate the newly planted seeds using water with added chemicals. In fact, he

will not even irrigate with council water, which is normally chlorinated to kill any bacteria. He will depend on natural rain or harvest and stored rainwater to use during dry months.

When the seeds have sprung up, and it's time to get rid of weeds, the conventional farmer will use weedicide to exterminate weeds. The organic farmer will not use such chemicals to get rid of the weed problem. Instead, he will physically weed out the farm, although it's very labor intensive. Better still, the organic farmer can use a flame weeder to exterminate weeds or use animals to eat away the weeds.When it comes to consumption, it's a no-brainer that anyone consuming products from the conventional farmer will absorb the pesticide and weedicide residues into the body, which could lead to developing dangerous diseases like cancer. People understand that health is important to them and that's why they are going organic in record numbers today.

Reasons For Organic Farming

The population of the planet is skyrocketing and providing food for the world is becoming extremely difficult. The need of the hour is sustainable cultivation and production of food for all. The Green Revolution and its chemical based technology are losing its appeal as dividends are falling and returns are unsustainable. Pollution and climate change are other negative externalities caused by use of fossil fuel based chemicals.In spite of our diet choices, organic food is the best choice you'll ever make, and this means embracing organic farming methods. Here are the reasons why we need to take up organic farming methods:

1. To accrue the benefits of nutrients

Foods from organic farms are loaded with nutrients such as vitamins, enzymes, minerals and other micro-nutrients compared to those from conventional farms. This is because organic farms are managed and nourished using sustainable practices. In fact, some past researchers collected and tested vegetables, fruits, and grains from both organic farms and conventional farms.The conclusion was that food items from organic farms had way more nutrients than those sourced from commercial or conventional farms. The study went further to substantiate that five servings of these fruits and vegetables from organic farms offered sufficient allowance of vitamin C. However, the same quantity of fruits and vegetable did not offer the same sufficient allowance.

2. Stay away from GMOs

Statistics show that genetically modified foods (GMOs) are contaminating natural foods sources at real scary pace, manifesting grave effects beyond our comprehension. What makes them a great threat is they are not even labelled. So, sticking to organic foods sourced from veritable sources is the only way to mitigate these grave effects of GMOs.

3. Natural and better taste

Those that have tasted organically farmed foods would attest to the fact that they have a natural and better taste. The natural and superior taste stems from the well balanced and nourished soil. Organic farmers always prioritize quality over quantity.

4. Direct support to farming

Purchasing foods items from organic farmers is a surefire investment in a cost-effective future. Conventional farming methods have enjoyed great subsidies and tax cuts from most governments over

the past years. This has led to the proliferation of commercially produced foods that have increased dangerous diseases like cancer. It's time governments invested in organic farming technologies to mitigates these problems and secure the future. It all starts with you buying food items from known organic sources.

5. To conserve agricultural diversity

These days, it normal to hear news about extinct species and this should be a major concern. In the last century alone, it is approximated that 75 percent of agricultural diversity of crops has been wiped out. Slanting towards one form of farming is a recipe for disaster in the future. A classic example is a potato. There were different varieties available in the marketplace. Today, only one species of potato dominate.This is a dangerous situation because if pests knock out the remaining potato specie available today, we will not have potatoes anymore. This is why we need organic farming methods that produce disease and pest resistant crops to guarantee a sustainable future.

6. To prevent antibiotics, drugs, and hormones in animal products

Commercial dairy and meat are highly susceptible to contamination by dangerous substances. A statistic in an American journal revealed that over 90% of chemicals the population consumes emanate from meat tissue and dairy products. According to a report by Environmental Protection Agency (EPA), a vast majority of pesticides are consumed by the population stem from poultry, meat, eggs, fish and dairy product since animals and birds that produce these products sit on top of the food chain.

This means they are fed foods loaded with chemicals and toxins. Drugs, antibiotics, and growth hormones are also injected into these animals and so, are directly transferred to meat and dairy products. Hormone supplementation fed to farmed fish, beef and dairy products contribute mightily to ingestion of chemicals. These chemicals only come with a lot of complications like genetic problems, cancer risks, growth of tumor and other complications at the outset of puberty.

Key Features of Organic Farming

a.Protecting soil quality using organic material and encouraging biological activity

b.Indirect provision of crop nutrients using soil microorganisms

c.Nitrogen fixation in soils using legumes

d.Weed and pest control based on methods like crop rotation, biological diversity, natural predators, organic manures and suitable chemical, thermal and biological intervention

e.Rearing of livestock, taking care of housing, nutrition, health, rearing and breeding

f.Care for the larger environment and conservation of natural habitats and wildlife

Four Principles of Organic Farming

a. Principle of Health: Organic agriculture must contribute to the health and well being of soil, plants, animals, humans and the earth. It is the sustenance of mental, physical, ecological and social well being. For instance, it provides pollution and chemical free, nutritious food items for humans.

b. Principle of Fairness: Fairness is evident in maintaining equity and justice of the shared planet both among humans and other living beings. Organic farming provides good quality of life and helps in reducing poverty. Natural resources must be judiciously used and preserved for future generations.

c. Principle of Ecological Balance: Organic farming must be modelled on living ecological systems. Organic farming methods must fit the ecological balances and cycles in nature.

d. Principle of Care: Organic agriculture should be practiced in a careful and responsible manner to benefit the present and future generations and the environment.

As opposed to modern and conventional agricultural methods, organic farming does not depend on synthetic chemicals. It utilizes natural, biological methods to build up soil fertility such as microbial activity boosting plant nutrition.

Secondly, multiple cropping practiced in organic farming boosts biodiversity which enhances productivity and resilience and contributes to a healthy farming system. Conventional farming systems use mono cropping that destroys the soil fertility.

Methods of Organic Farming:

"Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved..." stated by *International Federation of Organic Agriculture Movements.* Organic farming methods combine scientific knowledge of ecology and modern technology with traditional farming practices based on naturally occurring biological processes. Organic farming methods are studied in the field of agroecology. While conventional agriculture uses synthetic pesticides and water-soluble synthetically purified fertilizers, organic farmers are restricted by regulations to using natural pesticides and fertilizers. The principal methods of organic farming include crop rotation, green manuresand compost, biological pest control, and mechanical cultivation. These measures use the natural environment to enhance agricultural productivity: legumes are planted to fix nitrogen into the soil, natural insect predators are encouraged, crops are rotated to confuse pests and renew soil, and natural materials such as potassium bicarbonate and mulches are used to control disease and weeds. Genetically modified seeds and animals are excluded.

Advantages:

1. Crop diversity: Organic farming encourages Crop diversity. The science of agroecology has revealed the benefits of polyculture (multiple crops in the same space), which is often employed in organic farming. Planting a variety of vegetable crops supports a wider range of beneficial insects, soil microorganisms, and other factors that add up to overall farm health. Crop diversity helps environments thrive and protects species from going extinct.
2. Soil managements: Organic farming relies heavily on the natural breakdown of organic matter, using techniques like green manure and composting, to replace nutrients taken from the soil by previous crops. This biological process, driven by microorganisms such as mycorrhiza, allows the natural production of nutrients in the soil throughout the growing season, and has been referred to as *feeding the soil to feed the plant.* Organic farming uses a variety of methods to improve soil fertility, including crop rotation, cover cropping, reduced tillage, and application of compost. By reducing tillage, soil is not inverted and

exposed to air; less carbon is lost to the atmosphere resulting in more soil organic carbon. This has an added benefit of carbon sequestration, which can reduce green house gases and help reverse climate change.

3. Weed managements: Organic weed management promotes weed suppression, rather than weed elimination, by enhancing crop competition and phytotoxic effects on weeds. Organic farmers integrate cultural, biological, mechanical, physical and chemical tactics to manage weeds without synthetic herbicides.
4. Controlling other organisms: Organisms aside from weeds that cause problems on organic farms include arthropods (e.g., insects, mites), nematodes, fungi and bacteria.

- **i**. encouraging predatory beneficial insects to control pests by serving them nursery plants

  and/or an alternative habitat, usually in a form of a shelterbelt, hedgerow, or beetle bank.

- **ii.**rotating crops to different locations from year to year to interrupt pest reproduction cycles;
- **iii.**planting companion crops and pest-repelling plants that discourage or divert pests.

5. Livestock: Raising livestock and poultry, for meat, dairy and eggs, is another traditional farming activity that complements growing. Organic farms attempt to provide animals with natural living conditions and feed. They use organic feed for livestock.
6. It increases sustainability & openness.
7. It also increases self sufficiency.
8. It is safe for human health.
9. Food security increases .

Disadvantages:

Agriculture imposes negative externalities (uncompensated costs) upon society through public land and other public resource use, biodiversity loss, erosion, pesticides, nutrient runoff, subsidized water usage, subsidy payments and assorted other problems.

i. **Environmental impacts & emissions**: It has been observed that organic products are sometimes worse for the environment. Organic milk, cereals, and pork generated higher greenhouse gas emissions per product than conventional ones but organic beef and olives had lower emissions in most studies.

ii. **Nutrient leaching:** nitrogen leaching, nitrous oxide emissions, ammonia emissions, eutrophication potential and acidification potential were higher for organic products.Excess nutrients in lakes, rivers, and groundwater can cause algal blooms, eutrophication, and subsequent dead zones. In addition, nitrates are harmful to aquatic organisms by themselves.

iii. **Land use:** The Oxford meta-analysis of 71 studies found that organic farming requires 84% more land for an equivalent amount of harvest, mainly due to lack of nutrients but sometimes due to weeds, diseases or pests, lower yielding animals and land required for fertility building crops. While organic farming does not necessarily save land for wildlife habitats and forestry in all cases, the most modern breakthroughs in organic are addressing these issues with success. It uses less energy but more land than the conventional one.

Benefits of Organically Grown Food Items :

i. Better Nutrition: As compared to a longer time conventionally grown food, organic food is much richer in nutrients. Nutritional value of a food item is determined by its mineral and vitamin content. Organic farming enhances the nutrients of the soil which is passed on to the plants and animals.

ii. Helps us stay healthy: Organic foods do not contain any chemical. This is because organic farmers don't use chemicals at any stage of the food-growing process like their commercial counterparts. Organic farmers use natural farming techniques that don't harm humans and environment. These foods keep dangerous diseases like cancer and diabetes at bay.

iii. Free of poison: Organic farming does not make use of poisonous chemicals, pesticides and weedicides. Studies reveal that a large section of the population fed on toxic substances used in conventional agriculture have fallen prey to diseases like cancer. As organic farming avoids these toxins, it reduces the sickness and diseases due to them.

iv. Organic foods are highly authenticated: For any produce to qualify as organic food, it must undergo quality checks and the creation process rigorously investigated. The same rule applies to international markets. This is a great victory for consumers because they are getting the real organic foods. These quality checks and investigations weed out quacks who want to benefit from the organic food label by delivering commercially produced foods instead.

v. Lower prices: There is a big misconception that organic foods are relatively expensive. The truth is they are actually cheaper because they don't require application of expensive pesticides, insecticides, and weedicides. In fact, you can get organic foods direct from the source at really reasonable prices.

vi. Enhanced Taste: The quality of food is also determined by its taste. Organic food often tastes better than other food. The sugar content in organically grown fruits and vegetables provides them with extra taste. The quality of fruits and vegetables can be measured using Brix analysis.

vii. Organic farming methods are eco-friendly: In commercial farms, the chemicals applied infiltrate into the soil and severely contaminate it and nearby water sources. Plant life, animals, and humans are all impacted by this phenomenon. Organic farming does not utilize these harsh chemicals so; the environment remains protected.

viii. Longer shelf–life: Organic plants have greater metabolic and structural integrity in their cellular structure than conventional crops. This enables storage of organic food for a longer time.

Probable Questions:

1. Define Organic farming.

2. State the differences between Organic and Conventional Farming Methods

3. What are the reasons for introducing organic farming.

4. Write the key features of organic farming.

5. Describe four principles of organic farming.

6. What are the advantages of organic farming.

7. What are the disadvantages of organic farming.

Suggested Readings:

1. A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

Unit-XI

Insect pollinators in agriculture

Objectives: In this unit you will learn about the important role played by the insect pollinators in agriculture.

Introduction:

Animal-mediated pollination plays an important functional role in most terrestrial ecosystems and provides a key ecosystem service vital to the maintenance of both wild and agricultural plant communities as most angiosperms are pollen-limited and rely on animals for sexual reproduction. A large proportion of the human diet depends directly or indirectly on animal pollination. Insects are the primary animal-mediated pollinators of 80% of all plant species in world, including most fruits, many vegetables and some biofuel crops and include diverse species of Hymenoptera (bees, solitary species, bumblebees, pollen wasps and ants), Diptera (bee flies, houseflies, hoverflies), Lepidoptera (butterflies and moths), Coleoptera (flower beetles), and other insects. Insects especially are of enormous importance in the pollinations of many agriculturally important crops. Although gravity, wind, water, mollusks, birds, bats and humans are agents of pollination, it is often possible to manipulate insects in their performance on high value cropping systems. Presently insect pollinators are considered to be in a state of decline due to a range of recent and projected environmental changes, such as habitat loss and climate change, with unknown consequences for pollination service delivery.

Insect-Pollinated Flowers Classified According to Insect Adaptation

a. Pollen Flowers (Group I):

Flowers such as rose, poppy, elderberry and potato, have no nectar but may be scented, they are generally conspicuous, simple, regular, with pollen freely exposed and usually abundant. A great variety of color types are included. Insects involved are usually Syrphidae flies,soldier flies and pollen feeding beetles. Many pollen gathering bees, including honeybees, usually frequent these flowers. They are generally unattractive to Colletiidae bees, male bees, bee flies, moths, butterflies and hummingbirds.

b. Flowers with Exposed Nectar (Group II):

Maple, carrot, some elderberries, Euphorbia, poison oak, grapes and saxifrage flowers are included here. Their sparce nectar is freely exposed as droplets, the flowers are simple, open and regular, and the inflorescence is usually inconspicuous and greenish-white. They attract many kinds of wasps and short-tongued flies and bees. They are not very attractive to long-tongued bees or flies and Lepidoptera, but some are attractive to honeybees.

c. Flowers with Partly Concealed Nectar (Group III):

Examples are strawberry, cactus, raspberry, stone fruits, many cruciferous species and buttercups. Here the nectar is partly concealed by numerous stamens or hairs or overlapping petals. The flowers are usually completely open only in sunshine and may be moderately to quite conspicuous. White and yellow colors predominate, but pink can be common. Attracted insects are Syrphidae flies and short-tonged bees. Some Rosaceae are also attractive to long-tonged bees and honeybees. Sawflies are common on many species, and some beetles and butterflies may also be attracted.

d. Flowers with Concealed Nectar (but not sexual organs or deep narrow corollas) (Group IV):

Currant, onion, orange, mallow and blueberry are included here. The nectar is completely hidden in pouches or by hair tufts. The flowers usually have corolla tubes and may be somewhat irregular. They are generally conspicuous flowers with blue, red or violet predominating. Long-tongued bees and honeybees are attracted. Also some short-tonged bees, bee flies, long-tongued wasps, Lepidoptera. Rarely attracted are most wasps and short-tongued flies and beetles.

e. Social Flowers with Completely Concealed Nectar (Group V):

The Compositae such as dandelion, aster, sunflower and *Scabiosa* are included. The nectar is hidden in narrow but not deep corolla tubes, but access to nectar is blocked by the stigma and the cone of stamens. Pollen is very abundant. The inflorescence is conspicuous because of the grouping of flowers into heads. The color groups white and yellow, and red and blue, are attractive. This group is very attractive to short and long-tongued bees, many butterflies and polleniferous beetles and Syrphidae flies. Insects that visit white and yellow flowers in this group are akin to those visiting flowers with partly concealed nectar, while those visiting red, blue and purple flowers are akin to those visiting flowers with concealed nectar.

f. Hymenoptera Flowers (Group VI):

Violets, legumes, sages, mints, monkshood, *Delphinium*, iris and some lilies are included here. The nectar is concealed in bilaterally symmetrical flowers with slightly long corolla tubes closed at the throat. The sexual organs are usually partially concealed by modified petals that require operation of a special mechanism to expose them. They are usually positioned horizontally, with special landing structures for the pollinator. These flowers are visited primarily by medium to long-tongued bees that can operate the mechanisms to get at the pollen and nectar. Lepidoptera that visit these flowers generally do not operate the mechanism exposing pollen so they do not accomplish pollination. They are visited in the same manner by long-tongued Conopidae flies and bee flies. Many have such deep nectarines as to be accessible only to bumble bees and a few other insects. Others have tough tripping mechanisms that require large, powerful bees for pollination. Other bees may bite holes in the corollas to rob the nectar without pollinating. One group of Hymenoptera flower might be called "wasp flower." It has a ventral pouch filled with nectar and a dull red color.

g. Lepidoptera Flowers (Group VII):

This group includes such species as tobacco, trumpet flowers, honeysuckle, croc gentian, many orchids and some lilies. The flowers bear nectar at the base of long, narrow corolla tubes and spurs. They are rather large and conspicuous with a strong scent. Mainly Lepidopterapollinate these, but long-tongued Hymenoptera may frequent some species. In tropical areas stingless bees are able to crawl into the slender corollas and spurs. Long-tongued bee flies may also use them. Hummingbirds and honey birds are also important pollinators in tropical regions. Within the Lepidoptera butterfly and moth flowers differ. Butterfly flowers have variable colors and they usually open and are fragrant during daytime. On the other hand, moth flowers usually open and are fragrant only at night. They are generally white or pale colored.

h. Special Types of Flowers (Group VIII):

Nauseous flowers that are attractive to flies include some umbellifera, calla lilies, skunk cabbage and many types of saxifrage. They may give off odors of feces, carrion or ammonia. They are especially attractive to filth flies, dung beetles and others.

Pitfall flowers are also often nauseous. Included are Jack-in-the-pulpit, pitcher plants and Dutchman's pipe. They capture flies, holding them until they become covered with pollen, after which they are released before the stigma is receptive.

Pinch-trap flowers include the milkweeds and some orchids. The pollen born on "pollenia" fastens onto visitors and are later pulled off in stigmatic grooves of the pistil. These are attractive to flies, bees and wasps. Syrphid fly flowers include *Veratrum* and *Veronica*. The flowers bear radiating streaks that lead to small, definite centers. Two long stamens are able to dehisce on the back of the syrphid fly when grasped at the base. Only syrphids are able to accomplish this.

Small insect flowers include some aquatic species and euphorbias and figs. There is an array of minute flowers that are attractive to tiny insects. The flowers may be clustered in a hollow receptacle (as in the fig) with an opening to the inflorescence that is just large enough to accommodate the tiny insect.

Importance of Pollination to Agriculture

Insects in their pollination activities have a direct impact on the evolution of flora and fauna. It is believed that angiosperm plants and the more highly evolved insects evolved together. Primitive flowering plants are all insect pollinated. Therefore, grasses and all other angiosperms arose from plants dependent upon insects. Some beetles, most Hymenoptera, many Diptera and almost all Lepidoptera are dependent upon materials provided by flowers. Without angiosperms the evolution of mammals would certainly have been different. Rodents, herbivores and primates are especially dependent upon the products of flowering plants. Thus, angiosperms were a required forerunner to the stocks, which gave rise to humans, and insect pollination was necessary to the development of angiosperms.

There would be grave consequences for the flora and fauna were pollinating insects to disappear or cease pollinating. Many types of plants would most likely perish eventually because in time they would be dependent on insect pollination for competitive reproduction. These would embrace by far most of the angiosperms. Certain elements of flora would rapidly perish. Plants that are usually propagated by seed are dependent upon insects for adequate pollination. Included here would probably be over half of the existing species. Plants that usually propagate asexually could probably survive for many seasons or generations. But asexual propagants are very limited in powers of dissemination and those species would have a fixed genetics incapable of adjusting to changes, which would be expected to be rapid under such conditions. Self-fertile plants that are capable of auto-self pollination might be able to persist longer. However, most of these are dependent upon occasional crossing in order to retain vigor. All would require some crossing in order to retain the genetic plasticity necessary to adjust to changing environmental conditions.

Some plants might survive indefinitely without insect pollinators and some might increase in the absence of normal competition. These include many nut-bearing trees, grasses, all conifers, and various other wind pollinated plants such as poplars, birches, elms, alders, etc. Even so, many grasses and other plants most certainly depend upon the surrounding flora for their survival. Those plants that are produced as crops by humans and propagated by asexual means might also be unaffected. Breeding for disease resistance, for example, could be done with hand pollination. Nevertheless, there are many consequences of a drastic reduction and elimination of most floras. These include the loss of plants with nitrifying bacteria, soil erosion, a drastic curtailment of the human diet, loss in forage values for livestock, loss of many kinds of animals, loss of most kinds of wild flowers, and a general upset in the balance of nature, with unpredictable results.

Advanced agriculture manages the production of products that require pollination, which are primarily fruits and seeds. Seeds are used for general plant propagation and for bedded plants. Some plants like papaya require occasional seeding; alfalfa is seeded every few years and spinach is seeded annually. Alfalfa and forage grasses often require a large amount of seed, while tomatoes and melons need little seeding. Plant breeding by crossing, selfing and selecting is done with pollination and

planting with seeds. Plant products that are consumed directly include cereals, beans, nuts, oils, fruits, preserves and many vegetables. Seeds such as grains, oilcake and peanuts are also used for livestock feed. Many seeds are used as medicines, spices and flavorings. Seeds, fruit oils and seed fibers are deployed in industry for soaps, paints, plastics, explosives, alcohol and textiles.

