ALAGAPPA UNIVERSITY

[Accredited with 'A+' Grade by NAAC (CGPA:3.64) in the Third Cycle and Graded as Category–I University by MHRD-UGC]

KARAIKUDI – 630 003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc. [Zoology]
350 14

PRACTICAL

LAB I : ANIMAL DIVERSITY, BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

I - Semester

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PRACTICAL
LAB I : ANIMAL DIVERSITY, BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

Syllabi

ANIMAL DIVERSITY

1. **Museum specimen**: Invertebrates and vertebrates: Phylum wise (at least one from each phylum)
2. **Mounting**: Earthworm – Body and pineal setae, Honey bee – sting apparatus, Cockroach – Mouth parts, Prawn – Appendages and Shark - Placoid scales
3. **Dissections:** Understanding the anatomy of frog using an appropriate software package (Carolina™ Biolab® – Frog), Dissection of cockroach: Digestive, reproductive & nervous systems. Dissection of available fish: General anatomy (Viscera)

BIOCHEMISTRY

1. Preparation of solutions: Molarity, Normality and Percentage.
2. Buffer preparation and Determination of pH.
3. Estimation of glucose and total protein.

CELL AND MOLECULAR BIOLOGY

1. Cell organelles from slide preparation/images.
2. Buccal mucosal epithelium – Smear preparation to detect Barr bodies.
3. Isolation and detection of DNA from gel electrophoresis (Demo only).
4. Onion root tip – Squash preparation and study of mitosis.
5. Grasshopper testis - Squash preparation and study of meiosis (Demo only).
6. *Chironomus* larva - Squash preparation of giant chromosome.
7. Separation of amino acid by paper chromatography.
8. Separation of protein by electrophoresis - SDS and Native PAGE.

INTRODUCTION

NOTES

Animal diversity refers to the animal evolution that began in the ocean over 600 million years ago with tiny creatures that probably do not resemble any living organism today. The animal classification system characterizes animals based on their anatomy, morphology, evolutionary history, features of embryological development, and genetic makeup. Taxonomy is that branch of biology which deals with the identification and naming of organisms. The ancient Greek philosopher Aristotle apparently began the discussion on taxonomy. During the 1700s, Swedish botanist Carolus Linnaeus classified all then-known organisms into two large groups: the kingdoms Plantae and Animalia. The Linnaean hierarchical classification system of nomenclature is still used today — termed as the binomial system of genus and species — and established as a discipline taxonomy. Robert Whittaker in 1969 proposed five kingdoms: Plantae, Animalia, Fungi, Protista, and Monera. Other schemes involving an even greater number of kingdoms have lately been proposed, however most biologists employ Whittaker's five kingdoms. The vast panorama of animal life, how animals function, live, reproduce, and interact with their environment, is exciting, fascinating and inspiring. Zoology is the scientific study of animal life, its origins and relationships, and includes the study of genetics and biochemistry. The genetic study of DNA from various animals and plants can provide insights into their evolutionary history.

Biochemistry, also sometimes called biological chemistry, is the study of chemical processes within and relating to living organisms. Biochemical processes give rise to the complexity of life. A sub-discipline of both biology and chemistry, biochemistry can be divided in three fields, namely molecular genetics, protein science and metabolism. Principally, 'Biochemistry' is closely related to molecular biology, the study of the molecular mechanisms by which genetic information encoded in DNA. In addition, it deals with the structures, functions and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform many of the functions associated with life.

Cell biology is a branch of biology that studies the structure and function of the cell, which is the basic unit of life. Fundamentally, it is concerned with the physiological properties, metabolic processes, signalling pathways, life cycle, chemical composition and interactions of the cell with their environment. The most significant tool for modern cell biology is molecular biology, which deals with the molecular basis of biological activity.

Molecular biology is a branch of biology that concerns the molecular basis of biological activity between biomolecules in the various systems of a cell, including the interactions between DNA, RNA, proteins and their biosynthesis, as well as the regulation of these interactions.

This book, *Animal Diversity, Biochemistry, Cell and Molecular Biology*, deals with the practical aspects of qualitative and quantitative analysis of the techniques used in the laboratory.

ANIMAL DIVERSITY

GENERAL INSTRUCTIONS AND LABORATORY ETHICS

1. GENERAL INSTRUCTIONS

1. The students while coming to the laboratory for the practical class work, it should be check that you possess the following materials namely, practical note book, pencil, pencil eraser, sharpener, scale, brush and complete set of dissecting instruments.
2. The instruments should be sharp and according to the requirements.
3. To come prepared with the work you are supposed to do in the laboratory.
4. To keep your instruments, practical note book and seat well-arranged and tidy.
5. Do not encourage the habit of lending either to or from your class fellows and bring all the requirements of the day.
6. Listen carefully to the instructions given by your teacher before starting the work.
7. Ask and taken out your all difficulties from your teacher and do not consult your class fellows for any help.
8. Never rub your pencil on the floor or on the top of the working table and use the sharpener for the work.
9. To clean and arrange your seat before leaving the laboratory.
10. Maintain complete silence in the laboratory.

2. STUDENT'S BELONGINGS OR EQUIPMENT FOR LAB WORK

The students while coming to the laboratory for the practical work, is required to bring certain necessary equipment. However, it is not possible to list all what is required, but the following list is considered necessary:

1. The practical book or laboratory manual of practical and practical record book.
2. The drawing pencil for drawing of specimens, slides or equipment.
3. Pencil sharpener, pencil eraser and measuring scale.

4. Well maintained dissecting box with following instruments: scalpels, scissors, and forceps, dissecting needles, blow pipe, one edged safety razor or blade.
5. Brush, dropper (one), hand lens, piece of clean cloth and alpins.

Note: *Do not mind bringing all these things on every turn, you do not know when you may need them.*

3. INSTRUCTIONS TO STUDY AND DRAW THE MUSEUM SPECIMENS

1. Before leaving home for Zoology practical laboratory, check that you are equipped with a Zoology practical exercise book, a text book of practical Zoology, H.B. pencil, pencil sharpener, pencil eraser (good quality rubber) and a piece of soft cloth.
2. Try to obtain advance information about the museum specimens to be drawn so that you come prepared for their study.
3. Special care should be taken to give a very correct proportion about the dimensions (length and breadth) of the specimen.
4. Usually draw only two diagrams on one page, but these should be of the same class.
5. The classification should be written on the right top of the diagrams.
6. The line diagrams should be drawn only.
7. The shading should be avoided as far as possible.
8. The important features must be exhibited in the diagrams.
9. Each diagram must be fully labeled with the help of the Practical Notebook or Lab Manual.
10. The labeling should be horizontal and clear.
11. You must write, both zoological and common names of each specimen below the diagram.

4. PRACTICAL NOTE BOOK (PRACTICAL RECORD BOOK)

1. The practical note book should be neat, clean and up to date.
2. Write the date on the left hand comer of the page of the note book and details of the work on the top in centre.
3. The diagrams should be correctly drawn and well labeled.
4. The diagrams of all museum specimens, slides and dissections should be drawn and comments on all should be written.

Figure 1 illustrates the simple or dissecting microscope used in the Zoology Lab.

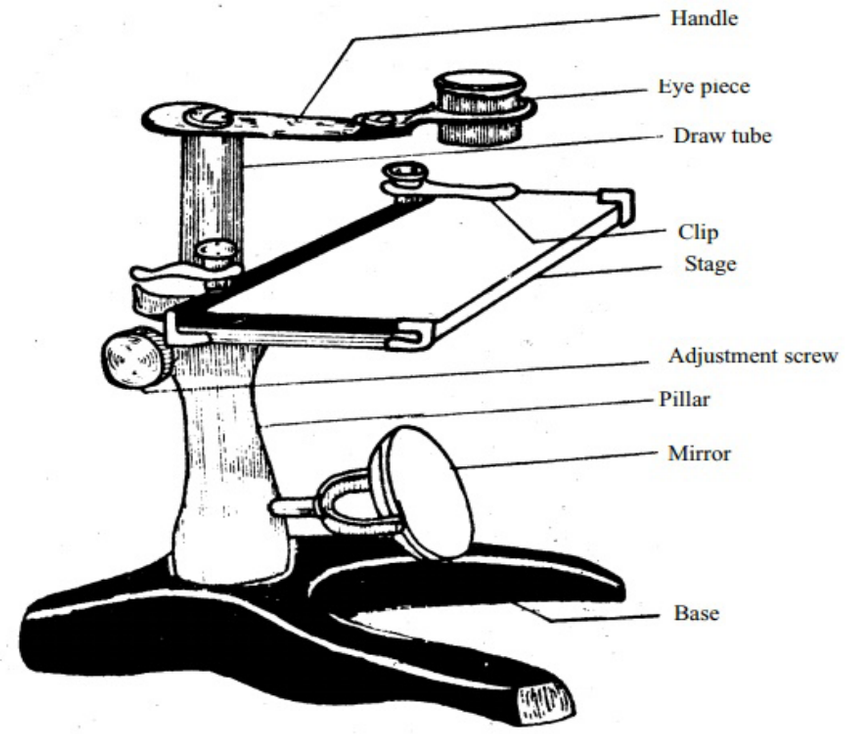

Fig. 1 *A Simple or Dissecting Microscope*

5. STUDY OF SPECIMENS, SLIDES AND TIPS FOR SPOTTING

During the practical examination the spotting is very important exercise to maintain the marks and merit. Students often face difficulty in spotting because of the lack of proper understanding of basic concepts and the method of commenting on a spot. Here are a few tips for good spotting.

1. First and foremost identify the spot and write the scientific name along with the Spot No.
2. If the spot is a whole specimen (and not a section or a part of it) write down the classification and mention Phylum, Class, Order and Genus of the Specimen.
3. Draw a well labeled line diagram of the spot.
4. The important comments can be highlighted in diagram.

5. While writing the comments let the first comment describe the common name if any, the next comment about phylum, the next comment describe the class and the next one the order.
6. The other comments should be specific to the genus.
7. The comments should be short and precise.
8. Always mention the special features of the spot.
9. In case of a section, for example, TS/LS/VS/CS of animals or organs or tissues, do not write the classification.
10. In case of a larval stage always mention the name of the adult animal of whose life cycle the larva is a part.
11. Follow the guidelines while writing scientific names. The generic name begins with a capital letter and the specific name begins with a small letter. Both names are underlined by separate lines or written in capital letters or italicized.

6. MOUNTINGS AND PREPARATION OF PERMANENT SLIDES

While making the permanent slides (mountings) the following instructions should be followed strictly:

1. Never keep your mounting material for less or more time than the desired time in an alcoholic grade or stain.
2. Keep the material for slightly more time in 90% alcohol and absolute alcohol for complete dehydration.
3. Ensure the complete dehydration after absolute alcohol by putting the material in Xylol or Benzene. If it gives turbidity with Xylol, then dehydrate it again.
4. Do the dehydration in closed specimen tubes or in covered cavity blocks.
5. Always use a brush and never the forceps for holding the mounting material.
6. Put the required amount of Canada Balsam or D.P.X. on the slides for mounting.

Figure 2 illustrates the simple laboratory light microscope.

Note: *Excess of Canada Balsam or D.P.X. makes the slides dirty.*

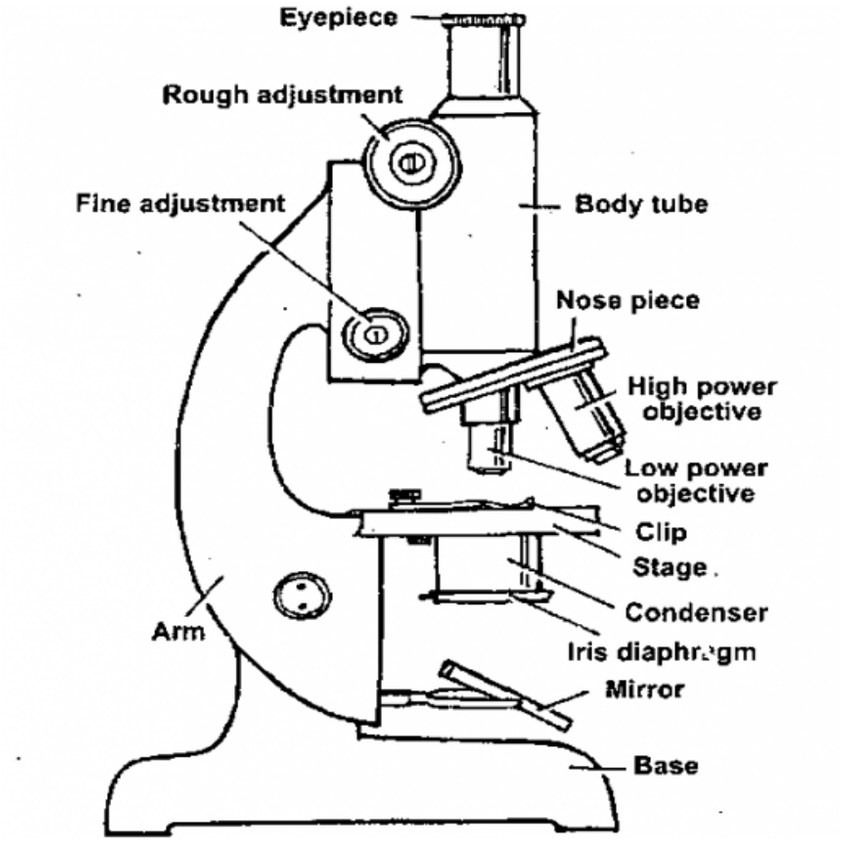

Fig. 2 *A Laboratory Light Microscope*

7. SIGNIFICANCE OF CLASSIFICATION AND ANIMALS NAMING

The classification is a human-made system for of arrangements of different animals. Nomenclature of animals provides a terminology that is essential in the exchange of knowledge and ideas in research and teaching. The name given to animals has specific meaning. The biological classification is based on the binomial system of nomenclature introduced by Linnaeus who is referred to as Father of Classification. International Rules for Zoological Nomenclature were prepared in 1931. Linnaeus subdivided plants and animals into groups or taxa as given below:

Phylum

Class

Order

Genus

Species

The above is called Linnaean Hierarchy. For details of International Rules of Nomenclature, student can refer taxonomy books. Here students must remember how to write generic and specific names. The first name is, the generic name. It should be written either in all capital letters or in all italic letters or underlined. The first letter must always be written with capital letter. Similarly, while writing the second name or specific name, either it should be written in capital letter or italicised or underlined, but the first letter is always written with a small letter. For example, the name of the tape worm can be written in any of the following three ways:

(1) TAENIA SOLIUM, (Both names (generic and specific) are written in capital letters).

(2) *Taenia solium,* (The generic name with capital letter and the specific name with small letter and italics).

(3) Taenia solium, (Both generic and specific names to be separately underlined always).

PHYLUM: PROTOZOA (GREEK, PROTOS = FIRST; ZOON = ANIMAL)

1. DEFINITION

The Protozoa may be defined as microscopic unicellular/ acellular animalcules existing singly or in colonies without tissues and organs, having one or more nuclei. When in colonies, they differ from Metazoa in having all the individuals alike except those engaged in reproductive activities.

2. GENERAL CHARACTERS

1. The protozoans are small, generally microscopic animalcules (tiny animal).
2. They are simple with protoplasmic grade of organisation.
3. These are primitive acellular or unicellular animals, without tissues and organs.
4. Body naked or covered by pellicle but in some forms body is covered with shells and often provided with internal skeleton.
5. Protozoans are solitary or colonial; in colonial forms the individuals are alike except those involved in reproduction.
6. Body shape variable; it may be spherical, oval, elongated or flattened.
7. Body protoplasm is differentiated into an outer ectoplasm and inner endoplasm.
8. Protozoans may have one or more nuclei; nuclei may be monomorphic or dimorphic.
9. Locomotory organelles are pseudopodia, flagella, cilia or none.
10. Nutrition may be holozoic (animal-like), holophytic (plant-like), saprozoic or parasitic.
11. Digestion intracellular, takes place inside the food vacuole.
12. Respiration occurs by diffusion through general body surface.
13. Excretion also occurs through general body surface but in some forms through a temporary opening in the ectoplasm or through a permanent pore, the cytopyge.
14. Contractile vacuoles perform osmoregulation in freshwater forms.

15. Reproduction can be asexual or sexual. Asexual reproduction occurs by budding, binary fission, multiple fission, or sporulation while sexual reproduction is performed by gamete formation or conjugation.

16. Life cycle often exhibits alternation of generation, i.e., it includes asexual and sexual stages.

17. Encystment usually occurs to tide over the unfavourable conditions and it also helps in asexual reproduction.

18. The single celled body of Protozoa performs all the vital activities of life and, therefore physiological division of labour is exhibited.

19. The protozoans exhibit mainly two modes of life, free-living inhabiting freshwater, salt and damp places, and parasitic living as ecto- and endo-parasites. They are also commensal.

3. *AMOEBA*

Classification

Phylum : Protozoa
Class : Rhizopoda
Order : Lobosa
Genus : *Amoeba*

Habitat

- *Amoeba* is a fresh-water acellular protozoan (Refer Figure 1).

Habits

- Amoeba has a biconvex nucleus, a spherical contractile vacuole and many food vacuoles.
- It is commonly called little Proteus.
- It shows amoeboid locomotion with the help of pseudopodia.
- It is omnivorous and holozoic in its nutrition.
- It undergoes only asexual reproduction which occurs by binary fission during favorable conditions and multiple fission during unfavorable conditions.
- It undergoes encystment during unfavourable conditions for perennation and dispersal.

Identification Points

- Irregular-shaped body due to presence of many lobopodia.
- Cytoplasm is differentiated into ectoplasm and endoplasm.
- Endoplasm is differentiated into outer denser plasmagel while ectoplasm is an inner fluidy plasmosol.

- The endoplasm has a biconvex nucleus, clear spherical contractile vacuole and many food vacuoles.

Economic Importance

- It is a Protoza, which is an important part of the soil ecosystem.

Viva Voce

- Why is *Amoeba* called little Proteus?
- Why protozoans are called acellular animals?
- What is function of contractile vacuole in *Amoeba*?

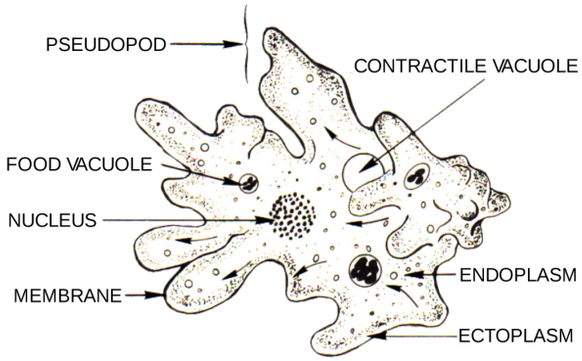

Fig. 1 *Amoeba*

4. *EUGLENA*

Classification

Phylum : Protozoa
Class : Mastigophora
Order : Euglenoidina
Genus : *Euglena*

Habitat

- *Euglena* is a fresh-water, acellular protozoan (Refer Figure 2).

Habit

- It shows gliding type (called metaboly) and swimming type (with the help of flagellum) locomotion.
- The mode of nutrition is mixotrophic nutrition.
- It shows only asexual reproduction by longitudinal binary fission during favorable conditions. During unfavorable conditions, it undergoes encystment and forms palmella stage.

Identification Points

- Spindle-shaped body covered by a definite pellicle.
- Anterior end has a flask-shaped cytopharynx leading into a reservoir. Single flagellum projects out of cytopharynx.
- Presence of single spherical contractile vacuole with many feeding canals near the reservoir.
- Endoplasm has a vesicular nucleus, radially-arranged chloroplasts and many paramylum bodies.

Economic Importance

- It is a type study specimen as connecting link between plant and animal.

Viva Voce

- What is mixotrophic nutrition?
- Why *Euglena* is called plant-animal?

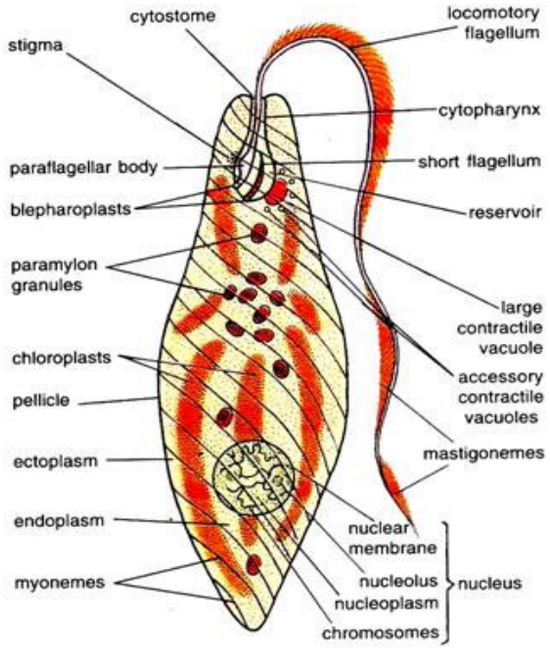

Fig. 2 *Euglena*

PHYLUM: PORIFERA (LATIN, PRORUS = PORE; FERRO = TO BEAR)

1. DEFINITION

In Latin, Porus means 'Pore' while Ferro means 'To Bear'.

The Porifera may be defined as "asymmetrical or radially symmetrical multicellular organisms with cellular grade of organisation without well-defined tissues and organs; exclusively aquatic; mostly marine, sedentary solitary or colonial animals with body perforated by pores canals and chambers through which water flows; with one or more internal cavities lined with choanocytes and with characteristic skeleton made of calcareous spicules, siliceous spicules or horny fibres of spongin".

2. GENERAL CHARACTERS

1. Porifera are all aquatic, mostly marine except one family Spongillidae which lives in freshwater.
2. They are sessile and sedentary and grow like plants.
3. The body surface is coated by numerous pores, through which the water enters body and one or more large pore, the oscula by which the water comes out.
4. The multicellular body consisting of outer ectoderm and inner endoderm with an intermediate layer of mesenchyme, therefore, porifers are diploblastic animials.
5. The interior space of the body is either hollow or permeated by numerous canals lined by choanocytes. The interior space of sponge body is called spongocoel.
6. The representative porifers comprising characteristic skeleton consisting of cither fine flexible spongin fibres, siliceous or calcareous spicules.
7. The mouth absent, digestion intracellular; excretory and respiratory organs absent.
8. The nervous and sensory cells are probably not differentiated.
9. The sponges are monoecious; reproduction both by asexual and sexual methods.

10. Asexual reproduction occurs by buds and gemmules formation. The sponges possess high power of regeneration.
11. Sexual reproduction occurs by ova and sperms, fertilization is internal.
12. Cleavage holoblastic, development indirect through a free-swimming ciliated larva amphiblastula or parenchymula.
13. The canal system organisation of sponges has been grouped into three main types, viz. ascon, sycon and leuconoid type due to simplicity in some forms and complexity in others.

3. *SYCON*

Classification

Phylum : Porifera
Class : Calcarea
Order : Heterocoela
Genus : *Sycon*

Habitat

- It is a marine, sedentary, colonial sponge found attached to rocks, etc., below low tide mark (Refer Figure 3).

Habits

- It is commonly called crown sponge or urn sponge.
- Locomotion is absent due to sedentary mode of life.
- It is omnivorous and feds upon planktons and organic particles. Water current is maintained by collar cells (choanocytes).
- Digestion is intracellular.
- Asexual occurs by external budding.
- *Sycon* is bisexual or hermaphrodite and reproduce by gamete formation. Fertilization is internal but always shows cross-fertilization due to protogyny.
- Development is indirect and includes a free swimming amphiblastula larva.

Identification Points

- *Sycon* has numerous small sized pores called dermal ostia on body surface and single large sized osculum at its tip.
- Osculum is guarded an oscular fringe of monaxon spicules.

Economic Importance

- It is a type study animal of Porifera.

Viva Voce

- Give three important characters of Porifera.
- What is amphiblastula larva?

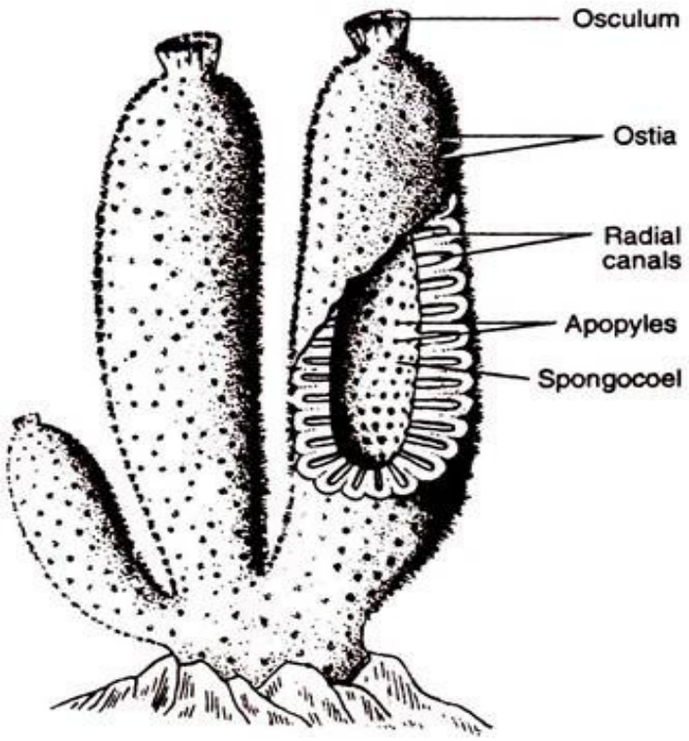

Fig. 3 *Sycon*

4. *EUSPONGIA*

Classification

Phylum : Porifera
Class : Demospongiae
Order : Keratosa
Genus : *Euspongia*

Habitat

- It is a marine, cosmopolitan, sedentary sponge found attached to rocks, etc. (Refer Figure 4).

Habits

- It is commonly called bath sponge.
- Locomotion is absent due to sedentary mode of life.

- It is omnivorous and feeds upon planktons.
- Digestion is intracellular.
- Asexual reproduction occurs by internal budding (called gemmulation). Gemmules help in dispersal and perennation.
- Sexes are separate means unisexual or dioecious with no sexual dimorphism. Fertilization is internal.
- Development is indirect and includes a stereo-gastrula larvae for dispersal which undergoes metamorphosis.

Identification Points

- Body surface has many small pores, called dermal ostia, and a few large sized apertures called osculum.
- Skeleton is formed of spongin protein fibres.

Economic Importance

- Their washed, dried and trimmed skeleton is used in bathing.

Viva Voce

- Why *Euspongia* is called bath sponge?
- What are gemmules?

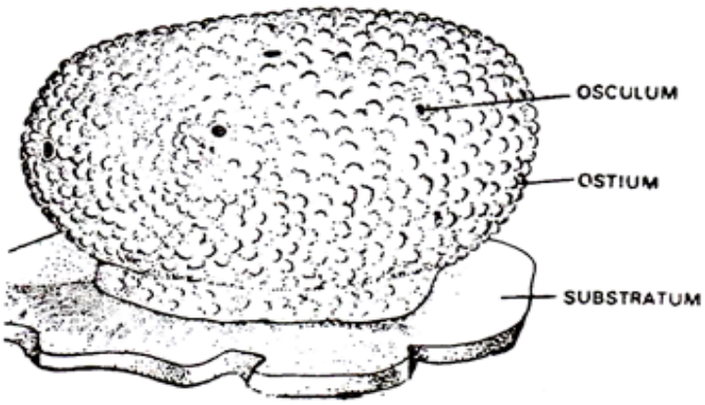

Fig. 4 *Euspongia*

PHYLUM: COELENTERATA (GREEK, KOILOS = HOLLOW; ENTERON = INTESTINE)

1. DEFINITION

The Coelenterata may be defined as "diploblastic Metazoa with tissue grade of construction having nematocysts and a single gastrovascular cavity or the coelenteron." The Coelenterata is an obsolete term about the animal Phyla Cnidaria (Coral animal, true Jellies, Sea Anemones).

