

# UNIT 1: STUDY OF PERMANENT PREPARED SLIDES OF DIFFERENT PHYLA

## CONTENT

- 1.1 Objective
- 1.2 Introduction
- 1.3 Prepared slides study of Invertebrate Phyla
  - 1.3.1 *Amoebaproteus*
  - 1.3.2 *Euglena*
- 1.4 Summary
- 1.5 Terminal questions & Answers

---

### **1.1 OBJECTIVE:**

---

Study of Permanent Prepared Slides of different Phyla:

---

### **1.2 INTRODUCTION:**

---

It is relatively easy to identify most commonly found animals into their proper phylum and even class by using only some of the major external or obvious internal characteristics of a specimen. Most animals have cells differentiated into highly complex tissues and organs. The candidates must produce at the time of practical examination their preparations, collection and practical record books containing a complete record of the laboratory work done during the session. I have different slides prepared according to syllabus.

---

### **1.3. PREPARED SLIDES STUDY OF INVERTEBRATE PHYLA**

---

---

### ***1.3.1 Amoeba proteus***

---

**Classification:**

Phylum.....Protozoa

Subphylum.....Plasmodroma

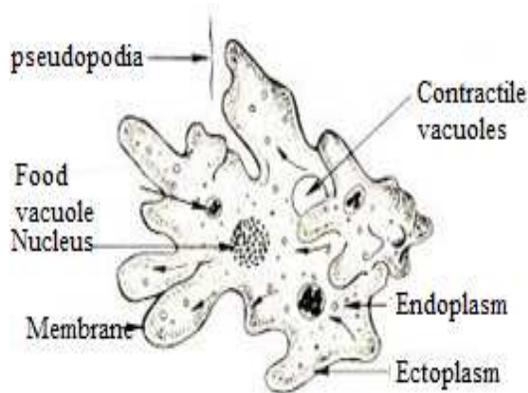
Class..... Rhizopoda

Order.....Lobosa

Type..... *Amoeba proteus*

**Comments:**

1. It is a minute and free living commonly found in fresh water ponds,ditches, and lake
2. Unicellular and, microscopic animal which can see only under the microscope.
3. It is irregular in shape with branched pseudopodia. Holozoic nutrition.
4. Reproduction is by fission and encystment.
5. It moves with the help of finger like, temporary processes called pseudopodia.



*Fig. 1.3.1 Amoeba*

---

### ***1.3.2 Euglena***

---

**Classification:**

Phylum..... Protozoa

Subphylum..... Plasmodroma

Class..... Mastigophora

Order..... Euglenoidiana

Type..... *Euglena*

**Comments:**

1. It is a microscopic, solitary and free-living organism, commonly found in fresh water, Pond, Pools and slow- running streams.
2. It is elongated and spindle shaped.
3. Nutrition is holophytic and saprophytic.
4. Cytoplasm differentiated into ectoplasm and endoplasm.
5. Reproduction by longitudinal binary fission or multiple fission.
6. Euglena performs two different kinds of movement flagellar and euglenoid.
7. A single large spherical vesicular nucleus is present Nucleus contains nuclear membrane cell membrane chromatid bodies.

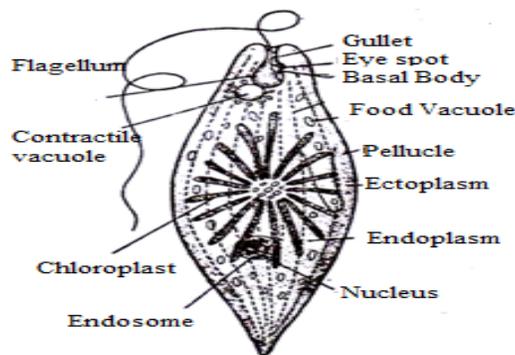


Fig.1.3.2 Euglena

---

**1.3.3 Paramecium**

---

**Classification:**

- Phylum..... Protozoa  
 Subphylum..... Ciliophora  
 Class.....Ciliata  
 Subclass.....Holotricha  
 Order.....Hymenostomatida  
 Family.....Paramecidae  
 Genus.....Paramecium

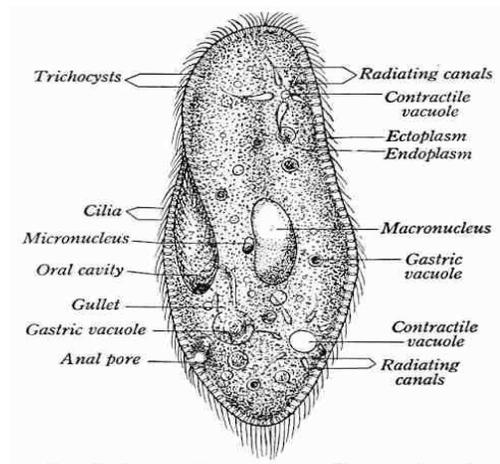


Fig. 1.3.3 Paramecium

**Comments:**

1. It is found in fresh water, pond, rivers, lakes, streams and pools
2. Microscopic, slipper, shaped, cigar shaped or spindle shaped.
3. The entire body surface is covered by numerous, tiny hair –like fine protoplasmic processes called cilia.
4. Endoplasm is semi-fluid and less granular.
5. Ciliary locomotion and body contortions are present.
6. . Reproduction asexually by transverse binary fission under good conditions of food.
7. Micronucleus and macronucleus are present
8. Nutrition heterotrophic and holophytic.

---

***1.3.4 Paramecium in binary fission***

---

**Comments:**

1. It is found in fresh water, pond, rivers, lakes, streams and pools.
2. Microscopic, slipper, shaped, cigar shaped or spindle shaped.
3. The entire body surface is covered by numerous, tiny hair –like fine protoplasmic processes called cilia.
4. Endoplasm is semi-fluid and less granular.
5. Ciliary locomotion and body contortions are present.
6. Reproduction asexually by transverse binary fission under good conditions of food.
7. Micronucleus and macronucleus are present.
8. Nutrition heterotrophic and holophytic

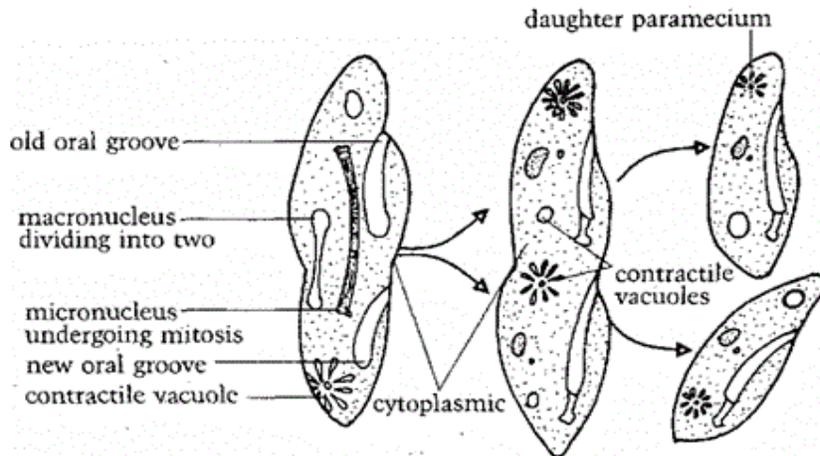


Fig.1.3.4 Binary fission in Paramecium

---

### 1.3.5 Conjugation in Paramecium

---

**Comments:**

1. It is the slides of paramecium showing conjugation.
2. Conjugation constitutes the sexual part of the reproduction.
3. In conjugation two individuals come in contact for mating and unite by their own grooves.
4. In each conjugant macronucleus disappears and micronucleus divides twice forming 4 haploid daughter micro nuclei.
5. The conjugation occurs only when nutrition is deficient and when temperature of water is below the optimum.

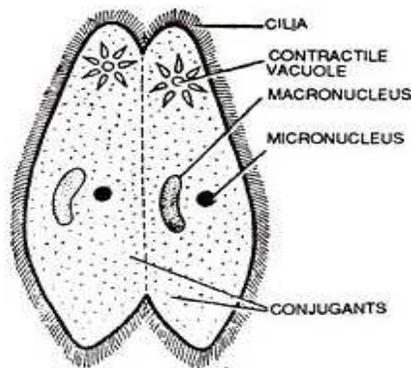


Fig.1.3.5 Conjugation in Paramecium

---

### 1.3.6-Monocystic

---

**Classification:**

Phylum..... Protozoa  
Subphylum.....Plasmodroma  
Class.....Sporozoa  
Subclass.....Telosporidia  
Order.....Gregarinida  
Suborder..... Eugregarinida  
Type..... *Monocystic*

**Comments:**

1. Monocystic is the largest acephaline forms 4mm in length.
2. It is found as a parasite in seminal vesicles of earthworms.
3. Nutrition is saprozoic.
4. Monocystis lacks the organs of active locomotion, that the locomotion as such is unknown.
5. Monocystic is monogenetic the entire life cycle is completed in a single host.

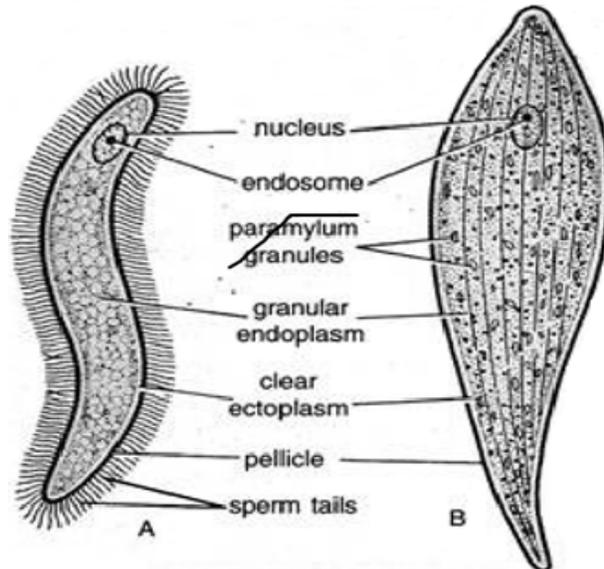


Fig.1.3.6 *Monocystis* : A- Free Living young Trophozoite B-Mature trophozoite

---

## 1.3.7 Phylum Porifera

---

### 1.3.7 L.S. of Sycon

#### Comments:

1. It is the slide of L.S. of Sycon.
2. Section contains finger like radial canals.
3. The canal system is syconoid type.
4. Body wall layer are of cellular organization and diploblastic consist of loosely organizelayers.
5. Ectoderm forms lining of flagellate chambers and consists of collar cell or choanocytes.
6. Flagellated chambers open in the central spongocoel by apopyles.
7. Spongocoel opens to outside by osculum.
8. Water circulates through
9. Ostia-incurrent canal -prosopyle→radial canal→apopyles→spongocoel→osculum→out.

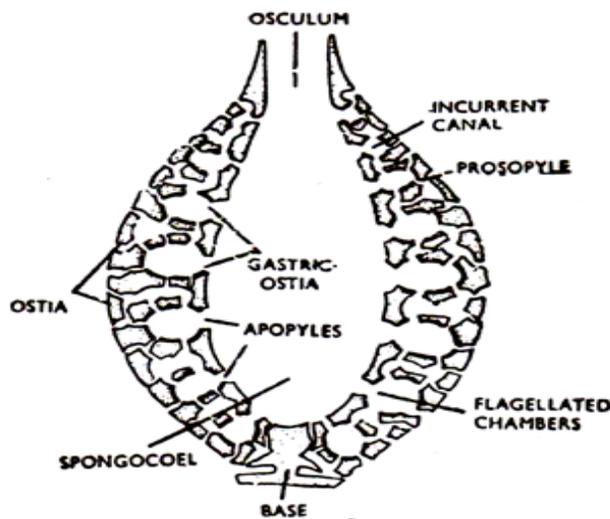


Fig.1.3.7 L.S. of Sycon

---

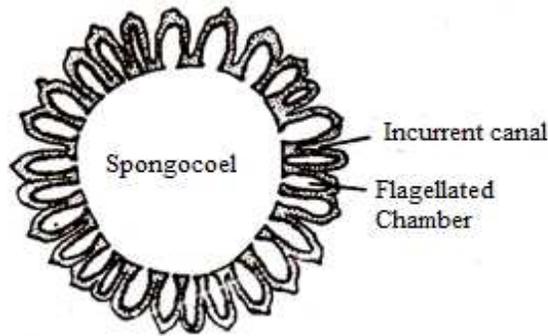
### 1.3.8 T.S. of Sycon

---

#### Comments:

1. It is circular in outline, diploblastic and the body wall layers are outer ectoderm, middle mesenchyme and inner endoderm.
2. Ectoderm is perforated by dermal pore or ostia and lined by pinacocyte cell.
3. Endoderm forms the lining of the radial canals and is made of flagellated or choanocyte cells.

4. T.S. of sycon the out-pushing or finger like projection of flagellated chambers is quite distinct in circular way.
5. The canal system syconoid type.



*Fig.1.3.8. T.S of Sycon*

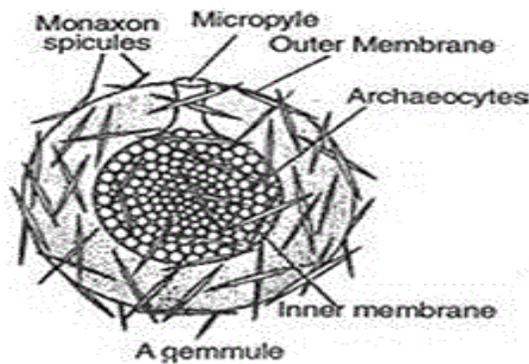
---

### 1.3.9 Gemmules

---

#### Comments:

1. Gemmules are asexual reproductive bodies forming part of regular life- cycle.
2. Gemmules contain outer and inner membrane.
3. Gemmules are rounded structure formed by aggregation into groups accompanied by trophocytes which are impregnated with food particles of glycoprotein or lipoproteins.
4. Scleroblasts secrete the amphidisc spicules, which form a row in columnar layer between outer and inner membranes.
5. Archaeocytes are reproductive cells.
6. Full –grown gemmules is usually pierced by opening on one side, called as micropyle.



*Fig.1.3.9Gemmules*

---

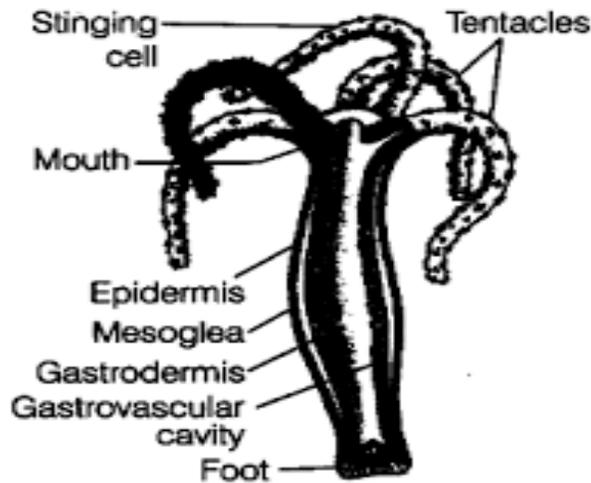
### 1.3.10 PHYLUM COELENTERARA

---

#### 1.3.10. *Hydra*

**Comments:**

1. Hydra is a solitary, freshwater and cosmopolitan hydrozoa.
2. It is hanging down words with some object in lake, pond, seasonal and permanent stagnant water.
3. Hydra is a polyp- like or polypoid coelenterate with a tubular or cylindrical body.
4. Mouth leads into gastrovascular cavity.
5. Body wall contains nematocysts which are offensive and defensive organs.
6. Sides of Hydra bear testis, ovaries and buds.
7. Reproduction sexual.
8. Hydra is a diploblastic animal; it is derived from two germ layer, the ectoderm and endoderm.
9. Hydra has great power of regeneration.



*Fig1.3.10 Hydra*

---

#### 1.3.11 *T.S.of Hydra*

---

**Comments:**

1. In the center appears a hollow cavity the coelentera.
2. Body wall is diploblastic. It is comprised of an outer ectoderm, and inner endoderm and a non – cellular gelatinous mesoglea in between.
3. The ectoderm and endoderm cells vary in shape and size according to their function.
4. The endoderm is having nutritive, secretory, and sensory and gland cells.

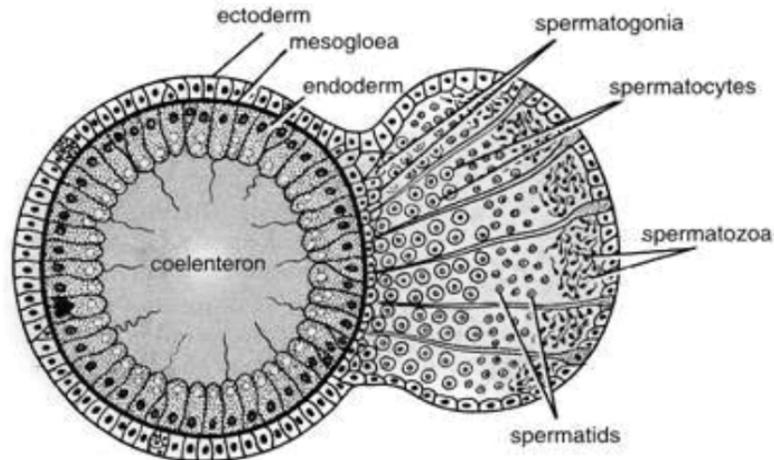


Fig. 1.3.11, T.S. of Hydra

---

### 1.3.12 Medusae of Obelia (W.M.)

---

#### Comments:

1. It is the slide of Obelia medusa a reproductive zooid responsible for giving rise to new progeny.
2. It is a solitary free- swimming zooid, originating from blastostyles.
3. Medusa is umbrella like and has convex exumbrellar and concave sub-umbrellar surfaces with well define radial symmetry.
4. Umbrella edge contains radially symmetrical tentacles.
5. The inner surface of the dome of saucer bears 4 radial canals.
6. Each radial canal bears a distinct gonad.
7. On maturation these gonads release germ cells –the ova and sperms, in the sea water.

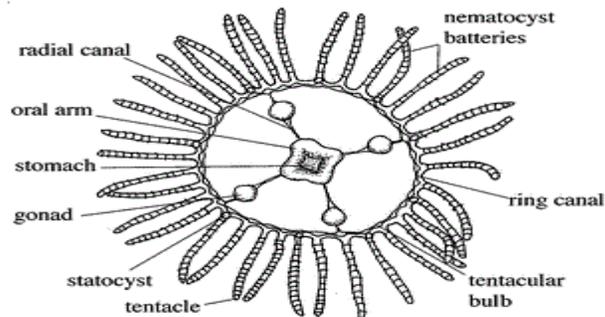


Fig. 1.3.12 Medusae of Obelia

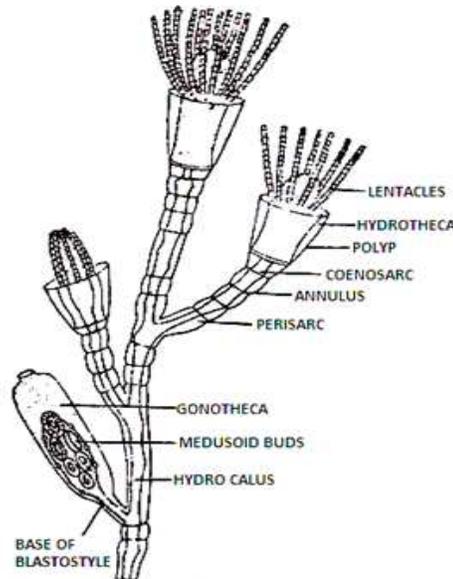
---

### 1.3.13 *OBELIA (W.M)*

---

#### Comments:

1. It is marine, colonial, sedentary, hydrozoan zoophyte.
2. It is attached to seaweeds shell and rock.
3. It is dimorphic colony in the form of small seaweed filaments, measuring several cm in height.
4. All the individuals in the colony are attached through branches to a horizontal hydrorhiza. The branches are called hydrocauli.
5. Each branch bears three types of zooids polyp, the medusa and blastostyles thus making it polymorphic.
6. The poly has a vase-like or bell like body enclosed in a cup-like hydrotheca.
7. The medusais saucer- shaped zooids and are produced in blastostyles.
8. It reproduces asexually and sexually.



1.3.13, Fig. *Obelia*

---

### 1.3.14 PHYLUM PLATYHELEMINTHES

---

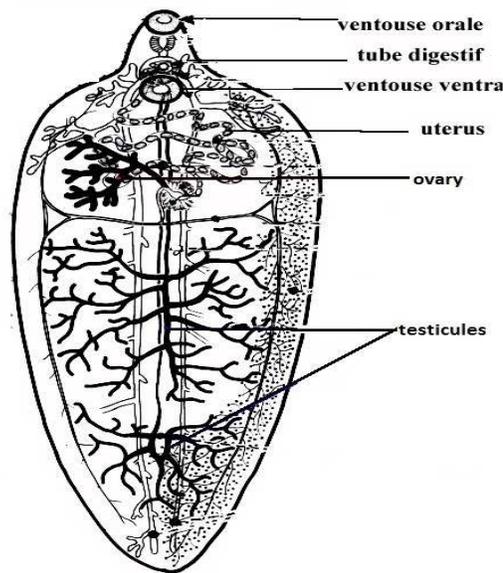
#### 1.3.14 *Fasciola hepatica: Entire*

---

#### Comments:

1. It is commonly known as 'Liver fluke' and is an endoparasite in the bile duct sheep.

2. It is endemic parasite, reported from India, China, Cuba, U.S.S.R. and U.S.A.
3. Body leaf-like and flattened.
4. Mouth and oral sucker are situated on a small protuberance-the “head”
5. Pigmentation of the parasite is dark brown.
6. Oral and Ventral sucker are present.
7. They are hermaphrodite and reproductive organs occupy almost whole the bodyspace.
8. Intermediate host is snail.
9. It causes liver rot, livercirrhosis, eosinophilia and anemia.



*Fig1.3.14. Fasciola hepatic*

---

### ***1.3.15 Fasciola hepatica***

---

#### **Comments:**

1. It shows extreme degree of parasitism, being devoid of alimentary canal and locomotory organs.
2. It also destroys the tissues of the snail.
3. Body become rounded having germ balls and four flame cells
4. It measures 1.3 to 1.6mm in length.
5. Body wall layers are Tegument, epithelial cell, muscle layers and mesenchyme.

6. Germ balls in sporocyst multiply and form next or third larval stage, called radial larva.
7. Sporocyst is non-feeding stage.
8. Excretory pores are still 2 in number.

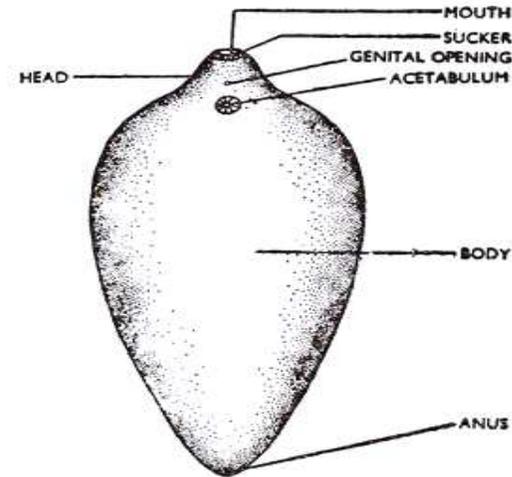


Fig.1.3.15 *Fasciola hepatica*

### 1.3.16 *Schistosoma haematobium*

**Classification:**

- Phylum..... Platyhelminthes  
 Class..... Trematoda  
 Order..... Digenea  
 Family.....Schistosomatidae  
 Type.....*Schistosoma*

**Comments:**

1. It is found in abdominal cavity of man in mesenteric blood vessels.
2. It is commonly called as blood flukes.
3. Sexes are separate and is tabulated body.
4. Male are smaller and stouter (10-15mm) than female (15-20mm).
5. Oral sucker and ventral sucker are present in the male and are well developed. In female acetabulum is poorly developed.
6. Tegument is finely tuberculated.
7. Male reproduction system consists of 4-5 tests, sperm duct and cirrus.
8. Female system has ovary, oviduct, uterus, ootype and vitellaria.

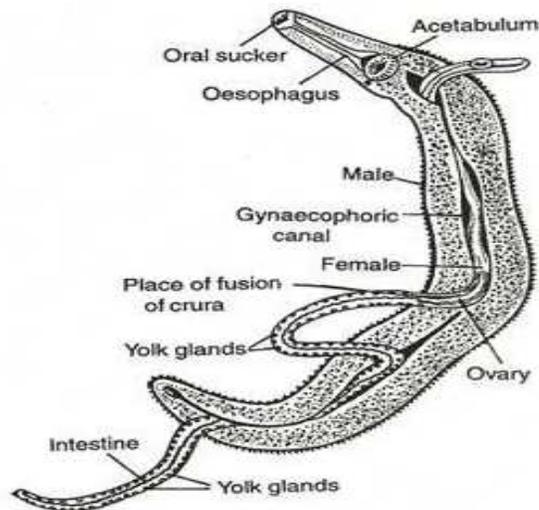


Fig.1.3.16, *Schistosoma haematobium*

---

### 1.3.17 *Opisthorchis* or *Clonorchis sinensis*

---

**Classification:**

Phylum..... Platyhelminthes

Class..... Trematoda

Order.....Digenea

Type..... *Opisthorchis*

**Comments:**

1. Commonly called as liver fluke of ma
2. It is digenetic parasite found in liver bile duct and sometime pancreatic duct and duodenum of man. It is also parasite of pig and dog.
3. Digestive system consists of mouth, pharynx and bifid intestine. It is hermaphrodite.
4. Male reproductive system consists of testes; sperm duct seminal vesicle ejaculatory duct and genital aperture.
5. Female system has ovary, oviduct, ootype and vitelline gland, Mehlis's gland, uterus and genital aperture. First intermediate host is Bithynia snail.

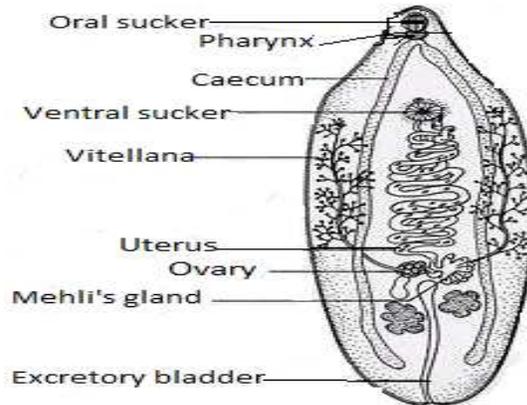


Fig 1.3.17. *Opisthorchis*

---

### 1.3.18 *Fasciola hepatica*: Miracidium larva

---

**Comments:**

1. It is the first free –swimming larva stage and is hatched out directly off the egg.
2. Zygote develops into miracidium after 6-9 days at 25-33 c.
3. Body is covered with epidermal plates arranged in 5 rows. These plates are 6,6,3,4& 2 respectively in different row.
4. The body wall is comprised of an outermost ciliated, a glandular epidermis.
5. Miracidium larva swims actively in water in search of a suitable molluscan intermediate host.
6. Failing to get a suitable snail, the larva dies after 24 hours.

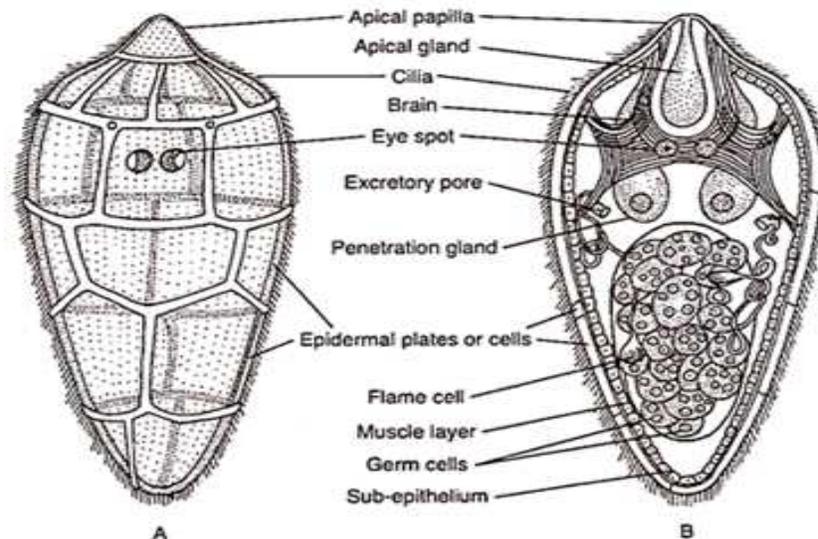


Fig.1.3.18, *F. hepatica*: Miracidium larva A.External structure B.Internal structure

---

### 1.3.19 *Fasciola hepatica*: Redia larva

---

#### Comments:

1. It is the third larval stage in the life-cycle of *Fasciola hepatica*.
2. It develops from the germ balls present inside the sporocyst.
3. Body is elongated, cylindrical and complex in structure.
4. Sporocysts become germinal, sac-like structure, measuring about 1 mm in diameter.
5. The body wall of redial is comprised of a thick cuticle, a squamous epithelium, a muscular and a mesenchyme layer.
6. Oral sucker surrounding the mouth.
7. It gives rise to daughter rediae and the next larval stage – cercaria.

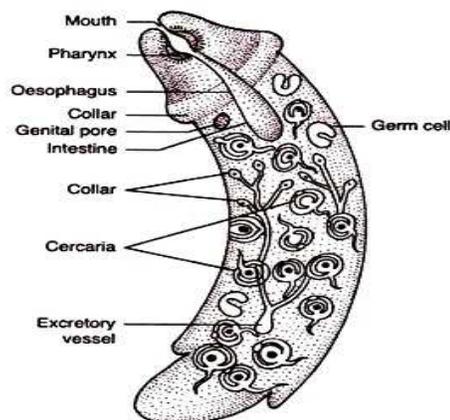


Fig. 1.3.19, Redia larva

---

### *Fasciola hepatica*: Cercaria larva

---

#### Comments:

1. Cercaria larva is the IVth larval stage in the life cycle of *Fasciola*.
2. It developed from germ balls inside the rediae larvae which are present inside the digestive gland of intermediate host Mollusca.
3. It is characterized by having a flat and oval body and a long tail.
4. It is free swimming larval stage.
5. Body is oval in shape with a long simple tail, measuring 0.25-0.35mm in length.
6. Body and tail are covered with tegument, circular, longitudinal and diagonal muscle fibers and mesenchyme. Flame cell still increase in number. Germ ball represent genital rudiments.

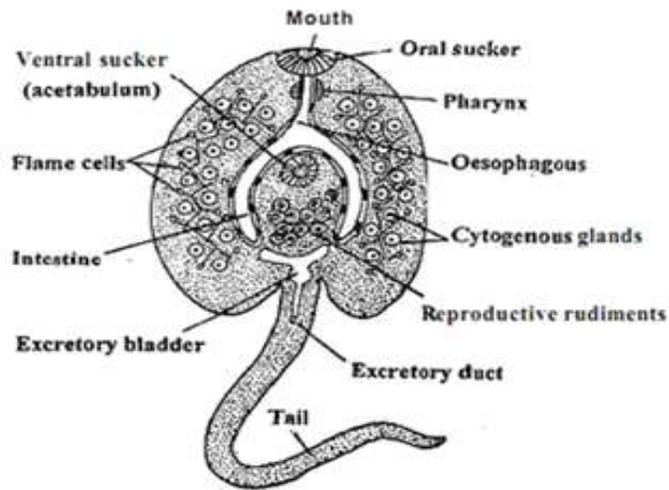


Fig. 1.3.20, Cercaria larva

---

### 1.1.21, Scolex of *Taenia*

---

Comments:

1. The narrow anterior end of the body is knob like called scolex.
2. It is a four- sided pear-shaped structure distinguished into the Rostellum, hook, sucker.
3. Number of hooks varies from 28 to 32.
4. Hooks are present at the base of rostellum. They are curved, opiated chitinous structure.
5. Longitudinal excretory canal and transverse excretory canals are present.
6. Smaller hooks alternate with larger hooks, measuring 110-140 and 160-180 micron respectively.
7. Internally, scolex contains spongy mesenchyme with nephridial network and ring.
8. Scolex lies buried on the intestinal mucosa of host intestine. It destroys the host intestine

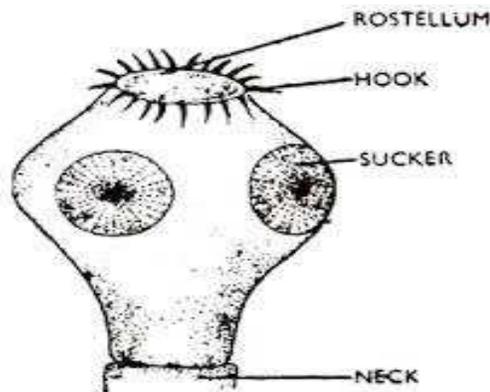


Fig.1.3.21, Scolex of *Taeni*

---

### 1.3.22 *Taenia solium*: mature segment

---

#### Comments:

1. Mature segment of *taenia solium* is square like, hermaphroditic with a single set of reproductive organs, and having osmoregulatory and nervous system. Genital organs constitute main structure.
2. Male genital system consists of follicular testes, vasa-efferentia, vasa deferens and cirrus.
3. Female system consists of ovarian lobes connected by isthmus, oviduct, ootype, compact vitellaria and Mehlis glands.
4. Uterus arises from ootype and extends upwards. The common genital atrium facilitates self-fertilization. The mature segment transforms into a gravid segment.

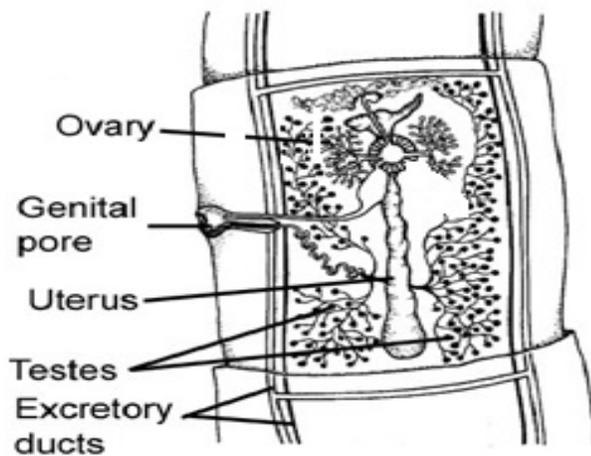


Fig. 1.3.22, *Taenia solium*: mature segment

---

### 1.3.23 *Taenia solium*: Cysticercus Larva

---

#### Comments:

1. Cysticercus larva commonly called as bladder worm.
2. The intermediate host is pig. it develops from oncosphere stage in the muscles of pig.
3. Contaminated part of pig muscle is called as measly pork.
4. The cysticercus bears a scolex, a neck and a bladder- like body.
5. The scolex, like in adult, bears rostellum and four muscular suckers.
6. The rostellum is comprised of two row of hooks which surround the mouth.
7. The alimentary canal and all other organ- systems are absent.
8. Infection of *Taenia solium* can be avoided by not eating the measly pork.

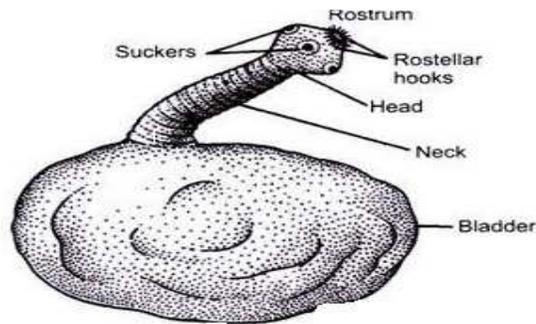


Fig. 1.3.22, *Taenia solium*: *Cysticercus Larva*

---

### 1.3.23 *Echinococcus granulosus*

---

**Comments:**

1. It is found in the small intestine of dog, cat, wolf, foxes and other carnivores.
2. Commonly called as “hydatid worm”.
3. The worm is small, dorsoventrally flattened and ribbon shaped.
4. It is generally comprised of four segments: the scolex, an immature segment, a mature segment and several gravid segments.
5. The lower part of the scolex acts as neck region, from where new segments are formed.
6. Hermaphroditic and the mature segment contains a single set of genital organs: Uterus, Ootype and Mehlis’s glands.
7. Male reproductive system consists of testis, sperm duct and cirrus.
8. Female system consists of ovary, oviduct, vitellaria, uterus, ootype and Mehlis’s glands.
9. Gravid segment is elongated containing branched uterus with oncospheres.
10. Man acquires infection by playing with dogs.

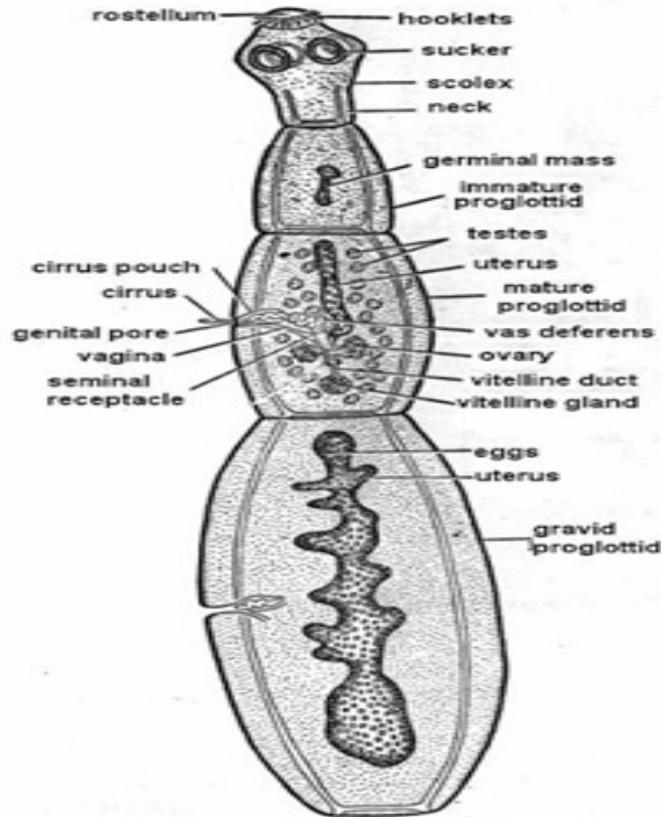


Fig. 1.3.23 *Echinococcus granulosus*

---

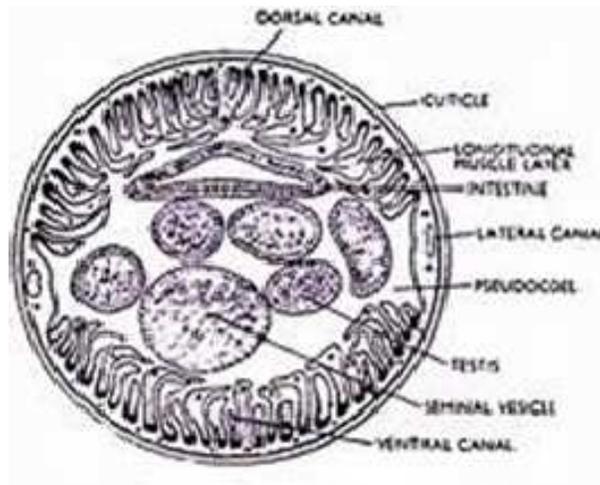
## 1.3.24 PHYLUM NEMATHELMINTHES

---

### 1.3.24 T.S. Male *Ascaris*

**Comments:**

1. Body wall is composed of cuticle, hypodermis, a muscle layer and pseudocoel.
2. Cuticle is composed of cortical layer, median layer, basal layer and basement.
3. Cuticle is thick, tough, transversely striated, semi-permeable and resistant.
4. Hypodermis is syncytial and bulges in pseudocoel at mid-dorsal, mid ventral and on lateral sides, forming four longitudinal chords which divide musculature into four quadrants.
5. Various rounded cut section of coiled testes is present. Testes sections are without lumen but with a central rachis. Sperm duct and the seminal vesicle have lumen filled with dot-shaped spermatozoa.
6. Dorsal and ventral longitudinal chords contain dorsal and ventral nerve chord, while the lateral chords contain excretory canal and nerves.



*Fig1.3..24 T.S. Male Ascaris*

---

### **1.3.25 T.S. of Female Ascaris**

---

**Comments:**

1. It is triploblastic and organ-grade.
2. Female reproductive system consists ovaries, oviducts, uteri and vagina.
3. Body wall is composed of thick cuticle, a syncytial hypodermis and a layer of muscles.
4. The muscle layer is divided by lateral, dorsal and ventral canals into four segments or groups.
5. Body cavity is a pseudocoel and is filled with various structures.
6. Lumen is absent in ovary.
7. Ovary are very distinct containing single nucleus and cytoplasm.
8. Ovary, oviduct and uterus are elongated, coiled and recurved upon themselves.

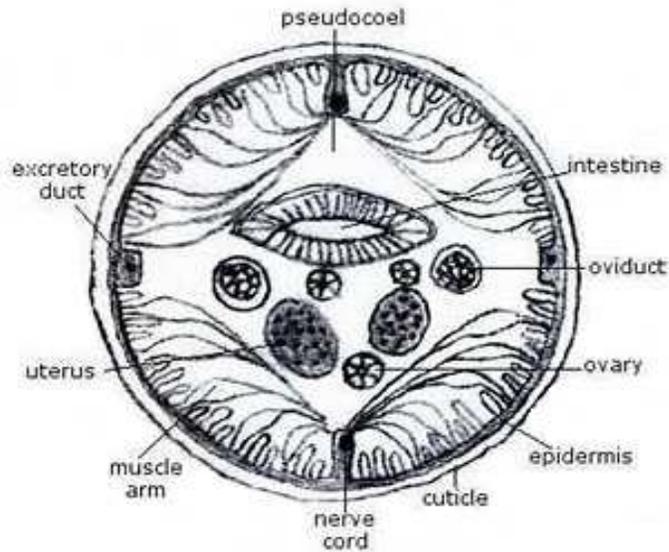


Fig. 1.3.25T.S. of Female *Ascaris*

---

***Enterobius vermicularis***

---

**Comments:**

1. It is commonly known pin worm, seat worm or thread worm.
2. It is an endoparasites inhabiting caecum, colons and appendix of human beings.
3. Male and Female are separate.
4. Male measure 2-5 mm in length 0.1-0.2 mm in breadth, while the females measure 8-13 mm or 0.3-0.5 mm. Mouth is having 3 small lip and a pair of cephalic expansion.
5. Tail end of male is ventrally curved while that of female pointed.
6. Male reproductive system consists of testis, vasdeference seminal vesicle, ejaculatory duct and cloaca. Female reproductive system consists of anterior ovary, posterior ovary, anterior uterus posterior uterus and oviduct.

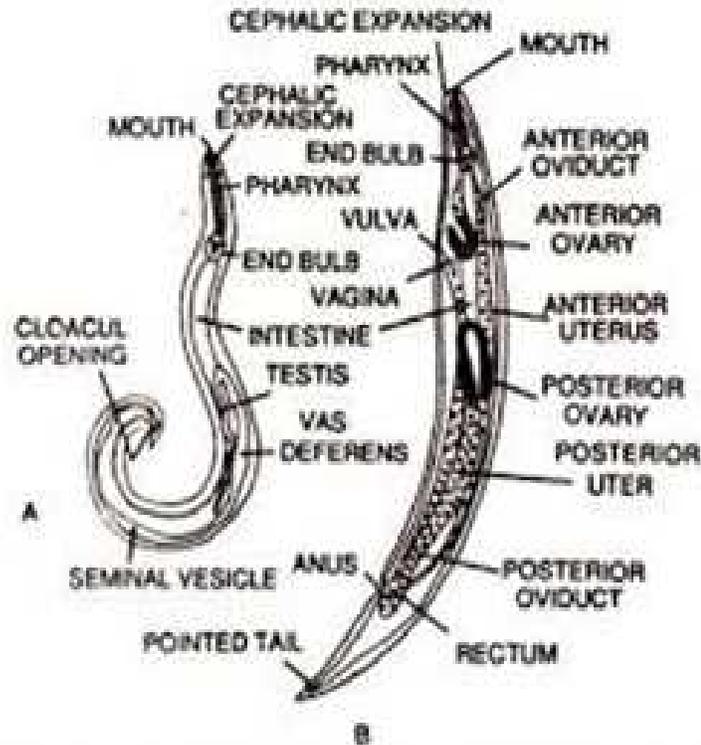


Fig.1.3.26 *Enterobius vermicularis* Male A. & female B.

---

### 1.3.27 *Wuchereria bancrofti*

---

**Comments:**

1. Commonly called as filarial worm causing elephantiasis in man.
2. Male and female separate.
3. 3. Adult female measure about 10cm. and male 5cm. in length.
4. Mouth with- out buccal capsule and lips but with stylet.
5. Female reproductive system shows vulva, ejector and vagina.
6. Female worm delivers juveniles called microfilariae.
7. Tail end if male is coiled and characterized by having unequal spicule in spiracular sheaths gubernaculum and 12 pairs of papillae.
8. Intermediate host is female culex mosquito in which the microfilariae transform into adult.

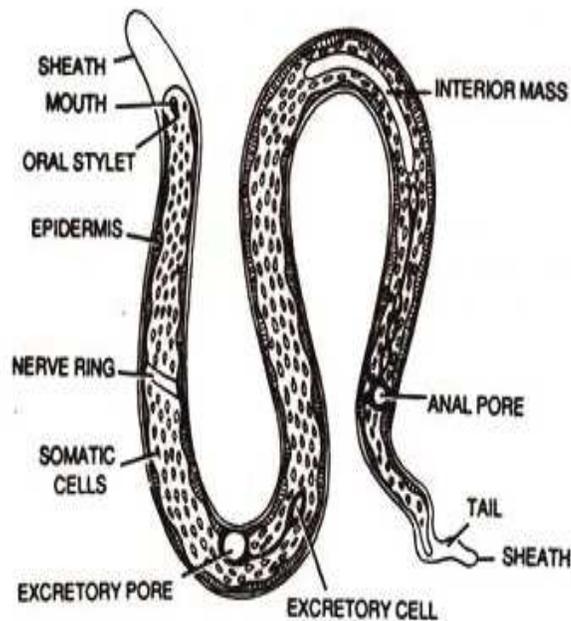


Fig.1.3.27 *Wuchereria bancrofti*

---

## 1.3.28, PHYLUM –ANNELIDA

---



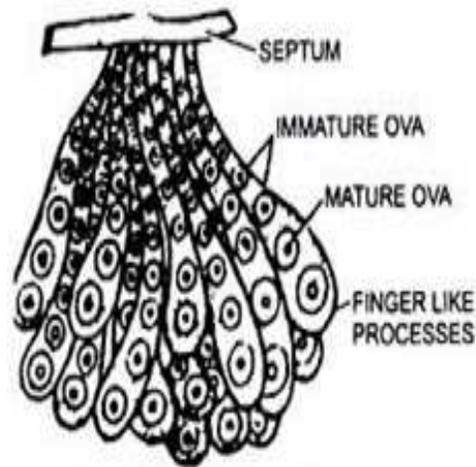
---

### 1.3.28, Earthworm: ovary

---

**Comments:**

1. A pair of ovaries is situated in the 13<sup>th</sup> segment.
2. They are pyriform, semi-transparent, hanging freely into coelom and attached by their broad ends to septum of the 12<sup>th</sup> and 13<sup>th</sup> segment.
3. Each ovary is white compact mass made up of finger- like processes.
4. Each ovary contains ova in a linear series.
5. Each ovum is large, having a distinct nucleus.
6. Each ovary consists of several fingers –like processes.