Biodiversity of insect pollinators:

a. Insect Pollinators Other Than Hymenoptera:

There are more insect species than all other animals and plants combined, the total number estimated to be over two million as of 2010. Joined appendages and an external skeleton characterize insects as part of the Arthropoda. Included are spiders, crustaceans, centipedes and scorpions. Insects are classified into 28 major orders, but seven comprise most of the species. These are, in order of increasing specialization and importance as pollinators, the Orthoptera (cockroaches, grasshoppers, crickets, walking sticks, praying mantis), Hemiptera (true bugs, cicadas, leafhoppers, scale insects, aphids), Thysanoptera (thrips), Coleoptera (beetles), Diptera (flies, gnats, mosquitoes), Lepidoptera (moths and butterflies), and Hymenoptera (ants, wasps, bees, sawflies, Ichneumon flies and chalcid flies). For the most part the Orthoptera of no importance as pollinators.

a.Hemiptera :

Only a few Hemiptera of value are Anthocoridae (minute pirate bugs), Phymatidae (ambush bugs) and Reduviidae (kissing bugs). The Anthocoride prey on thrips in flowers; a few Reduviidae prey on bees in flowers and most Phymatidae prey on bees and flies in flowers. Anthocoridae are found in almost any flowers that are visited by thrips. Phymartids and reduvids are found primarily on Compositae and flowers that are grouped into tight heads.

b. Coleoptera :

Except for a few flower-inhabiting forms, the Coleoptera are not as important pollinators as the Diptera, Lepidoptera and Hymenoptera. There are nine families of Coleoptera that are at times involved in the pollination of flowers. Most species of Cantharidae, the leather-winged beetles, that are predaceous as larvae occasionally pollinate.. Polleniferous species are also predaceous as adults. The majority of Meloidae, or blister beetles, occasionally are involved in pollination. The larvae of some species are parasitic in bee nests; others are parasitic on grasshopper egg masses. All adult Meloidae feed on pollen or on both nectar and pollen. The larvae of some species of Cleridae are flower inhabiting. They are mainly parasites in the nests of wasps and bees. The adults are predaceous, but they also feed on pollen. Most Melyridaeare predaceous as larvae and both predaceous and polleniferous as adults. One genus of Buprestidae, *Acmaeodera*) (flat-headed borer) is polleniferous. The larvae bore into wood and the adults feed on pollen. Many genera of Cerambycidae, or long-horned beetles and round-headed borers, can be involved as pollinators. The larvae bore into wood but the adults feed on pollen. Several genera of Scarabaeidae, or white grubs, visit flowers. They are primarily root-feeders as larvae, but they also feed on pollen as adults. Elateridae, or click beetles, are mostly root-feeders as larvae, but adults will feed on nectar and pollen. In the Dermestidae, the genus *Anthrenus* feed on decaying animal matter as larvae, but adults may also utilize pollen (especially *Anthrenus*). There are also other small families of Coleoptera, such as the Mordellidae, Oedemeridae, Lycidae and Rhipiphoridae, whose members have been observed to act as pollinators.

Most groups of flowers do not escape visits by beetles feeding on their petals as well as nectar and pollen. Some blister beetles will feed on legume petals in order to expose the pollen and nectar. Some very tiny flower-visiting beetles may crawl into the narrowest corollas or tightest keels. Nevertheless, only a few groups of flowers are visited regularly by a variety of

beetles. Examples are flowers with abundant pollen, social flowers with concealed nectar, flowers with exposed nectar and flowers with partially concealed nectar.

c. Diptera:

The adults of several large families of Diptera feed frequently on nectar or pollen or both, but the larvae are usually harmful to plants. Examples are found in the Anthomyidae (hovering house flies), Bombyliidae (bee flies), Calliphoridae (blow flies & bottle flies), Ceratopogonidae (biting midges) Conopidae (thick-headed flies), Cyrtidae (small-headed flies), Empididae (dance flies), Muscidae (house flies), Sarcophagidae (flesh flies), Stratiomyidae (soldier flies), Syrphidae (flower flies, syrphid flies, hover flies), Tabanidae males (horse flies), Tachinidae (tachinid flies), Tephritidae (fruit flies). These families might be considered in the following order of decreasing importance: Syrphidae, Muscidae, Calliphoridae, Sarcophagidae, Bombyliidae, Conopidae, Tachinidae, Empididae, Stratiomyiidae, Tabanidae, Tephritidae, Ceratopogonidae and Cyrtidae. However, this order may differ for any one-plant species. A few of the more important pollinating Diptera are discussed in the following.

Syrphidae have larvae with a wide variety of habit. They occur under bark, manure and liquid and are predatory on small insects such as aphids. The adults re almost all flower visitors. Most species feed on nectar and pollen or only nectar. Nectar-feeding species have a long, slender proboscis and generally visit the same group of flowers as the long-tongued bees. Those syrphids with short or moderate tongue length visit predominantly flowers of Groups I, Group II and Group III. Some also consume pollen on flowers of Group V.

Bombyliidae have larvae that either feed on grasshopper egg masses or those that feed on the larvae of wasps and wild bees. Adults of the latter group have a long, slender proboscis and visit flowers of Group III to Group VIII, but mostly Group III and Group IV. Although a few genera are intermediate, most have very a short proboscis and visit primarily flowers of Group II.

Muscidae have larvae with various habits. Some are internal parasites of other insects, while some feed on plant roots, and a great many feed on decaying animal and plant material. The adults of most species visit flowers and eat pollen and nectar. Flowers of Group II are favored, but a few others like onion in Group IV are also visited. Species in other families of Diptera will on rare occasions pollinate plants either directly or accidentally.

d. Lepidoptera :

Adults of most Lepidoptera feed mainly on nectar from flowers, while their larvae feed on herbage, some roots or stored food products and wool and are therefore pestiferous. Their preferred flowers are in Groups IV to VII. Encounters with Hymenopterid flowers (Group VI) often do not expose the pollen and therefore do not result in pollination.

The tongue lengths of Lepidoptera vary from 1 to 250 mm. Those with 4-10 mm. Tongues are most often seen on flower Groups IV & V, while those with longer tongues are most apt to be seen on Groups VI & VII.

Butterflies tend to frequent day-blooming flowers and moths visit constantly open or evening and night-blooming flowers. The entire suborder, Rhopalocera and 5 families of Heterocera that are numerous or specially adapted as pollinators are Arctiidae (tiger moths & wooly bears), Geometridae (loopers), Noctuidae (nun moths, cut worms), Pyralidae (snout moths), Rhopaloceridae (butterflies) and Sphingidae(hawk moths & horn worms).

Because investigations of visits to flowers have been made primarily in daylight, the value of moths as pollinators is probably underestimated. Butterflies often spend a lot of time on the same flowers

and they are regularly less effective than bees in pollination. Haw moths that fly in the evening or at night are assiduous flower visitors by darting rapidly from plant to plant. Their very long proboscis seems to be especially suited for the most highly developed Lepidoptera flowers that have musky odors, long and narrow corolla tubes or long spurs that contain nectar. Butterflies tend to prefer red flowers while moths prefer white flowers. Nun moths are similar to haw moths in rapid flight and long tongues. They are usually more abundant also. Many flowers are sometimes referred to as haw moth flowers, and where the corolla tube exceeds 25 mm. the term is deserved. But, hummingbirds and honeybirds contribute more effectively to the pollination of such flowers in some areas.

e. Thysanoptera (Thrips):

A small order, Thysanoptera are tiny but individual species occur in large numbers. Adults and larvae feed either mostly on honey and pollen or are predators of other thrips in flowers. It has been suggested that few indigenous flowers in Europe escape from occasional or frequent visits by thrips. Even though individual thrips may only convey pollen accidentally, their great abundance enhances their value for pollination. Nevertheless, they are generally thought to be ineffective in the pollination of many flower species and consequently they are rarely credited with much influence. They rarely migrate from plant to plant so that their role would be primarily self-pollination.

Generalizations on the use of non-hymenoptera pollinators

Among the Coleoptera, larvae of most species are destructive and not advisable for propagation. One genus of Cantharidae (*Chauliognathus*) are predators as larvae on aphids and as adults they feed on nectar and pollen. It is able to trip alfalfa and might be adaptable to mass production in insectaries and mass release in field crops.

The larvae of many genera of Diptera are destructive. Adults may pose a health hazard and are thus unsuitable for purposeful deployment. Muscidae may be useful in confinement for breeding work and small-scale increase of desirable plant stocks. There are may good pollinators among the Syrphidae, however. They could be increased rapidly and used as predaceous forms in insectaries. Although species may resemble bees and wasps, they are non-biting. Semi-aquatic species could be increased in field crops. The drone fly, e.g., is an efficient fruit pollinator and might be propagated in shallow tanks infused with organic material.

Most larvae of Lepidoptera are also destructive and thus the group is mostly unsuitable for deployment. There may be some exceptions, but any species considered would need to be carefully studied for any possible destructive tendency. *Vanessa cardin & V. atalanta* (L.) feed on thistles as larvae and might be considered for the pollination of some ornamentals. Sphinx moths are more destructive to weeds than crops (excluding grapes) and could be deployed to pollinate ornamental plants. Vanilla is usually hand-pollinated, and the search for a nondestructive Lepidoptera might be made. The possibilities for deploying Lepidoptera as pollinators are probably greatest for agriculture in tropical regions.

Pollinators enhance Crop Yield and Quality:

There are many examples of the major significance of pollinators to farmers, especially in terms of increasing the volume and quality of crops produced. One example is a project ran by CropLife India, which aimed to raise farmers' awareness of the importance of honey bees to their crop yields.31 The project used a number of approaches, such as providing subsidized honey bee hives and training farmers how to use them for managed crop pollination, as well as training in Integrated Pest

Management, the responsible use of pesticides32 and practical beekeeping. Overall, the project helped 230 farmers in Maharashtra district to increase their onion seed and pomegranate yields by 17 percent and almost 35 percent, respectively. Importantly, the farmers also saw their incomes increase, by 19 percent on average for onion farmers and a staggering 42 percent for those growing pomegranates. These increased profits were not only due to higher yields but also improved quality: For example, the color and shape of the pomegranates were more attractive to consumers. Moreover, spending on pesticides was reduced as farmers were taught to use them selectively and in a more targeted way. It is not just managed bees that influence crop quality. High pollinator diversity can improve several commerciallyimportant crops.33 For example, strawberries that have not been pollinated by a diverse group of insects like flies, solitary bees and honey bees can be smaller and irregular in shape, whereas those which are pollinated by these diverse insects are larger and have fewer deformations34, enabling farmers to charge a higher price. Coffee is another globallyimportant crop where it seems that a high diversity of pollinators can improve the quality.

Probable Questions:

1. How pollination occurs by Lepidopteran insects ? Cite suitable examples.

2. How pollination occurs by Hymenopteran insects ? Cite suitable examples.

3. How pollination occurs by Hemipteran insects ? Cite suitable examples

4. How pollination occurs by Coleopteran insects ? Cite suitable examples.

5. How pollination occurs by Dipteran insects ? Cite suitable examples.

6. How pollination occurs by Thysanoptera insects ? Cite suitable examples.

7. How Pollinators enhance Crop Yield and Quality ?

Suggested Readings:

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Current knowledge on Pisciculture

Unit-XII

Integrated Fish Farming

Objectives: In this unit you will learn about various methods used in integrated fish farming

Introduction:

Integrated farming is a whole organic farm management system which aims to deliver more sustainable agriculture. It is a dynamic approach which can be applied to any farming system around the world.Integrated fish farming is a system of producing fish in combination with other agricultural/livestock farming operations centered on the fish pond. The farming sub-systems e.g. fish, crop and livestock are linked to each other in such a way that the byproducts/wastes from one sub-system become the valuable inputs to another sub-system and thus ensures total utilization of land and water resources of the farm resulting in maximum and diversified farm output with minimum financial and labour costs. Integrated fish farming is based on the concept that 'there is no waste', and waste is only a misplaced resource which can become a valuable material for another product (FAO, 1977). In integrated farming, the basic principles involve the utilization of the synergetic effects of inter-related farm activities and the conservation, including the full utilization of farm wastes.

It is assumed that all the constituents of the system would benefit from such a combination. However, in most cases, the main beneficiary is the fishes which utilizes the animal and agricultural wastes directly or indirectly as food. As integrated farming involves the recycling of wastes, it has been considered an econo-mic and efficient means of environmental manage-ment.

Basic Principles of Integrated Fish Farming:

Integrated fish farming is based on the concept that 'there is no waste', and waste is only a misplaced resource which can become a valuable material for another product (FAO, 1977). In integrated farming, the basic principles involve the utilization of the synergetic effects of inter-related farm activities and the conservation, including the full utilization of farm wastes.

It is assumed that all the constituents of the system would benefit from such a combination. However, in most cases, the main beneficiary is the fishes which utilizes the animal and agricultural wastes directly or indirectly as food. As integrated farming involves the recycling of wastes, it has been considered an economic and efficient means of environmental management.

Types of Integrated Fish Farming:

A.Fish-Cum-Paddy Culture:

Rice fields which are water-logged for 3-8 months in a year, there is always small population of fishes that gain access to such waters. This probably had given rise to the practice of deliberate stocking of fishes and harvesting. The trapping of prawns and fishes with the help of 'gamcha or dhoti' in fallow paddy-fields has been an age old practice in India.

Paddy-field aquaculture provides additional income to the farmers. In areas where rice and fish form the staple food, paddy-field aquaculture makes available an essential diet for the people. As paddy and fish can be grown either simultaneously or alternately in the same water mass, it requires very little extra input by way of additional costs, particularly in management and labour. It provides off-season employment to the farmers and farm labours.

All fishes are not suitable for such a type of culture as paddy-fields provide special ecological conditions such as shallow turbid water with high temperature. Fishes that can adapt to shallow waters necessary for paddy crops. Fishes that can tolerate high temperature and also thrive on low dissolved oxygen, which is the characteristic of paddy-fields especially in tropical countries. Fishes that can tolerate fairly high turbidity. As the duration of culture is quite short, fishes that have high growth rate is to be selected, so that it can reach marketable size within these few months. Fishes that are cultured in such waters in India are *Mugil sp., Mystusgulio, Haplochromismellandi* (mollusc eating fish), *Mugil parsia, Puntius sp., Channasp*., prawns and shrimps. In India, limited experimental works have shown the suitability of Indian carps for such integrated farming

Fig 1: Fish-Cum-Paddy Culture

Objectives of Paddy-Field Aquaculture:

The objectives or advantages of paddy-field aqua-culture are as follows:

(1) Paddy-field aquaculture provides additional income to the farmers.

(2) In areas where rice and fish form the staple food, paddy-field aquaculture makes available an essential diet for the people.

(3) As paddy and fish can be grown either simultaneously or alternately in the same water mass, it requires very little extra input by way of additional costs, particularly in management and labour.

(4) It provides off-season employment to the farmers and farm labours.

(5) Combination of paddy and fish farming is mutually beneficial. Fish cultivation promotes better paddy production by way of exercising an effective control on unwanted weeds, mol-luscs, noxious insects and their larval stages.

Fishes Suitable for Paddy-Cum-Fish Culture:

All fishes are not suitable for such a type of culture as paddy-fields provide special ecological conditions such as shallow turbid water with high temperature.

Fishes having the following criteria are generally selected for paddy-cum-fish culture:

(1) Fishes that can adapt to shallow waters necessary for paddy crops.

(2) Fishes that can tolerate high temperature.

(3) Fishes that can thrive on low dissolved oxygen, which is the characteristic of paddy-fields especially in tropical countries.

(4) Fishes that can tolerate fairly high turbidity.

(5) As the duration of culture is quite short, fishes that have high growth rate is to be selected, so that it can reach marketable size within these few months.

(6) Fishes that can live in confinement and do not tend to escape from the cultivated area.

Fishes that are cultured in such waters in India are Mugil sp., *Mystusgulio*, *Haplochromismellandi* (mollusc eating fish), *Mugil parsia*, *Puntius sp*., *Channa* sp., prawns and shrimps. In India, limited experimental works have shown the suitability of Indian carps for such integrated farming.

Management of Paddy-Fields:

To increase the utility of paddy-fields as fish ponds, the following managements are required:

(1) A continuous flow of water in the field, with proper inlet and outlet is to be maintained.

(2) The water in the field is to be maintained at a desired level.

(3) Proper drainage of water from the field has to be made in case of flooding.

(4) At the point of entry and exit of water, some control means (such as screens) have to be provided to prevent the cultivated species from escaping and stopping the entry of wild fishes into the paddy-field.

(5) Deep pits or other devices has to be provided as shelter to the cultivated fishes at the time of distress.

Types of Paddy-Field Aquaculture:

Three major types of paddy-field aquaculture are generally practiced:

(i) Simultaneous or combined or synchronous farming.

(ii) Alternate or sequential farming or paddy- fish rotation.

(iii) Relay farming.

(i) Simultaneous or Combined or Synchronous Farms:

In such farming paddy and fish are grown together and harvesting the paddy and fish at the end of the rice-growing season.

Literature reveals that a variety of techniques and stocking rates have been employed in different countries on paddy-field aquaculture. In general, fishes are stocked not earlier than 5 days after transplantation of paddy seedlings, so as to enable the seedlings to form proper root. After transplantation of paddy, stocking in case of fry should be done after 10 days, while for fingerlings after 3 weeks.

The methods of culture adopted are for production of either fingerlings or fish for consumption. Farmers generally cultivate local varieties of paddy which takes up to 6 months to be harvested, so that more than one crop per year can be grown. Generally organic manures are preferred over the use of fertilisers in the preparation of the paddy-fields.

The advantages of simultaneous cultivation are:

(1) There is no additional cost for fish production.

(2) Fishes contribute to the enhancement of paddy production by destroying weeds, causing tillering and mineral enrichment by their digging activity, and for the fertilisation of soil by their excrement and also by the unutilised artificial feed.

(3) Due to fish cultivation paddy production gets increased by 5 to 15%.

The disadvantages are:
(1) The water depth being shallow hinders better fish raising.

(2) For fear of harmful effect on fishes, herbicides and insecticides uses in paddy-field is generally prevented which ultimately limits paddy production.

(3) Due to abrupt changes in temperature and dissolved oxygen, inadequate space and presence of piscivore birds, there is great loss of fishes which may be about 20-60%.

Progressive agricultural areas have slowly discarded paddy-fish cultivation in favour of the rotation of paddy and fish crops.

(ii) Alternate or sequential farming or paddy- fish rotation:

It is the simplest form, where flooded paddy-fields after harvest are used to raise one or more crops of fish or shrimp.

In rotation system the paddy-field has to be prepared for raising fishes after the harvest of rice. To maintain the required depth of water the bunds surrounding the field have to be raised. After harvesting the rice, the stubbles are not removed.

These submerged stubbles provide the substrate for the development of fish food organisms. These stubbles undergo decomposition, thereby fertilising the water and stimulating higher productivity.

After fish harvesting, the residues remain in the soil which in turn serves as fertiliser for the paddy crop.

Shrimp Production in Paddy-Fields:

Shrimp production in paddy-fields is generally practised in areas (generally in the west coast and deltaic areas of eastern India) where only one crop (July to September) of a salt-resistant variety of paddy is grown. Following the harvesting of rice, the bundhs are strengthened and sluice gates are installed to control the water supply.

At high tides, the fields are filled with tidal water, containing a large number of shrimp larvae. These larvae gain access to the fields, where they find food and shelter. To attract the larval shrimps, lamps are hung above the inlets.

By this process, natural stocking of shrimp larvae is done at every high tide for two to three months. To prevent the escape of juvenile shrimps at low tides, conical bag-nets are attached to the sluice gates.

Shrimp harvesting from these fields starts in December, as by this time the early stock of shrimps will have attained marketable size. Regular harvesting is done which results in thinning of the stock, leading to better growth rate and harvesting of larger shrimps. The total yield of shrimp is around 780-2100 kg per hectare.

Advantages of Paddy-Fish Rotation:
(1) There is no water depth limitation either to paddy or fish cultivation. Shallow water depth is maintained till the harvesting of paddy, after which the water depth is raised for the culture of fishes.

(2) Through this adequate water level management, suitable water temperature and dissolved oxygen content can be maintained.

(3) After harvesting of paddy, the submerged stubbles decompose and fertilise the water. This leads to the development of fish food organisms and ultimately stimulates higher productivity.

(4) The interval between paddy harvesting and fish stocking is sufficient enough to allow degradation of pesticides.

(5) Insect pest infestation gets reduced as their life-cycles are disrupted.

(6) Either monoculture or polyculture can be practised.

Disadvantages:
Due to presence of piscivorous birds like herons, cormorants, etc., considerable loss of fish (20-60%) takes place.

(iii) Relay Farming:
This system is much complicated and it ensures prolonged period of fish culture. It involves transferring the stock of fishes to specially prepared ditches, channels or pools at the time of paddy harvest and re-stocking them in the field for a further growing period. This system provides better growth rate than what is possible in such a short duration of one paddy crop.

For such a cultivation the farm should have additional holding or rearing facilities for the period between paddy harvesting and planting of new fish seedlings. The carps or shrimps can be grown to the required size through adequate feeding. This culture involves higher inputs including labour.

Advantages of Paddy cum fish farming:

There is no additional cost for fish production. Fishes contribute to the enhancement of paddy production by destroying weeds, causing tillering and mineral enrichment by their digging activity, and for the fertilization of soil by their excrement and also by the unutilized artificial feed. Due to fish cultivation paddy production gets increased by 5 to 15%.

Disadvantages of Paddy cum fish farming:

The water depth being shallow hinders better fish raising. For fear of harmful effect on fishes, herbicides and insecticides uses in paddy-field is generally prevented which ultimately limits paddy production. Due to abrupt changes in temperature and dissolved oxygen, inadequate space and presence of birds, there is great loss of fishes which may be about 20-60%.

B. Duck-Cum-Fish Farming:
Although duck and fish farming have been in practice in eastern Europe and China and their compatible nature being recognised since long, the interaction and benefits of the association have been understood only a few years back.

During the last decade suitable methods have been developed in various countries for raising ducks in fish ponds. Undoubtedly, such a combined culture is highly profitable as it greatly increases the production of protein in terms of fish and duck per unit area.

The combination of duck and fish farming is seen presently as a means of reducing the cost of feed for ducks, at one hand, and on the other, the excreta of ducks acts in an inexpensive way of fertilising ponds, which results in production of fish food organisms.