2. GENERAL CHARACTERS

1. Coelenterates are Metazoa or multicellular animals with tissue grade of organization.
2. They are aquatic, mostly marine except few freshwater forms like *Hydra*.
3. They are sedentary or free-swimming and solitary or colonial.
4. Individuals are radially or biradially symmetrical with a central gastrovascular cavity communicating to the exterior by the mouth.
5. These are diploblastic animals; body wall consists of an outer layer of cells called ectoderm, inner layer of cells, the endoderm cemented together by an intermediate layer of non-cellular mesogloea.
6. They are acoelomate animals because they do not possess a second body cavity, the coelom.
7. The slender tentacles encircle the mouth in one or more whorls. The tentacles are provided with nematocysts. The tentacles serve for food capture and its ingestion. These are also present on body layers and are called as adhesive organs.
8. They exhibit the phenomenon of polymorphism with very few exceptions; the main types of morphic forms are polyps and medusa.
9. Polyp is sessile and asexual zooid, while medusa is free-swimming and sexual zooid.
10. Skeleton, either exoskeleton or endoskeleton is of common occurrence.
11. They are usually carnivorous. Digestion is extracellular as well as intracellular. Anus is absent.

12. Nervous system consists of one or more networks or nerve-cells and neurites located in be ectoderm and endoderm.
13. Respiratory, circulatory and excretory systems are inadequate.
14. Reproduction is both by asexual and sexual methods.
15. Asexual reproduction occurs by budding and sexual reproduction by the formation of gametes. Reproduction external.
16. A ciliated planula larva usually present in the life history.
17. The life history exhibits the phenomena of alternation of generations or metagenesis in which the asexual polypoid, sessile generation alternates with sexual medusa, free-swimming.

3. *PHYSALIA*

Classification

Phylum	:	Coelenterata/Cnidaria
Class	:	Hydrozoa
Order	:	Siphonophora
Genus	:	*Physalia*

Habitat

- It is a marine, free living, pelagic, polymorphic hydrozoan found in warm seas (Refer Figure 5).

Habits

- It is commonly called Portuguese man-of-war.
- Locomotion is floating type, due to presence of air-filled pneumatophore which remain or float at the top.
- It is carnivorous and feeds upon crustaceans, fishes, etc., captured by dactylozooids and digested by gastrozooids.
- *Physalia* shows proto-cooperation with a fish, called *Nomeus*, which lives in its tentacles. *Physalia* protects the fish from enemies with its stinging cells.
- *Physalia* is a bisexual or hermaphrodite animal. Fertilization is external.
- Development is indirect and includes a planula larva which undergoes metamorphosis.

Identification Points

- It has an oval-shaped pneumatophore with a sail on aboral axis side.
- Coenosarc is flat disc-like with grooves of zooids called cormidia.

- A cormidium has gastrozooids, dactylozooids and branched blastostyles, called gonozooids or gonodendra, which bear male and female gonophores.

Economic Importance

- *Physalia* is eaten by some fish and crustaceans of commercial value. Thus keeping the balanced ecosystem.

Viva Voce

- Explain proto-cooperation in *Physalia*.

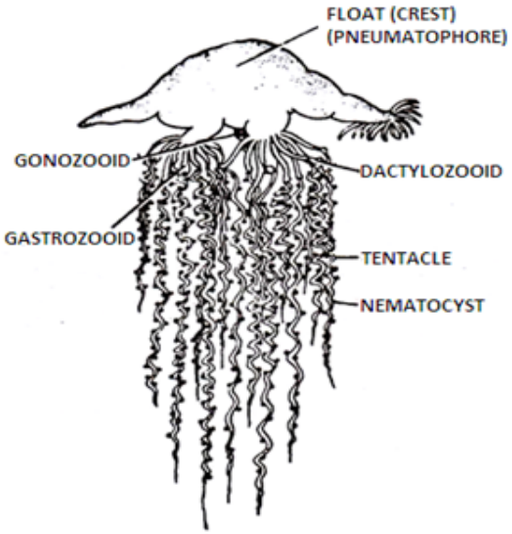

Fig. 5 *Physalia*

4. *METRIDIUM*

Classification

Phylum : Coelenterata/Cnidaria
Class : Anthozoa
Order : Actiniaria
Genus : *Metridiunm*

Habitat

- It is marine, cosmopolitan, solitary, sedentary anthozoan found attached to substratum in shallow water.

Habits

- It is commonly called sea anemone.
- Locomotion is slow creeping type with the help of pseudopodia arising from pedal disc.

- It is omnivorous and plankton feeder.
- It has a good power of regeneration.
- Asexual reproduction occurs by fission and fragmentation.
- It is unisexual or dioecious with no sexual dimorphism. Fertilization is external.
- Development indirect and includes a planula larva for dispersal.

Identification Points

- Body is differentiated into oral disc, column and pedal disc (for attachment to substratum).
- Oral disc is with mouth surrounded by numerous tentacles.
- Column is differentiated into upper scapus by a fold called collar.

Economic Importance

- Sea anemones are known for their pigmentation and beauty.

Viva Voce

- Explain proto-cooperation between Hermit-crab and sea anemone.

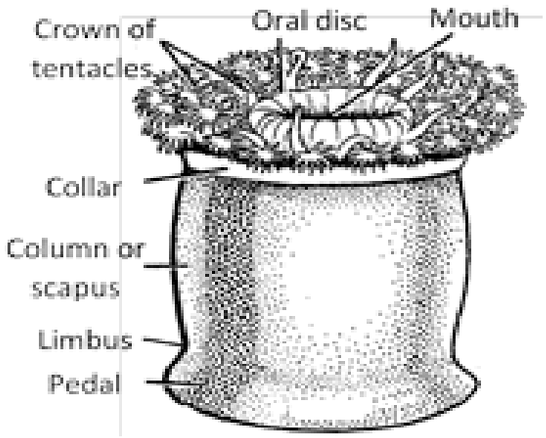

Fig. 6 *Metridium*

PHYLUM: PLATYHELMINTHES (GREEK, PLATYS = FLAT; HELMINS = WORMS)

1. DEFINITION

Platyhelminthes are triploblastic, bilaterally dorso-ventrally flattened, acoelomate flatworms with organ grade of construction without definite anus, circulatory, skeletal or respiratory systems but with protonephridial excretory system and mesenchyme filling the pace between the various organs of the body.

2. GENERAL CHARACTERS

1. Platyhelminthes are bilaterally symmetrical and dorso-ventrally flattened triploblastic worms.
2. Body shape generally worm-like but varies from moderately elongated flattened shape to long flat ribbon.
3. The flatworms are small to moderate in size varying from.
4. The majority of flatworms are white, colourless, some derive colour from host tissue or feeding materials, while the free-living forms are brown, grey, black or brilliantly coloured.
5. The anterior end of the body is differentiated into suctorial structure, the so called scolex in tapeworms and head with oral sucker in flukes.
6. The ventral surface bearing mouth and genital pores in trematodes.
7. The presence of great variety of adhesive secretions and organs of hooks are well marked in both trematodes and cestodes.
8. The body is covered with a cellular syncytial layered structure, partly ciliated parasitic in trematodes and cestodes, epidermis is lacking.
9. The exo- and endo-skeloton are completely absent, hence, the body is generally soft and is covered with one layered syncytium consisting of cuticle, spines, thorns, hooks, teeth, etc.
10. These are acoelomate, i.e., true coelom is absent.
11. The digestive system is totally absent in Acoela and tapeworms but in other consists of mouth, pharynx and blind intestine (anus absent).
12. The respiratory and circulatory systems are absent.

13. Excretory system consists of single or paired protonephridia with flame cells. In Acoela the protonephridia are absent.
14. The nervous system is primitive. The main nervous system comprising one pair ganglia or brain and one to three pairs of longitudinal nerve cords connected to transverse commeasures. This type of nervous system is called ladder type of nervous system.
15. Sense organs are of common in occurrence. Chemo- and Tango-receptors commonly occur in the form of ciliated grooves.
16. Sexes are united, i.e., hermaphrodite with very few exceptions parasitic forms.
17. Reproductive system is highly evolved or complex in most of the forms.
18. Asexual reproduction by fission occurs in many freshwater *Turbellaria.*
19. Fertilization is internal. The cross-fertilization in trematodes and self-fertilization in cestodes.
20. The life cycle complicated involves one or more hosts.
21. The parthenogenesis and polyembryony commonly occur in trematodes and some tapeworms propagate by endogenous or exogenous budding.
22. The flatworms are either free-living or ecto- or endo-commensals or parasitic.

3. *FASCIOLA*

Classification

Phylum : Platyhelminthes
Class : Trematoda
Order : Digenea
Genus : *Fasciola*

Habitat

- It is a cosmopolitan, endoparasitic trematode found attached inside the larger bile ducts of sheep and goats (primary Rost) while its larval stages are found inside the fresh water snails (*Limnaea*-secondary host) as shown in Figure 7.

Habits

- It is commonly called sheep liver fluke.
- Adult fluke shows wriggling movements inside bile duct by muscular contractions.
- It is holozoic and feeds upon blood, bile and epithelial cells of bile duct.

- It respires anaerobically.
- It is bisexual or hermaphrodite and shows both the self or cross fertilization.
- Development is indirect and complexed by the presence of larval stages miracidium, sporocyst, redia, cercariae and metacercariae.
- It shows delayed polyembryony and larval multiplication. Life cycle is digenetic.

Identification Points

- It is leaf-like with dorso-ventrally flat body (Refer Figure 7).
- It has oral sucker at anterior end surrounding mouth and a cup-shaped sucker, called acetabulum, on ventral side.

Economic Importance

- Heavy infection in sheep causes liver rot or fascioliasis.

Viva Voce

- What is habitat and pathogenicity of *Fasciola*?
- What is delayed polyembryony?

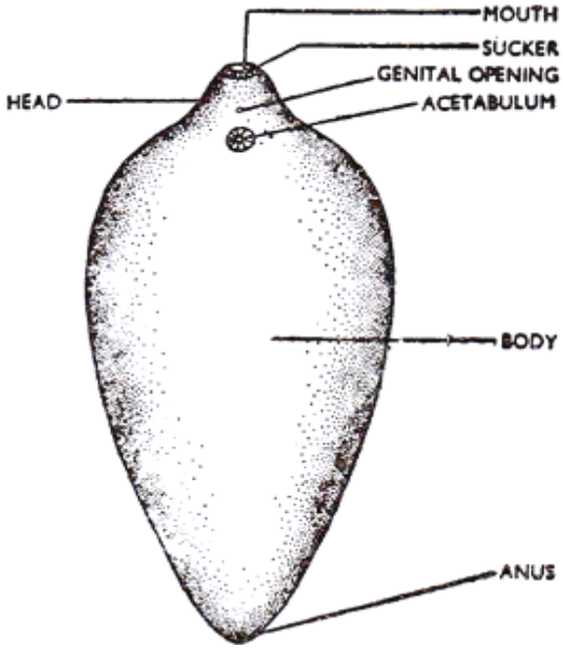

***Fig.** 7 Fasciola*

4. *TAENIA*

Classification

Phylum : Platyhelminthes
Class : Cestoda
Order : Cyclophyllidea
Genus : *Taenia*

Habitat

- It is a cosmopolitan, endoparasitic cestode. Adult is found attached to mucosa of intestine of man (primary host) while larval stages are found in pig muscles (secondary host).

Habits

- It is commonly called pork tape-worm.
- Locomotion is absent as adult is fixed to intestinal wall with the help of hooks and suckers.
- It is saprozoic or parasitic and takes the digested food from intestine through its body surface.
- It respires anaerobically.
- It is protandrous hermaphrodite, so there is always self fertilization between different mature proglottids of the same animal. Fertilization is internal.
- Development is indirect and includes a six-hooked hexacanth larva and a bladder worm or Cysticercus larva.
- Human infection is caused by taking undercooked measly pork. Life cycle is digenetic.

Identification Points

- Body is differentiated into scolex, neck and strobila (Refer Figure 8).
- Scolex has four suckers and rostellum with hooks for adhesion to intestinal mucosa.
- Strobila is formed of three types of proglottids: immature, mature and gravid proglottids.

Economic Importance

- Adult causes taeniasis in weak persons while bladder worm causes cysticercosis in man.

NOTES

Viva Voce

- What is the habitat of *Taenia*?
- What is the function of scolex of *Taenia*?
- How does human infection of *Taenia* occur?

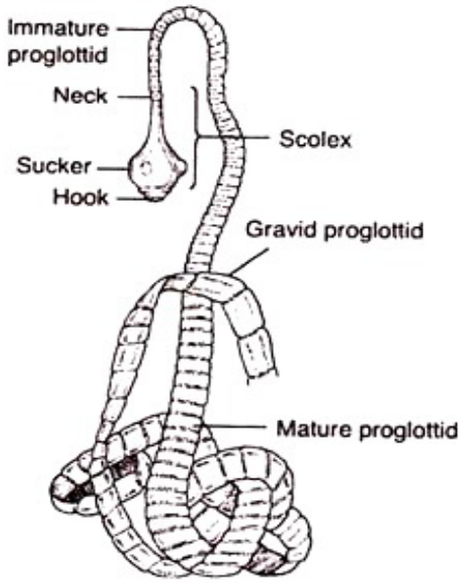

Fig. 8 *Taenia*

PHYLUM: ASCHELMINTHES (GREEK, ASKES = CAVITY; HELMINTHS = WORMS)

1. DEFINITION

Aschelminthes are pseudocoelomate, bilaterally symmetrical, triploblastic, unsegmented, vermiform, organ-system grade of construction with complete digestive tube.

2. GENERAL CHARACTERS

1. These are bilaterally symmetrical, unsegmented worms.
2. They are triploblastic and pseudocoelomate animals with organ-system grade of body organisation.
3. Body usually worm-like cylindrical or flattened.
4. Cuticle usually present and cilia absent.
5. The alimentary canal straight and complete with mouth and anus.
6. Respiratory and circulatory systems absent.
7. Excretory system includes a system of canals, protonephridia present in some forms.
8. Nervous system simple and consists of a circumenteric nerve ring having anterior cerebral ganglia or brain and lateral longitudinal nerves.
9. The sense organs are in the form of pits, papillae, bristles and eye spots.
10. The sexes are separate, i.e., dioecious and reproductive organs relatively simple.
11. The asexual reproduction does not occur.
12. The eggs shelled, cleavage determinate and spiral. The life cycle is simple or complicated usually with no special larval stages.
13. Heterogeneous group inhabiting aquatic and terrestrial environment, many are well equipped for parasitic life.

3. *ASCARIS*

Classification

Phylum : Aschelminthes
Class : Nematoda
Order : Ascaroidea
Genus : *Ascaris*

Habitat

- It is cosmopolitan, endoparasitic nematode found in small intestine of man, especially children.

Habits

- It is commonly called round worm.
- Though active organs of locomotion are absent yet it shows dorso-ventral undulations of body to counter peristalsis.
- It is holozoic and takes the digested food of host by the sucking action of pharynx.
- It is mainly respires anaerobically. It is a facultative anaerobe.
- Sexes are unisexual and show sexual dimorphism. Male is smaller in size with curved posterior end, with cloacal aperture and two penial setae.
- Fertilization is internal. Development is direct.
- Human infection is by taking cysts with second juveniles. Life cycle is monogenetic.

Identification Points

- Body is long cylindrical with mouth bounded by three lips at anterior end and anus (in female) or cloacal aperture (in male) near the posterior end (Refer Figure 9).
- There are four longitudinal lines on body surface.

Economic Importance

- Heavy infection causes ascariasis, blockage of intestine, etc. Its toxins may also cause appendicitis, pneunonia, enteritis, mental depression, etc.

Viva Voce

- What is the habitat of *Ascaris*?
- Give the differences between male and female *Ascaris*.

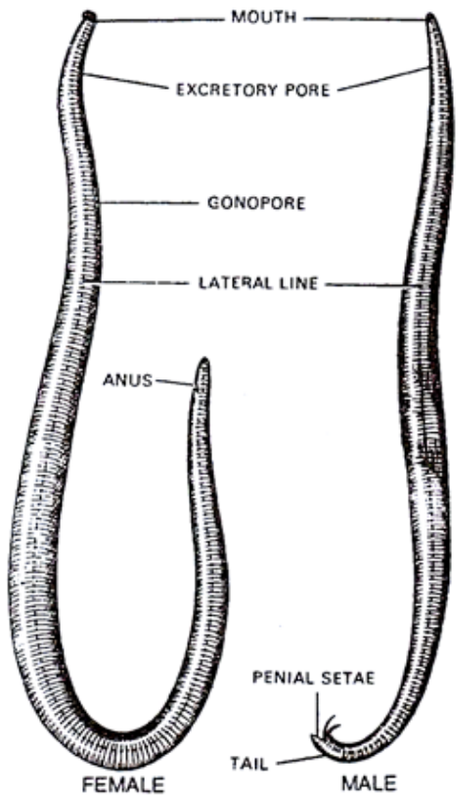

Fig. 9 *Ascaris*

4. *ANCYLOSTOMA*

Classification

Phylum : Aschelminthes
Class : Nematoda
Order : Strongyloidea
Genus : *Ancylostoma*

Habitat

- It is coelozoic endoparasitic nematode found in duodenum of small intestine of man.

Habits

- It is commonly called Hookworm (Refer Figure 10).
- Locomotion is absent as it is attached to mucosa of intestine.
- It feeds on the epithelial cells of duodenum with the help of cutting plates or teeth.

- Sexes are unisexual and show sexual dimorphism. Male hook worm is smaller in size and has a cloacal aperture, a copulatory bursa supported by muscular rays and two copulatory spicules near the posterior end.
- Fertilization is internal and development is indirect and includes rhabditiform larva and infective filariform larva.

Identification Points

- Male is long and cylindrical but smaller in size than female.
- Both sexes have a large buccal capsule with six cutting plates at anterior end.
- Male has cloacal aperture, copulatory bursa and two penial spicules near the posterior end.

Economic Importance

- Heavy infection causes ancylostomiasis which is characterized by gastro-intestinal dysfunctioning, anaemia, and physical and mental deficiency.

Viva Voce

- Why *Ancylostoma* is commonly called hook worm?
- State sexual dimorphism in hook worm.

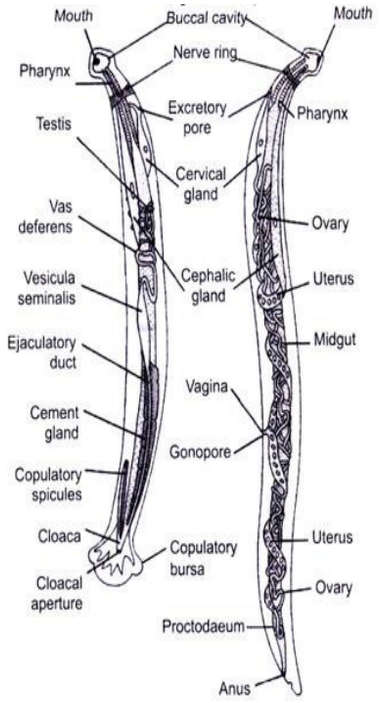

Fig. 10 *Ancylostoma*

PHYLUM: ANNELIDA (LATIN, ANNELUS = LITTLE RING)

1. DEFINITION

Annelids are triploblastic, bilaterally symmetrical, metamerically segmented, coelomate worms with a thin cuticle around the body.

2. GENERAL CHARACTERS

1. Annelida are mostly aquatic, marine or freshwater, terrestrial, burrowing or living in tubes, sedentary or free living, elongated and vermiform. Some are commensal and parasitic also.
2. The body triploblastic, bilaterally symmetrical.
3. The body metamerically segmented, externally by transverse grooves and internally by septa into a number of divisions, each division is called a segment, metamere or somite.
4. The organ-system grade of body organization usually divided into a large number of compartments by inter-segmental septa.
5. The outer covering of the body is cuticle secreted by the underlying epidermis.
6. Body wall is contractile, consisting of an outer epidermis, circular and longitudinal muscles.
7. Appendages when present are unjointed. Locomotory organs are segmentally arranged paired setae or chaetae in most of the cases.
8. Presence of a true schizocoelous coelom.
9. The alimentary canal is tube-like, complete extending from mouth to anus.
10. Respiration occurs through general body surface, in some cases by gills also.
11. The blood vascular system is closed type, blood is red due to the presence of or erythrocruorin found dissolved in the plasma.
12. The excretion by segmentally arranged nephridia which usually communicate the exterior.
13. Nervous system with a pair of cerebral ganglia, the brain and a double nerve cord and segmentally arranged ganglia.

14. The tactile organs, taste buds, statocysts, photoreceptor cells and eyes are present.
15. Usually monoecious, i.e., hermaphrodite; dioecious or unisexual forms also.
16. The development is direct in monoecious forms but indirect in dioecious forms.
17. A free-swimming trochophore larval stage is characteristic in case of indirect development, while in others this stage is passed during the development.
18. Asexual reproduction also occurs in some forms.

3. *PHERETIMA*

Classification

Phylum : Annelida
Class : Oligochaeta
Order : Neooligochaeta
Genus : *Pheretima*

Habitat

- It is a terrestrial, cosmopolitan and fossorial oligochaete commonly found in moist soil, rich in dead and decaying organic matter (hums).
- These are commonly seen in fields, pastures, lawns and gardens.

Habits:

- It is commonly called earthworm (Refer Figure 11).
- It is nocturnal.
- Due to burrowing or fossorial habit it lives in self-dug burrows made either by eating the soil or simply by pushing into the soil.
- Locomotion is of creeping type with muscle contraction aided by setae.
- It is omnivorous. It mainly eats humus but also takes algal filaments, pieces of leaves, seeds and even small insects.
- Ingestion is by sucking action of pharynx.
- The faeces are heaps of pellets called worm castings.
- It shows cutaneous respiration.

- It is bisexual or hermaphrodite but shows protandrous condition, so always involves cross-fertilization, Copulation occurs during rainy season and involves mutual-insemination.
- Fertilization is external and occurs in ootheca secreted by clitellum.
- Development is direct and there is no larva.

Identification Points

- Body is cylindrical and metamerically segmented.
- Body is dark brown on dorsal side while light brown on ventral side.
- A glandular band, clitellum, surrounds 14, 15 and 16 segments.
- Presence of single female gonopore on 14th; male gonopores on 18th and genital papillae on 17th and 19th segments.

Economic Importance

Earthworm is an animal of mixed blessing:

(a) Harmful

- Makes burrows in golf grounds.
- Promotes soil-erosion by making pores in slopes.
- Makes burrows in the banks of irrigation canals.

(b) Useful

- Used as a fish-bait.
- Improves soil-fertility as makes the soil porous for better irrigation, aeration and deeper penetration of roots. These bring sub-soil with minerals to the surface.
- Used as food for many useful animals.

Viva Voce

- What is the importance of earthworms?
- Why is *Pheretima* known as earthworm?

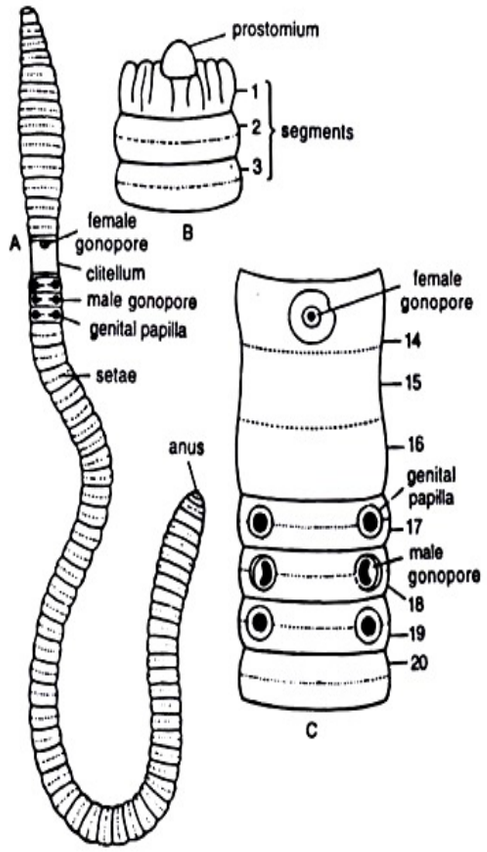

Fig. 11 *Pheretima: Ventral View (A), Dorsal Anterior Portion (B), Gonopores (C)*

4. *HETERONEREIS*

Classification

Phylum : Annelida

Class : Polychaeta

Order : Errantia

Type : Heteronereis

Habitat

- Heteronereis is a free swimming reproductive phase of *Nereis* which is a marine, cosmopolitan and fossorial Annelida Found between tide-marks.

Habits

- It commonly called clamworm (Refer Figure 12).
- It is of free swimming type with the help of highly modified parapodia with oar-like setae and foliaceous outgrowths.

- Feeding is carnivorous.
- Heteronereis is unisexual or dioecious but with no sexual dimorphism. Gonads are temporary and appear only in posterior epitokal segments.
- Two sexes swarm together at night during breeding season. Female is oviparous and fertilization is external.
- Development is indirect and includes a free-swimming trochophore larva which undergoes metamorphosis.

Identification Points

- Body is differentiated into anterior asexual atoke and posterior sexual epitoke.
- Parapodia of segments of epitoke have oar-like setae and foliaceous outgrowths.

Economic Importance

- It is a very nice example of epitoky.

Viva Voce

- What are the differences between *Nereis* and Heteronereis?
- What are the differences between atoke and epitoke?

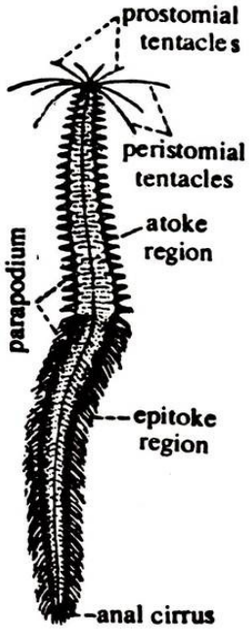

Fig. 12 *Heteronereis*

PHYLUM: MOLLUSCA (LATIN, MOLLIS = SOFT)

1. DEFINITION

Molluscs are soft-bodied, bilaterally symmetrical unsegmented, coelomate animals; usually shelled having a mantle, ventral foot, anterior head and a dorsal hump of visceral mass.

2. GENERAL CHARACTERS

1. Molluscs are essentially aquatic mostly marine, few freshwater and some are terrestrial forms.
2. The body is soft, unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass, the mantle cavity dorsal hump or dome and the lumen of the gonads and nephridia.
3. The body is clothed with one layered often ciliated organs except in Pelecypoda and Scaphopoda secreted by the mantle.
4. These are variously modified for creeping, burrowing and swimming.
5. Body is commonly protected by an exoskeletan calcareous shell.
6. Head is distinct, bearing the mouth and provided with eyes and tentacles.
7. Ventral body wall is modified into a muscular flat or plough-like structure.
8. The mantle or pallium is a fold of body wall.
9. Visceral mass contains the vital organs of the body in a compact form.
10. The body cavity is haemocoel. The true coelom is generally limited to the pericardial space.
11. The digestive tract is simple with an anterior mouth and posterior anus or in cephalopoda and gastropoda, it become “U” shaped and bringing mouth and anus together.
12. The pharynx contains a rasping organ, the radula, except in Pelecypoda.
13. The circulatory system is open except in cephalopods.
14. The respiratory organs consist of numerous gill or ctenidia usually provided osphradium at the base. Lung is developed in terrestrial forms. Respiratory pigment is haemocyanin.
15. The nervous system consists of paired cerebral, pleural, pedal and visceral ganglia, longitudinal and transverse connectives and nerves to joined ganglions.

16. The excretory system consists of a pair of metanephridia which are true coelomoducts communicate from pericardial cavity to the exterior by nephridiopore.
17. The sexes usually separate (dioecious) but some are hermaphroditic.
18. The fertilization is external or internal.
19. The development is either direct or indirect with metamorphosis through the trochophore stage called veliger larva or glochidium larva.

3. *LOLIGO*

Classification

Phylum : Mollusca
Class : Cephalopoda
Order : Decapoda
Genus : *Loligo*

Habitat

- It is a marine, cosmopolitan, free-swimming decapod mollusc found in coastal shallow or deep waters of warm seas.

Habits

- It is commonly called squid or sea arrow (Refer Figure 13).
- Locomotion is of swimming type by undulating movements of lateral fins or parapodia. It can swim forward as well as backward.
- It is carnivorous and feeds upon crabs, fishes etc. which are captured by sucker-bearing oral arms.
- It defends itself by: protective colouration so called metacritic and by discharging a smoke-screen of loligo-ink, as well as by backward darting with siphon on hydropropulsion principle.
- It is unisexual or dioecious. Sexual dimorphism is seen during breeding season when 3rd right arm of male, is modified into spoon-headed hectocotylised arm which is used to transfer spermatophore in female.
- Fertilization is internal. Female is oviparous and development is direct.

