

PRACTICAL MANUAL IN ZOOLOGY

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PREFACE

Animals have inhabited the earth for millions of years, growing from simple one-celled organisms to the diverse variety we see today. This manual encompasses an overview of the animal kingdom and classification suitable for both B.Sc. and M. Sc. Zoology. The collection of spotters and mountings related to the Functional Morphology and Paleontology of Invertebrates and Chordates. The spotters are presented in a simple way with taxonomical characters and salient features. It also emphasizes the microscopic study of live and preserved specimens. This manual is comprehensive in its representation of the major groups of animal phyla. The experimental parts related to the Genetics, Microbiology and Cell and Molecular Biology are presented in a simple way with a discussion on all subjects. It contains experiments, selection of which was governed by the following parameters: easy availability of resource material, chemicals and instruments; involvement of a new concept or technique; and reproducibility by the students.

This practical manual has a collection of spotters and experiments related to Biochemistry and Biophysics, Biotechnology, Biostatistics and Computer Applications. The experimental parts are presented in a simple way with a discussion on all subjects. It contains experiments, selection of which was governed by the following parameters: easy availability of resource material, chemicals and instruments; involvement of a new concept or technique; and reproducibility by the students.

The manual also has a collection of experiments related to the Developmental Biology, Immunology, Animal Physiology, Microtechnique and Environmental Biology. It contains experiments, selections of which are governed by the following parameters: easy availability of resource material, chemicals and instruments; involvement of a new concept or technique; and reproducibility by the students. The experimental parts are presented in a simple way with a discussion on all subjects. The model question papers are given at the end of this manual. We hope that the manual will be more useful to the post-graduate students in understanding the experiments. This practical manual

will be a boost for the learners. Designing of the manual is such that the students will be benefited as far as the knowledge and examination is concerned.

I express my deep sense of gratitude and heartfelt thanks to Janab S. Mohamed Aslam, Secretary and Dr. A. Mohamed Abdul Khader, Principal, Dr. S.V.S. Amanulla Hameed, HOD, Dr. P. Kumarasamy, Associate Professor and Dr. S. Raveendran, Associate Professor, Khadir Mohideen College, Adirampattinam-614701 for their encouragement and contributions to take up the assignment of writing a Practical Manual in Zoology for the welfare of the UG and PG student of Zoology community.

I welcome any corrections and constructive suggestions to improve this manual and those will be incorporated in the next edition.

Author

ABOUT THE AUTHOR

Dr. A. Amsath is the author of this manual. He is working as an Associate Professor in Zoology, Khadir Mohideen College, Adirampattinam. He has shown a keen interest to publish this manual. The other others were contributed and helped him to bring out the manual.

He completed his higher studies from B.Sc. to Ph.D. degree at Jamal Mohamed College, Tiruchirappalli. He has 20 years of teaching and research experiences. He is an author for many books like Basic Biotechnology, Immunology, Apiculture, Entomology and Practical Manual for B.Sc. Zoology, etc. He has published more than 25 research articles in National and International reputed Journals. He has attended and presented more than 30 papers in National and International Seminars/Symposia/Conferences. He has participated in 5 workshops and 4 refreshers courses. He has guided more than 25 M. Phil. Scholars. He has been guiding 7 Ph. D. scholars. One candidate submitted Ph.D. Thesis under his guidance. He has organized 2 National seminars.



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**FUNCTIONAL MORPHOLOGY AND
PALEONTOLOGY OF
INVERTEBRATES AND CHORDATES**

GENETICS

MICROBIOLOGY

CELL AND MOLECULAR BIOLOGY

PART - I. TAXONOMY

A. TAXONOMY OF INVERTEBRATES

PHYLUM- PROTOZOA

1. EUGLENA

Classification

1. Phylum: Protozoa.....: Microscopic and acellular.
2. Class.....: Mastigophora.....: Locomotory organs flagella.
3. Order.....: Euglenoidina.....: Body covered with soft and rigid pellicle; cytostome and cytopharynx or gullet present; flagella one or two; chromatophores few to numerous and green.
3. Genus.....: *Euglena*
4. Species.....: *viridis*.

Salient features

1. *Euglena* (Fig. 1) is oval, spindle-shaped measuring from 50 to 100 microns in length with a blunt anterior end and pointed posterior end.
2. Body is often covered by striated pellicle. The pellicle is flexible. Due to flexibility in pellicle *Euglena* is capable of euglenoid movement.
3. Anterior end of the body bears a small funnel-like cytostome and a tubular cytopharynx which leads into a large spherical reservoir.
4. Single whip-like flagellum arising from the base of the reservoir.
5. Nucleus is conspicuously large and spherical located towards the posterior region of the body.
6. The cytoplasm is differentiated into ectoplasm and endoplasm.
7. Chloroplasts, paramylum reserves, stigma and a large contractile vacuole surrounded by accessory contractile vacuoles are present in the endoplasm.
8. Nutrition is holophytic or saprophytic.
9. Reproduction is asexual by longitudinal binary fission. No sexual reproduction.
10. The species commonly met in India are *Euglena viridis*, *E. agilis*, *E. orientalis*, *E. gracilis*, *E. spirogyra* and *E. fusiformis*.

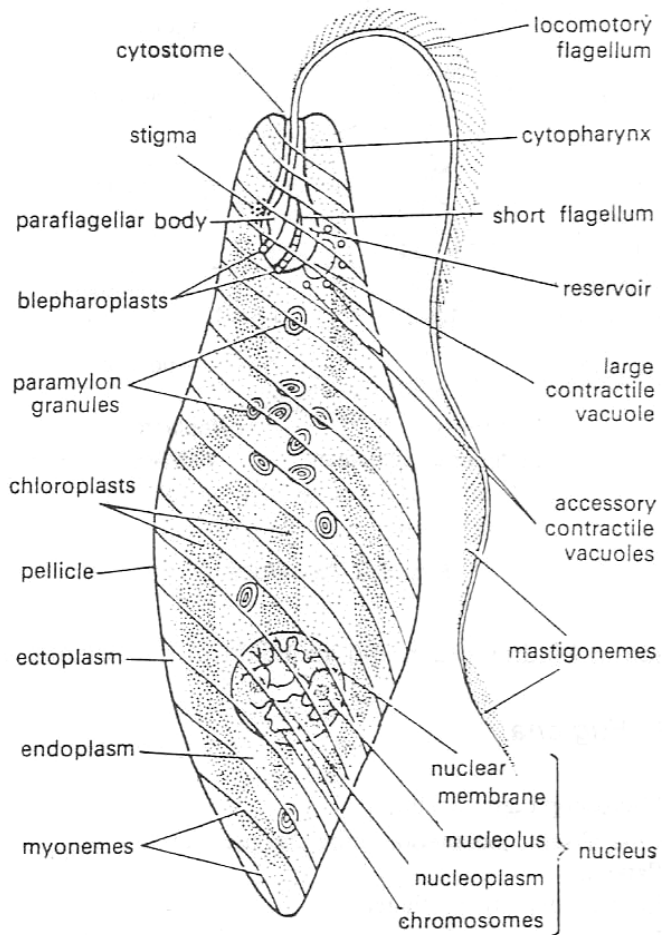


Fig. 1. *Euglena*.

2. AMOEBA

Classification

1. Phylum.....: Protozoa.....: Microscopic and acellular.
2. Class.....: Rhizopoda.....: Locomotory organs pseudopodia.

3. Order.....: Lobosa.....: Pseudopodia lobose or filose; ecto and endoplasm distinct.
4. Genus..... : *Amoeba*
5. Species.....: *proteus*.

Salient features

1. *Amoeba* (Fig. 2) is a minute organism measuring from 0.25 mm to 1.00 mm.
2. Shape of the body is irregular appears like a colourless gelatinous mass of protoplasm.
3. Body is covered externally by a very thin, clear relatively tough elastic and semi-permeable layer of protoplasm called plasmalemma.
4. Cytoplasm is distinguished into an outer clear dense ectoplasm and an inner granular more fluid portion the endoplasm.
5. Ectoplasm shows ectoplasmic ridges and a permanent posterior end as uroid.
6. Single small spherical nucleus, a large single contractile vacuole and a variable number of food vacuoles are present in the endoplasm.
7. Locomotion by short and blunt pseudopodia.
8. Nutrition is holozoic.
9. Reproduction by binary fission and multiple fission.

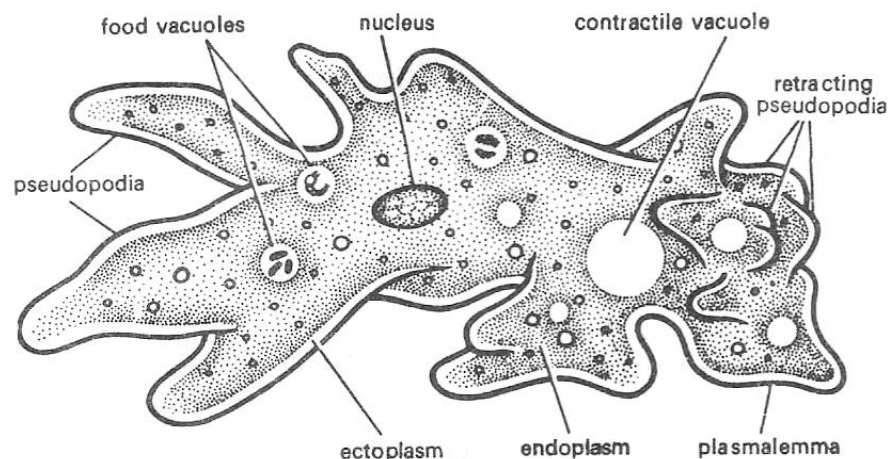


Fig. 2. Amoeba.

3. PLASMODIUM

Classification

1. Phylum : Protozoa.....: Microscopic and acellular.
2. Class.....: Sporozoa.....: Locomotory organs absent in adult; exclusively parasitic forms.
3. Order.....: Haemosporidia...: Trophozoite small, amoeboid and intracellular; sporozoites naked; zygote motile; parasites of blood of vertebrates.
4. Genus.....: *Plasmodium*
5. Species.....: *vivax*.

Salient features

1. *Plasmodium* (Fig. 3) is an intracellular blood parasite of man and other vertebrates and causes.
2. The life history of *Plasmodium* is completed in two hosts, viz., partly in definitive host, the man and partly in intermediate host, the female *Anopheles* mosquito.
3. When an Infected female *Anopheles* mosquito bites a man, sporozoites are introduced in the blood from where they reach in liver cells through blood streams and multiply to form merozygotes.
4. After a few schizogenous cycles in the liver, the merozoites enter the red blood corpuscles(R.B.C.) and feed on the contents of R.B.C.

5. After 2-8 schizogenous changes in the main blood stream the merozoites assume different shapes and known as gametocytes.

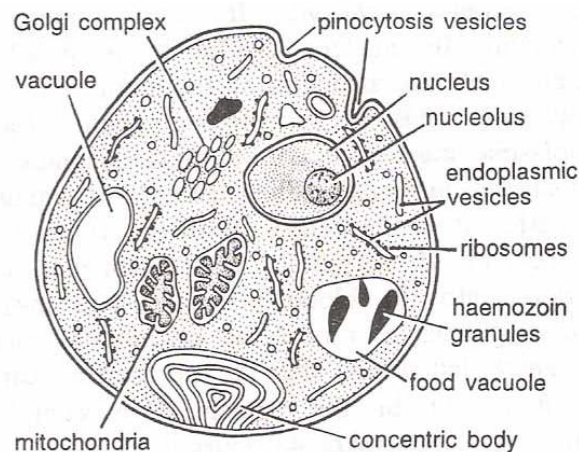


Fig. 3. *Plasmodium*.

6. Gametocytes cannot develop further in the blood of man, therefore, they wait for female.
7. *Anopheles* mosquito to suck them with the blood.
8. When gametocytes are sucked in by the female *Anopheles* with the blood of man, they undergo sporogony for further development. .
9. There are four species of *Plasmodium* causing different types of fever: *E.g. Plasmodium vivax* causes benign tertian fever.
10. *P. falciparum* causes malignant tertian fever; *P. malariae* causes quartan fever; *P. ovale* causes ovale or mild tertian fever.
11. Habit and habitat. *Plasmodium* is found as an intracellular parasite in the blood of vertebrates.
12. Distribution. *Plasmodium* is widely distributed in tropical and temperate countries of world but they are no longer a problem in the colder countries of the world. Countries like India, Sri Lanka, Bangla Desh, Nepal and Pakistan, etc., are worst affected. In India, states like Bihar and Uttar Pradesh suffer a great setback by the infection of this parasite. In fact, the infection of *Plasmodium* is a global problem.