Thus, ducks can be said to be ‘living manuring machines’. The duck droppings contain 25 per cent organic and 20 per cent inorganic substances along with a number of elements such as carbon, nitrogen, phosphorus, potassium, calcium, etc.

Moreover, ducks by agitating the shore areas of the pond, help to release nutrients. Besides this, ducks feed on a variety of organisms such as weeds, snails, unwanted harmful insects and their larvae (some being vectors of fish pathogenic organisms), tadpoles, frogs and water-borne disease-causing organisms infecting man, whose eradication is one of the important aspects of farm management.

(a) Ducks Culture and their Raising:

Ponds provide a clean and healthy environment for ducks. Special strains of ducks have been developed that are suited for pond raising. Such suitable strains when used, approximately 50-60 per cent of their droppings when falls into the pond, is sufficient to fertilise the water.

Reliable and timely supply of required strains of good quality ducklings are of great significance as it promotes successful farming. For supply of ducklings, small scale farmers have to depend on outside sources, while larger farms have their own breeding centres which is much convenient and profitable.

Raising of Ducks is done by one of the two ways:

(i) Extensive Raising:
In extensive raising ducks are stocked at approximately 150-500 per hectare of pond surface. Here small amount of supplementary feed is provided and the number of ducks is limited due to the food they can find in the pond water. As the number of ducks are limited, they contribute little amount of manure to the pond and its effect on fish yield is also limited. Such a type of method is usually employed in Europe.

(ii) Intensive Raising:
Here ducks are stocked at a much higher density, approximately 1000-2500 per hectare of pond surface and is usually employed in Africa. The ducks are fed at the same rates as on land. As ducks are held at a much higher density per unit area, higher amounts of manure are thus loaded into the fish pond which subsequently results in higher yields.

(b) Duck Keeping:

There are two basic ways of keeping ducks in fish ponds:

(i) Free-Range System:
Here the ducks are allowed to have free access to the whole pond (Fig. 6.43). As they are allowed to swim freely around the whole pond surface, a good proportion of their droppings fall directly into the pond water and are distributed more or less uniformly.

The ducks are able to forage around the whole pond. Adjacent to the ponds, small duck houses are built with facilities for providing them with supplementary feed. In this system, as considerable energy is spent by the ducks in swimming activity, it is believed to affect the feed conversion ratio and growth rate of the ducks.

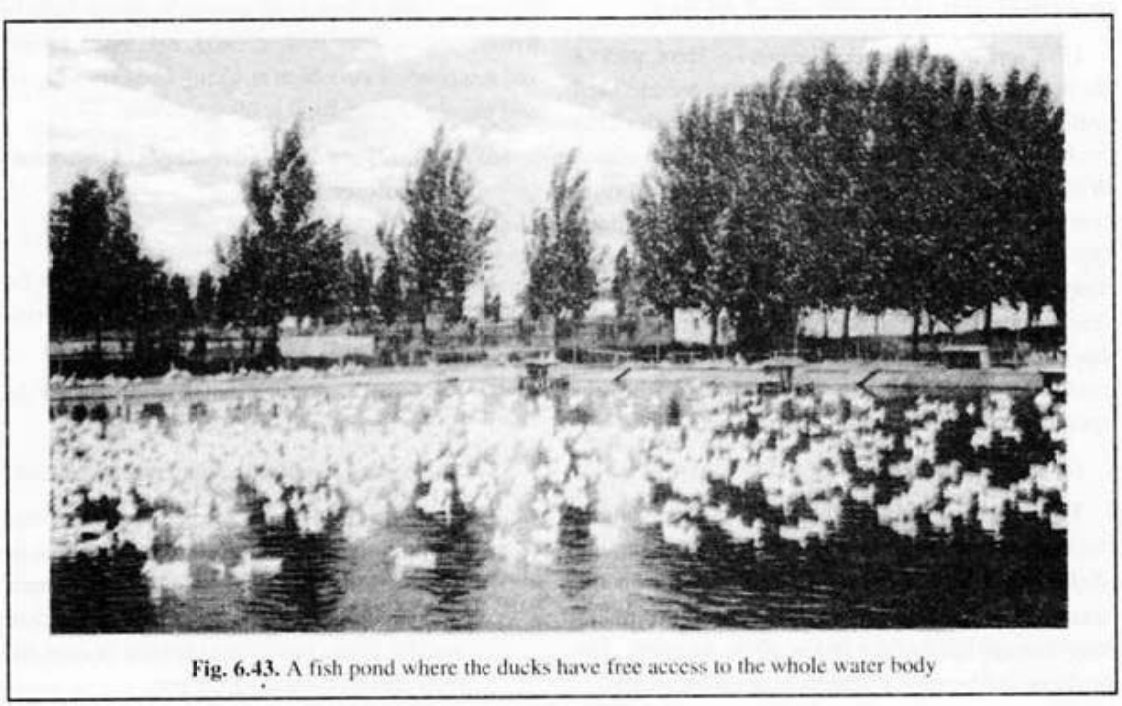

Fig. 6.43. A fish pond where the ducks have free access to the whole water body

Fig 2: Duck-Cum-Paddy Culture

(ii) Confinement in Enclosures:

Here, part of the pond area and the adjacent land area are enclosed with wire fences (Fig. 6.44). About one-fourth of the enclosure will be on the land and the rest in water. Within the wire fence suitable feeding and resting areas are made.Some of the droppings of the duck falls directly into the pond water, while the rest that drops on land is washed down into the pond. Under this system fish production is reported to be almost equal to the free-range system. Keeping ducks in enclosures is preferred by most farmers, who use special strains of ducks for better growth.

(c) Raising of Fish:

For duck-fish farming the most suitable pond is the barrage type of ponds. This is made by damming shallow valleys, so that the ducks can lie on the natural slopes. There is every likely-hood that ducks may damage the earthen dykes while foraging. The problem can be solved with proper maintenance.

Commonly the fish species cultured are herbivores and omnivores. The common carp was traditionally the main species but subsequently other species of Chinese carps are used to make full use of the food resources. Other important species used are male or hybrid tilapia, grey mullet, catla, eel, asian catfish and sea perch. Fairly high stocking rates are adopted and supplementary food is also provided.

(d) Advantages and Disadvantages of Fish-Cum- Duck Culture:

(i) Advantages:

(1) There is practically less additional cost for fish culture, as the excreta of the ducks fertilises the pond water.

(2) Ducks by agitating the shore areas of the ponds help to release nutrients.

(3) The cost of feed for the ducks gets reduced.

(4) The ducks help to eradicate vectors of fish pathogenic organisms and water borne diseases — causing organisms infecting man, as they feed on a variety of organisms such as weeds, snails, unwanted harmful insects and their larvae, tadpole, frog, etc.

(ii) Disadvantages:

(1) The fingerlings released should be of more than 10 cm size, otherwise the ducks may feed on the fingerlings.

(2) While foraging for food, ducks sometimes damages the earthen dykes. This problem can be solved with proper maintenance.

3. Fish-Cum-Poultry Farming*:*

Integrated fish farming with poultry is generally cultured as the poultry manure is a very efficient fertilizer for fish ponds. The poultry droppings comprises 2% nitrogen, 1.25% phosphoric acid and 0.75% potash. The low feeding cost per individual bird makes poultry farming along with fish, a common investment for poor farmers.

(a) Poultry Raising:

Both 'broilers' and 'layers' variety of chicken can be raised for fish-poultry farming. One day old chick are raised up to the pullet stage after which they are put in layer cages.

Fish culture with both intensive and extensive poultry productions have been integrated successfully. The most intensive type of poultry production is the battery type of housing, which is installed by the side of the pond. The floor of the house is cemented and is set up at a slope so that the eggs may roll forward.

For layers, the floor area required is about 30 cm^2 while for broilers, 15-20 cm^2. The usual floor space allotted for each bird is 20 x 30 x 40 cm. The birds are confined to cages which are made up of standard, stout, galvanised wire. The cages are kept on trays for collection of droppings. For further on poultry refer chapter 5.
For manuring one hectare pond water, the droppings of about 250 layers and four batches of 200 broilers each are adequate in a year's time.

(b) Fish Raising:

For fish raising the ponds are stocked with fingerlings of Catla, silver carp, common carp, murrels, tilapia, giant freshwater prawns, etc. The stocking density of fishes is related with that of poultry and also with the period of culture.

In one hectare pond area, when stocked with 5000 giant freshwater prawns (*Macrobrachiumrosenbergii*) and 1500 silver carp, and cultured for a period of four months, one can harvest 600 kg of prawns and an equal amount of fish, along with 250 culled birds.

For culturing over a period of one year, ponds may be stocked with fingerlings of catla, common carp, silver carp and grass carp at a density of 5000-6000 fingerlings per hectare. At the end of twelve months of fish-cum-poultry culture, fish yield of over 3900 kg per hectare can be normally obtained along with 42,000 eggs and 200 culled birds.

(c) Advantages and disadvantages of fish-cum- poultry culture:

(i) Advantages:

(1) Chicken manure is a very efficient fertiliser, so no chemical fertiliser is needed for fertilising the pond water. This cuts down the expenditure of rearing fishes.

(2) No supplementary fish feed is required.

(3) The purchase and feeding cost per bird is low.

(4) Chicks are readily available and their productivity can be improved with simple and cheap management.

(ii) Disadvantages:

(1) Chicks should be examined from time to time and diseased one should be isolated, otherwise they will destroy the entire stock.

(2) Sufficient time should be given from one stocking of chicks to the next for renovation of the house and disinfecting it.

Fig 3: Fish-Cum-Poultry Farming

C. Fish-Cum-Pig Culture:

In integrated fish farming with pig, the 'pig dung' is useful for conditioning the soil and providing the necessary nutrients required for fertilising the pond water. Fish-cum-pig culture is practised at large in China where pigs are considered as **"costless fertiliser factories".**
Pig dung contains about 70 percent digestible feed for fishes. The feed while passing down the pig's alimentary canal, gets mixed up with enzymes which continue to act even after defecation. Such undigested solids serve as direct food source for tilapia and common carp.In tropical fish ponds, weeds are a major problem in fish culture. Such vegetation's are considered as valuable food resource for pigs.

(a) Pig Rearing:

In fish-cum-pig culture the embankments of fish ponds are made wider (over 10 m in China) to facilitate the building of pig sites and also for growing vegetables, fruit trees or other crops. In the slopes, grasses can also be grown which is used as fodder for grass carp and for other farm animals.

Various aquatic plants, such as azolla, duck weed, Pistia, Wolffia, Lemna, and water hyacinth (Eichhor-nia) are grown in feeder channels and irrigation ditches associated with the pond farms. These and the foliage of other terrestrial plants (vegetables, rice, corn, etc.) are used for feeding the pigs.

In the water area of the pond, about 10 tons of aquatic plants can be produced, which are sufficient to feed 10 pigs. These plant materials are generally mixed with bananas, coconut meal, rice bran, soybean wastes, groundnut cakes, fish meal, etc.Pig sites or pen or sty are generally built on nearby land or on the pond embankment. The pen enclosure is built not only for pig raising but also special consideration is given to the needs for breeding, nursing and fattening activities. Pig pen generally have a system of channels for transferring the organic matter into the pond water.

Alternatively, the sty or pen may be constructed above the pond water. The structure is made of wood and provided with a lattice type of floor which permits the excreta and uneaten food to fall directly into the pond water.Modern practices are to avoid direct washing of the wastes into the pond. The urine and dung of pigs are first allowed to the oxidation tanks (digestion chambers) where sedimentation and fermentation of the manure take place. The supernatant liquid, at regular intervals, are then discharged into the fish ponds.

The sludge that remains is utilised as fertilisers in agriculture. Alternatively the pig manure may be kept in a heap on the pond embankment for later use. The chemical composition of pig wastes is depicted in Table 6.13.

Table 6.13 : Chemical composition of pig wastes

Source	Organic matter (%)	N (%)	P_2O_5 (%)	P (%)	K_2O (%)	K (%)
Fresh manure	15.00	0.60	0.40	0.18	0.44	0.37
Urine	2.00	0.30	0.12	0.05	1.00	0.83
Air-dried manure	34.32	2.12	0.98	0.43	2.45	2.03
Litter manure	34.00	0.48	0.24	0.11	0.63	0.52

The number of pigs to be raised per hectare and the manuring rates to be applied are based on years of experience. The production of manure depends upon the age and size of the pig. A piglet produces about 3.4 kg manure a day, while a one-year-old pig gives about 12.5 kg a day.

The average production of faeces and urine per pig is about 7.8-8 tons per annum. A density of 60-100 pigs has been found to be sufficient to fertilise a one hectare fish pond.

(b) Fish Rearing:

Polyculture is commonly practised in such integrated farming due to the variety of food that becomes available in the pond. Herbivorous and omnivorous fishes are used for culture; generally common and Chinese carps and less frequently catfishes (Pangasius), Indian carps and tilapia.Due to high productivity of the ponds, fairly high rates of stocking are generally practised — 60,000 fingerlings of different species (weighing 20-30 gm) per hectare.

(c) Production and Duration of Culture:

The duration of culture of fishes and pigs varies. Generally it is about one year, but culture for 6 months duration is also practised. The overall economics of combined fish and pig rearing depends on the local conditions. However, the pigs are generally sold when they have attained a weight of 90-100 kg. The production of fish generally varies between 2 and 18 tons per hectare per annum.

(d) Advantages and Disadvantages of Fish-Cum- Pig Culture:

(i) Advantages:
(1) Such integrated farming increases the productivity per area and thus, the farmers income becomes doubled or more.

(2) Pig dung conditions the soil of a new pond and provides ready-made organic matter, containing the necessary nutrients.

(3) Pig dung contains about 70 per cent digestible feed for fishes. The undigested solids present in the faeces of pig serves as direct feed source for tilapia and common carp.

(4) Pigs aptly plays a role in biological control of weeds, as weeds are considered as valuable food source for pigs.

(ii) Disadvantages:
(1) Addition of too much pig manure may lead to increased nutrient load resulting in pollution of the water body and mortality of the fishes.

(2) Considerable care and management skills are required to prevent pollution. It has been found that satisfactory fish production can be obtained with much lower manuring.

Fig 4: Fish-Cum-Pig Culture

D. Fish-cum-Horticulture:

The top, inner and outer dykes of ponds as well as adjoining areas can be best utilized for horticulture crops. Pond water is used for irrigation and silt, which is a high-quality manure is used for crops, vegetables and fruit bearing plants. The success of the system depends on the selection of plants. They should be of dwarf type, less shady, evergreen, seasonal and highly remunerative. Dwarf variety fruit bearing plants like mango, banana, papaya, coconut and lime are suitable, while pineapple, ginger, turmeric, chilli are grown as intercrops. Plantation of flower bearing plants like tuberose, rose, jasmine, gladiolus, marigold and chrysanthemum provide additional income to farmers.

Fig 5: Fish-Cum-Horticulture

Ideal management involves utilization of middle portion of the dyke. Residues of vegetables cultivated could be recycled into fishponds, particularly when stocked with fishes like grass carp. Grass carps can be stocked @ 1000/ha and addition of common carps are beneficial for utilizing feacal debris. In mixed culture of grass carps along with rohu, catla and mrigal, in 50: 15: 20: 15 ratio at a density of 5000 fish/ha. Similarly when banana or coconut is cultivated in rows in wetlands, the ditches made between such rows act as supply or drainage canals. These canals serve as fish culture systems owing to their round-the-clock supply of water and rich insect populations. Larvivorous air-breathing fish species such as snakeheads *C. marulius* and *C. striatus* and tilapia, *O. mossambicus* are ideal species for culturing in this system. This integrated system fetched 20-25% higher return compared to aquaculture alone.

E. Fish-cum-Sericulture:

In this integration system, mulberry is the producer; silkworm is the first consumer while fish is the secondary consumer, ingesting silkworm faeces directly. Inorganic nutrient in the silkworm faeces are utilized by phytoplankton, and filter-feeding fish in turn consumes heterotrophic bacteria. The optimum range of temperature and humidity is 15-32^{o}C and 50-90% respectively.

The Seri-fish system provides linkages between mulberry and pond sub-system. Harvested mulberry leaves are fed to silkworm and the waste material obtained from silkworm rearing enters fish-pond as a mixture of mulberry leaves and silkworm excrement. Mulberry dykes yield leaves at 30 tonnes/ha/year, when fed to silkworm 16-20 tonnes of waste is produced.

Fig 6: Fish-Cum-Sericulture

F. Fish-Cum-Dairy Farming:

Fish-cum-Dairy Farming is considered as an excellent innovation for the use of organic wastes. Cattle wastes and washings from the cattle sheds are conveyed through pipes into the ponds which acts as good fertilizer. Cattle wastes are generally collected in a pit for later use. In addition to fish yield, production of milk from cattle and beef adds to the economy.

Fig 7: Fish-Cum-Dairy farming

G. Fish-Cum-Rabbit Culture:

Rabbit farming has been found to be ideal for integration with small- scale fish culture. Rabbit manure have greater value as a direct food for fish compared to other livestock wastes.

Advantages of Integrated Fish Farming:

(1) Full utilization of farm wastes.

(2) Utilization of the cooperative effects of interrelated farm activities.

(3) It increases employment opportunities.

(4) It increases nutritional source for the farmer's family.

(5) It gives higher and stable farm productivity and there is less risk (biologically and economically).

(6) It increases the income of rural population.

(7) It is a means of land reclamation in certain areas.

(8) It is an efficient and economic means of environmental management.

Disadvantages of Integrated Fish Farming:

Recently, controversy has arisen among scientists on the public health aspects of integrated farming. Speculations are ripe that integrated fish farming with pigs and poultry may be a cause of influenza pandemic. This may be, as the pigs would act as 'mixing vessels' for avian and human influenza viruses, it can create new lethal strains of viruses by mutation.

In such an act, fishes themselves do not play any role. However, there is no conclusive evidence that integrated farming can become a public health hazard. For safety measures, pig-poultry combinations in integrated fish culture should thus be avoided.

Probable Questions:

1. Sate the basic principles of Integrated fish farming.

2. State the Objectives of Paddy-Field Aquaculture.

3. Which are the criteria for selecting fish species n paddy cum fish integrated culture?

4. What are the advantages and disadvantages of paddy-fish rotation.

5. what is relay farming?

6. Describe two basic ways of keeping ducks in fish ponds.

7. State the Advantages and Disadvantages of Fish-Cum- Duck Culture.

8. Describe the advantages and disadvantages of fish-cum- poultry culture.

9. Describe the advantages and Disadvantages of Fish-Cum- Pig Culture.

10. Write short notes on (a) Fish cum horticulture, (b) Fish cum sericulture

(c) Fish cum Dairy farming, (d) Fish cum rabbit culture

11. Write down the advantages of Integrated Fish Farming.

12. Write down the disadvantages of Integrated Fish Farming.

Suggested Readings:

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Unit-XIII

Induced Breeding

Objectives: In this unit you will learn about various aspects of Induced breeding of Fishes

Introduction:

Major carps are most important species from the point of view of their high food and nutritive values. Hence they have kept attention of scientists and aqua farmers. They have peculiar habit of breeding in running waters of rivers and streams where they have large space for movement.

During breeding season, water is sufficiently provided with minerals, O2 and food contents. This friendly aquatic environment provides stimulus for spawning. Carps do not breed in confined water of captivity even if their gonads are matured and ovulation might have taken place in natural environment. For the increasing production of carps it is necessary that they should be made to breed in confined water so that increasing demand of good quality fish and their seed could be available.This can be done by adopting induced breeding technique by which ripe or mature fishes breed in confined water when stimulated by injection of pituitary hormone. The pituitary hormone is an important gonadotropin, which is extracted from the hypophysis of a mammal or a mature fish.

Induced breeding of carps in captivity by the use of pituitary hormone injection has been successfully done. Many scientists are involved in doing research to improve induced breeding technique. Several attempts are being made to establish pituitary banks to meet out the need of pituitary hormones throughout the year. It is well known that the gonadotropic hormone (FSH and LH) secreted by the pituitary gland play an important role in maturation of gonads as well as spawning of fishes. The Brazilians were the first to succeed in induced breeding by injecting fish with pituitary extract. During breeding season, water is sufficiently provided with minerals, O_2 and food contents. This friendly aquatic environment provides stimulus for spawning. Carps do not breed in confined water of captivity even if their gonads are matured and ovulation might have taken place in natural environment.

Historical aspects

In Argentina, House (1930) was the pioneer of this artificial breeding technique. Then Russia as well as USA have also successfully applied this technique with the help of pituitary injections. In India, Khan(1938) was the first who successfully induced *Cirrhunusmrigala* to spawn by injecting mammalian pituitary hormone. After Dr. Hiralal Chaudhari (1955) adopted this technique more efficiently. Later, Chaudhari and Alikunhi (1957) further developed this technique by injecting pituitary extract. Now, this process is very popular in obtaining fish seed for commercial purpose.

Definition

The artificial process by means of which the extract of the pituitary is introduced inside the body of both the matured male and female fishes, then the fishes after being excited lay eggs in the pond water

and subsequently fertilization takes place and the process is called **induced breeding** of fishes. This process of breeding is also known as hypophysation.

Induced breeding technique has following steps:

a. Collection of Pituitary gland:

Pituitary gland is collected from a mature fish, which is called as a donor fish. Most widely used donor fish is the common carp, *Cyprinus carpio*. The best time for preparation of pituitary extract is May and June. The Indian major carps like *Catlacatla*, *Labeorohita*, *Labeokalbasu*, *Labeogonius* and *Cirrhinusmrigala* do not breed in confined water and have need to be subjected to induced breeding.