Identification Points

- Head with eight sucker-bearing arms and two long tentacles.
- Presence of Siphon or funnel on ventral side.
- Presence of fins or parapodia on sides of posterior half of trunk.

Economic Importance

- It is used as food by Chinese and Italians.

Viva Voce

- What is Metacritic?
- Why is *Loligo* placed in order Decapoda?

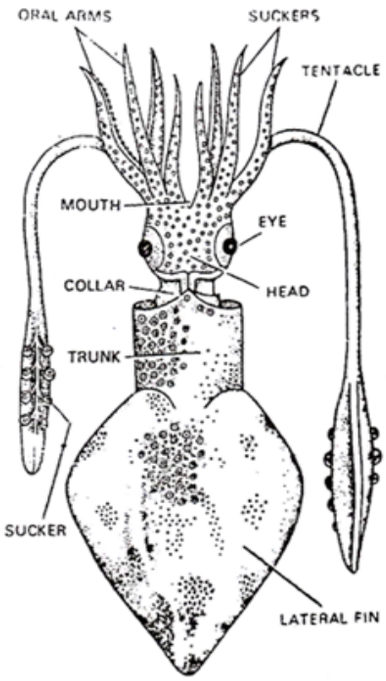

Fig. 13 *Loligo*

4. *OCTOPUS*

Classification

Phylum : Mollusca
Class : Cephalopoda
Order : Octopoda
Genus : *Octopus*

Habitat

- It is a marine cosmopolitan and benthonic cephalopod mollusc (Refer Figure 14).

Habits

- It is commonly called devil fish.
- It is nocturnal.

- Locomotion is crawling type (with its arms).
- Feeding is carnivorous and feeds upon crabs, fishes and other molluscs. The prey is killed with poisonous saliva.
- It is unisexual or dioecious and shows sexual dimorphism in breeding season.
- The 3rd right arm of male is modified and is called hactocotylus arm for transfer of sperms in females.
- Fertilization is internal. Female is oviparous and development is direct.
- It defends itself by forming a smoke-screen with an ink-gland and protective colouration as well as backward darting with the help of siphon (funnel).

Identification Points

- Bears eyes and a siphon.
- Body is globular and bears eight sucker-bearing equal sized arms.

Economic Importance

- It is harmful mollusc as voraciously eats the fishes and is used as sea food.

Viva Voce

- Why is *Octopus* placed in Octopoda?
- What is hectocotylus arm?

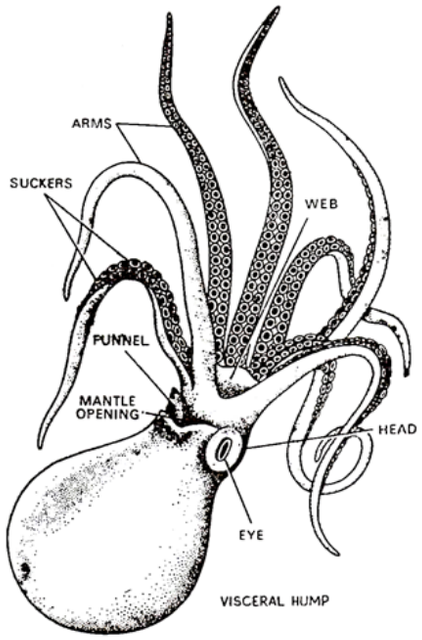

Fig. 14 *Octopus*

PHYLUM: ARTHROPODA (GREEK, ARTHROS = JOINTED; PODOS = FOOT)

1. DEFINITION

Arthropods are bilaterally symmetrical, triploblastic metamerically segmented animals with coelom which reduced and modified. Their body is covered externally in a chitinous exoskeleton which moults periodically and their appendages are jointed.

2. GENERAL CHARACTERS

1. The arthropods are triploblastic, bilaterally symmetrical, metamerically segmented animals.
2. The body is covered with a thick chitinous cuticle forming an exoskeleton.
3. The body segments usually bear paired lateral and jointed appendages.
4. The musculature is not continuous but comprises separate striped muscles.
5. The body cavity is haemocoel.
6. The digestive tract is complete; mouth and anus lie at opposite ends of the body.
7. The respiration through general body surface, by gills in aquatic forms, and tracheae or book lungs in terrestrial forms.
8. The circulatory system is open with dorsal heart and arteries but without capillaries.
9. True nephridia are absent, excretion by coelomoducts or Malpighian tubules or green or coxal glands.
10. The cilia are entirely absent from all parts of the body.
11. The sexes are generally separate and sexual dimorphism is often exhibited by several forms.
12. The parental care is also often well marked in many arthropods.
13. It is most diversified group inhabiting the land, water and air.
14. Fertilization is internal and development is usually indirect through larval stages, may be direct also.

3. *PERIPATUS*

Classification

Phylum : Arthropoda
Class : Onychophora
Genus : *Peripatus*

Habitat

- It is a terrestrial, worm-like segmented arthropod (onychophora) found in moist places, in crevices of rocks, under stones and logs, etc.

Habits

- It is commonly called walking worm (Refer Figure 15).
- It is nocturnal.
- Locomotion is of walking type with many pairs of unjointed legs.
- Feeding is carnivorous and is a predacious feeder of insects. The food is captured with a jet of slime.
- It is unisexual or dioecious and shows sexual dimorphism. Male is smaller than female.
- Fertilization is internal and female is viviparous and produces about 30 young ones in a year.
- Development is direct.

Identification Points

- Body is segmented and is similar to caterpillar.
- Head has one pair of each of antennae, eyes, jaws and oral papillae.
- Presence of many pairs of unjointed legs each ending into one pair of claws.

Economic Importance

- It acts as a connecting link between Arthropoda and Annelida. Its arthropod characters are presence of haemocoel, dorsal tubular heart with paired ostia, tracheal respiration and salivary glands. Its annelid characters are continuous muscle layers, structure of eye like Polychaetes, unjointed legs as parapodia and segmental nephridia.

Viva Voce

- What is evolutionary significance of *Peripatus*?
- Why is *Peripatus* called walking worm?

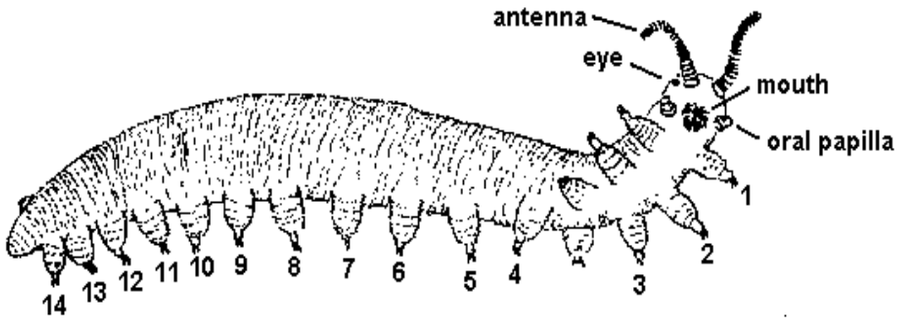

Fig. 15 *Peripatus*

4. *PERIPLANETA*

Classification

Phylum : Arthropoda
Class : Insecta
Order : Dictyoptera
Genus : *Periplaneta*

Habitat

- It is terrestrial and cosmopolitan insect found in places with warmth and food like kitchens, bakeries, grocer's shops, railway wagons, etc. Its native place is Africa but came to India through America along with shipment of wheat.

Habits

- It is commonly called Cockroach (Refer Figure 16).
- It is nocturnal.
- Locomotion is cursorial (fast runner) but rarely flies with the help of hind wings only.
- Feeding is omnivorous and also shows cannibalism with biting and chewing type mouth parts.
- Sexes are unisexual and show sexual dimorphism. Male has anal styles while female has a brood pouch.
- Fertilization is internal. Female is oviparous and lays sixteen eggs in an ootheca.
- Development is direct. Young, called nymph, undergoes gradual metamorphosis.

Identification Points

- Body is oval-shaped, reddish-brown colored and formed of three parts: Head, thorax and abdomen.
- Head has kidney-shaped compound eyes, long and cylindrical antennae, and biting and chewing type mouth parts.
- Thorax bears three pairs of legs and two pairs of wings. Forewings act as tegmina or wing covers.
- Abdomen has anal cerci in both sexes while male has anal styles and female has a brood pouch.

Economic Importance

It is an animal of mixed blessing:

(a) Harmful: It destroys human food and other food items and acts as vector of many pathogens.

(b) Useful: It is used in physiological and toxicological researches.

Viva Voce

- State two differences between male and female Cockroach.
- Which type of mouth parts are found in Cockroach?

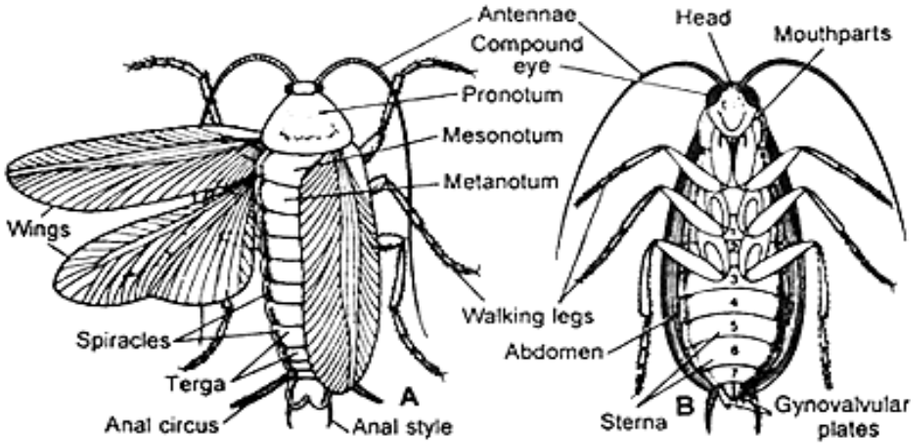

Fig. 16 *Periplaneta: Dorsal View (A), Ventral View (B)*

5. *MELANOPUS*

Classification

Phylum : Arthropoda

Class : Insecta

NOTES

Order : Orthoptera

Genus : *Melanopus*

Habitat

- It is a terrestrial, gregarious and cosmopolitan grasshopper commonly found on the plants.

Habits

- It is commonly called grasshopper (Refer Figure 17).
- It is a diurnal insect.
- It shows two type of locomotion: Jumping with the help of saltatorial meta legs and flying with the help of hind wings only.
- Feeding is herbivorous and a voracious feeder of leaves with the help of biting and chewing type mouth parts.
- Sexes are unisexual and show sexual dimorphism. Male is with rounded tip of abdomen while female has ovipositor at the tip of abdomen.
- Fertilization is internal. Female is oviparous. Eggs are laid in tunnels made in the soil.
- Development includes gradual metamorphosis (gradual change in body) or paurometa bolous.

Identification Points

- Body is yellow banded and divisible in three parts: head, thorax and abdomen.
- Head is hypognathous and bears antennae, compound eyes, three ocelli, and biting and chewing type mouth parts.
- Thorax is with three pairs of legs and two pairs of wings. Meta legs are saltatorial. Forewings act as tegmina.
- Abdomen is with respiratory spiracles and copulatory organs in male and ovipositor in female.

Economic Importance

- Used as food by animals.

Viva Voce

- What is hypognathous head?
- Define paurometaboly.

NOTES

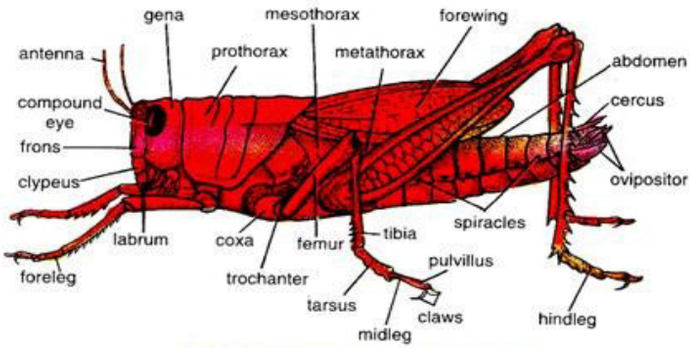

Fig. 17 *Melanopus*

PHYLUM: ECHINODERMATA (GREEK, ECHINUS = HEDGEHOG; DERMA = SKIN)

1. DEFINITION

Echinoderms are enterocoelous coelomates with pentamerous radial symmetry, without distinct head or brain, having a calcareous endoskeleton of separate plates or pieces and a peculiar water vascular system of coelomic origin with podia or tube-feet projecting out of the body.

2. GENERAL CHARACTERS

1. The echinoderms are exclusively marine and are among the most common and widely distributed of marine. They occur in all seas from the intertidal zone to the great depths.
2. The symmetry usually radial, nearly always pentamerous.
3. The body is triploblastic, coelomate with distinct oral and aboral surfaces and with definite head and segmentation.
4. They are of moderate to considerable size but none are microscopic.
5. The body shape rounded to cylindrical or star like with simple arms radiating from a disc or branched feathery arms arise from a central body. The radiating grooves may be composed of separate small ossicles.
6. The coelomic system develops from embryonic archenteron, i.e., enterocoel lined by peritoneum, occupied mainly by digestive and reproductive.
7. Alimentary tract is usually coiled tube extending from the mouth located on the oral surface to the anus on the aboral surface.
8. The outer surface is rarely smooth, typically it is covered by five symmetrically spaced ambulacra with five alternating inter-radii or inter-ambulacra.
9. The body wall consists of an outer epidermis, a middle dermis and an inner lining of endodermis.
10. The endoskeleton consists of closely fitted plates forming a shell usually called theca or test.

11. Presence of water vascular or ambulacral system is the most characteristic feature consists of tubes filled with a watery fluid.

12. The nervous system is primitive, consisting of networks concentrated into the radial ganglionated nerve cords.

13. Sense organs are poorly developed.

14. Circulatory or haemal or blood lacunar system is typically present.

15. Respiration tube feet by gills in sea urchins, brittle stars and cloacal respiratory trees in holothuria.

16. The excretory system is inadequate.

17. The sexes are usually separate (dioecious) with few exceptions. Gonads are simple with or without genital bursae.

18. Reproduction is usually sexual, few reproduce asexually or by regeneration.

19. Fertilization is external, while few echinoderms are viviparous.

20. The development is indeterminate including characteristic larvae which undergo metamorphosis into radially symmetrical adults.

3. *ASTERIAS*

Classification

Phylum : Echinodermata
Class : Asteroidea
Order : Forcipulata
Genus : *Asterias*

Habitat

- It is a marine and benthic echinodermata found in shallow waters in the seas of India and U.S.A.

Habits

- It is commonly called starfish or sea star (Refer Figure 18).
- It is nocturnal.
- Locomotion is of creeping type with the help of tube feet or podia.
- Feeding is carnivorous and prefers clams (bivalves) as food.
- Digestion is extra-corporeal.
- It is unisexual or dioecious with no sexual dimorphism.
- Female is oviparous and fertilization is external.
- Development is indirect and includes a free swimming and ciliated bilateral symmetrical bipinnaria larva which through Brachiolaria undergoes metamorphosis.
- It has good power of autotomy and regeneration.

Identification Points

- Body is star-shaped and is formed of a central disc and five arms.
- Central disc has mouth with parastomal teeth on oral side and madreporite and anus on aboral side.
- Oral side of each arm has an ambulacral groove with two rows of tube feet.

Economic Importance

- It is an animal of mixed blessing:
  (a) Harmful as it causes damage to clams, oysters, etc.
  (b) Useful as acts as scavenger, dried skeleton as fertilizer and a study animal.

Viva Voce

- Why is *Asterias* called starfish?
- State two peculiar features of Echinodermata.

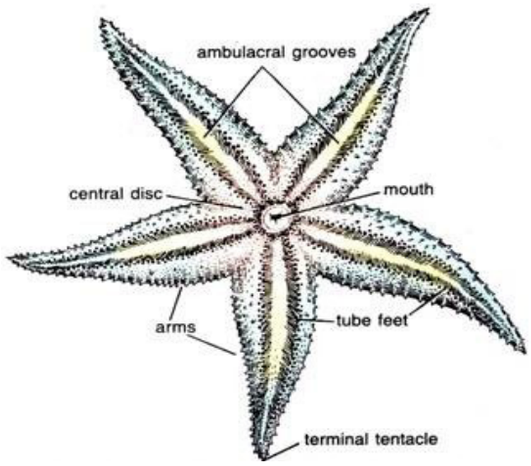

Fig. 18 *Asterias*

4. *CUCUMARIA*

Classification

Phylum : Echinodermata
Class : Holothroidea
Order : Dendorchirota
Genus : *Cucumaria*

Habitat

- It is marine benthic, cosmopolitan and fossorial echinoderm found in burrows on sea bottom.

Habits

- It is commonly called sea cucumber (Refer Figure 19).
- Locomotion is slow creeping type with the help of muscular undulations of body aided by tube feet.
- It is omnivorous and feeds upon planktons, bottom-debris, etc. captured with the help of mucus on tentacles.
- It respires with the help of respiratory tree. The water is drawn inside and expelled outside.
- It has very high power of autotomy and can eject most of viscera in self-defense. The viscera later regenerated.
- Sexes are separate, development indirect, includes an Auricularia larva.

Identification Points

- Body is long, cylindrical and leathery like a cucumber.
- Arms are absent.
- At oral end, mouth is surrounded by a ring of branched tentacles.
- Body surface has alternating ambulacral and inter-ambulacral zones.

Economic Importance

- It is used as food in some countries.

Viva Voce

- Explain autotomy in *Cucumaria*.

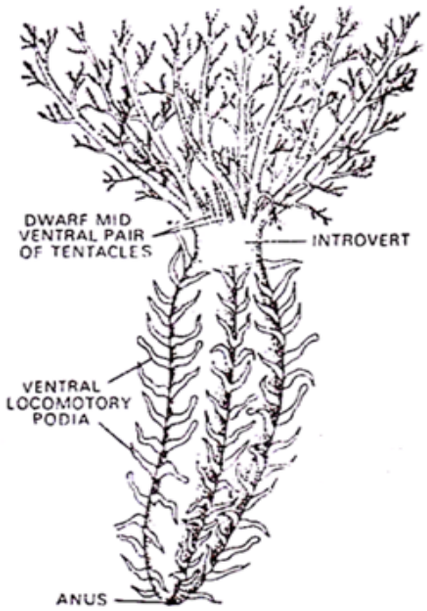

Fig. 19 *Cucumaria*

PHYLUM: HEMICHORDATA (GREEK, HEMI = HALF; CHORDE = CORD)

1. DEFINITION

Usually vermiform, solitary or colonial enterocoelous coelomate animal with intra-epidermal nervous system and a pre-oral gut with or without gill slits and without typical nephridia.

2. GENERAL CHARACTERS

1. Exclusively marine and soft-bodied forms.
2. Body is divisible into proboscis, collar and trunk.
3. Notochord occurs only in the anterior end of the body. Recently it has been called "buccal diverticulum" due to its doubtful nature.
4. Numerous paired gill-slits are present.
5. Nervous tissues lie embedded in the epidermis and occur both on the dorsal and ventral surfaces.
6. Coelom is usually divided into three distinct portions corresponding to the three regions.
7. Blood vascular system is simple.
8. Sexes are separate and the development may be direct or indirect.

11.3 *BALANOGLOSSUS*

Classification

Phylum : Hemichordata
Class : Enteropneusta
Genus : *Balanoglossus*

Habitat

- It is a marine, fossorial, tubicolous and cosmopolitan hemichordata found in U-shaped burrows in shallow coastal waters.

Habits

- It is commonly called acorn or tongue-worm (Refer Figure 20).
- Locomotion crawling type by peristaltic contractions of the trunk and cilia on body surface.

- Feeding is omnivorous, planktonic and ciliary feeder.
- Sexes are unisexual and show sexual dimorphism. Male and female differ in the color of gonads.
- Female is oviparous and fertilization is external.
- Development is indirect and includes a tornaria larva which undergoes metamorphosis.
- It has good power of regeneration.

Identification Points

- Body is divided in three regions: Proboscis, collar and trunk.
- Proboscis is conical and has a buccal diverticulum (stomochord).
- Collar has mouth and collar coelom.
- Trunk is differentiated into two parts: anterior branchial region with gills and posterior genital region with gonads.

Economic Importance

- It acts as connecting link between echinodermata and chordata.

Viva Voce

- What is evolutionary importance of *Balanoglossus*?
- Why is *Balanoglassus* called tongue-worm?

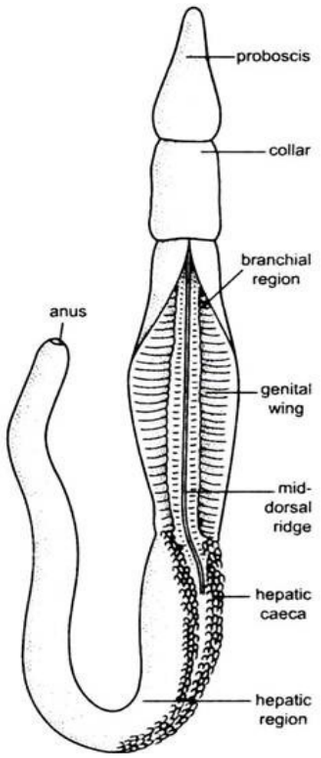

Fig. 20 *Balanoglossus*

PHYLUM: CHORDATA (GREEK, CHORDE = A STRING OR CORD; ATA = BEARING)

1. DEFINITION

The chordates are the animals comprising a stiff, supporting rod-like structure along the back, the notochord (Greek, Noton = Back; Latin, Chorda = Cord) pharyngeal gill slits and dorsal tubular nerve cord. Thus all the chordates possess three common fundamental characteristics at some stage in the life history. These three common fundamental morphological features are as follows: (i) Notochord (a longitudinal supporting rod-like structure); (ii) Dorsal tubular (hollow) nerve cord and (iii) Pharyngeal gill-slits.

2. GENERAL CHARACTERS

1. Chordates are aquatic, aerial or terrestrial. All are free living with no fully parasitic forms.
2. The body is small to large, bilaterally symmetrical and metamerically segmented.
3. A post anal tail usually projects beyond the anus at some stage of life.
4. The exoskeleton often present, well-developed in most vertebrates.
5. The body wall triploblastic, i.e., presence of three germinal layers ectoderm, mesoderm and endoderm.
6. These are coelomate animals, i.e., a well developed true coelom is always present and enterocoelic or schizocoelic in origin.
7. The notochord is always essentially present at some stage of life cycle but not persist in the adult. It is completely or partly replaced by vertebral column in majority of animals.
8. Cartilaginous or bony, living and jointed endoskeleton present in the major members (vertebrates).
9. Paired pharyngeal gill-slits are present on either side of the pharynx at some stage which may or may not be functional.
10. The digestive system is complete with digestive glands.
11. The blood vascular system is closed, heart ventral with dorsal and ventral blood vessels.
12. Hepatic portal system is present which is well developed.

13. The excretory system comprising proto- or meso- or meta-nephric kidneys.

14. The nerve cord is dorsal and tubular. Anterior end usually enlarged to form brain.

15. The sexes separate with rare exceptions.

3. *HERDMANIA*

Classification

Phylum : Chordata
Class : Ascidiacea
Order : Pleurogona
Genus : *Herdmania*

Habitat

- It is a solitary, sessile, free living marine protochordate.

Habits

- It can release water simultaneously or independently through the siphons. Hence, it is called sea squirt (Refer Figure 21).
- Body proper has two cylindrical projections, branchial siphon and atrial siphon.
- Water enters through branchial aperture and leaves through atrial aperture.
- The foot is made by the tunic and has foreign particles, such as sand grains, shell pieces, etc., attached to it.
- Foot helps in anchorage and in balancing. Its shape and size varies.
- Animal is hermaphrodite but cross fertilization occurs.
- Fertilization is external. Development includes ascidian tadpole larva.
- It shows retrogressive metamorphosis.

Identification Points

- *Herdmania* has a sac like unsegmented body covered by a protective tunic.
- Test or tunic is wrinkled and acts like an accessory respiratory organ.
- Body appears like a potato and can be divided into body proper and foot.
- *Herdmania* can suddenly contract its body.
- Notochord, nerve chord, tail and tail fins of the larva degenerate.

Economic Importance

- It is a study material for research.

Viva Voce

- Why is *Herdmania* called sea squirt?

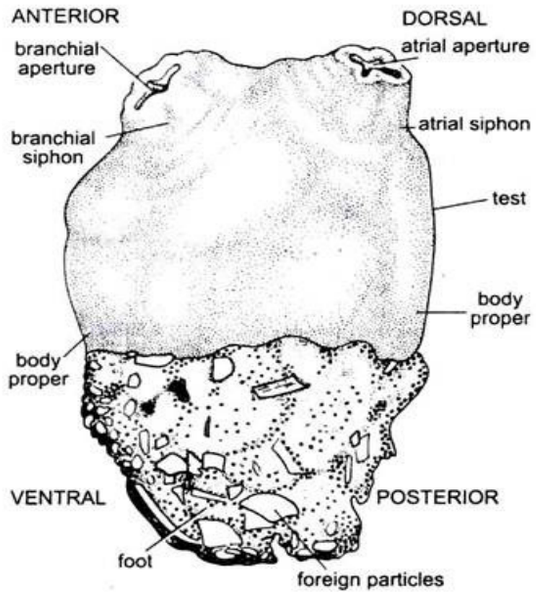

Fig. 21 *Herdmania*

4. *AMPHIOXUS*

Classification

Phylum : Chordata
Class : Leptocardii
Genus : *Amphioxus*

Habitat

- It is a marine animal, commonly found in shallow waters.

Habits

- Its common name is Lancelet (Refer Figure 22).
- It is a fossorial animal. Mostly it remains buried in sand.
- It is a ciliary feeder.
- Sexes are separate and fertilization is external.
- Development is indirect involving a free swimming larva.

Identification Points

- Amphioxus has transparent, whitish, laterally compressed and spindle shaped body.
- The anterior end is called rostrum. It is adapted for burrowing and swimming.
- Body is divisible into trunk and tail. A true head is absent.
- Trunk region bears three apertures, viz. mouth, atriopore and anus.
- Amphioxus bears three median fins a dorsal, ventral and caudal, which are unpaired.
- Two thin membranous folds hanging from ventro-lateral margin are called metapleural folds.
- On the lateral side of the body, a series of arrow shaped muscle bands are present called myotomes.

Economic Importance

- It is considered as a blue print of phylum chordata.

Viva Voce

- Why is amphioxus called Lancelet?

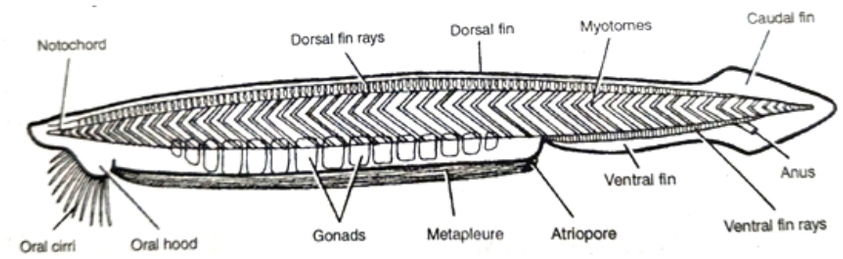

Fig. 22 *Amphioxus*

5. *MYXINE*

Classification

Phylum : Chordata
Class : Cyclostomata
Order : Myxinoidae
Genus : *Myxine*

Habitat

- It is a marine animal of coastal waters of Atlantic and Pacific Oceans. It is found buried in sea.

Habits

- It is commonly called common hag or slime eel or borer (Refer Figure 23).
- It swims by lateral undulations of muscular body.
- It is carnivorous and nocturnal feeder.
- It shows branchial respiration with the help of gills present in gill pouches.
- Sexes are unisexual and shows sexual dimorphism during breeding season only.
- Female is oviparous and fertilization is external.
- Development is direct.

Identification Points

- Body is long, pinkish and eel like.
- Body is soft, slimy and scale-less.
- Body is divisible into head, trunk and tail.
- Head bears a ventral mouth.
- Trunk is with large number of mucous glands on the lateral sides of the body and a pair of external branchial aperture. It also has a poorly developed median ventral fin.
- Tail bears a narrow and diphycercal caudal fin.

Economic Importance

- It is a serious threat to fisheries in some regions.