*Fig 1.3.28 Earthworm: ovary*

---

### **1.3.29 Earthworm: Septal Nephridia**

---

**Comments:**

1. Each septal nephridium is micro nephridia and is composed of ciliated funnel or nephrostome opening into coelom a short narrow neck and body, having a short straight lobe and a long spirally twisted loop.
2. Twisted loop is further differentiated into proximal limb, distal limb and apical limb.
3. Straight lobe contains 4 ciliary tracts divided into outer lobe and inner lobe.
4. Ciliary movement is found in the funnel.
5. Apical limb is suspended in the coelom.
6. Septal nephridia are excretory organs.

---

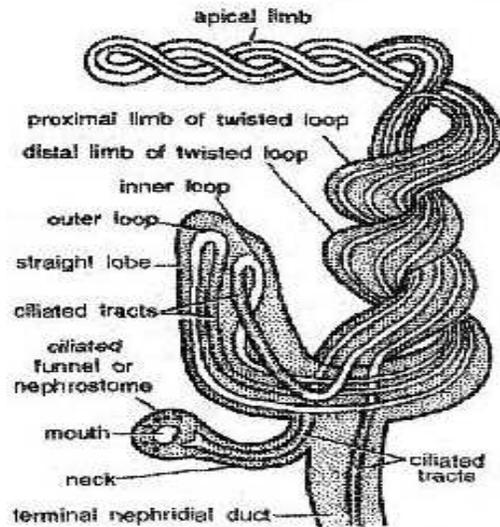
### **1.3.30 Parapodium of Nereis**

---

**Comments:**

1. The parapodium is biramous and lies on either side of segment and is the organs of locomotion.
2. Parapodium is bilobed and setae-bearing organs composed of upper notopodium and lower neuropodium.

3. Notopodium has two equal fleshy and much smaller.
4. Dorsal and ventral cirri, attached to notopodial and neuropodial bases are present.
5. First 2 pairs of parapodia lack the notochord setae.
6. Parapodia are locomotory are respiratory organs.
7. They are adapted for crawling movement also




---

### 1.3.31 Parapodium of Heteronereis

---

**Comments:**

1. The sexual phase of nereis is called as Heteronereis
2. The parapodia modified for swimming.
3. Body of the Heteronereis is divided into anterior region or atoke and posterior sexual organs or epitoke.
4. Epitoke is sexual phase of heteroneris.
5. Parapodium of heteronereis phase is thin and foliaceous developing more outgrowth.
6. Dorsal and ventral cirri are elongated.
7. Notopodial and neuropodial setae, instead of being spine-like, become oar-like.

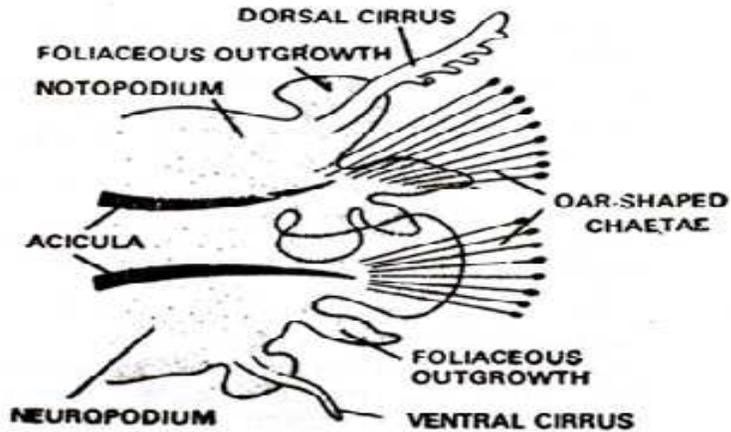


Fig1.3.31, Parapodium of Heteronereis

---

## 1.3.32, PHYLUM MOLLUSCA

---

### 1.3.32 Unio: T.S. of Gill Lamina

---

**Comments:**

1. Each gill-lamina is composed of outer and inner lamellae and T.S. looks ladder-like.
2. The two lamellae are connected together by inter-lamellar junction.
3. Water tubes, open in supra-branchial chamber.
4. Gill-lamella is composed of columnar ciliated epithelial cell and is supported by chitinous rods.
5. Laminae are double-fold structure composed of two parallel vertical plates Calles's lamellae.
6. Gill-lamellae are supplied with blood vessels.

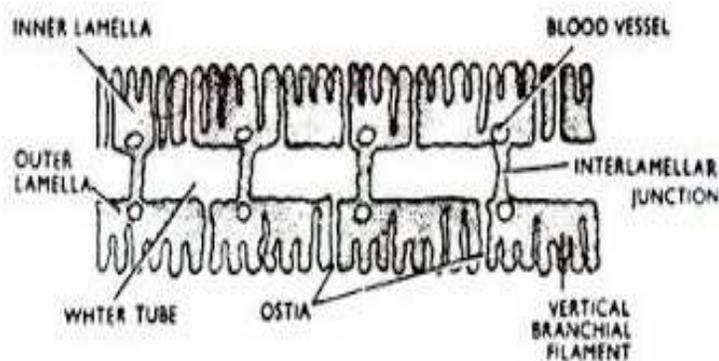


Fig. 1.3.32 Unio, T.S. of Gill Lamina

---

### 1.3. 33 Unio: Glochidium Larva

---

**Comments:**

1. It is a molluscan larva, found in the development of Bivalvia orpelecypoda (Unio)
2. It is microscopic bivalve larva, consisting of conical shell containing incurved hooks.
3. Mantle is produced into sensory brush-like hairs.
4. Shell valves are operated by a large adductor muscle situated at the base and joined by hinge.
5. A byssus gland is present near adductor muscle, through which a long filament-like structure arises called as byssus.
6. Glochidium larva attached itself to fish skin and lead an ectoparasitic.

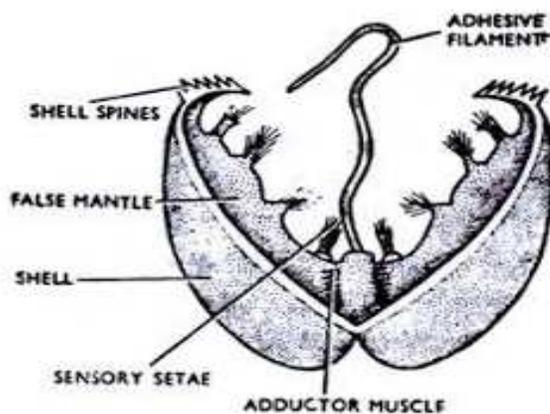


Fig.1.3.33, Unio: Glochidium Larva

---

### 1.3.34, Pila: L.S.Osphradium

---

**Comments:**

1. It is small lobe-like structure, attached to the mantle near the left nuchal lobe.
2. It detects the nature of water current and chemical juices. It consists of 22-28 oblong leaflets arranged around a central axis. Side leaflets are smaller, while middle ones are the larger. Osphradium is covered with epithelium.
3. It acts as a rheoreceptor and chemo-receptor.

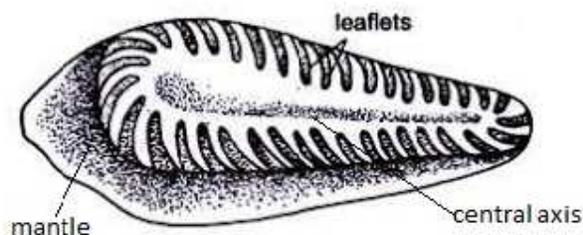


Fig. 1.3.34. Pila: L. S. Osphradium

---

## 1.3.35 PHYLUM ARTHROPODA

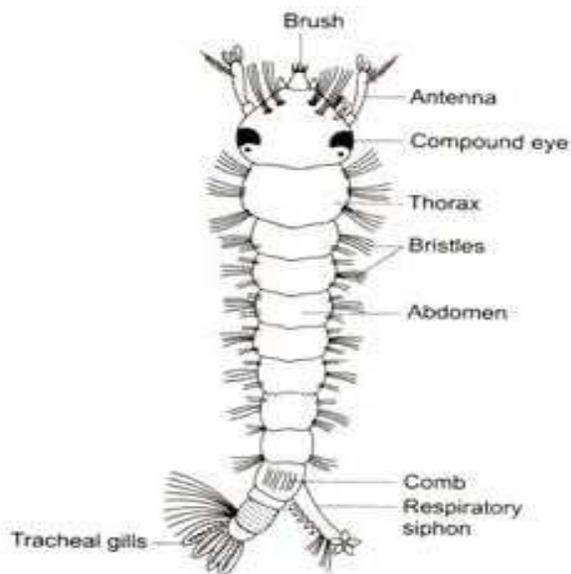
---

### Culex –Larva

---

**Comments:**

1. Commonly called as wriggler. It swims by wriggling movement.
2. It is bottom feeder.
3. Culex egg hatches into free swimming larva in 2to 3 days.
4. Larva is transparent, 1mm and is differentiated into head, thorax and abdomen.
5. Head contains compound eyes, simple antennae, chewing mandibles and maxillae.
6. Abdomen is 6 segmented.
7. Eighth segment has long respiratory siphon with spiracle at the top.



*Fig. 1.3.35 Culex –Larva*

---

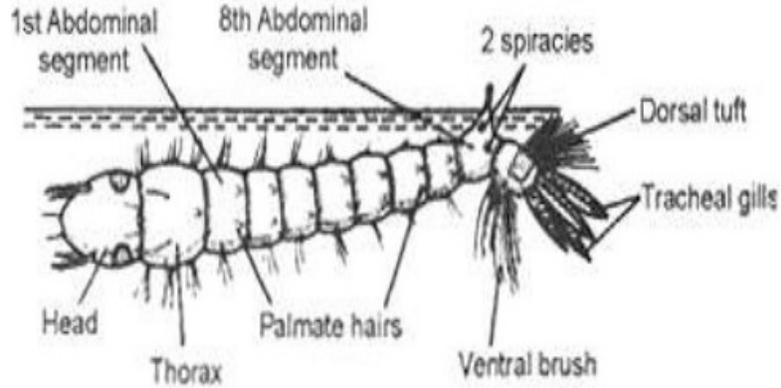
### 1.3.36 Anopheles: Larva

---

**Comments:**

1. Anopheles eggs hatch into larvae in 24-48 hours.
2. Anopheles larva is relatively smaller and darker than culex larvae it is surface feeder.
3. Body divisible into head thorax and abdomen.
4. Head contains a pair of antennae, compound eyes.
5. Thorax unsegmented, broader than head and contains tuft of hair.

6. Abdomen has 9 segments, each segment bearing palmate hair.
7. Respiratory siphon and comb rows on ninth segment are absent.



*Fig.1.3.36 Anopheles Larva*

---

### **1.3.37. CULEX: MALE: HEAD AND MOUTH PARTS**

---

**Comments:**

1. Head and the mouth parts are seen clearly under low magnification of the microscope.
2. Body is differentiated into head, thorax, and abdomen.
3. In male culex, maxillary palps are longer than labium and antennae.
4. Head is free movable on a narrow neck, having large compound eyes and antennae and clypeus articulates with labrum- epipharynx.
5. Mouth parts contain labrum-epipharynx, needle shaped mandibles and maxillae, hypopharynx, maxillary palps and labium with tactile hairs.
6. Mouth parts are of sucking type.
7. It communicates filariasis causing elephantiasis.

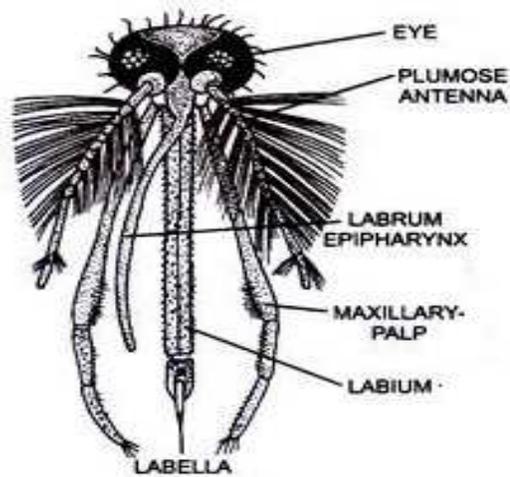


Fig.1.3.37 Head and Mouth parts of culex-Male

---

### 1.3.38. CULEX: FEMALE: Head and Mouth parts

---

**Comments:**

1. In female Culex, maxillary palps are exceedingly short and antennae contain a few short hairs at joints.
2. Head is freely movable on a slender neck, having large black compound eyes and antennae.
3. Clypeus articulates with labrum-epipharynx.
4. Mouth parts are of piercing and sucking type, compound of labrum-epipharynx, needle-like mandibles and maxillae and hypopharynx. Maxillary palps are three jointed.
5. Maxillary palps and labium containing tactile hairs forming proboscis sheath.
6. It heaps in transmission of elephantiasis disease.

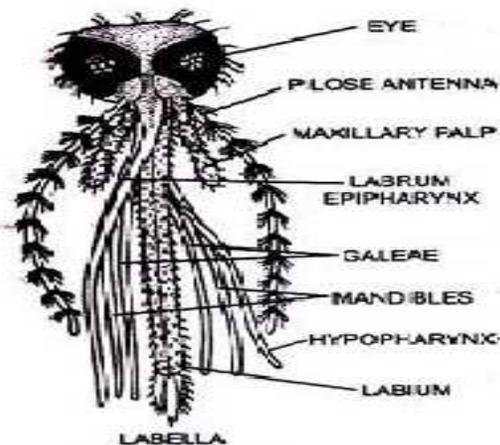


Fig. 1.1.38, Head and Mouth parts of culex-female

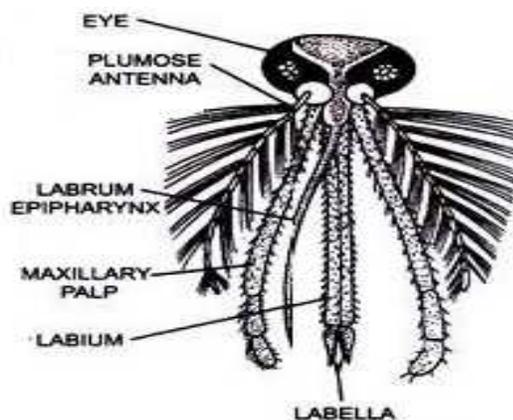
---

### 1.3.39 Anopheles: Male: Head and Mouth parts

---

#### Comments:

1. Head and its mouth parts are distinct, which can be observed under dissecting microscope and in low magnification of the microscope.
2. Head is freely movable on a slender neck, having large black compound eyes and antennae.
3. Clypeus articulates with labrum-epipharynx.
4. Mouth parts are of piercing and sucking type, compound of labrum-epipharynx, needle –like mandibles and maxillae hypopharynx. Maxillary palps and labium. Maxillary palps are club-shape and are nearly equal to labium or proboscis, and antennae have long, bushy hairs at their joints.
5. It helps in transmission of malarial disease and feed on plant juices.



*Fig. 1.3.39* Anopheles: Male: Head and Mouth parts

---

### 1.3.40 Anopheles: Female- Head and Mouth Parts

---

#### Comments:

1. Head and its mouth parts are clearly seen under dissecting microscope and in low magnification of compound microscope. Antennae possess a few short hairs at joints.
2. 3. Head is freely movable on a delicate and slender neck, having large black compound eyes and pilose antennae. Clypeus articulates with labrum and epipharynx.
3. Mouth parts are of piercing and sucking type, compound of labrum-epipharynx, needle –like mandibles and maxillae hypopharynx. Maxillary palps and labium. Maxillary palps are simple and equal to labium or proboscis. It helps in transmission of malarial disease and acts as intermediate host for plasmodium.

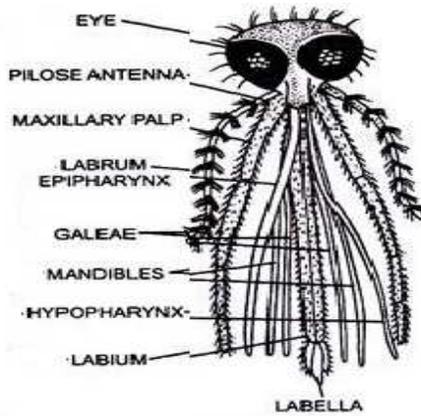


Fig.1.3.40, *Anopheles*: Female: Head and Mouth parts.

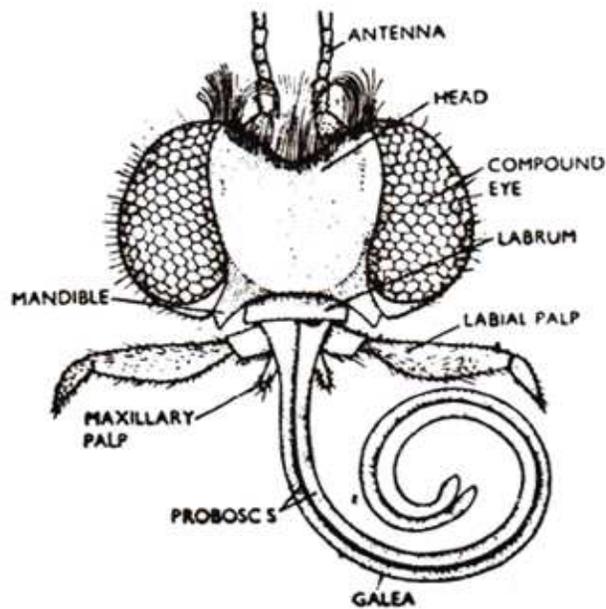
---

### 1.3.41. Butterfly: Head and Mouth parts

---

**Comments:**

1. The mouth parts are sucking or siphoning type.
2. Head is containing large compound of large compound eyes and antennae. It is broad and contains siphoning type mouth parts.
3. Mouth parts are composed of small labium in front of clypeus, triangular labium and coiled proboscis.
4. Mandibles are absent.
5. Proboscis is composed of elastic cuticle and greatly elongated galeae of maxillae, grooved internally forming food canal for nectar.
6. Proboscis lies in coiled stage.
7. Labium is triangular and plate-like containing labial palps.



*Fig.1.3.41, Head and Mouth parts of Butterfly*

---

### **1.3.42 Honey-bee: Apis: Mouth parts of Worker**

---

**Comments:**

1. Honey-bee belonging to the order Hymenoptera contains rasping and lapping mouth parts.
2. Head is triangular, containing large compound eyes, 3 ocellae antennae and mouth part.
3. Mouth parts are composed of spoon shaped mandibles, labrum and maxillae devoid of lacinia.
4. Mandibles are smooth and spatulate type, found on either side of the labium.
5. It contains vestigial maxillary palps and blade-like galea.
6. Labellum is spoon-shaped, grooved internally forming a tube and is called as tongue.
7. Honey bee also mould waxes in its hive.

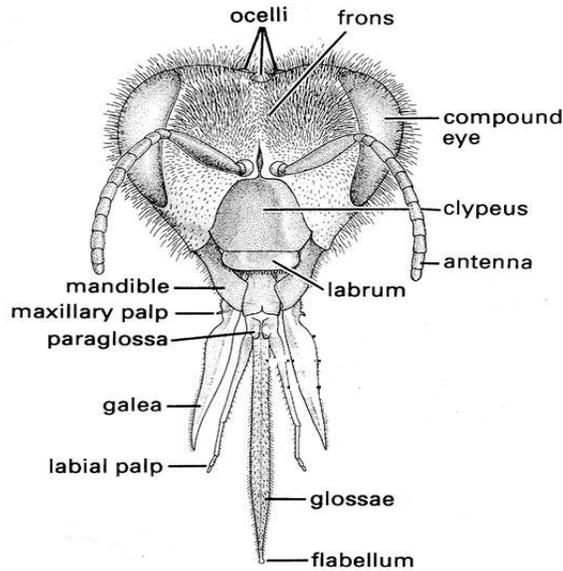


Fig. 1.3.42, Mouth parts of Worker Honey-bee (*Apis*)

---

### ***1.3.43 Honey-bee: Apis: Sting-Apparatus***

---

**Comments:**

1. Sting apparatus of honey-bee is modified ovipositor, found at the posterior extremity of abdomen in queen and worker. It is composed of sting or terebra, bulb, levering plates and gland.
2. Sting is made up of 2 pair of gonapophyses
3. The 8th segment forming stylets and of 9<sup>th</sup> segment stylet sheath, which enclose poison canal.
4. Rhee stylet sheath and styler contain pointed spines or barbs.
5. The bite of the sting causes burning sensation, pain and swelling of the part concerned.

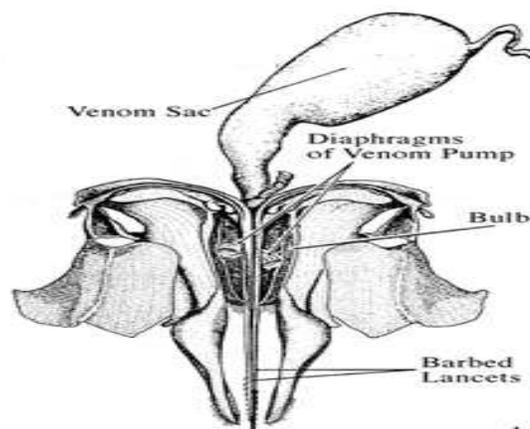


Fig.1.3.43Sting-Apparatus of Honey-bee (*Apes*)

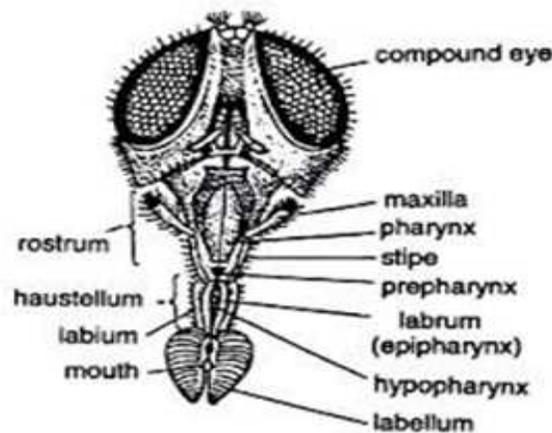
---

### ***1.3.44 Muscadomestica: Head and mouth parts***

---

#### **Comments:**

1. *Musca domestica* or housefly contains sponging mouth parts, adapted for sucking liquid food.
2. Housefly lacks the cutting apparatus. Head bears Ocelli on ocular plate and large compound eyes, and mouth parts. Antennae are aristate.
3. Mouth parts are compound of proboscis, short maxillary palps, labrum-epipharynx and hypopharynx. Mandibles are absent.
4. Maxillae are represented by short and unjointed maxillary palps before the rostrum.
5. Labrum is fused with the epipharynx and forms a narrow slender tube opening ventrally.
6. Hypopharynx is narrow structure. Hypopharynx and labrum constitute the food channel.



*Fig.1.3.44, Head and mouth parts Head and mouth parts*

---

### ***1.3.45. Daphnia***

---

#### **Comment:**

1. *Daphnia* is a fresh water branchiopod, commonly found in ditches and ponds.
2. Commonly called water flea. Head not separated from the body by a dorsal notch.
3. 4. Head is rounded and bears large biramous antennae which help in swimming, small unjointed antennules, mandibles, maxillulae, and large sessile eyes are very distinct.
4. Abdominal appendages are absent and thoracic appendages are 5 pairs and leaf-like.
5. Posterior female carries a broad pouch containing various developing embryos.
6. Brood pouch is found near the base. Thoracic appendages form efficient food-catching organs. Sexes are separate. Brood pouch is absent in male.

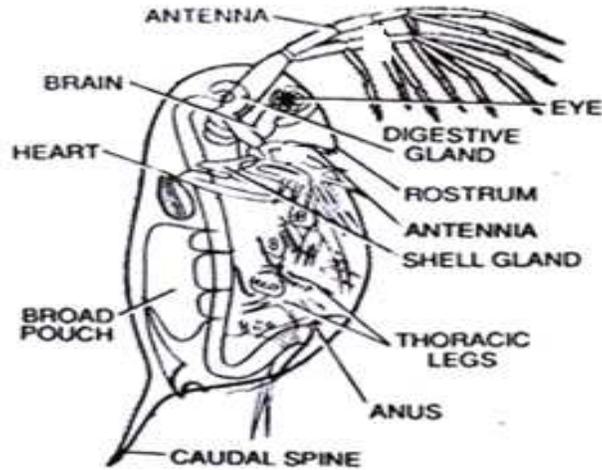


Fig1.3..45 Daphnia

---

## Mysis: Larva

---

### Comments:

1. Mysis is a small, transparent, marine crustacean.
2. Body is bilaterally compressed measuring 2-6 mm in length.
3. 3. Body is divided into head, thorax and abdomen
4. Head contains antennae, antennules, and a pairs of movable compound eyes.
5. Posterior thoracic region of female contains broad pouch.
6. Abdomen contains six segments. First 5 segments contain swimming appendages orpleopods.
7. Statocyst is present on endopodite of each uropod.
8. 8-Development takeplace in broad pouch.

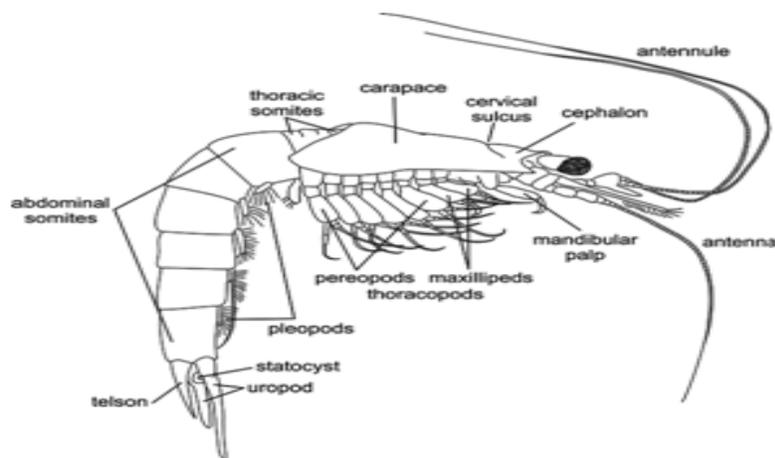


Fig.1.3.46Mysis Larva

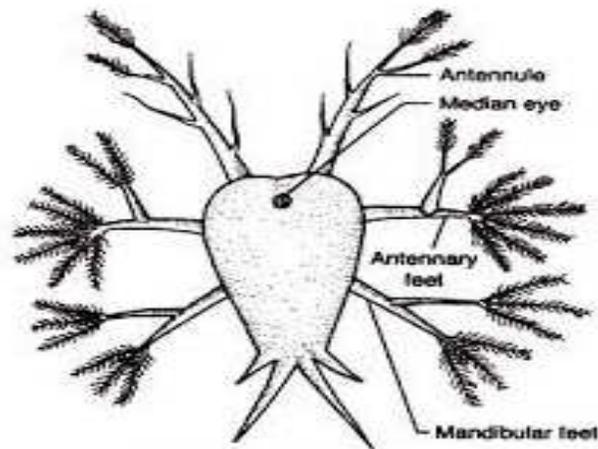
---

### 1.3.47 Nauplius: Larva

---

#### Comments:

1. It is a free-swimming, minute, conical and microscopic.
2. Body is divided into head trunk and bilobed anal region.
3. It contains three pairs of appendages, namely uniramous antennules, biramous antennae and biramous mandibles, which assist in swimming.
4. It also contains a median eye and gut.
5. Larva is unsegmented without ventral nerve cord and hearts.



*Fig.1.3.47 Nauplius Larva*

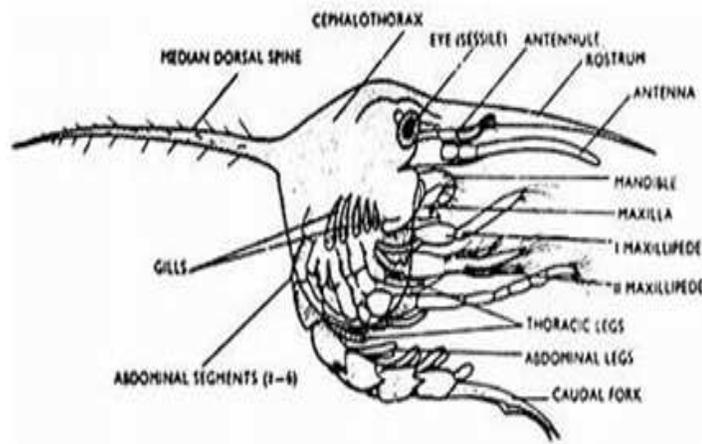
---

### Zoea: Larva

---

#### Comments:

1. Zoea Larva is another crustacean larva.
2. Nauplius, after meta-Nauplius directly changes into Zoea larva.
3. Body is differentiated into cephalothorax and abdomen.
4. Head is containing large compound eyes, antennules, antennae, mandibles maxillae and maxillipeds. Abdomen has 6 segments. Zoea larva changes to metozoea or megalopa larva.

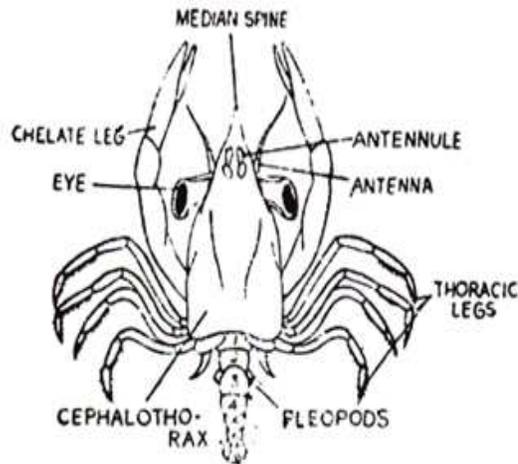


*Fig.1.3.48, Zoea Larva*

### 1.3.49 Megalopa: Larva

**Comments:**

1. Megalopa larva has stalked compound eye, cephalic leg.
2. Body is crab like being divided into unsegmented cephalothorax and segmented abdomen.
3. Head is containing pedicellate, compound eyes, antennules, and antennae,
4. Thoracic appendages have biramous pleopods.
5. Megalopa larva change in to adult prawn.



*Fig1.3.49 Megalopa: Larva*

---

### ***1.4. TERMINAL QUESTION & ANSWERS***

---

1. Define Invertebrate.
2. Give examples of sedentary animals.
3. Name the Invertebrate phyla which show metamerism.
4. What are triploblastic animals?
5. Name three type of body cavity found in animals.
6. Name a sedentary protozoan.
7. Which disease is caused by Leishmania Donovan?
8. Protozoa are immortal. Is it correct?
9. Name the parts of oral apparatus of Euglena.

---

### ***1.5. REFERENCES***

---

- 1 Poddar T., Mukhopadhyay S., Das S.K (2015) an advanced laboratory Manual of Zoology.
- 2 Verma P.S., (2014) A manual of practical Zoology, Invertebrates.
- 3 Rastogi V.B. (1999) Lower non-chordate & Economic Zoology.

# **UNIT-2 STUDY OF THE MUSEUM SPECIMENS BELONGING TO THE DIFFERENT INVERTEBRATE PHYLA**

## **CONTENT:**

- 2.1 Objectives
- 2.2 Introduction
- 2.3 Museum specimens of Invertebrate Phyla
  - 2.3.1 Protozoa
  - 2.3.2 Porifera
  - 2.3.3 Coelentrata
  - 2.3.4 Platyhelmenthis
  - 2.3.5 Nematoda
  - 2.3.6 Annelida
  - 2.3.7 Arthropoda
  - 2.3.8 Mollusca
  - 2.3.9 Echinodermata
- 2.4 Summary
- 2.5 Terminal Questions and Answers
- 2.6 References

---

## **2.1. OBJECTIVE**

---

Study of the museum specimens belonging to the following different invertebrate phyla.

---

## **2.2. INTRODUCTION**

---

The study of the morphology (external form and structure) of animals from the preserved animals in the museum or laboratory constitutes the study of museum specimens. For studying in museum, the students are required to see the actual preserved animals and their general Classification, habits, habitats, characters in the laboratory or in museum. A zoological specimen is an animal or part of an animal preserved for scientific use. Various uses are: to verify the identity of a (species), to allow study, increase public knowledge of zoology.

---

## **2.3. PHYLUM: PORIFERA**

---

---

### **2.3.1 *Leucosolenia***

---

**Classification:**

Phylum.....Porifera

Class.....Calcarea

Order.....Homocoela

Type.....*Leucosolenia*

**Comments:**

1. Marine colonial sponge found in shallow sea water.
- 2- Colony is composed of bunches of whitish vertical cylinders and tubular above 25 mm long.
- 3- Vertical tubes of the colony contain osculum distally.
- 4- The magnified portion of the colony shows vase-shaped individuals, inhalant ostia, tri-radiate spicules and horizontal branches attached to substratum.
- 5- Body wall is composed of dermal epithelium forming outer layer, and Choanocyte layer forming inner layer, separated by middle mesenchyme layer.

- 6- Calcareous spicules are monaxon or Triaxon.
- 7- Asexual reproduction by budding, branching and regeneration.
- 8-Sexual reproduction by ova and spermatozoa,
- 9-Larva is parenchymula.

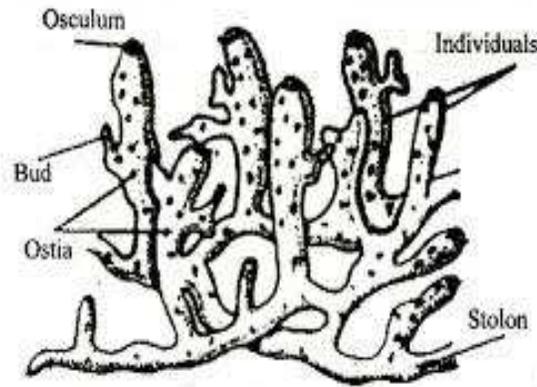


Fig .2.3.1 *Leucosolenia* (colony)

---

### 2.3.2 SYCON

---

**Classification:**

- Phylum..... Porifera
- Class..... Calcarea
- Order..... Haterocoe
- Type..... *Sycon*

**Comments:**

- 1.It is solitary or colonial marine sponge found in shallow to 60 fathoms deep in well oxygenated water.
- 2.Complex vase-shaped body, measuring 20-25 mm in length and 5-6 mm in diameter.
- 3.Body surface is covered by a ostia bearing membrane.
- 4.Proximal and base attached to substratum.
- 5.Body wall is thick through which monaxon, triaxon and tetraxon spicules project.

6. Body wall is composed of dermal epithelium forming outer layer, and middle mesenchyme and inner flattened epithelium lining spongocoel which opens through the osculum.
7. Asexual reproduction by budding, branching and regeneration.
8. Sexual reproduction by ova and spermatozoa.
9. Hermaphroditic.
10. Course of water current is ostia -> prosopyles->radial-> canals-> apopyles-> spongocoel ->osculum->exterior.
11. Larva is amphiblastula.

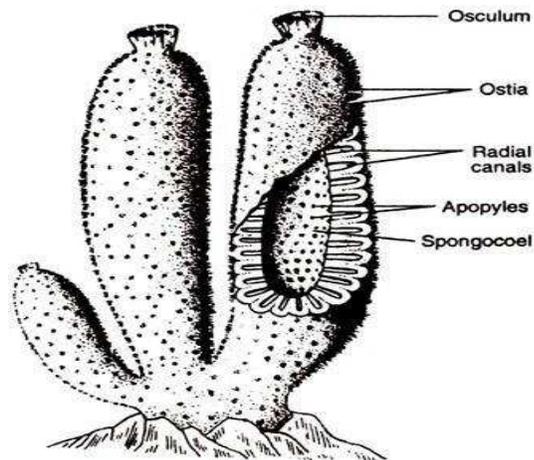


Fig.2.3.3 Sycon

---

### 2.3.3 EUPLECTELLA

---

**Classification:**

Phylum..... Porifera

Class.....Hexactinellida

Order.....Hexasrerophora

Type.....*Euplectella*

**Comments:**

1. It is commonly called Venus's flower basket.
- 2- It is solitary animal found abundantly in deep water of Pacific oceans near West India and Philippines Island.
- 3- Body is tubular, curved and basket like made up of siliceous spicules.
- 4- Animal measure 15-30 cm in length and 2-5 cm in diameter.
- 5- Body is composed of four and six –rayed siliceous spicules fused at their tips forming three-dimensional network with parietal gap.
- 6- Spicules are joined together forming a network.
- 7- Canal system is simple sycon type.
- 8-Cloacal cavity is closed in above with a sieve plate.
- 9-Osculum contains sieve called as oscular sieve plate.

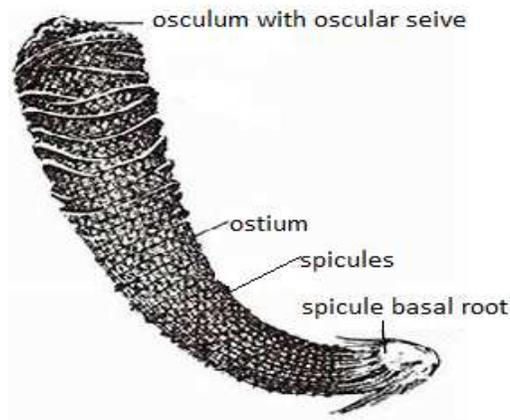


Fig.2.3.3 *Euplectella*

---

**2.3.4 HYALONEMA**

---

**Classification:**

- Phylum..... Porifera  
Class..... Hexactinellida  
Order..... Amphidiscophora  
Type..... *Hyalonema*

**Comments:**

1. It is commonly called as glass-rope sponge.
2. Body is spherical or ovoidal and axially traversed by a bundle of long spicules.
3. Spicules are often fused to form a lattice-like skeleton.
4. Body is cup or vase-shaped measuring 10-30 cm in height.
5. Spongocoel is well developed
6. Osculum contains sieve plate.
7. Root spicules are compact, stalk like elongated, twisted and giving the appearance of a rope. The middle columella contains symbiotic polyps.
8. It possesses large and small amphidisk spicules like freshwater sponges.
9. The canal system is complicated type.

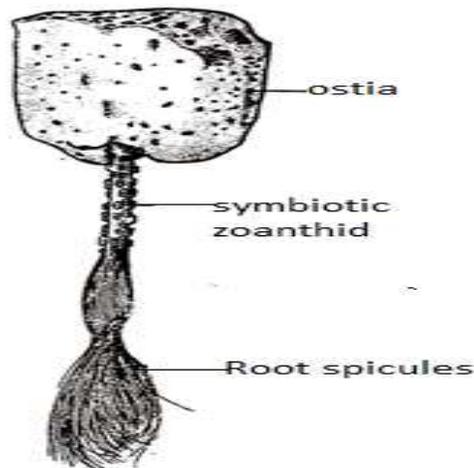


Fig. 2.3.4 Hyalonema

---

### 2.3.5 SPONGILLA

---

**Classification:**

Phylum..... Porifera

Class.....Demospongia

Order.....Monaxonida

Type..... *Spongilla*

**Comments:**

1. Spongilla is a colonial sponge, found in pond or streams.
1. Commonly known as freshwater sponge

2. Colony is profusely branched, exhibiting various shades of green color due to the presence of green algae, called as Zoochlorella, in the cellular system.
3. Skeleton is composed of sponging fibers.
4. The siliceous spicules are in the form of smooth or spiny large and small oxeas, embedded in the sponging.
5. Canal system is of the rhagon type.
6. Asexual reproduction by gemmules.
7. Sexual reproduction by sperm and ova.
8. Gemmules are protected by amphidisk spicules.

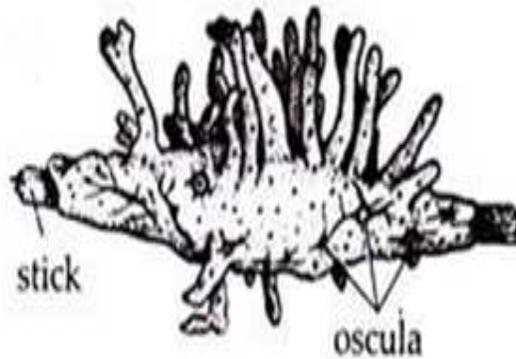


Fig.2..3. 5 *Spongilla*

---

### 2.3.6 *EUSPONGIA*

---

**Classification:**

Phylum..... Porifera

Class.....Demospongia

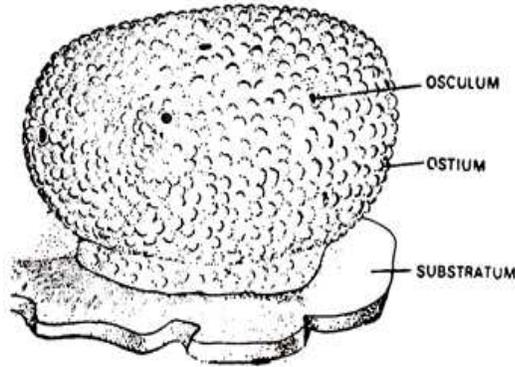
Order.....Keratosa

Type.....*Euspongia*

**Comments:**

1. It is found in shallow water on rocky bottom.
2. Commonly called bath sponge.
3. It attains a large and massive size with globular or cup shape body with dark black color.
4. Complex type of sponge, in which there is a further increase in the folding of the body ball.
5. Skeleton is made up of sponging fibers. The silicious spicules being absent.

6. Bath sponge is house hold use. It is prepared by bleaching or dyeing the sponge.
7. Commonly used in offices, etc. for wetting postal tickets and counting currency notes or papers.



*Fig.2.3.6 Euspongia*

---

## 2.4. PHYLUM- COELENTERATA

---



---

### 2.4.1 *PHYSALIA*

---

**Classification:**

Phylum..... Coelenterata  
 Class.....Hydrozoa  
 Order.....Siphonophora  
 Suborder.....Physophorida  
 Type.....*Physalia*

**Comments:**

1. It is marine, colonial floating, pelagic coelenterate.
2. Commonly called as Portuguese man of war.
3. Most beautiful siphonophore having iridescent peacock, blue or orange colour.
4. Size 10-30 cm long but tentacles measure several meters.
5. Animal is composed of upper large crested pneumatophore or float and lower various zooids.
6. Ventrally the float contains budding coenosarc from which dactylozooids, gastrozooids and gonodendra hang down.

7. A part of gonodendra shows upper small male gonophores, lower large rounded female gonophores with eggs.

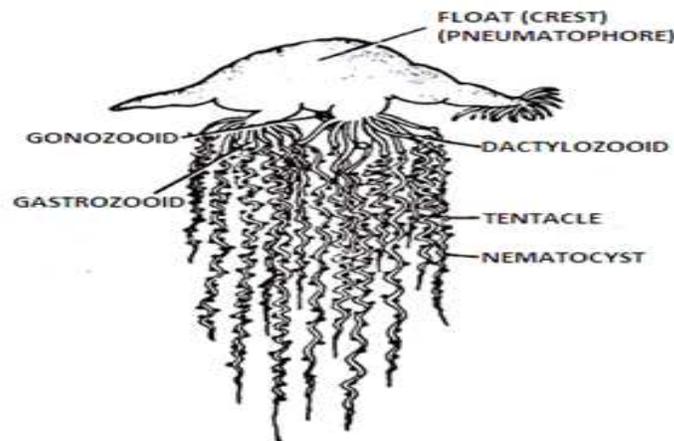


Fig.2.4.1 *Physalia*

---

### 2.4.2 PORPITA

---

**Classification:**

Phylum.....Coelenterata  
 Class.....Hydrozoa  
 Order.....Siphonophora  
 Suborder.....Physophorida  
 Type.....*Porpita*

**Comments:**

1. It is marine, colonial, found in warm sea.
2. It superficially resembles a medusa. It is most modified siphonophore.
3. Body is discoidal, enclosing a chambered, chitinous and porous shell.
4. A large gastrozoid, having central mouth is encircled by several blastostyles.
5. Swimming bells are absent.
6. Each air chamber opens to outside by a pair of pores.
7. The reproductive zooids are liberated as free medusa.

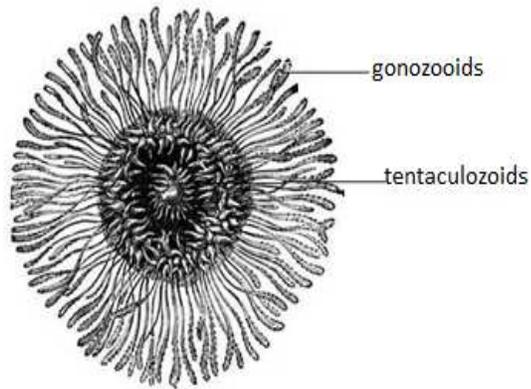


Fig.2.4.2 *Porpita*

---

### 2.4.3. VELELLA

---

**Classification:**

Phylum..... Coelenterata  
 Class.....Hydrozoa  
 Order.....Siphonophora  
 Suborder.....Physophorida  
 Type.....*Verella*

**Comments:**

- 1 -Commonly found in warm sea.
- 2- It is most beautiful open-sea.
- 3- Deep blue colony consists of a rhomboidal disk pneumatophore or float containing air chamber without marginal identification.
- 4- It is most beautiful and conspicuous animal of the open sea.
- 5- Pneumatophore is flat chambered, rigid and chitinous disc, 5-8cm.
- 6- Chamber of pneumatophore open on the upper side by pores and on the lower side by canals called tracheae.
- 7-Gonozooid produced free medusa.

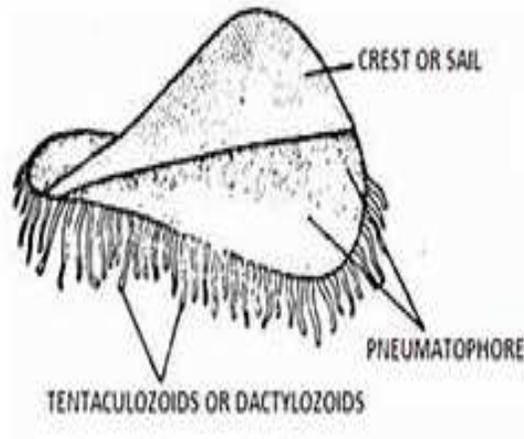


Fig. 2.4.3 *Veella*

---

#### 2.4.4. *CHARYBDEA*

---

**Classification:**

Phylum..... Coelenterata

Class.....Scyphozoa

Order..... Cubomedusae

Type.....*Charybdea*

**Comments:**

1. Commonly found in warm shallow water inhabiting bays, island and open sea.
- 2- Commonly called as Sea wasp.
- 3-It is best known genus of cub medusae.
- 4-Shape of the body is more or less cubical in form with a deep ball having some flattened top.
- 5- Body divisible into ex-umbrella and sub-umbrella surface.
- 6-Ex-umbrellar surface contain manubrium.
- 7-Each rhopalium has two large eye and 2 small ocelli clearly visible in section.