4. PARAMECIUM

Classification

1. Phylum.....: Protozoa.....: Unicellular,
2. Sub-phylum.: Ciliophora.....: Ciliary movement in all stages.
3. Class.....: Ciliata.....: Cilia present throughout life.
4. Subclass.....: Euciliata.....: Cytopharynx contractile, vacuole, mega- and micronucleus present.
5. Order.....: Holotricha.....: Equal Cilia.
6. Suborder.....: Trichostornata....: Mouth leads in cytopharynx.
7. Family..... : Paramecidae....: Oral groove present.
8. Genus..... : *Paramecium*

Salient features

1. Paramecium (Fig.4) is commonly called as **slipper animalcule**, being microscopic, elongated slipper-shaped, cigar-shaped or spindle shaped.
2. Paramecium is the most familiar and extensively studied protozoan genus.
3. Anterior end is bluntly rounded, while posterior end is pointed.
4. *P. caudatian* measures 80-350 microns, while *P. aurelia* 170-290 microns.

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

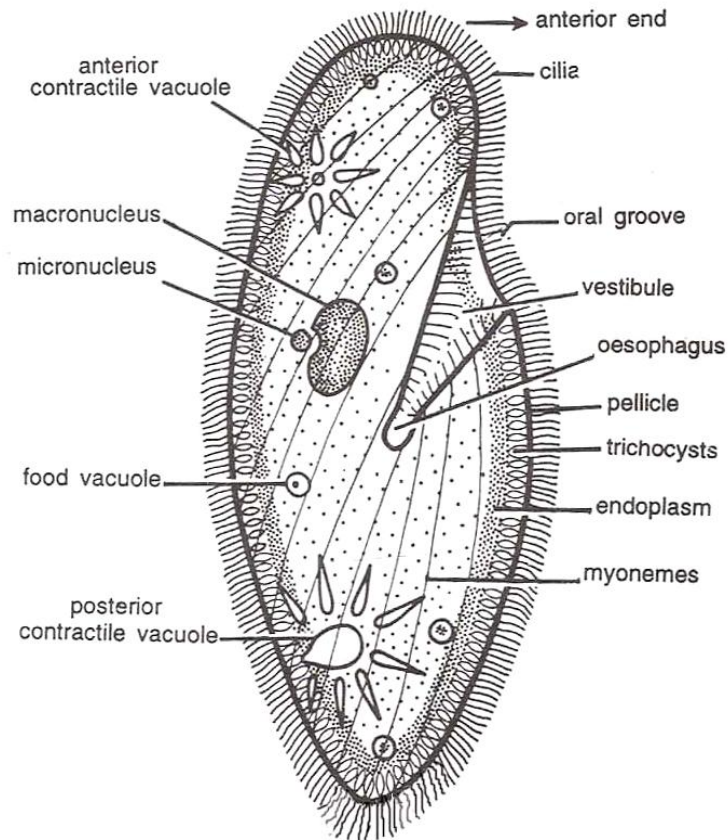


Fig. 4. *Paramecium*.

6. Cilia covers the entire animal. They are hair-like projections of uniform length, except at posterior end where they are longer and at cytopharynx where they form undulating membrane.
7. Infraciliary system consists of basal bodies and kinetodesmata.
8. Cytoplasm contains ecto- and endoplasm. Ectoplasm has myonemes and rod-shaped trichocysts. Endoplasm contains food vacuoles, granules, meganucleus, micronucleus, anterior contractile, posterior contractile vacuole, fat and glycogen.
9. Reproduction is by binary fission, conjugation, endomixis, hemixis and automixis.
10. Locomotion is ciliary. Nutrition is holozoic and it shows response to light and temperature, etc.
11. Habit and habitat: *Paramecium* is best known ciliate, found in fresh-water ponds, rivers, lakes, ditches, streams and pools, etc.
12. Distribution: It has cosmopolitan distribution.

5. PODAPHRYA

Classification

1. Phylum.....: Protozoa.....: Microscopic and acellular.
2. Sub-phylum.: Ciliophora...: Locomotory organs cilia.
3. Class.....: Suctorina: Body rounded, oval or conical; cilia absent in adult; mouth absent; single oval macronucleus and one to several micronuclei; contractile vacuole one to several.
4. Genus..... : *Podaphrya*
5. Species.....: *collini*.

Salient features

1. The body of *Podophrya* (Fig. 5) is globular, measures about 40-50 microns in diameter, provided with a stalk having a basal disc for the attachment with the substratum.
2. The body gives rise to 30-60 knobbed tentacles, giving a pin-cushion like appearance to the animal.
3. Tentacles and the surface of the body are covered by gelatinous sheath.
4. Endoplasm contains a large macronucleus in centre with 3-12 small micronuclei and a contractile vacuole.
5. Cytostome is absent.
6. Nutrition is holozoic. The food consists chiefly of *Paramecia* and other ciliates.
7. Habit and habitat: *Podophrya* is found in ponds having rich vegetation under decomposition.
8. Distribution: *Podophrya* is cosmopolitan.

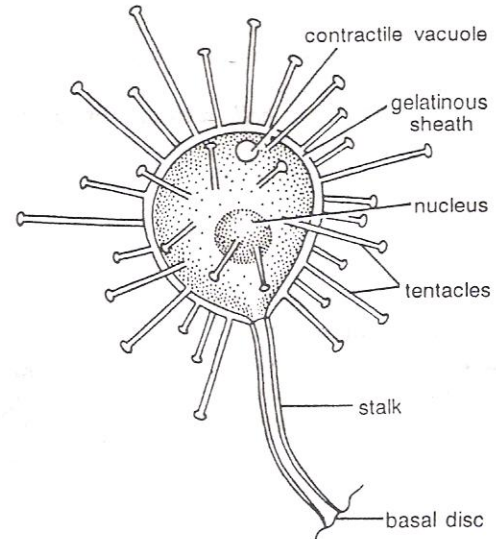


Fig. 5. *Podophrya*.

PHYLUM – PORIFERA

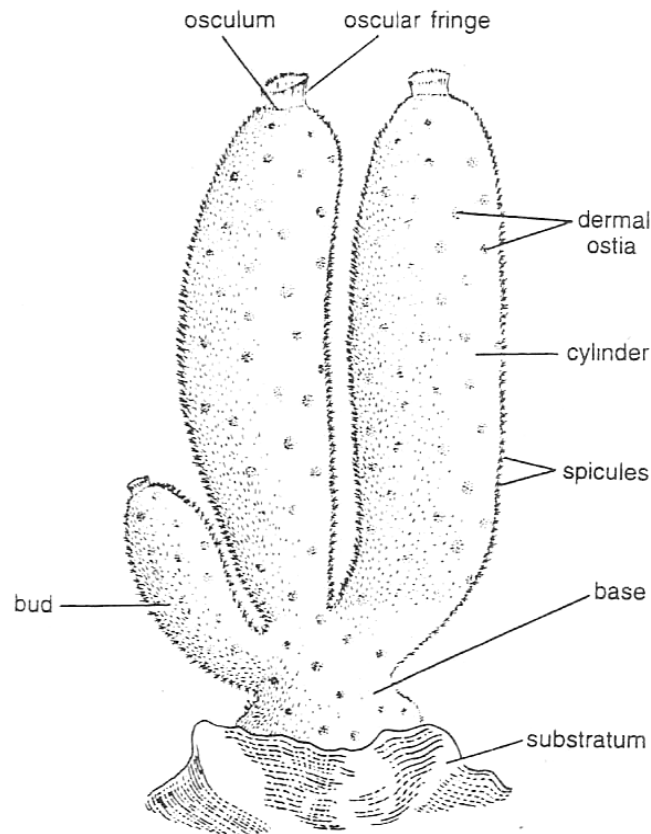
1. SYCON

Classification

1. Phylum.....: Porifera: Pore bearing; asymmetrical or symmetrical; cellular grade.
2. Class.....: Calcarea: Skeleton consists of calcareous spicules.
3. Order.....: Heterocoela: Canal system syconoid type
4. Genus.....: *Sycon* or *Sypha*

Salient features

1. The body of *Sycon* (Fig. 6) is slender vase-shaped cylinder measuring about 5-6 mm in length and 5-6 mm in diameter.
2. Each cylinder bulges in the middle and opens to the exterior by an osculum.
3. The osculum is encircled by a fringe of large giant monaxon spicules.
4. The surface of the body is perforated by numerous pores, the ostia or incurrent pores.
5. The body wall consists of an outer dermal epithelium and an inner flattened epithelium which lines the spongocoel separated by a middle layer of mesenchyme.
6. The choanocytes or flagellated cells are restricted only to the radial canals.
7. Skeleton comprises calcareous base spicules of monaxon, triaxon and tetraxon bud type.
8. Canal system syconoid type. Water enters the body by ostia and passes into the radial canal reaches into the spongocoel radial canals by prosopyles. The water from through the apopyles and passes out by an osculum.
9. Nutrition, respiration and excretion are performed by canal system.
10. Reproduction both asexual and sexual. Asexual by budding and regeneration and sexual by ova and sperms.
11. Habit and habitat: *Sycon* or *Scypha* is a small, solitary or colonial marine sponge, found attached to the rocks and other substrata in shallow waters.
12. Distribution: *Scypha* is widely distributed and found in abundance near North Atlantic shores.

Fig. 6. *Sycon*.

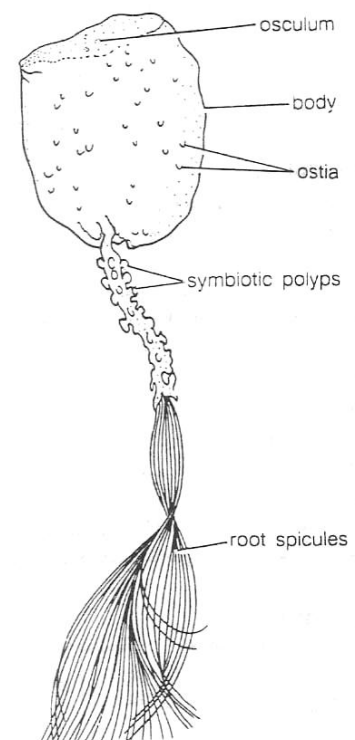
2. HYOLONEMA

Classification

1. Phylum.....: Porifera.....: Pore bearing; asymmetrical or symmetrical; cellular cellular grade.
2. Class.....: Hexactinellida: Skeleton consists of triaxon, six rayed siliceous spicules.
3. Order.....: Heterocoela.: Spicules are amphidiscs. No hexasters.
4. Genus.....: *Hyalonema*.

Salient features

1. *Hyalonema* (Fig. 7) is popularly known as **glass rope sponge**.
2. It has a rounded or oval body with a simple spirally twisted root tuft.
3. The spicules are often fused to form a lattice-like skeleton, giving the sponge a glass-like appearance.
4. The spicules of root tuft continue through the sponge body as an axis or columella and projecting above as gastral cone.
5. The root spicules are compact, stalk-like, elongated, twisted and giving the appearance of a rope.
6. The middle part of the columella commonly has symbiotic polyps (*Epizoanthus*) attached to it.
6. When the upper surface of the sponge body is depressed, the resulting cavity may be termed a spongocoel since the excurrent canals open into it, but where surface is extended into a gastral cone by upward projection of columella, no spongocoel exists.

Fig. 7. *Hyalonema*.

7. Skeleton consists mainly of small amphidiscs. Extending from all over the surface are small, branching, five-rayed spicules. These resemble small Christmas trees on cross-shaped bases.
8. Habit and habitat: *Hyalonema* is marine, found in 10-15 metres deep water of sea.
9. Distribution. *Hyalonema* is found along the New England coast.

3. SPONGILLA

Classification

1. Phylum.....: Porifera: Pore bearing; asymmetrical or symmetrical; cellular cellular grade.
2. Class.....: Demospongiae...: Skeleton consists of siliceous spicules or spongia fibres or a combination of siliceous spicules and spongin fibres or absent; canal system leuconoid type.
3. Subclass.....: Monaxonida.....: Skeleton consists of monaxon spicules with or without spongin; spicules distinguished into megascleres and microscleres.
4. Order.....: Haplosderina.....: Monaxon megascleres are diactinal; microscleres absent; spongin fibres generally present.
5. Genus..... : *Spongilla*

Salient features

1. *Spongilla* (Fig. 8) is probably the best known of **freshwater sponges**.
2. The colony of *Spongilla* is profusely branched exhibiting various shades of green colour due to the presence of *Zoochlorellae* a green alga in the tissues.
3. The body wall consists of very thin dermal membrane provided with dermal pores or ostia and several oscula.
4. The canal system is rhagon type.
5. Skeleton consists of siliceous spicules in the form of network of smooth or spiny large and small oxeas embedded in the spongin.
6. Reproduction both asexual and sexual.
Asexual reproduction by gemmules.
7. Sexual reproduction by way of unusual free swimming larva which is characteristic of *Spongilla*.
8. Gemmules are protected by the amphidisc spicules.
9. Habit and habitat: *Spongilla* is a colonial sponge. It is abundantly found in ponds, lakes and slow stream growing on submerged sticks and plants.