Viva Voce

- Why is *Myxine* called hag fish?
- How many external gill slits are present in *Myxine*?

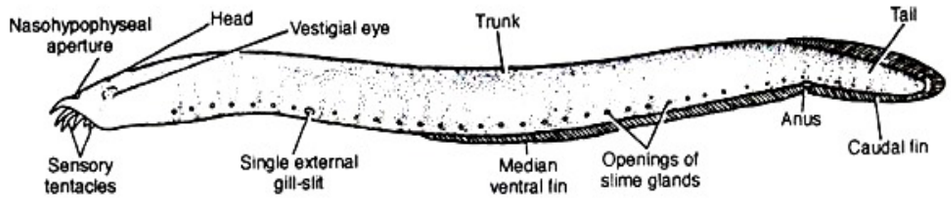

Fig. 23 *Myxine*

6. *PETROMYZON*

Classification

Phylum : Chordata
Class : Cyclostomata
Order : Petromyzon
Genus : *Petromyzon*

Habitat

- The lampreys are both marine and fresh-water forms. *Petromyzon marinus* is the marine lamprey. It inhabits Great Lakes between U.S.A. and Canada and along the Atlantic coasts of North America, West Africa and Europe.

Habits

- *Petromyzon* is commonly called as Lamprey (Refer Figure 24).
- *Petromyzon* is a Sanguivorous ectoparasite of a large healthy fish.
- It actively swims by lateral undulations of highly muscular body.
- It respires through gills.
- Its life cycle includes two phases the larval phase and adult phase. The larval phase, i.e., *Ammocoetes* larva is fresh water, microphagous and sedentary organism.
- In adults, sexual dimorphism is observed. The adults breed only once in life during early summer period. Fertilization is external.

Identification Points

- The adult *Petromyzon* is elongated, cylindrical and eel like. The body lacks scales.
- The upper surface of the body is dark and greenish brown in color, whereas lower surface is light, smooth and slimy.
- The body of is divisible into head, trunk and tail.
- Two prominent eyes are present on the lateral side of head.
- On the dorsal side of head, between the eyes, nostril or naris is present.
- Gill slits are small rounded opening on either side of head.
- At the junction of trunk and tail region, cloaca is present on ventral side.
- Tail bears caudal or tail fin supported by cartilaginous fin rays.

Economic Importance

- It is a sanguivorous ectoparasite of fishes.

Viva Voce

- Why is *Petromyzon* known as Lamprey?
- How many external gill apertures present in *Petromyzon*?
- Differentiate between *Myxine* and *Petromyzon*.

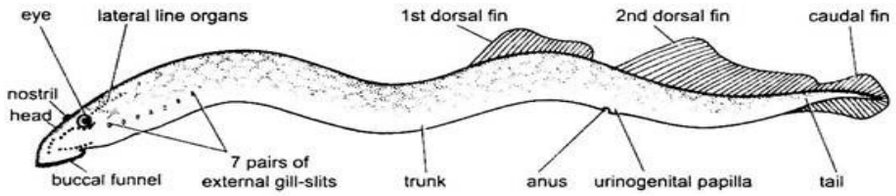

Fig. 24 *Petromyzon*

7. *TORPEDO*

Classification

Phylum : Chordata
Class : Chondrichthyes
Order : Hypotremata
Genus : *Torpedo*

Habitat

- It is a marine fish, found on muddy bottom.

Habits

- It is commonly called electric ray (Refer Figure 25).
- It is carnivorous.
- It swims by flapping action of pectoral fin.
- There is branchial respiration.
- It defends itself by producing an electric current of 50-60 volts.
- It is viviparous and produce live young ones.
- There is sexual dimorphism. Male has claspers. Fertilization is internal.

Identification Points

- Body is divisible into head, trunk and tail.
- Skin is smooth and without scales.

- Electric organs are situated on either side of the body between head and pectoral fin.
- Eyes and spiracles are closely placed dorsally above electric organs.
- Tail bears two dorsal fins and a caudal fin.

Economic Importance

- It is used as study specimen and as food also.

Viva Voce

- Why is *Torpedo* called electric ray?

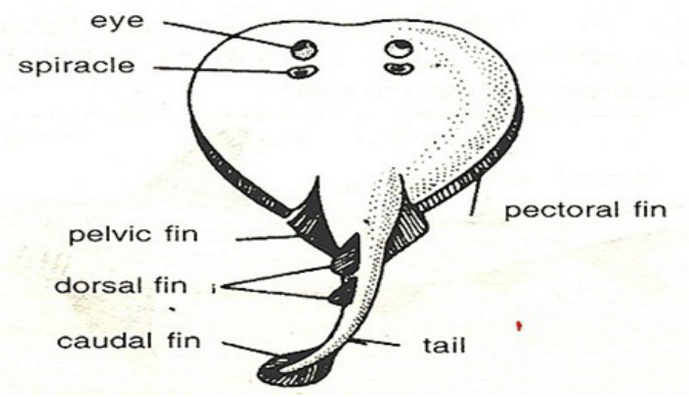

Fig. 25 *Torpedo*

8. *CATLA*

Classification

Phylum : Chordata
Class : Osteichthyes
Order : Cypriniformes
Genus : *Catla*

Habitat

- It is a fresh water bony fish. It is largest Indian carp found throughout India.

Habits

- It is commonly called Katla or Rohu (Refer Figure 26).
- It swims by undulations of body aided by fins.
- It is omnivorous and feeds on planktons and decaying vegetation.
- It shows branchial respiration.

- Sexes are separate but there is no sexual dimorphism.
- Female is oviparous and lays eggs in July and August.
- Development is indirect including a larval stage.

Identification Points

- Body is divisible into head, trunk and tail.
- Body is blackish grey dorsally and silvery on the sides.
- Head has terminal mouth with prominent lips. It also bears large rounded eyes.
- Trunk contains small-sized pectoral and pelvic fins. Dorsal fin is large sized.
- Trunk is covered by cycloid scales.
- Caudal fin is deeply forked.

Economic Importance

- *Catla* is considered as a major food fish.

Viva Voce

- What is the common name of *Catla*?
- What is habitat of *Catla*?

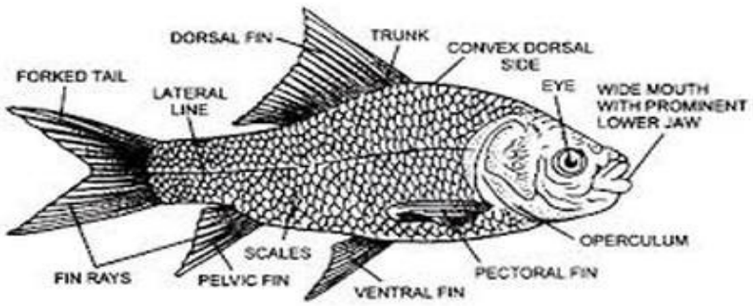

Fig. 26 *Catla*

9. *HIPPOCAMPUS*

Classification

Phylum : Chordata
Class : Osteichthyes
Order : Gasterosteiformes
Genus : *Hippocampus*

Habitat

- It is a marine fish found all over the world in and Atlantic sea.

Habits

- It is commonly called sea horse (Refer Figure 27).
- It swims upright swaying its tail and gyrating its trunk in graceful manner, holding a weed with its tail.
- It is herbivorous feeding upon sea weeds and minute organisms.
- It shows branchial respiration.
- It shows protective colouration.
- Though sexes are separate but it shows sexual dimorphism.
- Male has a broad pouch on the ventral side of abdomen.
- Fertilization is internal. Male shows parental care and carries eggs until young ones hatch out. Development is direct.

Identification Points

- Body is divisible into head, trunk and tail.
- Head bears snout with terminal and toothless mouth.
- Eyes are situated on the upper side of head.
- Body is covered by rigid exoskeletan armor of ring-like bony plates.
- Gill clefts are reduced to a small opening.
- Dorsal fin is single, ventral and caudal fins are absent. Pectoral fin is found on either side of head.

Economic Importance

- Its dried skeleton is used as an ornament.

Viva Voce

- What is common name of *Hippocampus*?
- Differentiate between male and female *Hippocampus*.

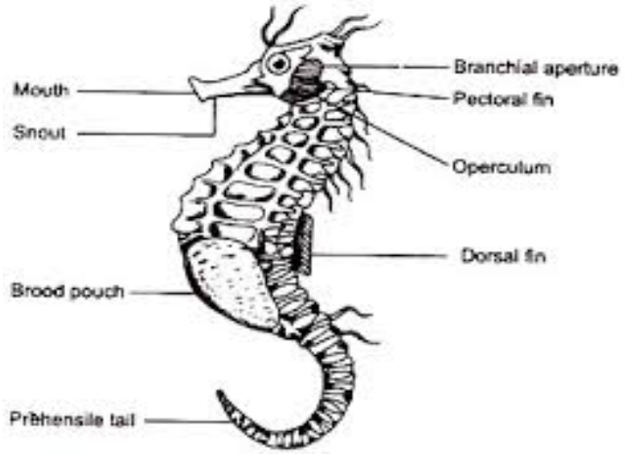

Fig. 27 *Hippocampus*

NOTES

10. *PROTOPTERUS*

Classification

Phylum : Chordata
Class : Osteichthyes
Order : Dipnoi
Genus : *Protopterus*

Habitat

- It is a fresh water bony fish, found in rivers and lakes of Western Africa.

Habits

- It is commonly called African lung fish (Refer Figure 28).
- The fishes are adapted for burrowing life. It comes to surface to engulf the air.
- It swims in water by body undulations aided by fins. On land, paired fins are used as legs for walking.
- It is carnivorous and voracious feeder. It also shows cannibalism.
- It shows branchial respiration in water and pulmonary respiration on land where swim bladders act as lungs.
- Fertilization is internal. Female is oviparous.
- Development is indirect including a larval stage with four pairs of external gills. It undergoes metamorphosis.

Identification Points

- Body is divisible into head, truck and tail.
- Head bears a wide and terminal mouth, one pair of nostrils and eyes.
- Dorsal, caudal and anal fins are continuous.
- Body is completely covered by small cycloid scales.

Economic Importance

- It acts as a connecting link between bony fishes and amphibians.

Viva Voce

- Why is *Protopterus* known as lung fish?

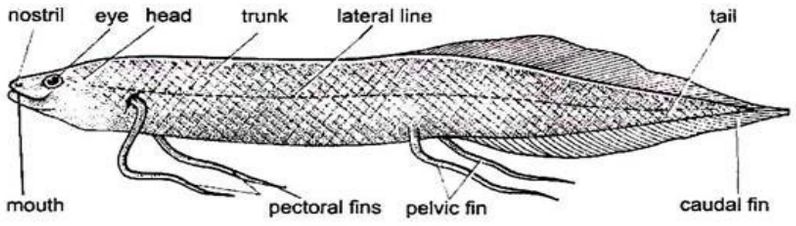

Fig. 28 *Protopterus*

11. *SALAMANDER*

Classification

Phylum : Chordata
Class : Amphibia
Order : Urodela
Genus : *Salamander*

Habitat

- It is terrestrial tailed amphibian found in Europe, Eastern Asia and North America.

Habits

- It is commonly called fire salamander.
- Animal shows two pairs of walking type and well developed limbs.
- It is a carnivorous animal and feeds upon insects and worms.
- Sexes are dioecious or unisexual but show sexual dimorphism.
- Female is longer than male. Female picks up spermatophore with its cloacal.
- Fertilization is internal. Female may viviparous or ovoviviparous.
- Development is indirect and includes a fish like larva which undergoes metamorphosis.

Identification Points

- Body is divisible in to head, trunk and tail.
- Body is coloured brilliantly black with irregular patches of yellow on back and limbs.
- Fore limbs and hind limbs are well developed. Each fore limb has four finger, while each hind limb has five toes.
- Head contains mouth, prominent eyes and nostrils.
- Tail is without tail fin.

Economic Importance

- It is valuable study material.

Viva Voce

- What is the common name of *Salmander*?

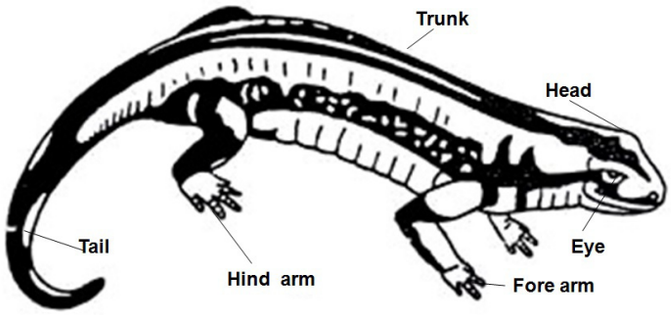

Fig. 29 *Salamander*

12. *RANA*

Classification

Phylum : Chordata
Class : Amphibia
Order : Anura
Genus : *Rana*

Habitat

- It is an amphibian found in and near fresh water bodies like ponds and lakes.

Habits

- It is called Bull frog of India (Refer Figure 30).
- It shows diurnal activity.
- It swims in water by webbed toes and leads son land by long hind limbs.
- It is carnivorous and feeds upon insects, worms and spiders etc. Which are captured with the help of bifid tongue. Prey is swallowed entire.
- Frog shows triple mode of respiration, which are Cutaneous respiration, Bucco-pharyngeal and Pulmonary respiration.
- Sexes are unisexual and show sexual dimorphism. Male has vocal sacs and nuptial pad to grasp the female during copulation.

- Female is oviparous and fertilization is external. Eggs are laid in an irregular gelatinous mass, called spawn.
- Development includes an aquatic tadpole larva which undergoes metamorphosis.

Identification Points

- Body is ovoid and depressed. It is divisible into head and trunk.
- Head bears snout, mouth, a pair of nostrils, large sized bulging eyes and ears are down just behind and below each eye.
- Nuptial pad is present in male.
- The trunk bears longitudinal wrinkles of skin, called dermal plicae on dorsal side and a cloacal aperture at the posterior end.

Economic Importance

- It controls insect population and is a good experimental animal.

Viva Voce

- Why is *Rana* known as bull frog?
- How many digits are present in fore arms and hind limbs of *Rana*?

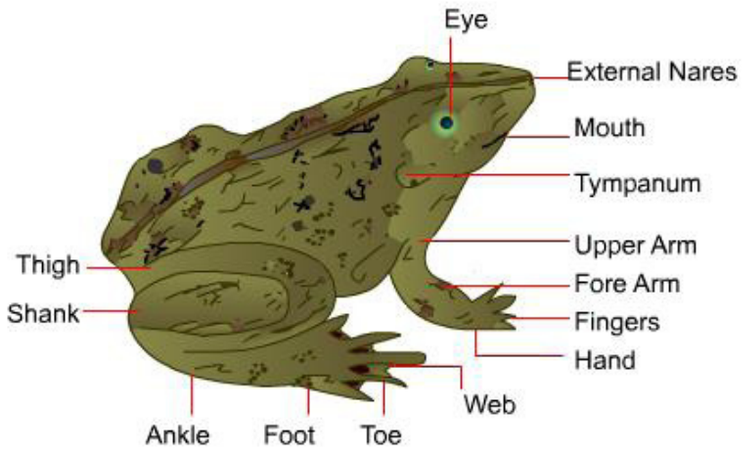

Fig. 30 *Rana*

13. *HEMIDACTYLUS*

Classification

Phylum : Chordata
Class : Reptilia
Order : Squamata
Genus : *Hemidactylus*

Habitat

- *Hemidactylus* is a common house lizard found in every house.

Habits

- It is called house lizard (Refer Figure 31).
- It is nocturnal in habit.
- During winter, it hibernate under wood, logs and crevices of the walls.
- They are adopted to walk on wall due to presence of adhesive pads.
- They feed on insects and small invertebrates.
- Sexes are unisexual and show sexual dimorphism. Male has a pair of copulatory organs, hemipenes and also femoral glands.
- Fertilization is internal and female is oviparous.
- Development is direct.

Identification Points

- Body is divisible into head, neck, trunk and tail.
- Head is triangular containing eyes, nostrils and external ear opening. Eyes lack moveable eyelids.
- Tongue is protrusible.
- Fore limbs and hind limbs are well developed.
- Quadrate bone is moveable.
- Vertebrae are amphicoelous.
- Egg shells calcified.

Economic Importance

- It is a useful lizard as it controls insect population.

Viva Voce

- What is the common name of *Hemidactylus*?

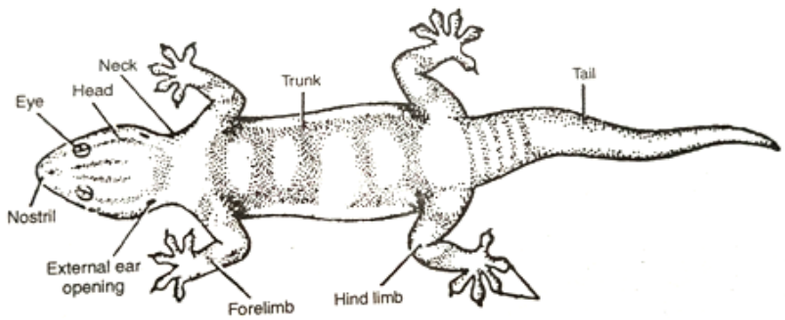

Fig. 31 *Hemidactylus*

14. *NAJA*

Classification

Phylum : Chordata
Class : Reptilia
Order : Squamata
Genus : *Naja*

Habitat

- It is a terrestrial snake, generally found in thick vegetation, under stones, rat-infested houses, etc. It is commonly found in South Asia and Africa.

Habits

- It is commonly called Cobra (Refer Figure 32).
- It is diurnal.
- It creeps very fast by lateral undulation of body.
- It is carnivorous and feeds upon frogs, lizards, birds and rats.
- It hibernates in winter.
- Its head is rigid, expands its neck as a hood, sways back and forth and hisses loudly through the nostrils.
- Sexes are unisexual. Fertilization is internal and female is oviparous. Development is direct.

Identification Points

- Body is divisible head, neck, trunk and tail. Head contains mouth, eyes and nostrils.
- Neck expands to form hood, which contains binocellate mark on dorsal surface.
- Eyes are with narrow pupil.
- Tail shields on the undersurface of the tail in a double row.
- Body is covered by smooth, oblique scales.
- Poison fangs are followed by 1-3 small teeth. Nostrils are large and vertically elliptical.

Economic Importance

- *Naja* is very common in India. It is worshiped on Nagpanchami day.

Viva Voce

- What is the common name of *Naja*?
- Differentiate between *Bungarus* and *Naja*.

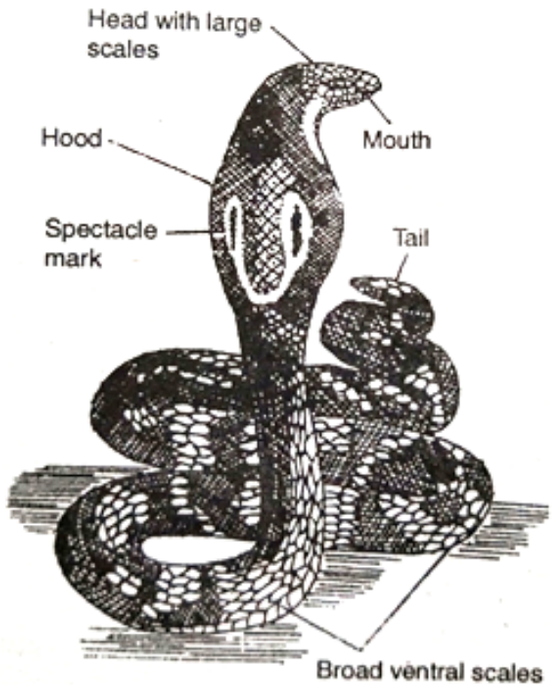

Fig. 32 *Naja*

15. *GAVIALIS*

Classification

Phylum : Chordata
Class : Reptilia
Order : Gaviiformes
Genus : *Gavialis*

Habitat

- It is an amphibious crocodilians reptile, commonly found in Asian rivers.

Habits

- It is commonly called ghariyal or naka (Refer Figure 33).
- It is diurnal.
- It walks on land and swims in water.
- It is carnivorous and predatory animal of fishes.
- It aestivates in summer season.
- Sexes are unisexual or dioecius and show sexual dimorphism. Male has a copulatory organ, penis.

- Fertilization is internal. Female is oviparous and development is direct.

Identification Points

- Body is divisible into head, neck, trunk and tail.
- Head contains eyes and elongated snout with nostrils at the tip.
- Body is covered with an exoskeleton of bony and epidermal horny scales.
- Fore limbs and hind limbs are short pentadactyl and ending in clawed toes with webs between.
- Vertebrae are proceļus.
- Tongue is not protrusible.
- Heart is four chambered with separate ventricles.
- Bladder is absent.
- Tail is strong and powerful and laterally compressed and containing vertical scutes.

Economic Importance

- It is carnivorous and predatory animal of fishes.

Viva Voce

- What is common name of *Gavialis*?
- How many chambered heart is found in ghariyal?

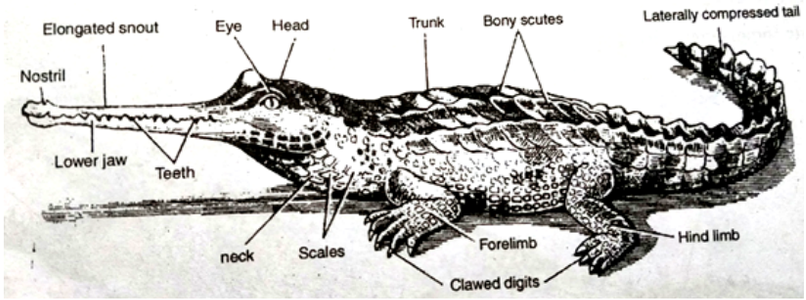

Fig. 33 *Gavialis*

16. *PAVO*

Classification

Phylum : Chordata
Class : Aves
Order : Galliformes
Genus : *Pavo*

Habitat

- It is a terrestrial game bird found in the forest as well as in the fields.

Habits

- It is commonly called peacock, more, mayor (Refer Figure 34).
- It shows diurnal activity.
- It is a slow flier. Feet are adapted for running.
- It is an omnivorous bird and feeds on grains as well as insects and reptiles. Feet are used for locating food.
- Sexes are dioecious and show sexual dimorphism.
- Male with a crest on head, brightly-coloured breast. Long oscillated tail feathers and a bony spur on legs.
- Male is polygamous and shows a peculiar dance during breeding season.
- Fertilization is internal. Female is oviparous and lays 3-5 eggs. Only the female incubates the eggs. Development is direct.

Identification Points

- Body is bright-colour in peacock and dull coloured in peahen.
- Body is divisible in to head, neck, trunk and tail.
- Head bears eyes, nostrils and mouth bounded by strong and crawled beak.
- Male has a crest on head.
- Wings are less developed.
- Tail feathers are long oscillated and erectile.

Economic Importance

- It is the National Bird of India.

Viva Voce

- What is the scientific name of peacock?
- Differentiate between male and female *Pavo*.
- Why is it called the national bird of India?

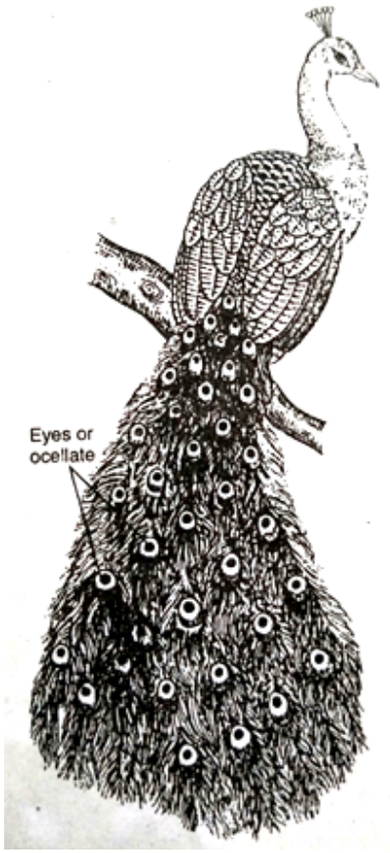

Fig. 34 *Pavo*

17. *EUDYNAMIS*

Classification:

Phylum : Chordata
Class : Aves
Order : Cuculiformes
Genus : *Eudynamys*

Habitat

- It is found on the tree of garden, groves large, leafy trees and is found in India, Pakistan, China, Philippines and Australia.

Habits

- It is commonly called as koel (Refer Figure 35).
- It shows diurnal activity.
- It flies with the help of wings.

- It is an omnivorous bird and feeds upon fruits, caterpillars and insects.
- Sexes are dioecious and show sexual dimorphism. Male is shining-black while female is dull brown colored with white spot.
- Male calls the female by its peculiar call Kuoo-Kuoo in summer and remains silent in winter season.
- Fertilization is internal. Female is oviparous and lays eggs in the nest of crow.

Identification Points

- Body is divisible into head, neck, breast and abdomen.
- Head contains eyes and beak. Beak is adapted for seed eating.
- Beak is pointed and curved downwards.
- Eyes are small with rounded pupil.
- Hind limbs bear reversible toes with two toes in front and two toes behind.
- Wings feathers are folded over the body and tail feathers are long.

Economic Importance

- Male is well known for sweet voice, Female does not sing.

Viva Voce

- Which koel is known well for singing?
- What is the colour of female koel?

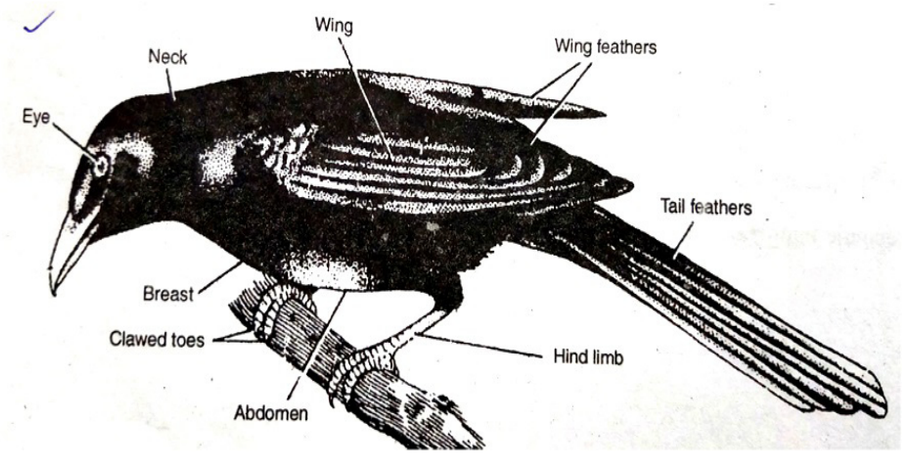

Fig. 35 *Eudynamys*

18. *ECHIDNA*

Classification

Phylum : Chordata
Class : Mammalia
Order : Monotremata
Genus : *Echidna*

Habitat

- It is a terrestrial mammal found in Tasmania, New Guinea and Australia.

Habits

- It is commonly called spiny ant eater (Refer Figure 36).
- It shows nocturnal activity.
- It walks with two pairs of pentadactyl limbs.
- It is insectivorous.
- When attacked by enemy it quickly digs a burrow and enter into it.
- Sexes are unisexual and show sexual dimorphism.
- Male has a horny spur on heel for defense.
- Fertilization is internal. Female is oviparous.
- Young ones develop on mother's milk. Male also has functional mammary gland.
- Development is direct.

Identification Points

- Body is divisible into head, neck, trunk and tail.
- Head is produced into a tubular snout having terminal mouth and has no pinnae.
- Tongue is long, sticky and protrusible.
- Trunk bears two pairs of pentadactyl limbs with sharp-clawed digits.
- Each heel has a bony spur in male.
- Hairs are modified into spines.

Economic Importance

- It acts as a connecting link between reptiles and mammals.

Viva Voce

- Differentiate between male and female *Echidna*?
- State about egg laying mammal.
- What is the common name of *Echidna*?