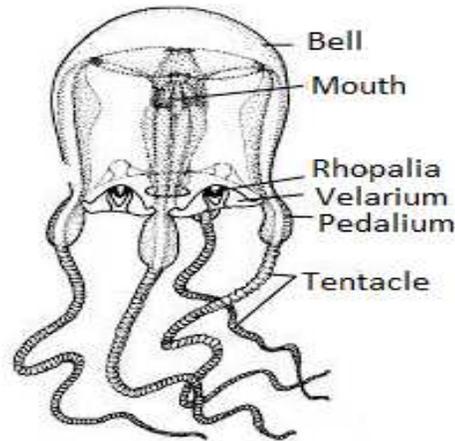


Fig. 2.3.4 Charybdea

---

### 2.4.5. AURELIA

---

**Classification:**

Phylum..... Coelenterata

Class.....Scyphozoa

Order.....Semaestomeae

Family.....Ulmariidae

Type.....*Aurelia*

**Comments:**

1-Commonly called as jelly fish.

2-Body is gelatinous, transparent, bluish, white, reddish and pink.

3-Saucer- shaped body is distinguished into convex ex-umbrella concave sub-umbrella surface.

4-Gasttic filaments, sub-genital pits and velarium are present.

5- Mouth 4-cornered. Each corner is drawn out into an oral arm along perradii.

6-Inter-radial, adradial and per-radial gastro-vascular canals open in circular present.

7- Male and female separate individuals.

8-Lifecycie shows alteration of generation.

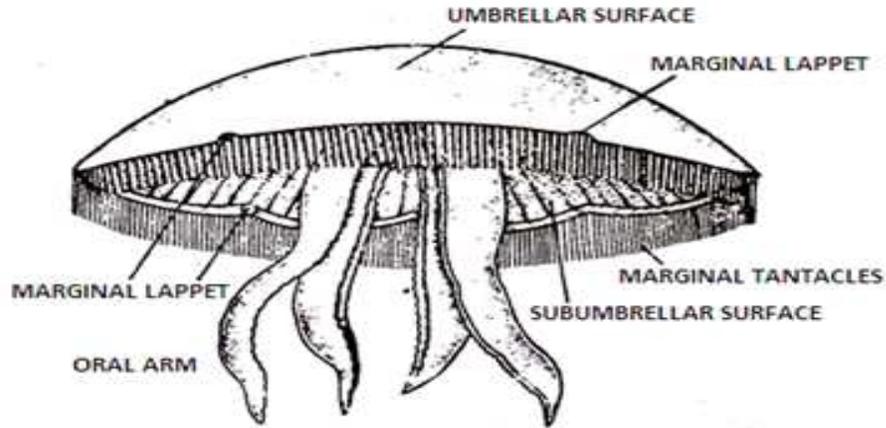


Fig. 2.4.5 Aurelia

---

### 2.4.6. *Gorgonia*

---

**Classification:**

Phylum..... Coelenterata

Class.....Anthozoa

Sub class.....Octocoralia

Order.....Gorgonacea

Sub order.....Scleraxonia

Family.....Gorgonida

Type..... *Gorgonia*

**Comments:**

1. Commonly called as Sea fan.
2. It is found in all sea from tide mark to over 4000 meters.
3. It forms erect, yellowish or reddish arborescent branches in one plane connected by cross connections in a feathery manner and contour of the body becomes fan-like.
4. Base of the colony is expanded to form a hold fast organ called as pedal disk.
5. Polyps emerge from branches and contain 8 pinnate tentacles, mouth, gastro-vascular cavity, 8 gastric filaments and mesenteries
6. Skeleton consists of hornlike material called embedded in mesoglea.
7. Sexes are separate.

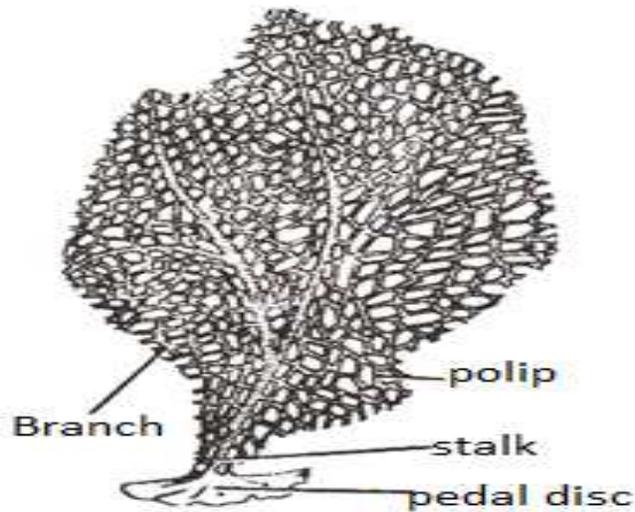


Fig .2.4.6Gorgonia

---

### 2.4.7. PENNATULA

---

**Classification:**

Phylum..... Coelenterata  
 Class..... Anthozoa  
 Sub class.....Octocorallia  
 Order.....Pennatulacea  
 Family.....Pennatulidae  
 Type..... *Pennatula*

**Comments:**

1. Commonly called as Sea fan or sea feather.
2. It is found in deep sea water in sandy and muddy bottom, 20-600 fathoms deep.
3. Body is divided into a proximal stalk peduncle devoid of anthocodia and distal rachis having secondary polyps.
4. Central axis or rachis posteriorly forms a peduncle with an end bulb.
5. Pinnules containing a series of 8-12 gastrozooids or polyps. Each polyp has pinnate tentacles.
6. Skeleton comprises of a long horny unbranched axis supporting rachis only. Rachis is deep red. Peduncle is rose colored. Base is whitish.
7. Gastrozooids are feeding zooids, while siphonozooids draw water current.

8. Sexes are separate and gametes develop in gastrozooids.

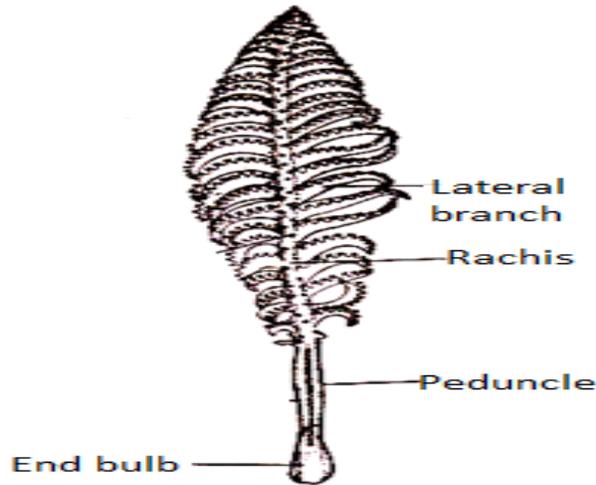


Fig .2.4.7Pennatula

---

### 2.4.8. METRIDIDIUM

---

**Classification:**

Phylum.....Coelenterata

Class.....Anthozoa

Sub class.....Hexacorallia

Order.....Actinaria

Type.....*Metridium*

**Comments:**

1. Commonly called as Sea-anemone.
2. Body is short cylindrical, radially symmetrical and longer than broad.
3. Body of the animal is divided into 3 region –Oral disk, column and pedal disk, column and pedal disk.
4. Oral disk is expanded as flat disk called as capitulum which is crowned with several marginal tentacles around mouth.
5. Column form major part of the body and is differentiated into 2 parts (i) upper delicate thin walled capitulum and (ii) lower thick walled scapus.
6. Wall of the scapus is perforated by small openings called as cinclides.
7. Sexes separate.
8. Asexual reproduction by budding and fragmentation.

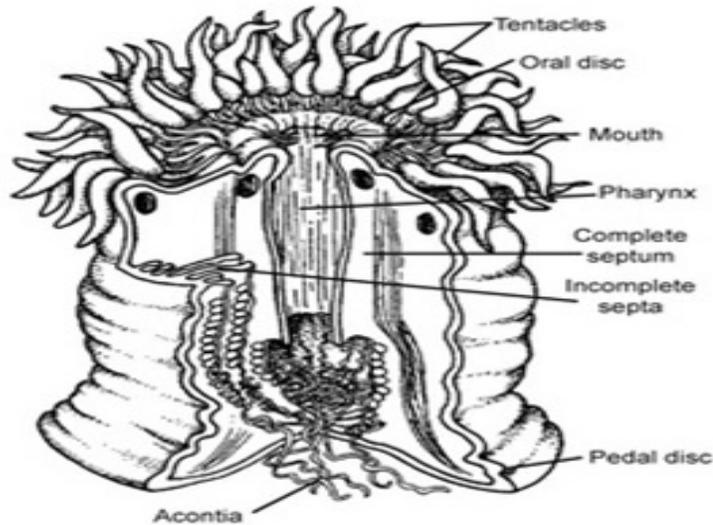


Fig.2.4.8 Metridium

---

## 2.4.9 MADREPORA

---

### Classification:

Phylum.....Coelenterata  
 Class.....Anthozoa  
 Sub class.....Hexacorallia  
 Order.....Madreporaria  
 Type.....*Madrepora*

### Comments:

1. Commonly called as Born coral.
2. It is colonial, symbiotic and marine coral.
3. It plays important role in coral-reef formation.
4. Colony is partly porous or reticulate covered by perisarc.
5. Colony consists of cylindrical cups or corallites or polyps in coenosarcs.
6. The corallite is secured by basal discs of polyps and is composed of calcium carbonate.
7. Polyps look like flowers. Terminal and lateral polyps possess 6 and 12 tentacles respectively, and are without central columella.
8. Internally mesenteries are arranged and coenosarcs contains a network of canals.

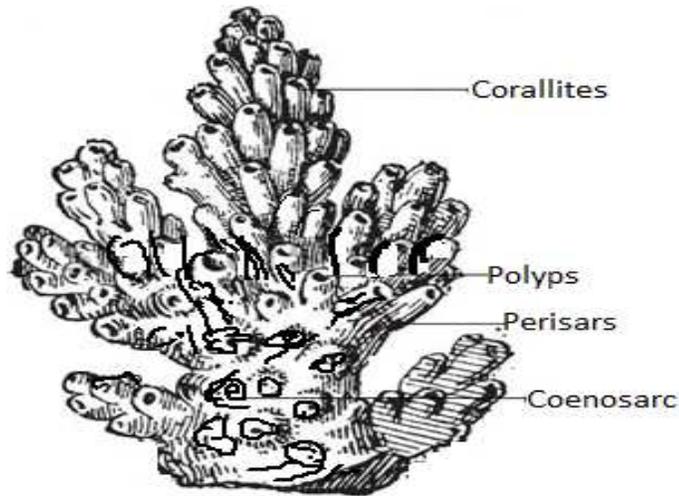


Fig .2.4.9Madrepora

---

## 2.5. PHYLUM PLATYHELMINTHES

---

### 2.5.1. PLANARIA (*DUGESIA*) *DOROTOCEPHALA*

---

**Classification:**

Phylum.....Platyhelminthes

Class.....Turbellaria

Order.....Tricladida

Family.....Planariidae

Type.....*Dugesia*

**Comments:**

1. It is found in fresh water, triclad found in streams, springs, ponds, lakes and caves, gliding over stones.
2. Commonly known as Planaria.
3. Body is dark brown elongated cylindrical measuring 15mm in length.
4. Anterior region is called a head.
5. Head is triangular containing 2 ear like auricles on side and two semicircular ocelli.
6. Body tapers posteriorly as a pointed end.
7. Digestive system, consists of mouth, proboscis, esophagus and intestine.
8. It is reproducing asexually and sexually.

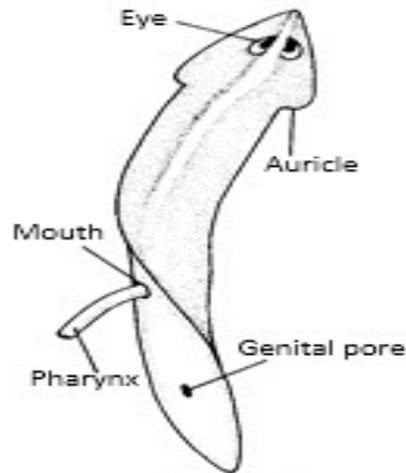


Fig. 2.5.1 *Dugesia* (Planaria.)

---

## 2.5.2. *FASCIOLA HEPATICA*

---

### Classification:

Phylum.....Platyhelminthes

Class.....Prematoda

Order.....Digenea

Family.....Fasciolidae

Type.....*Fasciola hepatica*

### Comments:

1. It is found in the bile duct of liver and biliary passages of sheep, ox, horse, dog, man, monkey, deer, an elephant and Kangaroo.
2. Commonly known as sheep fluke.
3. It is polyxenous and pathogenic parasite.
4. Body is leaf- like, dorsoventrally flattened measuring 18-51 mm in length and 4-15 mm in breadth.
5. Oral suckers are present on the mouth.
6. Life-cycle involves two hosts. Sheep and Limnea as intermediate host.
7. Life-cycle stages include zygote, Miracidium larva, Sporocyst larva, Redia, cercaria larva. Metacercaria. Metacercaria after ingestion by sheep changes into adult parasite inside the host.

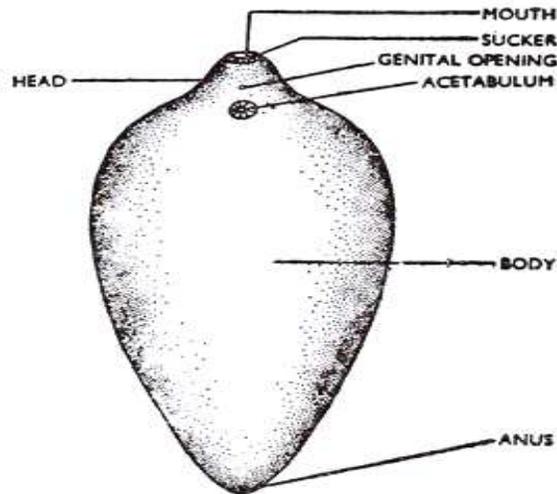


Fig. 2.5.2 *Fasciola hepatica*

---

### 2.5.3. TAENIA SOLIUM

---

**Classification:**

Phylum.....Platyhelminthes

Class.....Cestoda

Order.....Cyclophyllidea

Family..... Taeniidae

Type.....*Taenia solium*

**Comments:**

1. It is found in the intestine of man.
2. Commonly called as Pork tapeworm.
3. Body divided into scolex, neck, Immature, mature and gravid segment
4. Scolex serves as hold-fast organ. It contains 4 suckers and a rounded rostellum.
5. Rostellum at its base contains double row of 28-32 hooks.
6. Mature segment contains well developed hermaphrodite genital organ.
7. Gravid segment contains branched uterus filled with Oncospheres.
8. The lifecycle includes two host (i) Man and (ii) Pig.

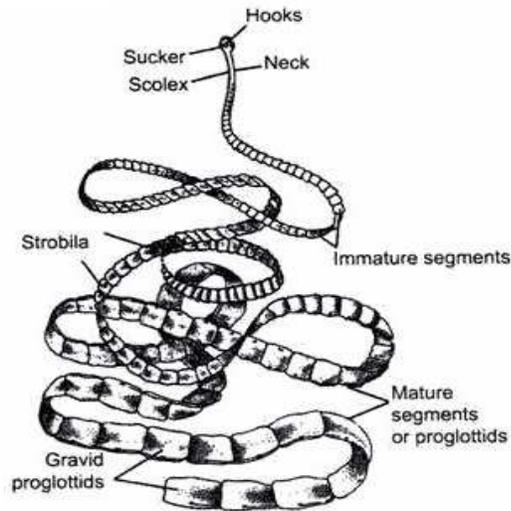


Fig.2.5.3 *Taenia solium*

---

## 2.6.PH YLUM ASCHELMIMTHES

---



---

### 2.6.1. ASCARIS LUMBRICOIDES

---

**Classification:**

Phylum.....Aschelminthes  
 Class.....Nematoda  
 Order.....Ascarioidea  
 Type..... *Ascaris lumbricoides*

**Comments:**

1. It is found in the intestine of man and the pig.
2. It is one of the most common nematodes found in all parts of the world India, China, Korea.
3. Commonly known as round worm.
4. It shows sexual dimorphism with separate male and female individuals.
5. Male measure 15-30cm in length and female 20-35 cm.
6. Tail end of female is bluntly pointed. Female genital opening or vulva found in anterior one third regions.
7. Anterior ends exhibit same structures in both male and female.
8. Body is elongated and cylindrical.

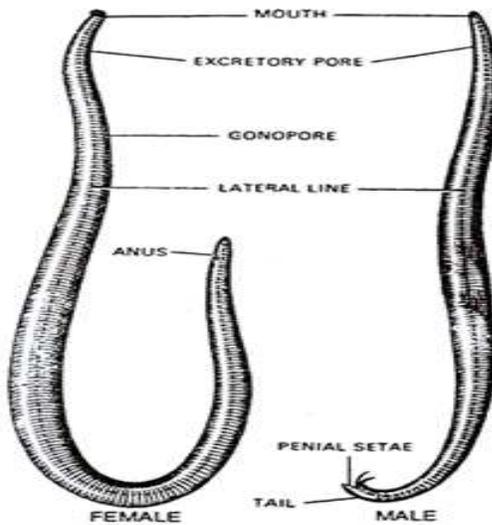


Fig .2.6.1 *Ascaris lumbricoides*

---

## 2.7. PHYLUM ANNELIDA

---

### 2.7.1. NEREIS

---

**Classification:**

Phylum.....Annelida

Class.....Polychaeta

Order.....Errantia

Type.....*Nereis*

**Comments:**

1. It is marine crawling type, in temporary burrows in sand, 200 meters in deep.
2. Commonly called as rag worm or clam worm and is the simplest annelid.
3. Head is composed of prostomium which carries prostomial tentacles, palps and ocelli; and (ii) peristomium which carries antero-laterally 4 pair of peristomal tentacles.
4. Mouth is found on the anterior surface of the peristomium.
5. Locomotory organ are parapodia.
6. Segments are also called as metameres and between two segments in intersegmental groove.
7. Parapodia also serve as respiratory and circulatory organs.
8. Anal segment contains a pair or anal cirri.

9. 9-. It is dioecious animals.

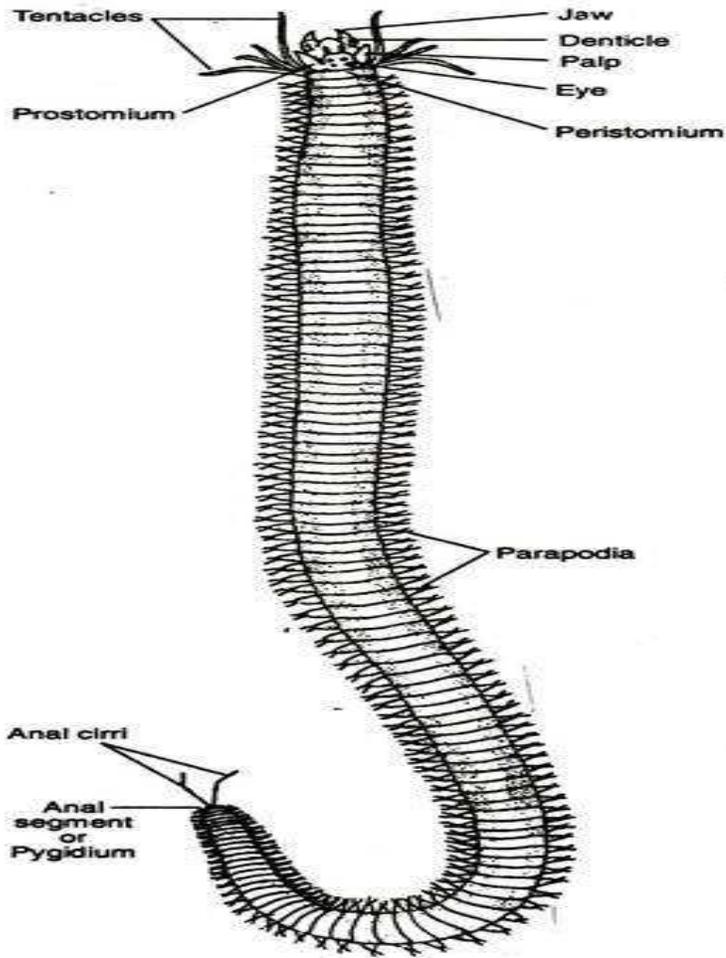


Fig .2.7.1 Nere

---

## 2.7.2. HETERONEREIS

---

### Classification:

Phylum.....Annelida  
Class.....Polychaeta  
Order.....Errantia  
Type.....*Heteronereis*

### Comments:

1. It is free-swimming worm found in sea.
2. It is sexual phase of Nereis.
3. During breeding season, clams worm leaves its tube and become free-swimming.

4. Body is differentiated into asexual anterior atoke and a posterior sexual epitoke which contains gametes.
5. Parapodia of heteronereis contain additional foliaceous lobes and setae become oar-like.
6. Peristomial cirri become large. Special sensory papillae develop on anal segment.
7. Prostomium contain prostomial tentacle, prostomial ocelli and prostomial palp.  
Peristomium contains peristomial tentacles.

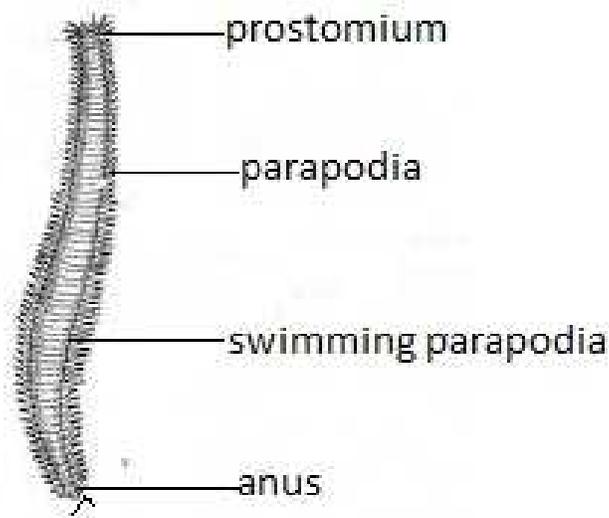


Fig .2.7.2 Heteronereis

---

### 2.7.3 APHRODITE

---

**Classification:**

Phylum.....Annelida

Class.....Polychaeta

Order.....Errantia

Type.....*Aphrodite*

**Comments:**

- 1- It is marine worm inhabiting the deep-water muddy bottom.
- 2- Commonly called as sea mouse measuring 12 cm in length and made up of 30-35 segments.

- 3- Body is covered dorsally by felt-like or blanket-like setae arising from the notopodium.
- 4- Shape of the animal is oval, and dorsoventrally flattened.
- 5- Anterior end contain a small head or prostomium, bearing a small median tentacle and 2 lateral palps.
- 6-Parapodial structures are greatly modified. Notopodia contain 3 kind of setae (1) stiff setae (II) soft setae, and (iii) iridescent setae.

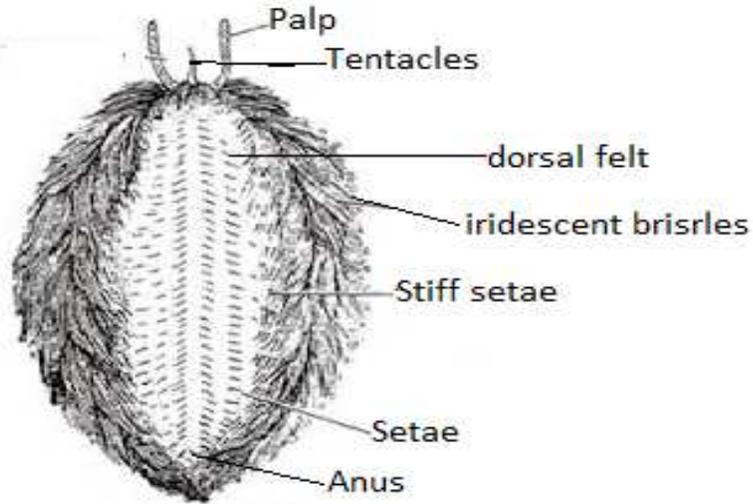


Fig.2.7.3Aphrodite

---

#### 2.7.4. CHAETOPTERUS

---

**Classification:**

Phylum.....Annelida  
 Class.....Polychaeta  
 Order.....Tubicola  
 Type.....*Chaetopterus*

**Comments:**

1. Commonly called as paddle worm having greatly modified segments.2-Tube is opaque, measuring 50 cm long and 1 cm in diameter.3-Body is white, delicate, 30 cm long and divided into anterior, middle and posterior region.4- Parapodia are variously modified as for water pumping fans, sucking discs or food ball organs.5- Anterior region comprise of 15-20 segment, having a funnel-shaped mouth surrounded by a collar-like peristomium and a pair of peristomial cirri in the first

segment.6-Middle region has fused segments.7-The notopodia of segment 14-16 are fused in mid line to form three fans.8-Posterior region is longer with a pair of parapodia in each segment, about 11-30 in number.

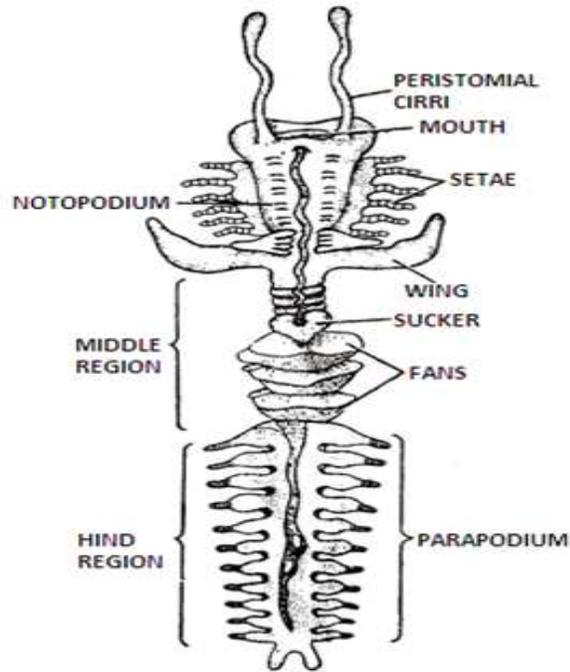


Fig. 2.7.4 Chaetopterus

---

### 2.7.5. ARENICOLA

---

**Classification:**

Phylum.....Annelida  
 Class.....Polychaeta  
 Order.....Sedendaria  
 Type.....*Arenicola*

**Comments:**

- 1-It is in a j-shaped burrow made of sand and mucus.
- 2-Body is stout, elongated, cylindrical and 15 cm long, brownish or greenish in colour and divided into metameres.
- 3 Head without appendages and with an unarmed proboscis.
- 4-Anterior region consist of a small trilobed prostomium with no eyes or tentacles, an achaetus peristomium and 6 segments bearing rudimentary parapodia.
- 5- Parapodia contains reduced notopodium and neutopodium.

6-Mouth lies ventral to the prostomium.7-Middle region has 13 segments, each bearing in addition to neupodia, a pair of extensively branched gill. Nephridia six pairs.8- Arenicola is generally used as bait for fish.

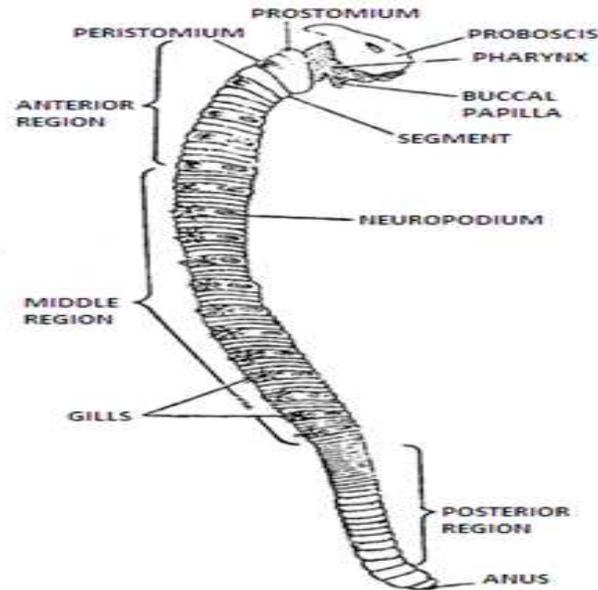


Fig. 2.7.5 *Arenicola*

---

## 2.7.6. AMPHITRITE

---

### Classification:

Phylum.....Annelida  
 Class.....Polychaeta  
 Order.....Sedentaria  
 Type.....*Amphitrite*

### Comments:

- 1-It is marine, sedentary polychaete found in Europe.
- 2-It is long cylindrical, and pink in colour measuring 20-30 cm in length.
- Body is divided into metameres and regionated into somewhat thickened anterior region (head), middle region (trunk) and posterior narrow region.
- 4-Prostomium forms the upper lip and peristomium lower lip of the mouth.
- 5-Eyes are absent and palps present in prostomium.
- 6-Peristomium is without appendages.
- 7-Gills contract rhythmically.
- 8-Abdomen is long and each segment contains notopodia and neuropodia.

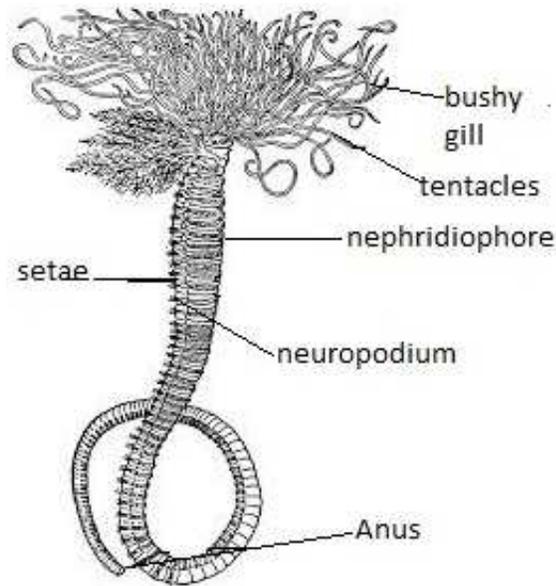


Fig. 2.7.6 *Amphitrite*

---

### 2.7.7. *TEREBELLA*

---

**Classification:**

Phylum.....Annelida

Class.....Polychaeta

Order.....Sedentaria

Type.....*Terebella*

**Comments:**

1-It is a marine, burrowing, sedentary animal found in Europe.

2-It is elongated, cylindrical, and divided into head and trunk.

3-Head is horseshoe-shaped, made up of prostomium and peristomium.

4-Prostomium contains number of long filiform bushy tentacles.

5-Trunk contains about 60 segments and is divided into 3 parts (i) anterior region (ii) middle region (iii) posterior region. 6-Setae of anterior segments are well developed while reduced in posterior segments.

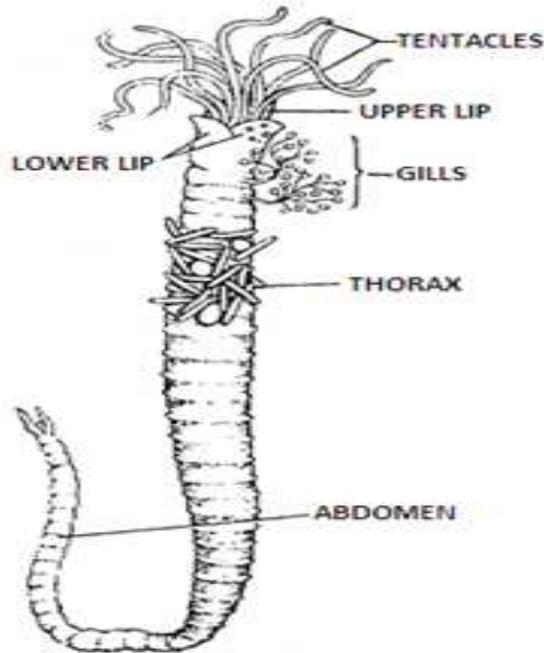


Fig . 2.7.7 *Terebella*

---

### 2.7.8. *PHERETIMA*: *EARTHWORM*

---

**Classification:**

Phylum.....Annelida

Class.....Oligochaeta

Order.....Neo-oligochaeta

Type.....*Pheretima*

**Comments:**

1-It is found in the soil but absent in sandy and humus deficient soil.

2-It is nocturnal animal.

3-It is hermaphroditic (monoecious).

4-Commonly called as earthworm.

5-Body consists of 100-120 rings like segment depicting true metamerism and measuring 150mm.

6-Both external and internal segmentations are distinct.

7-Clitellum is present 14-16 segment.

8-Spermathecal pore are found in the inter-segment groove of 5/6, 6/7, 7/8 segment.

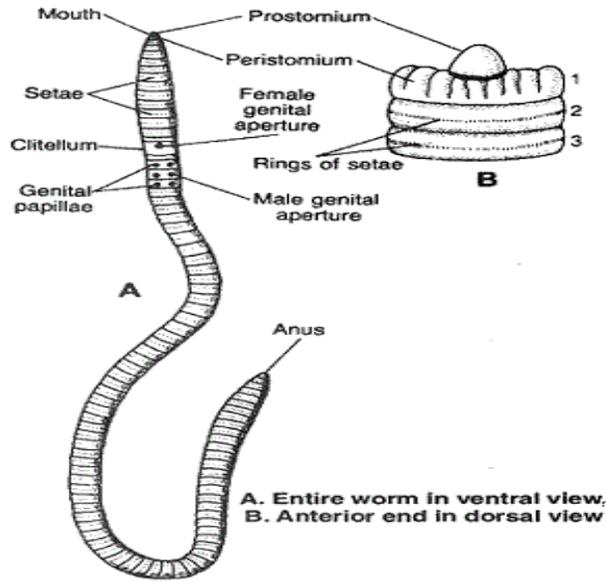


Fig .2.7.8 *Pheretima*: Earthworm

---

### 2.7.9. TUBIFEX

---

**Classification:**

Phylum.....Annelida

Class.....Oligochaeta

Order.....Archiologochaeta

Type.....*Tubifex*

**Comments:**

- 1-It is freshwater arch oligochaete, found abundantly on the bottom of deep lake.
- 2-Body is cylindrical, red colored; Meta numerically segmented 4cm in length.
- 3-Prostomium and peristomium are present on first and second segment.
- 4-Each segment contains 4 bundles of setae on dorsal and ventral sides.
- 5-Clitellum is found in 11<sup>th</sup> and 12<sup>th</sup> segment.6-Contractile heart is found in 8<sup>th</sup> segment.

7- Female-genital pores in the the11th segment and male-genital pore in the 12<sup>th</sup> segment.8- Hermaphroditic animal. It is reproducing sexually only.

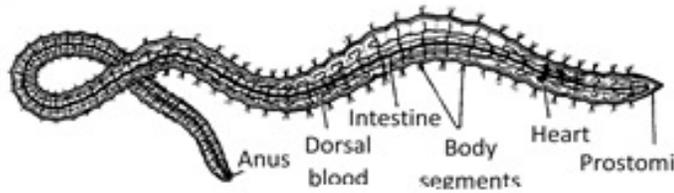


Fig .2.7.9 Tubifex

## 2.7.10. HIRUDO MEDICINALIS

### Classification:

Phylum.....Annelida

Class.....Hirudineas

Order.....Arhynchobdella

Type.....*Hirudo medicinalis*

### Comments:

1-It is found in pond, marshes and streams.

2-They are sanguivorous (blood sucking) ectoparasites on Submerged cattle's.

3- Commonly known as Indian cattle leech.

4-Body is cylindrically elongated dorsoventrally flattened having variable pigment on dorsal surface measuring 40cm.

5-Anterior and posterior sucker are well developed and are meant for attachment.

Hermaphroditic.7-Development takes place in cocoons or ootheca.

8-Male and female genital pores are on 10<sup>th</sup> and 11<sup>th</sup> segments respectively.

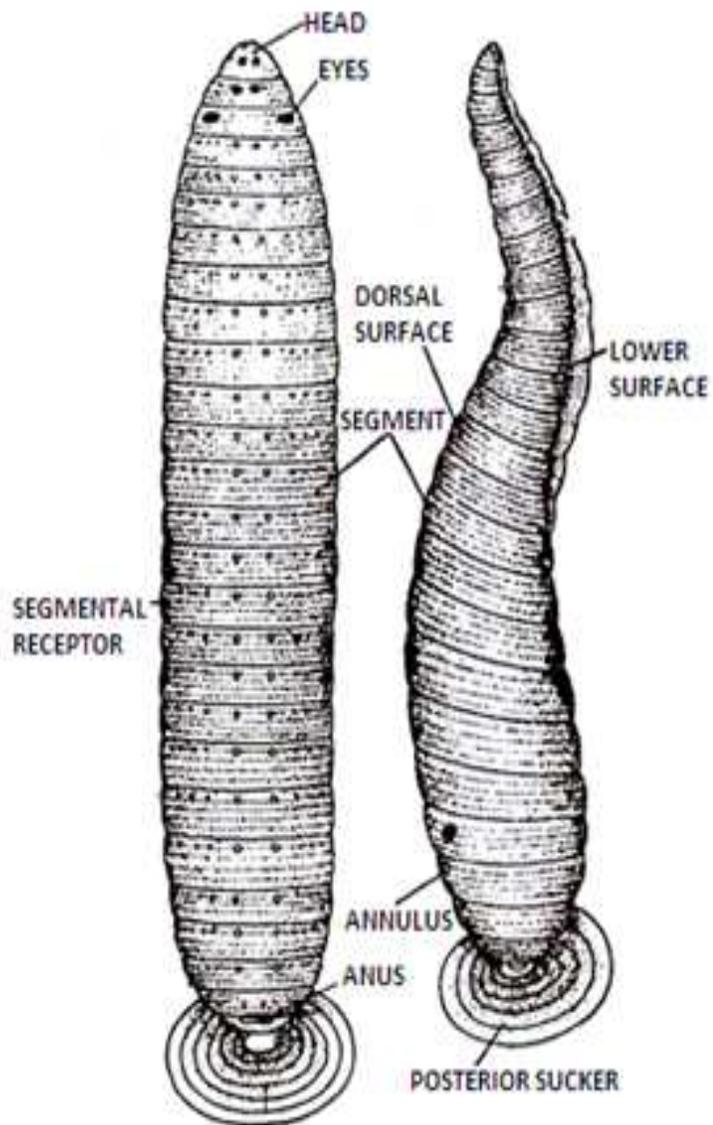


Fig. 2.7.10 *Hirudo medicinalis*

---

## 2.8. PHYLUM- MOLLUSCA

---

### 2.8.1. CHITON OR ISCHNOCHITON

**Classification:**

Phylum.....Mollusca

Class.....Amphineura

Order.....Polyplacophora

Type.....*Chiton*

Comments:

1-It is marine and sluggish slow-moving animals, attached to rocks, empty shell.

2-It is mostly nocturnal and remains concealed under rocks during day-time.

3-Commonly called as sea mouse.4-It measures 1 to 5 cm in length.

Body is elliptical, bilaterally symmetrical and dorsoventrally flattened and is differentiated into a small, indistinct head, a large flat foot and a dorsal mantle forming a roof-like covering.6-Head contains ventral mouth and labial palps.

7-Eyes and tentacles are absent. 8-Mouth and anus are opposite ends.

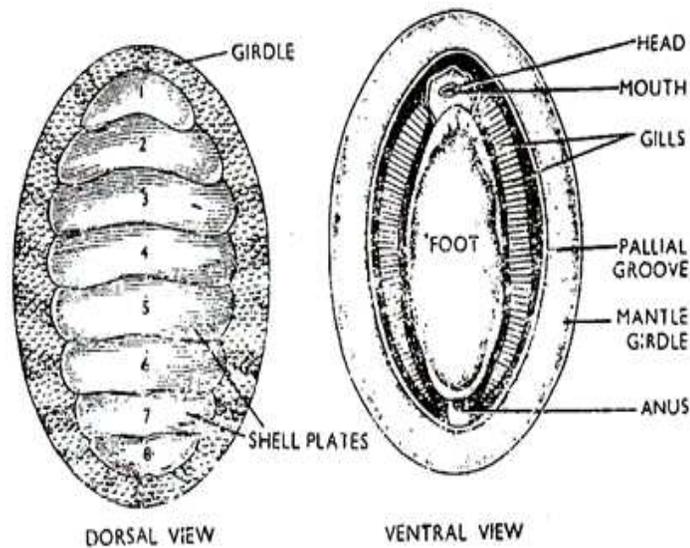


Fig .2.8.1 Chiton

---

## 2.8.2. CYPRAEA

---

### Classification:

Phylum.....Mollusca

Class.....Gastropoda

Sub class.....Prosobranchiata

Order.....Pectinibranchiata

Type.....*Cypraea*

Comments:

1-It is commonly found in shallow marine water.

2-It is commonly called as cowry.

- 3-Solid shell is very smooth, polished and often highly colored.
- 4-Mantle and the foot are more beautiful.
- 5-lateral fold of mantle are reflected over the shell and may completely cover it.
- 6-Eye and tentacles, pallial tentacle foot mantle and siphon are easily seen.
- 7-Cowry shell are much used as ornament, curious and mantle-piece decoration.

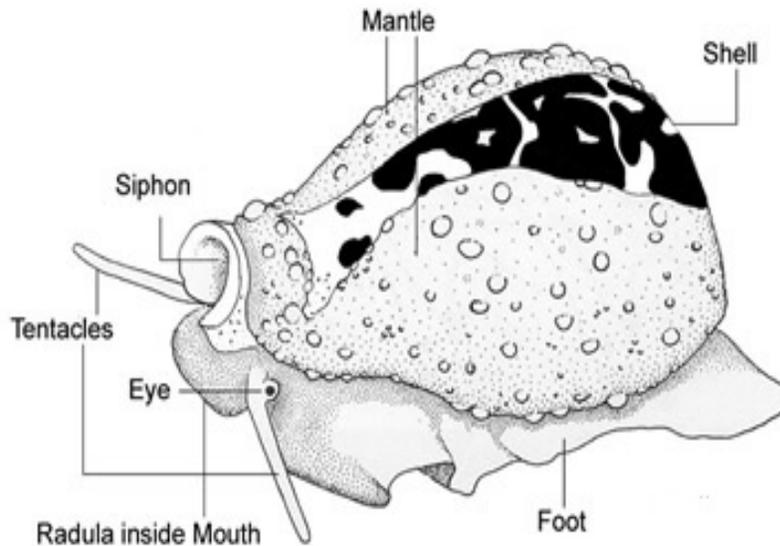


Fig .2.8.2Cypraea

---

### 2.8.3. *PILA*

---

**Classification:**

Phylum..... Mollusca

Class.....Gastropoda

Sub class.....Prosobranchiata

Order.....Pectinibranchiata

Type..... *Pila*

**Comments:**

- 1-It is commonly found in fresh water, ponds tank, rice- field.
- 2- Commonly called as fresh water apple snail.

- 3-Body is covered by a thick yellow-coloured or brown globular univalve shell.
- 4-Surface of the shell is marked by lines of growth.
- 5-Shell is spirally coiled round the axis, called the columella.
- 6-Head lies on the upper side and contains 1 pair of eyes.
- 7-Foot is highly muscular and act as creeping organ.
- 8-Sexes are separate but without sexual dimorphism.

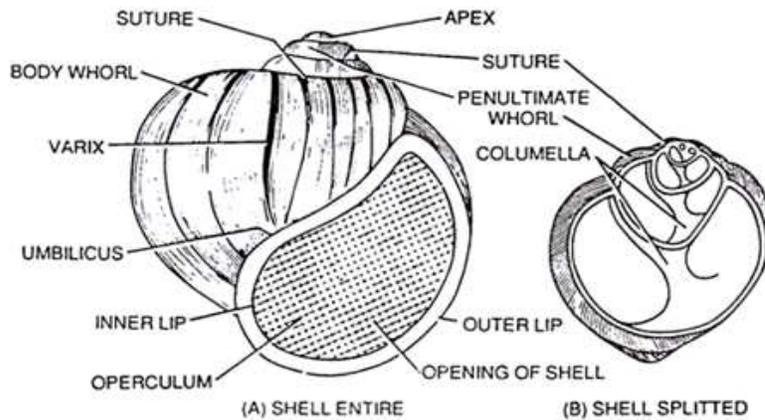


Fig .2.8.3Pila

---

#### 2.8.4. APLYSIA

---

**Classification:**

- Phylum.....Mollusca  
 Class.....Gastropoda  
 Sub class.....Euthyneura  
 Order.....Opisthobranchia  
 Type.....Aplysia

**Comments:**

- 1-It is a marine gastropod found crawling among seaweeds.
- 2- Commonly called as sea hare.
- 3- Body of the animal soft, slimy, fleshy and whitish or greenish in colour.
- 4-It is slug-like, with the anterior angles of the head extended into two large tentacular fold.
- 5-Beside anterior tentacular fold, there are pair of eye and behind eyes another tentacular fold like structure called as rhinophores.

6-Mentle cavity is open on the right side with backwardly pointing ctenidium through a longitudinal slit.7-Shell is internal and rudimentary.8-Animal is bisexual with a single Gono duct and a common genital opening.

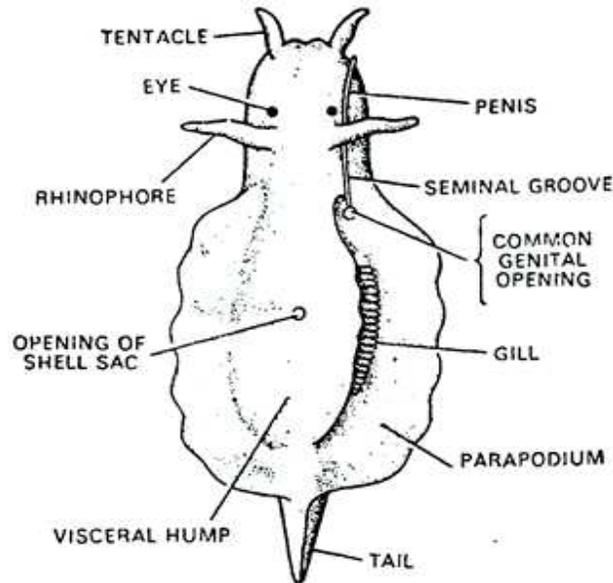


Fig .2.8.4 Aplysia

---

## 2.8.5. DORIS

---

### Classification:

Phylum.....Mollusca  
 Class.....Gastropoda  
 Sub class.....Euthyneura  
 Order.....Opisthobranchiata  
 Type.....*Doris*

### Comments:

- 1-It is a sluggish marine and curious gastropod found under stones at low tide mark and between weeds.
- 2- Commonly known as sea lemon.
- 3-Body consists of more or less ovoid mass with a convex warth dorsal side.
- 4- Colour is purplish brown.
- 5- Head bears a pair of short retractile tentacles or rhinophores beset with calcareous spicules.

6-Mantle is usually pigment and contains spicules or dorsal tubercles.

7-Ventral surface has mouth, head, tentacle and mantles.

8-Anus surrounded by branchia or gills.