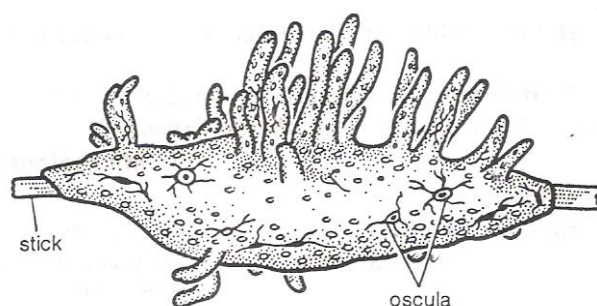


Fig. 8. *Spongilla*.

PHYLUM: COELENTERATA

1. HYDRA

Classification :

1. Phylum..... Coelenterata.....Multicellular; tissue grade; diploblastic and acoelomate.
2. Class..... Hydrozoa.....Hydroids bearing medusa with true velum.
3. Order..... .. HydroidaPolypoid generation well developed.
4. Suborder..... Anthomedusae..Hydrotheca and gonotheca absent.

5. Genus.....*Hydra*

Salient features

1. *Hydra* (Fig. 9) is elongated, cylindrical and like an elastic tube measures 1-3 cm in length.
2. Proximal end of the body is known as basal disc or foot, used for attachment with some objects or for locomotion.
3. The free distal end or oral end bears the mouth situated on a conical elevation called the hypostome.
4. The hypostome is encircled by 6-10 tentacles. The tentacles are hollow, slender finger like projections provided with nematocysts.
5. Body wall is diploblastic consists of an outer e ctoderm and an inner endoderm separated by mesogloea.
6. Body wall encloses a digestive cavity or gastrovascular cavity which extends into the tentacles.
7. Lateral buds maybe present on the sides of the body which may give rise to new individuals by asexual reproduction.
8. Gonads appear as buds on the sides of the body. Testes lie near the oral end, while the ovaries near the base.
9. Reproduction both asexual and sexual. Asexual by means of budding. Sexual by the fusion of gametes.
10. Habit and habitat. *Hydra* is a freshwater solitary animal. It is found attached to some objects in ponds, streams and lakes all over the world.
11. Distribution. Cosmopolitan, but most common in India, Canada and U.S.A. Fig.

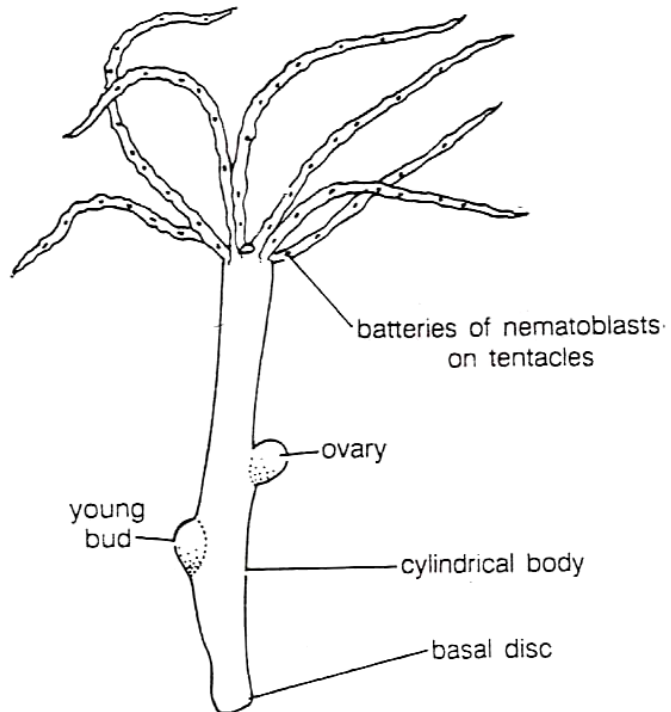


Fig. 9. *Hydra*.

2. PHYSALIA

Classification

1. Phylum.....: Coelenterata...: Multicellular; tissue grade; diploblastic and acoelomate.
2. Class.....: Hydrozoa.....: Hydroids bearing medusa with true velum.
3. Order.....: Siphonophora : Polymorphic, free-swimming or floating colonial; colony consists of several types of polypoid and medusoid individuals; polyps without tentacles; medusa always incomplete and rarely freed
4. Suborder.....: Physophorida.: Upper end of the colony bears a float or pneumatophore.
5. Genus..... : *Physalia*

Salient features

1. *Physalia* (Fig. 10) is a colonial hydroid commonly known as **Portuguese man of war**.
2. Colony has a large pneumatophore or float which is brightly coloured as blue or purple.
3. The float or pneumatophore is bladder-like, elongated pointed at both the ends, 6 to 12 cm long. The upper surface of the float is produced into a crest or sail.

4. A gas gland present inside the float secretes a gas of a composition similar to air. This helps the animal in floating over the surface of water.
5. The swimming bells or nectocalyces are absent.
6. Colony exhibits remarkable polymorphism and the phenomenon of division of labour.
7. Beneath the float are hanging down the three types of zooids and tentacles.
 - (i) Gastrozooids are simple polyps with mouth but without tentacles. These are nutritive in function.
 - (ii) Dactylozooids are of two types, large as well as small. These are provided with tentacles bearing numerous nematocysts. These catch the fishes and other prey.
 - (iii) Gonozooids are branching blastostyles bear clusters of medusae. Male medusae are reduced and remain attached. Female medusae are free-swimming.
8. Tentacles are large and bear stinging batteries or nematocysts to kill the large fishes and prey.
9. Habit and habitat: *Physalia* is a marine, colonial, swimming or floating pelagic animal.
10. Distribution: *Physalia* is found in tropical and subtropical seas.

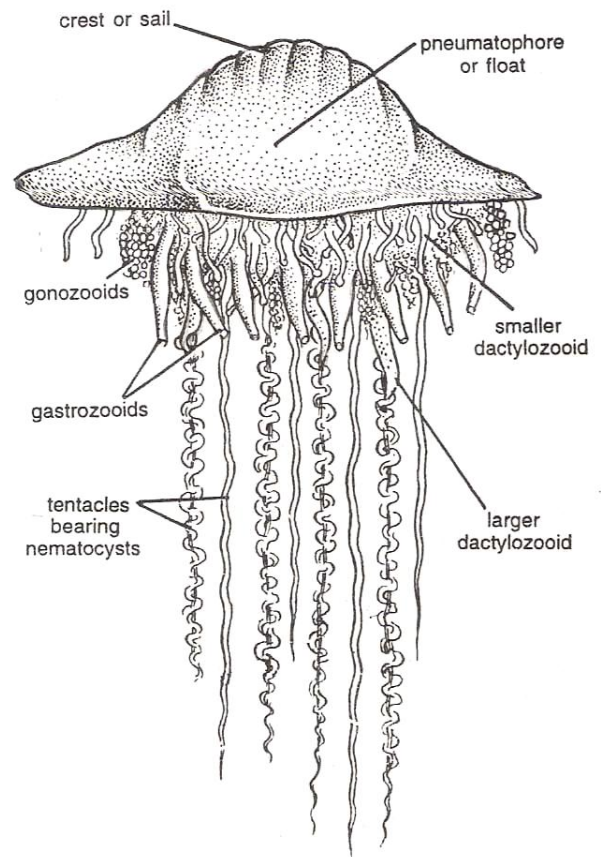


Fig. 10. *Physalia*.

3. AURELIA (JELLY-FISH)

Classification

1. Phylum.....: Coelenterata....: Multicellular; tissue grade; diploblastic and acoelomate.
2. Class.....: Scyphozoa ..: Jelly-fishes or true medusae: exclusively marine ; medusa hinge umbrella-shaped without true velum; free-swimming or attached by an aboral stalk; marginal sense organs tentaculocysts ; polypoid generation absent or small polyp; gastrovascular system without stomodaeum, with gastric filaments; mesogloea cellular; gonads endodermal.
3. Order....: Semaestomeae: Umbrella flat, saucer or bowl-shaped, mouth square, margin of umbrella fringed with hollow tentacles and eight or more tentaculocysts; gastric pouches and filaments are absent.
4. Genus.....: *Aurelia*

Salient features

1. *Aurelia* (Fig. 11) is the commonest jelly-fish.
2. The medusa is bowl or saucer-shaped having tetramerous radial symmetry, measuring about 7.5 -10 cm in diameter.
3. The medusa or umbrella has a slightly convex upper surface known as umbrellar surface and a lower concave, the subumbrellar surface.
4. The margin of the umbrella is divided into eight lobes or lappets by notches. Each notch contains a tentaculocyst or rhopalium enclosed by a pair of marginal lappets.
5. Numerous short, hollow tentacles are present all round along the margin of the umbrella and are known as marginal tentacles.

6. The mouth is four cornered situated on the short manubrium, which hangs down in the centre of subumbrellar surface.
7. Each corner of the mouth is drawn out into a long frilled, tapering process, the oral arm. The four oral arms lie along the four per radii.
8. Mouth leads into short gullet which opens into stomach. The stomach gives rise to four inter-radial gastric pouches.
9. Each gastric pouch gives off branched or unbranched radial canals which open into a circular canal situated along the margin of the umbrella.
10. On the subumbrellar surface lying between the oral arms are four rounded apertures leading into shallow pouches called sub genital pits.
11. It is unisexual. The four gonads (testes or ovaries) lie on the floor of the gastric pouches. Gonads are horse-shoe-shaped and reddish in colour.
12. The gametes (sperms or ova) are discharged into the stomach and passed out through the mouth.
13. The fertilized ovum develops into a free-swimming planula, and finally into fixed scyphistoma which gives rise to adult by transverse fission.
14. Habit and habitat: *Aurelia* is a solitary, marine jelly-fish.
15. Distribution: *Aurelia* is found in coastal waters of all oceans of the world.

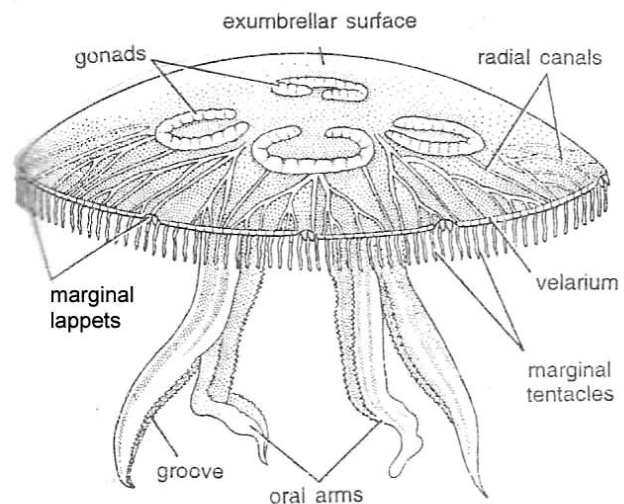


Fig. 11. *Aurelia*.

4. SEA ANEMONE

Classification

1. Phylum.....: Coelenterata...: Multicellular; tissue grade; diploblastic and acoelomate.
2. Class.....: Anthozoa ...: Exclusively marine and polypoid, medusoid stage absent; hexamerous, octomerous or polymerous; stomodaeum with one or more siphonoglyphs; gastrovascular cavity divided by complete or incomplete mesenteries.
3. Subclass...Hexacorallia.....: Polyps bear numerous tentacles and mesenteries arranged in the multiple of five and six but never eight; siphonoglyphs; polyps usually monomorphic.
4. Order.....: Actiniaria..... : Body cylindrical divided into oral disc, column and base, aboral end with a pedal disc; tentacles and mesenteries numerous and often arranged in the multiple of six; siphonoglyph one or more, skeleton absent.
5. Genus.....: *Metridium*.

Salient features

1. ***Metridium*** is commonly known as sea anemone (Fig. 12).
2. Body is short, cylindrical and radially symmetrical, divisible into three distinct regions, pedal disc, column and oral disc.
3. Pedal disc is muscular broad base or foot by which it is attached to the substratum.