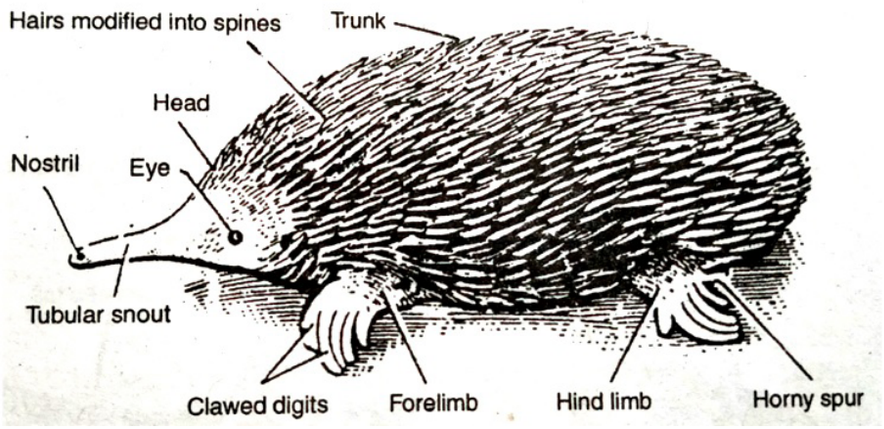

Fig. 36 *Echidna*

19. *FUNAMBULUS*

Classification

Phylum : Chordata
Class : Mammalia
Order : Rodentia
Genus : *Funambulus*

Habitat

- *Funambulus* is worldwide in distribution. It is found on all continents and islands.

Habits

- Its common name in Hindi is gilahari (Refer Figure 37).
- It shows diurnal activity.
- It is a fast runner and lives on trees.
- It is herbivorous and feeds on fruits and seeds.
- It builds its nest of twigs and leaves.
- Sexes are unisexual and show sexual dimorphism.
- Male has a penis, whereas female has mammy.

- Fertilization is internal and female is viviparous. There is maternal care.

Identification Points

- Body contains three white and grey stripes on dorsal side which are absent on neck.
- Body is divisible into head, neck, trunk and tail.
- Head contains snout with moustaches, nostrils and large eyes.
- Fore limbs and hind limbs are well developed with clawed toes.
- Tail is elongated and bushy.

Economic Importance

- Squirrel destroys fruits and crops. It is also used for experimental purposes. It is largely used in cancerous studies.

Viva Voce

- What is common name of *Funambulus*?
- Why *Funambulus* is a mammal?

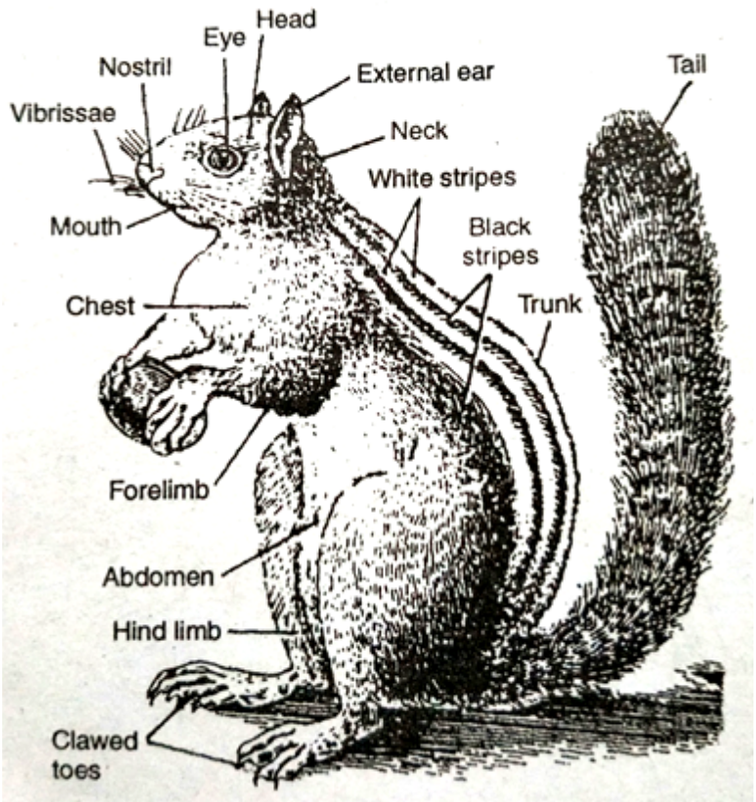

Fig. 37 *Funambulus*

NOTES

20. *PTEROPUS*

Classification

Phylum : Chordata
Class : Mammalia
Order : Chiroptera
Genus : *Pteropus*

Habitat

- It is a cosmopolitan mammal found in South-Eastern Asia, especially in India.

Habits

- It is commonly called Flying Fox in English and Chamgadar in Hindi (Refer Figure 38).
- It shows arboreal and aerial mode of life.
- It lives in groups and feed on fruits.
- They sleep on tree branches during the day. It shows nocturnal activity.
- The sexes are dioecious and show sexual dimorphism.
- Male has penis, whereas female has two thoracic nipples.
- Female is viviparous and give birth only to single young one at one time. Mother shows maternal care.
- Development is direct.

Identification Points

- Body is divisible into head, neck, trunk and tail.
- Body is dark brown coloured and shoulders are golden brown.
- It is capable of true flight.
- Head bears an elongated snout, fox like face, large eyes and short pinnae.
- Each wing is formed by a fold of patagium, supported by elongated fore limbs and 2-5 fingers.
- Only first and second fingers bear claws.
- Tail is absent.

Economic Importance

- They are used for experimental purposes.
- Faces of bats are used as fertilizer.

Viva Voce

- Why is flying fox a mammal?
- Differentiate between male and female chamgadar.

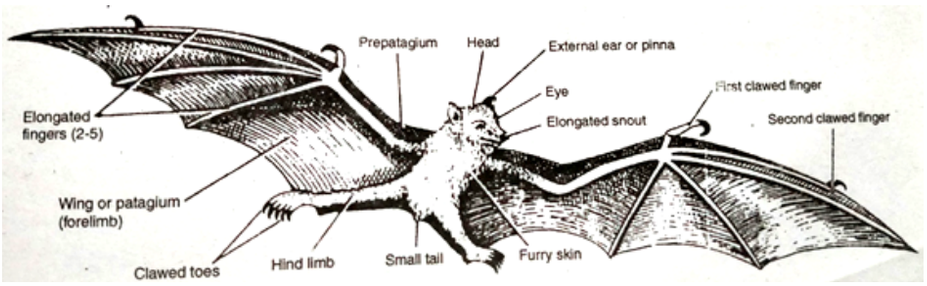

Fig. 38 *Pteropus*

NOTES

PERMANENT MOUNTING

1. T.S. OF EARTHWORM BODY PASSING THROUGH PHARYNGEAL REGION

Identification Points

- Body wall is formed of outer cuticle, single layered epidermis, outer layers of circular and inner bundles of longitudinal smooth muscles, and inner parietal peritoneum (Refer Figure 1).
- Presence of true coelom lined by peritoneal layers.
- Presence of section of pharynx in the centre.
- Pharynx is divided into dorsal and ventral chambers by pharyngeal shelves. It is attached to body wall by dilator muscles.
- Presence of sections of dorsal vessel, ventral vessel and ventral nerve cord.
- Pharyngeal bulb lies on the roof of pharynx.

Viva Voce

- What is the function of pharyngeal bulb?
- List the differences between true coelom and pseudocoel.
- Give the function of dilator muscles.

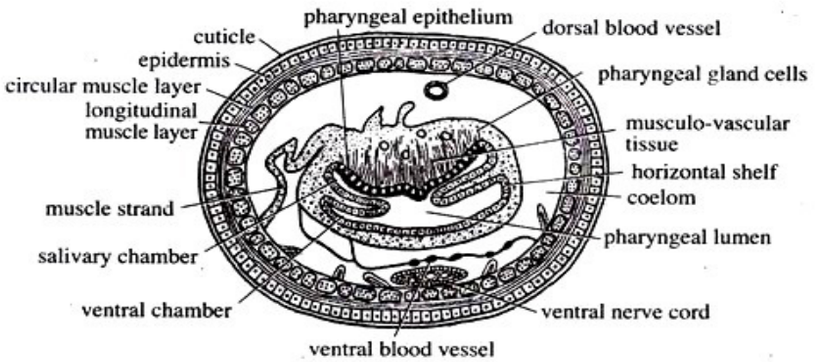

Fig. 1 *T.S. of Earthworm Body Passing Through Pharyngeal Region*

2. SETAE OF EARTHWORM WHOLE MOUNT (WM)

Identification Points

- Each seta is formed of 3 parts: base, body and neck (Refer Figure 2).

- Each seta is present in an epidermal setal sac and is secreted by a seta forming cell.
- Body of seta has a nodule.
- Setae are moved outward by two sets of protractor muscles and moved inward by single retractor muscle.

Viva Voce

- What is arrangement of setae in earthworm?
- What are the functions of setae?
- Name the segments in which setae are absent.

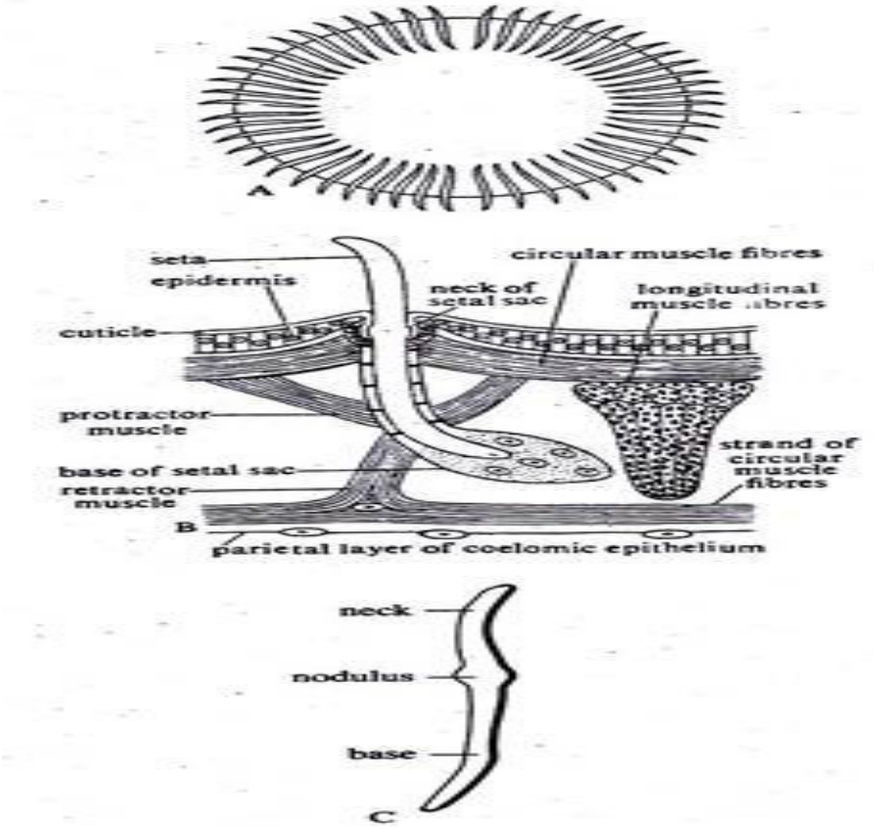

Fig. 2 *Setae of Earthworm Whole Mount (WM)*

3. HONEY BEE STING APPARATUS (WM)

Identification Points

- The sting of honey bee is made of three pairs of gonapophyses, one pair of segment 8 and two pairs of segment 9 (Refer Figure 3).

- The gonapophyses of segment 8 form two stylets lying parallel and enclosing a poison canal.
- One pair of gonapophyses of segment 9 fuse together to form a single stylet sheath, the other pair forms two stylet palps.
- The sting or terebra consists of two stylets articulated along their length to the hollow stylet sheath by a groove and rail arrangement.
- The stylets are held in place by this arrangement and they can move only up and down.
- The stylets and their sheath bear barbs at the tips to make a wound.
- Proximally the stylet sheaths have a dilated bulb, then they form arms which are associated with 3 pairs of plates bearing muscles.
- Attached to the stylets proximally is a median poison sac into which open two acid glands and one alkaline gland.
- In stinging, the muscles of plates drive stylets and stylets sheath into a victim, the secretions of two glands mix and pass down the poison canal into the wound.

Viva Voce

- What is stinging apparatus in honey bee?
- Which sex has stinging apparatus?
- What is the chemical nature of apitoxin?

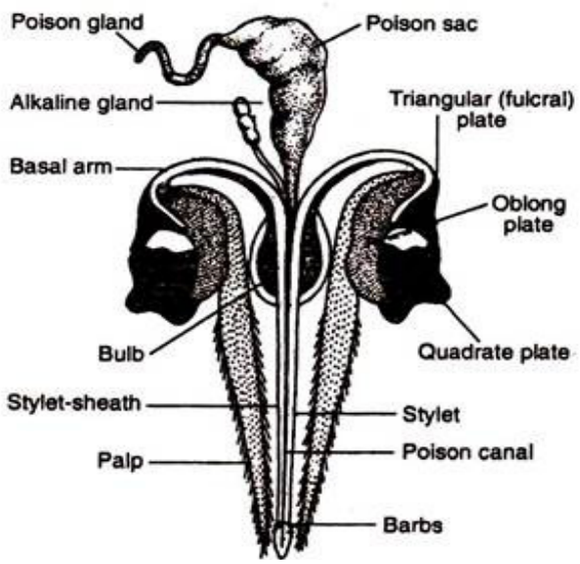

Fig. 3 *Honey Bee Sting Apparatus (WM)*

4. COCKROACH: MOUTH PARTS

Identification Points

- Biting and chewing type of mouth parts found in cockroach.
- Mouth parts are formed of labrum, hypopharynx, two mandibles, two first maxillae and a labium (Refer Figure 4).
- Labrum has a notch at its lower end and a sensory epipharynx on inner side.
- Each mandible has seven teeth.
- First maxilla is formed of protopodite, endopodite and 5-segmented exopodite.
- Labium is formed of submentum, mentum and prementum.
- Prementum gives glossa and paraglossa on inner side and 3-segmented labial palp on outer side.

Viva Voce

- Which type of mouth parts are found in cockroach?
- What are the functions of different mouth parts?

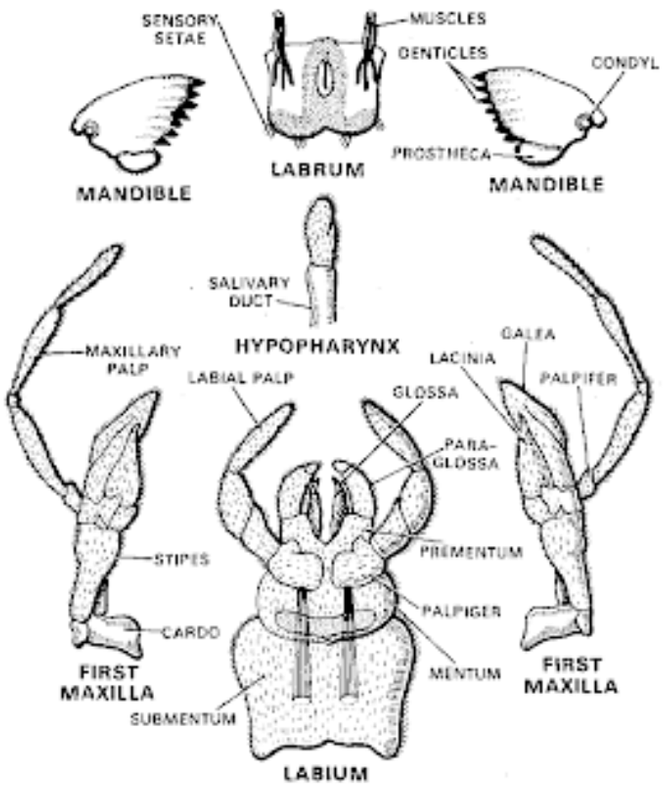

Fig. 4 *Cockroach: Mouth Parts*

5. PRAWN: CEPHALIC APPENDAGES

Identification Points

- Antennules are situated at the anterior end of the body.
- Each antennule is consisted of peduncle made up of three podomeres and a pair of slender many jointed feelers (Refer Figure 5).
- Precoxa of antennules is very large and is flattened dorso-ventrally. Coxa is short and cylindrical bearing setae along its inner margin. Basis is longer than coxa and carries two feelers.
- Antennae are situated immediately below and behind the antennules, consist of a peduncle composed of two podomeres (coxa and basis) (Refer Figure 5).
- Mandibles are short but stout appendages lying one on each side of the mouth.
- Each mandible is formed of the coxa of protopodite which is densely calcified to a powerful jaw. The head of mandible consists of a stout molar process and a flat plate-like incisor process (Refer Figure 5).
- Maxillulae lie close behind the labium on each side. It is thin and foliaceous and consists of a coxa and a basis.
- The maxillae are also thin and foliaceous. The coxa is very small while basis is comparatively larger and projects inwards to form a prominent forked gnathobase with stiff pointed setae at the inner end. The exopodite is large and forms fan-shaped structure called scaphognathite.

Viva Voce

- What are the parts of cephalic appendages of prawn?

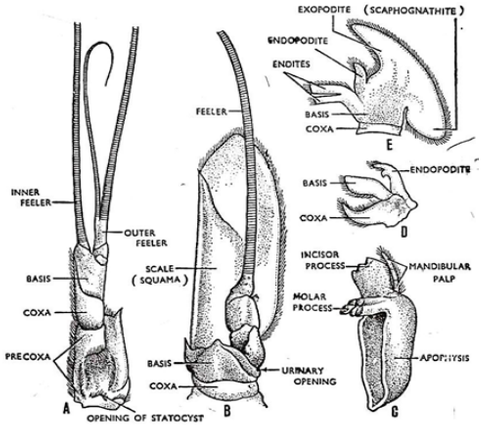

Fig. 5 *Prawn- Cephalic Appendages: Antennule (A), Antenna (B), Mandible (C), Maxillula (D), Maxilla (E)*

6. PRAWN: THORACIC APPENDAGES

Identification Points

- The thoracic appendages consist of the anterior three maxillipedes and posterior five walking legs (Refer Figure 6).
- The first maxillipede consists of coxa and basis. Both the coxa and basis are flattened and leaf-like and project inward to form two gnathobases bearing two rows of pointed spines along the median edges.
- The second masxillipede is less flattened than the first maxillipede and consists of coxa and basis. The coxa is short and covered with setae on its inner border and bears a small epipodite. The basis is short.
- The third maxillipede is leg-like in appearance and consists of coxa and basis. The coxa bears a small epipodite on its outer side. The basis bears a slender unjointed exopodite and an elongated endopodite.
- The three typical non chelate walking legs consist of seven podomeres arranged along a single axis and representing only the protopodite and endopodite of a typical appendage.
- The first chelate walking leg consist distally elongated propodus beyond its articulation with dacylus so that the distal elongation comes to be opposed to the dactylus, the two together forming a pair of chela with sharp terminal claws.
- The second chelate leg resembles the first chelate leg in general plan and structure except that all the podomeres are considerably larger in size.

Viva Voce

- What are the parts of thoracic appendages of prawn?

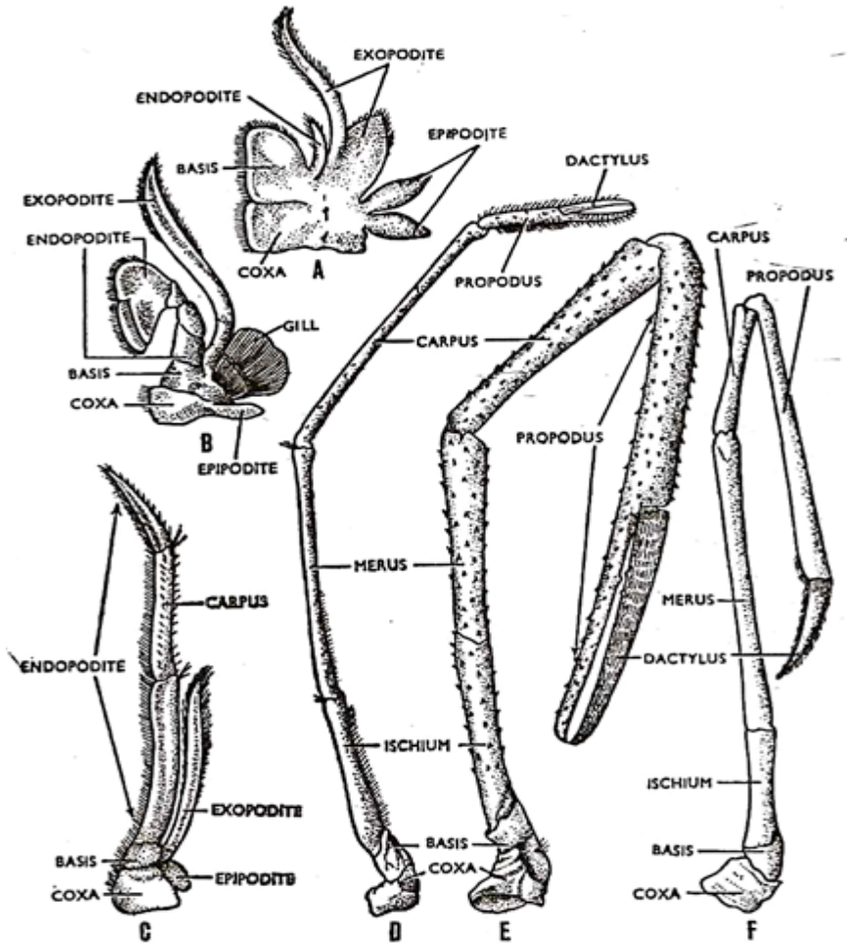

Fig. 6 *Prawn- Thoracic Appendages: 1st Maxillipede (A), 2nd Maxillipede (B), 3rd Maxillipede (C), 1st Walking Leg (D), 2nd Walking Leg (E), Non-Chelate Leg (F)*

7. PRAWN: ABDOMINAL APPENDAGES

Identification Points

- The six abdominal appendages are known as pleopods and present a typical biramous (Refer Figure 7).
- The third abdominal appendage may be taken as a typical. Its stalk or podomere consists of proximal coxa and distal basis. The coxa is short ring-like and does not bear any setae. The basis is long and cylindrical and bears a number of elongated setae on its outer surface.
- The first abdominal appendage resembles to third. The endopodite is considerably reduced. The appendix interna is totally absent.

- The second abdominal appendage has the typical structure in female but in male the appendix interna gives off on its inner side an additional process known as appendix masculine.
- The last abodominal appendage is the uropode. The protopodite of each uropod is short and thick and consists only of one triangular podomere which represents the coxa and basis fused together.
- The exopodite and endopodite of uropod are oval and oar-shaped. The exopodite is broad and large and is divided into two unequal parts by transverse suture. The endopodite is slightly smaller in size and undivided.

Viva Voce

- What are the parts of abdominal appendages of prawn?

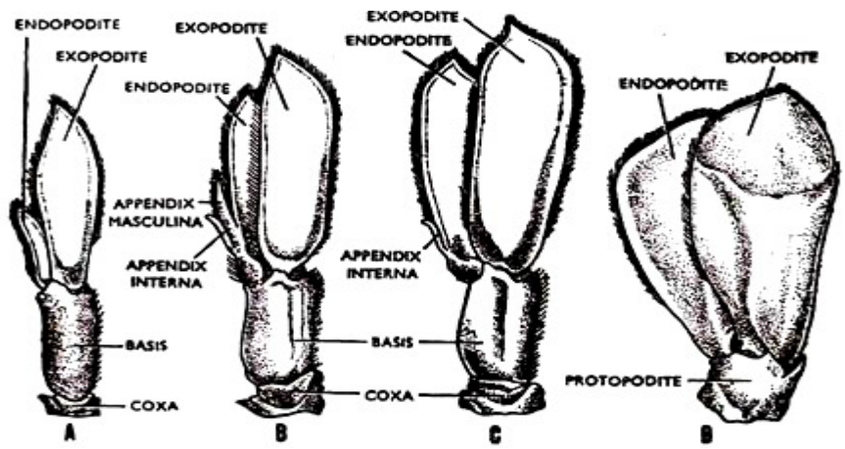

***Fig.** 7 Prawn- Abdominal Appendages: 1st Pleopod (A), 2nd Pleopod (B), 3rd Pleopod (C), Uropod (D). 4th and 5th Pleopods are Similar to 3rd Pleopod*

8. SHARK: PLACOID SCALES

Identification Points

- The placoid scales are arranged in regular oblique rows covering the entire surface of the body and form the exoskeleton of the shark (dogfish) (Refer Figure 8).
- Placoid scales are small pointed and tri-radiate denticles found embedded in the dermal layers of the skin.
- A typical placoid scale consists of a diamond-shaped or rhomboidal basal plate having an opening of the pulp cavity and flat trident spine.

- The basal plate remains embedded in the dermis held by Sharpey's and other connective tissue fibres and formed of a trabecular calcified tissue, the cement.
- The spine is composed of a hard calcareous substance, the dentine coated externally with a hard and dense enamel like vitrodentine.
- The pulp cavity of basal plate and spine contain dentine forming cells the odontoblasts, blood vessels, nerves and lymph channels.

Viva Voce

- Which fish contain placoid scales?
- How the placoid scale look like?
- What is the function of placoid scales?

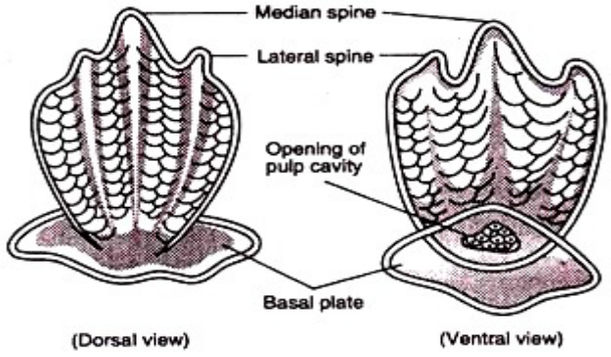

Fig. 8 *Shark: Placoid Scale*

VIRTUAL DISSECTIONS

1. DISSECTION OF COCKROACH: DIGESTIVE SYSTEM

Comments

- Digestive system of Cockroach (Refer Figure 1) is formed of 2 parts, i.e., (I) Alimentary canal and (ii) Digestive glands.
- **Alimentary Canal (Digestive Tract).** It is long coiled tube of varying diameter and is complete. It is formed of following parts:
  - **(a)** Mouth: It is a slit-like aperture bounded by biting and chewing type mouth parts. It is bounded anteriorly by labrum, posteriorly by labium and laterally by mandibles and first maxillae. It is for ingestion.
  - **(b)** Pharynx: It is narrow and tubular part which extends upto vertex.
  - **(c)** Oesophagus: It is narrow and tubular. It runs backward through neck.
  - **(d)** Crop or Ingluvies: It is thin walled, pear-shaped and distensible sac which occupies the whole of thorax and 2 abdominal segments. It stores the food.
  - **(e)** Gizzard: It is small, thick, muscular and conical structure which has six proventricular teeth for mastication of food. Its posterior funnel-shaped part is called stomodeal valve which prevents back-flow of food from midgut to gizzard.
  - **(f)** Mesenteron (Midgut): It is thin walled, tubular and glandular part. It gives rise to 6-8 finger-like processes, hepatic caecae, from its anterior end.
  - **(g)** Ileum: It is small, narrow and tubular part. It is with 60-90 unbranched thread-like Malpighian tubules (excretory in function).
  - **(h)** Colon: It is long, wide and coiled part.
  - **(i)** Rectum: It is terminal dilated part which stores the faeces and absorbs water from undigested food.
  - **(j)** Anus: It is a slit-like aperture present below 10th tergum.
- **Digestive Glands.** These secrete digestive juices. These are of following types:

(a) Salivary Glands: These are one pair of glands one on either side of crop in thorax. Each gland is formed of a bipartite glandular part and a sac-like reservoir. The glandular part is formed of numerous secretory lobules called acini. The salivary glands secrete saliva which is carried by a salivary duct which opens at the base of hypopharynx.

(b) Hepatic Caecae: These are 6-8 finger-like processes which arise from anterior end of midgut.

Viva Voce

- What are the components of digestive system of Cockroach?
- What is the function of gizzard?
- What is hepatic caeca?
- What is the function of Malpighian tubules?