To remove pituitary gland, the head of the fish is dissected and brain is exposed. The gland is immediately removed from the brain and stored in a refrigerator. It may be preserved in absolute alcohol at room temperature. The gland then is homogenized in distilled water. The homogenate is centrifuged and clear supernatant is used as source of hormone to which 0.3% sodium chloride solution is added to it. This extract is ready for the immediate use. If pituitary extract is to be stored for a longer period the glycerine or trichloroacetic acid may be used instead of sodium chloride.

b. Preservation of Pituitary Glands:

The collected glands must be preserved immediately because glycol- or muco- protein contained in them are degraded by the enzymatic action.The pituitary glands can be preserved by three methods:

1. Absolute Alcohol:

The glands after collection are immediately put in absolute alcohol for de fatting and dehydration. Each gland is kept in a separate phial marked serially to facilitate identification.After 24 hours, the glands are washed with absolute alcohol and kept again in fresh absolute alcohol in dark color bottles and stored either at room temperature or in a refrigerator.Occasional changing of alcohol helps in keeping the glands in good condition for longer periods.In order to prevent moisture from getting inside the phials, they may be kept inside desiccators containing some anhydrous calcium chloride.It is preferable to keep the glands in a refrigerator. They can be stored in refrigerator up to 2-3 years and at room temperature up to one year.Preservation of fish pituitary gland in absolute alcohol is preferred in India.

2. Acetone:

It is also a good preservative. In this method, soon after collection, the glands are kept in fresh acetone or in dry ice-chilled acetone inside a refrigerator at 100^0C for 36-48 hours. During this period, the acetone is changed 2-3 times at about 8-12 hours intervals for proper de-fatting and dehydration. The glands are then taken out of acetone, put on a filter paper and allowed to dry at room temperature for one hour. They are then stored in a refrigerator at 100^0C, preferably in desiccators charged with calcium chloride or any other drying agents.

3. Freezing

Soon after removal of gland, they should be stored inside the refrigerator. But this method is not commonly used.

c. Preparation of Pituitary extract:

Preserved glands are weighed. This is essential for accurate determination of the dose to be given according to the weight of the breeders. The weight of the gland may be taken individually or in a group. To get a more accurate weight, a gland should be weighed after two minutes of its removal from alcohol. The pituitary extract should be prepared just before the time of injection. The quantity of gland required for injection is at first calculated from the weight for the breeder to be injected. The glands are then selected and the required quantity of glands is taken out of the phials. The alcohol is allowed to evaporate, if the glands are alcohol preserved ones. Acetone-dried glands are straight away taken from the phials for maceration. The glands are then macerated in a tissue homogenizer by adding a measured quantity of distilled water or common salt solution or any physiological solution which is isotonic with the blood of the recipient fish. The most successful results of induced breeding in the Indian major carps have so far been obtained with distilled water and 0.3% common salt solution. The concentration of the extract is usually kept in the range of 1-4 mg of gland per 0.1 ml of the media i.e., at the rate of 20- 30 gm. of the gland in 1.0 ml of the media. After homogenization, the suspension is transferred into a centrifuge tube. While transferring, the homogenate should be shaken well so that settled down gland particles being mixed with the solution come into the centrifuge tube. The extract in the tube is centrifuged and the supernatant fluid is drawn into a hypodermic syringe for injection. The pituitary extract can also be prepared in bulk and preserved in glycerin (1 part of extract with 2 parts of glycerin) before the fish breeding season so that the preparing extract every time before injection is avoided. The stock extract should always be stored in a refrigerator or in ice.

d. Dosage of Pituitary Extract:

The dose of the pituitary gland is calculated in relation to the weight of the brooders to be injected. A single high dose has been found useful when the brooders are in ideal condition and the weather is favorable. Rohu seed production technologies responds well to two injections while catla and mrigal to both one and two injections. An initial dose as a Provocative Dose at the rate of 2-3 mg. of pituitary gland per kg body weight of fish is administered to the female breeder only. In case the condition of any one of the two males is not found in the freely oozing stage, an initial injection – Provocative Dose may be administered to the male at the rate of 2-3 mg/ kg body weight. After 6 hours, a second dose of 5-8 mg/kg body weight is given to the female, while both the males receive the first or second dose at the rate of 2-3 mg/kg body weight. Two males against each female make a breeding set. To make a good matching set, the weight of the males together should be equal to or more than the female. Slight alterations in doses may be made depending upon the condition of maturity of the brooders and the prevailing environmental factors. 1-3 pituitary glands are effective for a pair of fish.

e. Injection of Pituitary Extract:

To ensure higher success rate of fertilization it is important to coincide time of ovulation with the release of milt of male fish. For this purpose usually ratio of female and male 2:1 is maintained in every set. Dose of pituitary extract to be given is decided according to age, sex, weight size and state

of maturity of both donor and recipient. A dose 2 to 3 mg of gland per kg body weight is given to female breeder.

There is no need of injecting dose to the male breeder if it in a state of milt oozing. After 6 hours of the first dose of injection another dose of 5-8 mg of gland per kg of body weight may be given to female if needed. However, a dose of 2-3 mg per kg body weight is recommended for the male breeder. More than 2 injections should not be given.

Fig: Injection of Pituitary Extract

The injection given may be intra-macular at caudal peduncle or shoulder or intra-peritoneal at the bases of paired fins. The first injection should be given at the early hours of the day, while the second one in the evening. Weather should be rainy or cloudy for easy and early spawning. The fishes should be transferred to the breeding hapa after injecting the pituitary hormone.

f. Selection of Breeders:

Medium sized fully ripe and healthy fish of around 2 to 4 years of age is preferred for induced breeding. The weight should be 1 to 5 kg. Healthy male and female breeders should be identified and netted out before the breeding season and should be kept in spawning pools. They should be provided with supplementary food.

Spawning in Breeding Hapa:

A pair of breeder is released into the breeding hapa for spawning after injection of pituitary extract. The breeding hapa is a rectangular case of fine netting. For larger fishes its size is 8′ x 3′ x 3′, but for the smaller fishes it is 5’x 3’x 3′. It is held on four bamboo poles, one at each corner of the rectangular case. The roof of the hapa may be open or closed.

The hapa is made of mosquito net cloth through which laid eggs and milt cannot escape out. Three-fourth part of hapa is summarized in water whereas upper one-fourth part remains in air. After 3 to 6 hours of injection of pituitary extract spawning takes place.

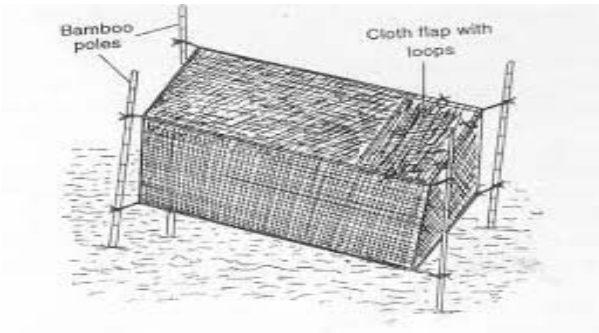

Fig: Breeding Hapa

The fertilized eggs are white and opaque whereas unfertilized eggs are transparent and bead-like. A hatching hapa is also rectangular and made of muslin or malmal cloth and is open from above. The mosquito net hapa is present inside the hatching hapa

Precautions for Induce Breeding:

(1) To avoid diseases and parasitic infections, breeders should be properly washed with $KMnO_4$ solution (0.5 g in 100 litres of water) for a few minutes. After this they should be kept in formalin (200 mg/ It of water) for one hour.

(2) Breeder should be protected from mechanical injuries during handling.

(3) Water condition should be favourable having temperature about 24 to 31°C and turbidity about 100 to 1000 ppm. Flowing water with higher O_2 content is of great use. The intensity and duration of light also affect the induced breeding and spawning. Pituitary glands taken from the same or related species as the recipient species are said to be more effective.

Advantages of Induced Breeding:

1. The seed spawn is timely available, its availability from natural sources is quite uncertain.

2. A pure spawn of a desired species is made available. The spawn obtained from the rivers are not pure. They are mixed with the spawns of other species and sorting of pure seed from the mixed spawn is not possible.

3. Any quantity of pure spawn can be made available.

4. Several carps attain sexual maturity in ponds but they do not breed in confined water. Such fish can be subjected to induced breeding and spawn can be collected.

5. It is economical to obtain a spawn from induced breeding experiments in comparison to its collection from the riverine sources.

6. The induced breeding technique is very simple and can be learnt even by a layman.

Disadvantage of Induced Breeding:

There is higher risk of various genetic disorders. Inbreeding depression (i.e. infertility, delayed maturity, slow growth, and decrease egg production) is also a major drawback of this artificial breeding system.

Probable questions:

1. State the precautions of Induced breeding.

2. State the advantages of induced breeding.

3. State the disadvantages of induced breeding.

4. How breeders for induced breeding is selected.

5. How pituitary extract for induced breeding is prepared and injected.

6. How pituitary in fishes is collected?

7. How pituitary gland is preserved for induced breeding.

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Medical Zoology

Unit-XIV

Genetics of Neurological Diseases: Pharmacogenetics and application

Objective: In this unit you will learn about Genetics of Neurological Diseases and Pharmacogenetics and its application

Introduction:

Pharmacogenetics is the study of inherited genetic differences in drug metabolic pathways (and other pharmacological principles, like enzymes, messengers and receptors) which can affect individual responses to drugs, both in terms of therapeutic effect as well as adverse effects.The term pharmacogenetics is often used interchangeably with the term pharmacogenomics which also investigates the role of acquired and inherited genetic differences in relation to drug response and drug behavior through a systematic examination of genes, gene products, and inter- and intra-individual variation in gene expression and function.

In oncology, pharmacogenetics historically is the study of germline mutations (e.g., single-nucleotide polymorphisms affecting genes coding for liver enzymes responsible for drug deposition and pharmacokinetics), whereas pharmacogenomics refers to somatic mutations in tumoral DNA leading to alteration in drug response (e.g., KRAS mutations in patients treated with anti-Her1 biologics).Pharmacogenetics is believed to account for inter-ethnic differences (e.g., between patients of Asian, Caucasian and African descent) in adverse events and efficacy profiles of many widely used drugs in cancer chemotherapy.

History :

The first observations of genetic variation in drug response date from the 1950s, involving the muscle relaxant suxamethonium chloride, and drugs metabolized by N-acetyltransferase. One in 3500 Caucasians has less efficient variant of the enzyme (butyrylcholinesterase) that metabolizes suxamethonium chloride. As a consequence, the drug's effect is prolonged, with slower recovery from surgical paralysis. Variation in the N-acetyltransferase gene divides people into "slow acetylators" and "fast acetylators", with very different half-lives and blood concentrations of such important drugs as isoniazid (antituberculosis) and procainamide (antiarrhythmic). As part of the inborn system for clearing the body of xenobiotics, the cytochrome P450 oxidases (CYPs) are heavily involved in drug metabolism, and genetic variations in CYPs affect large populations. One member of the CYP superfamily, CYP2D6, now has over 75 known allelic variations, some of which lead to no activity, and some to enhanced activity. An estimated 29% of people in parts of East Africa may have multiple copies of the gene, and will therefore not be adequately treated with standard doses of drugs such as the painkiller codeine (which is activated by the enzyme). The first study using Genome-wide association studies (GWAS) linked age-related macular degeneration (AMD) with a SNP located on chromosome 1 that increased one's risk of AMD. AMD is the most common cause of blindness,

affecting more than seven million Americans. Until this study in 2005, we only knew about the inflammation of the retinal tissue causing AMD, not the genes responsible.

Genetics of Neurological Disorders:

The central nervous system is very complex, and normal function of neurons is depending on the correct performance of thousands of genes and their products. A large number of genetic disorders are therefore affecting functions of the nervous system. There are several different types of inherited genetic disorders, and neurological disease can be caused by all these different mechanisms.

a. Chromosomal disorders:

This group of disorders is caused by structural mutations of one or several chromosomes. Chromosome abnormalities impair cell functions because of missing or extra chromosomal material (either whole chromosome or segments of chromosomes) or because a structural rearrangement interrupts a gene. Chromosome abnormalities are a relatively common cause of developmental disorders, occurring in 1 in 200 individuals. Chromosomal disorders are common syndromes with a complex phenotype and frequently involve intellectual disability. Thus, these syndromes are mostly diagnosed in childhood. However, neurologists are involved in the treatment for symptoms persisting into adulthood as epilepsy and spasticity. A diagnosis of a chromosomal disorder was traditionally based on cytogenetic testing using karyograms and fluorescent in situ hybridization (FISH). Due to recent advances in diagnostic techniques, including the development of array-based comparative genomic hybridization (aCGH), genomic copy number variations can be detected at a much higher resolution level than through traditional methods. This had led to the identification of a large number of chromosomal disorders in the last years. As an example, aCGH has been applied to identify the molecular causes of several types of epilepsy.

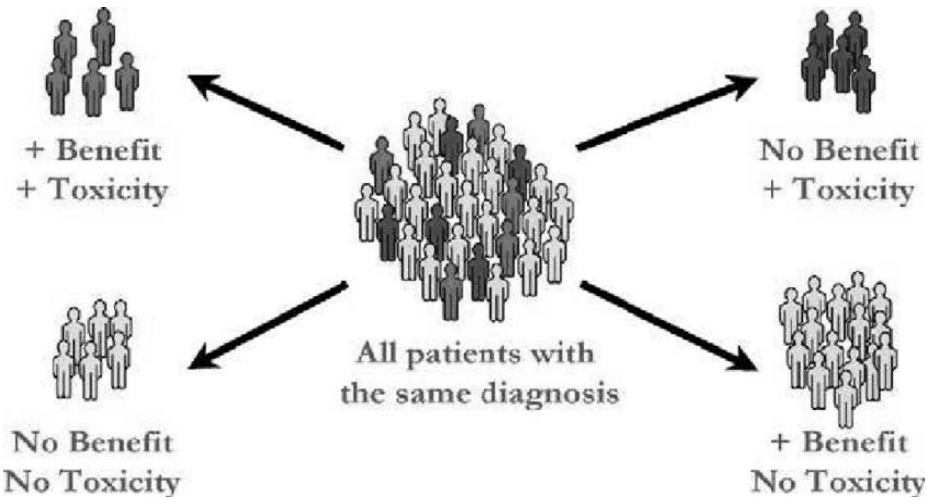
b. Mendelian disorders

These disorders are caused by a mutation in a single gene and are therefore also known as monogenic disorders. In Mendelian disorder one copy (for dominant genes) or two copies (for recessive genes) of the mutant gene inevitably lead to the development of the disease. Diseases with such simple inheritance patterns are each relatively rare and constitute a small proportion of all cases of neurological disease. However, the total frequency of all inherited neurological disorders is considerable. There are a many Mendelian neurological disorders, including neuropathies, myopathies, epilepsies, ataxias and other degenerative disorders of the brain and spinal cord. In 1993, one of the first molecular causes of a Mendelian disorder was found when an international consortium identified the gene causing Huntington's disease. Huntington's disease is caused by an expansion of a CAG trinucleotide repeat. A number of repeat disorders are caused by abnormal length of a repeated section within a gene, including Friedreich's ataxia, several spinocerebellar ataxias and myotonic dystrophy. Interestingly, a hexanucleotide repeat within the C9orf72 gene has recently been identified in a substantial proportion of both familial and sporadic forms of frontotemporal dementia and amyotrophic lateral sclerosis. This indicates that repeat disorders might be even more frequent than appreciated. The most common causes of Mendelian neurological disorders are however missense and nonsense mutations within the coding region of a gene. Many neurological phenotypes are genetically heterogeneous. Different mutations within a single gene can cause the same disease (allelic heterogeneity), and mutations in several different genes can be related to the same clinical expression (locus heterogeneity). For example, a large number of genes have been identified in neuropathies, myopathies and ataxias. In addition to these more traditional genetic disorders, Mendelian forms of disorders

that are normally occurring sporadically have also been identified. As an example, a proportion of patients with Parkinson's disease have inherited mutations in one of several disease genes. Even more complex is the aetiology of epilepsies, where a number of environmental causes are also known. In addition to improved diagnostic possibilities, these forms of disease have identified pathways that are involved in disease pathogenesis, providing information that is applicable also for the more common sporadic formsComplex disorders Complex disorders are presumably caused by interplay between a large number of genetic, environmental and stochastic factors. Genome-wide association studies (GWAS) have identified large numbers of loci that contribute to the genetic basis of complex traits. For example, a large international study of multiple sclerosis, which included Norwegian participants, identified 29 new risk loci in addition to the more than 20 previously known loci. Many of the identified genes are involved in immune response, in particular differentiation of T-helper cells, further emphasizing the role of the immune system in the pathogenesis of multiple sclerosis. Despite the identification of many susceptibility genes for complex disorders, these genes collectively contribute very little to disease risk of each individual patient. It was recently confirmed a number of disease associations in a large Scandinavian study of Parkinson's disease. However, the results from risk-profile analysis showed that the group of individuals with the largest number of risk alleles only had an about three times higher disease risk than individuals with few risk alleles. Thus, the most important contribution of genetic association studies is increased general insights, rather than individual predictions. There is therefore currently a very limited role for genetic testing of complex disorders. Nevertheless, detailed genetic and molecular characterizations might in the future translate into personalized and better treatment options for patients with these common disorders.

Applications of Pharmacogenetics:

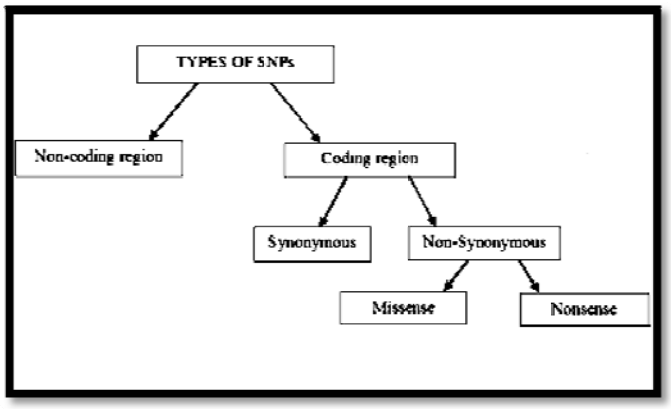
Pharmacogenetics has been defined as the study of variability in drug response due to heredity.Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to the differences in the efficacy and toxicity of many medications from one individual to another.



The history of pharmacogenetics stretches as far back as 510 B.C. when Pythagoras noted that ingestion of fava beans resulted in a potentially fatal reaction in some, but not all, individuals. Since then there have been numerous landmarks that have shaped this field of research, and have led to the current wave of interest.

Although there are a number of different types of polymorphic markers,which mainly includes **Single nucleotide polymorphisms (SNPs)**.

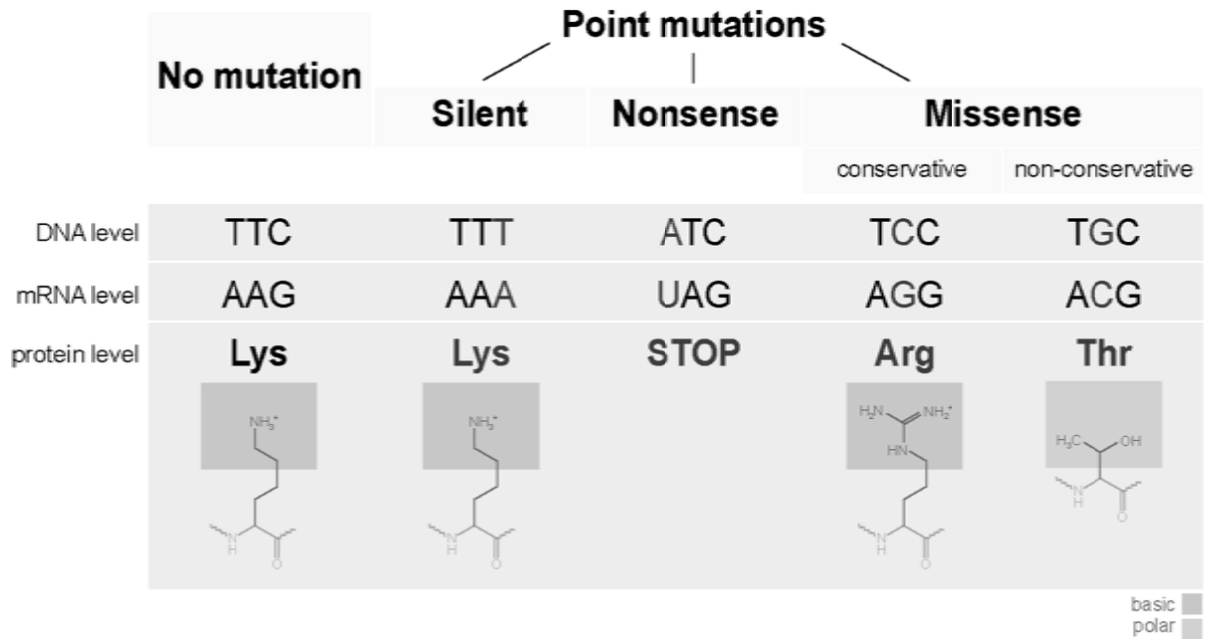
Single nucleotide polymorphisms (SNPs), is the variation in a single nucleotide that occurs in a specific position or locus (SNP locus) in the genome of an individual, which makes the genetic constituency of that individual, different from rest of the population.



SNPs in the coding region are of two types: **Synonymous** and **Nonsynonymous** SNPs. Synonymous SNPs do not affect the protein sequence, while nonsynonymous SNPs change the amino acid sequence of protein. The nonsynonymous SNPs are of two types: **missense** and **nonsense**.

SNPs occur at a frequency of 1% or greater in the population.Hence,SNP of a coding sequence may result in a different or varied translational product, and when this translational product is the protein that acts as metabolic enzyme or porter system responsible for the drug metabolism or transport, it results in a varied response of the encountered drug which may be dramatically different from one person to another.