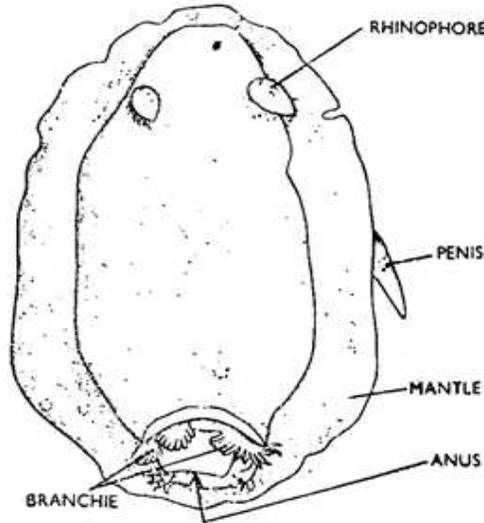


Fig. 2.8.5 Doris

---

## 2.8.6. MYTILUS

---

### Classification:

Phylum..... Mollusca

Class.....Pelecypoda

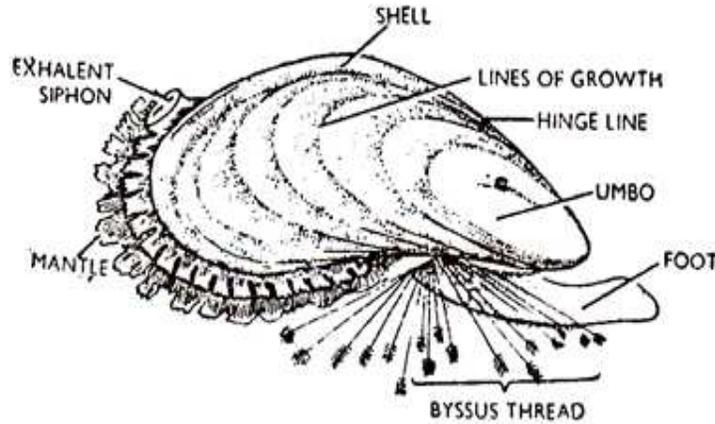
Order.....Filibranchia

Type..... *Mytilus*

### Comments:

1. It is found at a depth of 2 or 3 fathoms in low tide, attached to rock or wooden structure by its byssus threads.
2. Commonly called as sea mussel.
3. Shell is elongated equivalved with umbo and marked by lines of growth.
4. Hinge toothless but may bear crenulations.
5. Shell is marked with line of growth.
6. Byssal filaments found in a byssal cavity are formed by byssal gland or byssogenous apparatus.

7. After removing the shell internal structure such as lamelliform gill, foot, kidney, heart and alimentary canal enclosed by mantle lobes are visible.
8. Gills are lamelliform, i.e., the filaments are plate-like and united by ciliary junctions.
9. Sexes are separate, Gonads extend into the mantle.



*Fig. 2.8.6 Mytilus*

### **2.8.7. UNIO**

**Classification:**

- Phylum.....Mollusca  
 Class.....Pelecypoda  
 Order.....Eulamellibranchiata  
 Type.....Unio

**Comments:**

1. It is found in ponds, lakes, rivers and streams. The animal is usually buried in the mud.
2. Commonly called as fresh water mussel or calm.
3. Body is dark brown, unsegmented, bilaterally symmetrical and flattened from side to side measuring 5-10 cm in length.
4. Animal is completely enclosed in equal bivalve shells.
5. Mantle consists of two lobes, corresponding to two valves of the shell.
6. Ctenidia are W-shaped and eulamellibranch.
7. Foot is large muscular and Wedge-shaped and is used for burrowing.
8. Sexes are separate but the male and female shells are alike.

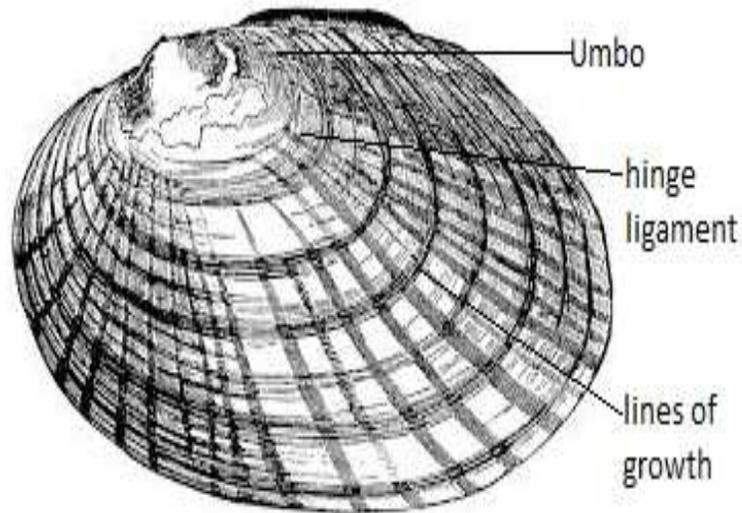


Fig .2.8.7Unio

---

### 2.8.8. *LOLIGO*

---

**Classification:**

Phylum.....Mollusca

Class.....Cephalopoda

Order.....Decapoda

Type.....*Loligo*

**Comments:**

1-It is found in worm seas and in coastal shallow or deep water.

2- Commonly called as squid.

3- Body is fleshy, dorsoventrally flattened and differentiated into 3 regions.

4- Head containing 10 oral arms and a pair of eyes with olfactory crest middle trunk and posterior region with lateral fins or parapodia.

5- Each one of 8 oral arms contain four rows of pedicellate sucker ventrally.

6.In males one such arm is also modified as copulatory organ.

7-Oral arms are modified of the foot.

8.Shell, internal horny and non-chambered and is used in maintenances of natural buoyancy.

9-Ventral siphon is formed by the modification of foot.

10-Sexes are separate.

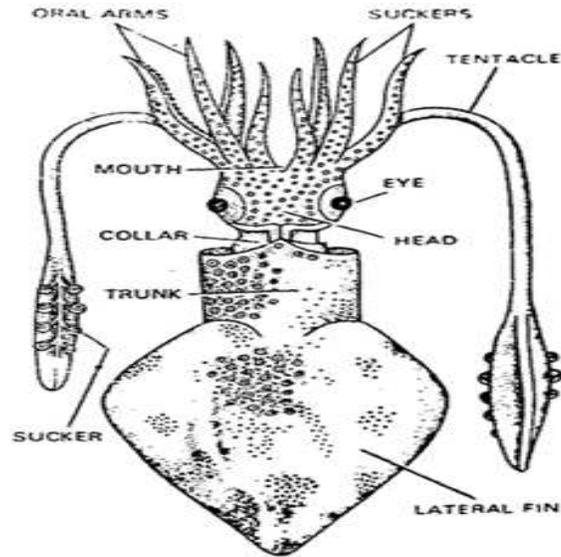


Fig. 2.8.8 *Loligo*

---

### 2.8.9. OCTOPUS

---

**Classification:**

Phylum.....Mollusca

Class.....Cephalopoda

Order.....Octopoda

Type..... *Octopus*

**Comments:**

1. It is a marine, nocturnal deep-sea form found at the bottom of the sea.
2. Commonly called as devil fish. Roundish or globose body is differentiated into a visceral hump and head.
3. Head contains eyes, siphon and 8 elongated arms, having 2 rows of sessile cupped, suctorial pockets or suckers on inner side.
4. One of the arms in male is modified as spoon-shaped intromittent organ or hectocotyli zed arm. The arms are elongated webbed and similar. Shell and nidamental glands absent.
5. Visceral mass and the mantle cavity are enclosed by mantle. Nervous system is well developed. Octopus is dibranchiate, having 2 gills, 2 auricles and 2 kidneys.

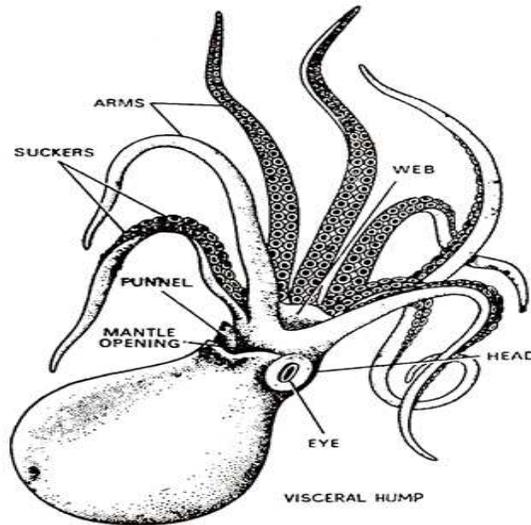


Fig .2.8.9 *Octopus*

---

## 2.9. PHYLUM ARTHROPODA

---



---

### 2.9.1. *SACCULINA*

---

**Classification:**

Phylum.....Arthropoda

Class.....Crustacea

Order.....Rhizocephala

Type..... *Sacculina*

**Comments:**

1. Commonly called as root-headed barnacle.
2. Parasitizes crabs. Zoologists were able to discover its real crustacean nature by studying its embryology having first stage Nauplius larva.
3. Adult loses all arthropodan characters and appears like a fleshy tumor attached the abdomen of the crab on ventral side and leads a parasitic life on decapods crustaceans.
4. *Sacculina* through peduncle sends root like processes like mycelium of fungus in each appendage of the crab and in the body to derive nutrition. Appendages, segmentation, mouth, anus, alimentation absent. Hermaphroditic

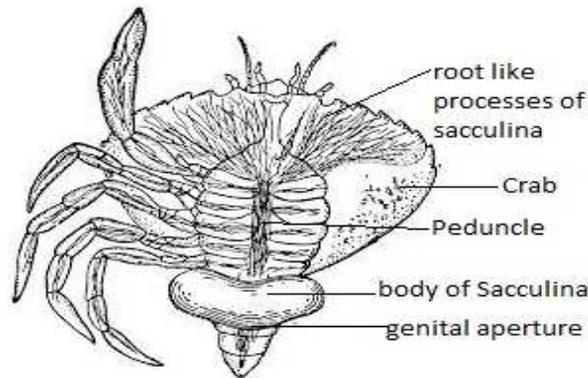


Fig .2.9.1 *Sacculina*

---

## 2.9.2. *SQUILLA*

---

### Classification:

Phylum.....Arthropoda

Class.....Crustacea

Order.....Stomatopoda

Type.....Squilla

### Comments:

1. It is large marine crustacean found in borrow in the sand or mud at the bottom of the sea.
2. Body is whitish, semi-transparent and is differentiated in cephalothorax, and abdomen measuring 25cm in length.
3. Head appendages are bilobed eyes, antennules and antennae.
4. First five pairs of uniramous thoracic appendages serve as maxillipeds, the second being the largest, subchelate and raptorial. The dactylus is armed with teeth on the inner margin. There are no oostegites. Last three thoracic segments bear walking legs.
5. Abdomen composed of 6 segments, is broader and elongated than cephalothorax. The first five abdominal segments with longitudinal ridges.
6. Heart is greatly elongated extending through the thoracic and abdominal regions. Larvae are pelagic and in their general form resemble the zoaea larva of crab.

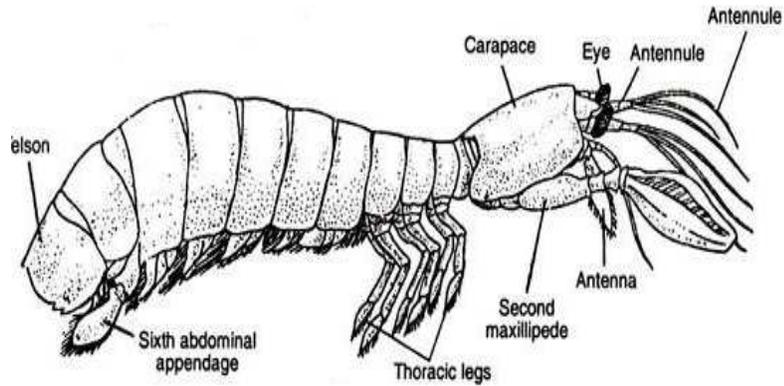


Fig. 2.9.2 Squilla

---

### 2.9.3. PALAEMON MALCOLMSONII

---

**Classification:**

Phylum.....Arthropoda

Class.....Crustacea

Order.....Decapoda

Type..... *Palaemon malcolmsonii*

**Comments:**

1. It is found in fresh water streams, rivers, ponds and lakes.
2. The animal is nocturnal hiding at the bottom during the day and coming to the surface at night in search of food.
3. Commonly called as prawn. Body is elongating, spindle-shaped, bilaterally symmetrical and deep orange coloured when preserved and measuring about 25cm in length.
4. Body is divided into three parts: cephalothorax, abdomen and telson. Cephalothorax is made up of 5 head and 8 thoracic segments. Carapace is anteriorly produced into saw-toothed rostrum. There are two prominent eyes on the head.
5. Abdomen is made up 6 segments. There are 19 pairs of appendages, one pair of each segment.

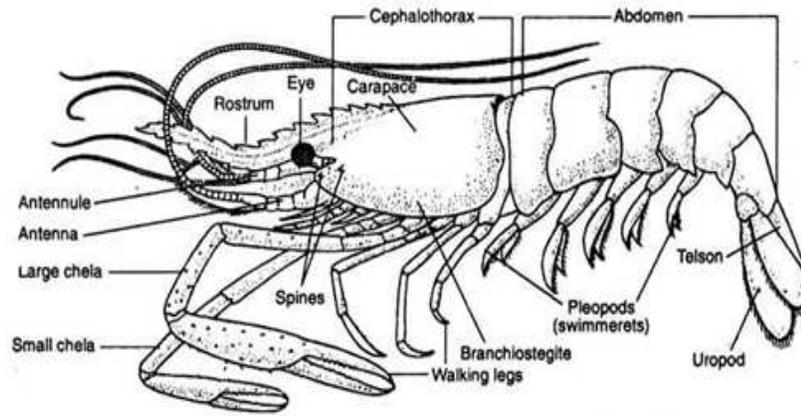


Fig .2.9.3 *Palaemon malcolmsonii*

---

## 2.9.4. *ASTACUS FLUVITILIS*

---

### Classification:

Phylum.....Arthropoda

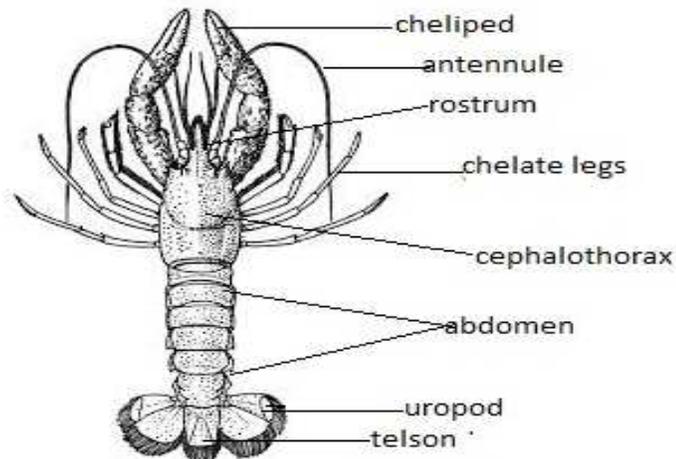
Class.....Crustacea

Order.....Decapoda

Type.....*Astacus fluviatilis*

### Comments:

1. It is commonly found in streams, rivers and lakes.
2. It is omnivorous feeding on any alive or dead matter.
3. Commonly called as cray-fish
4. Body is essentially sub-cylindrical in shape, small, about 9 cm in length and divided into anterior cephalothorax, middle flexible abdomen and posterior telson.
5. Cephalothorax comprise of head and thorax and is covered by carapace, which is produced into short and underrated rostrum and on sides covers the gills.
6. Eyes are stalked, antennules short and antennae elongated.
7. Appendages are 19 pairs-5 cephalic, 8 thoracic and 6 abdominal.
8. Walking legs are chelated. One pairs of legs are called as chelipeds.10-Telson forms a tail-fan together with uropods.



*Fig.2.9.4Astacus fluviatilis*

---

### **2.9.5. CARCINUS**

---

**Classification:**

Phylum.....Arthropoda

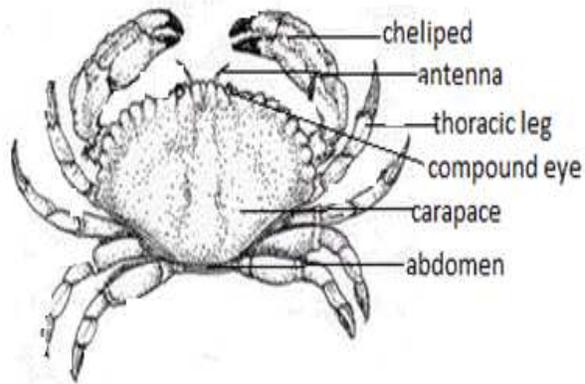
Class.....Crustacea

Order.....Decapoda

Type.....*Carcinus*

**Comments:**

1. It is found in buried among rock or mud in shallow water.
2. The crabs are highly specialized crustacean.
3. Commonly called as Rock crab or True crab.
4. Five pairs of thoracic legs are well developed.
5. Small antennules, antennae, and eye spots are contained in the sockets of the carapace.
6. Breeding season is spring.
7. Third maxillipeds are broad flat, valve like covering the other mouth parts on ventral surface. 8.Young hatches in the zoea stage and passes through a megalopa stage before reaching maturity. 9. Pleopods are greatly reduced.



*Fig .2.9.5Carcinus*

---

## **2.9.6. SCOLOPENDRA**

---

### **Classification:**

Phylum.....Arthropoda

Class.....Chilopoda

Order.....Scolopendromorpha

Type.....*Scolopendra*

### **Comments:**

1. It is tropical animal found in swampy places under bark, stones, decaying, wood.
2. In less than 100 percent humidity, it losses water through spiracles and dies.
3. Commonly called as centipede.Body segment have 21 pairs of walking legs.Scolopendra is harmful to mankind.
4. Body is elongated, dark greenish-brown in color, dorsoventrally flattened and is divided into a distinct head and a long, segmented trunk or body.Paired, oval spiracles or stigmata lies on the pleural area, above the leg bases on segments 4,6,9,11,13,15,17,19and 21.
5. There is a single pair of tracheal tufts opening to the exterior on the head. Mouth is guarded by labrum, mandibles and first maxillae.

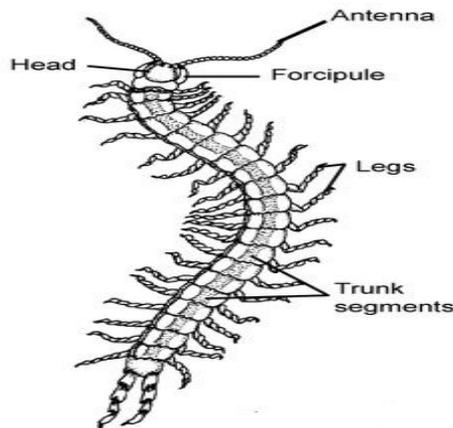


Fig . 2.9.6 Scolopendra

---

## 2.9.7. JULUS

---

### **Classification:**

Phylum.....Arthropoda

Class.....Diplopoda

Type.....*Julus*

### **Comments:**

1. *Julus* is also tropical, found in dark and damp places in meadows and gardens under stones and bark of dead trees. The animal is rolled up under stones. It is herbivorous. It is also borrowing into the soil.
2. Commonly called as wire worm. Body of the animal is differentiated into head, thorax and abdomen. There is no tracheal system. Animal moves very slowly in spite of its so many legs. the colour may be yellowish brown or reddish-chestnut.
3. Thorax ha four segments and each having one tergum, two small sterna, two pairs of walking legs. Sides of most terga have dark openings of odoriferous glands, secreting noxious substance.

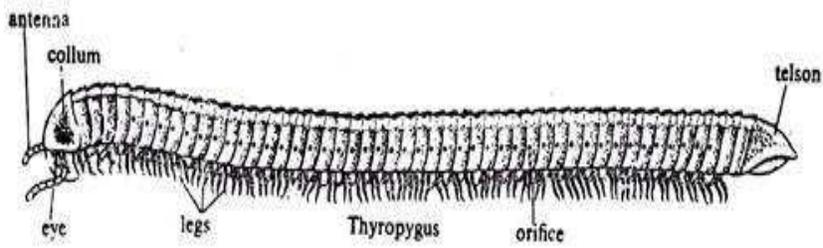


Fig. 2.9.7 *Julus*

---

## 2.9.8. PERIPATUS

---

### Classification:

Phylum.....Arthropoda  
 Class.....Onychopodra  
 Type.....*Peripatus*

### Comments:

1. It is a nocturnal carnivorous animal.
2. It is referred as living fossil.
3. Body of the animal is cylindrical and elongated caterpillar like and measuring 4-6 cm in length.
4. *Peripatus* is supposed to be the connecting link between Annelid and Arthropoda.
5. There is a head of three segments which is not clearly separate from the body.
6. Anterior end is marked by preantennae and ventral mouth and posterior end make anus.
7. Sexes are separate. 8. Each leg is unjointed but is ringed by ridges having tubercles and terminates in a foot.

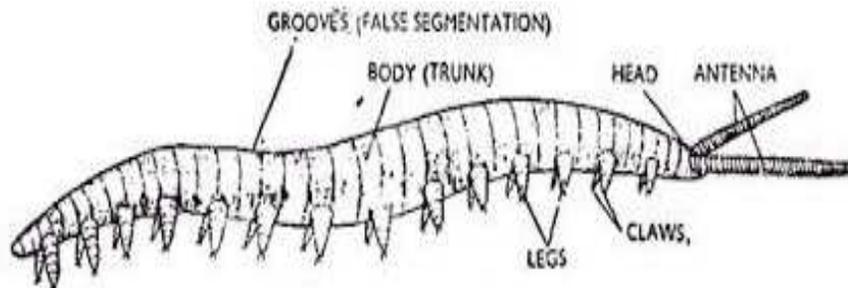


Fig. 2.9.8 *Peripatus*

---

## 2.9.9. PALAMNAEUS: SCORPON

---

### Classification:

Phylum.....Arthropoda

Class.....Arachnida

Order.....Scorpionida

Type.....*Palamnaeus (Scorpion)*

### Comments:

1. It is a nocturnal, found in sand, crevices and under stones and in bark of dead tree.
2. Commonly called as scorpion.
3. Body is elongated, segmented and differentiated into anterior and opisthosoma.
4. Opisthosoma is sub-divided into a broad anterior mesosoma and a narrow posterior metasoma, a pair of large chelate pedipalps, 4 pair of Walking legs and several ocelli.
5. Body is covering with a chitinous covering.
6. Mesosoma is composed of 7 broad segments and metasoma of 5 narrow segments.
7. It has cosmopolitain distribution and specially found in India, Europe and U.S.A
8. It feeds on insect and spiders which they often kill with the sting.

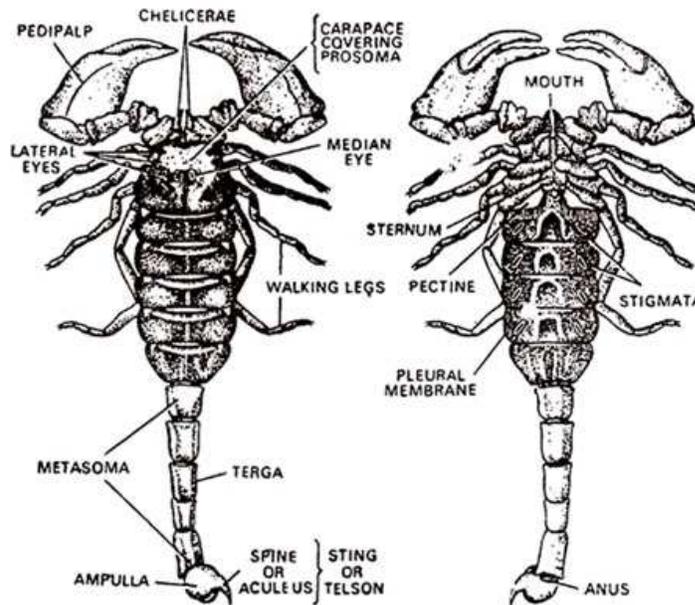


Fig. 2.9.9 *Palamnaeus (Scorpion)*

---

## 2.9.10. CARAUSIUS: STICK INSECT

---

**Classification:**

Phylum..... Arthropoda

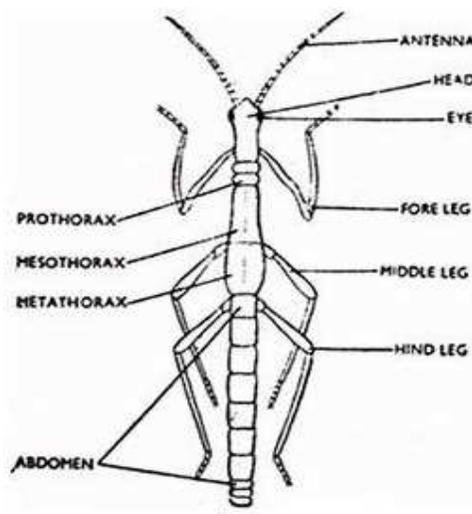
Class.....Insecta

Order.....Orthoptera

Type.....*Carausius*

**Comments:**

1. It is found tropical forests and thick vegetation. All the species vegetable feeders.
2. Commonly called as stick insects.
3. It is called as walking stick, because its slender body resembles a twig or stick and is a greatly modified orthopteran attaining 22.5-32.5cm in length.
4. This has great power of mimicry and shows changes in color like its environment and jumps swiftly. Body is differentiated into head, thorax and abdomen. Head is small containing antennae and a pair of compound eyes.
5. Walking legs are three pairs, simple and adapted for walking. The tarsi are usually 5 jointed. Sexes are separate. The male is small, active and winged and the female is large, sluggish and apterous. Ovipositor rudimentary.



*Fig. 2.9.10 Carausius*

---

## 2.9.11. MANTIS RELIGIOSA

---

### Classification

Phylum.....Arthropoda

Class.....Insecta

Order.....Orthoptera

Type.....*Mantis religiosa*

### Comments:

1. It is commonly found in green vegetation and other garden places. It is canibolic, pungnacious and predacious.
2. Commonly called as praying mantis.
3. Body of the animal is differentiated into head, thorax and abdomen and it is not dorsoventrally flattened.
4. Eyes are large and set on extremely mobile triangular head. There are three ocelli mouth parts of biting type.
5. Head contains compound eyes, a pair of antennae and biting mouth parts.
6. Pro-thorax is elongated and fused with the head;
7. Eggs are laid in ootheca formed by viscid secretion, which becomes hard and eggs are arranged in chamber.
8. Middle and hind legs elongated.
9. It is pincer-like or raptorial tibia of sub-chelate forelegs are modified for grasping prey.
10. 10-Wings are folded flat and overlapping the sides of the body.

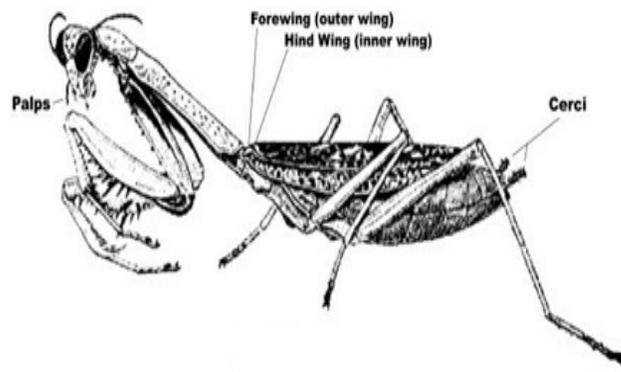


Fig . 2.9.11 *Mantis religiosa*

---

## 2.10. PHYLUM - ECHINODERMATA

---

---

### 2.10.1. ANTEDON

---

**Classification:**

Phylum.....Echinodermata

Sub-phylum.....Pelmatzoa

Class.....Crinoidea

Order.....Articulata

Type.....*Antedon*

**Comments:**

1. Antedon is marine and occurs about 2 fathoms deep and remains attached to rocks by cirri. Commonly called sea-lily or feather-star. On the aboral side calyx bears a knob-like structure called as strong of stalk. The calyx is differentiated into an upper convex oral surface, having mouth and anus and the lower flat aboral surface, into which arms and cirri are inserted. On the aboral side calyx bears a knob-like structure, called as strong of the stalk. The oral surface is covered by a leathery skin which leads into 5 food open grooves or ambulacral grooves, which divide into 10 as they reach near the edge and lead into arms. The mouth is surrounded by sensory tube feet or podia which are without suckers.
2. There are 10 arms having extensions of viscera and each bear numerous pinnules containing gonads. Sea-lily attaches to substratum by cirri. Anus on aboral surface.

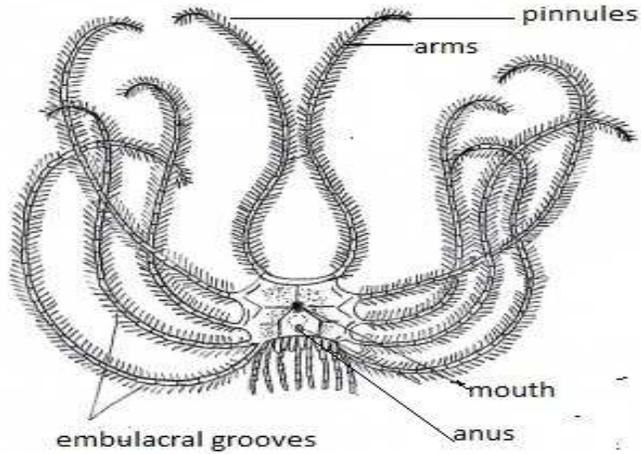


Fig.2.10.1 Antedon

---

### 2.10.2. PENTACEROS: SEA PENTAGON

---

**Classification:**

Phylum..... Echinodermata  
 Sub-phylum.....Eleutherozoa  
 Class.....Asreroidea  
 Order.....Phanerozonia  
 Type.....*Pentaceros (starfish)*

**Comments:**

- 1-It is most common eleutherozoan echinoderm found in sea from shallow water to 1,000 fathoms.
- 2- Commonly called as sea pentagon or starfish.
- 3-Body is enclosed in a tough, hard and leathery integument containing several ossicles.
- 4-Central disk and the arms fused together.
- 5-Arms contain extension of gonad, coelom and the gut.
- 6-Arms are 5 in number and symmetrically arranged in the form of star around the central disk.
- 7-Ambulacral groove contain double rows of locomotory organs of tube feet.
- 8-Sexes are separate. Development indirect which include bipinnaria larva.

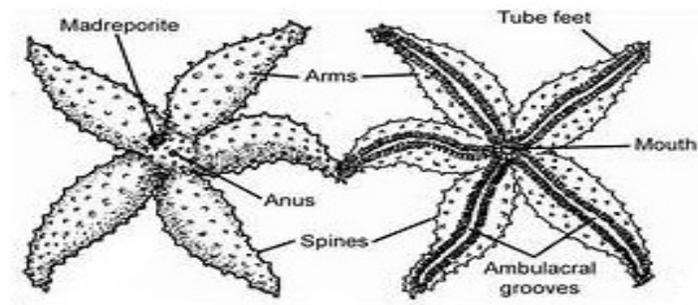


Fig.2.10.2 *Pentaceros* (starfish)

---

## **2.5 TERMINAL QUESTION & ANSWERS**

---

1. Describe the structure of sycon.
2. What is the common name of scypha?
3. Which type of canal system is found in *Leucosolenia*?
4. What is the zoological name of Portuguese man of war?
5. What is the common name of the *Gorgonia*?
6. Name the primary host of blood fluke.
7. What is the food of *Fasciola hepatica*?
8. Name the primary and secondary hosts of *fasciola hepatica*.
9. What is the name of the organ of attachment in tapeworm?
10. Name of two important nematode parasites of human beings.
11. How many appendages are present in cephalothorax of prawan?
12. What is the common name of *Pila*?
13. Why *Peripatus* is said to be a connecting link?
14. What is the common name of *Asterias*?

---

## ***2.6REFERENCES***

---

1. Agarwal V.K., (2016) Zoology for Degree student
2. Verma P.S., (2014) A manual of practical Zoology, Invertebrates.
3. Kotpal R.L.,(2005)Text book of zoology, Invertebrates.
4. Lal S.S., ( 2003 ) A Text Book of practical Zoology Invertebrates.

# **UNIT 3 PERMANENT SLIDE PREPARATIONS OF THE MATERIAL AVAILABLE/ PROVIDED**

## ***CONTENTS***

- 3.1 Objectives
- 3.2 Introduction
- 3.3 Method of Microscopic Preparations
- 3.4 Methods for slide preparation
  - 3.4.1 Protozoa (Paramecium)
  - 3.4.2- Coelenterate (Obelia Colony & Obelia Medusa)
- 3.5 Summary
- 3.6 Terminal Questions and Answers
- 3.7 References

3.3

---

### **3.1- OBJECTIVES**

---

The study of Permanent slide preparation of Paramecium and Obelia colony:

---

---

### **3.2-INTRODUCTION**

---

A microscope slide is a thin flat piece of glass, typically 75 by 26 mm (3 by 1 inch) and about 1 mm thick, used to hold objects for examination under a microscope. Typically, the object is placed or secured ("mounted") on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored in appropriate slide cases or folders. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips, slide clamps or a cross-table which is used to achieve precise, remote movement of the slide upon the microscope's stage.

The following points highlight the seven main processes involved in preparation of permanent slides. The processes are: 1. Killing 2. Fixing and Hardening 3. Staining 4. Dehydration 5. Clearing 6. Mounting 7. Labelling.

---

### **3.3- METHOD OF MICROSCOPIC PREPARATIONS**

---

For microscopic studies the specific material (tissue organs or small organism) is mounted on a glass slide. There are two methods of mounting the material on slide.

Temporary

Permanent

#### **Temporary mounting:**

The temporary mount is prepared either in glycerin, water or normal saline. The material is first washed in tap, then stained and differentiated. Drop of mounting medium (glycerine and water) is placed on center of the slide. The material is then transferred into that drop. It

is then covered neatly with a cover slip. The excess of glycerine or water is absorbed by piece of blotting paper. Mount prepared by this method can be used for study only for few hours, after which material loses its original form due to diffusion and other post mortem change

### **Permanent mounting:**

But for the study of microorganisms, smaller animals and histological studies of tissues, an elaborate technique is employed for making their permanent preparations. These smaller objects are mounted in balsam on a slide. There is a series of processes by which a living organism or its tissue is made fit for microscopic examination in a permanent state. The utility of permanent preparation is that the animal cell or tissue remains as such without undergoing major changes. The permanent preparation includes:

Killing and narcotization

Fixing

Washing

Staining

De-staining or removal of excess of stain.

Clearing or de-alcoholization.

Mounting on slide

### **Killing and Narcotization: -**

The first step in permanent preparation is killing instantaneously in order to prevent the change in form of the object as it has in living condition and immediately fixing the object. Sometimes killing is preceded by narcotization. The narcotics used are chloroform, menthol, ether, alcohol, acetone, etc. the purpose of narcotization and killing is important as to have the same form and chemically constructed tissue or organisms as it had during its lifetime. In certain cases, for smaller animals killing is heating done by the slide.

**Fixing:**

Fixing is done with various fixative agents for histological elements. Fixative is essential in every type of microscopic preparations, either for sections or for whole mounts and also in larger specimens. The function of fixation is manifold:

The tissues become hard and hardening resists further post-mortem changes.

Fixative agent coagulates and renders insoluble elements of tissues which are dissolved in further processing.

The fixative agent renders insoluble the various constituent elements of cells, alters their refractive indices and thus makes them optically differentiated under the microscope. Because of Brownian motion there is no possibility of material but we must bear in mind that fixed details are the coagulation artifact of the living structures.

Various fixative agents generally used are absolute alcohol, 90% alcohol plus glycerine, picric acid, corrosive sublimate, formal, osmium tetra oxide and nitric acid with or without water. Depending upon the material, corrosive sublimate or alcohol Carnoy's fluid for cytological studies and other fixative for histochemical studies.

**Washing:**

Washing is essential as by this process the uncombined and excess of fixative agent is removed. The presence of fixative agent in tissues or cells will inhibit good staining. The washing agent depends upon the type of fixative agent used. As alcoholic picric acid in water is removed by 70% alcohol. Formal and corrosive sublimate are washed with water distillate. Sublimate is washed in alcohol.

**Staining:**

The tissue or cell components are stained in various dyes. The dye makes the tissues distinct in its histological sphere. The various dyes are Orange G, Bordeaux red, Sudan's Congo red, methylene blue, neutral red, borax carmine, hematoxylin, e picro- indigo carmine, eosin and Gower's carmine. Mainly two

kinds of stains are used. Nuclear stains. Stains the nuclear parts of the cells, such as Delafield's or Ehrlich's haematoxylin. Cytoplasmic stains such as borax carmine, picro-indigo carmine, Gower's carmine and eosin, etc., which stain cytoplasm. For general staining borax carmine is used aqueous stains are prepared in water whereas alcoholic stains are prepared in alcohol. When a single stain is used the process is called as simple or single staining. In some cases, two stains, i.e., nuclear and cytoplasmic are used and this is called as double staining. Generally single stain is used for whole mounts but for protozoan's etc., both cytoplasmic and nuclear stains are used.

For general staining borax carmine is used aqueous stains are prepared in water whereas alcoholic stains are prepared in alcohol. When a single stain is used the process is called as simple or single staining. In some cases, two stains, i.e., nuclear and cytoplasmic are used and this is called as double staining. Generally single stain is used for whole mounts but for protozoan's etc., both cytoplasmic and nuclear stains are used.

**Destaining: -**

The removal of excess of stain is called as destaining or differentiation. De-staining agents are acid alcohol or acid water. The acid alcohol is used with alcoholic stains while acid water is used with aqueous stains.

**Dehydration: -**

This process is meant for removal of water from the tissues. The dehydration prevents putrefaction or decaying and maintains the same shape and size of tissues or cells. The moisture or water in tissues absorbs various germs of destructive nature so that the tissue may be destroyed, hence the passing the mounting material through various grades of alcohol, such as 30, 50, 70, 90 and 100% alcohols. The tissue is soaked in gradually increasing strengths of alcohol. The lower grades prepared either from 90% or absolute alcohol. The dehydration is carried out in corked or glass-stopped tubes.

## SUMMARY OF PERMANENT MOUNTING

### Single Staining

- ↓ Fix the material in fixative
- ↓ Wash in water to remove fixative
- ↓ Dehydrate in 30% alcohol (5 minutes)
- ↓ Dehydrate in 50% alcohol (5 minutes)
- ↓ Dehydrate in 70% alcohol (5 minutes)
- ↓ Stain in Borax carmine or Picro-indigo
- ↓ Carmine or Eosin (3-5 minutes)
- ↓ Destain in acid-alcohol (Few seconds)
- ↓ Dehydrate in 90% alcohol (5 minutes)
- ↓ Dehydrate in 100% alcohol (5-10 minutes)
- ↓ Xylol or Benzene or Clove oil (5 minutes)
- ↓ Mount in Canada balsam or D.P.X.

4

### Double staining

- ↓ Fix the material in fixative
- ↓ Wash in water to remove fixative
- ↓ Stain in hematoxylin (3-5 minutes)
- ↓ Wash in water
- ↓ Destain in acid water
- ↓ Wash in water
- ↓ Dehydrate in 30% alcohol (5 minutes)
- ↓ Dehydrate in 50% alcohol (5 minutes)
- ↓ Dehydrate in 70% alcohol (5 minutes)
- ↓ Stain in eosin (1-3 minutes)
- ↓ Dehydrate in 90% alcohol (5 minutes)
- ↓ Dehydrate in 100% alcohol (5-10 minutes)
- ↓ Xylol or Benzene or clove oil (5 minutes)
- ↓ Mount in Canada balsam or D.P.X.

5

6

(iv) Excess of mounting medium should not come out of the covers

### **De-alcoholization or clearing: -**

After dehydration, transparency in tissues is obtained by treating with a clearing agent, which removes alcohol and makes the tissue clear and transparent. The clearing agents are wood oil, clove oil, xylol and benzol, etc. Xylol is most commonly employed and it makes the tissues hard and brittle. Clove oil is a superior clearing agent especially in the whole mounts. It also possesses higher index of refraction than balsam mounting media.

### **Mounting:**

Mounting forms, the end of permanent preparation the choice of mounting media is not much but they should have the same refractive index as that of the cleared tissue. The refractive index of such a stained, dehydrated and cleared cells is 1.54. Canada balsam or D.P.X has almost the same refractive index. Mounting is an easy process. The tissue is kept over glass slide in a drop of balsam and cover-slip is lowered slightly. After mounting, the excess of balsam on the slide, as generally happens with beginners, should be removed with cotton soaked with the balsam has dried. For much better finishing the edge of the cover-glass may be ringed with cement such as gold seal or a varnish. The air bubbles present in balsam under cover-glass should be removed by gentle heating. During all the chemical bathing of tissues, two changes of each reagent are necessary. The time of keeping tissue in various reagents may vary from 5 to 15 minute.

### **ALCOHOLIC GRADES**

They are generally prepared from rectified spirit which contains 95% alcohol.

To prepare the required percentage, it is necessary that required percentage

should be minus from rectified spirit. For example, if 30% alcohol is to be prepared then  $95 - 30 = 65$  ml water is mixed with 30 ml of rectified spirit.

#### **30% Alcohol**

- |                     |       |
|---------------------|-------|
| 1. Rectified spirit | 30 ml |
| 2. Distilled water  | 65 ml |

#### **50% alcohol**

1. Rectified sprit	50 ml
2. Distilled water	45 ml
<b>70% alcohol</b>	
1. Rectified sprit	70 ml
2. Distilled water	25 ml
<b>90% alcohol</b>	
1. Rectified sprit	90 ml
2. Distilled water	5 ml

### **Precautions and Instructions:**

The articles, such as slide, cover slips and instruments should be perfectly cleaned.

The working place should be kept in order.

During dehydration, the tissues should be kept in tightly closed cork or glass stopper tubes. The opened tube will spoil material by absorbing moisture from atmosphere. Even breathing closely with dehydrating tube is undesirable. Change of solution is done very quickly, reducing time of exposure to atmosphere to minimum.

The chemicals used once should not be reutilized.

The Canada balsam used should be clean, dust-free and not viscous.

6.3  
**METHODS FOR SLIDE PREPARATION**  
6.4

---

**3.4.1- Protozoa (Paramecium)**

6.5

**Classification:**

Phylum.....Protozoa	Unicellular
Sub-Phylum .....Ciliophora	Ciliary movement in all stages.
Class ...Ciliata	Cilia present throughout life.
Sub Class.....Euciliata	Cytopharynx, contractile vacuole, meganucleus and micronucleus present
Order.....Holotricha	Equal cilia.
Sub-order ..... Trichostomata	Mouth leads in cytopharynx.
Family.....Paramecidae	Oral groove present
Genus..... Paramecium	

---

**CULTURE PREPARATION OF PARAMECIUM**  
6.6

---

It is found abundantly in the ponds and ditches in decaying vegetation. For culturing paramecia boil 20 grains of wheat plus 20-25 hay stems in 500 cc of distilled water for about 10 minutes. Keep it in dark and cool place for about four days and inoculate it with few paramecia by a micropipette, within little days. The culture will be found to contain numerous paramecia.

**Examination in living condition:**  
6.7

---

Take a clean slide. Through the micropipette put a drop of water from the culture medium of Paramecium. Examine the slide under low magnification of compound microscope. Observe the fast-moving Paramecia and their cytopharynx. Many protozoans move very fast. So, they must be slowed down for proper examination. This is done in three ways: 2. % sodium carboxymethyl cellulose solution is also good for slowing down protozoan movement. Boil 2gm of sodium methyl cellulose. Cool. Nickel sulphate acts as an anesthetic. By keeping the animal for 15 min can restrict their movement.

---

## ***PERMANENT PREPARATION:***

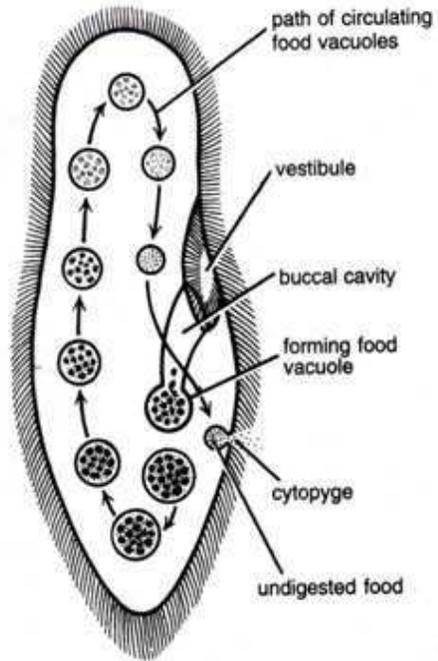
---

For the free living and fast-moving protozoans, they are first made non motile on a glass slide coated with albumin. Then the small drop of culture containing Paramecium is fixed with an equal drop of 1% of Agar solution melted (1 gm of Agar in 100 cc of water distillate) at 45<sup>0</sup>C. The solution becomes jelly like. The animal may survive for 30 min. They are fixed with 90% alcohol or by a drop of Schaudinn's fixative.

Pass the slide through descending grade of alcohol 90%, 70%, 50% and 30% and distilled water. Stain both nuclei and cytoplasm by double staining. Stain first with Ehrlich's haematoxylin. Destain in acid water and wash in tap water. Again, dehydrate in ascending grade of alcohol. After 90% alcohol stain in cytoplasm Eosin. Keep in 100% alcohol, Clear in xylol and mount on D.P.X.

**Feeding experiment:** As *Paramecium* is a ciliary and selective feeder. The cilia direct the food particles into the cytopharynx or gullet. Its food particles consist of bacterial etc. The food is collected into membranous vesicle which is formed just below the gullet. When the vesicle is filled with food it is detached and is called food vacuole. In paramecium food particle is circulated in the body by more or less definite path by slow streaming movement of endoplasm called cyclosis. Digestion and assimilation take place during the journey of food vesicle, first it is alkaline and then acidic and again alkaline.

**For observing cyclosis:** Take a drop of culture medium of Paramecia over a slide. Add a little yeast Congo red in a drop of water. The Congo red is taken into the food vacuole. Observe under low magnification along with the movement of Congo red in Food vacuole.

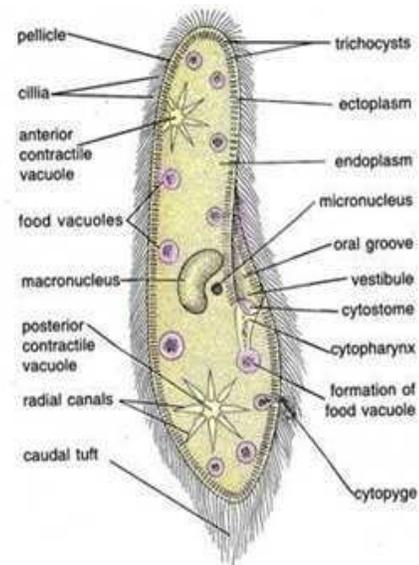


*Fig.11.1 Paramecium showing Cyclosis*

**Distribution:** It has cosmopolitan distribution.

**Habit and Habitat:**

*Paramecium caudatum* is commonly found in freshwater ponds, pools, ditches, streams, lakes, reservoirs and rivers. It is specially found in abundance in stagnant ponds rich in decaying matter, in organic infusions, and in the sewage water. *Paramecium caudatum* is a free-living organism and this species is worldwide in distribution.



PARAMECIUM

Fig.11.2

**Comments:**

Commonly called as slipper animalcule, being microscopic elongated slipper-shaped, cigar-shaped or spindle-shaped.

Most familiar and extensively studied protozoans.

Anterior end is bluntly rounded, while posterior end is pointed.