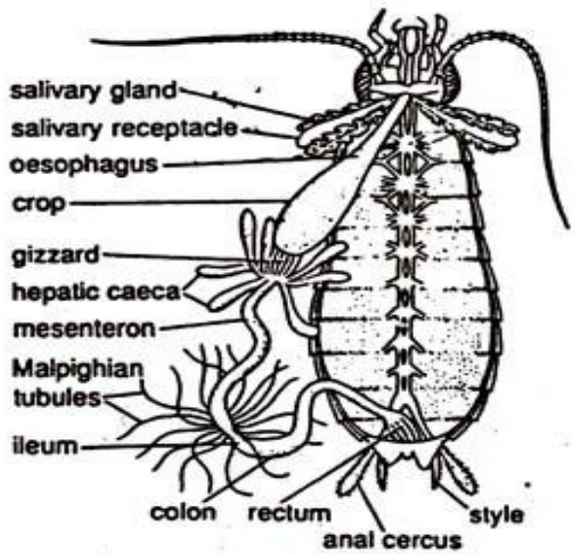

Fig. 1 *Cockroach: Digestive System*

2. DISSECTION OF COCKROACH: MALE REPRODUCTIVE SYSTEM

Comments

Figure 2 illustrates the male reproductive system of Cockroach.

- It is formed of the following parts:

(a) Testes: These are one pair of trilobed structures embedded in fat bodies along dorso-lateral sides of 5th and 6th abdominal segments. Each lobe of testis is formed of numerous follicles.

(b) Vasa Deferentia: These are one pair of thread-like tubules which arise from posterior side of testes, run backward downward and inward and finally open in ejaculatory duct.

(c) Ejaculatory Duct: It is thick, muscular, glandular and tubular structure which extends backward upto male gonopore.

(d) Male Gonopore: It lies below the anus and at the base of ventral phallomere.

(e) Phallic Gland: It is flat, leaf-like gland which is present in 6th abdominal segment. It leads into a phallic duct which opens out by phallic pore near male gonopore.

(f) Utricular gland: It is large sized, mushroom-shaped gland present at the junction of vasa deferentia and ejaculatory duct. It is formed of three types of tubules: small tubules, seminal vesicles and peripheral tubules.

(g) Gonapophyses: These are three irregular, chitinous structures which surround the male gonopore. These are right, left and ventral phallomere. These act as external genitalia.

Viva Voce

- What are the components of male reproductive system?
- What is the function of testes?

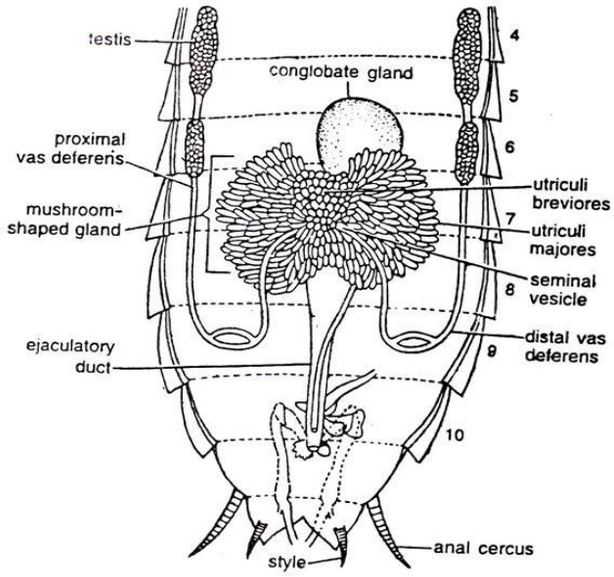

Fig. 2 *Cockroach: Male Reproductive System*

3. DISSECTION OF COCKROACH: FEMALE REPRODUCTIVE SYSTEM

NOTES

Comments

It is formed of following parts:

(a) Ovaries: These are one pair of yellowish structures embedded in fat-bodies along dorsolateral sides from 4th to 6th abdominal segments. Each ovary is formed of eight ovarioles. Each ovariole consists of upper filliform germarium and lower wider vitellarium. Eggs in each ovariole are present in acropetal order.

(b) Oviducts: These are small, muscular and tubular structures which arise from posterior sides of ovarioles.

(c) Common Oviduct: It is small, muscular and tubular structure formed by fusion of two oviducts. It runs backward and opens in blood pouch by female gonopore on 8th sternum.

(d) Brood Pouch: It is a boat-shaped formed from 7th, 8th and 9th sterna. It is divisible into anterior genital chamber and posterior oothecal chamber.

(e) Spermatheca: These are 2 in number. Left spermatheca is narrow and reduced. These store the sperms received during copulation.

(f) Collaterial Glands: These are two in number. Left collateral gland in large and more branched. These open in genital chamber and secrete secretion for ootheca formation.

(g) Gonapophyses: These are three pairs of irregular chitinous structures surrounding the female gonopore. These act as ovipositor.

Viva Voce

- What are the components of female reproductive system?
- What is the function of ovaries?

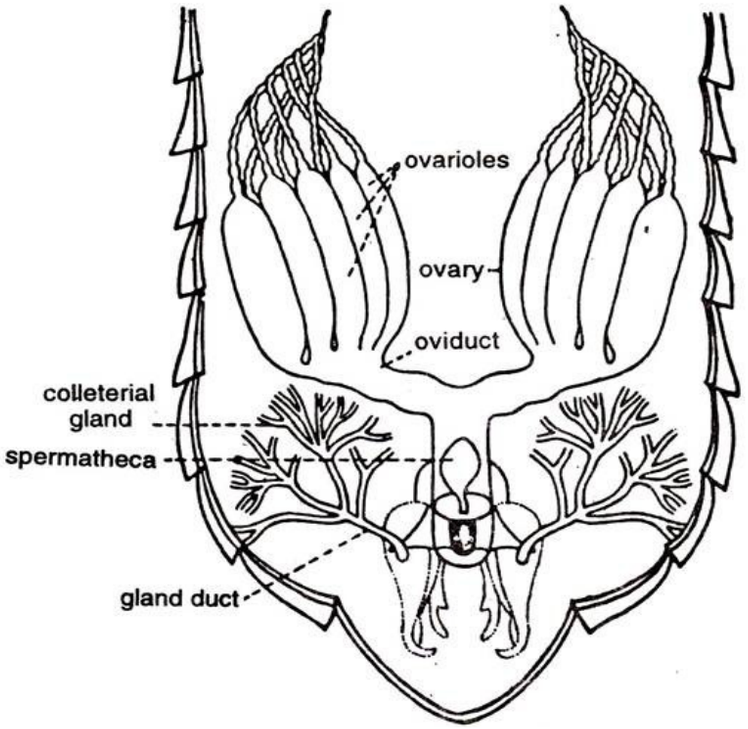

Fig. 3 *Cockroach: Female Reproductive System*

4. DISSECTION OF COCKROACH: NERVOUS SYSTEM

Comments

Central Nervous System (CNS) is present along ventral longitudinal axis and is formed of two parts (Refer Figure 4):

(a) Circum-Oesophageal Nerve Ring: It is formed of three parts:

- **i.** Cerebral Ganglia or Brain: It is a bilobed ganglionic mass present dorsal to oesophagus in the head.
- **ii.** Sub-Oesophageal Ganglionic Mass: It is a smaller bilobed mass present beneath the oesophagus in the head.
- **iii.** Circum-Oesophageal Connectives: These are one pair of thick bands which arise from cerebral ganglia, run downward and join sub-oseophageal ganglionic mass.

(b) Nerve Cord: It is double, ventral, long, solid and whitish cord which arises from sub-oesophageal ganglionic hass and runs backward beneath alimentary canal. It is ganglionated and bears nine ganglia, out of which three are thoracic ganglia (one in each thoracic segment)

and 6 are abdominal ganglia (one ganglion in each of 1st to 4th, 6th and 7th abdominal segment). Two nerve cords join only at the ganglia but remain separate in other parts.

Peripheral nervous system is formed of nerves extending between CNS and body parts:

(a) Cerebral Ganglia: Each cerebral ganglion give two nerves optic nerve to compound eye and antennary nerve to antenna.

(b) Sub-Oesophgeal Ganglia: Each sub-oesophageal ganglion gives four nerves: mandibuiar to mandible; maxillary to first maxilla; labial to labium and cervical to neck.

(c) Throacic Ganglia: Each thoracic ganglion gives several pairs of nerves to parts of same thoracic segment. One pair of metathoracic nerves also supply first abdominal segment.

(d) Abdominal Ganglia. Each of 1st to 4th abdominal ganglia gives one pair of nerves to 2nd to 5th abdominal segment. 5th abdominal ganglion supplies 6th abdominal segment while 6th abdominal ganglion gives nerves to abdominal segments from 7th to 10th abdominal segments.

Viva Voce

- What are the components of nervous system?
- What is the function of CNS?
- How many thoracic ganglia found in cockroach?

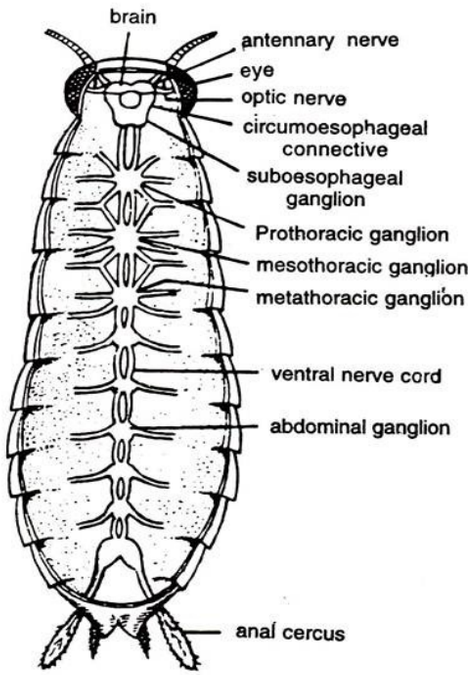

Fig. 4 *Cockroach: Nervous System*

NOTES

5. DISSECTION OF DOG FISH (SCOLIODON): GENERAL ANATOMY

Comments

When the dog fish is dissected then the following organs are seen (Refer Figure 5):

1. Digestive Organs

(a) Oesophagus is short opening anteriorly into pharynx and posteriorly into stomach.

(b) Stomach is J-shaped, consists of long proximal limb extending almost to the posterior end of the abdominal cavity and is called cardiac stomach, while the short narrow distal limb is called pyloric stomach. At the junction of cardiac and pyloric stomach a blind sac is present.

(c) Intestine is a wide tube about the diameter of cardiac stomach. It runs straight backward in the abdominal cavity. It has a longitudinal scroll valve in its lumen, which is the folding of internal wall of intestine.

(d) Rectum is formed by narrowing of the intestine. It opens into the cloaca.

(e) Rectal gland is a small diverticulum from the dorsal wall of the rectum.

(f) Liver is an elongated gland, lies below the cardiac stomach. It consists of two lobes which extend back along the greater part of the abdominal cavity.

(g) Gall bladder is V-shaped and thin-walled sac, embedded in the anterior portion of the right lobe of the liver.

(h) Pancreas is a compact bilobed gland situated in the angle between the two limbs of stomach.

(i) Spleen is a large brownish-red body attached to the loop of the pyloric stomach.

2. Vascular Organs

(a) Pericardial Cavity

(b) Heart (Atrium and Ventricle)

(c) Conus Arteriosus

(d) Ventral Aorta

3. Urinogenital Organs

(a) Female urino-genital organs include ovaries, shell glands, oviducts, uteri, epigonal organs, kidneys, ureters and cloaca.

(b) Male urino-genital organs include testes, vasa efferentia, vasa deferentia, vesicula seminalis, sperm sacs, kidneys, ureters and cloaca.

Viva Voce

- What are the components of digestive system?
- What is the function of pancreas in dog fish?
- State the organs of male urino-genital system of dog fish.
- How many parts comprises in female reproductive system of *Scoliodon*?

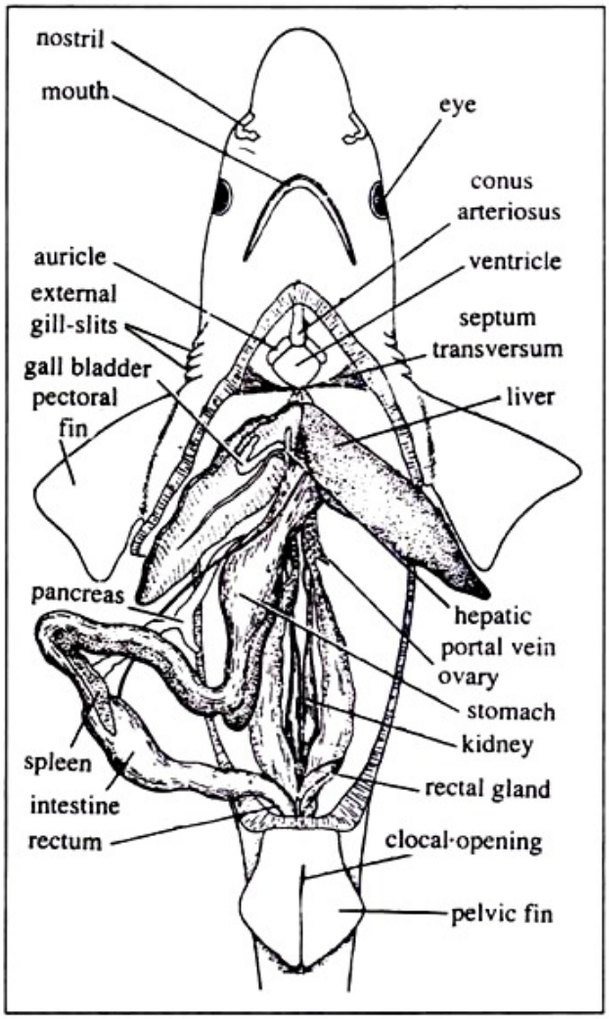

Fig. 5 *Shark: General Anatomy*

NOTES

BIOCHEMISTRY

PREPARATION OF SOLUTION

Solutions are homogenous mixtures of two or more substances in relative quantities upto the limit of solubility. The liquid is usually called the solvent and the substance dissolved in it is called solute. The concentration of any component in a solution may be expressed in following ways namely: i. Percentage by weight, ii. Percentage by volume, iii. Molar solution and iv. Normal solution.

Aim 1

To prepare a solution from provided solvent and solute.

Principle

A solution is a homogeneous mixture created by dissolving one or more solutes in a solvent. The chemical present in a smaller amount, the solute, is soluble in the solvent (the chemical present in a larger amount). Solutions with accurately known concentrations can be referred to as standard (stock) solutions. These solutions can be formed by dissolving the desired amount of solute into a volumetric flask of a specific volume. Stock solutions are frequently diluted to solutions of lesser concentration for experimental use in the laboratory.

Requirements

Weighing balance, solute, solvent, volumetric flask, funnel, measuring cylinder, weighing paper and stirring rod.

Procedure

1. At first the solid solute is weighed out on weighing paper or in a small container and then transferred directly to a volumetric flask (commonly called a "vol flask"). A funnel might be helpful when transferring the solid into the slim neck of the vol flask.
2. A small quantity of solvent is then added to the vol flask and the contents are swirled gently until the substance is completely dissolved.
3. More solvent is added until the meniscus of the liquid reaches the calibration mark on the neck of the vol flask (a process called "diluting to volume") or measuring cylinder.
4. The vol flask is then capped and inverted several times until the contents are mixed and completely dissolved.

Result

The solution of a known concentration is now ready for use in laboratory.

Disadvantage

The disadvantage of this method is that some of the weighed solid may adhere to the original container, weighing paper, or funnel. Also, solid may be spilled when it is transferred into the slim neck of the vol flask.

Aim 2

To prepare 10% solution (w/v) of sodium chloride.

Requirements

Weighing balance, sodium chloride (solute), distilled water (solvent), volumetric flask, funnel, measuring cylinder, weighing paper and stirring rod.

Procedure

1. Weigh 10g of sodium chloride using weighing balance on weighing paper.
2. Transfer the solute to a flask containing 50ml of distilled water through funnel.
3. Dissolve carefully using stirrer and make it up to 100ml.

Result

The prepared solution is 10% (w/v).

Aim 3

To prepare 30% solution (v/v) from a stock solution.

Requirements

Stock solution (polar solution), distilled water, volumetric flask, funnel and measuring cylinder.

Procedure

1. Take 30ml of stock solution using measuring cylinder.
2. Transfer to a flask containing 50ml of distilled water through funnel.
3. Make it up to 100ml using distilled water.

Result

The prepared solution is 30% (v/v).

Aim 4

To prepare 1M solution of sodium chloride.

Principle

A Molar solution contains 1g molecular weight of substance dissolved in 1 litre of solvent.

Requirements

Weighing balance, sodium chloride (solute), distilled water (solvent), volumetric flask, funnel, measuring cylinder, weighing paper and stirring rod.

Procedure

1. Weigh 58.5g of sodium chloride (mol. Weight of NaCl is 58.5) on weighing pane using weighing balance.
2. Carefully transfer to a flask containing 200 ml of distilled water.
3. Dissolve it by gentle stirring.
4. Make it up to 1 litre by distilled water.

Result

The prepared solution is 1M.

Note: *To get 0.2M solution of NaCl calculate the amount of NaCl to be dissolved in 1 litre of distilled water by the given formula:*

Mass of Solute (g) in 1 litre of Solvent = Mol. wt. x Conc.(for example, 0.2)

Aim 5

To prepare 1N solution of hydrochloric acid.

Principle

A Normal solution contains 1 gram equivalent weight of a compound in one litre of solvent. One gram equivalent weight is equal to gram molecular weight divided by the total valency of its positive or negative ions. For example, Gram molecular weight of = 36.5 and Total valency of positive ion = 1.

Requirements

Weighing balance, hydrochloric acid, distilled water (solvent), volumetric flask, funnel and stirring rod.

Procedure

1. Take 36.5g of hydrochloric acid.
2. Carefully transfer to a flask through the wall of glass ware, containing 500ml of distilled water.
3. Make it up to 1 litre by adding distilled water.

Result

The prepared solution is 1N.

Note: *For 1N H_2SO_4 solution= Mol. wt. / Valency = 98 / 2= 49g/1 litre water.*

BUFFER AND pH

Human blood contains a 'buffer' that allows it to maintain its pH at 7.35 to ensure normal functioning of cells. Buffers are solutions that resist change in pH on dilution or on the addition of small amounts of acids or alkali. A lot of biological and chemical reactions need a constant pH for the reaction to proceed. Buffers are extremely useful in these systems to maintain the pH at a constant value. Buffers are broadly divided into two types: acidic and alkaline buffer solutions. Acidic buffers are solutions that have a pH below 7 and contain a weak acid and one of its salts. For example, a mixture of acetic acid and sodium acetate acts as a buffer solution with a pH of about 4.75. Alkaline buffers, on the other hand, have a pH above 7 and contain a weak base and one of its salts. For example, a mixture of ammonium chloride and ammonium hydroxide acts as a buffer solution with a pH of about 9.25.

The pH is the potential of hydrogen ions concentration in a solution. The pH can also define as the negative log of hydrogen ion concentration. It is a scale of acidity from 0 to 14. It tells how acidic or alkaline a substance is. More acidic solutions have lower pH. More alkaline solutions have higher pH. Substances that aren't acidic or alkaline (that is, neutral solutions) usually have a pH of 7.

Aim 1

To prepare acetic acid-sodium acetate buffer of required pH.

Principle

Take mixture of Acetic Acid (CH_3COOH) and Sodium Acetate (CH_3COONa). Here, acetic acid is weakly ionized while sodium acetate is almost completely ionized. If you add a drop of a strong acid like HCl, the H^+ ions from HCl combine with CH_3COO^- to give feebly ionized CH_3COOH. Thus, there is a very slight change in the pH value. Now, if you add a drop of NaOH, the OH^- ions react with the free acid to give undissociated water molecules. In this way, the OH^- ions of NaOH are removed and the pH is almost unaltered.

Reagents Requirement

1. Acetic Acid 0.2M: (1.5 ml of glacial acetic acid is made upto 100ml with distilled water).
2. Sodium Acetate Solution: (0.64 gm of sodium acetate or 2.72gm of sodium acetate trihydrate is dissolved in 100ml distilled water).

Procedure

1. Pipette out exactly 36.2ml of sodium acetate solution into 100ml of standard flask.

2. Add 14.8ml of glacial acetic acid, make the volume 100ml using distilled water using distilled water.
3. This gives 0.2 M of acetic acid and sodium acetate buffer.
4. The pH is measured with pH meter.

Result

36.2ml Sodium Acetate and 14.8ml Glacial Acetic Acid were mixed and buffer was prepared. The initial reading observed was 4 which made upto 4.6 with 5N NaOH.

Aim 2

To prepare potassium phosphate buffer of required pH.

Principle

The buffer is a solution that resists the change in pH of solution after adding a small amount of weak acid and base. The pH is the potential of hydrogen ions concentration in a solution. The pH can also define as the negative log of hydrogen ion concentration.

Reagents Requirement

1. Dipotassium Hydrogen Phosphate (K_2HPO_4)
2. Potassium Dihydrogen Phosphate (KH_2PO_4)

Procedure

1. 174.18 g/mol dipotassium hydrogen phosphate and 136.09 g/mol potassium dihydrogen phosphate was taken flask.
2. About 100 ml of distilled water added in the same flask with continuous stirring.
3. It was made up to 200ml using distilled water with gentle stirring.
4. This gives the potassium buffer. It was standardized with pH meter using standard buffer.

Result

The buffer solution was prepared. The pH of the made solution was standardized using 1N HCl and 5N NaOH and the pH was found to be 6.5.

Aim 3

To test the pH of given sample using pH meter.

Principle

The pH meter measures an electrical potential developed by pair of electrode pins in a solution. For measurement of pH, an electrode system sensitive to change in H^+ ion concentration of solution is taken. The electrode system consists of sequence of electrode whose potential raise with pH (H^+ concentration of the solution).

Requirements

50- or 100-mL beakers, three pH buffer solutions (pH 4, 7, and 10), 100ml graduated cylinder, distilled water in a squeeze bottle, paper towels, masking tape, soft tissues, permanent marker, stirring rod or spoon, latex gloves and safety goggles and pH meter.

Overview

In order to measure the pH of your water sample using the pH meter you need to:

1. Prepare buffer solutions.
2. Calibrate the instruments.
3. Recheck your instrument by measuring the buffers in the field.
4. Measure the pH of your sample in the field.

Procedure

1. Rinse the electrode and the surrounding area with distilled water using the squeeze bottle. Blot the area dry with a soft tissue.
2. Fill a clean, dry 100ml beaker to the 50ml line with the water to be tested.
3. Immerse the electrode in the water. Be sure that the entire electrode is immersed, but avoid immersing it any further than necessary.
4. Stir once and then let the display value stabilize.
5. Once the display value is stable, read the pH value and record it in your record book.
6. Repeat steps 1 through 5 for another sample as a quality control check. The two pH values should agree to within 0.2 level of accuracy required.
7. Rinse the probe with distilled water, blot it dry with soft tissue, replace the cap on the probe, and turn the instrument off.
8. Take the average of pH values measured by the student groups.

Results

The pH of the given sample is ……..

CARBOHYDRATE AND PROTEIN

Carbohydrates are important macromolecules that consist of carbon, hydrogen, and oxygen. Thus carbohydrates are the hydrates of carbon n(CH_2O). They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain. Hence carbohydrates are the polyhydroxy ketones or polyhydroxy aldehyde. Carbohydrates are the most abundant organic compounds in living organisms and account for one of the four major biomolecular classes including proteins, lipids, and nucleic acids. They originate as products from carbon dioxide and water by photosynthesis. The general empirical structure for carbohydrates is (CH_2O)n. Monosaccharides, which are simple sugars that serve as fuel molecules as well as fundamental constituents of living organisms, are the simplest carbohydrates, and are required as energy sources. The most commonly known ones are perhaps glucose and fructose.

Proteins play a central role in biological processes and form the basis of living tissues. They consist of long, looping or folding chains of smaller compounds called amino acids. Enzymes, antibodies, and hemoglobin are examples of proteins. Proteins may be defined as the high molecular weight mixed polymers of α-amino acids joined together with peptide linkage (-CO-N H-). Proteins are the chief constituents of all living matter. They contain carbon, hydrogen, nitrogen and sulphur and some contain phosphorus also.

Aim 1

To test the given sample for the presence of carbohydrate (glucose) by Fehling's test.

Principles

Fehling's solution contains copper tartarate. It is blue in color. Fehling's test is based on the reduction of copper tartarate to red colored cuprous oxide by reducing sugars

Reagents Requirements

Fehling's solution A and B, glucose test solution (sample), pipettes, test tube, test tube holder and spirit lamp.

Procedure

1. Take 2 ml each of Fehling's solution A and B in a test tube and boil it. If a precipitate is formed the solution is rejected and fresh solution is used.
2. Take 2.5 ml each of Fehling's solution A and Fehling's solution B in a test tube.
3. Add 1 ml of glucose solution to the test tube.

NOTES

4. Boil the solution for two minutes.
5. When the experiment is complete cool the test tube under flowing water.

Result

A greenish, yellow or brick red precipitate is obtained confirms the presence of sugar.

Aim 2

To estimates the amount of glucose present in the given unknown solution using Benedict's quantitative reagent.

Principle

Benedict's quantitative reagent is a modification of qualitative aspects. It contains copper sulphate-sodium acetate and sodium carbonate. It also contains potassium thiocyanate and small amount of potassium ferricynate. The inclusion of acetate prevents the precipitation of copper by chelating Cu^{3+} ions. The thiocynate causes with the precipitation of white cuprous thiocynate rather than red cupric oxide. On the reduction of Cu^{3+} ions, which inhibits the end point of the titration digest the transition from blue to white to be readily observed. Methylene blue will be used as an additional indicator. The small amount of potassium ferricyanaide prevents the pre oxidation of copper. The reduction of Cu^{3+} ions by sugar is a non-stiochemetric equation and is only constant over a small range of sugar concentration. To obtain accurate results the volume of sugar added must be within 6-12ml for 10ml of Benedict's reagent.

Reagents Required

1. Standard Glucose Solution (2mg/ml): 200mg of glucose was weighed accurately and made up to 100ml with distance water.
2. Benedict's Quantitative Reagent: (100mg of sodium citrate and 62.5gm potassium thiocynate were dissolved in 300ml of distilled water by warming gently and filtered). 18% of copper sulphate is dissolved in 50ml of water, added with continuous stirring. 2.5ml of 5% potassium ferricyanide is added and volume is made up to 500ml with water.
3. Anhydrous sodium carbonate.

Procedure

1. Pipette out 5ml of Benedict's reagent was into a clean conical flask.
2. Add 600mg. of anhydrous sodium carbonate, to provide the required alkalinity with a few porcelain bits.
3. To heat to boiling over a moderate flame.
4. Standard glucose solution is taken in a burette.

5. When the Benedict's solution boils continuously, glucose solution is added drop by drop (1 drop/sec) till last trace of blue color disappears.
6. Note the volume of glucose rundown at the end of titrations. Repeat it to concordant values.
7. Made the given unknown sugar solution up to 100ml in a standard flask with distilled water.
8. Fill the burette with unknown sugar solution and titrate with the Benedict's reagent as before.
9. Note the volume of sugar solution rundown and titrations are repeated for concordant values.

Result

The amount of glucose present in 100ml of given unknown solution is mg.

Aim 3

To test the presence of protein in given sample by using Biuret test.

Principle

The biuret test is positive for all proteins and those substances that contain 2 Carbamyl groups (-$CONH_2$) joined either directly or through a single atom of N or C. Those substances that have a CH_2NH_2, $CSNH_2$, $CONH_2$ or C(NH) NH_2 also give a positive test. The purple violet color is due to the formation of a co-ordination complex between Cu^{2+} ions and nitrogen atoms.

Reagents Requirements

1. Test tubes, pipette, dropper, glass rod and Bunsen burner.
2. 10% NaOH, 0.5% $CuSO_4$.

Procedure

1. Take 3 ml test sample in a test tube.
2. Add 3 ml 10% NaOH solution and mix thoroughly.
3. Add few drops of 0.5% $CuSO_4$ solution. Mix well.

Result

A purplish-violet or pink color is produced indicates the presence of proteins.

Note: *If $CuSO_4$ solution is added in excess the color disappears.*

Aim 4

To estimates the amount of Protein present in given unknown solution.

Principle

Alkaline $CuSO_4$ catalyses the oxidation of aromatic amino acids with subsequent reduction of sodium potassium molybdate tungstate of Folin's reagent giving a purple color complex the intensity of the color is directly

proposition to the concentration of the aromatic amino acid in the given sample solution.