Moreover, a phylogenetic gene constituency greatly influences the functionality of a drug. This is mainly due to the **haplotype** that changes from one family to another. **Haplotype** is a set of specific

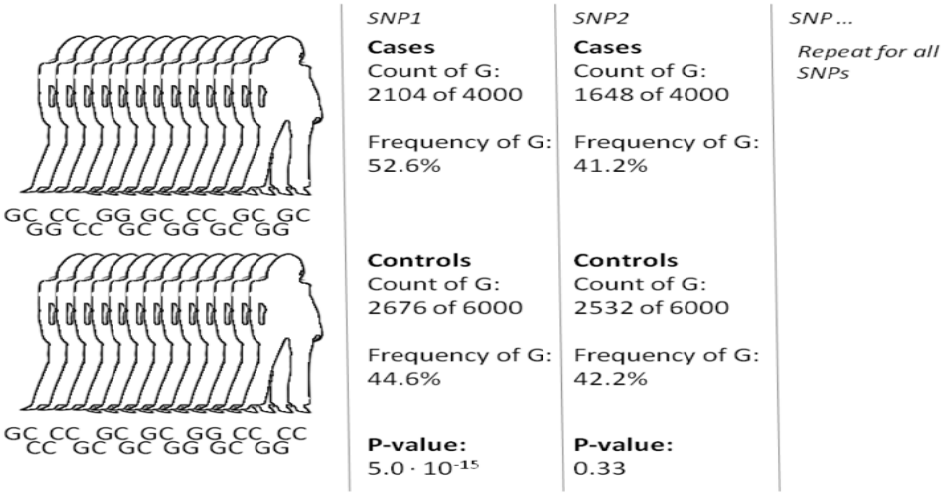


SNP alleles at particular SNP loci that are closely positioned in one small region of a chromosome, so in any particular family, these haplotypes are rarely scrambled by genetic recombination.

Methods involved in Pharmacogenetics:

Pharmacogenomics, similar to other areas of human genetics, has adopted a new strategy for the identification of genetic variation associated with clinical end points: primarily that of **genome-wide association studies (GWAS)**.GWAS, also known as whole **genome association study (WGAS),** is an observational study of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait of any major human disease. GWASs typically focus on associations between single-nucleotide polymorphisms (SNPs) of a person and traits like major human diseases.The most common approach of GWA studies is the **case-control setup**, which compares two large groups of individuals, one healthy control group and one case group affected by a disease. All individuals in each group are genotyped for the majority of common known SNPs. For each of these SNPs it is then investigated if the allele frequency is significantly altered between the case and the control group.In such setups, the fundamental unit for reporting effect sizes is the odds ratio (i.e., how strongly the presence or absence of property "A" is linked with the presence or absence of property "B").

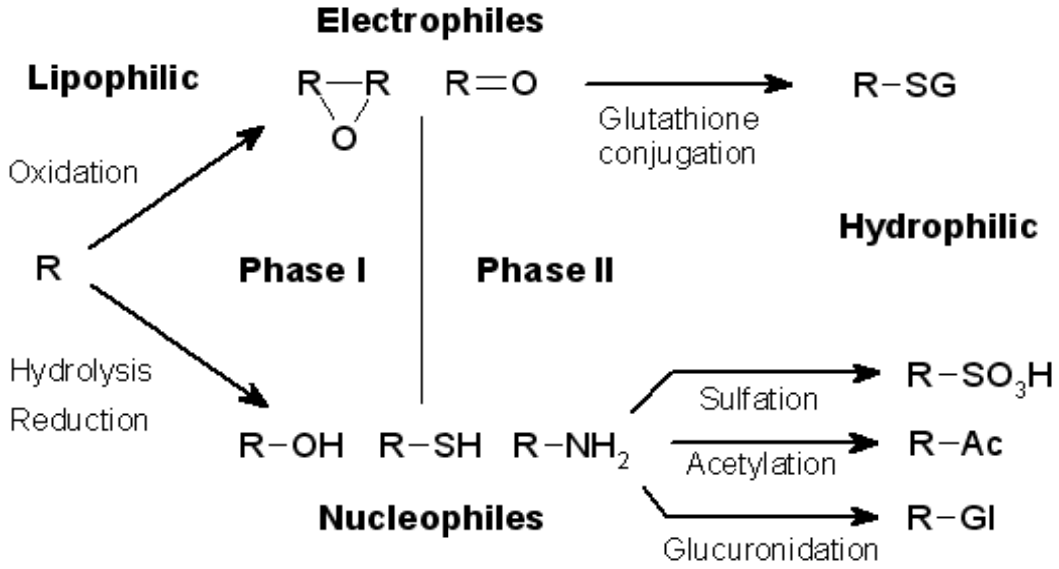
The odds ratio is the ratio of two odds, which in the context of GWA studies are the odds of disease for individuals having a specific allele and the odds of disease for individuals who do not have that same allele.Calculations are typically done using bioinformatics software such as SNPTEST and PLINK, which also include support for many of these alternative statistics.[Earlier GWAS focused on the effect of individual SNPs. However, the recent data shows that complex interactions among two or more SNPs, epistasis, might contribute to complex diseases. Moreover, the researchers try to integrate GWA data with other biological data such as protein-protein interaction network to extract more informative results



Above diagram illustrating the methodology of a case-control GWA study. The allele count of each measured SNP is evaluated—in this case with a chi-squared test—to identify variants associated with the trait in question. The numbers in this example are taken from a 2007 study of coronary artery disease (CAD) that showed that the individuals with the G-allele of SNP1 (rs1333049) were overrepresented amongst CAD-patients.

Molecular Mechanism:

Drug Metabolism:
In order to understand the molecular mechanisms of pharmacogenic conditions, we first need to get an overview of metabolism and excretion of xenobiotic components by our body.



The metabolism of xenobiotics is often divided into three phases:- modification, conjugation, and excretion. These reactions act in concert to detoxify xenobiotics and remove them from cells.

. Phase I – modification
In phase I, a variety of enzymes act to introduce reactive and polar groups into their substrates. One of the most common modifications is hydroxylation catalyzed by the cytochrome P-450-dependent mixed-function oxidase system. These enzyme complexes act to incorporate an atom of oxygen into nonactivated hydrocarbons, which can result in either the introduction of hydroxyl groups or N-, O- and S-dealkylation of substrates

$$\mathbf{O_2 + NADPH + H^+ + RH \rightarrow NADP^+ + H_2O + ROH}$$

Phase I reactions may occur by oxidation, reduction, hydrolysis, cyclization, decyclization, and addition of oxygen or removal of hydrogen, carried out by mixed function oxidases, often in the liver.

b. Phase II – conjugation
In subsequent phase II reactions, these activated xenobiotic metabolites are conjugated with charged species such as glutathione (GSH), sulfate, glycine, or glucuronic acid. Sites on drugs where conjugation reactions occur include carboxyl (-COOH), hydroxyl (-OH), amino (NH_2), and sulfhydryl (-SH) groups. Products of conjugation reactions have increased molecular weight and tend to be less active than their substrates, unlike Phase I reactions which often produce active metabolites. The

addition of large anionic groups (such as GSH) detoxifies reactive electrophiles and produces more polar metabolites that cannot diffuse across membranes, and may, therefore, be actively transported.Examples given in the table below.

Mechanism	Involved enzyme	Co-factor	Location
methylation	methyltransferase	S-adenosyl-L-methionine	liver, kidney, lung, CNS
acetylation	N-acetyltransferases bile acid-CoA:amino acid N-acyltransferases	acetyl coenzyme A	liver, lung, spleen, gastric mucosa, RBCs, lymphocytes
glutathione conjugation	glutathione S-transferases	glutathione	liver, kidney

c. Phase III – further modification and excretion

After phase II reactions, the xenobiotic conjugates may be further metabolized. A common example is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates. Here, the γ-glutamate and glycine residues in the glutathione molecule are removed by Gamma-glutamyltranspeptidase and dipeptidases. In the final step, the cystine residue in the conjugate is acetylated.
Conjugates and their metabolites can be excreted from cells in phase III of their metabolism, with the anionic groups acting as affinity tags for a variety of membrane transporters of the multidrug resistance protein (MRP) family. These proteins are members of the family of ATP-binding cassette transporters and can catalyze the ATP-dependent transport of a huge variety of hydrophobic anions,and thus act to remove phase II products to the extracellular medium, where they may be further metabolized or excreted.

Examples of Pharmacogenetic diseases:

1. **Glucose-6-phosphate dehydrogenase** deficiency is a genetic disorder that occurs almost exclusively in males. This condition mainly affects red blood cells, which carry oxygen from the lungs to tissues throughout the body. In affected individuals, a defect in an enzyme called glucose-6-phosphate dehydrogenase causes red blood cells to break down prematurely. This destruction of red blood cells is called haemolysis.

   The most common medical problem associated with glucose-6-phosphate dehydrogenase deficiency is haemolytic anaemia, which occurs when red blood cells are destroyed faster than the body can replace them. This type of anaemia leads to paleness, yellowing of the skin and whites of the eyes (jaundice), dark urine, fatigue, shortness of breath, and a rapid heart rate. In people with glucose-6-phosphate dehydrogenase deficiency, haemolytic anaemia is most often triggered by bacterial or viral infections or by certain drugs (such as some antibiotics and medications used to treat malaria). Haemolytic anaemia can also occur after eating fava beans or inhaling pollen from fava plants (a reaction called favism).

2. **Malignant hyperpyrexia** is a dangerous complication of general anesthesia occurring in individuals with an underlying disease of muscle. The essential clinical features of the syndrome are a drastic and sustained rise in body temperature, metabolic acidosis, and widespread muscular rigidity. Triggered by exposure to certain drugs used for general anesthesia (Ex. halothane)

Ethical issues regarding Pharmacogenetics:

Pharmacogenetics has become a controversial issue in the area of bioethics. It's a new topic to the medical field, as well as the public. This new technique will have a huge impact on society, influencing the treatment of both common and rare diseases. As a new topic in the medical field the ethics behind it are still not clear. However, ethical issues and their possible solutions are already being addressed.

There are three main ethical issues that have risen from pharmacogenetics. First, would there be a type equity at both drug development and the accessibility to tests. The concern of accessibility to the test is whether it is going to be available directly to patients via the internet, or over the counter. The second concern regards the confidentiality of storage and usage of genetic information. Thirdly, would patients have the control over being tested.

One concern that has risen is the ethical decision health providers must take with respect to educating the patient of the risks and benefits of medicine developed by this new technology. Pharmacogenetics is a new process that may increase the benefits of medicine while decreasing the risk. However clinicians have been unsuccessful in educating patients regarding the concept of benefits over risk. The Nuffield Council reported that patients and health professionals have adequate information about pharmacogenetics tests and medicine. Health care providers will also encounter an ethical decision in deciding to tell their patients that only certain individuals will benefit from the new medicine due to their genetic make-up. Another ethical concern is that patients who have not taken the test be able to have access to this type of medicine. If access is given by the doctor the medicine could negatively impact the patient's health. The ethical issues behind pharmacogenetics tests, as well as medicine, are still a concern and policies will need to be implemented in the future.

Limitations of Pharmacogenetics:

1. Complex targeting due to multiple gene involvement.
2. Difficult and time consuming to identify small variations in genes.
3. Interaction with other drugs and environment to be determined.

Probable Questions:

1. What is pharmacogenetics? How it is developed?

2. What are the applications of Pharmacogenetics ?

3. what are the limitations of Pharmacogenetics ?

4. What are different phases involved in drug designing ?

5. Describe the methods of Pharmacogenetics.

6. Describe ethical issues regarding application of Pharmacogenetics.

7. describe two pharmacogenetic diseases.

Suggested Readings:

1. https://en.wikipedia.org/wiki/Pharmacogenetics.
2. Pharmaceutical Biotechnology by Sambamurthy and Kar. New Age Publication
3. Pharmaceutical Microbiology by Purohit, Saluja and Kakrani. Student Edition.

Unit-XV

Preliminary knowledge on Zoonotic Diseases

Objective: In this unit you will learn about various aspects of zoonotic diseases.

Introduction:

Zoonotic disease, also called **zoonosis**, any of a group of diseases that can be transmitted to humans by nonhuman vertebrate animals, such as mammals, birds, reptiles, amphibians, and fish. A large number of domestic and wild animals are sources of zoonotic disease, and there are numerous means of transmission. Public healthveterinarians have a critical role in zoonotic disease surveillance, prevention, and control, but risk reduction increasingly requires multidisciplinary teams and a unified concept of medicine in humans and other animal species.

Every year, tens of thousands of Americans will get sick from diseases spread between animals and people. These are known as zoonotic diseases. Zoonotic means infectious diseases that are spread between animals and people. Because these diseases can cause sickness or death in people, CDC is always tracking and reporting them.

Animals provide many benefits to people. Many people interact with animals in their daily lives, both at home and away from home. Pets offer companionship and entertainment, with millions of households having one or more pets. We might come into close contact with animals at a county fair or petting zoo, or encounter wildlife while enjoying outdoor activities. Also, animals are an important food source and provide meat, dairy, and eggs.

However, some animals can carry harmful germs that can be shared with people and cause illness – these are known as zoonotic diseases or zoonoses. Zoonotic diseases are caused by harmful germs like viruses, bacterial, parasites, and fungi. These germs can cause many different types of illnesses in people and animals ranging from mild to serious illness and even death. Some animals can appear healthy even when they are carrying germs that can make people sick.

Zoonotic diseases are very common, both in the United States and around the world. Scientists estimate that more than 6 out of every 10 known infectious diseases in people are spread from animals, and 3 out of every 4 new or emerging infectious diseases in people are spread from animals. Every year, tens of thousands of Americans will get sick from harmful germs spread between animals and people. Because of this, CDC works 24/7 to protect people from zoonotic diseases.

History:

During most of human prehistory groups of hunter-gatherers were probably very small. Such groups probably made contact with other such bands only rarely. Such isolation would have caused epidemic diseases to be restricted to any given local population, because propagation and expansion of epidemics depend on frequent contact with other individuals who have not yet developed an adequate immune response. To persist in such a population, a pathogen either had to be a chronic infection, staying present and potentially infectious in the infected host for long periods, or it had to have other additional species as reservoir where it can maintain itself until further susceptible hosts are contacted and infected. In fact, for many 'human' diseases, the human is actually better viewed as an accidental or incidental victim and a dead-end host. Examples include rabies, anthrax, tularemia and West Nile virus. Thus, much of human exposure to infectious disease has been zoonotic.

Many modern diseases, even epidemic diseases, started out as zoonotic diseases. It is hard to establish with certainty which diseases jumped from other animals to humans, but there is increasing evidence from DNA and RNA sequencing, that measles, smallpox, influenza, HIV, and diphtheria came to

humans this way. Various forms of the common cold and tuberculosis also are adaptations of strains originating in other species.
Zoonoses are of interest because they are often previously unrecognized diseases or have increased virulence in populations lacking immunity. The West Nile virus appeared in the United States in 1999 in the New York City area, and moved through the country in the summer of 2002, causing much distress. Bubonic plague is a zoonotic disease,as are salmonellosis, Rocky Mountain spotted fever, and Lyme disease.
A major factor contributing to the appearance of new zoonotic pathogens in human populations is increased contact between humans and wildlife. This can be caused either by encroachment of human activity into wilderness areas or by movement of wild animals into areas of human activity. An example of this is the outbreak of Nipah virus in peninsular Malaysia in 1999, when intensive pig farming began on the habitat of infected fruit bats. Unidentified infection of the pigs amplified the force of infection, eventually transmitting the virus to farmers and causing 105 human deaths.
Similarly, in recent times avian influenza and West Nile virus have spilled over into human populations probably due to interactions between the carrier host and domestic animals. Highly mobile animals such as bats and birds may present a greater risk of zoonotic transmission than other animals due to the ease with which they can move into areas of human habitation.Because they depend on the human host for part of their life-cycle, diseases such as African schistosomiasis, river blindness, and elephantiasis are not defined as zoonotic, even though they may depend on transmission by insects or other vectors.

Zoonotic Disease Classification :

All classes of disease agents cause zoonotic disease, including bacteria, viruses, parasites, and fungi. Although zoonotic diseases can be classified according to their infectious agents, they also can be subdivided into those diseases that are transmitted from nonhuman animals to humans or from humans to nonhuman animals. Examples of the complex pathways of transmission among zoonotic diseases include the spread of *Mycobacterium tuberculosis* from humans to cattle and elephants and the transmission of methacillin-resistant *Staphylococcus aureus* (MRSA) from humans to horses and back to humans. Some diseases are considered to be zoonotic even though they are rarely transmitted between nonhuman animals and humans; an example is foot-and-mouth disease in cattle.
Zoonotic diseases also can be classified according to their life cycle. Diseases that are transmitted directly (e.g., through direct contact or a mechanical vector) and that are maintained in nature in a single vertebrate host species are known as **orthozoonoses**; an example is rabies, which is maintained by canids. **Cyclozoonoses,** such as echinococcosis, require more than one vertebrate host for development. **Metazoonoses** require both a vertebrate host and an invertebrate host; an example is trypanosomiasis. Zoonotic diseases that require a vertebrate host and another type of environmental reservoir (e.g., food or soil) are known as **saprozoonoses**. Listeriosis and histoplasmosis are examples of saprozoonoses.

How do germs spread between animals and people?

Because of the close connection between people and animals, it’s important to be aware of the common ways people can get infected with germs that can cause zoonotic diseases. These can include:

Direct contact: Coming into contact with the saliva, blood, urine, mucous, feces, or other body fluids of an infected animal. Examples include petting or touching animals, and bites or scratches.

Indirect contact: Coming into contact with areas where animals live and roam, or objects or surfaces that have been contaminated with germs. Examples include aquarium tank water, pet habitats, chicken coops, plants, and soil, as well as pet food and water dishes.

Vector-borne: Being bitten by a tick, or an insect like a mosquito or a flea.

Foodborne: Each year, 1 in 6 Americans get sick from eating contaminated food. Eating or drinking something unsafe (such as unpasteurized milk, undercooked meat or eggs, or raw fruits and vegetables that are contaminated with feces from an infected animal).

Zoonotic Disease Control :

Zoonotic diseases are difficult to control, particularly because of their animal reservoirs. Indeed, unlike diseases such as smallpox and polio, most zoonotic diseases cannot be eradicated through intensive human vaccination campaigns. Their successful control relies instead on strategies aimed at reducing the burden of disease among wild animals. In the case of rabies, for example, the distribution of baits containing oral rabies vaccine has led to the near-elimination or eradication of variant rabies (e.g., the Arctic fox and red fox variants) from regional wildlife reservoirs (e.g., foxes and raccoons).

Zoonotic disease risk is increased when humans live in close proximity to domestic animals such as poultry and livestock. Although the practice allows for the efficient use of limited land resources and constant care and protection of the animals, it also increases the risk of humans' becoming infected with disease agents such as HPAI (highly pathogenic avian influenza—e.g., H5N1 virus). Pets, which often live in human homes, are common sources of zoonotic disease. For example, *Salmonella* infections (sometimes with multidrug-resistant strains) can occur as a result of contact with pet reptiles and amphibians (e.g., turtles, iguanas, and snakes), exotic pets (e.g., hedgehogs and sugar gliders), pocket pets (e.g., hamsters, mice, and rats), pet birds (e.g., chicks and ducklings), and dogs and cats. Pet treats and other pet-associated environmental factors may also be sources of *Salmonella*.

Even in areas with separation between human homes and animal facilities, zoonotic diseases still pose a risk to humans, largely because of human contact with animals. Large *E. coli* O157:H7 outbreaks, for example, have been associated with dairy farms, children's day camps conducted in farm settings, social events in buildings previously used for animal exhibitions, fair petting zoos, and contaminated fair water systems. Critical control methods in homes and public settings include animal management to reduce disease burden, management of animal and human contacts, and education to reduce exposure (particularly by handwashing). Limiting contact between humans and wild animals is critical to reducing the risk of zoonotic disease transmission. Many human rabies deaths are due to bites from bats, frequently in home settings. Although the human immunodeficiency virus (HIV), which causes AIDS, is not zoonotic, it is thought to have evolved from similar monkey viruses that jumped to humans through the practice of hunting and consuming bush meat (monkeys). Contact with rodent feces is associated with hantavirus infection, and plagueinfection is associated with activities that bring people into contact with wild rodents and their fleas. The risk of zoonotic disease in humans can be further reduced by limiting contact between wild and domestic animals.

Because zoonotic disease agents can be found in humans, animals, the environment, and vectors, management requires the collaboration of many types of health and disease-control specialists. Disease control may include vector-control programs for ticks, fleas, or mosquitoes, and environmental cleanup or protection may be required to address disease agents that remain viable from days to years on surfaces, in soils, or in the water. In most state health agencies, public health veterinarians are available to assist in disease-control coordination.

The control of zoonotic disease has been challenged by increases in human-associated movement of animals. For example, raccoon rabies was introduced to the central east coast of the United States by deliberate human movement of raccoons from the south-eastern United States for hunting purposes. The disease subsequently spread throughout the east coast and into the midwestern United States and parts of Canada. Monkeypox virus infection of African rodents imported for the pet trade led to cases of the disease in pet prairie dogs and humans, resulting in the introduction of restrictions on the pet prairie dog trade. The spread of H5N1 bird flu in Asia, the Middle East, and Europe likely was the result of human movement of infected domestic birds as well as wild bird migration.

Zoonotic diseases continue to be of significant concern for public health. In the early 21st century, an estimated 60 percent of novel human pathogens were zoonotic in origin, and increasing numbers of zoonotic diseases were spreading into areas where they previously had not occurred. In addition, several zoonotic disease agents were identified as candidates for use in bioterrorism attacks.

Who is at a higher risk of serious illness from zoonotic diseases?

Anyone can become sick from a zoonotic disease, including healthy people. However, some people may be more at risk than others and should take steps to protect themselves or family members. These people are more likely than others to get really sick, and even die, from infection with certain diseases. These groups of people include:

a. Children younger than 5

b. Adults older than 65

c. People with weakened immune systems.