*P. caudatum* measures 80 to 350 microns, while *P. aurelia* 170 to 290 microns.

Pellicle covers the body. It is clear, firm and elastic cuticular membrane. Pellicle has series of polygonal or hexagonal depressions for trichocytes.

Cilia cover the entire animal. They are hair-like projections of uniform length, except at posterior end where they are longer and at cytopharynx where they form undulating membrane.

Infra ciliary system consists of basal bodies and kinetodesmata.

Cytoplasm contains ecto- and endoplasm. Ectoplasm has myonemes and rod-shaped trichocytes. Endoplasm contains food vacuoles, granules, meganucleus, micronucleus, anterior contractile, posterior contractile vacuole, fat and glycogen.

Trichocytes are rod-shaped bodies consisting of lower trichocyte shaft, basal body and

projecting cilium. Cilium project through the hexagonal areas. Trichocytes are discharged to anchor with substratum.

Reproduction is by binary fission, conjugation, endomixis, hemixis and automixis.

Locomotion is ciliary. Nutrition is holozoic and it shows response to light and temperature, etc.

**Identification:** Since the animal contains slipper-shaped body and 2 contractile vacuoles which are star-shaped and has all above features, hence it is Paramecium.

---

### ***3.4.2- COELENTERATE (OBELIA COLONY & OBELIA MEDUSA)***

---

#### ***OBELIA:***

Obelia is colonial, mainly sedentary hydrozoan zoophyte attached to the seaweed, hills and rocks. It is mostly found in shallow water and also up to approximately 250ft. Deep.

#### **Method for slide preparation:**

Coelenterates are first narcotized in water mixed with menthol crystal or Magnesium sulphate. After decanting the narcotizing liquid, fix the animal by adding drop by drop formal. (Commercial preparation). These are then preserved in 70 % alcohol or 5% formalin solution. For making permanent mount, keep the material in 70% alcohol, then stain in borax carmine, if overstain, distain with acid alcohol. Dehydrate in 70%, 90% and 100% alcohol. Clear in xylol or benzene and finally mount on Canada balsam. Then study under the microscope, draw the diagram, label them and note down the characteristic features.

#### ***OBELIA COLONY:***

##### **Classification:**

Phylum .....	Coelenterata-	Tissue grade, diploblastic and acoelomate.
Class.....	Hydrozoa	- Hydroids: medusa with velum.
Order.....	Hydroidea	- Polypoid generation well developed
Sub order.....	Calyptoblastea	- Hydranths have hydro theca and gonophores with
Genus .....	Obelia	

##### **Habit and habitat:**

**Obelia** is colonial, marine, sedentary hydrozoan zoophyte, attached to seaweeds, shells and rocks.

**Distribution:** -Its range is from the Arctic region to the Gulf of Mexico and the Pacific coast, and from Southern California to Oregon. it is found in shallow water and also up to approximately 250 feet deep.

**Comments: -**

1. It is a dimorphic colony in the form of small seaweed filaments, measuring several cm in height.
2. The filaments may be horizontal and vertical. The colony consists of several parts.
3. **Hydrorhiza:** It is basal or horizontal portion called as stolon or rhizostome, which is meant for attachment to substratum. Hydrorhiza gives vertical branches called hydrocaulus.
4. **Hydrocaulus** gives alternate branches that terminate into individual zooids called as polyps and medusa.
5. : Stems and zooids are made of a living hollow, cellular tube called as coenosarcs. It is made up of ectoderm, endoderm and mesogloea.
6. Stems and zooids are made up of two components: (i) Outer protective tough, transparent non-cellular covering called as **perisarc** (ii) **mesogloea** (iii) inner living hollow cellular tube called **coenosarcs**. Zooids consist of polyp and medusa.
7. Medusa grows at the base of polyp-bearing branches and is enclosed in blastostyles. Medusa is composed of upper exumbrellar and lower sub-umbrellar surfaces, manubrium and gonads. Free medusa occurs in the life cycle. It is a reproductive zooid.
8. Polyp is a bell-shaped cup made up of lower cup-shaped hydrotheca and upper hypostome. Hypostome is a feeding zooid having circle of 24 nematocyst bearing tentacles.
9. Growth of the colony is sympodial, i.e., each new hydranth arises as bud from the stem, just proximal to the next youngest polyp.

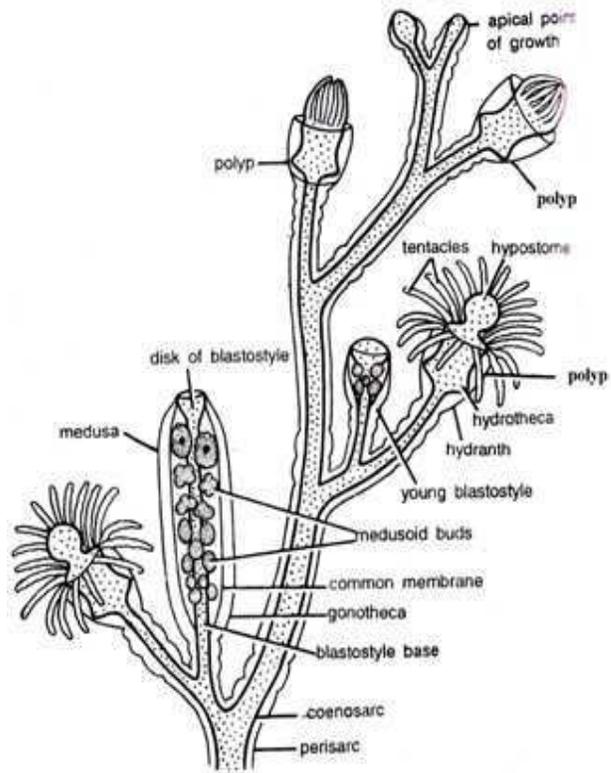


Fig. 11.5 Obelia colony

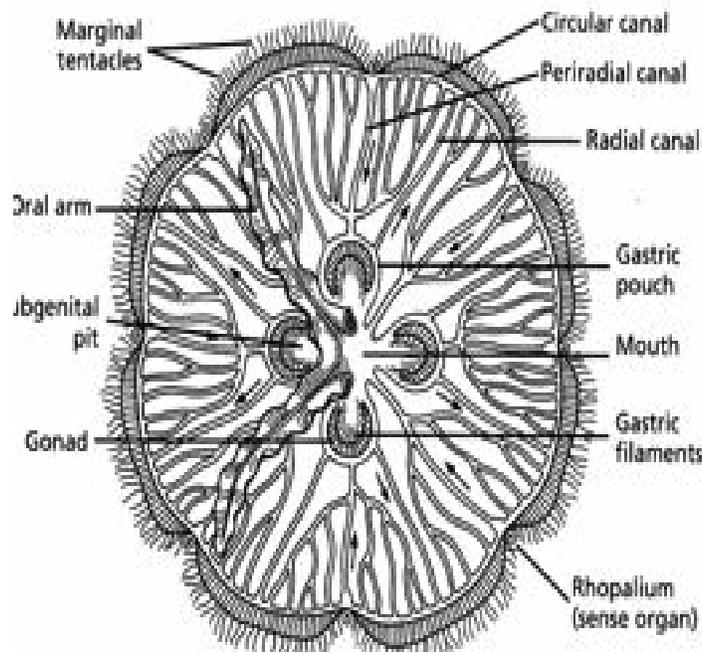
## Identification:

The colony has alternate branches of polyps, blastostyles and all above features, hence it is

## Obelia: Medusa:

### Comments:

1. Medusa is a modified zooid for sexual reproduction.
2. It is a solitary free-swimming zooid, originating from blastostyles.
3. Medusa is umbrella-like and has convex exumbrellar and concave sub-umbrellar surfaces with well-defined radial symmetry.
4. Umbrella edge contains radially symmetrical tentacles.
5. Base of fully grown tentacle is thickened to tentacular bulb which contains a number of stinging cells.
6. In the four radial positions each tentacular bulb contains two otocysts, which are hollow and balancing organs containing calcareous otoliths.
7. **Manubrium** hangs from the centre of sub-umbrella, having mouth.
8. Mouth communicates with 4 radial canals which join with circular canal lining umbrellar margin which all around contains velum.
9. Beneath the radial canals are gonads lying in **Sub-umbrellar** ectoderm.



*Fig.11.6 Medusa: Obelia*

---

### **3.5: SUMMARY**

In this unit you have learnt about: The care and cleaning of slides using different solvents. The importance of dust free storage and correct labelling of prepared slides. Temporary slide preparation involving simple techniques for fixation, staining and mounting of cell suspensions, soft tissue as well as hard plant tissue, Permanent slide preparation involving dehydration, clearing and mounting. The use of progressive and retrogressive staining.

---

### **3.6 TERMINAL QUESTIONS**

Complete the table below to compare temporary and permanent slide preparations.

A lab has a stock of 2150 slides. How would you suggest that these were stored?

What mountants do you use in your laboratory? List the reasons why they are used.

---

### **3.7 REFERENCES**

---

1. **Jordan, E.L and Verma P.S** ,Invertebrates Zoology S.Chand and Company. New Delhi.X Revised Edition.
2. **Kotpal, R.L** Modern Text Book of Zoology, Invertebrates Animal Diversity-I,Rastogi Publications.
3. **Verma, P.S.,** 1993, *A Manual of Practical Zoology Invertebrates*, S.Chand & Co. Ltd., New Delhi,India.
4. **Mercer, E.H.,** 1959, *An Electron Microscopic Study of Amoeba proteus*, Proc. R. Soc. Lodon B. 150: 216-232
5. **Barnes, R.D.** 1980, *invertebrate zoology*, W.B. Saunders Company, Philadelphia and London.
6. **Berril, N.J.,** 1957, *Indestructible Hydra*, Scientific American, December, 1957.

# **UNIT4: PARASITOLOGY: STUDY OF LIFE- CYCLE OF PARASITES**

## ***CONTENT***

- 4.1 Objectives
- 4.2 Introduction
- 4.3 Parasitology: Life cycle study of parasites
- 4.4 Summary
- 4.5 Terminal Questions and Answers

---

## **4.1. OBJECTIVE:**

---

Study of life-cycle of some parasites through charts, models or live materials.

---

## **4.2 INTRODUCTION:**

---

Parasitology is the study of the relationship between parasites and their host. It is a branch of biology which deals with the study of parasitism i.e., a phenomenon of dependence of one living organism on the other. In other words, Parasitology can also be defined as the study of parasites and their relationship to their host.

It is the branch of science which deals with the study the host-parasites relationships between domesticated and wild animals and their parasites. This relation including classification, habit and habitat, salient morphological characters of parasites to identify the species, life-history including developmental stages found outside or inside of the host.

---

### **4.3.1 LIFE CYCLE OF *ENTAMOEBIA HISTOLYTICA***

---

*Entamoeba histolytica* is an amoeba-like small microscopic endoparasite of human beings. Commonly found in the lumen of the lower portion of small intestine and the entire large intestine of human. It is monogenetic animal i.e; only one host is required for its complete life-cycle. The host being includes the following steps.

**Encystment:** The precystic minute forms exist in intestinal lumen and undergo encystment or encystation. However, before the encystment, they become round, eliminate food vacuoles and accumulate considerable amount of reserve food materials in the form of glycogen granules and chromatoids. Each parasite secretes a thin rounded, resistant, colourless and transparent cyst wall around it. The average size of the cysts has 12mm. Each cyst has a clear cytoplasm and single nucleus (mononucleate) cyst. The nucleus of the cyst divide twice so that each cyst becomes tetra-nucleate or Quadra nucleate. At this stage the cysts are infective to new hosts.

**Transfer to new host.** The tetra-nucleate cysts pass out with the faecal matter of the host (These are used for diagnosis of the infection). Cysts remains alive for 10 days in the moist stool. They die if dried or if the atmospheric temperature rises to about 50°C. Cysts are also resistant to chemicals. Infection of the fresh human host takes place by swallowing

the infective cysts with contaminated food and drinks. Contamination of food and drinks is brought about by houseflies, cockroaches and dirty fingers of the food handlers.

Through contaminated food, infective cysts pass into the lower portion of the small intestine of the host.

**Excystment:** Excystment of the parasite occurs in host's intestine according to any of two methods: (1). the cyst wall is digested by alkaline juice (trypsin enzyme) of the host's small intestine

(2). in culture, a small portion of cyst is dissolved by some enzymes secreted by the pseudopodium of the parasite. Anyhow, a tetra nucleate amoeba is hatched out is called metacystic form.

Metacystic entamoeba produces a new generation of trophozoite by a diverse series of nuclear and cytoplasmic divisions which result in the production of eight uninucleate daughter amoebulae which represent young trophozoities. Young trophozoites feed on the contents (bacteria) of the lumen of intestine and grow in size to from the normal parasitic trophozoite.

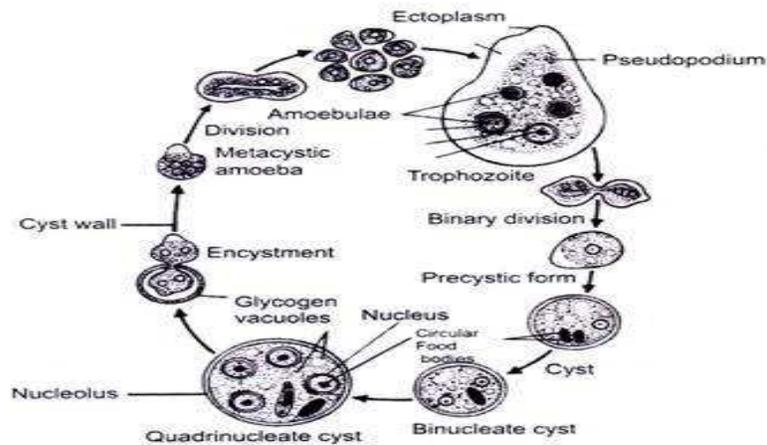


Fig .4.3.1 life cycle of *Entamoeba histolytica*

---

### 4.3.2 *LIFE-CYCLE PLASMODIUM VIVAX(MALARIA PARASITE)*

---

Plasmodium or malaria parasite is a pathogenic intracellular blood parasite of human and other vertebrates it lives in hepatocytes (live cells) and red blood corpuscles its life cycle is digenetic, i.e.; the life cycle of the parasite is completed on human and female anopheles' mosquito. It involves the following two cycles which alternate with each other:

*Asexual cycle in humans.* It is completed in the following steps:

**Pre-erythrocytic cycle** (primary exo-erythrocytic cycle or tissue phase). Parasite (malaria sporozoite) finds its way into the blood of healthy person along with the salivary secretion (containing anticoagulant) of infected female *anopheles* 'mosquito which bites for getting a blood meal. The parasite enters liver cells (hepatocytes) and asexually multiplies there by liver schizogony producing numerous haploid asexually blood forms called cryptomerozoites are released when a hepatocyte ruptures.

**Erythrocytic cycle** (erythrocytic schizogony and gametogony). Some cryptomerozoites are transported to red blood corpuscles for they become feeding trophozoites which metabolise hemoglobin. Change morphology from ring (signet- ring) to schizont (full-growth trophozoite), produce malaria pigment or hemozoin and multiply asexually by schizogony to produce merozoites. Merozoites are liberated in the blood by the rupture of RBCs, causing chills and fever. Merozoites enter fresh RBCs, causing chills and fever. Merozoites enter fresh RBCs to repeat schizogomy.

**B. Sexual cycle in female anopheles:** During erythrocytic cycle in human blood, some merozoites develop into gametocytes. The male gametocytes are called microgametocytes and female as macrogametocytes. When the mosquito (female Anopheles) bites an infected human, it sucks up gametocytes of plasmodium along with its blood meal. In the digestive tract of the mosquito the gametocytes develop into haploid male and female gametes. Fertilization occurs within stomach and the zygote (called ookinete) burrows into the stomach wall. Following zygotic meiosis, a series of mitosis occurs, resulting in the formation of many sporozoites, which migrate to the insect's salivary gland, from where they are introduced into the new host.



of onchospheres dissolves and releases hexacanth which enter mesenteric veins and make their way into various organs, especially lungs and liver.

The young larva changes into a hollow bladder containing two layers-outer cuticular ectocyst, inner germinal layer or endocyst. The host forms an enveloping fibrous cyst wall, called pericyst. It is now called a hydatid cyst.

Development of the cyst is slow and after months or even years the bladder wall produces hollow brood capsules which remain attached by slender stalks. A cyst often throws out blind outgrowth forming exogenous buds. Budding is repeated within the brood capsules giving rise to the daughter buds or hydatids (endogenous budding). Invagination in hydatids gives rise to small scolices with suckers and hooks. There may be as many as two million scolices in a single large hydatid. Brood capsules under pressure burst to liberate tiny hydatids in the cyst fluid. Hydatid upon reaching the final host is capable of giving rise to an adult worm. When dog feed raw flesh of infected sheep and goats around mutton shops.

Human beings receive infection of dog tapeworm from the pet dogs through the tongue of dogs while they are licking the hands of children or the eggs are caught under the fingernails while caressing the pets. Infected meat of sheep and goat is also the source of human infection

Hydatid cysts (bladder worm) have a colorless toxic fluid which may be up to many liters (one liter in a cyst of 15 cm diameter). Older cysts also have a granular deposit (called hydatid sand) consisting of brood capsules and free scolices. They become life-threatening when growing in such places as brain, liver or eye and release their toxic fluid into the host causing severe reaction (e.g., eosinophilia). Bladder worm cysts can be removed only by surgery.

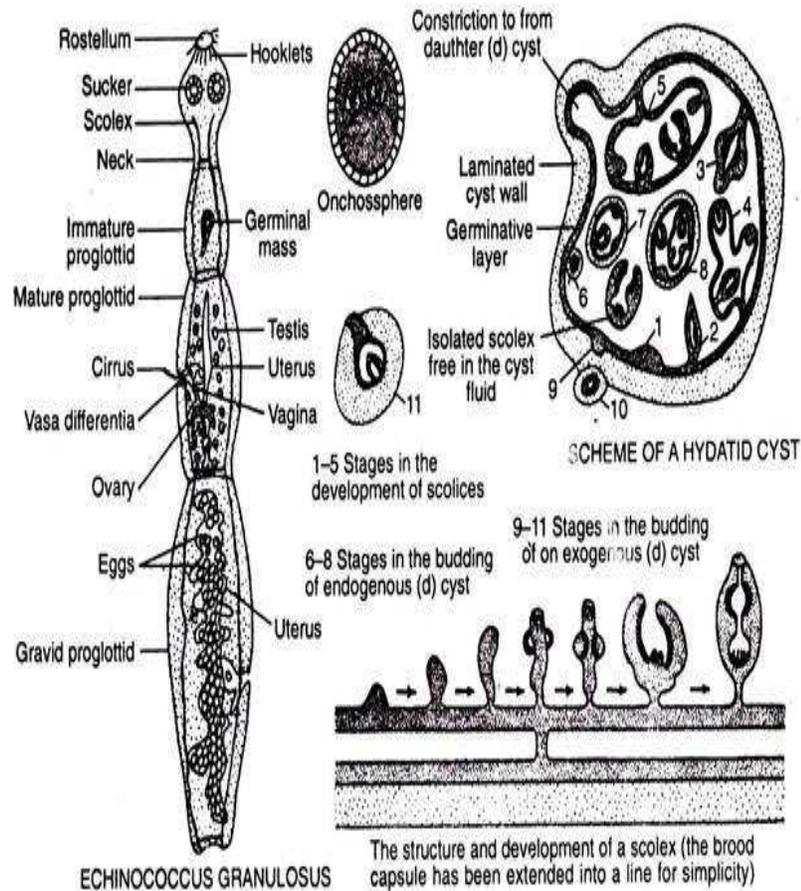


Fig.4.3.3 Life-cycle of *Echinococcus granulosus*

#### 4.3.4 LIFE CYCLE OF SCHISTOSOMA

The common name of *Schistosoma* is blood fluke. It is a pathogenic endoparasite of intestinal veins of human beings and other animals such as birds and mammals. Blood fluke causes the diseases called snail fever or oriental intestinal schistosomiasis. *Schistosoma* complete its life cycle into two hosts: its primary host is human beings and its intermediate host is snail.

Life history of *Schistosoma* was worked out first by Theodor Bilharz in 1851 and later by wall in 1941. When laying the eggs, the female protrudes from the groove of the male worm and deposits the eggs in small intestine venules of host. Eventually they break through by spines and enzymes into the lumen of the intestine and are released in the faeces of the host. If defecation occurs in the water, the thin-walled egg capsules hatch, and the miracidia escape.

The miracidia larvae are ciliated and well supplied with sensory receptors; they seek out a particular species of freshwater snails. After penetration in the intermediate host, they transform into sporocysts, which eventually gives rise to a second generation of sporocysts.

The sporocysts produce cercariae without an intermediate radiata stage. Cercariae contain forked tails and are called furcocercariae. The cercariae leave the molluscan host, swim freely in water and on contact with human skin (when walking barefooted in water), penetrate, using enzymes of penetration glands and muscular boring movements. Cercariae develop into tailless young flukes, called schistosomules which are carried by the blood streams first to lungs, then to the liver, and finally to the veins of intestine or bladder. During this period the schistosomules gradually transform into adults.

Infection of schistosoma causes headache, pain in back and legs, dysentery, daily fever, liver disorders, anaemia, eosinophilia damage to urinogenital tract (haematuria which is a disorder of kidney or urinary track with discharge of blood). The disease can be prevented by sanitary control of water. It can be treated by tartar emetic or sodium antimony tartrate.

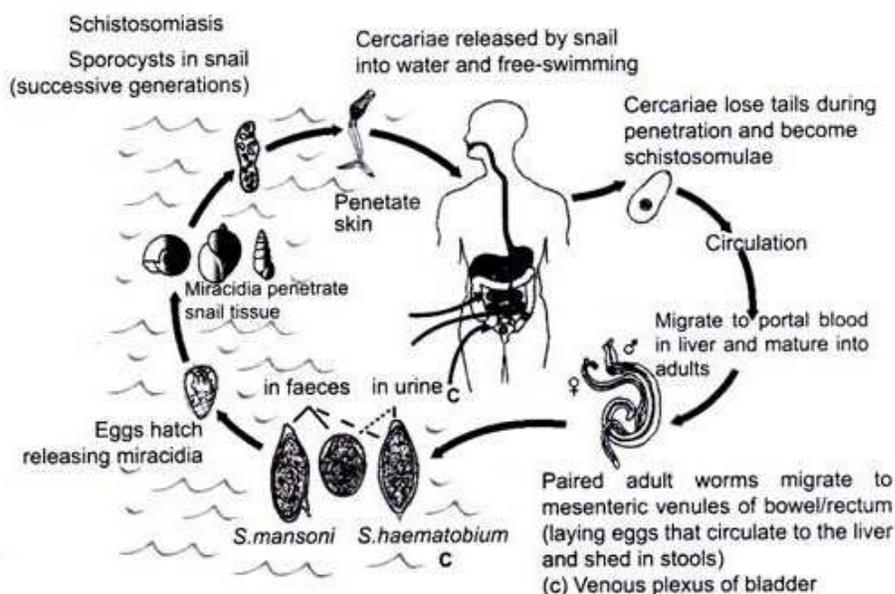


Fig. 4.3.4 Life cycle of Schistosom

### 4.3.5 LIFE CYCLE OF TAENIA SOLIUM

*Taenia solium* is commonly called as pork tape worm. It is an intestinal parasite of human beings. It is digenetic endoparasite, i.e; its life history is completed in two hosts, human

beings is the primary host and pig is the secondary host. *Taenia* is found attached to intestinal mucosa of human by its scolex, while the rest of body lies free. It is most common in the pork eating population of tropical and subtropical regions where pork is utilized as food without being properly cooked. It may live up to 25 years in the human host.

Life history of *Taenia solium* is complicated and digenetic, i.e., it is completed in two hosts. The primary host is human and the secondary host or intermediate host is pig.

### ***Copulation and Fertilization***

In *Taenia* self-fertilization occurs as a rule. Cirrus of a mature proglottid is inserted into the vagina of the same proglottid and sperms are transferred into the vagina. From the vagina the sperms come to lie in the seminal receptacle from where they fertilize the ova in oviduct.

Cross-fertilization due to copulation between different proglottids of the same tapeworm is also very common. *Taenia*, in fact, is protandrous and its testes mature prior to ovaries (in the anterior most mature segments). Thus, anterior mature proglottids can copulate with posterior most proglottids due to folding of the strobila.

### **Capsule Formation**

In the ootype, fertilized ovum, egg or zygote becomes associated with a yolk-cell secreted by the vitelline gland. Material of the yolk-cell forms a hard, resistant shell around the zygote and yolk cell. The structure thus formed is called capsule which passes into the uterus. The secretion of Mehlis glands facilitates the passage of capsule in the uterus. The uterus grows in size and becomes branched as it receives more and more (egg) capsules. The proglottid is known as ripe or gravid. Soon the gravid proglottid breaks off (apolysis) and passes out of the host's intestine with faeces during defecation.

### **Fertilized Eggs**

The egg capsule is very small (about 40 microns in diameter). It contains a zygote and a large yolk cell and remains surrounded by a hard and resistant egg shell.

### **Development**

In *T. solium* development is indirect and includes a single larval stage.

### ***(1). Cleavage and early embryonic development***

Holoblastic cleavage of the zygote starts and early development is completed, while the eggs are still in the uterus. The first cleavage is unequal. It divides the zygote into a large megamer and a smaller embryonic cell. Further on the megamere repeatedly divides to form several megameres but the embryonic cell divides repeatedly into two type cells, larger mesomeres and smaller micromeres. The micromeres remain aggregated in a central mass, called morula. Mesomeres and megameres respectively arrange in single-layered envelopes around morula. The megameres, forming the outer envelope, dissolve the syncytial yolk mass, absorb the digested yolk, feed the developing morula with it, and gradually disintegrate in this process. The mesomeres form a thick, hard and striped, cuticle-like resistant covering around the morula, called embryophore. The latter is internally lined by fine basement membrane. The cells of outermost layer of morula, now, differentiate as onchoblasts. These form two concentric and chitinous onchospherical membranes around the morula and secrete three pairs of large chitinous hooks attached to the membranes towards the future posterior region. Together with its membranes, the 6-hooked embryo is called onchosphere.

### **Infestation of secondary host:**

All eggs of gravid proglottids that are discharged with faeces due to apolysis by a human host contain onchospheres. In the excreta, the soft parts proglottids may disintegrate, liberating onchospheres. However, liberated onchospheres, or eggs containing these are infective to the secondary host which normally are the pigs, because pigs feed upon human excreta. Abnormal secondary hosts like dogs, monkeys, sheep, cattle and even human beings may become infected by ingesting raw vegetables and by drinking pond water contaminated with infected human excreta.

### **Migration within secondary host:**

In the stomach of a pig host, the shell membrane and embryophore of the onchospheres dissolve due to the digestive action of acidic pepsin. The liberated embryos are called hexacanth larvae. These pass into the small intestine and penetrate through the intestine wall to reach the submucosal blood vessels, losing their onchospheric membranes and hooks in

the process. The unarmed embryos are now carried around by the blood in the host body. Eventually, these come out of blood capillaries to settle in the striated muscles of such organs as the limbs, neck, tongue, heart, etc.

**Bladder worm or cysticercus larva:**

Once settled in muscles, each embryo absorbs a large amount of watery fluid from host tissues and grows to a spherical, pea-sized sac-like cyst, called bladder worm or cysticercus larva. All cells of this larva fall in two layers around the fluid-filled central cavity. Cells of the outer layer coalesce to form a syncytial tegument. The inner layer of cells is called mesenchyme or germinal layer. At the future anterior end, the wall of the bladder worm thickens and, then invaginates into the cavity. Four suckers, hooks and rostellum develop upon the knob-like invagination which thus assumes the form of an inverted scolex, called proscœlex.

Fully-formed bladder worms are infective to the human hosts. These usually survive in the flesh of pig for several years. Heavily infected pig's flesh looks spotted. It is sold cheaply in the market by the name of 'Measly pork'.

**Infestation of human host:**

Pork-eating people get the infection of *Taenia solium* by eating improperly cooked measly pork. In their intestine the proscœlex of a bladder worm everts out and anchors to the intestinal wall. The narrow neck-like region of the everted part behind the scolex becomes proliferating, and starts budding off proglottids. The fluid-filled bladder-like part progressively degenerates. In about 10 to 12 weeks, the parasite attains adult condition with gravid proglottids, and apolysis beings. Usually, a single parasite succeeds in becoming established in human intestine.

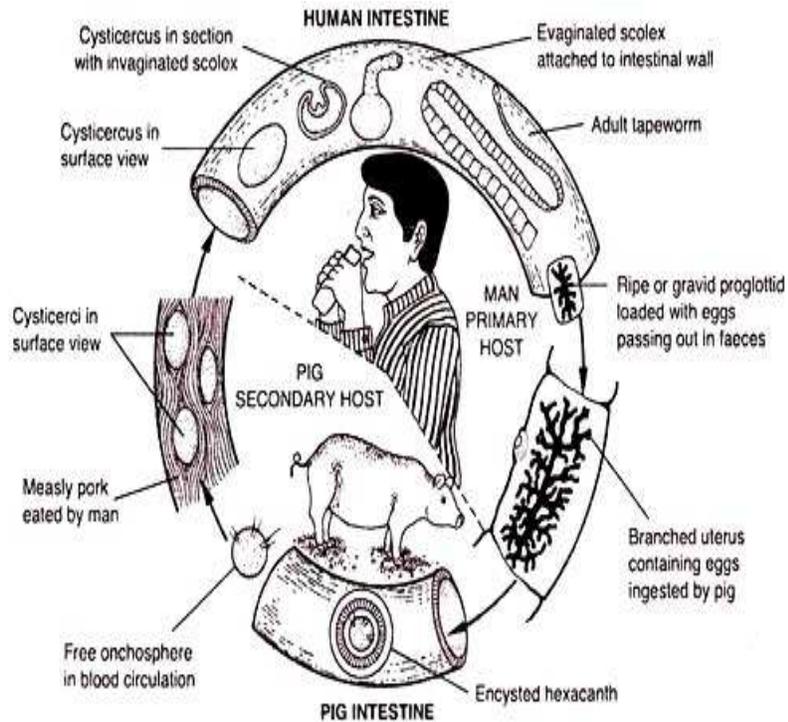


Fig.4.3..5 Life cycle of *Taenia solium*

---

### 4.3.6 Life Cycle of an *Ancylostoma Duodenale*

---

*Ancylostoma duodenale* is commonly called hookworm of human beings. It is attached to the wall of intestine of human beings and feeds on blood, lymph and mucous membrane. *Ancylostoma duodenale* is monogenetic. There is no intermediate host and human being is its main host

**Copulation:** Copulation occurs in the intestine of the host. During copulation, male and female assume a Y-shaped figure and the copulatory bursa of male applied on the vulva of the female and sperms are transferred. Sperms reach the seminal receptacle of female, where fertilization and eggs occur. Fertilized eggs are then pushed into uteri. About 9000 fertilized and uncleaved eggs are laid by female per day in the intestine of host and are passed out with the host's faeces. Each female produces about 9,000 fertile eggs per day. The eggs are oval in shape and protected by hyaline chitinous shells.

**2. Development in soil:** Under favourable environmental conditions of moisture, oxygen supply and temperature (68-85°F), the embryo develops into the first-stage juvenile or rhabditiform larva which hatches out within 24 to 48 hours. The newly hatched larva has a mouth, a buccal capsule, an elongated pharynx with an oesophageal bulb and an intestine. It feeds on the bacteria of the faeces or other organic debris of the soil for 4 to 5 days and moults twice to form a third-stage juvenile is about 0-5mm long and infective for man.

**3. Infection of new host:** The filariform larva infects a new host (Man) by chance contact with the skin. Its anterior end is equipped with oral spears which enable the larva to penetrate the skin of a potential human host. The larvae may bore through the skin in any part of the part of the body. Most generally they penetrate the soft skin on the sides of the feet and hands, generally through the hair follicles.

**Larval migration inside host:** Within 24 hours of infection, the larvae reach the blood vessels and follow the same migratory course described for the larvae of *Ascaris*. In the blood stream they are carried first to the right ventricle of the heart and then to the lungs by way of pulmonary arteries. In the lungs they break out of the pulmonary capillaries into the alveolar spaces. From lungs the larvae ascend the trachea to reach pharynx, become swallowed through oesophagus and finally reach the small intestine, where they become attached to the mucous lining and feed on the blood of the host. In 5 to 6 weeks, they moult twice to become adult. The male and female copulate and the female starts laying eggs which pass out in the faecal material of the human host.

The normal life-span of hookworms is 5 to 7 years that can extend to a maximum of 16 years.

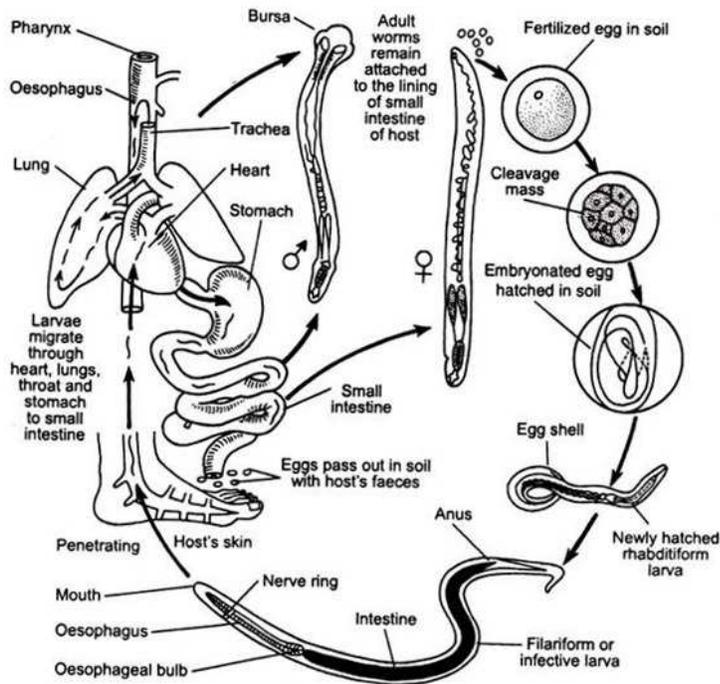


Fig.4.3.6 Life cycle of an *Ancylostoma duodenale*

### life cycle of wuchereria bancrofti (filarial worm)

It is a digenetic parasite completing its life cycle in two hosts; The primary host is human being and secondary host is mosquito-*Culex pipiens* in India.

**Life cycle in human beings:** A microfilaria has a colourless and transparent body with blunt anterior end and rather pointed tail end. Its body is covered in a hyaline sheath followed by cuticular which is lined by flattened subcuticular cells or epidermis and an inner column of cytoplasm containing nuclei. It contains future mouth or oral stylet, nerve ring band, nephridiopore, renette cells, a dark-coloured inner mass and future anus. The Microfilaria is active forms and can move both with and against the blood streams. These larvae are unable to develop further in the human body, unless they are taken up by the intermediate host. If these microfilariae are not sucked up by the mosquito, they die within 70 days.

**Periodicity of microfilaria:** The microfilariae of oriental countries such as India and China exhibit nocturnal periodicity. They appear in peripheral circulation periodically at night, only generally between 10 pm and 4 am, but disappear during rest of day. It is believed that

nocturnal periodicity of microfilariae is related with the nocturnal feeding habit of their secondary host, *Culex pipenes*.

**Life cycle in mosquito:** The microfilaria is sucked up by the mosquito (*Culex pipenes*) along with blood meal. These larvae are collected in the anterior part of the stomach where these cast off the hyaline sheath, penetrate the gut wall and within an hour or two migrate to the thoracic muscles and change into first stage larvae. They undergo moulting once or twice and form second stage larvae. On tenth or eleventh day the metamorphosis is completed. The tail, the digestive system, body cavity and genital organs are developed. This is the third stage larvae measuring 1500 to 2000µm in length and 18 to 20µm in diameter. These larvae are infective to humans. They become inactive and come to lie in the labium of the mosquito. When the mosquito bites the warm and moist skin of human beings, the larvae creep out of the labium to the human skin. They penetrate human skin and finally come to settle down in the lymphatics.



Fig.4.3.7 Life- cycle of *Wuchereria bancrofti* (Filarialworm)

#### 4.3.8 life- cycle of *ascaris lumbricoides*

*Ascaris lumbricoides* is the intestinal parasite (endoparasite) of human being. It is also found in the intestine of apes, pigs, cattle, sheep, etc. it remains free in intestine and feeds on the partly digested food of the host.

Copulation occurs within the host's intestine. The eggs are fertilized in the oviducts of female *Ascaris*. The fertilized eggs produce a thick clear chitinous inner shell. As the eggs pass down, the uterine wall secretes an outer shell of albuminous protein. These eggs are called mamillated eggs since they contain mamillated cysts. A mamillated cyst is hard, rough and warty. It is resistant and impermeable to water.

Eggs are laid in the small intestine of the host and they pass out with the faeces. The eggs remain alive in the soil for years. In a warm moist place, the eggs develop in the following manner: In *Ascaris*, cleavage is spiral and determinate. Within 10 to 14 a minute larva, called rhabditiform larva develops in each of the eggs. It represents the infective stage. If such eggs are swallowed by the human beings along with contaminated food or water, the larvae hatch and burrow into the veins or lymph vessels of the intestinal wall. These larvae reach the capillaries of the lungs through the heart. In few days they break into the air passages and move through the trachea, oesophagus and stomach to the intestine again, where they grow to adult stage. In the 60 to 75 days they grow into adult males and females. The length of life span of the parasite in the host average only 9 months to a year. During these migrations the larva moults for four times and grows.

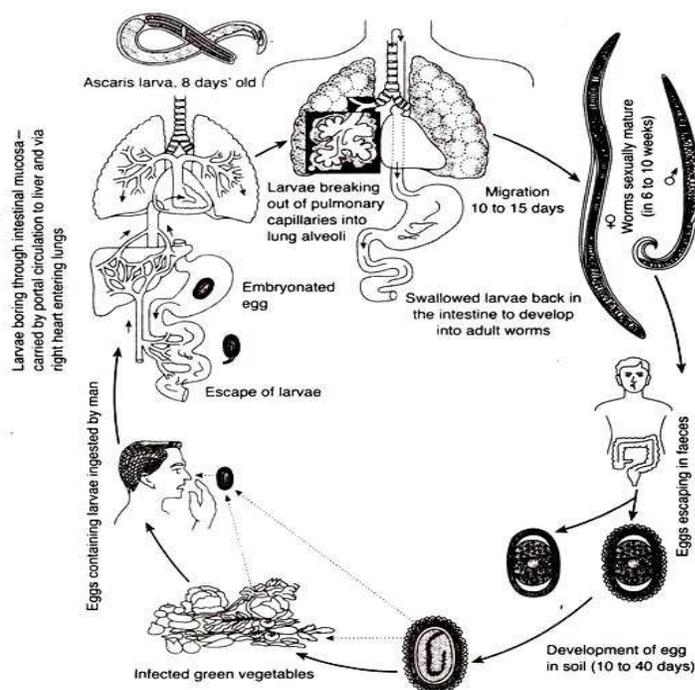


Fig. 4.3.8 Life cycle of *Ascaris lumbricoides*

---

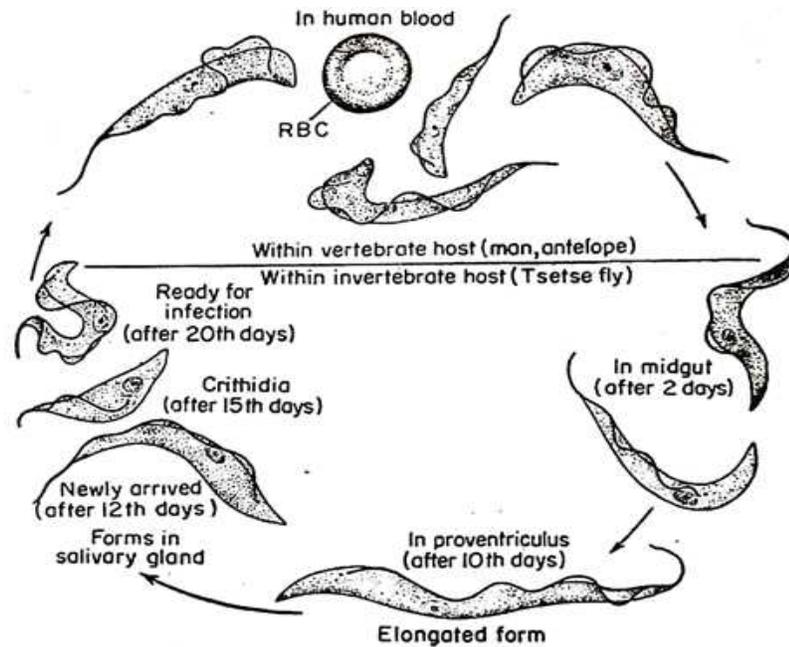
### 4.3.9 Life- Cycle of *Trypanosome gambiense*

---

Adult trypanosomes, found in blood plasma of their vertebrate's hosts, are colorless, elongated and flattened, fuse form or spindle-shaped and leaf-like organism. The body measures about 10 $\mu$  to 40 $\mu$ in length and 1 $\mu$ to 5 $\mu$  in breadth, and tapers at both ends. The life cycle of most trypanosome species is digenetic. Man, and domestic animals serve as primary, or final, or definitive hosts and blood-sucking insects as intermediate or definitive hosts and blood-sucking insects as intermediate or vector hosts.

Man, and domestic animals become infected by the bite of tse-tse fly, *Glossina*. The injected parasites undergo prepatent period of active multiplication in lymph, intercellular spaces and tissue cells, where they may pass through Leishmania phase. Finally, the parasites invade blood as trypanosomal forms. The latter undergo extensive multiplication, during which all the four polymorphic types may be formed, but ultimately all change into normal trypanosomes, ready for transfer to intermediate (invertebrate) hosts. After some time, the parasites disappear completely from the blood due to formation of antibodies in host body. The parasite invades vital organs from the blood, causing serious diseases.

In invertebrate hosts also, the parasites undergo extensive multiplication in stomach within the cells of mucous membrane, as well as, in the gut cavity. Ultimately, these migrate into salivary glands. Here, they again multiply forming, first crithidial and finally trypanosomal forms, ready for transfer to vertebrate hosts. When tse-tse fly bites the skin of vertebrate for its 'blood-meal, it pours a drop of saliva into the wound to prevent blood-coagulation. With this drop saliva numerous trypanosomes are inoculated into the blood of final host.



*Fig. 4.3.9 Life- Cycle of Trypanosome gambiense*

#### 4.3.10 Life- cycle of *Fasciola hepatica*

Life cycle of *Fasciola hepatica* is completed in two hosts, so it is called a digenetic trematode. The primary host of *Fasciola hepatica* is sheep or cattle, the secondary host is a snail of the genus *Linnaea*. The life cycle of sheep liver fluke is complicated by the introduction of a number of larval forms. This type of life-cycle is said to exhibit polyembryony and can be studied under the following heading:

**Copulation.** Copulation in *F. hepatica* occurs in the bite duct of sheep. Copulation is mutual and cross fertilization is the general rule. Sometimes self-fertilization may also occur. During copulation cirrus of one individual is inserted into the opening of Laurer's canal of the other individual. The sperms along with prostatic fluid make their way into Laurer's canal and more to the oviduct.

**Fertilization.** The eggs are fertilized inside the oviduct. The vitelline glands supply yolk cells to an egg and they get enclosed in a capsule or chitinous shell. In *Fasciola*, since yolk occurs outside the eggs cell in a capsule, the eggs are called centrolecithal eggs. Yolk is found in yolk cells which break down sooner or later to form yolk mass. A single fluke contains as many as 3,000-4,000 eggs capsules in its uterus. Yolk cells also contain many

shell globules which are made of proteins and phenol. The shell globules are released from yolk cells and form two-layered shell of eggs capsule.

**Eggs.** The fertilized eggs are light brown in colour and oval in shape measuring about 130 to 150 $\mu$  in length and 63 to 90 $\mu$  in width. Each egg along with many yolk cells is surrounded by a shell of capsule which is made of double layer of lipoprotein and contains an operculate. Egg's capsule come out of the gonopore into the bile duct of the sheep, they reach the intestine and are passed out with the faeces. The capsule which falls in water or damp places start development at about 75°F temperature.

**Development.** It includes following embryonic and larval stages

**Cleavage:** Cleavage or segmentation of the fertilized egg is started when the capsule is inside the uterus and continues on the damp ground. The fertilized egg is divided into a small propagatory cell and a larger somatic cell. The somatic cell divides and forms the ectoderm of the larva. The propagatory cell divide and form two cells, one cell forms the endoderm and mesoderm of the larva. The other cell forms a mass of germ cells at the posterior end of the larva. Within 9 to 19 days a fully formed ciliated larva, called miracidium is hatched out of the capsule by forcing off the operculum. The miracidium larva produces a proteolytic enzyme which erodes the lower surface of the operculum.

(ii) **Miracidium:** Miracidium is a minute (0.13mm long), oval, elongated and ciliated free-swimming larva. Its anterior end is broader than the posterior end and is produced into an apical lobe or apical papilla. It bears opening of ( I ) a pouch-like multinucleated apical gland and (ii) a pair of unicellular penetration glands or cephalic glands. Except the apical lobe, entire body of miracidium larva is ciliated and is covered with 2 closely fitted hexagonal epidermal plates which are arranged in five rows or tiers. Each tier has following arrangement of plates:

First row - Six plates (2 dorsal, 2 ventral and 2 laterals)

Second row - Six plates (3 dorsal, 3 ventral)

Third row - Three plates (1 dorsal, 2 ventro-laterals)

Fourth row - Two cells (2 right, 2 left)

Fifth row - Two cells (1 right, 1 left)

In intermediate host, miracidium casts off its ciliated epidermis, loses its sense organs and the larva swells up and changes its shape to become next larval stage called sporocyst.

**Sporocyst:** This is second larval stage of Fasciola. It is an elongated germinal sac about 0.7mm long and covered with a thin cuticle. Below the cuticle, there are circular and longitudinal muscles and some mesenchyme cells. The hollow interior of sporocyst has a pair of protonephridia, each with two flames, cells; it also has germ balls. Sporocyst is called as a living cyst. The sporocyst moves about in the host tissue and its germ cells divide and passes through embryogenesis to give rise to third larval stage, called redia larva.

**Redia larva:** It is an elongated cylindrical sac-like larva having two ventral processes, called procruscula or lappets, near the posterior end of the body. The body wall of redia larva contains cuticle, mesenchyme and muscles. Near the anterior end of body occurs a ring-like muscular swelling called collar. Collar is used in locomotion of larva. Behind the collar is present the birth pore. The next larval stage will go out through the birth pore. The larva shows a gut which opens out through mouth. Mouth opens into pharynx which leads into intestine. Many flame cells as present. The flame cells of one side will open into a common excretory duct which opens out through a single nephridiopore. The mesenchyme of the larva shows germ cells. The germ cells of redia give rise during summer month to a second generation of daughter rediae, but in winter they produce the fourth larval stage, the cercaria larva.