Reagents Required

1. Stock Solution (1mg/ml): Bovine Serum Albumin of 100mg is weighed accurately and dissolved in 100ml of distilled water in a standard flask.
2. Working Standard (0.1mg/ml): The 10ml of stock solution is distilled to 100ml with distilled water in a standard flask.
3. Folin's Phenol Reagent: Folin's phenol reagent is mixed with distilled water in the ratio 1:2.
4. Alkaline Copper Reagent: Solution A: 2% sodium carbonate in 0.1N sodium hydroxide; Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate. Solution A, B is mixed in the proportion of 1:0.5.
5. Unknown Preparation: The unknown protein is made upto 100ml with distilled water.

Procedure

1. Working standard of 0.2 -1ml is pipette out into clean test tube and labeled as S1-S5.
2. Test solution of 0.2ml is taken into test tube and labeled as T1.
3. The volume is made upto 1ml of distilled water. Distill water of 1ml serve as blank.
4. To all the test tube 4.5ml of alkaline $CuSO_4$ reagent is added and incubated at room temperature for 10 minutes.
5. Add 0.5ml of Folin's phenol reagent in all the test tube.
6. The contents are mixed well to develop the blue color which is read in colorimeter at 640A^o after 15 minutes.
7. From the standard graph the amount of protein in the given unknown solution is calculated by plotting the values over that.

Result

The amount of protein present in the given unknown solution is …….. mg/ml.

CELL AND MOLECULAR BIOLOGY

1. Cell and Cell Organelles

The cell is the structural and functional unit of all living organisms, and is sometimes called the 'building block of life.' Some organisms, such as bacteria, are unicellular, consisting of a single cell. Other organisms, such as humans, are multicellular, (humans have an estimated 100 trillion cells; a typical cell size is 10μm, a typical cell mass 1 ng). The largest known cell is an ostrich egg. Each cell is at least somewhat self-contained and self-maintaining: it can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Each cell stores its own set of instructions for carrying out each of these activities. There are two types of cells, eukaryotic and prokaryotic. Prokaryotic cells are usually singletons, while eukaryotic cells are usually found in multi-cellular organisms.

Cell biology is the study of cell structure and function, and it revolves around the concept that the cell is the fundamental unit of life. Focusing on the cell permits a detailed understanding of the tissues and organisms that cells compose. The starting point for this discipline might be considered the 1830s. Though scientists had been using microscopes for centuries, they were not always sure what they were looking at. Robert Hooke's initial observation in 1665 of plant-cell walls in slices of cork was followed shortly by Antonie van Leeuwenhoek's first descriptions of live cells with visibly moving parts. In the 1830s two scientists Schleiden, looking at plant cells, and Schwann, looking first at animal cells, provided the first clearly stated definition of the cell known as cell theory. Their definition stated that that all living creatures, both simple and complex, are made out of one or more cells, and the cell is the structural and functional unit of life.

Aim 1

To comment upon the permanent preparations or images showing ultra structures of cells and some cell organelles like mitochondria, Golgi body, chloroplast and nucleus.

A. Plant Cell

Comments

1. A plant cell is more or less a polyhedral structure limited on the outside by a rigid limiting membrane called cell wall (Refer Figure 1).
2. Inside, it contains the protoplast. The content of each living cell is known by the name protoplast.

3. A membrane which bounds the protoplasm of the cell is known as plasma membrane or cell membrane. It is made of lipids and proteins. It is found in all plants and animals.
4. The basic substance, protoplasm, is not homogeneous.
5. Embedded in the protoplasm is a fairly large, spherical body, the nucleus.
6. The protoplasm surrounding the nucleus of the cell is usually referred to as the cytoplasm.
7. There are other smaller disc shaped bodies embedded in the protoplasm called the plastids.
8. The studies under the electron microscope have revealed that the cytoplasm contains a number of other much smaller cell organelles like mitochondria, endoplasmic reticulum, Golgi apparatus and ribosomes which usually occur in groups called the polysomes.
9. In very young conditions, the cell lumen or cavity is filled with protoplasm. But as the cell matures a corresponding amount of protoplasm is not synthesized to keep pace with the increase in its volume and so vacuoles appear which is filled with watery fluid and delimited by tonoplast.

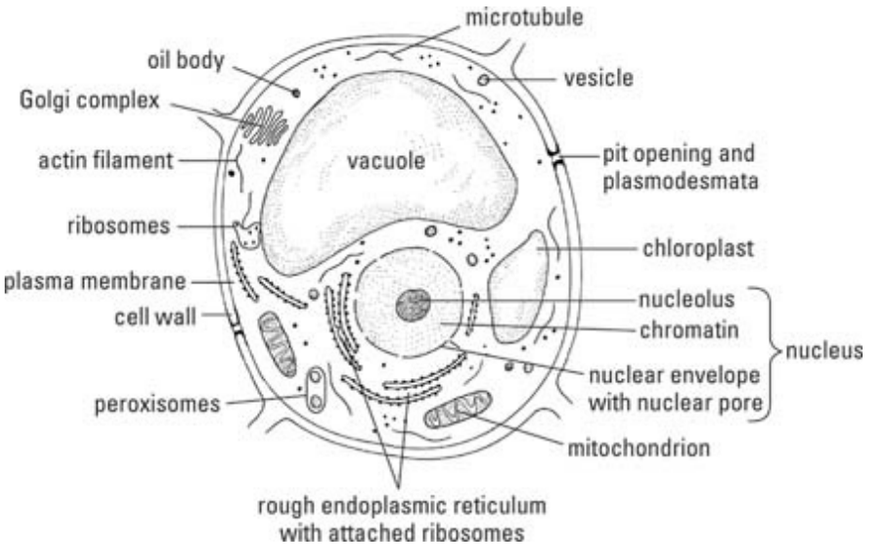

Fig. 1 *A Typical Plant Cell*

B. Animal Cell

Comments

1. A thin semipermeable membrane surrounding the cell is known as cell membrane (Refer Figure 2).

2. Nuclear membrane is the double membrane that surrounds the nucleus.
3. A celestial body, the nucleus present contains several organelles including the nucleolus. It contains DNA and other cell's hereditary information.
4. Centrosomes are small organelle found near to the nucleus which has a thick center and radiating tubules.
5. The lysosomes are round organelle surrounded by a membrane comprising of digestive enzymes which help in digestion, excretion and in cell renewal process.
6. A jelly-like substance found outside the cell nucleus in which the organelles are located called cytoplasm
7. A flat smooth layered, sac-like organelle, Golgi body is present, located near the nucleus and involved in manufacturing, storing, packing and transporting the particles throughout the cell.
8. The spherical to rod-shaped organelles with a double membrane present called mitochondria. They are the powerhouse of a cell.
9. Ribosomes are small organelles made up of RNA-rich cytoplasmic granules and they are the sites of protein synthesis.

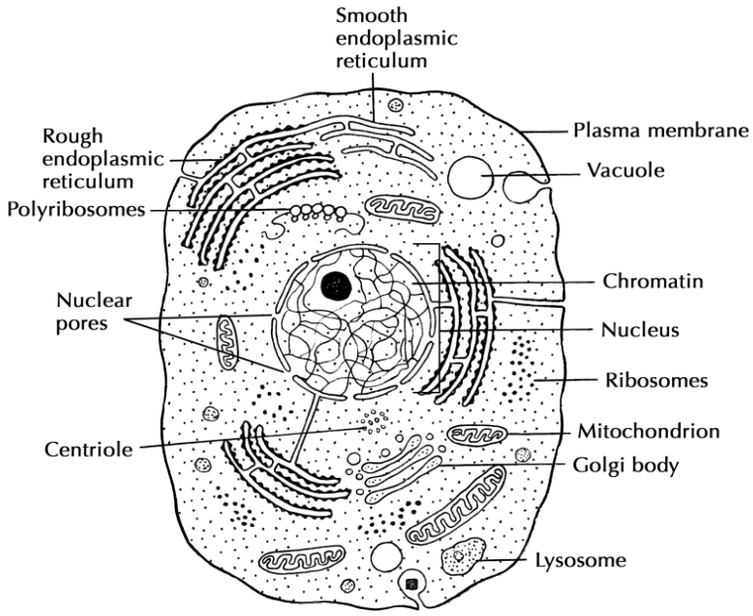

Fig. 2 *A Typical Animal Cell*

C. Mitochondria

Comments

1. Mitochondria are the powerhouse of cells (Refer Figure 3).
2. Its length is 3 to 4 micrometer & its diameter is 0.5 to 1 micrometer.
3. Most of the cellular respiration takes place in mitochondria.
4. Mitochondria have tow membrane: an outer membrane and inner Membrane.
5. There is a space between the inner membrane and outer membrane called, inter membrane space.
6. The outer membrane covers the mitochondria which is semipermeable and smooth.
7. The inner membrane of mitochondria forms infolding or finger like folding. These folds are called Cristae. The electron transport system is a series of protein imbedded on the cristae of mitochondria.
8. The cristae bear several F1 particles. These cristae are important because they make more surface area where chemical reaction can take space.
9. The area inside the cristae comprises a fluid called the matrix that has water and proteins (enzymes).
10. The Krebs cycle occurs in the matrix while electron takes place on the cristae.

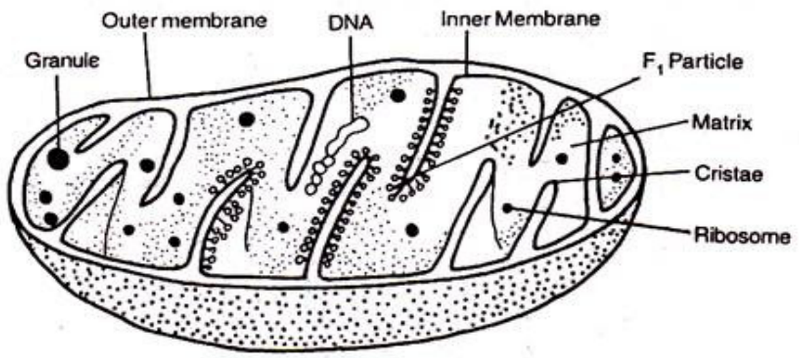

Fig. 3 *Mitochondria to Show Inner Structure*

D. Golgi Body

Comments

1. Golgi body is a complex cytoplasmic structure made up of smooth membrane saccules or cistemae, a network of tubules with vesicles

and vacuoles, which takes part in membrane transformation, secretion and production of complex biochemicals (Refer Figure 4).

2. It is present in all eukaryotic cells except sieve tubes of plants, sperms of bryophytes and pteridophytes and red blood corpuscles of mammals.
3. Golgi complex consists of a stack of generally 4-8 (range 3-20) membrane bound saccules or cisternae. Unicistemal dictyosomes are found in fungi.
4. The saccules are frequently curved to give a definite polarity to the Golgi apparatus. One face of the apparatus is convex while the other is concave. The convex side is called forming face or cis-face while the concave side of the apparatus is known as maturing face or trans-face.
5. Tubules form a complicated network towards the periphery and maturing face of the apparatus. Actually tubules arise due to fenestrations of the cisternae. The tubules interconnect the different cisternae.
6. Vesicles are small sacs of 20-80 nm diameters. The vesicles are found attached to the tips of tubules at various levels in the network.
7. There are two types of vesicles, smooth and coated. The coated vesicles have a rough surface and elaborate membrane proteins. The smooth vesicles have a smooth surface and contain secretory substances and are hence known as secretion vesicles.
8. Golgi complex brings about membrane transformation and recycling of plasma membrane. Production of hormones by endocrine glands, acrosome synthesis during spermiogenesis is mediated through Golgi body.

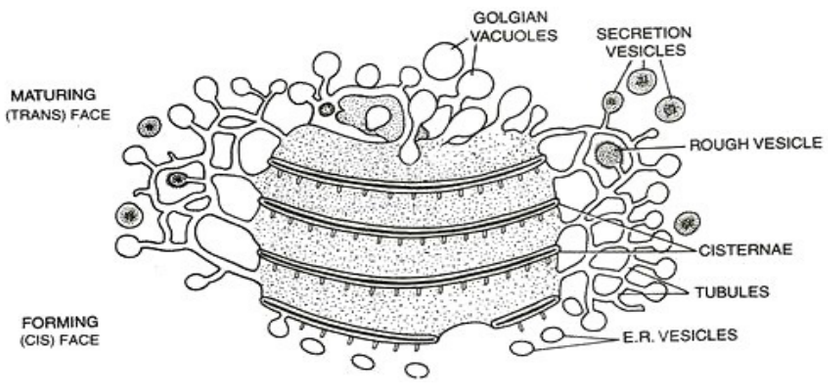

Fig. 4 *Golgi Body*

E. Chloroplast

Comments

1. Chloroplasts are found in the cells of green plants (Refer Figure 5).
2. It is a kind of chlorophyll-bearing plastid.

3. These are spheroidal or oval in the leaves, but may be club-shaped, stellate, collar-like or spiral band in algae.
4. On an average in higher plants measure 4-5 micrometer in diameter.
5. Each chloroplast is covered by a double membrane.
6. The space between these membranes called periplastidial space.
7. The inner matrix of the chloroplast known as stroma.
8. Several chlorophyll bearing double membrane lamellae are found scattered throughout chloroplast bearing disc like structure piled up, called grana.
9. Each granum consists of about fifty superimposed membranous compartments, the thylakoids.
10. Chloroplasts carry out photosynthesis by trapping solar energy.

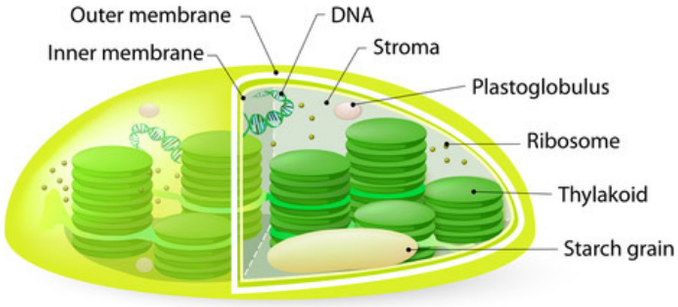

Fig. 5 *Chloroplast*

CHROMOSOME AND BARR BODY

Polytene chromosome is one of the giant chromosomes found in animals and plants. In animals it is found in the salivary glands, Malpighian tubules, the epithelial cell lining of the gut and in the fatty cells of the larvae of certain Diptera. The polytene chromosomes of salivary glands in *Chironomous* fly larvae can be demonstrated easily as laboratory (Refer Figure 1).

Aim 1

Preparation of Polytene chromosomes from the larva of *Chironomous*.

Collection of Larva

Chironomous larvae are found in abundance in ponds, spools, ditches and in drains. These are easily identified due to their red colored body because they possess haemoglobin in their blood (hence also called bloodworm). Collect these larvae in a jar with a little amount of water from any of the sources listed above. Separate the larvae from water and place them in normal saline. Identify its anterior and posterior ends by the presence of blood gills at its posterior end.

Dissection of 3rd Instar Larva for Salivary Gland

1. Take a few drops of saline on a clean slide and put the 3rd instar larva in it.
2. Locate the junction of thorax and abdomen.
3. Take two needles, one in each hand. Press the first needle firmly on the posterior end of thorax and other needle at the junction of thorax and abdomen.
4. Pull the second needle so that abdomen is separated from head and thorax.
5. Then press the thorax with a needle and observe that the salivary glands are seen floating in the saline water on the slide.

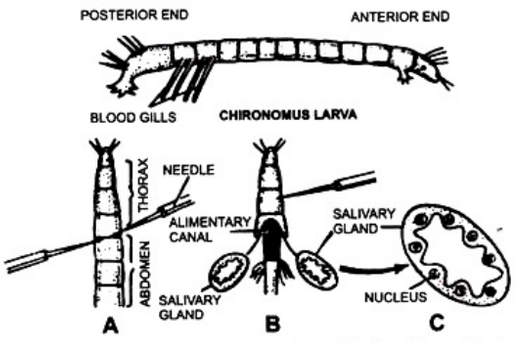

Fig. 1 *Dissection of Chironomous Larva for its Salivary Gland*

Preparation of Slide

1. Take a clean slide, put a drop of acetocamine on it.
2. Transfer the salivary glands in acetocarmine on slide and cover it with a cover-slip.
3. Leave it for 10 minutes and then warm it gently and prepare squash.
4. Observe the slide under microscope and for details observe under high power of microscope.

Comments

1. These are large-sized, hence, called giant chromosomes.
2. These chromosomes present alternate pattern of dark bonds and light inter bands.
3. The dark bonds contain rich amount of DNA and RNA, and composed of much coiled chromosomal thread.
4. The light bands contain large amount of proteins and little amount of DNA and RNA.
5. A polytene chromosome is multi-stranded; it is formed of large number of chromosomal threads or strands.
6. A polytene chromosome exhibits puffs and Balbiani rings at certain points. The puffs are made of lateral extensions of bonds of chromosomal strands into side loops (Refer Figure 2).
7. These chromosomes help in the synthesis of proteins, nucleic acids and formation of nuclear material. These were discovered by Balbiani in 1881.

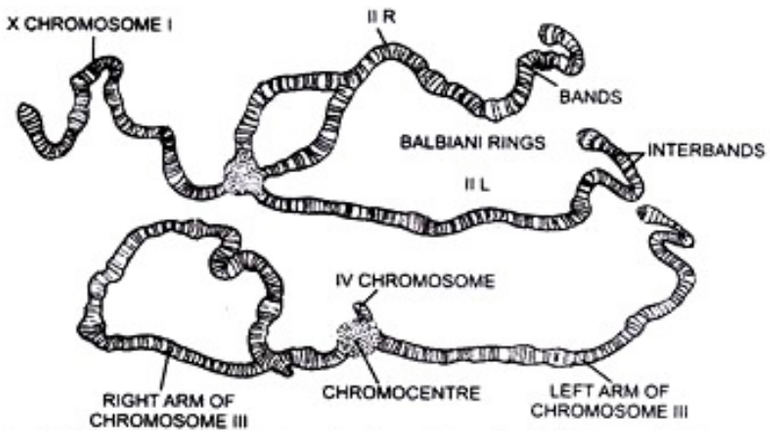

Fig. 2 *Polytene Chromosome*

Aim 2

To identify the presence of Barr body in buccal epithelial cells of female.

Principle

Buccal epithelial cells especially have Barr body structure, which are considered to play a major role for sex determination. This small round Barr body is located either in the border of nuclear membrane or sometimes inside of nucleus. This Barr body may be single or more in number in some cases. These structures are present only in the female sex (Refer Figure 3).

Requirements

Pre-cleaned slides, Methylene blue, Microscope, Epithelial cells (sample).

Procedure

1. Wash your mouth with sterile water to prepare mucous.
2. Take a sterilized slide and scrap epithelial cells superficially from the inner side skin of the mouth.
3. Keep the sample on the center of the pre sterilized glass slides and dry it for few minutes.
4. Then add few drops of Leishman stain on the smear and incubate for 5-10 minutes.
5. After incubation, wash it or remove the excess stain using water by keeping the in slant position.
6. Eventually, blot the water using tissue paper.
7. The smear is now ready for microscopic observation.

Result

In female epithelial cells dark stained heterochromatin (Barr body) is observed usually periphery of the nucleus, clearly under high power microscope.

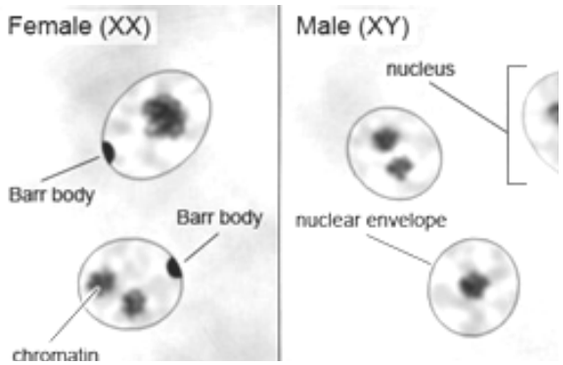

Fig. 3 *Barr Body Present in Female and Absent in Male*

CELL DIVISION

Cell division is especially rapid in the growing root tip; therefore, it is easier to observe each stage of mitosis than in slowly growing tissues. The young tips will be harvested, fix them, digest them in acid, treat them with a reagent which stains chromosomes, and view them under a microscope. Then estimate the proportion of time that cells in actively dividing tissues, such as the root tip, actually spend in M-phase and cytokinesis.

Aim 1

To prepare squash of onion root tip cells to observe mitosis and the cell cycle.

Principle

Cell division in flowering plants takes place in particular regions of the plant called meristems. Cells in meristems are not specialized for any particular function and divide repeatedly by mitosis. Some of the daughter cells remain meristematic others cease dividing and become differentiated into appropriate cell types depending on their position. The root tip meristem is usually a denser white and more rounded than the cut end. Chromosomes in root tip tissue are made visible with the stain. Dividing cells (if present) will show up clearly with chromosomes in different forms according to the stage of mitosis. Individual chromosomes (as tightly-coiled threads) are visible during anaphase. The links between the cellulose walls of plant cells are broken down by the treatment with hydrochloric acid. This ensures that the stain can penetrate the cells and allows the tissue to be squashed out one cell thick.

Requirements

Carnoy's Fixative, 1N HCl, Feulgen Stain, 45% HOAc (Acetic Acid), Freshly Sprouted Onion (About 2-3 cm Long Root Tip), Eppendorfs, Pasteur Pipettes, 60°C Water Bath, Microscope Slides, Razor Blades, Cover Slips.

Procedure

1. Cut off the last 6 mm (1/4 inch) of root tip from sprouting onions. Place 5 of them in the labeled Eppendorf tube.
2. Add 1 ml Carnoy's fixative and make sure that all tips are immersed.
3. Close tube and incubate for 24 hours.
4. Remove your root tips from the Carnoy's fixative and immerse in a new tube filled with 1 ml 1N HCl. Incubate for 12 minutes at 60°C.
5. Remove the HCl with a Pasteur pipette and discard in the drain with running cold tap water.
6. Add 0.5 ml Feulgen stain.

Note: *This stain does not look brightly colored, but stains strongly; keep it away from your clothes, books, etc.*

7. Let the root tip stain in Feulgen for about 10 minutes, or until the very tip of the root shows distinct dark coloring.
8. Put one drop of 45% HOAc on the slide. Place the root tip in the HOAc on the slide. With a scalpel or razor blade, remove all but the red-stained very tip of the root.
9. Add the cover slip on top of the root tip. Place the slide on a white piece of paper on your bench.
10. Tap gently and straight down with the eraser of a pencil until the stained tip is spread out to a faint purple monolayer.

Note: *Do not smear the cover slip sideways, this will shear the chromosomes.*

11. Examine your spread under the microscope at low power to ensure that the cells are spread to a monolayer. If not, squish the cover slip some more.
12. Once you have spread your cells into a nice monolayer, switch to oil immersion.
13. Spend some time identifying the different stages of the cell cycle visible in root section squashes.

Results

Different mitotic stages (prophase, metaphase, anaphase and telophase) observed in the slide (Refer Figure 1).

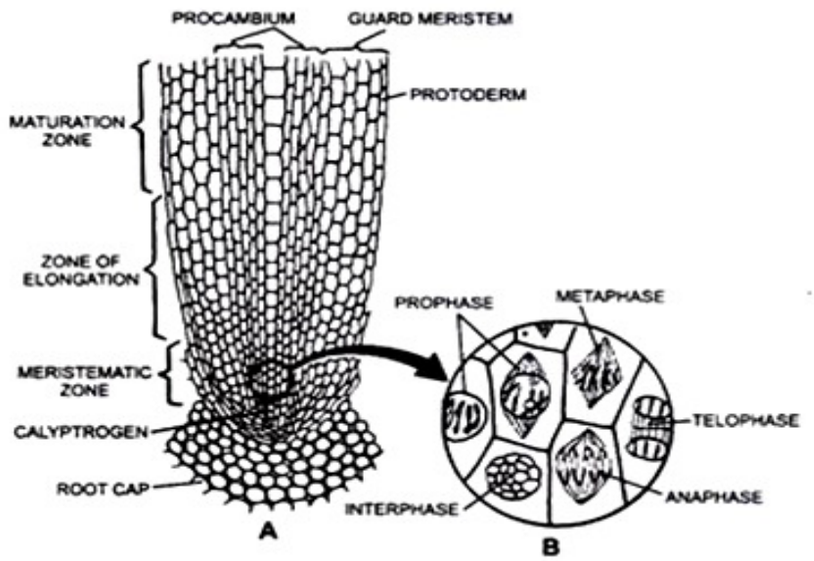

Fig. 1 *Squash of Onion Root Tip Showing Different Stages of Mitosis*

Aim 2

To perform meiotic cell division in the given sample (grasshopper testis follicles).

Principle

Meiosis is a process where a single cell divides twice to produce four cells containing half of the original amount of genetic information. These cells are sex cells, sperm in males and ovum in females. In males the process of meiosis called spermatogenesis and produce sperms while in females, the process of meiosis is called oogenesis, since it produces oocytes and ultimately yields mature ova (eggs). The meiosis occurs in the primordial germ cells, cells specified for sexual reproduction and separate from the body›s normal somatic cells.

Requirements

Acetocarmine, Glass Slide, Cover Slips, Lab Needle Sample (Grasshopper Testis Follicles), Eppendorfs, Pasteur Pipettes, Watch Glass, Razor Blades and Dissection Box.

Procedure

1. Identify the male grass hopper, which has lean and lengthy tail whereas, female has short and blunt tail.
2. Turn the dorsal side of grasshopper and count the 7th segment from the tail which is abdominal portion of the grasshopper.
3. Using sharp scalpel cut at the 7th segment and press gently to release a yellow fluid clumps (which is genital organ covered with yellow fat bodies).
4. Transfer the clump carefully to the vessel containing saline for washing.
5. Shake gently with lab needle so the fat bodies and follicles (white fibers in elongated shape) get separated.
6. Transfer this follicle bunch to a Petri dish and separate it into individual follicles.
7. Take a pre-cleaned slide and transfer a follicle to the centre of the slide.
8. Add a drop of acetocarmine incubate it for 5 minutes.
9. If it is dehydrated add another drop of acetocarmine and cover with cover slip.
10. Using your forefinger press gently and prepare squash carefully.
11. Remove the excess stain by blotting technique.
12. Focus at 10x and examine at 40x for sharp image using light microscope.

Results

Different stages of meiotic cell division could be visible. Refer Figure 2 and see that Interphase: condensed chromosome; Prophase thread like chromosome structure; Metaphase: spindle fibers and distinct chromosome at center of the cell; Anaphase: stretching of chromatids towards opposite poles; Telophase: cell undergoes equal division.

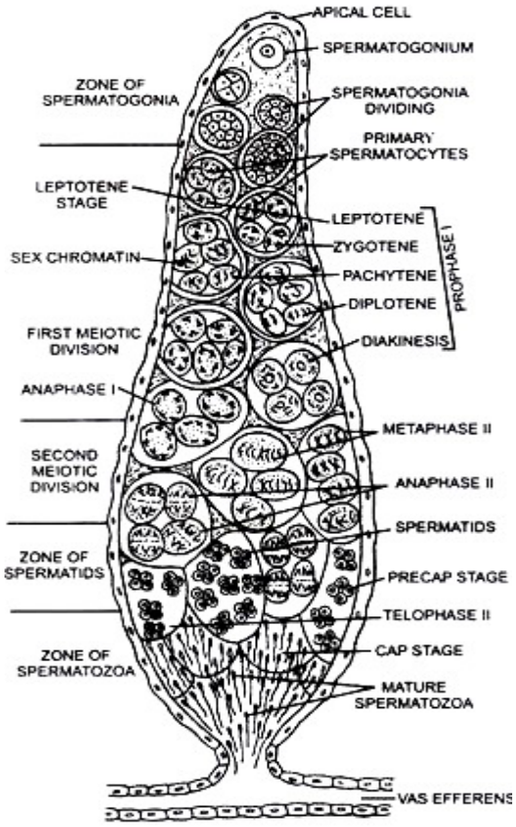

Fig. 2 *T.S. of Testis of Grasshopper to Show Meiosis*

ELECTROPHORESIS

The proteins, DNA and RNA (biomolecules) have electric charges which depend on molecule to molecule and the conditions of the medium (pH of buffer in which dissolved). Charged molecules can be separated by electrophoresis in gels. Agarose gel electrophoresis is chiefly used for DNA and RNA while Polyacrylamide gel is mainly used for protein isolation. Due to the differences in amino acid composition proteins have unique mass and charge. Hence proteins have net negative charge and net positive charge or isoelectric point (no charge) at a given pH of buffer. Sodium Dodecyl Sulphate- PolyAcrylamide Gel Electrophoresis (SDS-PAGE) is a high resolution method used universally for analyzing the mixture of proteins according to their respective size. SDS solubilised in soluble proteins makes possible the analysis of the other insoluble mixtures. Separation of the proteins does not occur due to similar charge: mass ratio (z/m). Therefore, such proteins are treated first with an ionic detergent called Sodium Dodecyl Sulphate (SDS) before the start and during the course of electrophoresis (PAGE). Therefore, such electrophoresis is called SDS- PAGE.