Common zoonotic diseases:

a. Rabies : Rabies is a disease that affects the nervous system of mammals. It is usually caused by a virus and is transmitted if an infected animal bites a person or other animal.Rabies is almost always fatal once symptoms appear. However, rabies vaccines exist and are commonly available.

b. Lyme disease and Rocky Mountain spotted fever: Lyme disease is transmitted through tick bites. Symptoms can range from mild to severe, but it can be treated using antibiotics.

c. Dengue, malaria, and chikungunya : These are mosquito-borne diseases and are more common in certain areas, such as the Caribbean.Symptoms include fever, vomiting, and headaches. It is vital to treat these conditions as soon as possible, as they can be fatal.

d. Salmonella infection : Salmonella is often caused by handling reptiles or amphibians that carry Salmonella, or by handling baby chicks or ducks.The illness usually lasts for between 4 and 7 days, and symptoms include diarrhoea, fever, and abdominal cramps. People can usually recover without medical treatment, although conservative measures are recommended.

e. E. coli infection: This infection is often caused by touching infected animals or handling contaminated food. Cows also have E. coli germs on their udders.Often associated with food poisoning, salmonella can cause vomiting, abdominal cramps, and diarrhoea. It is essential that infected people rest and drink plenty of fluids.

f. Psittacosis : Also known as ornithosis or parrot fever, psittacosis is a bacterial disease that most often affects birds. Humans can get it from feathers, secretions, and droppings.Symptoms include fever, headache, and dry cough. In serious cases, it may cause pneumonia and require a hospital visit.

Preventive measures from zoonotic diseases:

People can come into contact with animals in many places. This includes at home and away from home, in places like petting zoos, fairs, schools, stores, and parks. Insects, like mosquitoes and fleas, and ticks bite people and animals day and night. Thankfully, there are things you can do to protect yourself and your family from zoonotic diseases.

- Washing your hands right after being around animals, even if you didn't touch any animals, is one of the most important steps you can take to avoid getting sick and spreading germs to others. Always wash your hands after being around animals, even if you didn't touch the animals. Many germs are spread by not washing hands with soap and clean, running water. If clean, running water is not accessible, use soap and available water. If soap and water are unavailable, use an alcohol-based hand sanitizer that contains at least 60% alcohol to clean hands. Because hand sanitizers do not eliminate all types of germs, it is important to wash your hands as soon as soap and water are available.

- Know the simple things you can do to stay safe around your pets.
- Prevent bites from mosquitoes, ticks, and fleas.
- Learn more about ways to handle food safely—whether it's for yourself or your family, your pet, or other animals.
- Be aware of zoonotic diseases both at home, away from home (such as at petting zoos or other animal exhibits), in child care settings or schools and when you travel.
- Avoid bites and scratches from animals.

Probable questions:

1. Define Zoonosis.
2. Classify zoonosis according to their lifecycle with suitable examples.
3. How do germs spread between animals and people?
4. Who is at a higher risk of serious illness from zoonotic diseases?
5. How zoonotic diseases can be controlled?
6. Describe preventive measures of zoonotic diseases.
7. Give some common examples of zoonotic diseases with suitable examples.

Suggested Readings:

1) Animal Parasitology by J. D. Smyth .

2) Parasitology by William C. Marquuardt and Richard S. Demaree, jr.

3) Foundation of Parasitology by Roberts and Janovy.

4) Fundamentals of Parasitic Zoonoses by Dr. K.M.L. PATHAK.

5) Medical microbiology by GilbartA.Castro.

6) The physiology of Trematodes , by J.D.Smyth and D. W. Halton

7) Advances in Parasitology by J.D.Smyth .

8) Introduction to Parasitology Nobel and Nobel.

Unit-XVI

Immunodiagnostics: Basic of Immunology and its application

Objective: In this unit you will earn about basic of immunology and its application.

Introduction:

Immunology deals with the study of immunity and immune systems of vertebrates. Immunity (immunis literally means exempt/free from burden) broadly involves the resistance shown, and protection offered by the host organism against the infectious diseases.

The immune system consists of a complex network of cells and molecules, and their interactions. It is specifically designed to eliminate infectious organisms from the body. This is possible since the organism is capable of distinguishing the self from non-self, and eliminate non-self.

The Immune System:
The immune system represents the third and most potent defence mechanism of the body. Acquired (adaptive or specific) immunity is capable of specifically recognizing and eliminating the invading microorganisms and foreign molecules (antigens).

In contrast to innate immunity, the acquired immunity displays four distinct characteristics:
i. Antigen specificity

ii. Recognition diversity

iii. Immunological memory

iv. Discrimination between self and non-self.

The body possess tremendous capability to specifically identify various antigens (antigen is a foreign substance, usually a protein or a carbohydrate that elicits immune response). Exposure to an antigen leads to the development of immunological memory.

As a result, a second encounter of the body to the same antigen results in a heightened state of immune response. The immune system recognizes and responds to foreign antigens as it is capable of distinguishing self and non-self. Autoimmune diseases are caused due to a failure to discriminate self and non-self-antigens.

Organization of Immune System:
The immune system consists of several organs distributed throughout the body (Fig. 68.1). These lymphoid organs are categorized as primary and secondary.

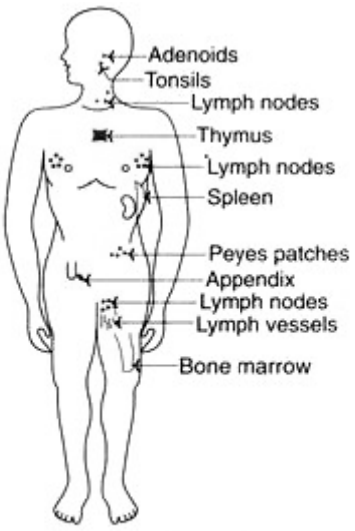


Fig. 68.1 : A diagrammatic representation of human lymphatic system.

Primary lymphoid organs:
These organs provide appropriate micro- environment for the development and maturation of antigen-sensitive lymphocytes (a type of white blood cells). The thymus (situated above the heart) and bone marrow are the central or primary lymphoid organs. T-lymphocyte maturation occurs in the thymus while B-lymphocyte maturation takes place in the bone marrow.

Secondary lymphoid organs:
These are the sites for the initiation of immune response, e.g. spleen, tonsils, lymph nodes, appendix, Peyers patches in the gut. Secondary lymphoid organs provide the microenvironment for interaction between antigens and mature lymphocytes.

Cells of the Immune System:
wo types of lymphocytes namely B-cells and T-cells are critical for the immune system. In addition, several accessory cells and effector cells also participate.

B-lymphocytes:
The site of development and maturation of B-cells occurs in bursa fabricus in birds, and bone marrow in mammals. During the course of immune response. B-cells mature into plasma cells and secrete antibodies (immunoglobulin's). The B-cells possess the capability to specifically recognize each antigen and produce antibodies against it. B-lymphocytes are intimately associated with humoral immunity.

T-lymphocytes:
The maturation of T-cells occurs in the thymus, hence the name. The T-cells can identify viruses and microorganisms from the antigens displayed on their surfaces.

There are at least four different types of T-cells:
i. Inducer T-cells that mediate the development of T-cells in the thymus.

ii. Cytotoxic T-cells (T_c), capable of recognizing and killing the infected or abnormal cells.

iii. Helper T-cells (T_H) that initiate immune responses.

iv. Suppressor T-cells mediate the suppression of immune response.

T-lymphocytes are responsible for the cell- mediated immunity.

Immunoglobulin:

The humoral immunity is mediated by a special group of proteins called immunoglobulin's or antibodies, produced by B-lymphocytes.

Structure of Immunoglobulin:

All the immunoglobulin (Ig) molecules basically consist of two identical heavy (H) chains (mol. wt. 53,000 to 75,000 each) and two identical light (L) chains (mol. wt. 23,000 each) held together by disulphide linkages and non-covalent interactions (Fig. 68.2).

Thus, immunoglobulin is a Y-shaped tetramer (H_2L_2). Each heavy chain contains approximately 450 amino acids while each light chain has 212 amino acids. The heavy chains of Ig are linked to carbohydrates, hence immunoglobulin's are glycoproteins.

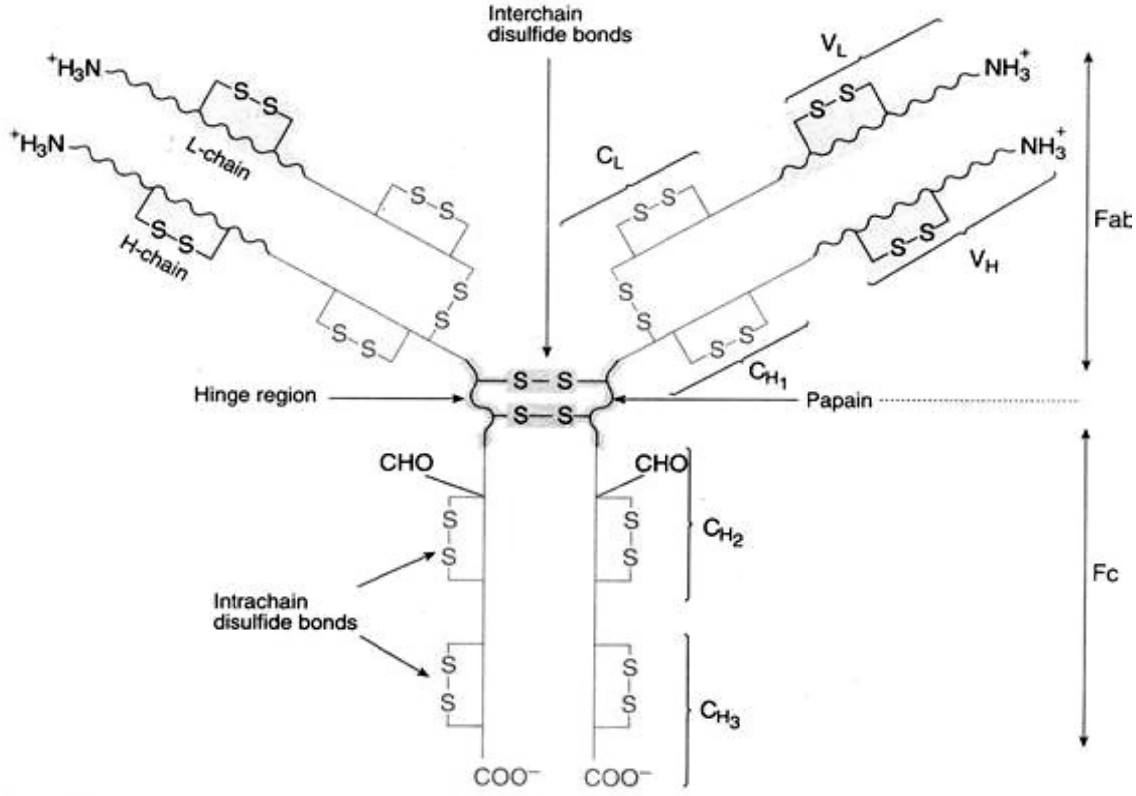


Fig. 68.2 : Diagrammatic representation of human IgG molecule (V—Variable region, C—Constant region; CHO—Carbohydrate; Each heavy chain is composed of four units—V_H, C_{H_1}, C_{H_2}, C_{H_3} while light chain consists of two units—V_L, C_L).

Constant and variable regions:

Each chain (L or H) of Ig has two regions (domains), namely the constant and the variable. The amino terminal half of the light chain is the variable region (V_L) while the carboxy terminal half is the constant region (C_L). As regards heavy chain, approximately one-quarter of the amino terminal region

is variable (V_H) while the remaining three-quarters is constant (C_{H1}, C_{H2}, C_{H3}). The amino acid sequence (with its tertiary structure) of variable regions of light and heavy chains is responsible for the specific binding of immunoglobulin (antibody) with antigen.

Classes of Immunoglobulin:

Humans have five classes of immunoglobulin's— namely IgG, IgA, IgM, IgD and IgE— containing the heavy chains γ, α, μ, δ and ε, respectively. The type of heavy chain ultimately determines the class and the function of a given Ig.

Two types of light chains — namely kappa (k) and lambda (λ) — are found in immunoglobulin's. They differ in their structure in $C_L$regions. An immunoglobulin (of any class) contains two k or two λ light chains and never a mixture. The occurrence of k chains is more common in human immunoglobulin's than λ chains.

The characteristics of the 5 classes of human immunoglobulin's are given in Table 68.1.

Table 68.1 Characteristics of human immunoglobulins

Type	*H-Chain*	*L-Chains*	*Molecular formula*	*Molecular weight*	*Percentage carbohydrate*	*Serum conc. mg/dl*	*Major function(s)*
IgG	γ	κ or λ	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	~150,000	3	800–1,500	Mostly responsible for humoral immunity
IgA	α	κ or λ	$(\alpha_2\kappa_2)_{1-3}$ or $(\alpha_2\lambda_2)_{1-3}$	~$(160,000)_{1-3}$	8	150–400	Protects the body surfaces
IgM	μ	κ or λ	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	~900,000	12	50–200	Humoral immunity, serves as first line of defense
IgD	δ	κ or λ	($\delta_2\kappa_2$ or $\delta_2\lambda_2$)	~180,000	13	1–10	B-cell receptor?
IgE	ε	κ or λ	$\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$	~190,000	12	0.02–0.05	Humoral sensitivity and histamine release.

a. Immunoglobulin G (IgG):
IgG is the most abundant (75-80%) class of immunoglobulin's. IgG is composed of a single Y-shaped unit (monomer). It can traverse blood vessels readily. IgG is the only immunoglobulin that can cross the placenta and transfer the mother's immunity to the developing fetus. IgG triggers foreign cell destruction mediated by complement system.

b. Immunoglobulin A (IgA):
IgA occurs as a single (monomer) or double unit (dimer) held together by J chain. It is mostly found in the body secretions such as saliva, tears, sweat, milk and the walls of intestine. IgA is the most predominant antibody in the colostrum, the initial secretion from the mother's breast after a baby is born. The IgA molecules bind with bacterial antigens present on the body (outer epithelial) surfaces and remove them. In this way, IgA prevents the foreign substances from entering the body cells.

c. Immunoglobulin M (IgM):
IgM is the largest immunoglobulin composed of 5 Y-shaped units (IgG type) held together by a J polypeptide chain. Thus IgM is a pentamer. Due to its large size, IgM cannot traverse blood vessels; hence it is restricted to the blood stream. IgM is the first antibody to be produced in response to an antigen and is the most effective against invading microorganisms.

d. Immunoglobulin D (IgD):

IgD is composed of a single Y-shaped unit and is present in a low concentration in the circulation. IgD molecules are present on the surface of B cells. Their function, however, is not known for certain. Some workers believe that IgD may function as B-cell receptor.

e. Immunoglobulin E (IgE):

IgE is a single Y-shaped monomer. It is normally present in minute concentration in blood. IgE levels are elevated in individuals with allergies as it is associated with the body's allergic responses. The IgE molecules tightly bind with mast cells which release histamine and cause allergy.

Synthesis of Immunoglobulin:

There are millions of different antigens. It was a big puzzle for a long time how an individual can produce so many antibodies to protect against antigens. It is now recognized that a gene rearrangement involving a combination of several genes is responsible for generating an extremely large number of antibodies.

Major Histocompatibility Complex:

The major histocompatibility complex (MHC) represents a special group of proteins, present on the cell surfaces of T-lymphocytes. MHC is involved in the recognition of antigens on T-cells. It may be noted here that the B-cell receptors (antibodies) can recognize antigens and on their own, while T-cells can do so through the mediation of MHC. In humans, the MHC proteins are encoded by a cluster of genes located on chromosome 6 (it is on chromosome 17 for mice). The major histocompatibility complex in humans is referred to as human leukocyte antigen (HLA). Three classes of MHC molecules (chemically glycoproteins) are known in human. Class I molecules are found on almost all the nucleated cells of the body. Class II molecules are associated only with leukocytes involved in cell-mediated immune response. Class III molecules are the secreted proteins possessing immune functions e.g. complement components (C_2, C_4), tumour necrosis factor.

The Immune Response:

The immune response refers to the series of reactions carried out by the immune system in the body against the foreign invader. When an infection takes place or when an antigen enters the body, it is trapped by the macrophages in lymphoid organs. The phagocytic cells which are guarding the body by constant patrolling engulf and digest the foreign substance. However, the partially digested antigen (i.e. processed antigen) with antigenic epitopes attaches to lymphocytes.

T-helper cells (T_H) play a key role the immune response (Fig. 68.3). This is brought out through the participation of antigen presenting cell (APC), usually a macrophage. Receptors of T_H cell bind to class II MHC-antigen complex displayed on the surface of APC. APC secretes interleukin-l, which activates the T_H cell. This activated T_H cell actively grows and divides to produce clones of T_H cells.

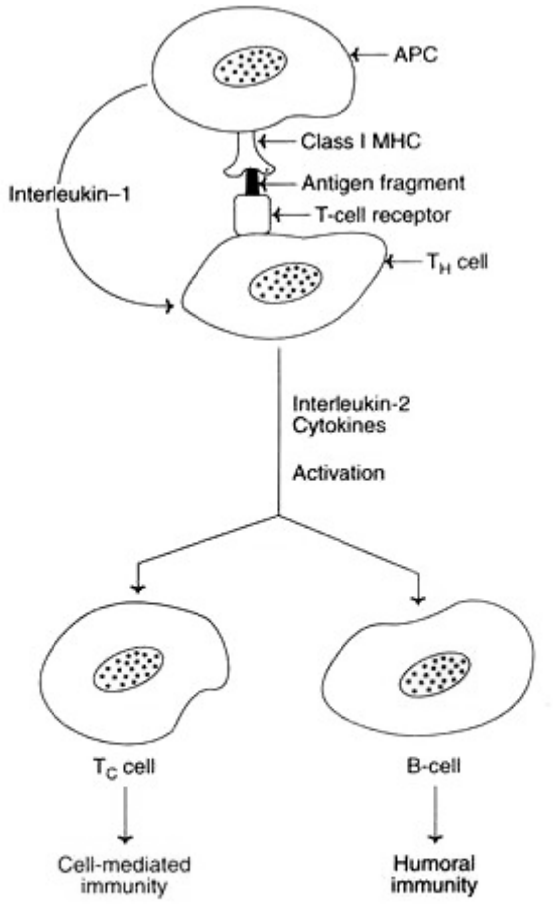


Fig. 68.3 : A diagrammatic representation of the central role of helper T cells (T_H cells) in immune response (APC—Antigen presenting cell; T_C cell—Cytotoxic T–cell).

All the T_H cells possess receptors that are specific for the MHC-antigen complex. This facilitates triggering of immune response in an exponential manner. The T_H cells secrete interleukin-2 which promotes the proliferation of cytotoxic T cells (T_C cells) to attack the infected cells through cell-mediated immunity. Further, interleukin-2 also activates B-cells to produce immunoglobulin's which perform humoral immunity.

Cytokines:

Cytokines are a group of proteins that bring about communication between different cell types involved in immunity. They are low molecular weight glycoproteins and are produced by lymphoid and non-lymphoid cells during the course of immune response. Cytokines may be regarded as soluble messenger molecules of immune system.

They can act as short messengers between the cells or long range messengers by circulating in the blood and affecting cells at far off sites. The latter function is comparable to that of hormones.

The term interleukin (IL) is frequently used to represent cytokines. There are more than a dozen interleukins (IL-I…… IL_{12})/ produced by different cells with wide range of functions. The main function (directly or indirectly) of cytokines is to amplify immune responses and inflammatory responses.

Therapeutic uses of cytokines:

It is now possible to produce cytokines in vitro. Some of the cytokines have potential applications in the practice of medicine. For instance, IL-2 is used in cancer immunotherapy, and in the treatment of immunodeficiency diseases. IL-2 induces the proliferation and differentiation of T-and B-cells, besides increasing the cytotoxic capacity of natural killer cells. A group of cytokines namely interferon's can combat viral infection by inhibiting their replication.

Antibody-Mediated (Humoral) Immunity :

The antibody-mediated or humoral immunity is that where the B-lymphocytes synthesize antibodies response to the detection of antigens and these antibodies counteract with those antigens. Antibody-mediated immunity is often referred to as humoral immunity because the antibody molecules flow extracellularly through the blood and other vital body fluids which are called humors in Greek.

The precursors of B-lymphocytes, which originate from the stem cells of bone marrow, processed within thymus-independent tissues of the lymphatic system (the spleen, tonsils, intestine, appendix, and lymph nodes) and become immunologically competent. In response to antigenic stimulation, the immunologically competent B-lymphocytes convert into two different cell populations primary B-lymphocytes and secondary B-lymphocytes. The primary B-lymphocytes show response to the first antigenic stimulation and immediately enter into the process of their conversion into plasma cells. In contrast, the secondary B-lymphocytes do not show any response to the first antigenic stimulation and constitute memory cells which transform into plasma cells in response to subsequent exposure to antigens, perhaps years later. However, these are the plasma cells which secrete antibodies. Once thought to be totally independent system, the conversion of immunologically competent B-lymphocytes into antibody producing plasma cells is cooperated by helper T-lymphocytes (helper T-cells). An antigen with at-least two different antigenic determinants first attaches on the surface of a macrophage cell which then migrates to lymphatic tissues and interact with helper T-lymphocyte.Once the helper T-lymphocyte binds with the antigen present on the surface of microphage cell, it releases interleukin-2 that stimulates multiplication of helper T-lymphocytes and also the release of B-lymphocyte growth factor, which in turn enhances the division of immunologically competent B-lymphocytes and their conversion into plasma cells. However, the antibodies secreted by plasma cells clump together (agglutinate) with antigens present in body's circulatory system forming antibody-antigen-complexes which are up-taken by scavenger white blood cells. The diagrammatic representation of the process of antibody-mediated (humoral) immunity is given in Fig. 41.3.