4. Column is differentiated into two portions a distal thin-walled short capitulum and a proximal thick-walled scapus by a groove and collar.
5. The wall of the scapus is perforated by small openings called cinclides.
6. Oral disc is lobed and flat having a slit-like mouth in the centre which is surrounded by numerous short, hollow marginal tentacles arranged in a number of circles.
7. Mouth leads into a short gullet which finally opens into the gastrovascular cavity. Gullet or stomodaeum is provided usually with one or two siphonoglyphs. Gastrovascular cavity is divided into compartments usually by six pairs of mesenteries.
8. Sexes are separate. Gonads are borne on the mesenteries.
9. Asexual reproduction by fragmentation and budding.
10. Habit and habitat: *Metridium* is a large sessile, brightly coloured, solitary, flower-like form. It is a marine form, found attached to the rocks, piles of wharves and solid objects from tide pools to a depth of 90 fathoms.

Distribution: *Metridium* is found on the Atlantic coast, New Jersey to Labrador, Pacific coast and Euro

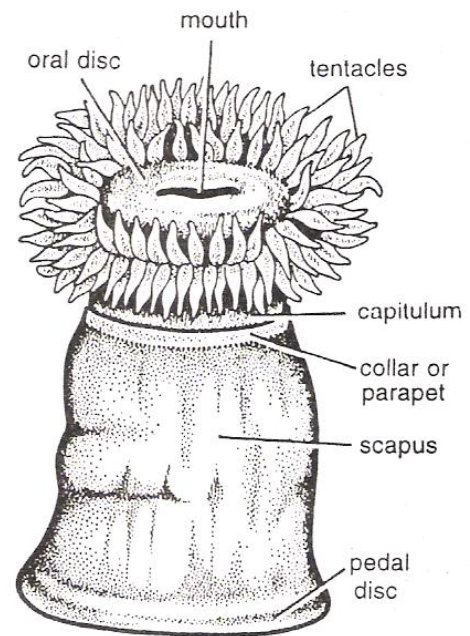


Fig. 12. Sea anemone.

PHYLUM : PLATYHELMINTHES

1. PLANARIA

Classification

1. Phylum.....: Platyhelminthes...: Acoelomate; organ grade and flatworms.
2. Class.....: Turbellaria.....: Epidermis provided with rhabdoids; adhesive organs present.
3. Order.....: Tricladida: Pharynx plicate usually intestine backwards, intestine with three branches; gonopore single
4. Subclass Paludicola.....: Eyes two to many or absent, bursa present.
5. Genus.....: *Planaria* (*Dugesia*)

Salient features

11. Body of *Planaria* or *Dugesia* is elongated, bilaterally symmetrical and dorsoventrally flattened (Fig. 13).
1. They are 2-15 mm in length and brown to black colour.
2. Head is triangular with conspicuous auricles and two eyes.
3. Digestive system consists of mouth, proboscis, pharynx and branched intestine.
4. 5. Mouth is situated on the ventral surface behind the middle of the worm.
5. Proboscis is enclosed in the proboscis sheath.
6. Pharynx plicate directed backwards.
8. Intestine forks into three diverticulated branches, one anterior and two posterior.

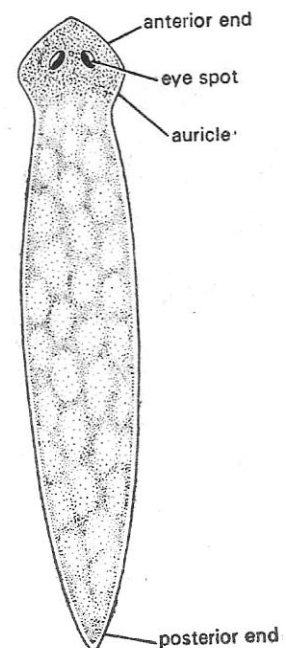


Fig. 13. *Planaria*.

7. Genital pore is situated a little posterior to the mouth.
8. Reproduction sexual, asexual and by regeneration.
9. Planarians are extensively used for the experimental purpose, e.g., regeneration and grafting, etc.
10. Habit and habitat: *Planaria (Dugesia)* is a freshwater triclad. It is found in the freshwater streams, springs, ponds and lakes under the stones throughout the temperate zones.
11. Distribution: *Planaria* is found in India, Myanmar, U.K., U.S.A. and U.S.S.R.

2. FASCIOLA

Classification

1. Phylum.....: Platyhelminthes...: Acoelomate; organ grade and flatworms.
2. Class.....: Trematoda: Ecto or endoparasitic; body wall without epidermis and well developed suckers present.
3. Order.....: Digenea: Endoparasitic; mostly with two suckers without hooks.
5. Genus.....: *Fasciola*
6. Species....: *hepatica*.

Salient features

1. *Fasciola* (Fig. 14) is commonly known as **liver fluke**.
2. Body is leaf-like, dorso-ventrally flattened measures 25-30 mm in length and 4-5 mm in breadth.
3. Anterior end is small and conical, while the posterior end is large more rounded in front than behind.
4. An oral sucker is situated apically and a larger highly muscular ventral sucker (acetabulum) is located a little posterior to the oral sucker.
5. Mouth is situated at the anterior end and is surrounded by the oral sucker.
6. Digestive system is simple; pharynx is muscular, oesophagus short and branched and diverticulated intestine.
7. Between the oral and ventral sucker is a median genital pore through which eggs pass to the exterior.
8. Excretory pore lies at the extreme posterior end of the body.
9. Hermaphroditic. Male system consists of testes, vasa deferentia, seminal vesicle, ejaculatory duct and penis, while "female system comprises ovary, uterus and vitelline glands.
10. Life-cycle is complicated includes an intermediate host, *Lymnaea*, a mollusc.
11. Liver-fluke causes a disease known as **liver rot**.
12. Habit and habitat: *Fasciola hepatica* is found as an endoparasite in the bile ducts of liver of sheep.
13. Distribution: *Fasciola hepatica* is cosmopolitan in distribution throughout sheep-raising areas. In U.S.A. and India it is endemic.

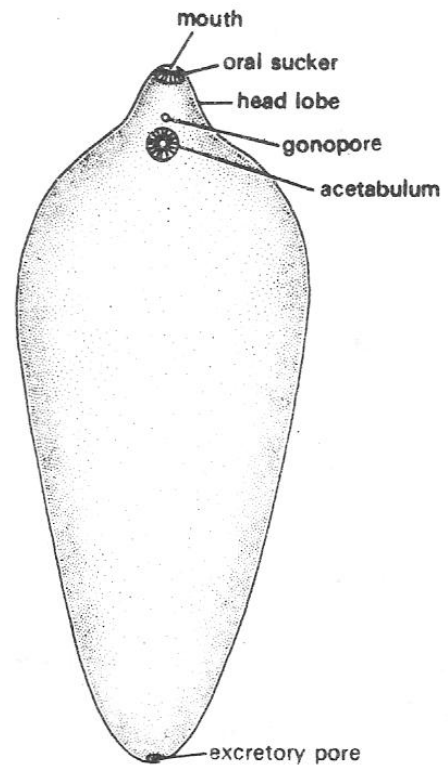


Fig. 14. *Fasciola*.

3. TAENIA (TAPEWORM)

Classification

1. Phylum.....: Platyhelminthes...: Acoelomate; organ grade and flatworms.
2. Class.....: Cestoda... : Endoparasitic in the intestines of vertebrates, Body divided into few to many segments (Proglottids); Anterior end with hooks and suckers.
3. Subclass.....: Eucestoda: Body elongated and ribbon-like, Anterior end bears an extended scolex, each proglottid with more than one set of reproductive organ.
4. Order.....: Taenioidea.....: Endoparasitic in the intestines of birds and mammals; scolex with four suckers often with an apical rostellum armed with hooks; yolk gland single and compact.
5. Genus.....: *Taenia*
6. Species.....: *solium*

Salient features

1. *Taenia* (Fig. 15) is commonly known as tapeworm.
2. Body consists of scolex or head, neck and strobila or body segments.
3. Scolex is smaller than the head of a pin, about 1 mm in diameter. It is the organ of attachment, bears four suckers and a rostellum which has a double circlet of hooks about 28 to 32 in number.
4. Behind the scolex is a thin unsegmented neck.
5. Strobila or body consists of large number of segments about 800 or more in number. Each segment is termed a **proglottid**.
6. Each proglottid contains a set of male and female reproductive organs, a part of excretory and nervous system and a lateral genital opening.
7. Life-cycle is complicated involves an intermediate host, pig.
8. The intermediate host of *Taenia solium* is pig in which infective cysticercus larva or the bladder worm is found encysted.
9. Pig is infected by bladder worm after eating contaminated human faeces. Man in turn gets infection by consuming measly pork.
10. Habit and habitat: *Taenia solium* is commonly found in the intestine of man in places where pork is eaten as food.
11. Distribution: *Taenia solium* is cosmopolitan in distribution.

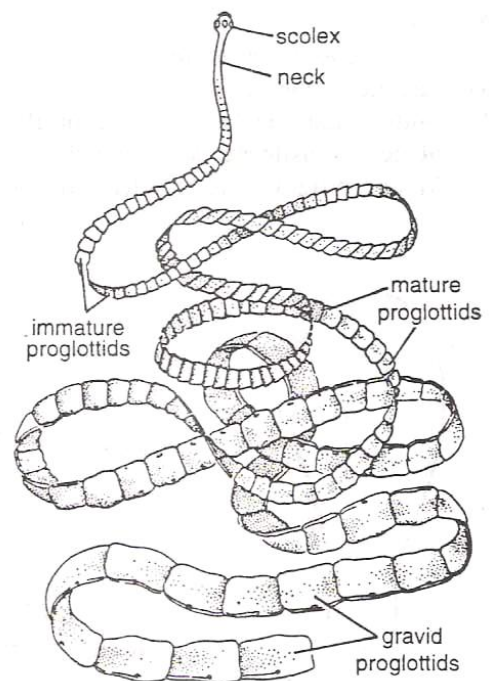


Fig. 15. *Taenia*.

SCOLEX OF TAENIA

Classification

1. Phylum.....: Platyhelminthes...: Acoelomate; organ grade and flatworms.
2. Class.....: Cestoda... : Endoparasitic in the intestines of vertebrates, Body divided into few to many segments (Proglottids); Anterior end with hooks and suckers.

3. Subclass.....: Eucestoda: Body elongated and ribbon-like, Anterior end bears an extended scolex, each proglottid with more than one set of reproductive organ.
4. Order.....: Taenioidea.....: Endoparasitic in the intestines of birds and mammals; scolex with four suckers often with an apical rostellum armed with hooks; yolk gland single and compact.
5. Genus.....: *Taenia*
6. Species.....: *solium*.

Salient features

1. Scolex (Fig. 16) is the cephalic portion of the body.
2. It is like, smaller than the head of a pin measuring about 1 mm in diameter.
3. It is the organ of attachment and also contains the brain and nephridial canals,
4. It is roughly quadrate in structure and has two distinct parts, the proximal and distal
5. The proximal part bears four adhesive suckers.
6. The distal part contains a small retractile prominence in the middle which is known as rostellum.
7. Rostellum has double circle of hooks about 28-32 in number, with larger hooks, measuring 110 to 140 and 160 to 180 microns respectively.
8. Internally scolex contains spongy mesenchyme having nephridial network and nerve ring.
9. Scolex lies buried in the intestinal mucosa of host's intestine. It destroys the tissues of host's intestine.

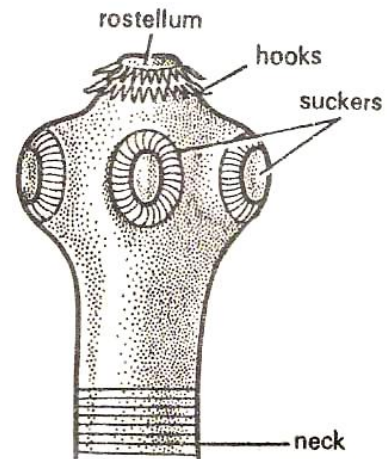


Fig.16. Scolex.

PHYLUM : ASCHELMINTHES

1. ASCARIS

Classification

1. Phylum.....: Aschelminthes..: Pseudocoelomate, unsegmented, unisexual nematodes.
2. Class.....: Nematoda: Round worms, alimentary canal straight.
3. Order.....: Ascarioidea: Buccal capsule absent, mouth with three lips.
5. Genus.....: *Ascaris*
6. Species...: *lumbricoides*.

Salient features

1. *Ascaris* (Fig. 17) is commonly known as round worm. It causes ascariasis in man especially in children.
2. Body is elongated and cylindrical and shows sexual dimorphism with separate male and female individuals. Males measure 15-30 cm in length and female 20-35 cm.
3. Tail end of male ventrally curved containing cloacal aperture, through which two equal isopscules project. Tail end of female bluntly pointed. However, anterior ends exhibit same structures in both male and female.
4. Mouth situated at the anterior extremity is guarded by one dorsal and two subventral lips.
5. Amphids are found in sub-ventral lips. Excretory pore lies at a distance of 2 mm from anterior end.