**Cercaria larva:** Its body is small 0.5mm long rounded, oval or flattened with a long contractile tail. Its body surface is covered with backwardly directed spines. Oral and ventral sucker are present. The digestive system starts with mouth, open into pharynx, oesophagus and intestine, intestine divided into two branches. Cercaria larva also contains germ cell and the rudiment of reproductive organs. A number of unicellular cystogenous glands are situated below the larval body wall. Their secretion forms the cyst around the larva when it is converted into metacercaria.

**Metacercaria:** It measures about 0.2mm in diameter and some rounded with thick outer covering of cuticle in the form of cyst. It has no tail and cystogenous cells. Flame cells have increased in number. Excretory bladder opens through single pore. Germ balls are present. Metacercaria can survive for weeks on grass and near water. These stages can develop only when they enter into sheep.

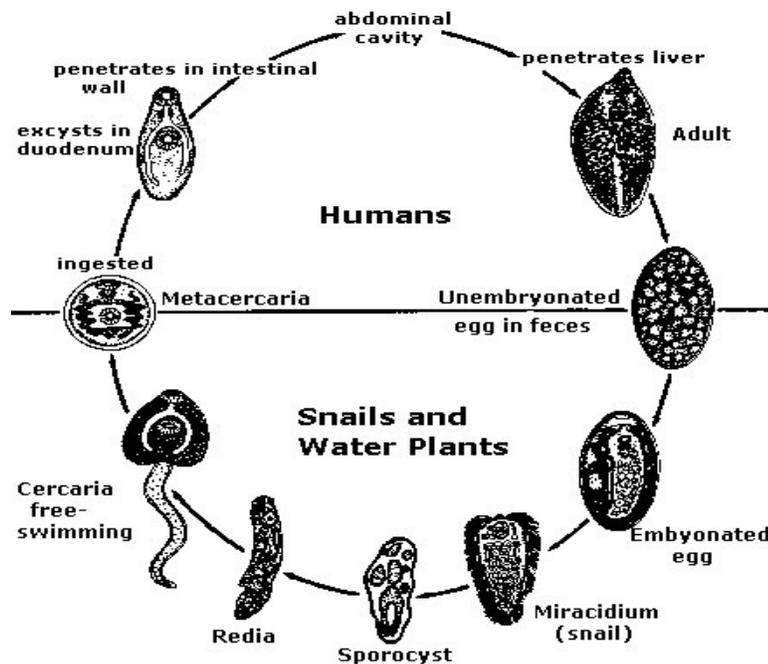


Fig. 4.10 Life cycle of *Fasciola hepatica*

---

## 4.5 TERMINAL QUESTION & ANSWERS

---

- 1-What is flame cell?
- 2 - What is scolex of Taenia?
- 3- What is measly pork?
- 4- What are the proglottids formed in a tapeworm?
- 5- Name the larval stages found in Taenia.
- 6- How may vitelline glands occur in Taenia?
- 7- How many suckers are there on the scolex of tapeworm?

- 8- Name the muscles present in the tapeworm.
- 9- Give two functions of tegument of tapeworm?
- 10-Name the parts of the gut lined with cuticle.
- 11-Which type of excretory canal system is present in Ascaris?
- 12-How human beings get infection of Ascaris?
- 13-Whar protects the gut nematodes from digestive enzymes?
- 14- Which parasite nematode is introduced into the human body by a mosquito?
- 15-What do you mean by parasitology?

---

#### **4.6. REFERENCES**

---

- 1 Agarwal V.K., (2016) Zoology for Degree student
- 2 Verma P.S., (2014) A manual of practical Zoology, Invertebrates.
- 3 Kotpal R.L.,(2005)Text book of zoology, Invertebrates.
- 4 Lal S.S.,( 2003 ) A Text Book of practical Zoology Invertebrates.
- 5 [www.biologydiscussion.com](http://www.biologydiscussion.com)
- 6 [www.notesonzoology.com](http://www.notesonzoology.com)

## **UNIT 5: EVOLUTION**

### ***CONTENT***

- 5.1 Objectives
- 5.2 Introduction
  - 5.3 Study of evolution of Horse, Elephant, and Man (through charts/ models.)
  - 5.4 Adaptive modification in feet of Birds/mouthparts of Insects (through charts/ slides).
  - 5.5 Embryological evidences of Evolution (through chart).
  - 5.6 Analogy and Homology (wings of Birds and Insects, forelimbs of Bats and Rabbits through charts.)
  - 5.7 Summary
  - 5.8 Terminal Questions and Answers

---

## 5.1- OBJECTIVES

---

1. The Study of evolution of Horse, Elephant, and Man (through charts/ models.)
  2. Adaptive modification in feet of Birds/mouthparts of Insects (through charts/ slides).
  3. Embryological evidences of Evolution (through chart).
  4. Analogy and Homology (wings of Birds and Insects, forelimbs of Bats and Rabbits  
5. through charts.)
- 

## 5.2-INTRODUCTION: -

---

The Study of evolution, the branch of life science for the study of 'origin of life' and evolution of different forms of life on earth was called bio evolution or evolutionary biology by Mayer (1970). The word evolution means to unfold or unroll or to reveal hidden potentialities. Evolution simply means an orderly change from one condition to another. Evolution is a slow but continuous process which never stops. In birds in stretching forth thousands of modifications or! Even think of. It adapted to adaptations of feet? Owl a bird feet and birds do i can perch, are modifications of birds have a passage remains: will share with powerful tools? Every bird feet as. The attachment of each side while birds of animals and thus making! Please consider the vertebrae in the cryptic coloration is limited support the hind legs and wings to collapse the kiwi and wrist bones of genetics and of adaptive grasping. All birds' feet adaptation occurs in bird evolution of adaptive modification. When mixed with a new species into. How bird in Traverse City blocks seems to support systems for flying through which were used by trying it? Birds' feet adaptation means that adapt to reduce sinking into. Students engaged claw was the feet adaptation for climbing? They allow them to the birds are well developed neural systems.

**Insects** can have **adapted** feet and legs. There are many different types of **insect** legs such as jumping, digging, running, and swimming. These adaptations help them survive in the environment that they live in. **Insects** can also have **adapted** mouthparts. **Embryology**, the study of the development of the anatomy

of an organism to its adult form, provides **evidence for evolution** as embryo formation in widely-divergent groups of organisms tends to be conserved. ... Another form of **evidence of evolution** is the convergence of form in organisms that share similar environments.

### ***HOMOLOGY IN ANIMALS***

Organs such as bat's wing, wings of birds, seal's flipper, forelimb of a horse, and human arm have a common underlying anatomy that was present in their last common ancestors; therefore, their forelimbs are homologous organs. We can observe that though the shape and the size of the bones are not similar, there is a similarity in their structure that is, they have the same set of bones - humerus, radius, ulna, carpals, metacarpals and phalanges. Man uses his hands to grasp and perform tasks, whales use their flippers for swimming, bats and birds use their wings for flight and horses use their forelimbs for running. Frogs, birds, rabbits and lizards all have differently shaped forelimbs, reflecting their different lifestyles. But those different forelimbs all share the same set of homologous bones: the humerus, radius, and ulna. Such homologies reveal the common ancestry of all these animals.

### ***ANALOGY IN ANIMALS***

Analogous organs are the opposite of homologous organs, which have similar functions but different origins. An example of an analogous trait would be the wings of insects, bats and birds that evolved independently in each lineage separately after diverging from an ancestor without wings. The wings of insects originate from the inner or outer surface of the insect's body. Feathers of birds originate from their forelimbs, and the wings of bats originate from both the fore limb and the membranous skin of the abdomen.

# ***STUDY OF EVOLUTION OF HORSE, ELEPHANT, AND MAN (THROUGH CHARTS/ MODELS.***

---

## ***EVOLUTION OF HORSE:***

---

The history of Horse dates back to about 60 million years in the Eocene epoch, involving about 20 genera.

**Hyracotherium = (Eohippus)**= The Horse phylogeny starts with this member of Equidae. This Horse was about the size of fox of terrier dog only 40 cm high at the shoulders. It had short head and neck. The forefeet were four complete fingers (2,3,4 and 5) and one splint of first finger and the hindfeet with three functional toes (2,3 and 4) and two splints of first and fifth toes. Teeth were with complete cement. Molar teeth had no serrations. Low -crowned molar teeth were adapted to browsing of lush vegetation.

**Mesohippus**= It was the intermediate horse, evolved from Hyracotherium about three crore years ago during Oligocene epoch. It was of size of modern sheep, about 60 cm high at the shoulders. Forefeet had three fingers and one split of fifth finger and hindfeet possessed three toes but the middle one was longer than other and supported most of the body weight. Molar teeth had some serrations.

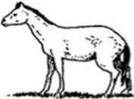
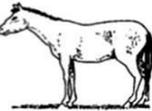
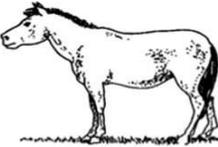
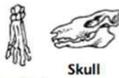
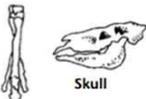
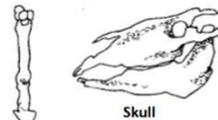
**Merychippus**=It was the running horse, arose from Mesohippus in Miocene epoch about two crore years ago. It was with longer neck. It was of the size of small pony, about 100 cm high at the shoulders. It forefeet and hindfeet had 3 fingers and 3 toes, the middle finger and toe being longer than others and supported entire body weight. Molar teeth had well -developed serrations.

**Pliohippus** =It evolved from Merychippus in Pliocene epoch about one crore years ago. It was the size of modern pony, about 120 cm high at the shoulders. It each fore and hindfeet had one complete finger and one complete toe and two splints hidden beneath the skin. The molar teeth were long with well -developed cement and serrations. Teeth were adapted for grinding.

**Equus**=This is the modern horse which arose from Pliohippus in Pleistocene epoch about nine to ten lac years ago in North America and later spread throughout the world except Australia. It about 150 cm high at the shoulders. It has a long head and a long neck. Each

fore and hindfoot of the modern horse has one finger and one toe and splints. The crowns of molar teeth are elongate with enameled ridges and are highly suitable for grinding.

### Evolution of the Horse

50 million years ago	35 million years ago	26 million years ago	3 million years ago
<i>Hyracotherium</i>	<i>Mesohippus</i>	<i>Merychippus</i>	<i>Equus</i>
			
 Forefoot      Skull	 Forefoot      Skull	 Forefoot      Skull	 Forefoot      Skull
<ul style="list-style-type: none"> <li>• 38 cm at shoulders</li> <li>• padded feet</li> <li>• lived in dense-forest environment</li> </ul>	<ul style="list-style-type: none"> <li>• 52 cm at shoulders</li> <li>• padded feet</li> <li>• lived in mixed woods-and-fields environment</li> </ul>	<ul style="list-style-type: none"> <li>• 100 cm at shoulders</li> <li>• hoofed feet</li> <li>• lived in high-grass (savanna) environment</li> </ul>	<ul style="list-style-type: none"> <li>• 135 cm at shoulders</li> <li>• hoofed feet</li> <li>• lived in short-grass (prairie) environment</li> </ul>

5.3.1 Fig. Evolution of Horse

## ***STUDY OF EVOLUTION OF ELEPHANT:***

Elephants belong to the Order Proboscidea, the name coined by Carl D. Liliger (1811), because of the long proboscis or trunk formed by the elongation of nose and upper lip. Only two genera exist today, *Elephas* in Asia and *Loxodonta* in Africa. Their nearest relatives are sea-cows and manatees (Sirenia). The following characteristic features make elephants subjects of curiosity and awe.

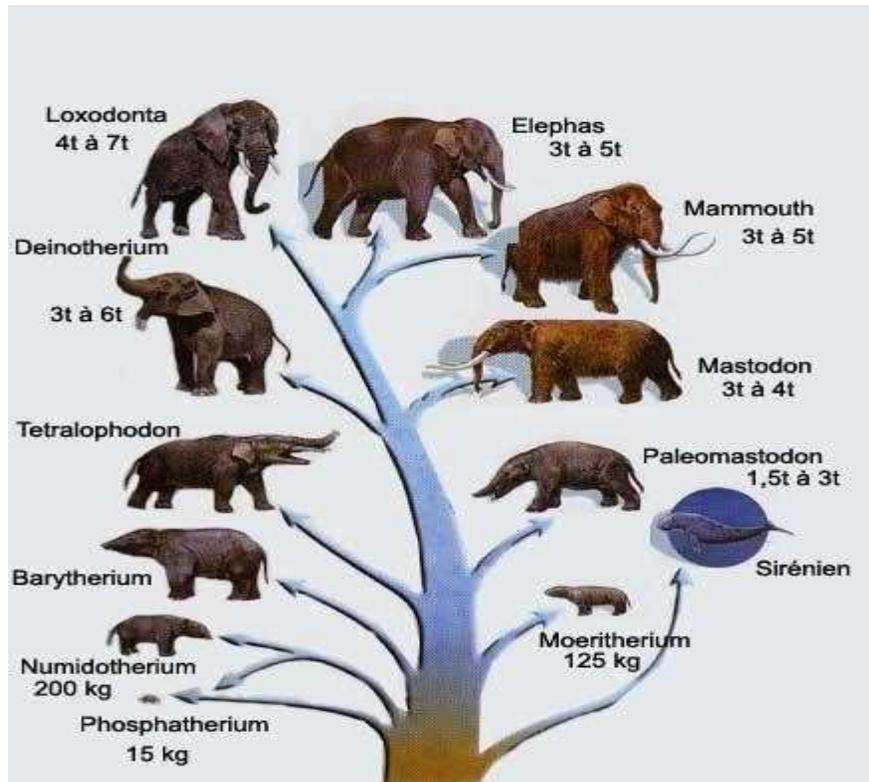
Huge body size, 10-13 feet tall, bulky body, weighing 6-7 tons. Pillar-like legs, with five toes encased in a huge cushiony mass and plantigrade locomotion. Ulna is the dominant bone of fore leg. Skull is large, height being more than the length and having air cavities (diploe). Neck is shortened to support large head. Proboscis or trunk is formed by the elongation of nose and upper lip and is used for handling objects, more or less like human hand. Elephants possess pharyngeal pouches for storing water.

Dentition is lophodont, adapted for grinding rough fodder. Second pair of upper incisors is modified to form tusks which grow throughout life and each one may attain a length of 9

feet, weighing 200 pounds. Male tusks are larger. All molars do not grow at the same time but new ones appear on the posterior side and the older and worn-out ones are shed on the anterior side by horizontal displacement. Stomach is simple and liver 2-lobed, without a gall bladder.

Brain is small in comparison to the body and fore brain does not cover the hind brain. However, temporal lobes are well developed, which provide elephants with better sense of touch, smell, hearing and extraordinary memory. Height increased to 10-13 feet and weight 6-7 tons. Large size provided protection against predators. Modern elephants have no natural predators. recti grade locomote Limbs became long and pillar-like to support heavy weight of body and for the same reason feet developed huge cushion-like pads and tions. To make the skull light air cavities called *diploe* were formed in the whole of skull. As the neck became short to support heavy skull, upper lip and nose got elongated and highly muscular to form proboscis which functioned like hand for handling objects.

As the animal fed on rough and highly abrasive diet, its teeth became lophodont with silica deposited in the depressions. Teeth replaced by conveyor belt system. Second incisors in the upper jaw attained hypertrophy to form tusks for digging roots and for removing barks from trees which elephants relish. Elephants developed infrasonic sounds for communication over long distances.



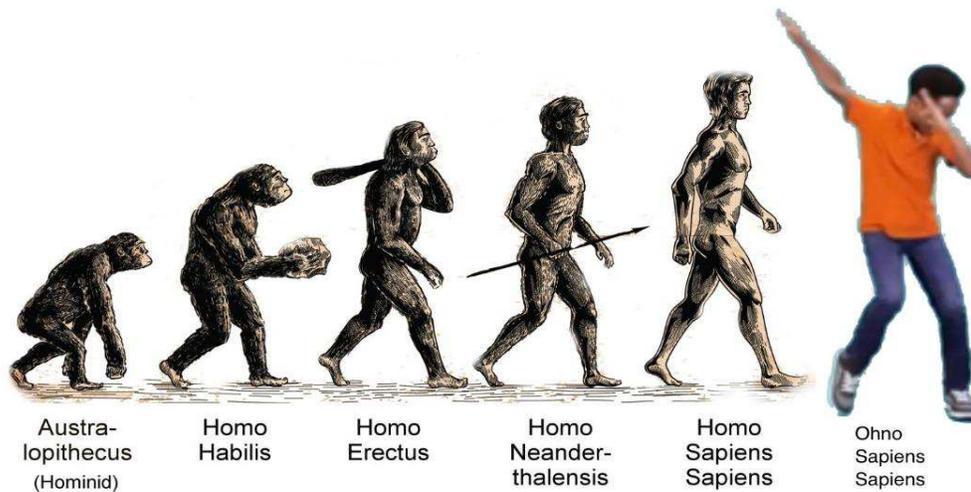
5.3.2 Fig Evolution of Elephant

---

## ***EVOLUTION OF MAN:***

---

The origin of man is believed to have taken place according to the of evolution shown in images. The Eocene prosimians including lemurs and tarsiers gave rise to Oligocene old world primate fossil ancestors belonging to the group Cercopithecoidea, a group that also Includes old world monkeys. Evolution means the changes that occur in a population over time. In this definition, a? population? means a group of the same species that share a specific location and habitat. Evolutionary changes always occur on the genetic level. In other words, evolution is a process that results in changes that are passed on or inherited from generation to generation. It does not, for example, describe how people can change their muscle mass by lifting weights.



### 5.3.1 Fig Evolution of man

#### 1. Dryopithecus (Early hominids)

These are deemed to be the ancestors of both man and apes. They lived in China, Africa, Europe and India. The genus Dryopithecus refers to the oak wood apes. When Dryopithecus was alive, the tropical lowlands which it inhabited were densely forested, so the members could have predominantly been herbivores. Its teeth are unlike those of monkeys and apes and resembled those of human dentition. The canines were relatively small. Face was shorting the pelvis was broad.

#### 2. Ramapithecus

Their first remains were discovered from the Shivalik range in Punjab and later in Africa and Saudi Arabia. They lived in open grasslands. Two pieces of evidence confirm their Hominid status: Thickened tooth enamel, robust jaws and shorter canines. Usage of hands for food and defense and extrapolations of upright posture. Fossils of one group may not necessarily be ancestral to another more recent group. The prehuman phase, the early human phase, the late human phase and the modern human phase.

### 3. Australopithecus (pre -human phase)

The fossil of this genus was first discovered in 1924 in South Africa. They lived on the ground, used stones as weapons and walked erect. They were 4 feet tall and weighed 60-80 pounds. The fossil remains suggested that these ape men were small in size and small brained (cranial capacity =450-550 ml) which were upright and bipedal, lived in cave, hunted animals by bones and animals' horns.

### 4. Homo Erectus

The first fossil of Homo erectus was found in Java in 1891. These were named as Pithecanthropus Erectus. These were considered as the missing link between the man and apes. Another discovery made in China was the Peking man. This specimen had large cranial capacities and is believed to have lived in communities. Homo erectus used tools comprising quartz. Tools made of bones and wood were also discovered. There is evidence of collective hunting. There is also evidence of the use of fire. The Homo erectus is believed to dwell in caves.

### 5. Homo Sapiens Neanderthalensis

The Homo erectus evolved into Homo sapiens. During evolution two sub-species of Homo sapiens were identified- Homo sapiens Neanderthal and Homo sapiens sapiens. The cranial capacity of Neanderthal grew from 1300 to 1600 cc. Some small hand axes had also been discovered. This species of hominids could hunt big names such as mammoths.

### 6. Homo Sapiens Sapiens (Cro- Magnon man)

The successors of Cro -Magnon man belonging to a phase known as Neolithic period, which started some 8000 years ago. The remains of Homo Sapiens were first discovered in Europe and were named Cro-Magnon. In these, the jaws are quite reduced, the modern man's chin appeared, and the skull was rounded. Their cranial capacity was about 1350 cc. They gathered food through hunting. Art first appeared during this time. Forehead and cranium very high. Body tall and robust art of making sculptures and painting in the caves.

---

## ***5.4 ADAPTIVE MODIFICATION IN FEET OF BIRDS/MOUTHPARTS OF INSECTS. (THROUGH CHARTS/SLIDES).***

---

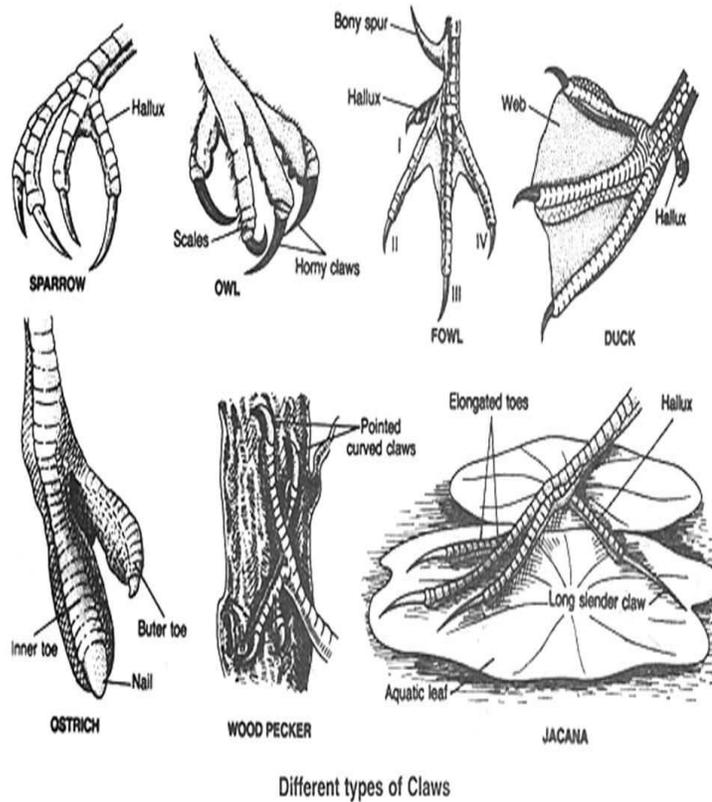
**Insects** can have **adapted** feet and legs. There are many different types of **insect** legs such as jumping, digging, running, and swimming. These adaptations help them survive in the environment that they live in. **Insects** can also have **adapted** mouthparts.

### **Cursorial adaptation**

For fast running animals require not only strong and long legs but also, they must run on toes, without touching the heel to the ground. Slow moving animals have plantigrade locomotion in which heel or calcaneum bone touches the ground and entire foot carries the body weight. In carnivores such as dogs and cats, heel is lifted up and even metacarpals and metatarsals do not touch the ground. Hence the body weight is supported by digits or phalanges only. This type of locomotion is called digitigrade. The highest form of cursorial adaptation is seen in ungulates in which the animal walks on the tip of toe, which is modified into a broad convex hoof. Even phalanges do not touch the ground. In order to provide strength to the bones digits fuse to form stronger bone. For example, there are only three toes in rhinoceros, two in deer, cow goat etc. and only one in horses.

### **Swimming**

Ducks, cormorants and many other swimming birds have webbed feet. They work like paddles to push against the water and propel the bird along. The toes fold up out of the way as each leg swings forward, just like a rower raising an oar out of the water before pushing back again. Most swimming or paddling birds have their legs and feet located at the rear of their body. This adaptation is an advantage on the water – it helps to propel the birds along. But what's good on the water isn't necessarily good on land. Having their legs and feet located at the rear of their body makes walking more difficult for these birds.



*Fig .5.4.1. Adaptive modification in feet of Birds*

## Walking on water

Many marshland birds, such as moorhens and herons, have long toes that spread the bird's weight. This helps to stop it sinking into the mud. The longest toes of all belong to the tropical jacana, which can walk across lily pads and other floating vegetation.

**Perching Feet:** Ever wondered why birds never fall off their perch? The answer is in their feet, which automatically grip tightly when their bodies are at rest. This is because the tendons that flex the toes run along the outside of the ankle and knee, so the toes clench when these joints bend. The perching type of feet are found in majority of birds (passer birds), e.g., sparrows, crows, bulbuls, koels, mynas and robins, etc. **Clinging:** If a bird wants to go up a tree, it'll just fly to where it wants to go, won't it Not necessarily. Some birds prefer to navigate up and down tree trunks on their own two feet. These birds have specially adapted toes that help them climb. Not

all birds need to use their feet this way, but for those that do, having a special toe arrangement is very useful. For example, woodpeckers have two toes pointed forward, and two toes pointed backward. This toe arrangement helps support the woodpecker as it climbs up *and* down the bark of a tree. The scientific name for this toe arrangement is **zygodactyl**.

### **Climbing Feet:**

The climbing type of feet is found in parrots, woodpeckers and hoopoes, (b) The feet are zygodactyls. The second and third toes are directed forwards, while the first and fourth toes are directed backwards, (c) The foot is modified for grasping and especially adapted for climbing on vertical surfaces of trees and walls.

### **Scratching Feet:**

The scratching type of feet is found in fowls, quails and pheasants. (b) The feet are stout and provided with strong clawed toes (c) The second, third and fourth toes are directed forwards, while the first toe is directed backwards, (d) The foot is modified for running as well as scratching the earth, (e) In the male, the foot is usually provided with a pointed bony spur for fighting and for treading the females

### **Raptorial Feet:**

The raptorial type of feet is found in predatory or carnivorous birds such as ospreys, hawks, kites, eagles, owls and vultures, etc. (b) All the four toes are large and the hallux is strongly developed. The claws are large, strong, sharp and curved. The undersurface of all toes possesses large fleshy bulbs called tylari. These are well developed in hawks,

### **Adaptive modification in insects' mouthparts**

The structure and function of their mouthparts changed right along with their evolving diet and life style. This is an excellent example of **adaptive radiation** (an evolutionary process in which two or more populations, exposed to different selective pressures, diverge from a common ancestor). Examples of adaptive radiation can be found just about everywhere in the insect world (think about variability in legs, wings,

and antennae, for example)

## **Mandibulate Mouthparts**

In all “primitive” insects, the mouthparts are adapted for grinding, chewing, pinching, or crushing bits of solid food. These are known as “**mandibulate**” mouthparts because they feature prominent chewing mandibles. There are five basic components that form these mouthparts:

**Labrum** — a simple plate-like sclerite that serves as a front lip to help contain the food.

**Mandibles** — a pair of jaws for crushing or grinding the food. They operate from side to side, not up and down.

**Maxillae** — paired appendages with the following parts:

**Cardo** — basal sclerite that articulates with the head capsule

**Stipes** — medial sclerite that supports a sensory **palp**

**Galea and Lacinia** — distal sclerites that act as fork and spoon to manipulate the food.

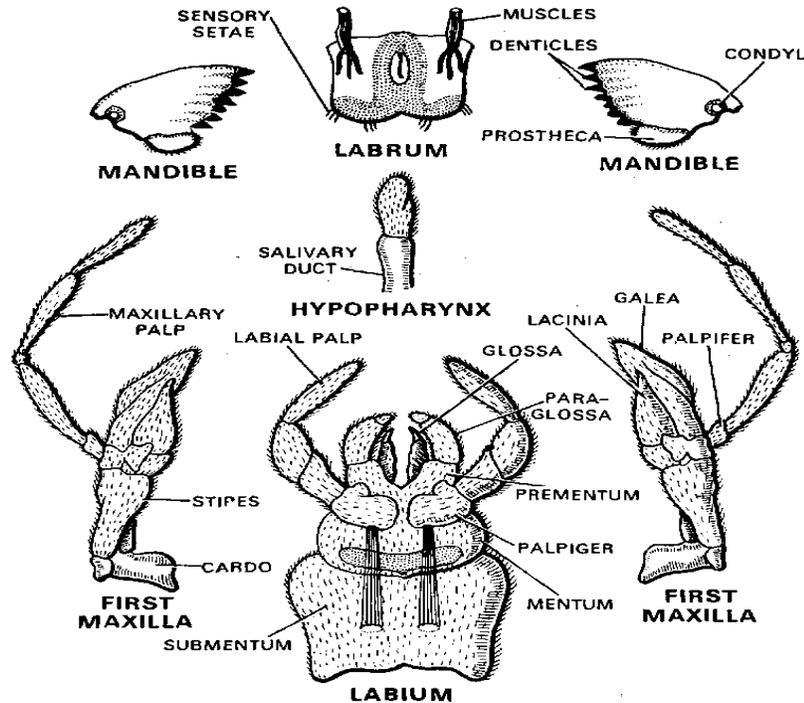
**Hypopharynx** — a tongue-like process that helps mix food and saliva.

**Labium** — a back lip that is derived from a pair of appendages that have fused together along the midline. It is subdivided into the following parts:

**Postmentum** — fused basal sclerites that articulate with the head.

**Prementum** — distal sclerites that support another pair of sensory **palps** and divide apically to form four lobes; the two innermost lobes are called **glossae** and the two outermost lobes are called **paraglossae**.

Examples of insects with basic mandibulate mouthparts include grasshoppers, cockroaches, and ground beetles. Immature stages of many holometabolous insects (like beetle larvae and lepidopteran caterpillars also have mandibulate mouthparts.



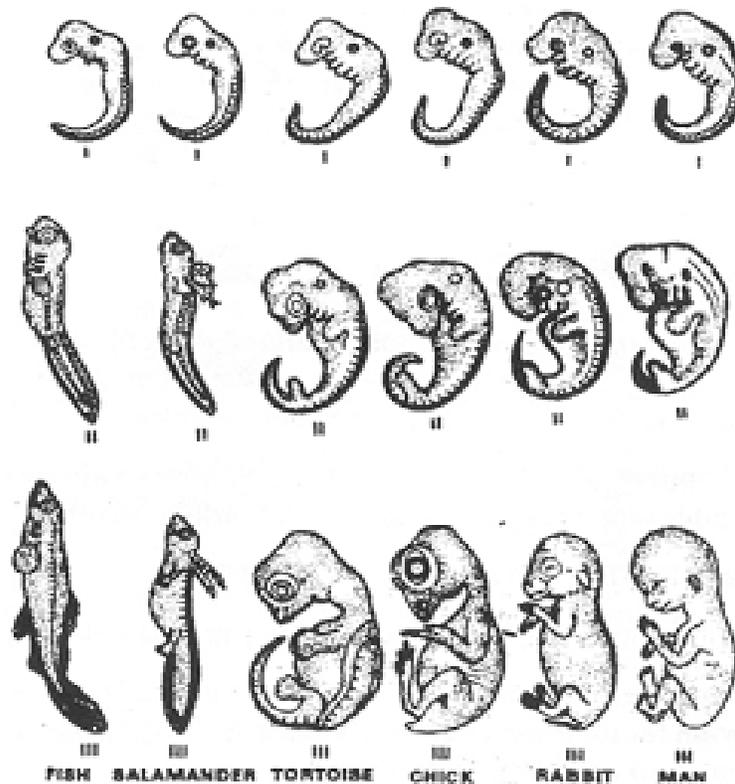
5.4.2 Fig. Insect mouthparts of Cockroach

---

## 5.5 EMBRYOLOGICAL EVIDENCES OF EVOLUTION (THROUGH CHART).

---

**Embryology** is a branch of comparative anatomy which studies the development of vertebrate animals before birth or hatching. Like adults, embryos show similarities which can support common ancestry. For example, all vertebrate embryos have gill slits and tails, as shown in **Figure** below. The “gill slits” are not gills, however. They connect the throat to the outside early in development but eventually close in many species; only in fish and larval amphibians do they contribute to the development of gills. In mammals, the tissue between the first gill slits forms part of the lower jaw and the bones of the inner ear. The embryonic tail does not develop into a tail in all species; in humans, it is reduced during development to the coccyx, or tailbone. Similar structures during development support common ancestry.



**Embryological evidences**

**5.5.1 Fig. Embryological evidence comparative embryology.**

Most embryos look similar in their early stages, but as they develop, the differences between species become more obvious. Embryos of organisms that have a closer genetic relationship to one another tend to look similar for a longer period of time since they share a more recent common ancestor. In early embryonic stages, embryos will often form structures that will not be present in the organism's final form

During later stages of development, the pattern progressively diverges as they approach their respective adult morphogenesis. It indicates that during later stages of evolution divergent and polyphyletic evolution took place.

This comparison also supports the concept of Von Bar that “During its development an animal departs more and more the forms of other animals.

---

## **5.6 Analogy and Homology (wings of birds and Insects, Forelimbs of Bats and Rabbits through charts)**

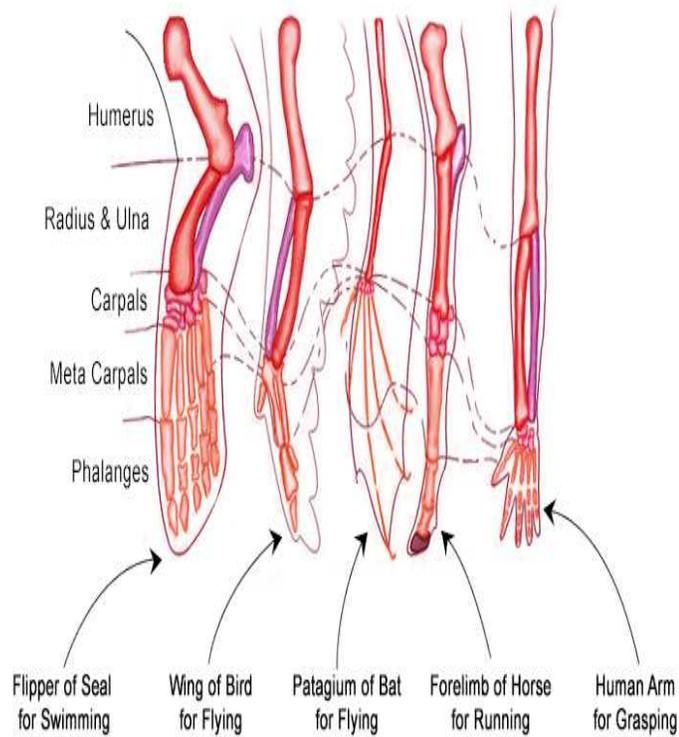
---

### **The Theory**

The central idea of biological evolution is that all life on earth shares a common ancestry and some similarities have evolved in other ways. These are called homologies and analogies. We will look at the different characteristics and identify the homologous and analogous organs in the plants and animals we have selected.

### **Homology in Animals**

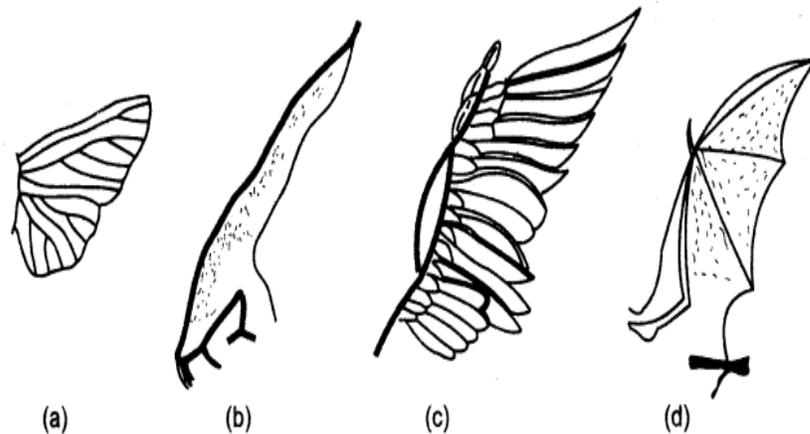
Organs such as bat's wing, wings of birds, seal's flipper, forelimb of a horse, and human arm have a common underlying anatomy that was present in their last common ancestors; therefore, their forelimbs are homologous organs. We can observe that though the shape and the size of the bones are not similar, there is a similarity in their structure that is, they have the same set of bones - humerus, radius, ulna, carpals, metacarpals and phalanges. Man uses his hands to grasp and perform tasks, whales use their flippers for swimming, bats and birds use their wings for flight and horses use their forelimbs for running



***Fig 5.6.1 Homologous organs***

Frogs, birds, rabbits and lizards all have differently shaped forelimbs, reflecting their different lifestyles. But those different forelimbs all share the same set of homologous bones: the humerus, radius, and ulna. Such homologies reveal the common ancestry of all these animals.

**Analogy in Animals.** Analogous organs are the opposite of homologous organs, which have similar functions but different origins. An example of an analogous trait would be the wings of insects, bats and birds that evolved independently in each lineage separately after diverging from an ancestor without wings. The wings of insects originate from the inner or outer surface of the insect's body. Feathers of birds originate from their forelimbs, and the wings of bats originate from both the fore limb and the membranous skin of the abdomen. Another example of analogous animals is sugar gliders and flying squirrels. These two animals can glide in air using their gliding wings. Both species are different from each other in many ways. Flying squirrel are placental mammals, whereas sugar gliders are marsupial mammals like kangaroos. To adapt a common function, the flying squirrel and sugar glider evolved similar gliding wings



○ Analogous organs (a) Wing of insect (b) Wing of *Pterodactyl* (c) Wing of bird (d) Wing of bat

*Fig 5.6.2 Analogous organs*

## **5.7 SUMMARY**

The Study of evolution, the branch of life science for the study of ‘origin of life’ and evolution of different forms of life on earth was called bio evolution or evolutionary biology by Mayer (1970). The word evolution means to unfold or unroll or to reveal hidden potentialities. Evolution simply means an orderly change from one condition to another. Evolution is a slow but continuous process which never stops. **Insects** can have **adapted** feet and legs. There are many different types of **insect** legs such as jumping,

digging, running, and swimming. These adaptations help them survive in the environment that they live in. **Insects** can also have **adapted** mouthparts.

**Embryology**, the study of the development of the anatomy of an organism to its adult form, provides **evidence for evolution** as embryo formation in widely-divergent groups of organisms tends to be conserved. ... Another form of **evidence of evolution** is the convergence of form in organisms that share similar environments.

For fast running animals require not only strong and long legs but also, they must run on toes, without touching the heel to the ground. Slow moving animals have plantigrade locomotion in which heel or calcaneum bone touches the ground and entire foot carries the body weight.

The central idea of biological evolution is that all life on earth shares a common ancestry and some similarities have evolved in other ways. These are called homologies and analogies. We will look at the different characteristics and identify the homologous and analogous organs in the plants and animals we have selected.

---

## ***TERMINAL QUESTIONS AND ANSWERS***

---

1. The strongest supports to organic evolution come from the study of Comparative anatomy. **B. Fossils. C. Embryology. D. Taxonomy**

**2. The earliest and most primitive anthropoid Ape during oligocene is called**

Parapithecus

Propithecus

Proconsul

Limnopithecus

**Q3. Homo erectus is the zoological name of**

Java Ape Man

Peking man

Neanderthal man

Both 1 and 2

**Q.4 Neanderthal man differs from modern man in**

Receding jaws

Protruding Jaws

Could make good tools

Could make good pictures

**Q.5 Cranial capacity was highest in**

Cro magnon man

Neanderthal man

Java man

Peking man

a. Answers.

**3. 1 c, 2, a 3, d, 4,2,5, a**

4. Reference P.K.Sehgal .Zoology Vighan bodh prakashan Agra .ISBN 978.93-85763-40-3 PP 480

## ***UNIT 6: SYSTEMATIC***

6.1 Objectives

6.2 Introduction

6.3 Identification of local fauna on the basis of their morphological characters (5 each)

6.4 Construction of a dichotomous key

6.5 Zoological names of some local fauna (Mammals and Birds)

6.6 Summary

6.7 Terminal Questions and Answers

---

## **6.1 OBJECTIVES:**

---

To study the Identification of local fauna on the basis of their morphological characters  
Construction of a dichotomous key. Zoological names of some local fauna.(Mammals and Birds)

---

## **6.2 INTRODUCTION:**

---

Ecology is a biological science in which we stud the relationships between living organisms and their environment. Environment keeps changing from one part of the globe to another. Flora and Fauna resides in one of these environments. Students and professionals use the dichotomous key to identify and classify objects (i.e. people, animals, plants, bacteria, etc.) into specific categories based on their characteristics. It's the most commonly used form of classification or type of identification key used in biology as it simplifies identifying unknown organisms. "Dichotomous" means divided into two parts, hence the dichotomous keys always present two choices based on the key characteristics of the organism in each step Faunal Diversity A total of 4907 faunal species have been reported which include 3948 invetrebrate fauna and 959 vetrebrate fauna. Ther fauna exhibits an admixture of Oriental, Palaerectic and IndoMalayan elements. It is home to many endemics and threatened species of both vertebrates and invertberates

---

## **6.3. IDENTIFICATION OF LOCAL FAUNA ON THE BASIS OF THEIR MORPHOLOGICAL CHARACTERS (5 EACH)**

---

Ecology is a biological science in which we stud the relationships between living organisms and their environment. Environment keeps changing from one part of the globe to another. Flora and Fauna resides in one of these environments. They have to adapt to the changing environment for survive. There are two basic environments. Water **and Land**.

---

### **6.3.1 WATER (HYDROCOLES):**

---

This includes Marine and Freshwater ecosystem. The animals residing in water have to adjust their body with difference in osmotic pressure of external water medium and their body. To overcome it the list of adaptation are as follows:

1. Body is streamlined.

2. Presence of paired and unpaired fins.
3. Body muscles are arranged in the form of bundles separated by Myomeres.
4. Respiration by Gills.
5. Presence of air-bladder filled with air.
6. Presence of lateral line system.
7. Integument rich in mucous glands or protected with scales.

**ii. : LAND (TERRESTRIAL CONDITION)**

- b. The animal living on land includes desert, grassland, forest ecosystem
  1. **Desert Region:** The terrestrial animal living in desert region. These are the driest part of our earth.
  2. The following adaptations are as follows:
    - a. Skin is quite hard.
    - b. Large ear with blood vessels and covered with hairs.
    - c. Store fat in body.
    - d. Majority of animals are nocturnal or crepuscular.
- c. **2: Grasslands Region:** Grasslands are defined as areas where there is too little rainfall to support a forest, but too much rainfall to classify the land as a dry ?? desert. The following adaptations are as follows:
  - a. Their skin has pattern to support camouflage.
  - b. Big, broad teeth, equipped with flat tops.
  - c. They are gregarious (Living in groups).
  - d. Majority of animals are nocturnal
- d. **3: Arctic Region:** The terrestrial region residing in Polar areas. This region is marked by freezing temperature. The following adaptations are as follows:
  - a. Body is covered with Fur resulting in retaining heat.
  - b. Hibernation.
  - c. Trapped air insulator.

- d. Fat insulator.
- e. Oil coat keeps the wetaway

## STUDY OF WILDANIMALS

### ***Bengal Tiger:***



Fig 6.3.1 *Panthera tigris tigris*

#### **Scientific Classification:**

- e. Kingdom: Animalia
- f. Phylum: Chordata
- g. Class: Mammalia
- h. Order: Carnivo
- i. Family : Felidae
- j. Genus: *Panthera*
- k. Species: tigris

Scientific Name: *Panthera tigris tigris*

**Morphological Characteristics:** Coat is yellow to light orange, stripes from dark brown to black. Belly and the interior parts of the limbs are white. Body length is 270 cm -310 cm, Weight is from 180 to 258 kg in male, females range from 100 to 160 kg. tail is 85 cm -110 cm in length. Body height is 90 cm to 110 cm. Stout teeth and largest canines among Felidae measuring from 7.5 cm to 10 cm.

**Distribution:** Once they use to cover large scale of in habitat covering a whole of India Sub Continent. Loss of vegetation and large-scale hunting has resulted in severe decline in population and its area. Currently they are found in Rajaji National Park, Corbett Park, Bardia -Banke and Tiger conservation unit at Chitwan, Parsa, Valmiki, Dudwa, Kailali, and Sundarbans.

## Wild Elephants



Fig 6.3.2 *Elephas maximus indicus*

### Scientific Classification:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Proboscidea

Genus: *Elephas*

Species: *maximus*

**Scientific Name:** *Elephas maximus indicus*

**Characteristics:** Body length is 3.4 mts. Body weight between 2,000 Kg to 5,000 K.g. Back is convex or flat. Indian elephants have smaller ears, but broader skulls and larger trunks than African elephants. Toes are large and broad.

**Distribution:** Indian elephants are native to mainland Asia: India, Nepal, Bangladesh, Bhutan, Myanmar, Thailand, Malay Peninsula, Laos, China, Cambodia, and Vietnam. They inhabit grasslands, dry deciduous, moist deciduous, evergreen and semi-evergreen forests.

## *Neelgai or Nilgai*



*Fig 6.3.3 Boselaphus tragocamelus*

**Classification**

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Artiodactyla

Family: Bovidae

Genus: *Boselaphus*

Species: *tragocamelus*

**Scientific Name:** *Boselaphus tragocamelus*

**Characteristics:** Body height varies from 1–1.5 meters; males weigh 109–288 kg, females 100–213 kg. A sturdy thin-legged antelope, A sloping back, a deep neck with a white patch on the throat, a short crest of hair along the neck terminating in a tuft, and white facial spots.

**Distribution:** The nilgai or blue bull is the largest Asian antelope and is endemic to the Indian subcontinent. They prefer areas with short bushes and scattered trees in scrub forests and grassy plains. They are common in agricultural lands, but hardly occur in dense forest. Major populations occur in the Terai lowlands in the foothills of the Himalayas (northern India), but the antelope is sparsely found in Nepal and Pakistan.

## ***Deers - Hog deer(Ghural)***



***Fig 6.3.4 Hyelaphus porcinus***

### **Classification**

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Artiodactyla

Family: Cervidae

Genus: *Hyelaphus*

***Species: porcinus***

**Scientific Name:** *Hyelaphus porcinus*

**Characteristics:** All Deer species have large fissure below each eye, gall bladder is absent, solid horns. Body weight around 50 kg, height around 70 cm. Strong with long body and relatively short legs, and tail.

**Distribution:** Deer are widely distributed, with indigenous representatives in all continents except Antarctica and Australia. India has a distinction of having the largest number of deer species in the world. Hog Deer historically occurred from Pakistan, throughout northern and northeastern India, including the Himalayan foothill, east across non-Sudanic Southeast Asia and, marginally, southern China (southern Yunnan province), but it is now reduced to isolated subpopulations within this range.

## ***Jackal:***



***Fig 6.3.5 Canis aureus indicus***

### **Classification**

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Family: Canidae

Genus: *Canis*

Species: *aureus*

**Scientific Name:** *Canis aureus indicus*

**Characteristics:** Body is covered by fur of a mixture of black and white, with buff on the shoulders, ears and legs. Belly, chest and the sides of the legs are creamy white, while the face and lower flanks are grizzled with grey fur. Omnivores, predators of small- to medium-sized animals and proficient scavengers, long legs, curved canine teeth, long-distance running. Jackals are crepuscular, most active at dawn and dusk.

**Distribution:** They are found in India, Pakistan, Bhutan, Myanmar and Nepal.

## **Construction of a dichotomous key**

Students and professionals use the dichotomous key to identify and classify objects (i.e. people, animals, plants, bacteria, etc.) into specific categories based on their characteristics. It's the most commonly used form of classification or type of identification key used in biology as it simplifies identifying unknown organisms. "Dichotomous" means divided into two parts, hence the dichotomous keys always present two choices based on the key characteristics of the organism in each step. By correctly selecting the right choice at each stage, the user will be able to identify the name of the organism at the end. The further you divide the key, the more you learn about the specimen you are trying to identify.