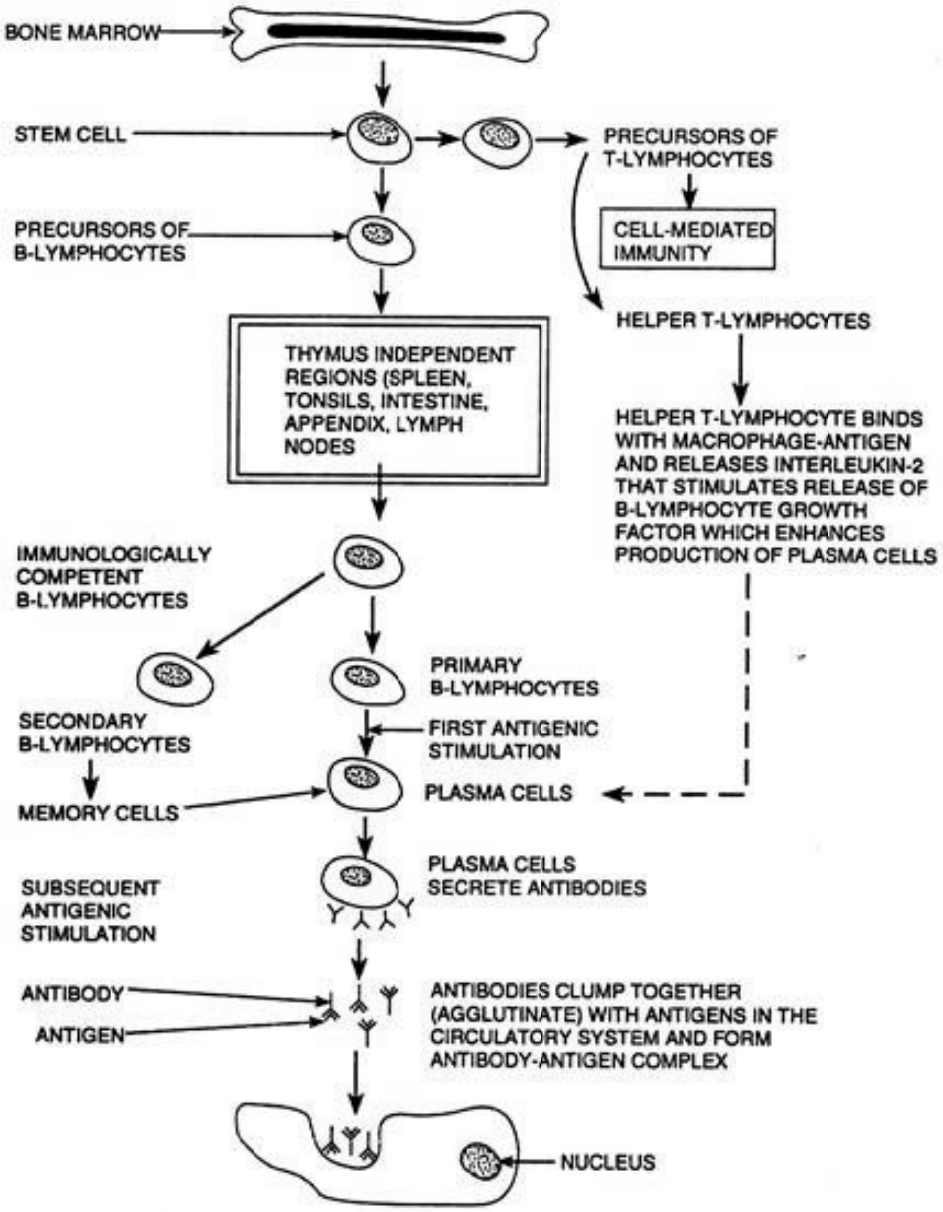


FIG. 41.3. Diagrammatic representation of antibody-mediated (humoral) immunity.

Cell-Mediated (Cellular) Immunity

The cell-mediated or cellular immunity is that where the T-lymphocytes destroy other cells having antigens on their surface without any mediation by antibodies. The precursors of T-lymphocytes produced by stem cells of bone marrow pass through liver and spleen before reaching the thymus where they are processed, hence called thymus-dependent (T) lymphocytes.

These lymphocytes come under the influence of the hormone "thymosin" and become immunologically competent and are called lymplioblasts. When stimulated by an appropriate antigen, the lymphoblasts divide and differentiate into cytotoxic T-lymphocyte (killer T-Iymphocytes), helper T-cells, and suppressor T-cells.

The cytotoxic T-lymphocytes, in addition with other T-lymphocytes, release biologically active soluble factors collectively called lymphokines which act as a biochemical mediators of cellular immunity.

Unlike B-lymphocytes which are normally stimulated by free antigens in the circulatory system of the body, the cytotoxic T-lymphocytes possess specific cell surface proteins, called T-cell receptors, on their surface and respond to only major histocompatibility complex antigens (MHC-antigens) bound to the surface of other cells.After the interaction between T-cell receptor and MHC-antigen is established and the cytotoxic T-lymphocyte cells binds the MHC-antigen containing cell, the latter undergoes lysis and is phagocytised (Fig. 41.2).

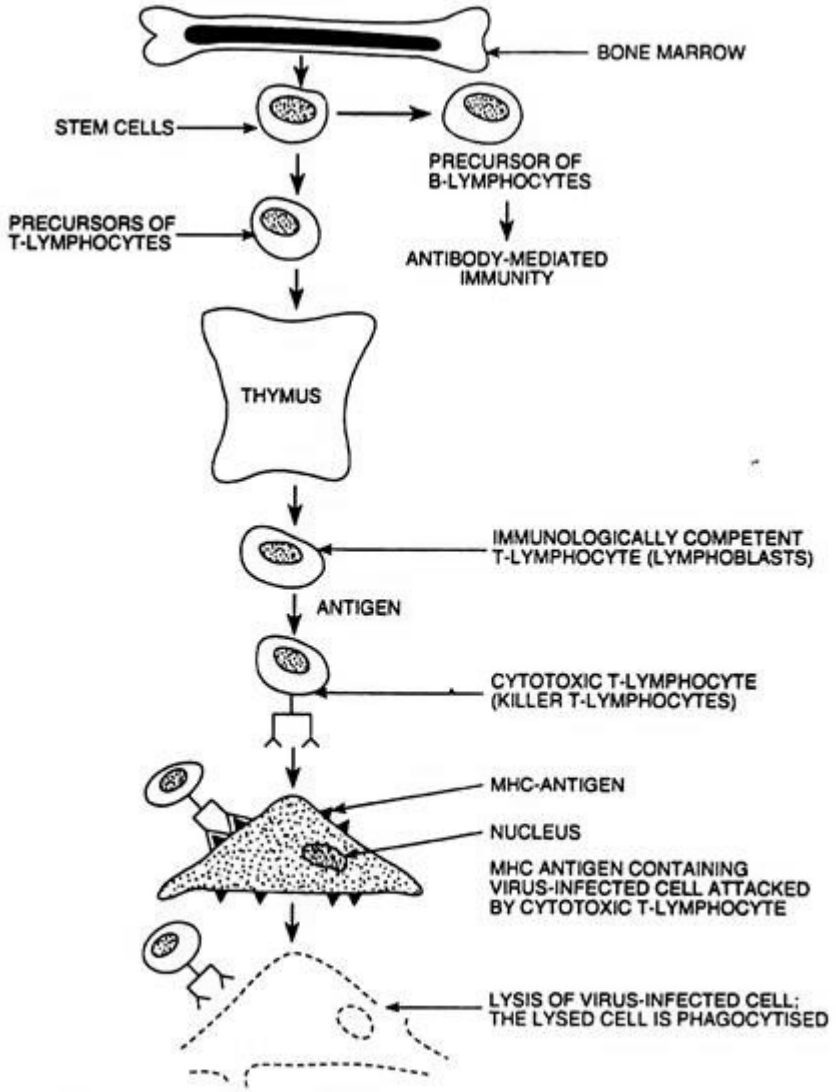


FIG. 41.2. Diagrammatic representation of cell-mediated immunity (cellular immunity).

The cell-mediated immunity is important in controlling those infections where the pathogens are intracellular and reproduce within the infected cells (e.g., viruses, rickettsia, chlamydia, some protozoans like Trypanosomes, etc.).

In such infections the antibodies (hence the antibody-mediated or humoral immunity) prove to be ineffective because the antibodies are unable to penetrate and attack intracellular pathogens multiplying within the host cells.In addition, the cellular immunity is considered to play an important role in monitoring and regulating the proliferation of abnormal type of cells, (e.g., would be tumour cells), and thus, inhibiting the tumour development.

The actions of the different types of T-cell are summarised below:

a. **Helper cells** react by producing small peptide molecules called lymphokines. The lymphokines promote proliferation of more T-cells, stimulate B cells to produce antibodies and also help in accumulating macrophages in the inflamed tissues and by promoting phagocytosis.

b. **Cytotoxic cells or Killer cells** kill cells infected by viruses, cancerous cells and transplants.

c. **Suppressor cells,** the third type of T-cells produce lymphokines that suppress the action of the phagocytes and the different types of WBC cells. They play an important role in immunotolerance.

d. Some of the cells remain as the memory cells that get lodged in the lymphoid tissue throughout the body. These on subsequent exposure to the same antigen can cause an immune response more rapidly than the first exposure.

Antigen Antibody Interactions: Principles and Application.

Antibody affinity is a measure of binding strength that indicates combined strength of non- covalent interactions (Hydrogen bond, Hydrophobic interaction, Vander Waals forces,, ionic bonds) between a single antigenic epitope and paratope/ antigenic determinant on immunoglobulin.

Cross- Reactivity

Although Ag- Ab reactions are highly specific, in some cases, antibody elicited by one antigen can cross react with antigenic epitope of an un-related antigen. Such cross- reactivity occurs if two different antigens share an identical or very similar epitope. In such cases, the antibody′s affinity for the cross reacting epitope is usually less than that for the original epitope.

A. Precipitation Reactions :

Antibody and soluble antigen interacting in aqueous solution form a multimolecular lattice that eventually develops into a visible precipitate due to low solubility. Antibodies that aggregate soluble antigens are called precipitins. So its principle depends on cross-linking of polyvalent antigens in which multivalent antigen combine with bivalent antibodies in optimal proportions.

Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:

- The antibody must be bivalent, a precipitate will not form with monovalent Fab fragments.
- The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope or have different epitopes that react with different antibodies present in polyclonal antisera.

Techniques of precipitation

1.Simple precipitation test

2.Gel diffusion test

3.Immuno-electrophoresis

A. Simple precipitation test:

Ring test- In this test a solution of test antigen is slowly layered on to a solution of antisera in a narrow test tube. Following a period of incubation, a white ring of precipitate develops at the junction of two solutions. This test is applied in tests for C reactive protein.

B. Immune diffusion/Gel diffusion test

- Precipitation reactions in gels yield visible precipitin lines.
- Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in semi solid agar medium or when antibody is incorporated into the agar and antigen diffuses into the antibody containing matrix, a visible line of precipitation will form in the zone of equivalence where antigens and antibodies meet in appropriate proportion, but no visible precipitate forms in regions of antibody or antigen excess.
- Two types of immune diffusion reactions can be used to determine relative concentrations of antibodies or antigens , to compare antigens or to determine the relative purity of an antigen preparation. They are **radial immunodiffusion** (the Mancini method) and **double immunodiffusion** (the Ouchterlony method), both are carried out in a semisolid medium such as agar.

In **radial immunodiffusion**, an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As antigen diffuses into agar, the region of equivalence is established as a ring of precipitation , in such way a precipitin ring is formed around the well. The area of precipitin ring is directly proportional to the concentration of added antigen. By comparing

the area of precipitin curve with a standard curve, concentration of the antigenic sample can be determined.

In **Ouchterlony double diffusion**, both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached , a visible line of precipitation is formed .

The pattern of precipitin line that is formed will indicate whether antigens placed in adjacent wells have similar epitopes or not.

single curved line of identity- If two antigens placed in adjacent wells share identical epitopes, the line of precipitin grow toward each other and fuse to give a single curved line of identity at the equivalent zone.

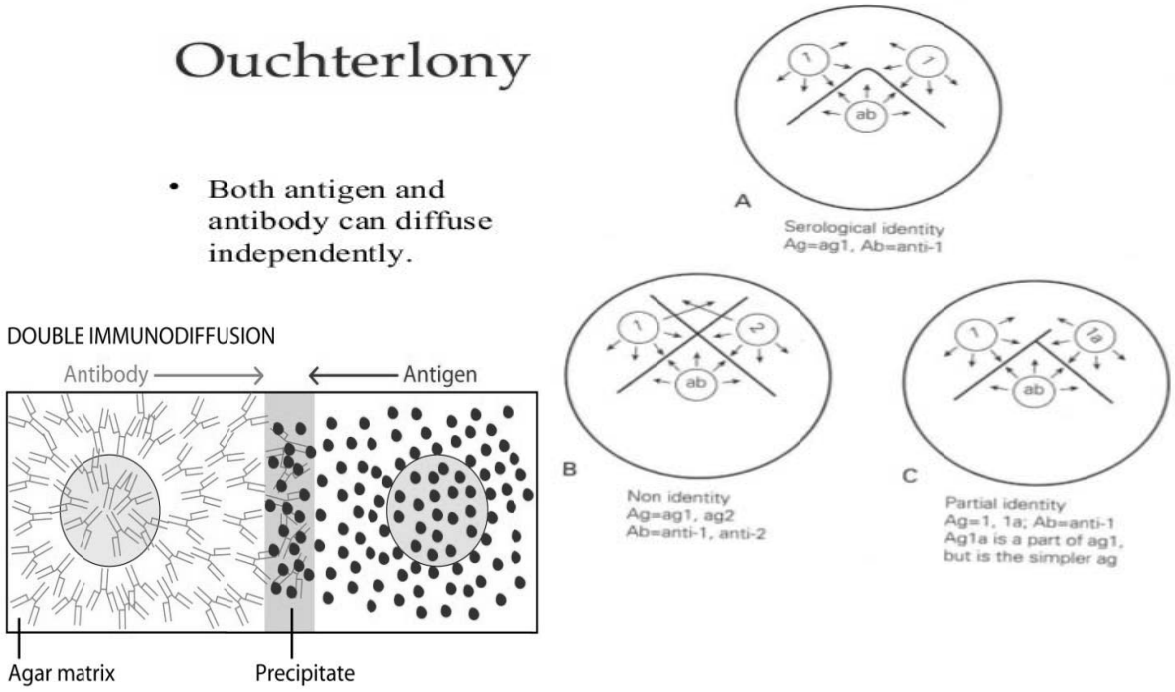


Fig: Schematic diagram of Ouchterlony method

Nonidentity- When two antigens are unrelated , having no common epitipes , the antiserum form independent precipitin line with each antigen and the two lines cross. This is reffered to as non-identity.

Partial identity-

If two antigens share some common epitopes and some non-identical epitopes then antiserum forms a line of identity with the common epitopes and a curved spur with the non identical epitopes. This is referred to as partial identity.

This method was used in diagnosis of small pox and can be used to determine the purity of antigens.

c. Immunoelectrophoresis- This technique combines electrophoresis with double immunodiffusion.

In Immunoelectrophoresis, the antigenic mixture is first electrophoresed to separate its components by charge in an given electric field.

In second step, troughs are then cut into the agar gel parallel to the direction of electric field and then antiserum is added to the troughs to detect antigen of interest in given sample.

Then antigen and antibody diffuse toward each other produce lines of precipitation (precipitin arcs)where they meet in appropriate proportions.

This technique is useful for determination of presence or absence of serum proteins(albumin, immunoglobulin) and detection of unusual proteins such as human myeloma protein. However, it is strictly a qualitative technique that can detect relatively high antibody concentrations.

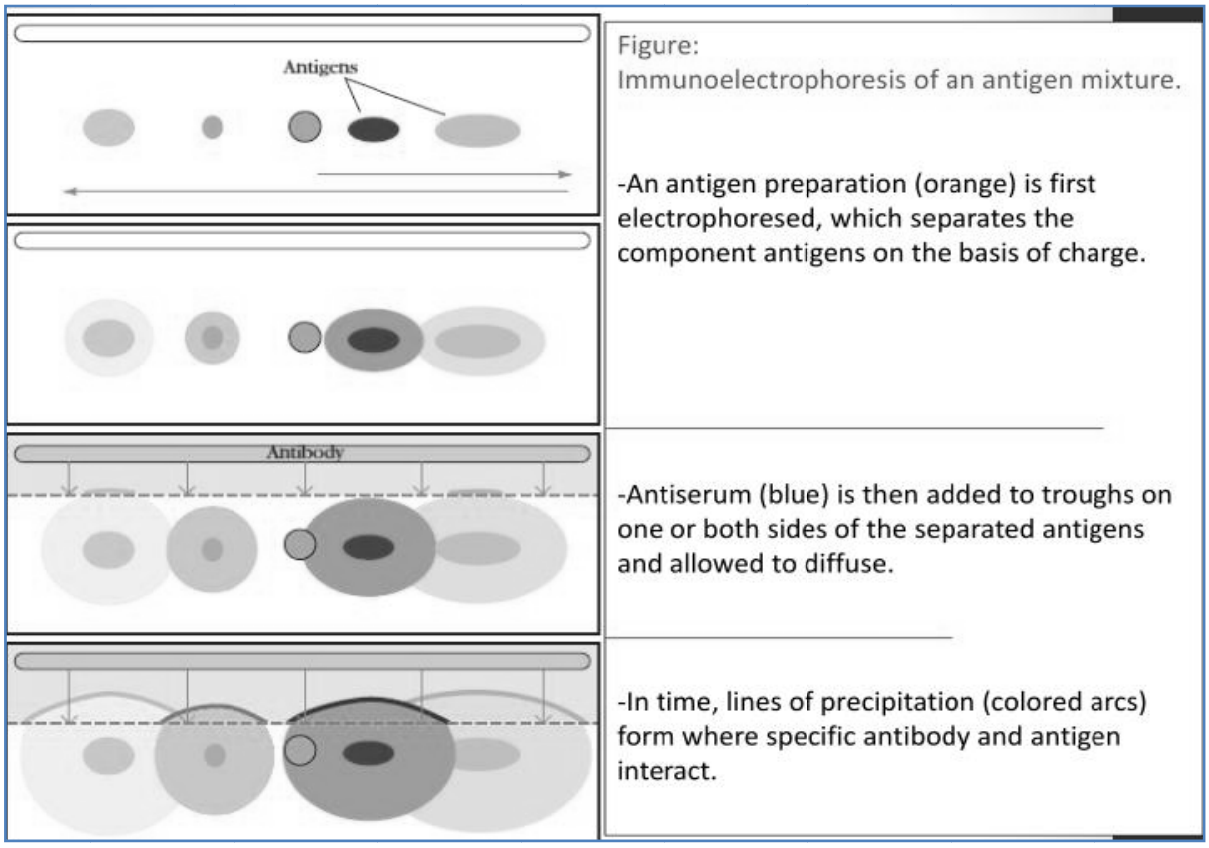


Fig : Schematic diagram of Immunoelectrophoresis

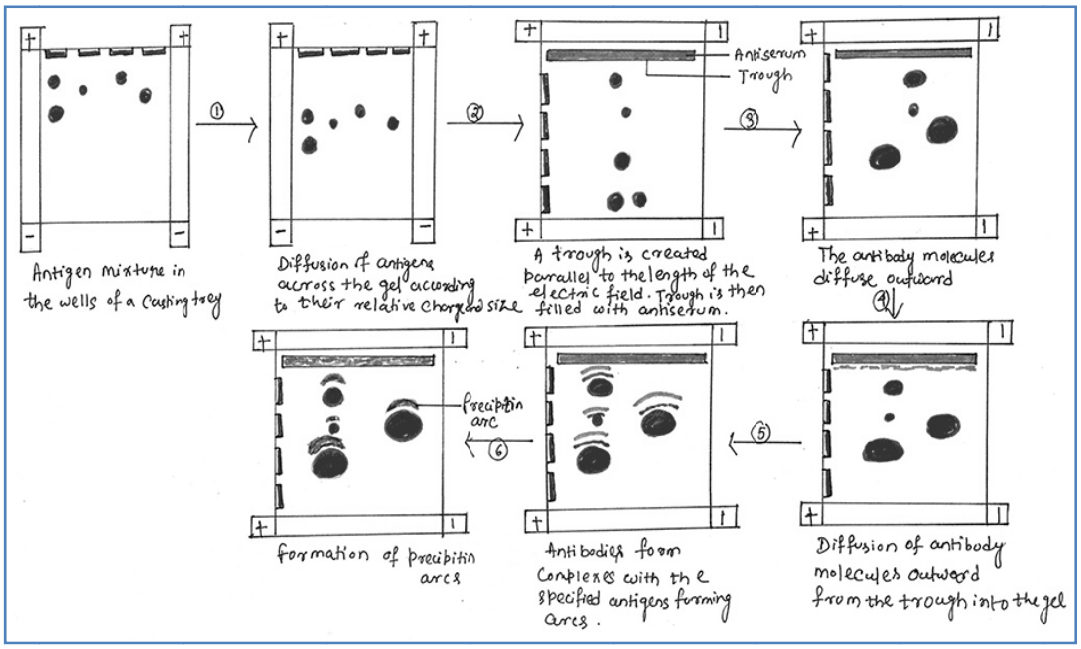
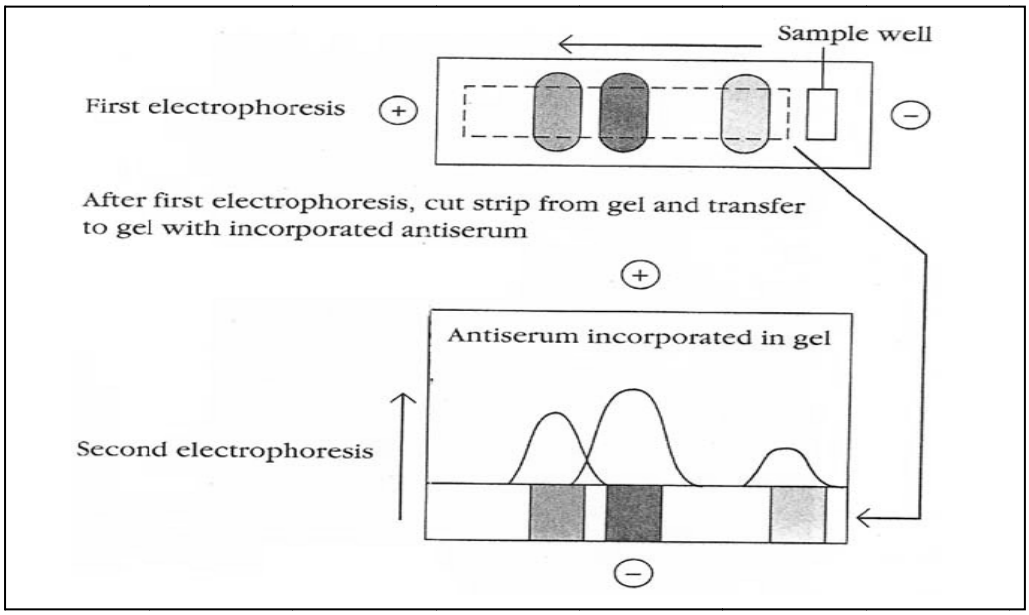


Fig: Different steps of Immunoelectrophoresis

Crossed Immunoelectrophoresis: It is also called two-dimensional quantitative Immunoelectrophoresis. In this method the proteins are first separated during the first dimension electrophoresis, then instead of the diffusion towards the antibodies, the proteins are electrophoresed into an antibody-containing gel in the second dimension. Immunoprecipitation will take place during the second dimension electrophoresis and the immunoprecipitates have a characteristic bell-shape, each precipitate representing one antigen, the position of the precipitate being dependent on the amount of protein as well as the amount of specific antibody in the gel, so relative quantification can be performed. The sensitivity and resolving power of crossed Immunoelectrophoresis is than that of the classical immunoelectrophoretic analysis and there are multiple variations of the technique useful for various purposes. Crossed Immunoelectrophoresis has been used for studies of proteins in biological fluids, particularly human serum, and biological extracts.