6. Two lateral, one mid-dorsal and one mid-ventral longitudinal chords extent from anterior to posterior end.
7. No intermediate host in life history. Infection occurs by eating raw and uncooked vegetables.
8. Pathogenesis: Causes haemorrhage, haemoptysis, insomnia, iappendicitis, peritonitis, tumour, ulcer, diarrhoea, eosinophilia and death. Ascaris infection also causes disturbances in the nucleic acid, sugar, protein and fat metabolism of the host.
9. Prevention: infection can be avoided by not eating raw and uncooked vegetables especially grown on human night soil fertilizer. Contaminated water should not be taken.
10. Identification: Since the animal has an unsegmented cylindrical body hence it is Ascaris.
11. Habit and habitat: Found in intestine of man and pig. Two specimens are called as *Ascaris lumbricoides* variety *humanis* (found in man) and *A. lumbricoides* variety *sum* (found in pig). Both forms are morphologically identical but two different physiological strains. Infective eggs from man's Ascaris will not develop into the pig and vice-versa.
12. Distribution : One of the most common nematodes found in ali parts of the world especially in India, China, Philippines, Korea and Pacific Islands.

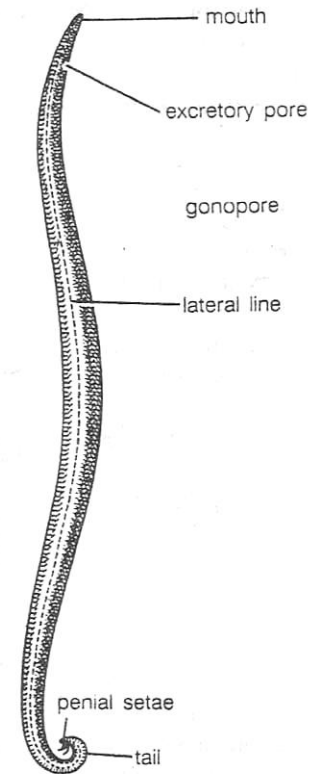


Fig. 17. Ascaris.

PHYLUM: ANNELIDA

1. NEREIS

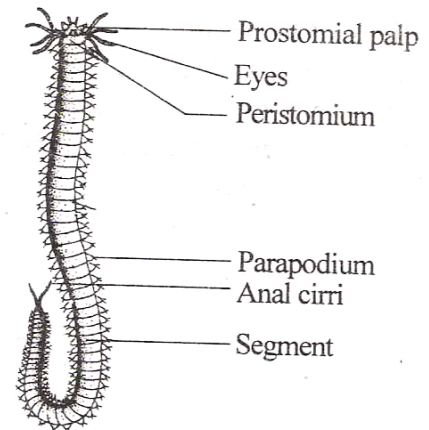
Classification

1. Phylum.....Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed, body somites.
2. Class....Polychaeta: Setae numerous; clitellum absent.
3. Order.....Errantia.....: Free-swimming often Pharynx, pelagic, protrusible and armed with chitinous jaws and hooks.
5. Genus.....*Nereis*
6. Species...: *diversicolor*.

Salient features

1. *Nereis* (Fig. 18) is commonly known as **rag worm** or **clam worm**.
2. The body is long, slender and dorso-ventrally flattened reaching a length of 5-30 cm.
3. The body consists of a distinct head followed by a number of similar segments.
4. Head consists of two parts: a roughly triangular anterior lobe, the prostomium and a posterior ring-like portion, the peristomium.
5. Prostomium bears a pair of terminal tentacles, dorsally two pairs of eyes and ventrally a pair of short two jointed palps.
6. Peristomium bears four slender cylindrical tentacles on each side and a transverse aperture, the mouth on the ventral surface.
7. Each segment of the body behind the peristomium bears a pair of lateral parapodia which are locomotory organs.

8. The last or anal segment is without parapodia but bears a pair of appendages known as anal cirri and a terminal anus.
9. At the bases of the parapodia on the ventral side in each segment are the paired openings of the nephridia, the nephridiopores.
10. Sexes separate, gonads develop temporarily during reproductive season.
11. Habit and habitat: *Nereis* is found in burrows in sand or mud of the sea shore at tide level.
12. Distribution: *Nereis* is cosmopolitan in distribution and found in coastal waters of Pacific and North Atlantic oceans, Europe and U.S.A.

Fig. 18. *Nereis*.

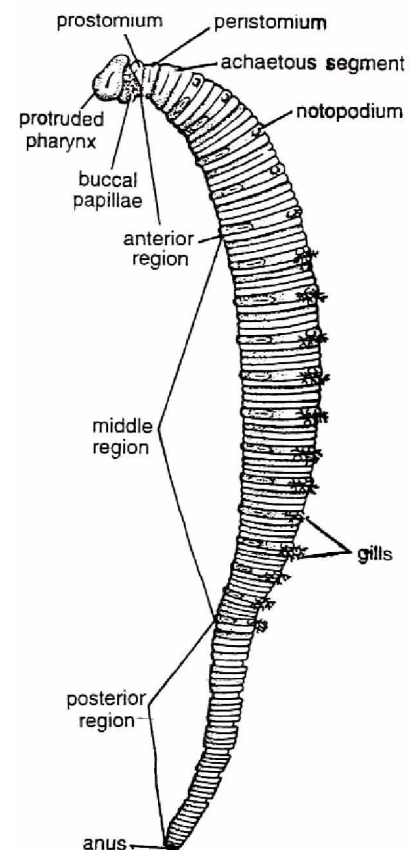
2. ARENICOLA

Classification

1. Phylum..Annelida.....Triploblastic, vermiform, setae coelomate, metamerically segmented appendages unjointed, body somites.
2. Class.....Polychaeta..Setae numerous; clitellum absent.
3. Order..... Sedentaria..Burrowing and tube-dwelling; pharynx non-protrusible devoid of jaws and teeth; feeding on plankton.
13. Genus..... *Arenicola*.

Salient features

1. *Arenicola* (Fig. 19) is commonly known as **lug-worm** or **lobe-worm**.
2. Body is stout, elongated, cylindrical worm-like measuring up to 20 to 25 cm in length and brownish or greenish in colour.
3. Body is divisible into three regions, anterior, middle and posterior.
4. Anterior region or prebranchial region comprises small trilobed prostomium with no eyes or tentacles, a peristomium, an achaetous segment and six segments bearing chaetae and parapodia.
5. Middle region or branchial region comprises thirteen segments bearing parapodia and branched gills.
6. Posterior region or post branchial region is much thinner comprising of variable number of segments devoid of parapodia, setae and gills.
7. Mouth lies ventral to the prostomium.
8. The buccal region and pharynx protrude as proboscis which is covered by chitinised papillae.
9. The anus opens through the last segment.
10. Nephridia are six pairs.
11. *Arenicola* is generally used as bait in fishing.
12. Habit and habitat. *Arenicola* is a marine worm, lives in U-shaped burrows of sand and mucus. It is found in Mediterranean and European shores.

Fig. 19. *Arenicola*.

3. CHAETOPTERUS

Classification

1. Phylum...Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed, body somites.
2. Class.....Polychaeta: Setae numerous; clitellum absent.
3. Order.....Sedentaria.....: Burrowing and tube-dwelling; pharynx non-protrusible devoid of jaws and teeth; feeding on plankton.

Genus... *Chaetopterus*.

Salient features

1. *Chaetopterus* (Fig. 20.) is commonly known as **paddle worm**.
2. *Chaetopterus* is usually 15 to 35 cm length.
3. The body is divisible into three distinct regions, anterior, middle and posterior
4. The anterior region is flat and bears usually nine pairs of simple parapodia which are large expanded notopodia, a small prostomium and a funnel-shaped peristomial collar with a pair of peristomial cirri and mouth.
5. The middle region comprises five segments, first anterior most is produced laterally into great wings directed forwards, next segment carries a pair of sucker and the rest three segments carry membranous folds so-called fans formed by the fusion of the notopodia.
6. The posterior region comprises thirty similar segments which are devoid of setae.
7. Mouth is wide and funnel-shaped.
8. The food comprises mainly small organisms, which are carried in by the currents of water set up by fans. It is a true filter feeder.
9. *Chaetopterus* is highly phosphorescent emits blue-green light.
10. Reproduction is usually asexual and by transverse fission.
11. *Chaetopterus* possesses great power of regeneration. The whole body can be regenerated from a single segment.
12. Habit and habitat. *Chaetopterus* is tubicolous, lives in parment-like U shaped tubes open at both the ends embedded in the mud encrusted with sand and debris.

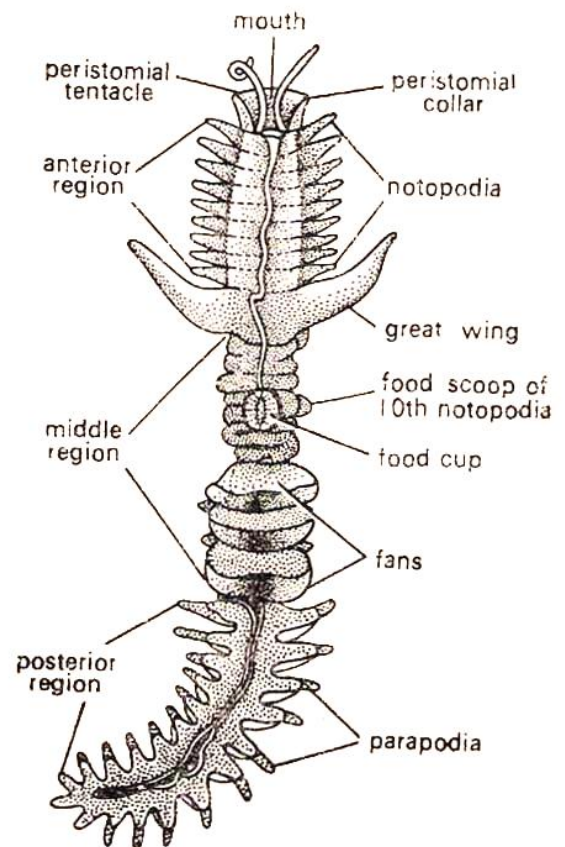


Fig. 20. *Chaetopterus*.

4. TOMOPTERIS

Classification

1. Phylum...Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed, body somites.
2. Class.....Polychaeta: Setae numerous; clitellum absent.
3. Order.....Errantia: Free-swimming often Pharynx, pelagic, protrusible and armed with chitinous jaws and hooks.
4. Genus....*Tomopteris*.

Salient features

1. Body of *Tomopteris* (Fig. 21) is colourless, transparent, having 18-20 segments.
2. Parapodia are large, bilobed and without setae.
3. Each lobe of parapodia bears a yellow rosette-shaped or spherical photogenic or light-producing organ.
4. The prostomium is hammer-shaped and bears a pair of eyes and two pairs of chaetigerous processes or tentacular cirri, of which the posterior are larger.
5. Mouth is devoid of proboscis or jaws.
6. Blood vascular system is absent.
7. Habit and habitat: *Tomopteris* is a marine pelagic polychaete.
8. Distribution: It is found in U.S.A. and European countries..

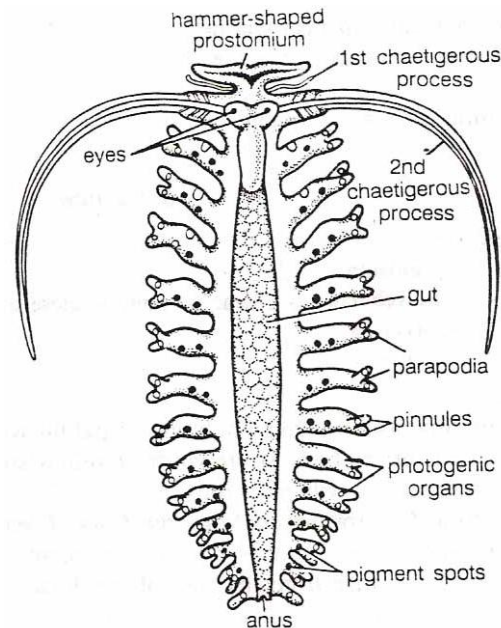


Fig. 21. *Tomopteris*.

5. EARTHWORM

Classification

1. Phylum.....:Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed.
2. Class.....:Oligochaeta: Parapodia absent; clitellum usually present; pharynx not eversible and without jaws
3. Order.....: Neoligochaeta: Terrestrial forms; setae arranged in lumbricine manner; gizzard well developed.
5. Genus.....: *Pheretima*
6. Species...: *posthuma*.