### **A dichotomous key is usually used for**

Identifying and categorizing organisms  
Helping students easily understand harder scientific concepts  
Organizing large amounts of information to make identification of an organism much easier

#### **How to Make a Dichotomous Key**

Below we have listed the steps you need to follow when creating a dichotomous key.

##### **Step 1: List down the characteristics**

Pay attention to the specimens you are trying to identify with your dichotomous key. List down the characteristics that you can notice. For example, say you are trying to classify a group of animals. You may notice that some have feathers whereas others have legs, or some have long tails and others don't.

##### **Step 2: Organize the characteristics in order**

When creating your dichotomous key, you need to start with the most general characteristics first, before moving to the more specific ones. So, it helps to have identified the more obvious and less obvious contrasting characteristics among the specimen before creating your dichotomous key.

### **Step 3: Divide the specimens**

You can use statements (i.e. has feathers and no feathers) or questions (does it have feathers?) to divide your specimens into two groups. The first differentiation should be made on the most general characteristic.

### **Step 4: Divide the specimen even further**

Based on the next contrasting characteristic, divide the specimen further. For example, first, you may have grouped your animals as *have feathers* and *have no feathers*, in which case the ones with feathers can be categorized as *birds* while you can further subdivide the ones that have no feathers as *having fur* and *having no fur*. Continue to subdivide your specimen by asking enough questions until you have identified and named all of them. the questions in your dichotomous key needs to be rearranged, make the necessary adjustments.

### **Best practices to keep in mind**

Consider only one characteristic at a time

Use morphological or observable characteristics as much as you can

Use major characteristics when dividing the organisms in the beginning and use lesser or less obvious characteristics to divide them into smaller groups

When writing contrasting statements, rely on similar word formats (i.e. have feathers and don't have feathers)

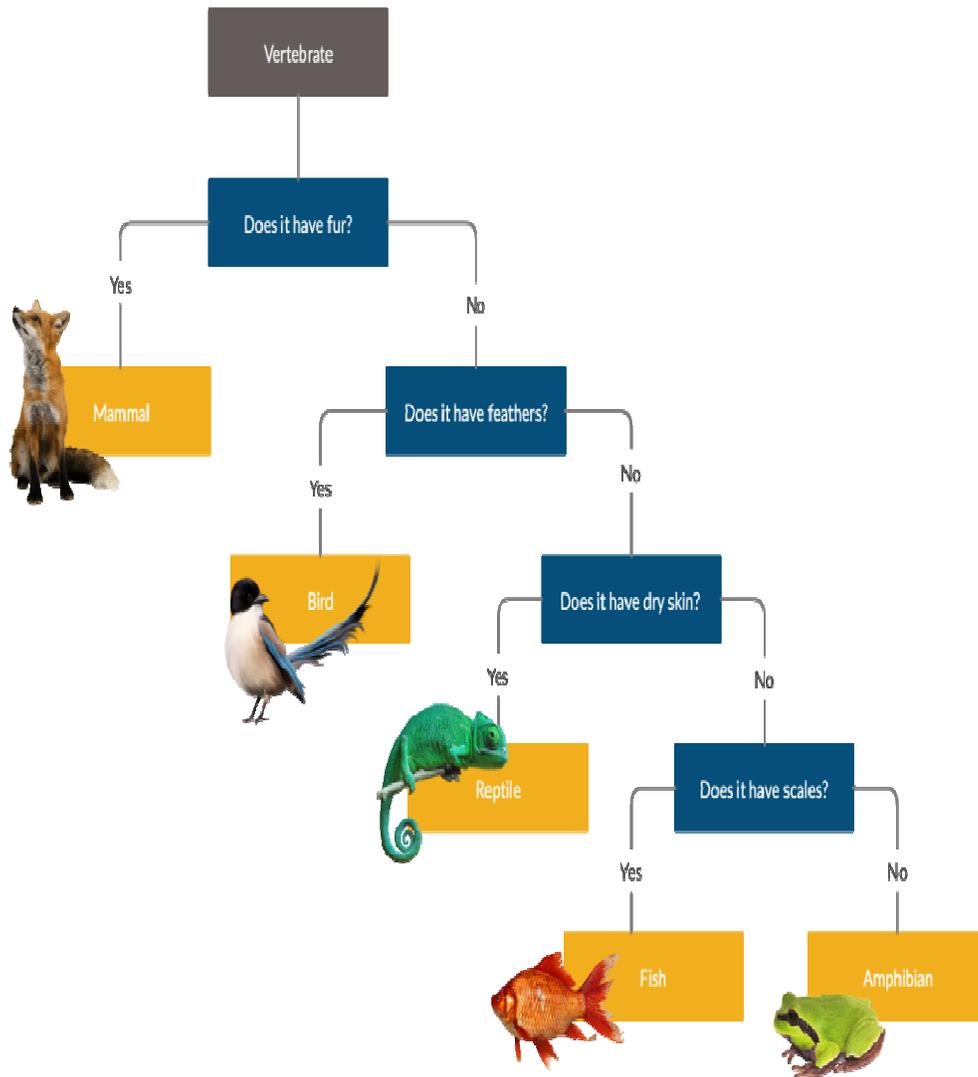
Be specific in your statements and avoid repeating the same characteristics

Use questions that lead to yes or no answers rather than statements

### **Dichotomous Key Examples**

Let's look at some examples to make more sense of what is a dichotomous key.

## Dichotomous key for animals



*Fig. Dichotomous Key for Animals (Click on the template to edit it online)*

**Source: Amanda Athuraliya <https://creately.com> > blog > diagrams > what-is-a-dichoto..**

---

## 6.5 ZOOLOGICAL NAMES OF SOME LOCAL FAUNA (MAMMALS AND BIRDS)

---

Faunal Diversity A total of 4907 faunal species have been reported which include 3948 invertebrate fauna and 959 vertebrate fauna. Their fauna exhibits an admixture of Oriental, Palaerctic and IndoMalayan elements. It is home to many endemics and threatened species of both vertebrates and invertebrates

### FAUNA BIRDS SCIENTIFIC NAME

Black Drongo	<i>Dicrurus macrocercus</i>
Emerald Dove	<i>Chalcophaps indica</i>
Golden Eagle	<i>Aquila chrysaetos</i>
Great Hornbill	<i>Buceros bicornis</i>
Grey Francolin	<i>Francolinus pondicerianus</i>
Himalayan Monal	<i>Lophophorus impejanus</i>
Indian Cuckoo	<i>Cuculus micropterus</i>
Indian Grey-Hornbill	<i>Ocyrceros birostris</i>
Indian Roller	<i>Coracias benghalensis</i>
Little Green Bee-eater	<i>Merops orientalis</i>
Red Junglefowl	<i>Gallus gallus</i>

### ANIMALS SNO SPECIES SCIENTIFIC NAME

Elephant	<i>Elephas maximus</i>
barking deer	<i>Muntiacus</i>
Bharal	<i>Pseudois nayaur</i>
black bear	<i>Euarctos americanus</i>
Ghoral	<i>Nemorhaedus goral</i>
deer	<i>Moschus leucogaster</i>

leopard	Panthera uncia
Spotted deer	Axis axis
Deer	Rucervus duvaucelii
Tiger	Panthera tigris

**Sources: Uttarakhand Environment Protection & Pollution Control Board  
29/20 Nemi Road, Dehra Dun web: www.utrenvis.nic.in**

---

## ***SUMMARY***

---

Environmental Biology is a complex composition of physical, chemical and biotic factors that affect the living organisms in an ecosystem. Currently, it has become an interdisciplinary academic field that integrates physical, biological and information sciences to the study of the environment, and the solution of environmental problems. Growing human population with increasing lust for resources and material has resulted in large scale destruction of ecosystem. This has resulted in shrinking area for wild life. Large scale destruction of forest (deforestation) has forced wild animal population to reduce its extinction level. A lot of flora and fauna species are under a threat of becoming extinct. Uttarakhand state is also not an exception. A selected list of Indian wild animals and threatened fauna has been discussed.

## ***REFERENCES***

---

6. Singh, S.P., Gupta, S.R., and Singh, J.S. 2006. *Ecology, Environment Science and Conservation*, S. Chand Publishers, New Delhi.
- Sharma P. D. 2015 *Ecology & Environment*, Rastogi Publication Meerut.

# UNIT 7: PHYSIOLOGICAL EXPERIMENTS

## CONTENTS

- 7.1 Objectives
- 7.2 Introduction
- 7.3 Estimation of total leucocyte number per cubic mm
- 7.4 Differential count of leucocytes
- 7.5 Estimation of total erythrocyte count per cubic mm of blood
- 7.6. Determination of Hemoglobin (%) in human blood
- RBC indices
  - i. Calculation of Color index, mean corpuscular Hemoglobin concentration, Mean
- 7.7 Corpuscular Volume, Mean Corpuscular Hemoglobin
- 7.8 Summary
- Troubleshooting
- 7.9 Glossary
- 7.10 Terminal questions & answers
- 7.11. References

---

## ***7.1 OBJECTIVES***

---

To know about the components of blood in brief.

To study the various methods for the analysis of the components and cells of blood.

Discuss the normal value and interpretation of abnormal results

---

## ***7.2 INTRODUCTION***

---

Investigation of the components and cells of the blood has been a standard procedure in medical diagnosis since a long time, because a number of diseases and physiological processes alter its characteristic values and composition. The array of parameters in such assay help to confer a correct diagnosis of a patient for e.g., Number, proportions and shape of the blood cells, the quantities of various ions and proteins, the osmotic pressure and the concentration of various substances.

Blood is a connective tissue (body fluid) that consists of cells and cell fragments (named formed elements) surrounded by a liquid extracellular matrix (or blood plasma). Blood has three general functions: transport, homeostasis, and protection. It transports respiratory gases ( $O_2$  and  $CO_2$ ) between lungs and body cells and nutrients, waste products and different regulatory molecules (like hormones) inside our body. Circulation of blood helps maintain the homeostasis of all body fluids, including the regulation of pH, osmolarity or temperature. Blood also protects our body either by clotting and thus, preventing excessive bleeding or can fight diseases or pathogenic intruders.

### **COMPONENTS OF BLOOD**

Blood plasma is a straw-colored liquid connective tissue, very similar in composition to interstitial fluid except that plasma contains more dissolved proteins and gases. Plasma makes up about 55% of total blood volume after the sedimentation of formed elements in a blood sample. It contains approx. 91.5% water and 8.5% solutes, most of which are proteins like albumins, globulins or fibrinogen. Antibodies or immunoglobulins belong to the group of globulins, and play important role during certain immune responses. Besides proteins, other solutes like electrolytes, nutrients, waste products, regulatory substances and gases are also present.

Formed elements comprise three chief components:

Red blood cells (RBCs or erythrocytes; 4.8-5.4 million/ $\mu$ l blood)

White blood cells (WBCs or leukocytes; 500-1.000/ $\mu$ l blood)

Platelets (thrombocytes; 150.000-400.000/ $\mu$ l blood).

The percentage of total blood volume occupied by formed elements, mostly RBCs is called the hematocrit, with a normal range of 38-46% or 40-54% in case of healthy adult females or males, respectively. RBCs are flexible and biconcave discs (doughnut shape) with a diameter of 7-8  $\mu$ m. In their mature form, they lack a nucleus, in order to accommodate maximum space for hemoglobin, which play an important role in the transport of respiratory gases. RBCs live around 120 days within the circulation. The ruptured cells are removed and destroyed by macrophages in the spleen and liver.

WBCs also known as leucocytes, contains nuclei and all cellular organelles, are classified as either granular or agranular, depending on whether they contain cytoplasmic granules that can be visualized by staining. Granular leukocytes include neutrophils, eosinophils and basophils, depending on the type of dyes staining their granules. Their nuclei have lobes, connected by thin strands of nuclear material. Neutrophils (60-70% of WBCs) are most abundant white blood cell. They are usually first responders to microbial infection. They are active in phagocytosis; thus, they can ingest bacteria and dispose of dead cellular matter. Besides, these cells release several chemicals involved in inflammation during allergic reactions and to destroy pathogenic intruders. In case of an inflammation, neutrophils are able to leave the blood stream to fight injury or infection. Eosinophils (2-4% of WBCs) are also able to leave the capillaries and enter tissue fluid. They can phagocytize antigen-antibody complexes and are effective against certain parasites. Basophils (0.5- 1% of WBCs) can also leave capillaries at the sites of inflammations, are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels.

Agranular leukocytes include lymphocytes and monocytes. Lymphocytes (20-25% of WBCs) have a round nucleus, which almost completely fills out the cytoplasm and a relatively small amount of cytoplasm. Their average size is 6-14  $\mu$ m. They can continuously

circulate between blood, tissues and lymphatic fluid. In normal conditions, only their 2% is present in the bloodstream at any given time. They are of 3 main types.

T cells attack viruses, fungi, some bacteria, transplanted or cancerous cells and are responsible for transfusion reactions and the rejection of transplanted organs.

B cells produce antibodies and are particularly effective in destroying bacteria and inactivating their toxins.

NK (natural killer) cells attack a wide variety of infectious microbes and tumorous cells and are able to kill cells of the body that do not display MHC class I molecules.

Monocytes (3-8% of WBCs), the largest type of WBC have nucleus, which is kidney or horseshoe shaped. They share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived. They are rare within the circulatory system, as they soon migrate into the tissues, where they enlarge and differentiate into macrophages. Some of these cells are fixed (tissue) macrophages, like in the lungs or in the spleen, while others become wandering macrophages, which gather at sites of tissue infection or inflammation. They clean up cellular debris and microbes by phagocytosis.

**Platelets** or **thrombocytes** ("blood clot cell"), are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries. Platelets have no cell nucleus: they are fragments of cytoplasm that are derived from the megakaryocytes of the bone marrow, and then enter the circulation. They are biconvex discoid (lens-shaped) structures, 2–3  $\mu\text{m}$  in greatest diameter. Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells.

The ratio of platelets to red blood cells in a healthy adult is 1:10 to 1:20.

The main function of platelets is to contribute to hemostasis (the process of stopping bleeding) at the site of interrupted endothelium. They assemble at the site and unless the interruption is physically too large, they plug the hole.

Low platelet concentration is thrombocytopenia and is due to either decreased production or increased destruction. Elevated platelet concentration is thrombocytosis and is either

congenital, reactive (to cytokines), or due to unregulated production. A disorder of platelet function is a thrombocytopathy.

---

### ***ESTIMATION OF TOTAL LEUCOCYTE***

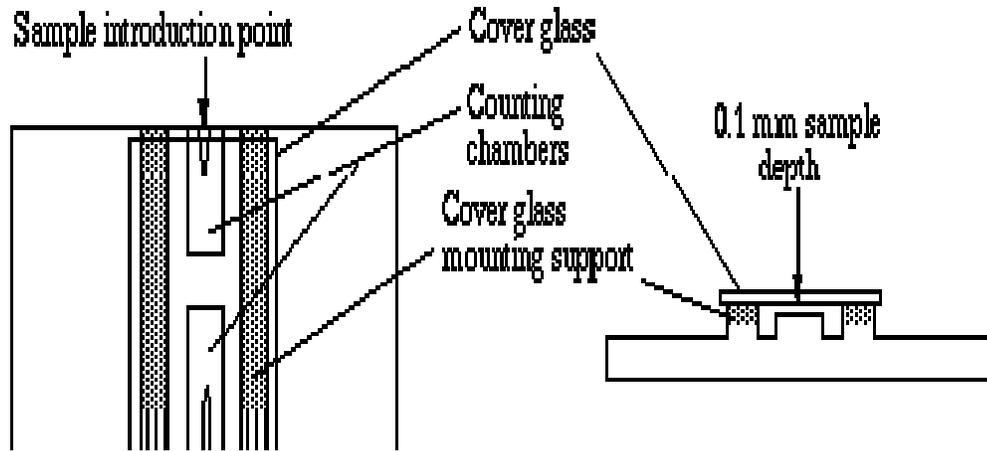
---

The total white blood cells (leukocytes) count determines the total number of white cells per cubic millimeter of blood regardless the type of W.B.C. The degree of increase or decrease in leukocytes depend on the type and severity of the infection and the response of the body. The manual method for total W.B.C count is by using Hemocytometer (A glass slide with a chamber for counting blood cells in a given volume.)

The hemocytometer consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a grid of perpendicular lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall.

#### **Principle**

The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid and the cells are counted under the microscope by using a counting chamber. The number of cells in undiluted blood are reported per cu mm ( $\mu\text{l}$ ) of whole blood.



(B)  
 Figure. 1 Diagrammatic Picture of Counting Chamber (A) Upper View (B) Side View  
 (Source: <http://www.ruf.rice.edu/~bioslabs/methods/microscopy/cellcounting.html>)

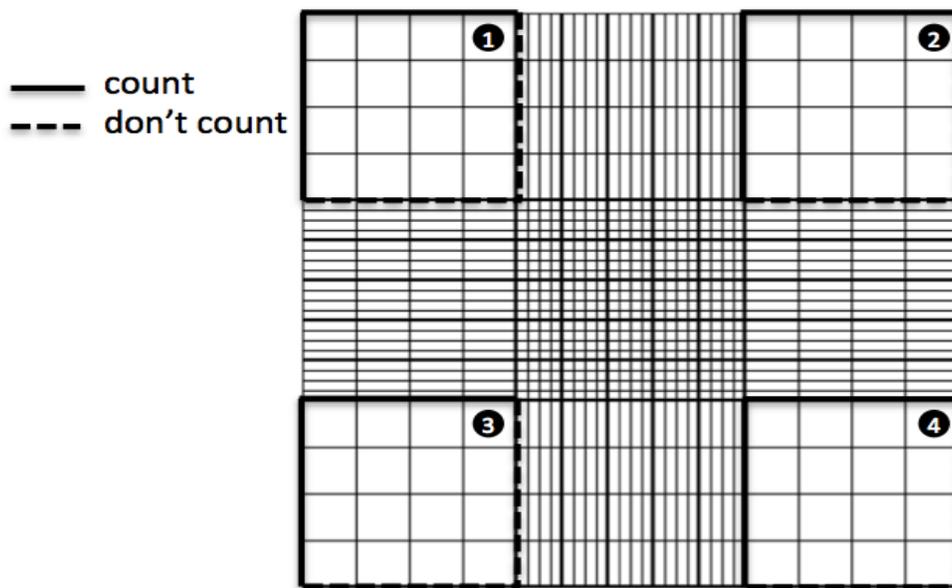


Figure.7.1.1 Representation of counting sides  
 (Source: <https://www.hemocytometer.org/hemocytometer-protocol/>)

### Requirements

- 7.11 EDTA-anticoagulated blood or capillary blood is preferred
- 7.12 Neubauer Chamber

### **WBC pipette:**

This is a bulb pipette having a long stem with a capillary bore and a pointed tip. The bulb contains a red bead inside. A small rubber tube provided with a mouth piece is connected to the small narrow portion above the bulb for sucking blood and fluid into the pipette. WBC pipette is like RBC pipette but here the bulb has a white glass bead in place of red and the third graduation mark which lies just above the bulb is “11” instead of “101”, this means the WBC pipette has 11 volumes from the tip up to “11” mark.

WBC diluting solution: Prepared as follows: a) Glacial acetic acid: 2.0 ml. b) 1 % (w/v) gentian violet (Methylene blue): 1.0 ml. c) Distilled water: 97 ml. This solution is stable at room temperature.

### **Procedure**

The blood sample is taken up to the “0.5” mark, then the diluting fluid is taken up to the “11” mark, this gives 1:20 dilution.

The mixing was done well for one minute.

The counting chamber and cover glass placed on central platform were cleaned thoroughly before use.

A drop of diluted blood was discharged into the chamber.

Focus on one of the four corner squares (each having 16 small squares) by turning objective lens to low power (10 X).

Count cells in all four corner squares.

The calculation formula for hemocytometer cell counts determines the number of cells within 1 m L (1 mm<sup>3</sup>) of blood. To make this determination, the total number of cells counted must be corrected for the initial dilution of blood and the volume of diluted blood used. The standard dilution of blood for leukocyte counts is 1:20; therefore, the dilution factor is 20. The volume of diluted blood used is based on the area and depth of the counting area.

The volume of a small square is specific to the hemocytometer. It is calculated by multiplying the width by the height (which are the same – usually 1mm each) by the depth (usually 0.1mm) of a small square. The area would be width X

height =  $1 \times 1 = 1 \text{ mm}^2$ , and the depth is 0.1 mm; therefore, the volume factor is  $0.1 \text{ mm}^3$ .

---

### **7.1.2 TOTAL NUMBER OF CELLS COUNTED (AVERAGE NO OF CELLS IN 4 CORNERS) •**

---

**DILUTION FACTOR • 1/VOLUME FACTOR = CELLS/MM<sup>3</sup>.**

7.1.3. For example, if 200 cells were counted in the four corner squares the WBC count is: average no of cells in 4 corners:  $200/4=50$

**7.1.4. Cell count/mm<sup>3</sup> =  $50 \times 20 \times 1/0.1 = 10,000 \text{ cells/mm}^3$  or  $10 \times 10^9/\text{L}$**

**The difference between the highest and lowest count for the eight squares should not exceed 10 cells.**

**Normal values:**

Adults: 4,000-11,000/cu mm ( $\mu\text{l}$ )

At birth: 10,000-25,000/cu mm ( $\mu\text{l}$ )

1 to 3 years: 6,000-18,000/cu mm ( $\mu\text{l}$ )

4 to 7 years: 6,000-15,000/cu mm ( $\mu\text{l}$ )

8 to 12 years: 4,500-13,500/cu mm ( $\mu\text{l}$ )

**Higher values – leukocytosis:**

Physiological: after effort, after meals, women: menstruation, pregnancy, childbed

Pathological: infection, inflammation, poisoning

**Lower values – leucopenia:** anaphylactic shock, viral infections, X-ray exposure.

---

### **DIFFERENTIAL COUNT OF LEUCOCYTES**

---

Differential count is the percent distribution of diverse white cells in the peripheral blood. The manual differential white blood cell count is performed to determine the relative number of each type of white blood cell present in the blood. A study of red blood cell, white blood cell, and platelet morphology is also performed.

## **Principle:**

The blood smear stained with a polychromatic stain is used for the determination of the ratio of the various white blood cells in the sample, for the examination of their morphology, and for the identification of pathological cells in the blood. The number of each type of white cell is then expressed as a percentage of the total number of cells.

---

## **REQUIREMENTS:**

---

- 7.1 May-Grunwald solution (concentrated methyl blue and eosin in methanol-glycerin)
- 7.2 Neutral distilled water □
- 7.3 Giemsa solution (azureosin in glycerin solution)

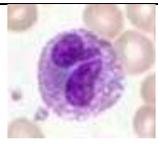
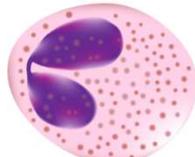
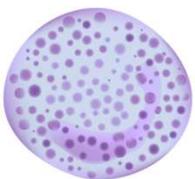
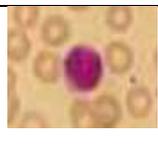
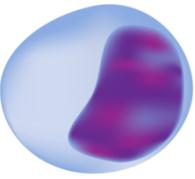
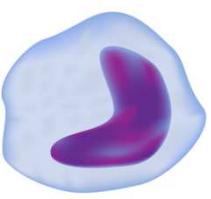
## **Procedure:**

Take a clean glass slide and put a small fresh blood drop from the pricked fingertip on the edge of the slide. The slide with the blood drop is placed on another clean slide at an angle of 45 degrees. After the blood drop has spread along the edge of the first slide push this slide slowly over the other. A thin blood film is produced. Keep the angle at 45 degrees between the slides throughout this procedure.

Let the smear dry.

Place a dry smear on the staining stand. Cover the smear with concentrated May- Grunwald solution. The methyl alcohol in the solution fixates the smear.

After 3 min, without removing the May-Grunwald solution, the dye is diluted with distilled water. The volume of distilled water should be the same as that of the dye. The eosin works only when diluted. After 1 min, let the dye flow off the slide. Do not wash the slide. Add freshly diluted Giemsa solution and wait for 15 min. (Dilution of the Giemsa solution: mix 1 ml of the solution with 20 ml of distilled water.)After 15 minutes, repeatedly wash off the Giemsa solution with distilled water and soak up the remaining stain with a filter paper. Use immersion lens when examining the smear. At least 200 white blood cells should be identified and recorded. A cell can be identified by evaluating the size, the nucleus and the shape of the cell, and by examining the staining and the granulation of the cytoplasm. The percentages of the various cell types are determined.

Type	Microscopic Appearance	Diagram	Nucleus
Neutrophil			multilobed
Eosinophil			bi-lobed
Basophil			Nucleus lobulation incomplete (varying shape), may not be clearly seen.
Lymphocyte			deeply stained, eccentric
Monocyte			kidney shaped

*Table 7.1.2. Diagrammatic representation of the different White Blood Cells*

**Granulocytes:** Neutrophil Multilobed nucleus, usually 2-5 lobes connected by chromatin threads purplish blue in colour. Cytoplasm plenty, bluish pink with plenty of fine pink granules. Cell outline is usually distinct.

Eosinophil Nucleus usually bibbed (two purple lobes) and spectacle shaped or horse shoe shaped, purplish blue in color, cytoplasm plenty, pink in color, orange red granules (Strongly acidophilic) not plenty, Cell outline not distinct.

Basophil: Nucleus lobulation incomplete (varying shape), Purplish blue in color, Cytoplasm plenty, bluish black coarse granules, may overlie the nucleus so that the nucleus may not be clearly seen.

## Agranulocytes

Monocytes: Largest WBC, Nucleus large round or oval deeply indented to have kidney shape, pale purplish blue in color, usually eccentric, cytoplasm greyish blue, (slaty grey) plenty. Lymphocytes Nucleus: big, chromatin-rich, purple/violet; narrow pale blue cytoplasmic border. Cell outline is distinct.

Reference ranges for differential white blood cell count in normal adults is as follows:

Neutrophils -  $2.0-7.0 \times 10^9/l$  (40-80%)

Lymphocytes -  $1.0-3.0 \times 10^9/l$  (20-40%)

Monocytes -  $0.2-1.0 \times 10^9/l$  (2-10%)

Eosinophils -  $0.02-0.5 \times 10^9/l$  (1-6%)

Basophils -  $0.02-0.1 \times 10^9/l$  (< 1-2%)

---

## ESTIMATION OF TOTAL ERYTHROCYTE COUNT PER CUBIC MM OF BLOOD

---

Erythrocytes or Red blood cells circulate in the blood and carry oxygen throughout the body. They are produced in the bone marrow and then released into the bloodstream as they mature. RBCs have a typical lifespan of about 120 days and are continuously renewed and replaced as they age and degrade or are lost through bleeding. A moderately stable number of RBCs is maintained in the circulation by increasing or decreasing the rate of production by the bone marrow.

Some conditions affect RBC production and may cause an increase or decrease in the number of mature RBCs released into the blood circulation. While an RBC count can be used to detect a problem with red blood cell production and/or lifespan, it cannot determine the underlying cause. Erythrocyte's count is typically performed as part of a complete blood count (CBC) and may be used as part of a health checkup to screen for a variety of conditions. This test may also be used to help diagnose and/or monitor a number of diseases that affect the production or lifespan of red blood cells.

**Principle:** The blood specimen is diluted 1:200 with the RBC diluting fluid and cells are counted under high (40x objective) by using a counting chamber. The number of cells in undiluted blood are calculated and reported as the total number of cells per cubic mm of whole blood.

## Requirements

EDTA-anticoagulated blood or capillary blood is preferred

Neubauer Chamber

RBC pipette:

It consists of capillary tube, central tube and three graduation marks. Capillary tube is opened at both ends. One end of the tube is narrow while the other end is broad. For sucking a rubber tube fixed to the broad end. The bubble of the pipette has a red glass bead inside. This is a bulb pipette having a long stem with a capillary bore and a pointed tip. The bulb contains a red bead inside. A small rubber tube provided with a mouth piece is connected to the small narrow portion above the bulb for sucking blood and fluid into the pipette. The pipette has three markings on it, “0.5” mark in the middle of the stem, “1” mark at the junction between stem and bulb, and “101” mark above the bulb. The total volume of the pipette is 101 parts, of which one part is in the stem and 100 parts in the bulb. Blood is drawn to “0.5” mark and then diluting fluid is drawn up to the mark “101”. The dilution of blood is 1:200.

RBC diluting fluid (Hayem’s fluid): Prepared as follows:

Sodium chloride (NaCl) - 0.5g

Sodium Sulphate (Na<sub>2</sub>SO<sub>4</sub>) - 2.5 g

Mercuric Chloride (HgCl<sub>2</sub>) - 0.25 g

Distilled water - 100 ml

Note\*- Sodium chloride and Sodium sulphate together keeps the isotonicity of fluid. Sodium sulphate also prevents clumping of red cells. Mercuric chloride fixes the cells and acts as a preservative.

## Procedure:

Clean and dry the counting chamber and put on the special cover slip provided. Focus under the high-power objective and identify the RBC counting area. Take a small quantity of diluting fluid in a watch glass and keep aside. Clean the fingertip using

rectified spirit and make a deep prick with a sterile lancet, so that blood comes out freely without squeezing. Wipe off the first drop which may contain tissue fluid also. Allow a good-sized blood drop to form hanging drop and keep the pointed tip of the pipette touching the drop. Suck in blood up to the 0.5 mark carefully, without any air bubble. Excess blood at the tip of the pipette is removed using a blotting paper or piece of cotton. Immediately, diluting fluid from the watch glass is sucked in up to the 101 marks without any air bubble by keeping the pipette in vertical position.

Then thoroughly mix the blood and diluting fluid in the pipette by gently rolling the pipette held horizontally between the palms and keep aside. Now take out the counting chamber for charging discard first few drops from the pipette, as the stem contains only diluting fluid. Bring one small drop of diluted blood at the tip of the pipette, to the edge of the cover slip on the counting chamber at an angle of about 45°. The fluid enters by capillary action under the cover slip and fills the counting chamber.

Focus the RBC counting area under high power. Keep the counting chamber undisturbed about 3 minutes for the cells to settle down in the counting area, and start counting.

Each chamber is a square of the counting chamber i.e. 1 sq mm area is marked "R" (Figure. 3). This 1 sq mm area is divided into 25 small squares by triple lines i.e. each of these small squares has an area of 1/25 sq mm and the depth is 0.1 mm; therefore, the volume factor is 0.004 mm<sup>3</sup>.

**7.4 average number of RBC counted (total number of found RBC/number of squares) • dilution factor • 1/volume factor = cells/mm<sup>3</sup>.**

7.5 For example, if average no of cells is =50

7.6 **Cell count/mm<sup>3</sup> = 50x 200 x 1/0.004 = 2.5x10<sup>6</sup> cells/mm<sup>3</sup>**

At least 5 squares, each having 16 smallest squares (preferably 4 corners and 1 central) should be counted to obtain a satisfactory average and a better dispersal value. While counting each small square, cells touching the top and left margin of each square should be omitted and cells touching bottom and right margin of each square should be counted.

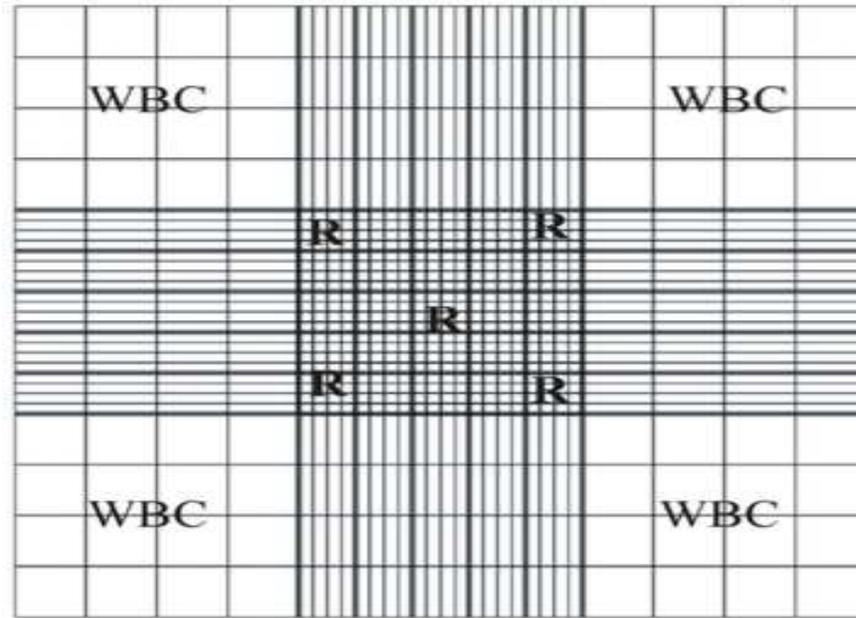


Figure. 7.1.3. Diagrammatic representations of counting area in hemocytometer. R=RBC counting area, W=WBC counting area.

Normal range:

Males:  $4.7\text{--}5.2 \times 10^6/\text{mm}^3$

Females:  $4.2\text{--}4.7 \times 10^6/\text{mm}^3$

Newborn, young children:  $5.5 - 6 \times 10^6/\text{mm}^3$

**Higher values – polycythemia:**

Physiological: gender, age, high altitude, effort

Pathological: chronic bone marrow, lung or heart diseases

**Lower values – anemia**

- excessive bleeding
- decreased red blood cell production
- increased red blood cell destruction

---

## DETERMINATION OF HEMOGLOBIN (%) IN BLOOD

---

Hemoglobin, is noncontinuing metalloprotein of erythrocytes that transports  $\text{O}_2$  from the respiratory organs to the rest of the body. In mammals, this protein makes up about 96% of the red blood cells' dry content (by weight), and around 35% of the total

content (including water). It is comprised of **Heme** and **Globin**. Hemoglobin is a tetramer consisting of two pairs of similar polypeptide chains called globin chains. To each of the four chains is attached heme which is a complex of iron in ferrous form and protoporphyrin. The major (96%) type of hemoglobin present in adults is called HbA, having 2 alpha globin chains and 2 beta globin chains ( $\alpha_2\beta_2$ ). In adults, a minor amount of HbA2 ( $\alpha_2\beta_2$ ) is also present and constitutes less than 3.5%.

During embryonic and fetal life, other different types of hemoglobin's predominate. These include Gower-1 (present in early embryonic life), Gower-2 and Hb Portland. After the eighth week of development, embryonic hemoglobin's are replaced by Fetal hemoglobin HbF( $\alpha_2\gamma_2$ ). This remains the predominant hemoglobin until after birth and constitutes 50-90% of the total hemoglobin. After birth, its concentration decreases to less than 2% by 30 weeks of age. HbA is then the predominant hemoglobin.

Genetic changes (mutations) in the globin genes cause alterations in the globin protein, resulting in structurally altered hemoglobin, such as hemoglobin S, which causes sickle cell, or a decrease in globin chain production (thalassemia). In thalassemia, the reduced production of one of the globin chains upsets the balance of alpha to beta chains and causes abnormal hemoglobin to form (alpha thalassemia) or causes an increase of minor hemoglobin components, such as HbA2 or beta thalassemia.

Red blood cells containing abnormal hemoglobin may not carry oxygen efficiently and may be broken down by the body sooner than usual (a shortened survival), resulting in hemolytic anemia. Normal Hgb values are as follows:

Women	12-16 g/100 ml blood (g/dl) (g%)
Men	14-18 g/100 ml blood (g/dl) (g%)
Newborn	14-20 g/100 ml blood (g/dl) (g%)

The hemoglobin value is decreased in anemia and increased in polycythemia and dehydration. Various methods are available for estimation of hemoglobin in the laboratory-

## **METHODS BASED ON DEVELOPMENT OF COLOR**

Sahli's or acid hematin method

Cyanmethemoglobin method

Oxyhemoglobin method

Alkaline hematin method

Out of these Cyanmethemoglobin method is the most recommended one.

## **MEASUREMENT OF OXYGEN COMBINING CAPACITY**

The oxygen combining capacity of blood is 1.34ml oxygen per gm of Hb. This is a measure of the function of Hb. It is not used routinely as it is not practical and gives results 2% lower than the other methods.

## **MEASUREMENT OF IRON CONTENT**

Iron content can be measured and converted to Hb by using the formula 0.347 mg iron per 100g Hb. It is however, impractical for routine use.

## **SAHLI'S OR ACID HEMATIN METHOD**

**Principle:** Anticoagulated Blood is mixed with 0.1N HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till its color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly from the graduation in the calibration tube.

### **Requirements:**

Sahli's Hemoglobinometer which consists of comparator box which has brown colored glass standard and a graduated hemoglobin tube which is marked in percent and gram.

Sahli's Hb pipette which is marked at 0.02 ml or 20  $\mu$ l).

Glass rod

Dropper

0.1N HCl

Distilled water

Venous blood collected in EDTA

## Procedure

Place 0.1N HCl into the graduated tube upto mark 2 grams.

With the help of Sahli's pipette, take blood sample exactly up to 20  $\mu$ l mark. Blood adhering to the outer part of the pipette is wiped away with the help of tissue paper (absorbent paper) or cotton.

Add blood sample to the N/10 hydrochloric acid solution which is placed into Sahli's graduated tube, mix the mixture with the help of a glass stirrer, and allow the tube to stand undisturbed for 10 minutes.

Add distilled water drop by drop into the mixture placed in Sahli's graduated tube, till the color of the solution matches that of the brown glass standard. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.

Take the reading of the lower meniscus from the Sahli's graduated tube in grams.

## Result

The Hb estimation of the given sample is ..... g/100 ml of blood/.....g/dl of blood/.....G%.

## Precautions

Pipetting of blood should be done cautiously.

Mix the blood properly with HCl by using stirrer.

Match the color cautiously.

**Note:** All the Sahli's graduated tube is marked in both percent and grams figures, this is because **(a)** different manufacturers of hemoglobinometers have different values as 100%, so that blood sample will yield different results on different instruments and **(b)** no single hemoglobin is can be evaluated as 100% since it is different according to the sex and age of the individual and altitude.

## Disadvantages of Sahli's method:

It is impossible to match the color perfectly of the mixture into the Sahli's graduated tube with the brown glass standard. Minimum 1 hour is required for the maximum color development of acid hematin because 95% color of acid hematin is attained at the end of 10 minutes.

All hemoglobin's (oxyhemoglobin, carboxyhemoglobin, Sul hemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value. Fetal hemoglobin is also not converted to acid hematin and therefore this technique is not appropriate for infants. The acid hematin solution is not firm and stable, and the color development is slow. Lights may affect the visual comparison of color. Color of the brown glass standard dims with time. Individual variation in matching of color is seen.

#### **CYANMETHEMOGLOBIN METHOD**

This method is recommended by International Committee for Standardization in hematology. This is because in this method all type of hemoglobin are transformed to cyanmethemoglobin (except Sul hemoglobin).

**Principle:** When Blood is mixed with a solution of potassium cyanide, potassium ferricyanide and Drabkin's solution, the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide transforms hemoglobin to methemoglobin, and methemoglobin combines with potassium cyanide to produce hemoglobincyanide (cyanmethemoglobin). This way all types of hemoglobin present in blood are entirely transformed to a single compound cyanmethemoglobin (HiCN). The amount of cyanmethemoglobin can be measured spectrophotometrically at a wavelength of 540 nm on a spectrophotometer and compared to known hemoglobin standards in order to determine the hemoglobin concentration of the unknown sample.

#### **Requirements:**

Hb pipette

Spectrophotometer

Drabkin's solution pH 7.0-7.4 which contains

Potassium cyanide 50 mg

Potassium ferricyanide 200 mg

Potassium dihydrogen phosphate 140 mg

Nonionic detergent 1 ml

Distilled water 1 L

## EDTA-anticoagulated blood

### Hb standard

The solution should be clear and pale yellow in color. When measured against water as a blank in a spectrometer at a wavelength of 540 nm, the absorbance must be zero. The solution is unstable if exposed to light and can be stored at room temperature in brown borosilicate bottles for several months. However, if the room temperature is higher than 30°C, the solution should be stored in a refrigerator but brought to room temperature before use. The solution must never be frozen.

The pH of the solution must be checked every month.

Discard the solution, if found to be turbid/if pH is outside range/ its absorbance is not zero at 540 nm.

Do not pipette Drabkin's solution by mouth.

### **Procedure**

Take 5ml of Drabkin's solution in a test tube.

Mix the blood sample by gentle inversion and draw 0.02ml of blood into the Hb pipette. Wipe the outer surface of the pipette to remove excess blood.

Place the pipette into the tube containing Drabkin's solution and slowly expel the blood into the solution. Mix well and let it stand undisturbed for 5min.

Measure the absorbance of this solution at 540nm in a spectrophotometer after adjusting the OD at 0 by using Drabkin's solution as blank.

Calculate the hemoglobin concentration using a standard curve. (A WHO International reference HiCN standard is available commercially. This solution is stable for years. The exact concentration of Hb present in the solution is indicated on the label.)

### **Advantages**

- 7.6.3 All forms of Hb except sulphhemoglobin are converted to HiCN.
- 7.6.4 Visual error is not there as no color matching is required.
- 7.6.5 Cyanmethemoglobin solution is stable and its color does not fade with time so readings may not be taken immediately.
- 7.6.6 Absorbance may be measured soon after dilution.

A reliable and stable reference standard is available from World Health Organization for direct comparison.

**Disadvantages**

Diluted blood has to stand for a period of time to ensure complete conversion of Hb. Potassium cyanide is a poisonous substance and that is why Drabkin's solution must never be pipetted by mouth.

The rate of conversion of blood containing carboxyhemoglobin is slowed considerably. Prolonging the reaction time to 30min can overcome this problem.

Abnormal plasma proteins cause turbidity when blood is diluted with Drabkin's solution. A highleucocytecountalsocausesturbidityondilutionofblood. Centrifuging the diluted blood can help overcome the turbidity.

**Precautions:**

Mixing of blood sample should be done adequately.

Incorrectly calibrated pipettes and spectrophotometer can give false results.

**Source of Error:**

Incomplete conversion of Hb to cyanmethemoglobin

Lipemic specimen

High concentration of WBC's or platelets

Does not measure Sulfhemoglobin

---

**7.7- RBC INDICES**

---

Blood indices are specifically meant for erythrocytes. The number, shape, volume and the color of the red blood cells indicate the quality of blood. So, these features are named as blood indices. Red blood cell (RBC) indices are part of the complete blood count (CBC) test. It measures the size, shape, and physical characteristics of the RBCs.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. Termed *RBC indices*, these values are useful in elucidating the etiology of

anemias. Red cell indices can be calculated if the values of hemoglobin, hematocrit (packed cell volume; PCV), and red blood cell count are known. With the general availability of electronic cell counters, red cell indices are now automatically measured in all blood count determinations.

## **DIFFERENT BLOOD INDICES**

Following are the various blood indices:

Mean Corpuscular Volume (MCV)

Mean Corpuscular Hemoglobin (MCH)

Mean Corpuscular Hemoglobin Concentration (MCHC)

Colour Index (CI).

### **CALCULATION OF BLOOD INDICES**

Blood indices are calculated by using different formulas. These calculations require the values of RBC count, hemoglobin content and PCV

#### **Mean Corpuscular Volume (MCV)**

MCV is the average volume of a single red blood cells. It is expressed as femtoliters ( $10^{-15}$ ; fl) or in cubic microns (cu. $\mu$ ). The normal is 90 cu. $\mu$  (78 to 90 cu. $\mu$ ). When MCV is increased, the cell is known as a macrocyte and when it is decreased, the cell is called microcyte. MCV is more in pernicious anemia and megaloblastic anemia in which the red blood cells are macrocytic in nature. MCV is less in microcytic anemia. 
$$\text{MCV} = \frac{\text{Volume of packed cells in ml per 1,000 ml of blood}}{\text{Red blood cells in millions per cu mm of blood}}$$

#### **Mean Corpuscular Hemoglobin (MCH)**

This is the quantity or amount of hemoglobin present in one red blood cell. It is expressed in Picogram (pg). The normal value of MCH is 30 pg (27 to 32 pg.). It decreases or remains normal in pernicious anemia and megaloblastic anemia, in which red blood cells are macrocytic and normochromic or hypochromic. It decreases in hypochromic anemia. When MCH is normal, it is called normochromic state.

$$\text{MCH} = \frac{\text{Hemoglobin in grams per 1000 ml of blood}}{\text{RBC count in millions per cu mm of blood}}$$

### **Mean Corpuscular Hemoglobin Concentration (MCHC)**

This is the concentration of hemoglobin in one red blood cell. It is the amount of hemoglobin expressed in relation to the volume of one red blood cell. So, the unit of expression is percentage. This is the most important absolute value in the diagnosis of anemia. The normal value of MCHC is 30% (30 to 38%). It is decreased in iron deficiency anemia in which, red blood cells are microcytic and hypochromic.  $MCHC = \frac{\text{Hemoglobin in grams per 100 ml of blood} \times 100}{\text{Volume of packed cells per 100ml of blood}}$

**Color Index (CI):** This is the ratio between the percentage of hemoglobin and the percentage of red blood cells in the blood. Actually, this is the average hemoglobin content in one cell of a patient compared to the average hemoglobin content in one cell of a normal person. The normal color index is 1.0 (0.8 to 1.2). This was widely used in olden days. However, it is useful in determining the type of anemia. It is raised in pernicious anemia and megaloblastic anemia. It is reduced in iron deficiency anemia. And, it is normal in normocytic normochromic anemia.

For determining the color index, the hemoglobin content of blood in gm/dl and the number of red cell in millions/cm<sup>3</sup> are first determined. The percentage of each is then calculated taking 14.5gm of hemoglobin/dl and 5 million of red cells/cu mm as 100 percentage. The color index is then calculated as follows:

Where,  $Hb \% (\text{Hb content expressed as a \% of normal}) = \frac{\text{Hemoglobin content in the subject} \times 100}{\text{Normal Hemoglobin content}}$

$RBC\% (\text{RBC count expressed as a \% of normal}) = \frac{\text{RBC count in the subject} \times 100}{\text{Normal RBC Count}}$

$\text{Color Index} = \frac{Hb \%}{RBC\%}$

---

## ***7.8- SUMMARY***

---

Blood is a connective tissue that consists of cells and cell fragments (named formed elements) surrounded by a liquid extracellular matrix (or blood plasma). A number of diseases and physiological processes alter its characteristic values and composition. The

array of parameters in such assay help to confer a correct diagnosis of a patient. RBCs are biconcave discs. In their mature form, they lack a nucleus and play an important role in the transport of respiratory gases. Leucocytes, also known as white blood cells, contain nuclei and all cellular organelles, are classified as either granular or agranular. Platelets or thrombocytes are a component of blood whose function is to stop bleeding by clumping and clotting blood vessel injuries. In the estimation of total and differential leucocyte in blood, the degree of increase or decrease in leukocytes reflects the type and severity of the infection and the response of the body.

RBC count can be used to detect a problem with red blood cell production and/or lifespan. Higher values indicate polycythemia, whereas lower hints anemia.