Rocket electrophoresis- A related quantitative technique , does permit measurement of antigen levels. At first, a negatively charged antigen is electrophoresed in a gel containing antibody. The precipitate formed between antigen and antibody has the shape of a rocket , the height of which is proportional to the concentration of antigen in the well. One limitation of rocket electrophoresis is need for the antigen to be negatively charged for electrophoretic movement .

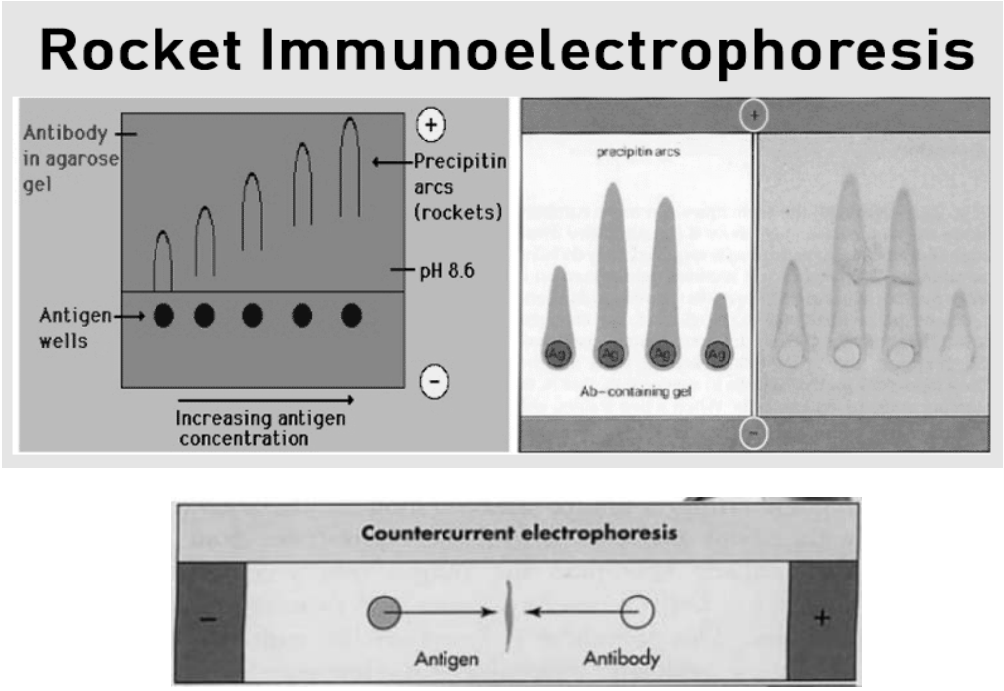
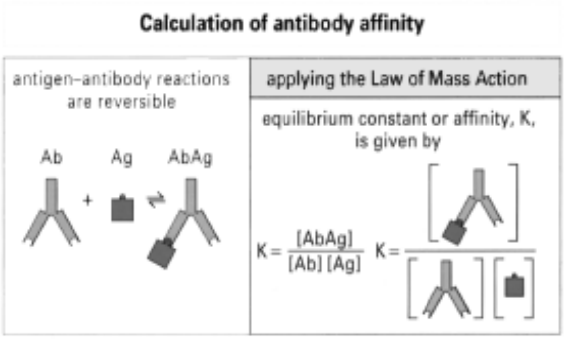
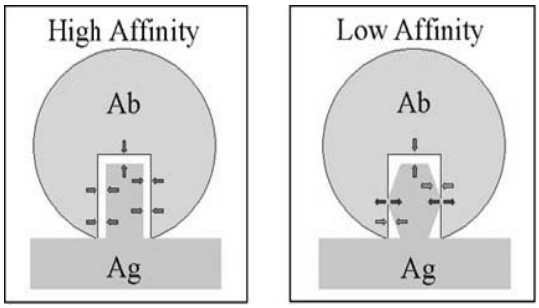


Fig : Schematic diagram of Rocket Electrophoresis

Counter current Immunoelectrophoresis- It relies on movement of antigen and antibody in opposite direction.Antigen carrying negative charge migrates towards the positive pole and positively charged antibody move toward the negative pole. Antigen and homologous antibody are placed in separate cells in agar gel through which electric current is passed. Line of precipitin appear at a point where the two lines meet in optimal proportions. This test is more faster and more sensitive than double diffusion technique. It is useful in detection of hepatitis B antigen and antibodies.

Affinity Immunoelectrophoresis is based on changes in the electrophoretic pattern of proteins through specific interaction or complex formation with other macromolecules or ligands. Affinity Immunoelectrophoresis has been used for estimation of binding constants, as for instance with lectins or for characterization of proteins with specific features like glycan content or ligand binding. Some variants of affinity Immunoelectrophoresis are similar to affinity chromatography by use of immobilized ligands.





NOTE: Two factors determine that immunoelectrophoretic methods are not widely used. First they are rather work intensive and require some manual expertise. Second they require rather large amounts of polyclonal antibodies. Today gel electrophoresis followed by electroblotting is the preferred method for protein characterization because its ease of operation, its high sensitivity, and its low requirement for specific antibodies. In addition proteins are separated by gel electrophoresis on the basis of their apparent molecular weight, which is not accomplished by Immunoelectrophoresis, but nevertheless immunoelectrophoretic methods are still useful when non-reducing conditions are needed.

B. AgglutinationReactions-

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions called agglutinin, its principle is same as precipitation. It depend on cross-linking of polyvalent antigens. But at concentration of antibody, precipitation is not possible, in which antibodies interact with antigen univalently instead of multivalently, this effect is also known as Protozone effect and antibodies that lack the agglutinating activity known as incomplete antibodies. Another reason for lack of agglutinating activity of incomplete antibody is restricted flexibility in hinge region as a result Fab arms can not assume required angle for optimal crosslinking of epitope on two or more particulate antigens.

a. RIA (Radioimmunoassay):

One of the most sensitive technic for detecting antigen or antibody is radioimmunoassay (RIA). The technic was first developed in 1960 by two endocrinologists, S.A. Berson and Rosalyn Yalow, to determine levels of insulin- anti-insulin complexes in diabetics. Although this technique encountered some scepticism, it soon proved its value for measuring hormones, serum proteins, drugs, and vitamins at concentrations of 0.001 micrograms per milliliter or less.

Principle of RIA-

It involves competitive binding of radiolabeled antigen and unlabeled antigen to a high affinity antibody At first, labeled antigen is mixed with antibody at a concentration that saturates the antigen binding sites of the antibody. Then test samples , containing unlabeled antigen of unknown concentration are added in progressively large amounts. But antibody does not distinguish labelled antigen from unlabeled antigen, so two kinds of antigens compete with each other for occupying available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labelled antigen will be displaced from the biding sites. Then decrease in the amount of radiolabeled antigen bound to specific antibody in presence of the test sample is measured in order to determine the amount of antigen present in the test sample.

The antigen is generally labelled with a gamma-emitting isotope such as ^{125}**I** but beta –emitting isotopes such as tritium **(^{3}H)** are also routinely used as labels. The radiolabeled antigen is part of the assay mixture; the test sample may be a complex mixture such as serum or other body fluids that contain unlabeled antigen.

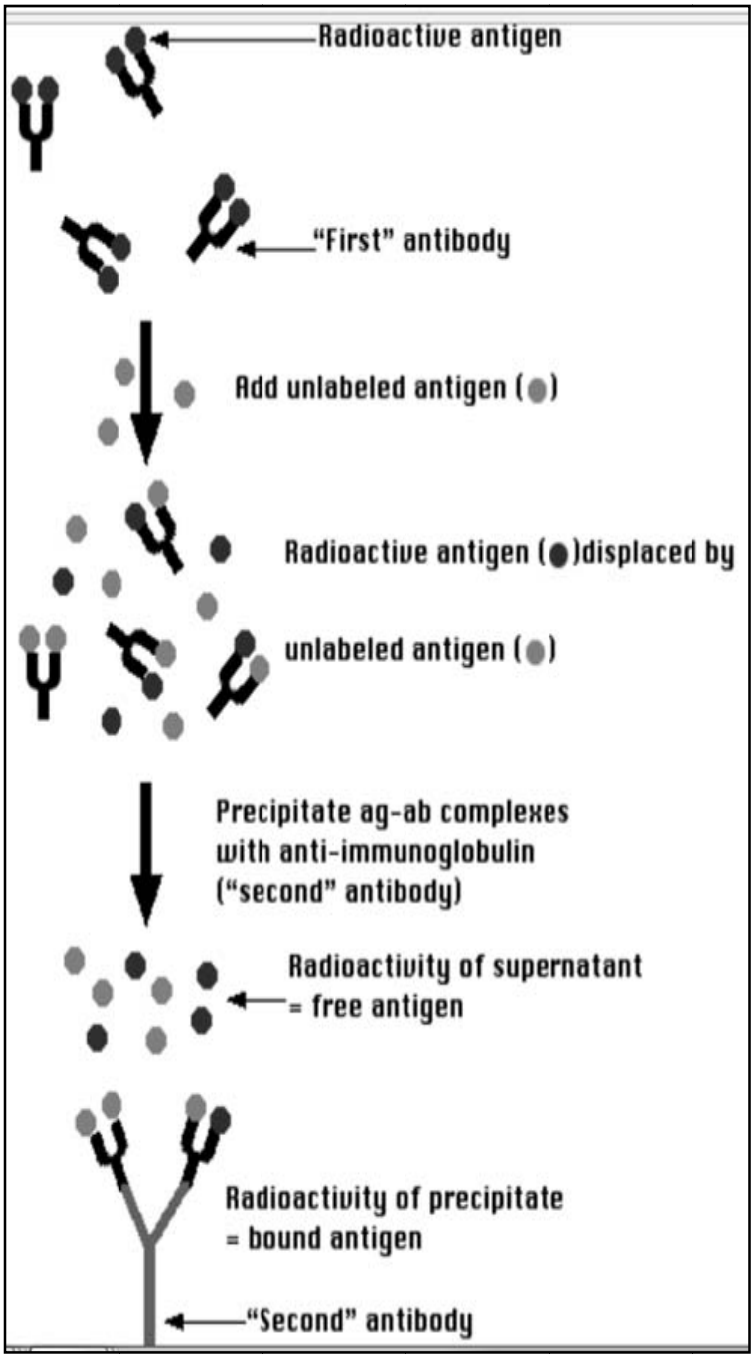
Steps- The First step in setting up an RIA is to determine the amount of antibody , needed to bind 50% to 70% of a fixed quantity of radioactive antigen (Ag*) in the assay mixture. This ratio of antibody to antigen is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites.

Consequently, unlabeled antigen added to the sample mixture will compete with radiolabeled antigen for the limited supply of antibody. So decrease in the amount of radiolabeled antigen will be proportional to the amount of unlabeled antigen added.

To determine the amount of labelled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen, and radioactivity in precipitate is then measured.

A standard curve can be generated by plotting increasing concentration of unlabeled antigen to concentration of bound labeled Ag- Ab complex and from this plot amount of antigen present in the test mixture may be precisely determined.

For example, a microtiter RIA has been widely used to screen for the presence of the hepatitis B virusRIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in recipients of blood transfusions.



b. ELISA (Enzyme linked immunosorbent Assay) :

Enzyme linked immunosorbent assay is commonly known as ELISA, its principle is similar in principle to RIA but depends on enzyme rather than a radioactive label. Enzyme conjugated antibody is used that reacts with colorless substrate to generate a colored reaction product , such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, Horseradish peroxidase, and β- galactosidase.

i. Indirect ELISA

Antibody can be detected and quantitively determined with an indirect ELISA.
Following steps of indirect ELISA are given below-
Serum or sample containing primary antibody (Ab_1) is added to an antigen coated microtiter well and allowed to react with the antigen attached to the well.

↓

After any free Ab1 is washed away , the presence of antibody bound to antigen is detected by adding enzyme conjugated secondary antibody (Ab2) that bind to the primary antibody.

↓

After washing the free secondary antibody, chromogenic substrate is added that generate colored reaction product , deposited at the site of reaction, measured by specialized by spectrophotometric plate readers which can measure the absorbance of all of the wells of a 96 well plate in seconds.
Indirect ELISA is method of choice to detect the presence of serum antibodies against human immunodeficiency virus(HIV) causative agents of AIDs.

ii. Sandwich ELISA

Antigen can be detected by sandwich ELISA, in this technique, the antibody rather than antigen is immobilized on a microtiter well .

↓

Sample containing antigen is added and allowed to react with the immobilized antibody.

↓

After well is washed , a second enzyme linked antibody specific for different epitope on the antigen is added and allowed to react with the bound antigen.

↓

After any free secondary antibody is removed by washing, chromogenic substrate is added and then colored reaction product is measured by spectrophotometric plate readers.

iii. Competitive ELISA

Another variation for measuring the slight amount of antigen is competitive ELISA . In this technique , antibody is first incubated in solution with a sample containing antigen.

↓

Then Ag-Ab complex is added to an antigen coated microtiter well.

↓

The more antigen present in the sample, the less free antibody will be available to bind to the antigen coated well .

↓

Enzyme conjugated secondary antibody (Ab2), specific for isotype of primary antibody is added to determine the amount of primary antibody bound to the well as in an indirect ELISA.
In competitive assay, higher the concentration of antigen in the original sample , the lower the absorbance

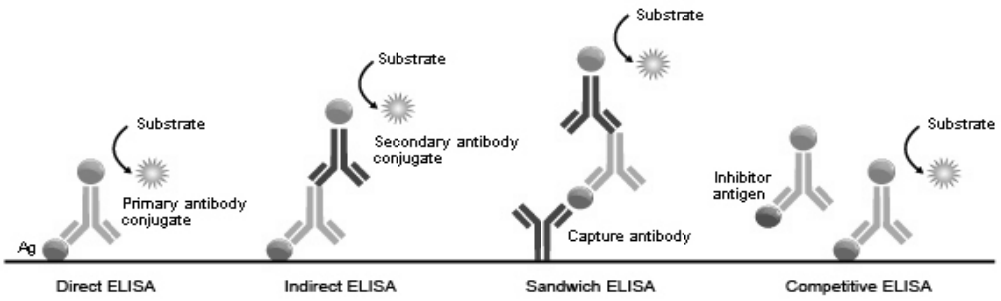


Fig: Schematic diagram of different types of ELISA

c. Immunofluorescence

In 1944, Albert coons showed that antibodies could be labelled with molecules that have the property of fluorescence. Fluorescence molecules absorb light at shorter wavelength and emit light at longer wave length . In this technique , antibody molecules are tagged with fluorescent dye , can be conjugated to Fc region of antibody molecules without affecting antigen binding specificity of the antibody.

If antibody molecules are tagged with a fluorescent dye or fluorochrome, immune complexes containing these fluorescently labelled antibodies in cells or tissue section can be detected by colored light emission when excited by light of appropriate wavelength. Then emitted light beam can be viewed with a fluorescence microscope equippedwith UV light source. Most commonly used fluorescent dyes are Fluorescein, Rhodamine, Phycoerythrin.

d. Western blotting

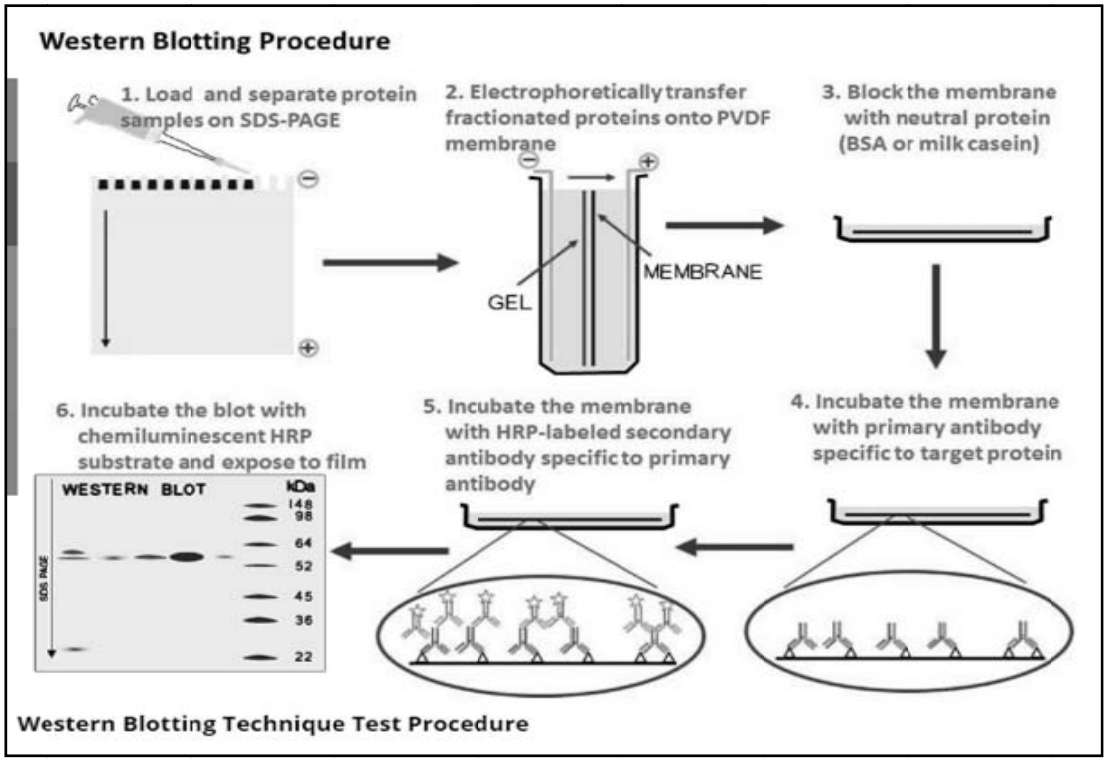
Identification of specific protein in complex mixture of proteins can be accomplished by a technique known as western blotting. In western blotting , a protein mixture is separated electrophoretically on an SDS-polyacrylamide gel(SDS-PAGE), a slab gel infused with sodium dodecyl sulphate (SDS), a dissociating agent.

↓

The protein bands are transferred to a nitrocellulose membrane by electrophoresis and then individual protein bands are identified by flooding the membrane with radio-labelled or enzyme linked polyclonal or monoclonal antibody specific for the protein of interest .

↓

Position of antibody is visualized by exposing the membrane to a sheet of X ray film, a procedure called- autoradiography in association with another detection procedure is ELISA, alternatively , a chemiluminescent ELISA can be used by using luxogenic substrate instead of chromogenic substrate , to generate light that is readily detected by exposure of the blot to photographic film.



Probable Questions:

1. Write short notes on primary lymphoid organs and secondary lymphoid organs.
2. Name 4 different types of T-cell and state their functions.
3. Describe the structure of immunoglobin.
4. Write a note on different types of immunoglobin.
5. Write note on Major Histocompatibility complex.
6. What is Humoral immune response. State its importance.
7. What is Cell mediated immune response. State its importance.
8. What is cytokines. State its role in therapeutics.
9. What is the difference between precipitation and agglutination.
10. Describe the different types of ELISA with diagrams.
11. State the principle and steps involved in RIA.
12. What is Ouchterlony method. Describe the method with suitable diagram.
13. What is Immunoelectrophoresis. Describe the method with diagram.
14. Write short notes on a) Rocket Immunoelectrophoresis
    b) Affinity Immunoelectrophoresis
    c) cross Immunoelectrophoresis
    d) Counter current Immunoelectrophoresis
    e) Immunofluorescence
15. Describe Western Blotting with suitable diagram.

Suggested Readings:

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
2. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt's Essential Immunology. Hoboken, NJ: Wiley-Blackwell.
3. Murphy, K., Travers, P., Walport, M., & Janeway, C. 2008. Janeway's immunobiology. New York: Garland Science.
4. Abbas, A. K., Lichtman, A. H., & Pillai, S. 2010. Cellular and molecular immunology. Philadelphia: Saunders/Elsevier.

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e sources.