Salient features

1. ***Pheretima*** is commonly known as earthworm (Fig. 22).
2. Body is long, narrow and cylindrical measuring up to 150 mm in length, brown in colour.
3. Anterior end is pointed, while the posterior end is more or less blunt.
4. Body is divided by circular furrows into a series of 100 to 120 ring-like segments or metameres.
5. Each segment (except the first and last) is provided with setae arranged in a ring embedded in the setal sac,
6. Mouth is crescentic aperture situated at the anterior end ventral to the prostomium.
7. Clitellum, a circular band of glandular tissue, is present in 14th to 16th segments,
10. A pair of male genital pore is situated ventrally in the eighteenth segment.

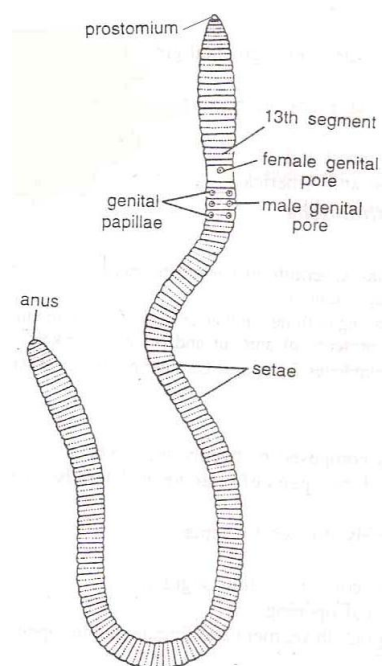


Fig. 22. Earthworm.

11. Two pairs of genital papillae lie on the ventral surface in the seventeenth and nineteenth segments.
12. Last segment bears the anus and is called anal segment.
13. Reproduction is usually sexual. Hermaphrodite. Female genital pore lies on the ventral surface in the fourteenth segment. Development takes place in cocoons.
15. Earthworm is economically very important as (i) it is used as a fishing bait, (ii) it has medicinal, educational and experimental value, (iii) it is beneficial to agriculture, (iv) it is cultured for preparing the organic fertilizers.
16. Charles Darwin has estimated that an acre of earth is inhabited by nearly 50,000 earthworms (a recent estimate suggests that their number may reach up to 25,00,000 per acre) which may bring more than 18 tons of deeper subsoil to the surface in one year.
17. Habit and habitat: *Pheretima* is found in moist soil. Earthworms usually live in the upper layers of slightly damp soils, lawns, gardens and up to the depth of 30-45 cm in burrows. The earthworms are nocturnal in habit.
18. Distribution: Found all over world.

6. LEECH

Classification

1. Phylum.....: Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed.
2. Class.....: Hirudinea: Body having definite number of segments; par apodia and seta-e are absent; presence of anterior and posterior suckers.
3. Order.....: Gnathobdellida : Anterior sucker with three jaws; proboscis absent

5. Genus.....: *Hirudinaria*

6. Species...: *granulose*.

Salient features

1. ***Hirudinaria*** is commonly known as **Indian cattle leech** (Fig. 23).
2. Body is elongated, dorso-ventrally flattened, measuring 30-35 cm in length with dorsal surface green and ventral surface orange yellow in colour.
3. Body is divided metamerically into 33 somites or segments. Each segment is further divided into rings or annuli.
4. Anterior and posterior suckers are well developed.
5. Anterior sucker is oval and ventral bearing triradiate mouth and is formed by the fusion of prostomium and few anterior segments.
6. Posterior sucker is circular and forms a highly muscular disc at the posterior end. It serves as a powerful organ of adhesion and locomotion.
7. Dorsal five segments bear five pairs of eyes.
8. Segmental receptor organs are four pairs on the dorsal surface and three pairs on the ventral surface.
9. Alimentary canal is straight tube. Anus is mid-dorsal placed on the 25th segment.

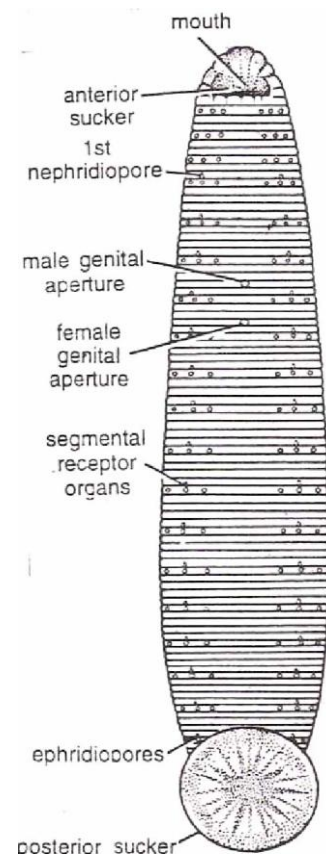


Fig. 23. Leech.

10. Nephridia are seventeen pairs segmentally arranged from six to twenty-two segments, opening by nephridiopores on the ventral surface.
11. Hermaphrodite. Male organs consist of testis sacs, vasa efferentia, vasa deferentia, epididymes, ejaculatory ducts and atrium. Female organs consist of ovisacs, ovaries, oviducts, common oviducts and vagina.
12. Male genital aperture is situated mid-ventrally in the second and third annuli of 10th segment and female genital aperture between second and third annuli of 11th segment. Sexual reproduction is common.
13. Habit and habitat: *Hirudinaria* is found in freshwater ponds, lakes, slow running streams and swamps. It is sanguivorous (blood sucking) in habit.
13. Distribution: *Hirudinaria* has cosmopolitan or worldwide distribution specially found in India and Myanmar.

7. PROTODRILUS

Classification

1. Phylum.....: Annelida.....: Triploblastic, vermiform, setae coelomate; metamerically segmented; appendages unjointed.
2. Class.....: Archannelida: Setae and parapodia are usually absent.
3. Genus.....: *Protodrilus*.

Salient features

- a. Body of *Protodrilus* (Fig. 24) is narrow, elongated and cylindrical.
2. Body segments are very indistinct and marked off by ciliated rings.
3. There is a median longitudinal ciliated groove on the ventral surface.
4. Parapodia, setae, cirri and gills are entirely absent.
5. Prostomium is small, bears a pair of large ciliated tentacles.
6. Peristomium is large and contains a pair of sensory ciliated pits.
7. Mouth is ventral situated in the first segment and anal opening is in the last segment.
8. Excretory system comprises paired segmental simple nephridia.
9. Hermaphrodite. Ovaries develop in the first seven segments and testes in some of the following segments.

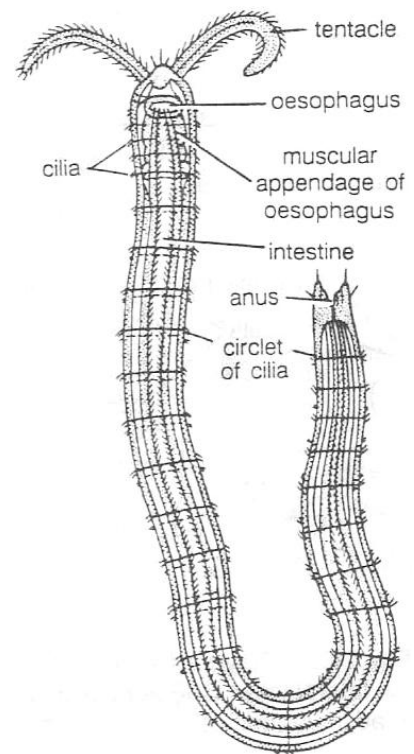


Fig. 24. *Protodrilus*.

10. Development is direct without metamorphosis and larval stages.
11. Habit and habitat: *Protodrilus* is a marine worm.
12. Distribution: *Protodrilus* is found in sand of an inland sea lake at Faro near Messina.

PHYLUM: ARTHROPODA

1. PRAWN (PENAEUS)

Classification

1. Phylum.....: Arthropoda.....: Triploblastic; metamerically segmented; jointed appendages, body cavity haemocoel.
2. Class.....: Crustacea: Thick exoskeleton; head fused with the thorax, to form cephalothorax.

3. Order.....: Decapoda.....: Carapace covers the entire thorax; thoracic appendages modified as three pairs of maxillipedes and five pairs of walking legs; statocyst present.

5. Genus.....: *Penaeus*

6. Species...: *monodon*.

Salient features

- a. *Penaeus* (Fig. 25) is the common marine prawn.
2. The general colour of the body is reddish grey, which becomes orange-pink on preservation of the animal.
3. Externally the body is covered by hard exoskeleton, which protects it.
4. The body is divided into an anterior rigid cephalothorax and a posterior flexible abdomen.
5. The cephalothorax is covered on all sides except ventrally by a strong shield or carapace produced anteriorly into a long serrated and pointed extension the rostrum.
6. The head exhibits no external sign of segmentation and no segmentation is visible dorsally or laterally on the thorax, but it can be traced on its ventral surface.
7. On each side of the carapace is a V-shaped cervical groove that represents the line of demarcation between the head and thorax.
8. Two prominent stalked eyes project on both sides of the rostrum.
9. The abdomen consists of six segments which are distinguished both dorsally and externally. It terminates with a small tail piece or telson which bears no appendages but a number of minute spines on both side and the anus opens on its ventral surface.
10. There are 19 pairs of appendages, one pair to each segment. There are 13 pairs of appendages in the cephalothorax and 6 pairs of appendages in the abdomen.
11. Sexes are separate. Development includes nauplius, protozoaea and zoea stages.
12. Habit and habitat: *Penaeus* is a marine and deep sea form found in the sea.
13. Distribution: *Penaeus* is found in American waters.

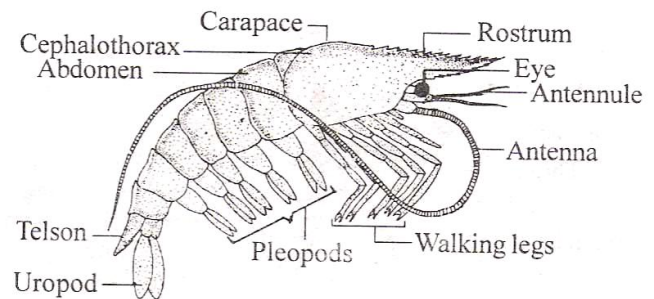


Fig. 25. *Penaeus*.

2. LIMULUS

Classification :

1. Phylum Arthropoda..... Triploblastic; metamerically segmented; jointed appendages; body cavity haemocoel
2. Class Arachnida..... Air-breathing; mostly terrestrial; body divisible into two regions, prosoma and opisthosoma; four pairs of legs; antennae and true jaws.
3. OrderXiphosura..... Prosoma convex, covered by a broad carapace bears six appendages; abdomen bears operculum; respiration by gills or book-gills.
4. Genus..... Limulus
5. Species..... *Polyphenus*.

Salient features

1. *Limulus* (Fig. 26) is commonly known as **king-crab**.
2. Body consists of anterior prosoma and a posterior opisthosoma terminating in a long spike-like telson.
3. Prosoma is covered by an unsegmented, semicircular, or horse-shoe-shaped carapace.

4. Prosoma is convex above with sloping sides and bears three longitudinal ridges one median and two lateral.
5. A pair of simple median eyes and a pair of lateral compound eyes are placed on the dorsal surface of prosoma.
6. Prosoma bears six pairs of appendages grouped round the mouth, the first pair of chelate chelicerae, four pairs of chelate legs and a last pair of non-chelate legs.
7. Opisthosoma is hexagonal, movably articulated with prosoma by a transverse hinge. It is composed by the fusion of an anterior large 6-segmented mesosoma and a posterior small 3-segmented metasoma.
8. The mesosoma is covered by a dorsal shield bearing a single median row of three prominent spines and two lateral rows of six small pits.
9. Each lateral side of mesosoma is serrated, produced into six short immovable spines and carries six short movable spines.
10. The metasoma is much reduced. It bears the mid-ventral anus.
11. Opisthosoma bears six pairs of appendages, first pair forms genital operculum-4 the remaining five pairs carry book-lungs.
12. Respiration by book-gills.
13. Excretion by coxal or brick red glands.
14. Larva trilobite.
15. The king-crab is of interest because it is a living member of a very ancient fossil group of animals, hence, sometimes called a living fossil.
16. Habit and habitat: *Limulus* is a marine form, found burrowing in the sand. They live comparatively in shallow waters along sandy and muddy shores of sheltered bays and estuaries. These sluggish creatures spend most of the time burrowing in sand or mud for worms, soft molluscs and small animals on which they feed.
17. Distribution: *Limulus polyphenus*, the American horse-shoe-crab, is restricted to North-western Atlantic coast, Gulf of Mexico and West Indies. All the other members are found along the South-east Asian Pacific coasts, from Japan and Korea through East Indies and Philippines.