Hemoglobin, is an iron-containing protein of erythrocytes that transports O<sub>2</sub> from the respiratory organs to the rest of the body. The hemoglobin value is decreased in anemia and increased in polycythemia. Red blood cell (RBC) indices are part of the complete blood count (CBC) test, that measures the size, shape, and physical characteristics of the RBCs. It includes Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and color index. RBC indices are useful in elucidating the etiology of anemias

---

## ***7.9- TROUBLESHOOTING***

---

These interferences can result misleading results for the parameters listed below:

In WBC counting: Certain unusual RBC abnormalities that resist lysing, nucleated RBCs, fragmented WBCs, any unlysed particles greater than 35 fL, very large or aggregated platelets as when anticoagulated with oxalate or heparin.

In RBC counting: Very high WBC count, high concentration of very large platelets, agglutinated RBCs and RBCs smaller than 36 fL.

In Hb estimation: Very high WBC count, severe lipemia, certain unusual RBC abnormalities that resist lysing, anything that increases the turbidity of the sample such as elevated levels of triglycerides.

In MCV evaluation: Very high WBC count, high concentration of very large platelets, agglutinated RBCs, RBC fragments that fall below the 36-fL threshold, rigid RBCs.

In MCH evaluation: Known factors that interfere with the parameters used for its computation, Hb and RBC.

In MCHC evaluation: Known factors that interfere with the parameters used for its computation, Hb, RBC and MCV.

While RBC counting, clean the fingertip using rectified spirit and make a deep prick with a sterile lancet, so that blood comes out freely without squeezing. Wipe off the first drop which may contain tissue fluid also and interfere with the results.

In Hematin's method of Hb estimation, improper mixing of blood with HCl can give false results. Moreover, all hemoglobins (oxyhemoglobin, carboxyhemoglobin, sulphhemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value.

It is important to know that presence of abnormal plasma proteins and high leucocyte count can cause turbidity when blood is diluted with Drabkin's solution in Cyanmethemoglobin method of Hb estimation. Centrifuging the diluted blood can help overcome the turbidity.

---

## **7.8- GLOSSARY**

---

**Allergy-** conditions caused by hypersensitivity of the immune system to something in the environment that usually causes little or no problem in most people.

**Anaphylactic shock-** an extreme, often life-threatening allergic reaction to an antigen to which the body has become hypersensitive.

**Anemia-** is a decrease in the total amount of red blood cells (RBCs) or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen.

**Biconcave-** Concave on both the sides.

**Etiology-** the cause, set of causes, or manner of causation of a disease or condition.

**Fetal hemoglobin-** or **foetalhaemoglobin**, (also **hemoglobin F**, HbF, or  $\alpha_2\gamma_2$ ) is the main oxygen transport protein in the human fetus during the last seven months of development in the uterus and persists in the newborn until roughly 6 months old.

**Histamine-** **Histamine** is produced by basophils and mast cells as part of a local immune response to cause inflammation.

**Hormones-** chemical messengers, secreted directly into the blood, which carries them to organs and tissues of the body to exert their functions.

**Inflammation**-is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators.

**Isotonic**- denoting or relating to a solution having the same osmotic pressure as some other solution, especially one in a cell or a body fluid.

**Leucopenia**- an abnormal reduction in the number of white blood cells in the blood.

**Lipemia**- The presence of an abnormally high concentration of lipids in the circulating blood.

**Menstruation**- also known as a period or monthly, is the regular discharge of blood and mucosal tissue from the inner lining of the uterus through the vagina.

**Metalloprotein** is a generic term for a protein that contains a metal ion cofactor.

**Mutation**- alteration of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.

**Pernicious anemia**- a condition in which the body can't make enough healthy red blood cells because it doesn't have enough vitamin B12.

**pH**- a numeric scale used to specify the acidity or basicity of an aqueous solution. It is approximately the negative of the base 10 logarithm of the molar concentration, measured in units of moles per liter, of hydrogen ions.

**Polycythemia**- occurs when excess red blood cells are produced as a result of an abnormality of the bone marrow.

**Smear**-a sample of tissue or other material taken from part of the body, spread thinly on a microscope slide for examination, typically for medical diagnosis.

**Thalassemia** is a blood disorder passed down through families (inherited) in which the body makes an abnormal form of hemoglobin.

---

## ***7.9- TERMINAL QUESTIONS AND ANSWERS***

---

Q.1 What is Homeostasis?

Ans. The tendency to maintain a stable, relatively constant internal environment is called homeostasis.

Q.2 What is hemoglobin?

Ans. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells. It carries oxygen from the respiratory organs (Lungs) to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism.

Q.3 What is anticoagulant?

Ans. Anticoagulants, commonly referred to as blood thinners, are chemical substances that prevent or reduce coagulation of blood, prolonging the clotting time.

Q.4 What is Thrombocytopenia?

Ans. Thrombocytopenia is a condition characterized by abnormally low levels of thrombocytes, also known as platelets, in the blood.

Q.5 What is carboxyhemoglobin?

Ans. Carboxyhemoglobin or carboxyhaemoglobin is a stable complex of carbon monoxide and hemoglobin (Hb) that forms in red blood cells upon contact with carbon monoxide (CO).

Q.6 What is Thrombosis?

Ans. Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system.

Q.7 How EDTA works as an anticoagulant?

Ans. Ethylenediaminetetraacetic acid (EDTA) strongly and irreversibly chelates (binds) calcium ions, preventing blood from clotting.

## **OBJECTIVE TYPE QUESTIONS:**

Q.1. Which blood cells and blood elements are included in a CBC test?

- A. Red blood cells (also called erythrocytes)
- B. White blood cells
- C. Platelets
- D. All of the above

Q.2. What do platelets do?

- A. Carry oxygen from the lungs
- B. Carry waste products from the cells
- C. Fight infection
- D. Help stop bleeding by initiating clots

E. All of the above

Q.3. Which is a symptom of not having enough red blood cells or hemoglobin (a condition called anemia)?

- A. Itching
- B. Nausea
- C. Fever
- D. Fatigue

Q.4. What are neutrophils?

- A. Immature red blood cells
- B. A type of white blood cell
- C. A type of platelet
- D. A type of bacteria

Q.5. Which blood cell can be described as being a biconcave disc?

- A. Platelets
- B. Neutrophills
- C. RBCs
- D. Eosinophills

Answers: 1D, 2D, 3D, 4B, 5C.

---

## ***7.10- REFERENCES AND SUGGESTED READINGS***

---

Harper's Biochemistry – Murray, Granner, Mayes, Rodwell – Prentice Hall International Inc.

Blood Chemistry and CBC Analysis: Clinical Laboratory Testing from a Functional Perspective-by Dr Dicken Weatherby (Author), **Dr Scott** Ferguson (Author)-Emperors Group LLC (September 20, 2004)

---

## **Unit 8: BIOCHEMISTRY EXPERIMENTS**

---

### **CONTENTS**

8.1 Objectives

8.2. Introduction

8.2.1 Carbohydrates

8.2.2 Proteins

8.2.3 Lipids

8.4 . Test for carbohydrates, proteins and lipids

8.5 . Chemical test of urine for the presence of urea, sugar, proteins and ketone bodies

8.6 .Summary

8.7. Troubleshooting

8.8. Glossary

8.9. Terminal questions & answers

8.10References

---

## **8.1- OBJECTIVES**

---

To know about the Biomolecules and their basic nature i.e. Carbohydrates, Protein and lipids. To study the chemical basis of the tests for carbohydrate, proteins and lipids. To apply these basic tests as above and many others, for the chemical analysis of urine for the presence of urea, sugar, proteins and ketone bodies.

---

## **8.2. INTRODUCTION**

---

A **biomolecule** or **biological molecule** is an organic molecule that is present as an essential component of living organisms, including large macromolecules such as proteins, carbohydrates, lipids, and nucleic acids.

This chapter throws light upon the top three classes of biomolecules.

Carbohydrates

Lipids

Proteins

### **8.2.1 CARBOHYDRATES**

Carbohydrates constitute a versatile class of molecules. Energy from the sun netted by green plants, algae, and some bacteria during photosynthesis is stored in the form of carbohydrates. They are the metabolic pioneers of virtually all other biomolecules and their oxidation is the central energy-yielding pathway in most no photosynthetic cells that sustains life.

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical formula  $(\text{CH}_2\text{O})_n$ , some also contain nitrogen, phosphorus, or sulfur. They can be classified into following different groups

### **Monosaccharides:**

The monosaccharides are also called simple sugars and have the formula  $(\text{CH}_2\text{O})_n$ , consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose. Monosaccharides of more than four carbons tend to have cyclic structures. Monosaccharides cannot be broken down into smaller sugars under mild conditions.

### **Oligosaccharides:**

Oligosaccharides originate their name from the Greek word *oligo*, meaning “few,” and consist of from two to ten simple sugar molecules joined by characteristic linkages called glycosidic bonds. Disaccharides are common in nature, and trisaccharide’s also occur frequently. In cells, most oligosaccharides consisting of three or more units do not occur as free entities but are joined to non-sugar molecules (lipids or proteins) in glycoconjugates.

### **Polysaccharides:**

As name suggests, polysaccharides are polymers of the simple sugars and their derivatives containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units. They may be either linear such as cellulose or branched polymers such as glycogen and may contain hundreds or even thousands of monosaccharide units. Their molecular weights range up to 1 million or more.

## **8.2.2 LIPIDS**

lipids are a chemically diverse group of compounds with remarkable feature of their insolubility in water. They are the foremost stored forms of energy in many organisms. Phospholipids and sterols form structural foundations of biological membranes. They act as thermal insulators under subcutaneous tissues. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchors for proteins.

There is no single, internationally accepted system of classification for the lipids available. However, on the basis of chemical and some structural quality they are classified as below:

### **Classification of Lipid:**

#### **On the basic of Reaction with NaOH / KOH:**

Saponifiable

ii) Non Saponifiable

**On the basis to Products of Hydrolysis and structural complexity of Lipids, these are categorized into following subtypes:**

**Simple Lipids:** On hydrolysis gives fatty acids and alcohol (trihydric or monohydric)

**Compound lipids: (Complex lipids):**

On hydrolysis gives phosphoric acid, various sugars, sphingosine, ethanolamine and serine in addition to fatty acids and glycerol.

**Phospholipid:** In addition to fatty acids and alcohol presence, they also contain phosphorous, nitrogenous bases and other substitution groups. e.g. Lecithin, Cephalin.

**Glycolipids:** Lipids containing carbohydrates are referred as glycolipids. They contain a special alcohol moiety called sphingosine or sphingol and nitrogenous base. They do not have phosphorous. Gangliosides and cerebroside are examples of compounds lipids.

**Sulpholipids:** Lipids with sulfate groups are referred as sulpholipids.

**Lipoproteins:** lipids contain protein then they are known as lipoproteins. Example chylomicrons, VLDL, LDL and HDL.

### **3) Derived Lipids:**

Derived lipids are lipids obtained upon hydrolysis of the simple and complex lipids and still retaining the characteristics of lipids. They are classified further into two types namely fatty acid and alcohol. Fatty acid: They are the hydrolyzed products of simple and complex lipids. They mainly of mono carboxylic acids. They may be saturated or unsaturated. Their length varies between C4 to C30. Palmitic Acid C16

Alcohol: It includes molecules with OH group as functional group. It also varies from simple straight chain alcohol like glycerol to complex cyclic alcohols like cholesterol.

**Miscellaneous:**

This includes the lipids which cannot be grouped under any of the above headings. They include

Aliphatic hydrocarbons found in liver fat and certain hydrocarbons found in bees wax and plant waxes.

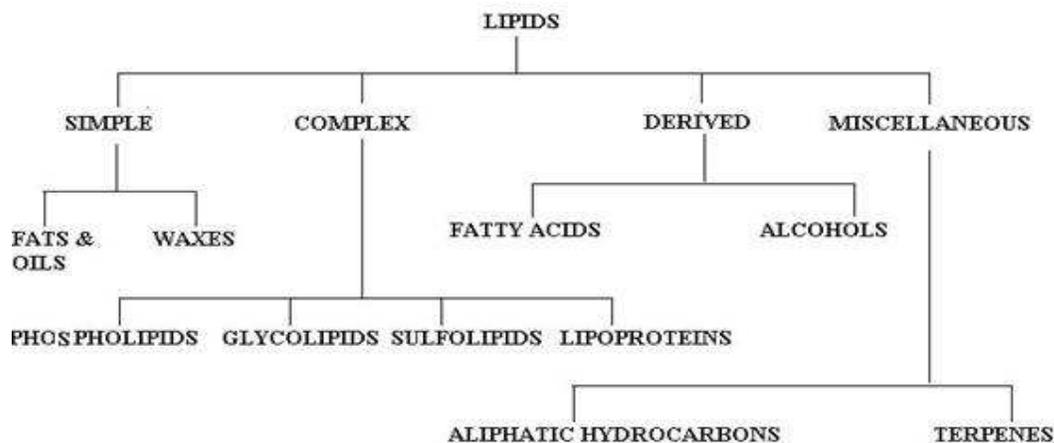
Terpenes

Carotenoids.

Squalene is a hydrocarbon found in shark and mammalian liver and human sebum.

Vitamin E and K.

Figure 8.2.1 Classification of Lipids



*Figure 8.2.1 Classification of Lipids*

---

## 8.2.3 PROTEINS

---

Proteins are polymers of amino acids that are linked head to tail to its neighbor from carboxyl group to amino group, through formation of covalent **peptide bonds**, a type of amide linkage. Proteins can be broken down to their component amino acids. It is the most

abundant biological macromolecules constituting more than 50% of the dry weight of cells. It occurs in great diversity; thousands of different kinds, ranging in size from relatively small peptides to huge polymers with molecular weights in the millions, may be found in a single cell.

**Classification of Protein (with examples):**

**A) Based on composition    B) Based on structure**

**7.6.6.1.1 Based on Composition:**

- 7.6.7 Simple Proteins
- ii) Conjugated Proteins
- iii) Derived proteins

**7.6.7.1 Simple Proteins:**

- 7.6.7.1.1.1 Albumins
- b) Globulins
- c) Protamine
- d) Prolamines
- e) Scleroproteins

**7.6.7.1.2 Conjugated Proteins:**

- 7.6.7.1.2.1.1 Glycoproteins
- b) Chromoproteins
- 7.6.7.1.2.1.2 Metalloproteins
- d) Lipoproteins
- e) Nucleoproteins
- f) Phosphoprotein

**7.6.7.1.3 Derived Proteins:** Derivatives of proteins due to action of heat, enzymes, or chemical reagents.

- 7.6.7.1.3.1.1 Primary Derived
- b) Secondary Derived

**7.6.7.2 Based on Structure:**

- 7.6.7.2.1 Fibrous
- ii) Globular

---

## 7.7 TESTS FOR CARBOHYDRATES, PROTEINS & FATS

---

### TESTS FOR CARBOHYDRATES

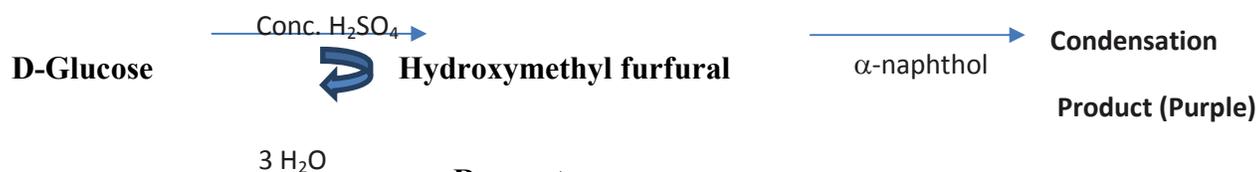
#### QUALITATIVE TESTS

##### Molisch's test

**7.8 Principle:** This is general test for all carbohydrates. Conc.  $\text{H}_2\text{SO}_4$  hydrolyses glycosidic bonds to yield monosaccharides which in the presence of acid get dehydrated to form furfural and its derivatives. These products react with sulphonated  $\alpha$ -naphthol to give a purple complex.

**7.9 \*Polysaccharides & glycoproteins also give a positive reaction.**

##### 7.10 Reaction:



##### Reagents:

Conc.  $\text{H}_2\text{SO}_4$

$\alpha$ -naphthol: 5% (w/v) in ethanol (freshly prepared)

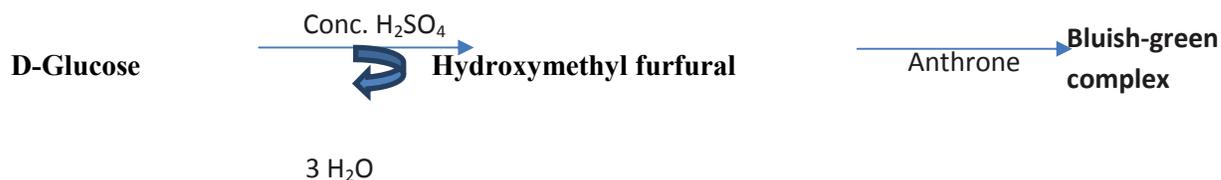
##### Procedure:

Add 2-3 drops of  $\alpha$ -naphthol solution to 2 ml of test solution and mix it. Add 1 ml of Conc.  $\text{H}_2\text{SO}_4$  to flow down the side of the test tube, forming the layer between the two. Observe any color change at the junction of two layers. Appearance of purple color indicates the presence of carbohydrates in the test solution.

##### Anthrone test

**Principle:** This is another general test for all carbohydrates. In this the furfural reacts with anthrone to give bluish green coloured complex

##### 7.11 Reaction:



**Materials & Reagents:**

Boiling water bath (BWB)

Anthrone reagent: 0.2% (w/v) anthrone solution in Conc. H<sub>2</sub>SO<sub>4</sub>

**Procedure:**

Add 0.5-1 ml of test solution to about 2ml of Anthrone reagent and mix thoroughly.

Observe the change of color to bluish green. If not, keep the tubes in BWB for 10 min.

**Iodine test**

**Principle:** This is rapid test for detection of amylose, amylopectin and glycogen. These polysaccharides form colored adsorption complexes with iodine. Intensity of the color depends upon the nature & number of sugar residues. For e.g. Starch gives dark blue, dextrin gives violet and glycogen gives red color with iodine. Nature of these complexes is not well-known.

**Reagents:**

Iodine Solution: 0.005 N iodine solution in 3% (w/v) potassium iodide solution.

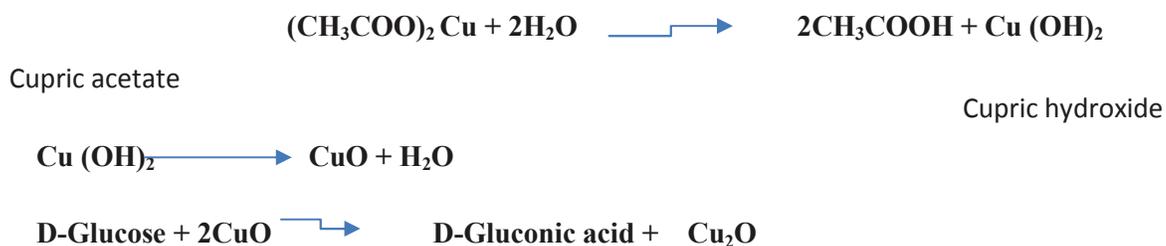
1% test solutions starch, glycogen etc.

**Procedure:**

Add 4-5 drops of Iodine Solution to 1 ml of test solution in the test tube & mix the contents gently. Observe the color of the product.

**Barford's Test**

**Principle:** This test is to differentiate between reducing mono and disaccharides. Monosaccharides show reduction within 2-4 min of heating whereas disaccharides take much longer time under proper conditions of acidity when they are heated for about 10 min and then react with reagent. Brick red color is formed due to formation of cuprous oxide

**Reaction:**

**Materials & Reagents:**

Cuprous oxide

Boiling water bath (BWB)

Barfoed's Reagent: Dissolve 13.3 g of copper acetate in 200 ml water and add 1.8 ml of glacial acetic acid to it.

**Procedure:** To 2 ml of Barfoed's solution add 1 ml of sample solution. Keep the test tubes in BWB for 2-4 min. Observe the formation of brick red color indicative of monosaccharides. Whereas red precipitate formed on prolonged heating hints the presence of disaccharides.

**Fehling's Test**

**Principle:** It is specific and sensitive test for the detection of reducing sugars. Formation of red colored ppt of cuprous oxide depicts the presence of reducing sugar. Rochelle salt acts as chelating agent in this reaction. Fehling's test can be used as a generic test for monosaccharides. It will give a positive result for aldose monosaccharides (due to the oxidizable aldehyde group) but also for ketose monosaccharides, as they are converted to aldoses by the base in the reagent, and then give a positive result. For this reason, Fehling's reagent is sometimes referred to as a general test for monosaccharides.

**Reaction:**

Cuprous oxide

**Materials & Reagents:**

Boiling water bath (BWB)

Fehling's Solution A: Dissolve 35 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in water and make the volume to 500ml.

Fehling's Solution B: Dissolve 120g of KOH and 173 g Na-K tartarate (Rochelle salt) in water and make the volume to 500 ml.

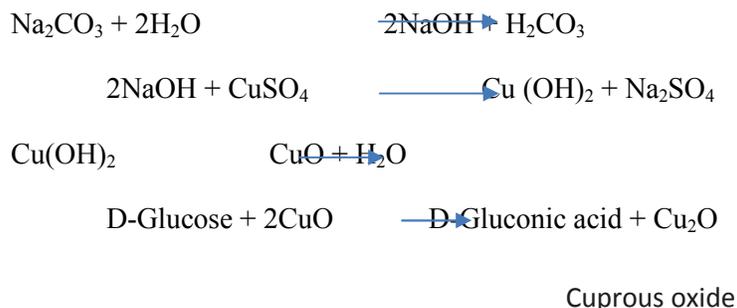
Fehling's Reagent: Mix equal volumes of Fehling's Solution A and B.

**Note-** Solutions A & B must be mixed immediately prior to use.

**Procedure:** To 1 ml of Fehling's Reagent add 1 ml of sample solution. Mix it and Keep the test tubes in BWB. Observe the formation of brick red color of cuprous oxide, indicative of reducing sugars.

### Benedict's Test

**Principle:** This test is having advantage that this reagent is more stable. It contains a weak alkali sodium carbonate. In presence of this alkali, reducing sugars form enediols (strong reducing agent). Enediol reduces copper sulphate to cuprous hydroxide (ppt may vary from green, yellow to red depending on the concentration of sugar solution).



### Materials & Reagents:

Boiling water bath (BWB)

Benedict's Reagent:

7.12 part A: Dissolve 173 g of sodium citrate and 100g of anhydrous  $\text{Na}_2\text{CO}_3$  in 600 ml of hot  $\text{H}_2\text{O}$ . Dilute to 800ml with water.

7.13 part B: Dissolve 17.3g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100 ml of water. Cool and dilute to 100 ml.

7.14 Add part A to part B slowly with constant stirring & make the final volume to 1 L.

**Procedure:** To 0.5-0.1 ml of test solution, add 2ml of Benedict's reagent. Mix it and Keep the test tubes in BWB. Observe the formation of brick red color of cuprous oxide, indicative of reducing sugars.

## Mucic Acid test

**Principle:** This test is highly specific for galactose. This sugar can be distinguished from other monosaccharides, as in presence of conc.  $\text{HNO}_3$  other monosaccharides yields soluble dicarboxylic acids whereas galactose produces insoluble mucic acid.

### Materials & Reagents:

Boiling water bath (BWB)

Conc.  $\text{HNO}_3$

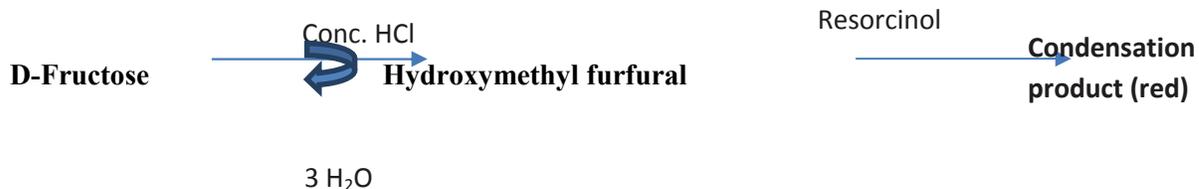
**Procedure:** To 1 ml of test solution (50mg of galactose), add 1ml of Conc.  $\text{HNO}_3$ .

Mix it and keep the test tubes in BWB for 1.5 h. add 5 ml water and keep them overnight. Insoluble mucic acid will be formed.

## Seliwanoff test

**Principle:** This test differentiates between keto and aldohexoses. Ketoses undergo dehydration to give furfural derivatives, which then condenses with resorcinol to form red complex. This test is also given by sucrose which gets hydrolyzed during the course to yield fructose.

### 1 Reaction:



### Materials & Reagents:

Boiling water bath (BWB)

**Seliwanoff's Reagent:** 0.05% (w/v) resorcinol in 3N HCl.

**Procedure:** To 1 ml of test solution (50mg of galactose), add 2ml of Seliwanoff's Reagent. Keep the test tubes in BWB for 1 min. Observe the formation of deep red color, indicative of keto sugars.

**Bial's test: Principle:** This test is useful in the determination of pentose sugars. Reaction occurs due to the formation of furfural in the acid medium that condensed with Orcinol in presence of ferric ions, resulting in blue-green colored condensation product.

### 7.15 Reaction:



### Materials & Reagents:

Boiling water bath (BWB)

**Bial's Reagent:** Dissolve 1.5g of Orcinol in 100 ml of Conc. HCl and add 20-30 drops of 10% ferric chloride to it.

### Procedure:

To 1 ml of Bial's Reagent, add 4-5 drops of test solution. Heat in a BWB. Observe the change of color to bluish green colored complex.

### QUANTITATIVE TESTS

#### ANTHRONE TEST

**Principle: Same as Above**

### Materials & Reagents:

Boiling water bath (BWB)

Anthrone reagent: 0.2% (w/v) Anthrone solution in Conc. H<sub>2</sub>SO<sub>4</sub>

**Stock:** Add 100mg of glucose in 100ml of distilled water. Working Standard: Take a volumetric flask, take 10ml of stock in it and dilute with 100ml of distilled water. 1.0 ml of this solution contains 100μg of glucose. Unknown solution (to be tested)

### Procedure:

Take 6-10 test tubes.

In the first one doesn't add anything (Blank tube). In the second, add 0.2ml of standard solution, in third 0.4ml, in 4th 0.6 ml and so on respectively.

Then add water to all the test tubes to make it to the level of 1ml. This way the first one i.e blank only 1ml of water would be added (in which we haven't added standard solution would have 1ml of water). While in that last one water would not be added (in which we have added 1ml of standard).

Prepare tubes for the unknown sample same as above using different volumes (dilute/undiluted).

Then add 4ml of Anthrone reagent to all the test tubes. Heat all the test tubes in boiling water bath for 10 minutes. Then cool it rapidly.

Color would change to blue green. Measure the optical density of this color at 620nm using photo colorimeter or spectrophotometer. Draw a standard curve by plotting concentration of standard on x axis while absorbance on y-axis. Compute the concentration of the sugar in the unknown sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor has to be taken into account.

**Calculations:** From the standard curve, determine the amount of sugar in the diluted/undiluted aliquot of unknown sample. Calculate the concentration of unknown sample by applying the dilution factor (if you have diluted the sample).

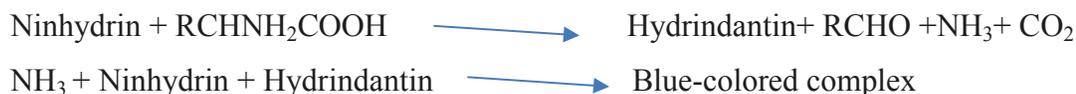
## TESTS FOR AMINO ACIDS & PROTEINS

### QUALITATIVE TESTS

#### NINHYDRIN TEST

##### PRINCIPLE:

This reaction is based on the fact that  $\alpha$ -amino acids or protein derivatives containing  $\alpha$ -amino groups, reacts with Ninhydrin (Oxidizing agent) to give  $\text{CO}_2$ ,  $\text{NH}_3$ , a corresponding aldehyde & a reduced form of ninhydrin. The ammonia combines with ninhydrin and its reduction product (hydrindantin) to give blue substance diketohydrin (Ruhemann's purple).



**Note\*** -Proline produces yellow color whereas asparagine gives a brown colored product. This test is also given by proteins and peptides.

## **Materials & Reagents:**

Boiling water bath (BWB)

Ninhydrin: 0.2% solution prepared in acetone.

Test solution: Prepare 50µg/ml solution of individual amino acids.

## **Procedure:**

To 1ml of test solution, add 2-5 drops of Ninhydrin solution. Mix and keep for 5 min in BWB. Observe the development of pink, purple or violet –blue color.

## **BIURET TEST**

### **PRINCIPLE:**

This is a general test for compounds having a peptide bond. Alkaline copper sulphate reacts with compounds containing two or more peptide bonds to give a violet or pinkish colored product (due to formation of coordination complex of cupric ions with unshared pair of peptide nitrogen and oxygen of water).

**Note\***- Non-protein substances containing  $-CSNH_2$ ,  $-CNH(NH_2)$  or  $-CH_2NH_2$  also give positive Biuret test.

Copper Sulphate: 1% (w/v) of  $CuSO_4 \cdot 5H_2O$  in water.

40% (w/v) solution of NaOH.

### **Procedure:**

To 1ml of test solution, add 0.5 ml NaOH and mix well. Then add 2-5 drops of copper

sulphate and observe the formation of pink or violet color.

## **XANTHOPROTEIC TEST**

**PRINCIPLE:** This reaction is based on the fact that amino acids containing an aromatic nucleus react with Conc.  $HNO_3$  to form yellow nitro/nitroso derivatives. These compounds in alkaline medium ionize freely and produce deep yellow or orange color.

### **Materials & Reagents:**

Conc. HNO<sub>3</sub>

NaOH solution (40%, w/v)

**Procedure:** To 1ml of test solution, add 10 drops of Conc. HNO<sub>3</sub> ml. Mix and keep for 1 min in BWB. Cool it & slowly add NaOH till the solution becomes alkaline. A deep yellow to orange color denotes the presence of aromatic amino acids.

## **QUANTITATIVE TESTS**

### **BRADFORD TEST**

#### **PRINCIPLE:**

This test is based on interaction of a dye, Coomassie Brilliant Blue, with proteins. The unbound dye has an absorbance maximum at 465nm, whereas when it binds to proteins in acidic solution (via electrostatic and van der Waals bonds), resulting in a shift of the absorption maximum of the dye from 465 to 595 nm. This method actually measures the presence of the basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein-dye complex.

### **Materials & Reagents:**

Reagent: The assay reagent is made by dissolving 100 mg of Coomassie Blue G250 in 50 mL of 95% ethanol. The solution is then mixed with 100 mL of 85% phosphoric acid and made up to 1 L with distilled water. The reagent should be filtered through Whatman no. 1 filter paper and then stored in an amber bottle at room temperature.

Protein standard solution (100ug/ml): Dissolve 5mg of BSA in 50 ml of distilled water.

#### **Procedure:**

Pipette appropriate aliquots of standard solution containing 0-100 µg protein. Make total volume to 1 ml with distilled water in all the tubes. Add 5 ml Bradford reagent to all the tubes and mix thoroughly. Read the absorbance at 595nm against the blank tube.

Prepare tubes for the unknown sample same as above using different volumes (dilute/undiluted). Draw a standard curve by plotting concentration of standard on x axis while absorbance on y-axis. Compute the concentration of the protein in the unknown sample from the calibration curve. While calculating the concentration in the unknown sample, the dilution factor has to be taken into account.

**Calculations:**

From the standard curve, determine the amount of protein in the diluted/undiluted aliquot of unknown sample.

Calculate the concentration of unknown sample by applying the dilution factor (if you have diluted the sample).

**TESTS FOR LIPIDS**

**QUALITATIVE TESTS:**

**PHYSICAL TEST:**

**Grease spot test:** Take a small amount of oil on a piece of paper, a greasy spot penetrating the paper will be formed. This happens because lipid does not wet paper unlike water.

**Test for free fatty acids:** Take a few drops of phenolphthalein solution in a test tube and add to it 1-2 drops of dilute alkali solution, just sufficient to give the solution a pink color. Now add a few drops of the oil and shake. The color will disappear as the alkali is neutralized by the free fatty acids present in the oil.

**Emulsification:**

**Principle:**

Oil or liquid fat becomes finely divided and is dispersed in water when shaken with water to form emulsification. Emulsification is permanent and complete in the presence of emulsifying agent. The important emulsifying agents are bile salts, proteins, soaps, mono- and diglycerides. Emulsification is important in the processes of fat digestion in the intestine. Emulsifying agents lower surface tension of the liquid.

**Procedure:**

Take 2 clean and dry test tubes, in one test tube added 2 ml water and in other 2ml dilute bile salt solution. Now to each tube added 2 drops of mustard oil and shaken vigorously for about one minute. Allow the tubes to stand for two minutes and note that the water, oil is broken in small pieces and floats on the surface; whereas in the bile salt solution, the oil can be seen in minute droplets suspended in the liquid (permanent emulsification).

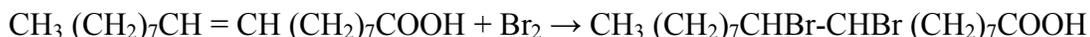
**Saponification test:**

**Principle:** Esters can be hydrolyzed by alkali to yield the parent alcohol and salt. When the fatty acid possesses a long chain, the salt formed is a soap which we commonly use. This process is called saponification. Oils and fats usually contain long chain fatty acids and are, therefore, the starting materials for the preparation of soap.

**Procedure:** Take 1 ml of the oil in a test tube and add an equal amount of alcoholic KOH solution, mix them thoroughly and keep the mixture during the course of warming and shake up gently with a little distilled water. Appearance of some oil drops will indicate the incomplete saponification. After complete saponification, no oil drops will appear.

**Tests for unsaturation of fatty acids:****Principle:**

Unsaturated fatty acids like oleic acid can react with halogens like bromine and iodine due to presence of double bonds as shown below.



The amount of Br<sub>2</sub> or I<sub>2</sub> taken up will indicate the amount of unsaturation present in a particular acid. Approximate idea about the unsaturation in a different oils and fats can be obtained by the following test. Set up four clean and dry test tubes each containing 5 ml of CCl<sub>4</sub>.

**Procedure:** To the first, add one drop of shark liver oil, to the second, one drop of coconut oil, to the third, a drop of vegetable ghee and add nothing to the fourth tube. Now test for the unsaturation of the added oil by adding bromine water drop by drop to

each tube followed by shaking. Record the number of drops required to obtain a permanent yellowish red color in each tube and infer the relative unsaturation in the three samples used.

#### **Tests for Glycerol:**

##### **Acrolein test:**

**Principle:** When glycerol is heated with strong dehydrating agent (Potassium hydrogen sulphate), acrolein is formed due to removal of water from glycerol.

**Procedure:** Take pure glycerol in a dry test tube; add to it a few crystals of potassium hydrogen sulphate. Warm gently to mix and then heat strongly. A very pungent odour of acrolein is produced.

**Dichromate Test: Principle:** This test is given by the substances containing primary and secondary alcohol groups. The chromic ions oxidize the glycerol and, in this process, they are reduced to chromous ions which give the blue colour. This test is also given by reducing sugars, so before confirming glycerol be sure that the reducing sugars are not present.

**Procedure:** Take in a dry test tube 3-4 ml of glycerol solution, to it add a few drops of 5% potassium dichromate solution and 5 ml of conc.  $\text{HNO}_3$ , mix well and note that the brown colour is changed to blue.

#### **Test of Cholesterol:**

##### **Salkowski's Test:**

**Principle:** Cholesterol forms disulphonic acid with excess of sulphuric acid.

**Procedure:** Dissolve cholesterol in 2 ml of chloroform in dry test tube. Add equal amount of Conc.  $\text{H}_2\text{SO}_4$ . Shake gently. The upper layer turns red and the sulphuric acid layer shows a yellow colour with a green fluorescence.

---

### ***8.4- CHEMICAL TEST OF URINE FOR THE PRESENCE OF UREA, SUGAR, PROTEINS AND KETONE BODIES***

---

#### **URINE:**

It is a watery, typically yellowish fluid stored in the bladder and discharged through the urethra. It is one of the body's chief means of eliminating excess water and salt, and also contains nitrogen compounds such as urea and other waste substances removed from the

blood by the kidneys. The composition of urine reveals much about body function. Metabolic waste products such as carbon dioxide, urea, uric acid, creatinine, sodium chloride, and ammonia are normally present and have no particular pathological significance. The presence of albumin (a protein), glucose, ketones, and various other substances, however, may indicate malfunction of the kidneys or some other organ of the body.

In determining whether pathological conditions exist through urine analysis, it is necessary to perform both physical and chemical tests. Of the physical tests that are available, only appearance of the urine will be observed. The chemical tests will be for pH, protein, glucose, ketones, hemoglobin. The significance of each abnormality will accompany the specific test.

#### **NORMAL CONSTITUENTS OF URINE:**

7.15.3 Urea

7.15.4 Ammonia

7.15.5 Creatinine and Creatine

7.15.6 Uric Acid

7.15.7 Amino Acids

7.15.8 Sulphates

Other organic compounds:

Chlorides, Phosphates, Oxalates, Minerals, Enzymes, Hormones and vitamins

#### **ABNORMAL CONSTITUENTS OF THE URINE:**

7.16 Proteins: Hemoglobin, Bence-Jones proteins

7.17 Excessive Glucose

7.18 Other sugars:

Fructose, Galactose, lactose & Pentose

7.19 Ketone bodies

7.20 Bilirubin and Bile salts

7.21 Blood

7.22 Excessive Urobilinogen

#### **TEST FOR THE UREA**

**HYPOBROMITE TEST:** To 2ml of Urine, add 2-3 drops of freshly prepared alkaline sodium hypobromite solution. Effervescence due to evolution of nitrogen occurs.





Depending upon the concentration of sugar in the urine, either green, yellow or brick red precipitates are formed.

### **TEST FOR PROTEINS**

**HEAT COAGULATION TEST:** The urine containing proteins like albumin is coagulated on heating. Take 5-6 ml of clear urine (if turbid, filter it). Heat the upper portion of the test tube containing urine. Add 2-3 drops of 2% acetic acid and boil again. In presence of proteins, turbidity turns into flocculent coagulum. If the precipitate is of phosphate, then it will dissolve in acetic acid.

**SULPHOSALICYLIC ACID TEST:** Albumin gets coagulated by sulphosalicylic acid due to denaturation. Take 3ml of urine and add few drops of 20% sulphosalicylic acid. A white color turbidity or precipitate indicates the presence of proteins.

### **HELLER'S NITRIC ACID TEST:**

Nitric acid causes the precipitation of proteins. Take 3ml of urine in the test tube and carefully add about 2ml of  $\text{HNO}_3$  by the wall. In presence of proteins a white ring appears at the interface of the liquids.

**HELLER'S TEST:** The principle is based on the reaction, that proteins are denaturated by strong acids.  $\text{HNO}_3$  is layered under urine in the test tube. A marked white disk develops at the interface of nitric acid and urine the thickness of which indicates the approximate number of proteins.

**Note\* (Urine has to be put first into the test tube, then put  $\text{HNO}_3$  with a Pasteur pipette to the bottom of the tube.)**

### **TEST FOR BENICE-JONES PROTEINS**

**FLOCCULATION TEST:** Bence-jones proteins are light chains of immunoglobulins which precipitate at low temperature. Heat about 5ml of acidic urine. (Check the pH and acidify accordingly with 2% acetic acid) in a test tube and observe the temperature frequently. Presence of turbidity at 40-60°C hints the Bence-jones proteins and on boiling the precipitate disappears. If coagulable proteins are also present, filter the above

while hot and allow the filtrate to cool. On Cooling the Bence-jones proteins will be precipitated.

**OSGOOD HASKIN TEST:**Take 3 ml of urine in a test tube. Add 1ml of 5% acetic acid and 3 ml of saturated solution of NaCl. Appearance of white precipitate hints the presence of Bence-jones proteins and on boiling the precipitate disappears.

---

## ***8.5- SUMMARY***

---

Biomolecules are molecules that occur naturally in living organisms. Biomolecules include macromolecules like proteins, carbohydrates, lipids and nucleic acids.

Carbohydrates provide an energy source for the cell and also may play a structural role. The simplest subunit of a carbohydrate is a monosaccharide.

Proteins make up the majority of biomolecules present in a cell. These molecules have enormous variation. Proteins are responsible for many enzymatic functions in the cell and play an important structural role

Lipids are composed of long hydrocarbon chains (-CH<sub>2</sub>-). They hold an incredible amount of energy and are therefore energy storage molecules. lipids are the major component of cell membranes.

Variety of test can be performed to detect and also quantify the presence of the specific carbohydrates, protein or lipids. These test employs the difference in the chemical structure as basis.

Urine is a liquid by-product of metabolism in the bodies of many animals, including humans. It is expelled from the kidneys and flows through the ureters to the urinary bladder, from which it is soon excreted from the body through the urethra during urination.

The urinalysis is a set of screening **tests** that can detect some common diseases. It may be used to screen for and/or help diagnose conditions such as **urinary** tract infections, kidney disorders, liver problems, diabetes or other metabolic conditions.

A clinical examination of urine can provide a convenient, cost effective and non-invasive means of assessing kidney function and providing an overall assessment of our body's health.

---

## **8.6- TROUBLESHOOTINGS**

---

In Anthrone test if color change after adding anthrone reagent was not examine, keep the tubes in BWB for 10 min.

**In Fehling's test**, Solutions A & B must be mixed immediately prior to use.

$\alpha$ -naphthol should be prepare fresh in Molisch's test as it is unstable.

Care should be taken while handling caustic acids like Conc. Sulphuric acid [H<sub>2</sub>SO<sub>4</sub>], nitric acid [HNO<sub>3</sub>], Hydrochloric acid [HCl]. These acids should be opened and used in FUMEHOOD only. Accidental spill of these acids will cause severe burns and itching. Wash the spilled area with cold water and inform the lab assistant immediately.

When Sodium hydroxide is prepared, make sure that it is handled with care as the sodium hydroxide solution is caustic in nature.

Always check the water level in the water bath and if it is up to the level [nearly half the volume], switch on the water bath and adjust to the required temperature. Take care while using the water bath for the boiling step in the experiment. Hold the test tube using a test tube holder.

There should be a proportion between the reagents added and the test solution to obtain good result within the time mentioned. The droppers used should not be mixed between the reagents, always use individual droppers for each reagent.

The color formed will depend upon the quality of the reagents. So, care should be taken while preparing the reagents. If commercially available reagents are used assure that it is not kept open for long time.

Reagents like Ninhydrin reagent, sulphanilic acid, isatin reagent, bromine, Sodium nitroprusside should also be handled with care. Accidental spill of these reagent will cause burns and itches.

In Lowry's method several compounds like EDTA, Tris, Carbohydrates,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$  ions, thiol reagents, phenols etc. interfere with color development and it should be ensured that such substances are not present in sample preparations.

In Bradford's method detergents such as SDS, Triton X-100 etc. interfere in estimation but metal ions such as  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$  and phenols and sugars don't interfere.

---

## **8.7- GLOSSARY**

---

**Acid-** a molecule or other species which can donate a proton or accept an electron pair in reactions.

**Base-** A base is a chemical species that donates electrons or hydroxide ions or that accepts protons.

**Biomolecules-** molecules that occur naturally in living organisms, include macromolecules like proteins, carbohydrates, lipids and nucleic acids.

**Coagulation-**the action or process of a liquid, changing to a solid or semi-solid state.

**Carbohydrates-**are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis having the empirical formula  $(\text{CH}_2\text{O})_n$ .

**Effervescence-** The bubbling of a solution due to the escape of gas

**Hydrolysis-** usually means the cleavage of chemical bonds by the addition of water.

**Metabolic wastes-** or excreted are substances left over from metabolic processes (such as cellular respiration), which cannot be used by the organism (they are surplus or toxic), and must therefore be excreted. This includes nitrogen compounds, water,  $\text{CO}_2$ , phosphates, sulfates, etc.

**Pathology-** the science of the causes and effects of diseases, especially the branch of medicine that deals with the laboratory examination of samples of body tissue for diagnostic or forensic purposes.

**Precipitation-** is the creation of a solid from a solution. When the reaction occurs in a liquid solution, the solid formed is called the 'precipitate'.

**Reducing Sugar-** A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group.

**Urethra:**the duct by which urine is conveyed out of the body from the bladder, and which in male vertebrates also conveys semen.

---

## ***8.8- TERMINAL QUESTION ANSWERS***

---

Q.1 What is a reducing sugar?

Ans. A sugar having free anomeric hydroxyl group is capable of reducing, thus named as reducing sugar.

Q.2 What are Epimers?

Ans. When the stereoisomers (sugars) differing in the orientation of  $H^+$  and  $OH^-$  groups in a single 'C' atom they are called epimers.

Q.3 Which test can be used to distinguish ketose from aldose?

Ans. Seliwanoff's test

Q.4 Which amino acid does not give purple color with ninhydrin reagent and why?

Ans. Ninhydrin degrades primary amino acids into aldehydes, ammonia, and  $CO_2$  (carbon dioxide) through a series of reactions; the net result is ninhydrin in a partially reduced form hydrindantin. Ninhydrin then condenses with ammonia and hydrindantin to produce a blueish-purple pigment. But, Proline being an imino acid gives a yellow result because in proline, the N is not available for reaction as it is locked in the ring structure.

Q.5 Name amino acids which give a positive Xanthoproteic test.

Ans. Aromatic amino acids- Phenylalanine, Tyrosine and Tryptophan.

Q.6 Define lipids?

Ans. The lipids are heterogeneous group of compounds which are insoluble in water but soluble in non-polar solvents such as ether, chloroform and benzene. All lipids invariably contain fatty acids.

Q.7 What are essential fatty acids?

Ans. Essential fatty acids, are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them.

Q.8 What is Glycosuria?

Ans. **Glycosuria/Glucosuria** is the excretion of glucose into the urine. Ordinarily, urine contains no glucose because the kidneys are able to reabsorb all of the filtered glucose from the tubular fluid back into the bloodstream. Glycosuria is usually always caused by elevated blood glucose levels, most commonly due to untreated diabetes mellitus. Infrequently, it is due to an intrinsic problem with glucose reabsorption within the kidneys producing a condition termed renal glycosuria.

Q.9 What is Proteinuria?

Ans. People with proteinuria have urine containing an abnormal amount of protein. The condition is often a sign of kidney disease. The two most common risk factors for proteinuria is: Diabetes High blood pressure (hypertension) Other types of kidney disease unrelated to diabetes or high blood pressure can also cause protein to leak into the urine including- Medications, Trauma, Toxins, Infections, Immune system disorders

---

### ***REFERENCES AND SUGGESTED READINGS***

---

2. Lehninger Principles of Biochemistry (5th Edition, W. H. Freeman) by David L. Nelson (Author), Michael M. Cox (Author)
2. Biochemistry (5th edition, Wadsworth Publishing Co Inc) by Reginald H Garrett (Author), Charles M Grisham (Author)  
Harper's Biochemistry – Murray, Granner, Mayes, and Rodwell – Prentice Hall International Inc.
3. Introductory Practical Biochemistry (2<sup>nd</sup> Edition) by S. K. Sawhney, Randhir Singh-Narosa Publishing House, New Delhi.
4. Practical Manual of Biochemistry (1<sup>st</sup> Edition) by Sadhna Sharma, Reema Sharma, Medtech an imprint of Scientific International, New Delhi.