The identical proteins are denatured by SDS resulting in their sub-units. The polypeptide chains get opened and extended. On the basis of their mass but not the charge, the molecules are separated. Electrophoretic separation is normally used for following reasons:

- Gel acts as molecular sieves hence separate the molecules on the basis of their size.
- Gel suppresses conventional current produced by small temperature gradient which improves the resolution.
- Agarose gel is used to separate large sized macromolecules like DNA and RNA.
- Polyacrylamide gel is used for this purpose due to its good nature (chemically inert, stable over a wide range of pH, temperature, ionic strength and transparent).

Aim 1

To isolate the genomic DNA from animal tissue using CTAB (Cetyl Trimethyl Ammonium Bromide) method.

Principle

DNA is a high molecular weight macromolecule. It is suitable for digestion with restriction enzymes. Hence, it is very important for the studies of

molecular biology. Several methods are used for the isolation of genomic DNA from tissues of organisms. But isolation of quality DNA from animal tissue is rather difficult. Due to low yield of DNA high amount of tissues are required, hence old method is not preferred. Therefore, CTAB (Cetyltrimethyl Ammonium Bromide) method is commonly used due to less expensive and high yield of genomic DNA.

Requirements

Water bath incubator (60^{o}C), refrigerated high speed centrifuge animal tissue, mortar and pestle, isopropanol and DelylTrimethylAmmonium Bromide (CTAB).

Preparation of Reagents

The following reagents must be prepared carefully:

1. Chloroform: Isoamyl Alcohol (24: 1, v/v) (prepare fresh and keep in a dark bottle).
2. 7.5M Ammonium Acetate (57.8g 100ml, pH 7.7).
3. RNase (10mg/ml with distilled water) or TE buffer (store in -20^{o}C).
4. Tris-EDTA (TE) buffer [Tris-HC1 10mM (pH74) and EDTA 1mM (pH- 8.0)].
5. 10mM Ammoniumacetate (0.077g/100ml, pH 7.7, autoclave and store in refrigerator).
6. 70% Ethanol.

Preparation of Isolation Buffer

It is prepared as below:

1. 10mM Tris-HCI (1M stock solution; 12.11g/100 ml, pH 8.0).
2. 0.2% (v/v) Mercaptoethanal solution (should be added freshly).
3. 5M NaCl (29.22g dissolve in 100ml to get stock solution).
4. 0.5MmM EDTA (18.62g/100ml pH 8.0 to get stock solution).
5. 20% (w/v) CTAB (20g/100ml to get stock solution).

Mix these chemicals fresh in a proper ratio to prepare isolation buffer. For making 10ml isolation buffer mix these stock solutions in the following volumes and make upto 10ml:

10 mM Tris-HCI:	0.1ml
0.2% (v/v) Mercaptoethanol (fresh):	0.02ml
1.4M NaCI:	2.8ml
20 mM EDTA:	0.4ml
2% w/v CTAB:	1.0ml

Note: *EDTA, NaCl, CTAB, Tris-HCl, ammonium acetate and all glassware should also be autoclaved. Chloroform: Isoamylalcohol (24: 1) should be freshly prepared and stored in a dark bottle.*

NOTES

Procedure

1. Collect fresh tissue, weigh 1.0g and grind in liquid nitrogen to make powder or paste keeping in pre chilled pestle and mortar (liquid nitrogen may also be used). The tissues should not have been ground earlier.
2. Warm 10ml isolation buffer in a centrifuge tube at 60-65°C in a water bath.
3. Mix powder or paste of tissue in preheated isolation buffer. Incubate at 65°C in a water bath with gentle swirling.
4. Extract with equal volume (24 : l) of chloroform: İsoamyl alcohol and mix gently.
5. Centrifuge at 10,000 rpm for 20-25 minutes at room temperature.
6. Collect the clear aqueous phase (supernatant) very carefully using a wide bore pipette in a fresh centrifuge tube. Note the volume of aqueous phase (the same process of chloroform: isoamyl alcohol extraction is repeated if the supernatant is not clear).
7. Gently add 2/3 volume of isopropanol to aqueous phase and mix properly (the fibres of nucleic acid become visible).
8. Then centrifuge immediately for 10-15 minutes at 10,000 rpm.
9. Remove the supernatant and collect over tissue paper so that isopropanol could be removed.
10. Wash the pellet twice with 70% ethanol centrifuging at 6000 mm for 5 minutes at room temperature.
11. Dry the plates at 37^{o}C for 30 minutes till completely dried.
12. Pour 100-200ml TE buffer (for DNA isolation about 100ml for 1g tissue) or sterile distil water so that the pellets could be dissolved. Add 10ml RNase from the stock (10mg/ml). Incubate for 30-60 minutes at 37°C.
13. Then add 2 ml of sterile distilled water or TE buffer to dilute the sample. Gently add 7.5M ammonium acetate to get the final concentration of 2.5M. Also add 2.5ml of cold ethanol for precipitating the DNA.
14. Incubate it overnight at -20°C (deep freeze) and centrifuge DNA at 10,000 rpm for 15 min at 4°C.
15. Dry the pellets in air and re-suspended in 100-150ml sterile distilled water or TE buffer.

Results

A white/milky precipitate of DNA will be observed on the sides/bottom of centrifuge tubes after precipitating with isopropanol and centrifugation.

Aim 2

To detect the presence of DNA by Agarose Gel Electrophoresis.

Principle: Electrophoresis refers to movement of charged molecules under the electric field (Refer Figure 1). Electrophoresis is carried out by using agarose or polyacrylamide gel. Agarose gels are more porous (100-300nm) than that of acrylamide. However, pore size depends on the concentration of agarose. Hence, agarose is used to separate large sized macromolecules like DNA and RNA. Agrose gel concentration depends on the size of DNA, RNA and plasmid molecules. Higher molecular weight of DNA, RNA or plasmid requires lower concentration of agarose gel. The DNA and RNA molecules are negatively charged and moves toward anode (positive charged) when an electric field is applied. This movement depends on the size i.e. molecular weight of nucleic acids. Small sized molecules move faster than the large sized molecules. A dye called ethidium bromide is used to detect the DNA It intercalates with DNA. Therefore, location of DNA can he detected and visualized when fluoresces under UV light.

Requirements

1. Genomic DNA (isolated as described in earlier experiment), Agarose Gel, Boric Acid, Tris, EDTA, Sucrose, Distilled Water, Ethidium Bromide, Glycerol. Bromophenol Blue, Eppendorf Tube, Micropipette, Electrophoretic Apparatus with Power Supply, Microwave Oven, Magnatic Stirrer.
2. Running buffer [the two buffers are commonly used e.g. Tris-Borate-EDTA (TBE) and Tris-Acetate-EDTA (TAE)]. The stock solutions are prepared as follows: 5X TBE 1 litre [boric acid (27.5g), Tris (54g) and 0.5M EDTA (20ml), pH8.0] and 5X TAE 1 litre [Tris-base (24.2g), glacial acetic acid (5.71ml) and 0.5M EDTA (10ml), pH8.0].
3. EDTA (0.5M, pH5.0, 100ml).
4. Ethidium bromide stock solution (dissolve 10mg/ml in water. wrap in an aluminium foil and store at 4^{o}C.

Note: *Always wear glove while working with this dye because it is mutagenic.*

5. 6X gel-loading stock (0.15% bromophenol blue, 0.15% cyanol, 30% (v/v) glycerol in water. Alternatively prepare loading dye of 10X (66mg sucrose, 4.2mg bromophenol blue and 1ml buffer; use 1X loading dye).

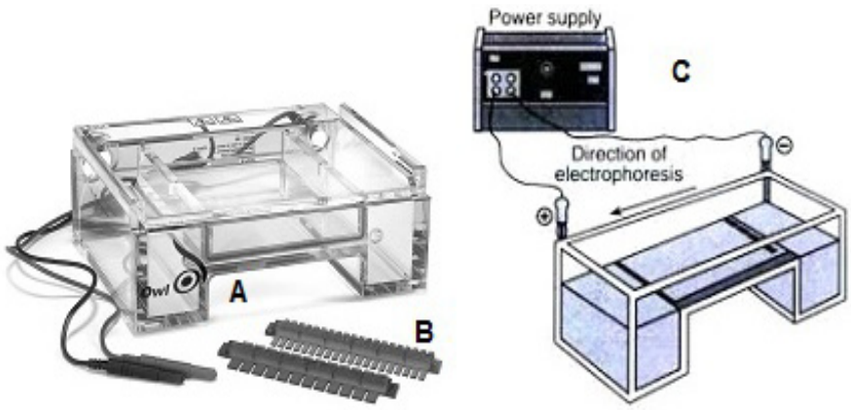

Fig. 1 *Electrophoresis Chamber (A), Comb (B), Typical Electrophoretic Apparatus Setup with Power Supply (C)*

Procedure

1. Dissolve 0.8g agarose in 100ml of 1X TBE by gentle heating on magnetic stirrer with hot plate (or microwave). This will result in 0.8% agarose gel.
2. Cool the contents to 60^{o}C and add ethidium bromide to get a final concentration of 0.5ug/ml. Mix the contents properly.
3. Using a tape seal both the open sides of gel tray.
4. Insert the comb in such a way that 1mm gap between the teeth and the surface of tray could be made.
5. To get the thickness of gel to about 4-5mm, pour the agarose solution into the tray. Care should be taken that no air bubble must be trapped in the gel. Now hold this for about 30-40 minutes.
6. Gently remove the comb when gel has been solidified. Remove the tapes and keep the gel tray into the electrophoresis tank.
7. Pour TAE into the tank so that gel may get immersed by about 5mm.
8. Carefully load DNA samples into the slots of submersed gel. The samples must be settled at the bottom of slot.
9. Connect the electric lead in such a way that the negative terminal should be at the end where sample has been loaded.
10. Run electrophoresis at 60-100V until bromophenol blue migrate to the other end of the gel.
11. Turn the button off and disconnect the electric leads. Take out the agarose gel from the electrophoresis tank.

12. Observe it in a UV trans-illuminator. The separated genomic DNA as bands fluoresces under UV light which makes it presence positive (Refer Figure 2).

Result

While running the genomic DNA on agarose, the bands must appear distinct without smear. At the end of the gel a prominent, RNA contamination is visualized if DNA is not treated with RNase or treatment is incomplete. RNA moves faster than the DNA because of being small sized.

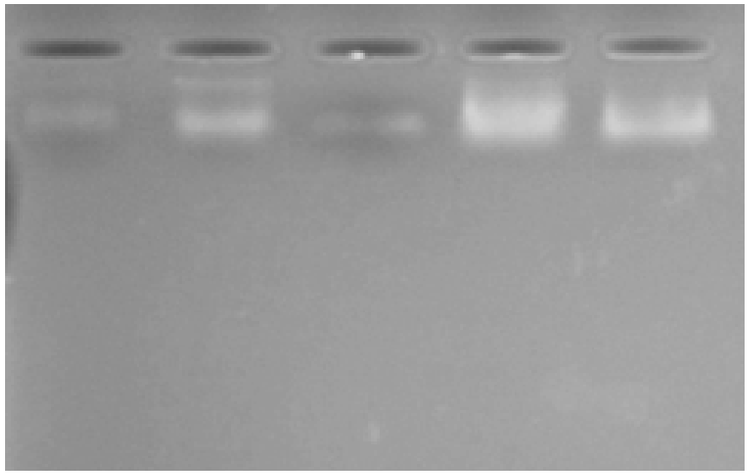

Fig. 2 *Genomic DNA on Agarose Gel*

Aim 3

To analyze the protein by SDS-PAGE under denaturing conditions.

Principle

The proteins are denatured and have a negative charge with a uniform charge to mass ratio (z/m) when treated with SDS (anioic detergent). Proteins migrate toward anode at alkaline pH through polyacrylamide gel during electrophoresis. The smaller polypeptides moves faster followed by the larger polypeptides. Therefore, the intrinsic charge on proteins is masked in SDS-PAGE. Hence, the separation is based on the size. Mercaptoethanol reduces interpolypeptide disulfide bridge and separates the sub-units of a polymeric protein. Molecular weight of the separated protein can be analyzed by comparing the molecular weight of the standard protein and its mobility.

Requirements

1. TEMED (Tetramethylethylenediamine), Ammonium Persulphate (10%), Coomassie Brilliant Blue (0.3%).

2. Destaining mixture, gel staining dish, electrophoresis apparatus with supply.
3. Running Buffer, SDS (10%), 1.5M Tris-HCl (pH 8.8), 0.5M Tris-HCI (pH 6.8), Laemmli buffer, stock acrylamide-bis-acrylamide solution.
4. Stock Acrylamide (bis-acrylamide solution): 29.2g acrylamide, 0.8g bis-acrylamide and final volume raised to 100ml).
5. 1.5M Tris-HCI, pH 8.8: Dissolve 18.15g of Tris in 50ml of distilled water, adjust the pH to 8.8 with HCl, make the final volume to 100ml).
6. 0.5 M Tris-HCI, pH 6.8: Dissolve 6g Tris in 60 ml distilled water, adjust pH to 6.8 with HCI, make the final volume to 100ml.
7. 10% SDS: Dissolve 1g SDS in 5ml distilled and raise the final volume equal to 10ml.
8. Gel Running Buffer: Dissolve 14.4g glycine, 1g SDS in 500ml distilled water, adjust pH to 8.3 by adding solid Tris, make the final volume to 1 litre.
9. Ammonium Persulphate (APS) (10%): Dissolve 500 mg of solid APS in 5ml of distilled water (Note: always use freshly prepared APS solution only).
10. Coomassie Brilliant Blue R250: Dissolve 600mg of Coomassie brilliant Blue R250 (CBBR 250) in 80ml methanol, add 20ml glacial acetic acid and make the final volume to 200ml with distilled water.
11. Destaining Solution: Mix 400ml methanol, 100ml glacial acetic acid and 500ml distilled to get 1 litre of this solution.
12. Laemmli Buffer: 62.5mM Tris-HCl, pH 6.8 (use diluted 0.5M Tris-HCI pH 6.8), 10% glycerol, 5% Mercaptoethanol, 2% SDS.

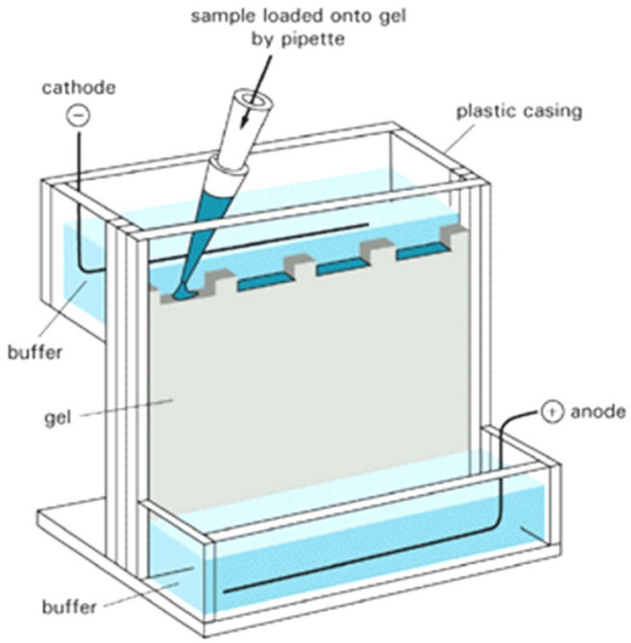

Fig. 3 *A Typical SDS-PAGE Cassette (Vertical)*

Procedure

1. Make 7.5% uniform concentration of SDS resolving gel. The capacity of the apparatus governs the volume of the final gel solution. Accordingly, read carefully the instruction manual supplied with the electrophoresis unit before deciding the volume of the gel.
2. According to manufacturer's instruction, assemble the gel electrophoresis apparatus (Refer Figure 3).
3. Make gel solution of the separating and stacking gels (find out the volume of solution needed by the apparatus).

Prepare fresh solution just before use as per the following table:

Reagents	***Stacking Gel (4%) 0.125M Tris, pH6.8***	***Separating Gel (7.5%) 0.375M Tris, pH8.8***
Acrylamide-Bis	1.3ml	25.0ml
Distilled Water	6.1ml	48.5ml
1.5M Tris-HCl, pH 8.8	—	25.0ml
0.5M Tris-HCl, pH 6.8	2.5ml	—
10% (w/v) Sterile Distilled Water	100ul	1ml
10% Ammonium Persulphate[#]	50ul	500ul
TEMED[*]	10ul	50ul
Total Monomer	10ml	100ul

Note: *APS (fresh) and PEMED are added to the rest of the solution just before pouring the gel solution into the glass sandwich.*

4. Fill up the glass sandwich with pipette upto mark with the separating gel solution prepared (Note: the body must not make contact with chemicals. The acrylamide solution in unpolymerized state is neurotoxic).
5. To make the gel surface straight after polymerization, overlay the acrylamide solution with water and the gel should be allowed to polymerize for 30 minutes (Note: when the polyacrylamide layer becomes distinct below the water layer, polymerization is complete).
6. Remove the distilled water by using the filter paper carefully. Thereafter, add the requisite amount of 4% stacking gel solution (freshly mixed). Then insert the comb for polymerization
7. When the gel polymerized place the glass sandwich into the electrophoretic chamber and add running buffer to level in both cathodic and anodie chambers. To prevent the sample wells from deforming, remove the comb under buffer.
8. With the help of micro-pipette, load the denatured protein solution into the well (Note: protein sample should be denatured in Laemmli buffer by boiling for 5 minutes).
9. Add standard molecular weight marker proteins in one lane (Note: for detection by CBB dye generally 20 to 50µg protein is sufficient).
10. Tightly connecting the electrodes of the apparatus with the power supply. Run the gel at constant current of 20mA.
11. Track the mobility of sample in the matrix with the dye (generally bromophenol blue is added to the Laemmli buffer). After completion, switch off the button and disconnect the apparatus.
12. Transfer the gel to the staining tray containing the gel staining dye.
13. Under shaking conditions on a rocking shaker, stain the gel for at least 2 hours or overnight (Note: at this stage, the whole gel turns blue).
14. Carefully transfer the gel destaining solution and shake on a rocker shaker for 30 minutes. Add fresh destaining solution.

Note: *Repeat these steps until the bands are clearly visible in the gel.*

15. Capture the photograph of the gel. The gel is clearly visible with bands and analyze the photographated gel (Refer Figure 4).

Results

- Several distinct blue coloured bands can be seen in the gel.
- Each band represents to a single or multiple bands in the lane.
- Depending on the amount of the polypeptide present in the protein solution loaded in the gel, the intensity of these bands varies.

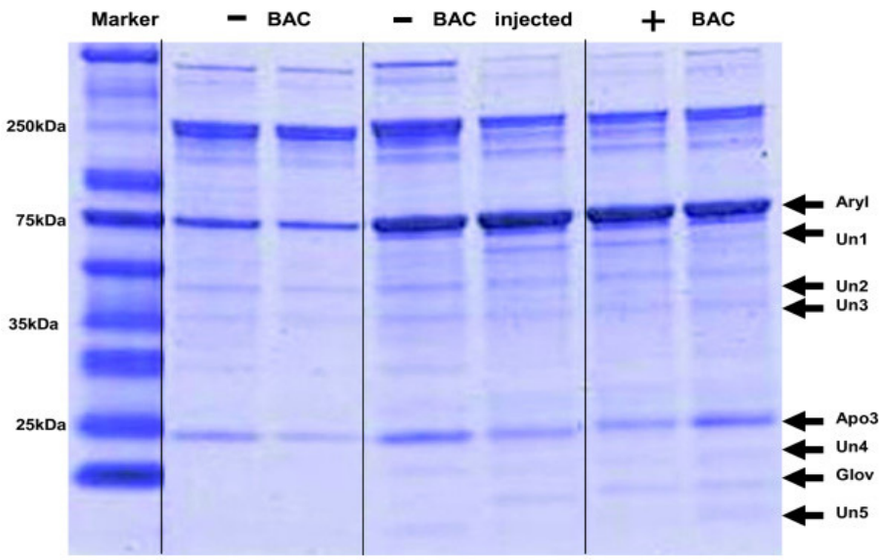

Fig. 4 *Acrylamide Gel with Protein Bands After SDS-PAGE*

CHROMATOGRPAHY

Chromatography is a procedure through which various substances in a mixture are separated from each other and identified. Separation involves the use of two phases, one of which is stationary and the other mobile. Separation depends upon the differential movement of the test substances between two phases. In a simple language chromatography can be defined as an analytical technique for separating compounds on the basis of differences in affinity for a stationary and mobile phase (Refer Figure 1).

Chromatographic Techniques

1. **Partition Column Chromatography:** In this technique, the column is packed with a porous solid of high surface area, e.g., silica gel and cellulose, coated with water (stationary phase). The components of a mixture are separated by passing an organic solvent (mobile phase) through the column.
2. **Paper Chromatography:** In this technique, the dissolved substances are applied as a small spot on a cellulose-bound filter paper which is then kept in a container dipped in an organic solvent. The mixtures are partitioned between (stationary phase) and organic solvent (mobile phase).
3. **Thin Layer Chromatography:** In this technique, the adsorbent (stationary phase) is spread over a glass plate in the form of a thin film of even thickness. The solvent (mobile phase) moves up the plate by capillary action and, thus, effects separation.
4. **Gel-Filtration Chromatography:** Sephadex contains the aqueous (stationary phase) which is distinguished from the mobile phase by its immobilization. The components of the mixture are separated according to their size, by virtue of differential distribution between easily displaceable water present in the interbed space.
5. **Gas Liquid Chromatography:** In this technique, a column is packed with a porous inert solid with a thin layer of a nonvolatile liquid as the stationary phase. Components of the mixture are separated by being partitioned between this phase and a gaseous (mobile phase).
6. **Adsorption Column Chromatography:** In this technique, the separation of a mixture is determined by the differential adsorption of the components on an active solid (alumina, silica gel, stationary phase) as an organic solvent (mobile phase) containing them which passes over and affects separation.
7. **Ion Exchange Chromatography:** In this technique, ionized compounds are separated in aqueous solution (mobile phase) by virtue

of their differences in affinity for ionized compounds which are an integral part of the insoluble solid phase (stationary phase).

RF Value

The RF value expresses the relative rate of movement of solute and solvent. The RF value is defined as the ratio of the distance travelled by the compound at its maximum concentration to the distance travelled by the solvent. Both the distances are measured from the point of application of the sample. The RF value has no unit.

$$RF = \frac{\text{Distance travelled by the substance from origin}}{\text{Distance travelled by the solvent from origin}}$$

Aim 1

To detect the presence of amino acid in the given sample by paper chromatography.

Principle

It involves the separation of substances based on their differential affinity for two phases: a mobile phase and a stationary phase. In this experiment the chromatographic paper is the stationary phase and the solvent is the mobile phase (Refer Figure 1).

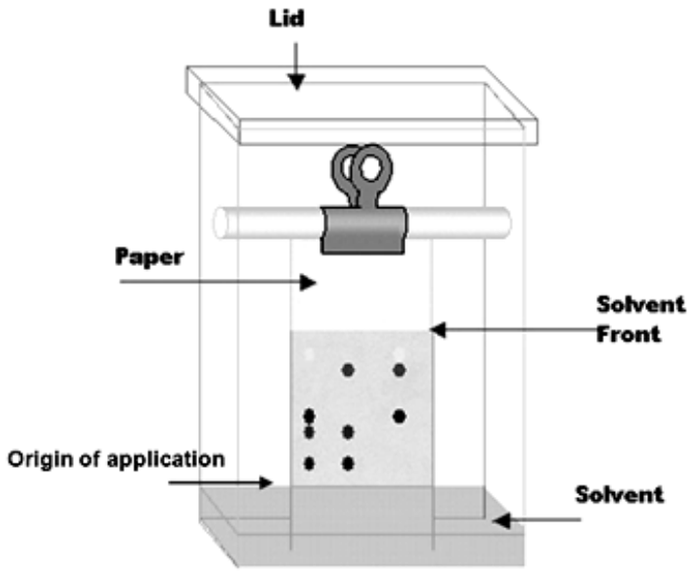

Fig. 1 *Paper Chromatography Chamber (Ascending)*

Requirements

Standard Amino Acid Solutions, Ninhydrin, Acetone, N-Butanol, Acetic Acid, Distilled Water, HB-Pencil, Ruler, Glass Capillaries, Unknown Sample, Atomizer, Whatman No-1 Chromatography Paper, Measuring Cylinder, Watch Glass.

Procedure

1. Whatman No-1 chromatography paper was cut into pieces of 20x20cm^2.
2. A line was drawn 5cm from the bottom edge of the chromatography paper along the width of the paper with an HB-pencil.
3. Spots were marked along the line.
4. A few drops of different amino acids solutions were applied to each spot with different capillary tubes.
5. A drop of unknown sample was applied to one of the spots.
6. The solvent was prepared using N-Butanol: Acetic Acid: Distilled Water in the ratio of 4: 1: 2, respectively.
7. The solvent was poured in a measuring cylinder to a depth of 4cm (Note: the solvent must not touch the pencil line).
8. The chromatographic paper was rolled into a cylinder and both ends were secured with staples or paper clips.
9. The roll of paper was placed in the measuring cylinder and the mouth of the cylinder was covered with a watch glass.
10. The chromatogram was removed from measuring cylinder after 3 hours or when the solvent runs bout the 95% of the paper length.
11. The position of the solvent front was marked immediately and the chromatogram was dried in air.
12. 1% Ninhydrin solution (w/v) in acetone was sprayed with an atomizer on the dried chromatogram.
13. The paper was dried at room temperature for 1 hour and followed for 2-5 minutes at 40-45^{o}C in hot air oven.
14. The position of the colored dots or spots was marked immediately with an HB-pencil.
15. The distance between the point of loading and the middle of the colored dot/ spot as well as solvent front was measured.
16. The RF value of standard amino acids was calculated using the formula as follows:

$$RF = \frac{\text{Distance travelled by the substance from origin}}{\text{Distance travelled by the solvent from origin}}$$

17. The amino acid composition of the unknown sample was determined by comparing the RF value of the spots obtained from sample with the RF values of standard or known amino acids on same chromatogram.

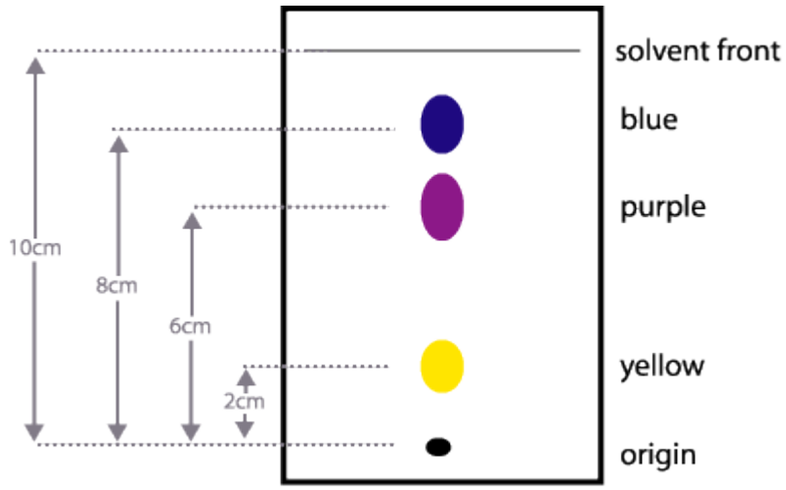

Fig. 2 *A Typical Chromatogram with Distance Travelled by Solute and Solvents*

Result

The presence of amino acids in unknown was determined by paper chromatography.

Precautions

1. Do not touch the paper with bare hands.
2. Use different capillaries for each amino acid and sample.
3. Ensure that the solvent does not touch the sample loading line.
4. Ninhydrin is a suspected carcinogen. Handle it carefully.

M.Sc. [Zoology]

350 14

PRACTICAL

LAB I : ANIMAL DIVERSITY, BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

I - Semester

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