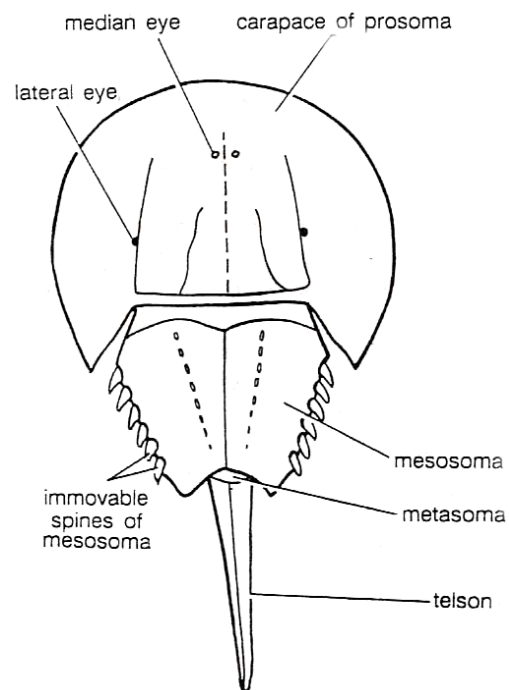


Fig. 26. *Limulus*.

3. PERIPATUS

Classification

1. Phylum.....: Arthropoda.....: Triploblastic; metamerically segmented; jointed appendages; body cavity haemocoel.
2. Class.....: Onychophora.: Body slender without external segmentation; appendages not jointed but lobe-like fleshy outgrowths of body; head bears a pair of antennae, a pair of jaws and a pair of simple eyes; slime-glands present; respiration by tracheae; excretion by paired nephridia; sexes separate; Onychophora is a connecting link between Annelida and Arthropoda.
3. Genus.....: *Peripatus*.

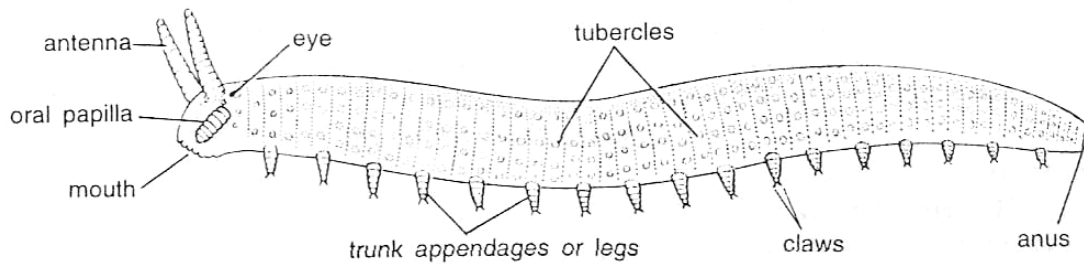


Fig. 27. *Peripatus*.

Salient features

1. Body of *Peripatus* (Fig. 27) is elongated, cylindrical caterpillar like without external segmentation, measuring 1.5 to 15.00 cm in length.
2. Outer covering or skin is velvety-like which is thrown into transverse wrinkles bearing numerous small papillae armed with spines.
3. Head is not distinct. The anterior end bears a pair of antennae, a pair of jaws, a pair of oral papillae and a pair of simple eyes.
4. Mouth is ventral on the anterior side.
5. Trunk bears a series of paired short stumpy appendages, which vary in number 14 to 43 pairs.
6. Anus lies at the posterior end of the body and behind the last pair of legs.
7. Slime glands open on the surface of oral papillae.
8. Respiration by tracheae.
9. Excretion by paired nephridia.
10. Sexes are separate and gonads are paired. The genital pore is situated on the ventral surface between the last pair of legs just in front of anus. Mostly viviparous, while some are oviparous.
11. Carnivorous. It feeds on insects, etc.
12. *Peripatus* is a connecting-link between Annelida and Arthropoda.
13. Habit and habitat: *Peripatus* is nocturnal, lives in crevices of rocks, under bark and stones and in other dark moist places and beneath fallen leaves. It is generally common to humid habitats.
14. Distribution: *Peripatus* has discontinuous distribution. The genus *Peripatus* is found restricted in the various places of the world such as South Africa, Australia, New Zealand, Tasmania, New Britain, South America and West Indies, Malaya, Equatorial Africa and Chile.

4. CENTIPEDE (SCOLOPENDRA)

Classification

1. Phylum.....: Arthropoda.....: Triploblastic; metamerically segmented; jointed appendages, body cavity haemocoel.
2. Class.....: Myriapoda.....: Terrestrial; air breathing; body elongated with numerous segments each bearing one or two pairs of legs.
- 4....Subclass...: Opisthogoneata.: Genital apertures situated at the posterior extremity of the body.
3. Order.....: Chilopoda: Numerous trunk segments, each bearing a single pair of poison jaws present.
5. Genus.....: *Scolopendra*.

Salient features

1. *Scolopendra* (Fig. 28) is commonly called centipede.

2. Body is elongated, dark greenish brown in colour, and dorsoventrally flattened with numerous segments. Body is divisible into head and trunk.

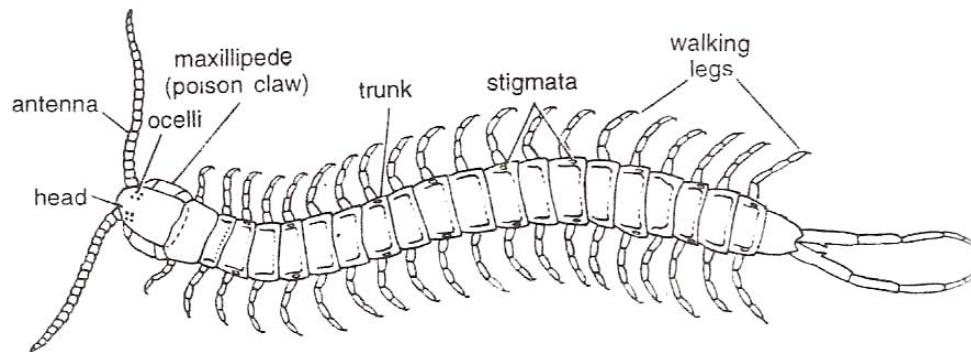


Fig. 28. centipede.

3. Head is distinct and bears a pair of antennae, a pair of mandibles and two pairs of maxillae.
4. Trunk segments are 22 in number and are nearly all alike.
5. Each trunk segment from 2-22 carries one pair of walking legs.
6. First pair of trunk appendages or maxillipeds bears a sharp claw connected with the poison gland.
7. Paired oval spiracles or stigmata lie on the pleura areas, above the leg bases, on segments 4, 6, 9, 11, 13, 15.
8. Sexes are separate. Genital opening situated in the last segment. Carnivorous, feeding on insects, spiders, worms slugs, etc
9. *Scolopendra* is harmful to mankind.
10. Habit and habitat. *Scolopendra* commonly occurs under stones, in rotten logs and in houses in damp places.
11. Distribution. *Scolopendra* is found in India and U.S.A.

5. MILLIPEDE

Classification

1. Phylum.....: Arthropoda.....: Triploblastic; metamerically segmented; jointed appendages, body haemocoel.
2. Class.....: Myriapoda.....: Terrestrial; air breathing; body elongated with numerous segments each bearing one or two pairs of legs.
3. Order.....: Diplopoda: Trunk bears numerous segments, each of which bears two pairs of legs.
4. Genus *Julus*.

Salient features

2. ***Julus*** is commonly called as **millipede** (Fig. 29) or **wire worm**.
3. Body is elongated and cylindrical, consisting of large number of segments; the colour may be yellowish brown or reddish chestnut.
4. Body is divisible into head, thorax and abdomen.
5. Head consists of five segments, thorax of four segments and abdomen of 20-100 segments.
6. Head bears a pair of short seven jointed antennae, a pair of mandibles and a pair of maxillae forming gnathochilarium.

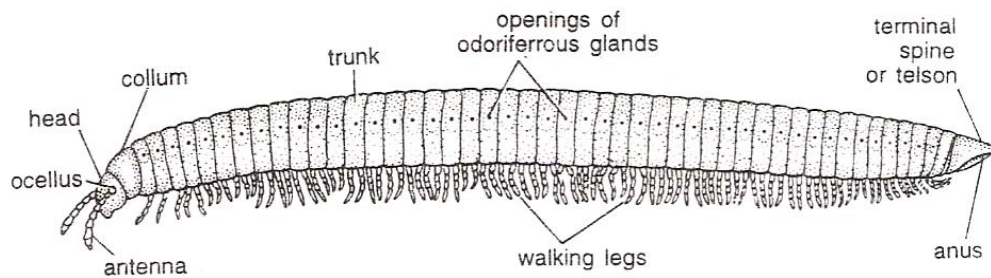


Fig. 29. Millipede.

7. Thoracic segments with one pair of legs in each, while abdominal segments bear two pairs of legs.
8. Poison claws are absent.
9. Stink glands present along the sides of the body, secreting noxious substance.
10. Sexes are separate. Gonopores mid-ventrally situated on 3rd abdominal segment.
11. Herbivorous in food habit.
12. Habit and habitat: *Julus* is found hidden usually in dark and damp places under stones or wood or in decaying leaves. The animal is found rolled up under stones.
13. Distribution: *Julus* is cosmopolitan in distribution; found in India; Europe and U.S.A.

6. GRASSHOPPER

Classification

1. Phylum.....: Arthropoda.....: Metamerically segmented; jointed appendages, body cavity haemocoel.
2. Class.....: Insecta.....: Air breathing, terrestrial or aquatic; body divisible into head, thorax and abdomen; three pair of legs; two pairs of wings.
3. Subclass.....: Pterygota.....: Wings usually present; abdomen devoid of appendages except genitalia and cerci; metamorphosis simple or complex.
4. Order.....: Orthoptera ...: Mouth parts strong biting and chewing; short or long, simple or segmented cerci.
5. Genus.....: *Melanopus* or *Poecillocerus*
6. Species.....: *picta*

Salient features

1. ***Melanopus*** is commonly called grasshopper (Fig. 30).
2. It is green in colour.
3. Body is divided into head, thorax and abdomen.
4. Head bears short antennae and well-developed compound eyes.
5. Mouth parts are biting and chewing type.
6. Thorax bears 3 pairs of legs and 2 pairs of wings.
7. Fore-wings are elongated, thick, leathery and straight called tegmina, while hind-wings are membranous.
8. Auditory organs are well-developed. It has stridulating organs also.
9. Abdomen is composed of 10 segments.
10. Sexes are separate. Female is provided with a long ovipositor. Eggs are laid in groups.
11. Nymphs are devoid of wings.
12. Grasshoppers are destructive to the crops and are harmful to mankind.

13. Habit and habitat: Grasshoppers are commonly found in the green fields, vegetation and trees.
14. Distribution: Grasshoppers are cosmopolitan and found throughout the world.

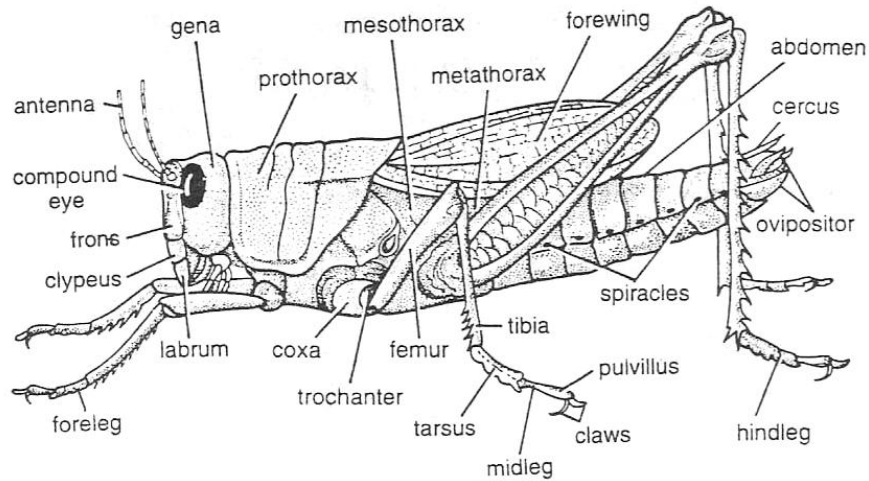


Fig. 30. Grasshopper.

7. SCORPION

Classification

1. Phylum.....: Arthropoda.....: Triploblastic; metamerically segmented; jointed appendages, body cavity haemocoel.
2. Class.....: Arachnida: Air breathing; mostly terrestrial; body divisible into two regions, prosoma and opisthosoma; four pairs of legs; antennae and true jaws.
3. Order.....: Scorpionidea.....: Body consists of prosoma, mesosoma and metasoma; a pair of comblike pectines occur on the second segment of the metasoma, respiration by four pairs of book-lungs.
4. Genus.....: *Palamnaeus*.

Salient features

1. ***Palamnaeus*** is commonly known as scorpion (Fig. 31).
2. Body is elongated, segmented and divisible into anterior prosoma (cephalothorax), middle mesosoma and posterior metasoma.
3. Prosoma is six segmented and bears a pair of median eyes, 2-5 pairs of lateral eyes and six pairs of appendages, *i. e.*, one pair of chelicerae, one pair of pedipalpi and four pairs of walking legs.
4. Mesosoma is seven segmented, sternum of first segment bears a genital opening, the sternum of second segment bears a pair of pectines and the sterna of third, fourth, fifth and sixth segments have each laterally a pair of stigmata, openings of book-lungs.
5. Metasoma composed of five segments without appendages.

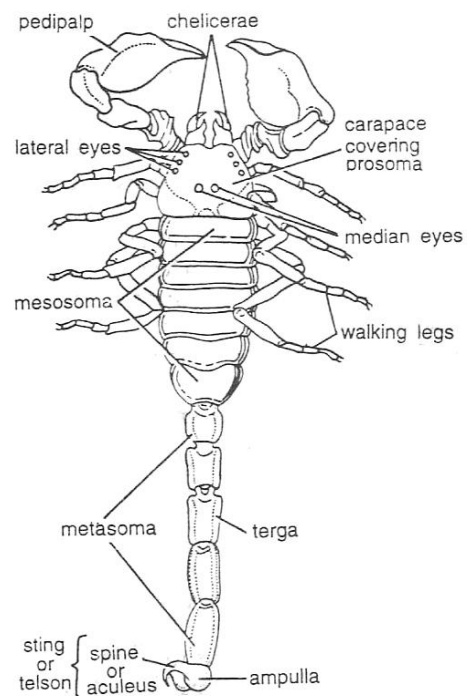


Fig. 31. Scorpion.

6. Last segment of metasoma bears a sting consisting of an ampulla and a spine.
7. Respiration by book-lungs.
8. Excretion by Malpighian tubules and coxal glands.
9. Sexes are separate gonads like a network.
10. Viviparous.
11. Scorpions are harmful to mankind; its sting causes extreme pain, fever and in some cases collapse.
12. Habit and habitat. *Palamnaeus* (Scorpion) is nocturnal animal, found under stones, bas of trees or in burrows in tropical and subtropical countries.
14. Distribution. Scorpions are inhabitants of tropical and subtropical countries of the world. They are fairly common in India, especially on the slopes of hills; and are most prevalent in coastal areas of Maharashtra, Assam, Rajasthan, Saurashtra and the Deccan. Most of India's 36 known species belong to genera *Palamnaeus* and *Buthus*.

PHYLUM: MOLLUSCA

1. CHAETODERMA

Classification

1. Phylum.....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Aplacophora.....: Worm-like, cylindrical, head, mantle, foot and nephridia are absent.
3. Order: Chaetodermatoidea..: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
4. Genus: Chaetoderma.

Salient features

1. Body of *Chaetoderma* (Fig. 32) is worm-like, bilaterally symmetrical and cylindrical.
2. Body is covered with several layers of calcareous spicules.
1. Shell, mantle, foot and nephridia are absent.
2. Mouth and anus are terminal and at opposite ends.
3. Mid-ventral longitudinal groove or pedal groove is absent.
4. Gills are reduced to a pair found in the buccal cavity.
5. Nervous system is well-developed having brain and ganglia. 8. Sexes are separate.
6. Habit and habitat: *Chaetoderma* is a marine form, found at great depths. It is carnivorous.
7. Distribution: *Chaetoderma* is found in Europe and U.S.A.

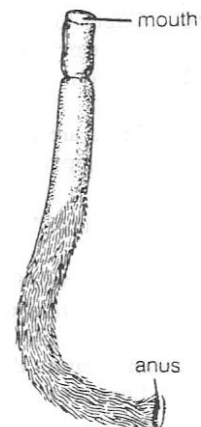


Fig. 32. *Chaetoderma*.

2. NEOPILINA

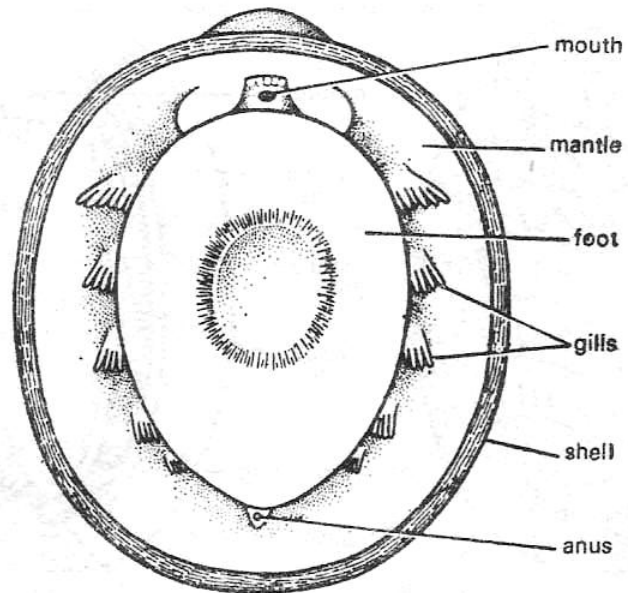
Classification

1. Phylum....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Monoplocophora.....: Single Shell.
3. Genus: *Neopilina*
4. Species...: *galathea*.

Salient features

1. *Neopilina* (Fig. 33) is one of very few living members of the Monoplacophora.
2. *Neopilina* is a deep sea mollusc of special interest, because it illustrates the primitive occurrence of metamerism in molluscs. *Neopilina* was discovered in 1952.

3. In *Neopilina* the single symmetrical shell is low, somewhat patelliform and exogastrically coiled. The largest specimen known is 37 mm long, 35 mm wide and 13 mm high.
4. *Neopilina* appears somewhat chiton-like from the ventral surface. The central circular foot is separated from the encircling mantle by a pallial groove which contains five pairs of lamellated gills with a ciliated epithelium.
5. The head is inconspicuous with two small pre-oral tentacles and two oral tentacle tufts around the mouth. Anterior to the mouth is a velar ridge with two flaps.
6. Unpaired anterior and posterior lips border the mouth. A feeding furrow extends between velar ridge and tentacle tufts. At the posterior end is the anus. Pharynx contains a radula. The stomach contains a crystalline style.
7. The muscular system is complex.
8. Six pairs of nephridia are located in the pallial fold.
9. Sexes are separate. The food of *Neopilina* consists of radiolarians and other materials of the bottom.

Fig. 33. *Neopilina*.

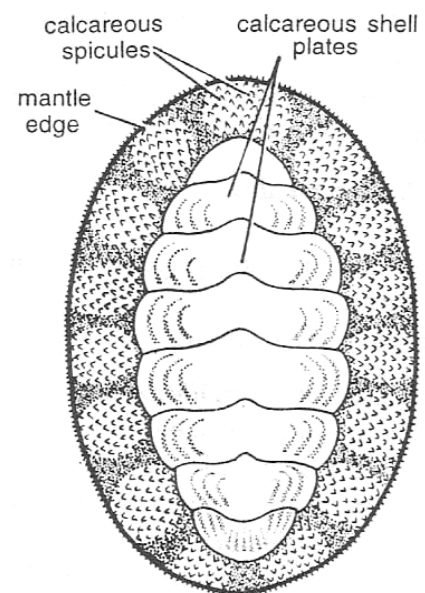
3. CHITON

Classification

1. Phylum....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Polyplacophora.....: Shell composed of a series of eight calcareous pieces; foot flat and ventral.
3. Order: Chitonina.....: Valves of the shell with insertion plates and teeth.
4. Genus: *Chiton*.

Salient features

1. Body of *Chiton* (Fig. 34) is elliptical, bilaterally symmetrical and dorsoventrally compressed and consists of shell, foot, mantle and the visceral mass. Mantle Shell is calcareous, side and composed of eight overlapping plates.
1. Head is small and indistinct. Eyes and tentacles are absent.
2. Foot is ventral, muscular with a flat sole extending along the whole length of the body. It serves for creeping and adhering to the substratum.
3. Mantle covers greater part of the body and partly covers the edges of the shell plates.
4. Mouth and anus are at opposite ends.
5. Numerous pairs of bipectinate ctenidia lying on either side of the body in the mantle groove.
6. Sexes are separate, gonad is single and median and gonoducts are paired.

Fig. 34. *Chiton*.

7. Development includes a trochophore larva.
8. Chitons are used as food and their shells are used for decoration.
9. Habit and habitat: *Chiton* is a sluggish, marine animal found attached to the rocks, empty shells and corals between tide marks. It is herbivorous feeding chiefly on algae.
10. Distribution: *Chiton* is found all over the world.

4. APLYSIA

Classification

1. Phylum....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Gastropoda.....: Asymmetrical spirally coiled shell, visceral mass spirally coiled exhibit torsion.
3. Subclass.: Opisthobranchia.....: Shell often reduced or wanting; operculum usually absent; single gill often replaced by secondary branchiae.
3. Order: Anaspeidea: Parapodial lobes well-developed; anterior end bears a pair of tentacles, a pair of rhinophores and a pair of eyes.
4. Genus: *Aplysia*.

Salient features

1. *Aplysia* (Fig. 35) is commonly known as **sea hare**.
2. The body is soft and lumpy with a thin flexible plate-like shell almost completely covered by mantle. The shell is internal.
3. Head bears two pairs of tentacles: the anterior being larger and ear-like, while the posterior pair is olfactory rhinophores, each bearing an eye at its base.
4. Mantle cavity opens on the right side with the ctenidium pointing backwards.
5. Visceral mass is raised into a prominent hump.
6. Foot is broad, muscular and ventral and bears a pair of lateral fleshy outgrowths, the parapodia which help in swimming.
7. Anus lies at the posterior end.
8. Hermaphrodite with a single generative duct and the single aperture.
9. In the wall of the mantle is a gland which secretes a purple pigment.
10. *Aplysia* feeds mainly on the sea weeds.
11. Habit and habitat: *Aplysia* is a marine gastropod found crawling in sea weeds. It is able to change its colour according to the colour of sea weeds on which it lives.
12. Distribution: *Aplysia* is found in most parts of the world from the Arctic to the Antarctic. It is found in West Indies, Florida Coast, Asia and India.

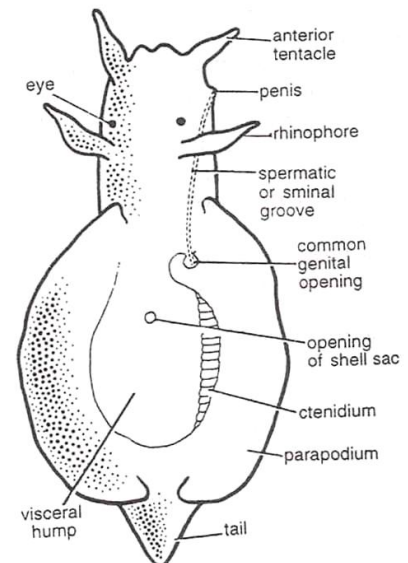


Fig. 35. *Aplysia*.

5. DENTALIUM

Classification

1. Phylum....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Scaphopoda.....: Shell composed of a series of eight calcareous pieces; foot flat and ventral.
3. Genus: *Dentalium*.

Salient features

1. *Dentalium* (Fig. 36) is commonly known as **tusk shell**. Since the shell of *Dentalium* resembles miniature elephant tusks, therefore, it is called elephant's tusk shell.
2. Body is bilaterally symmetrical and enclosed in a tubular shell open at both ends.
5. Mantle folds are fused ventrally to - form a tube enclosing the body.
6. Head is vestigial, bearing the mouth which is surrounded by a circlet of retractile tentacles, the captacula with sucker-like ends.
7. 5. Foot is long and conical, protrudes through the anterior opening of the shell and is used in burrowing.
8. Well developed radula is present.
9. Anus lies behind the base of foot.
10. Gills are absent. Respiration by transverse folds in the lining of mantle.
11. A pair of nephridia is present with their external openings on either side of the arm.
12. Vascular system is poorly developed without distinct heart.
13. Nervous system simple and consists of cerebral, pleural and pedal ganglia.
14. Eyes absent. Otocysts present.
15. Sexes are separate.
16. Development includes Veliger larva.
17. Habit and habitat: *Dentalium* is marine and found in the sand at great depth. They feed on minute organisms such as foraminifera and diatoms.
18. Distribution: They are found in all seas except polar. They are commonly found in Europe.

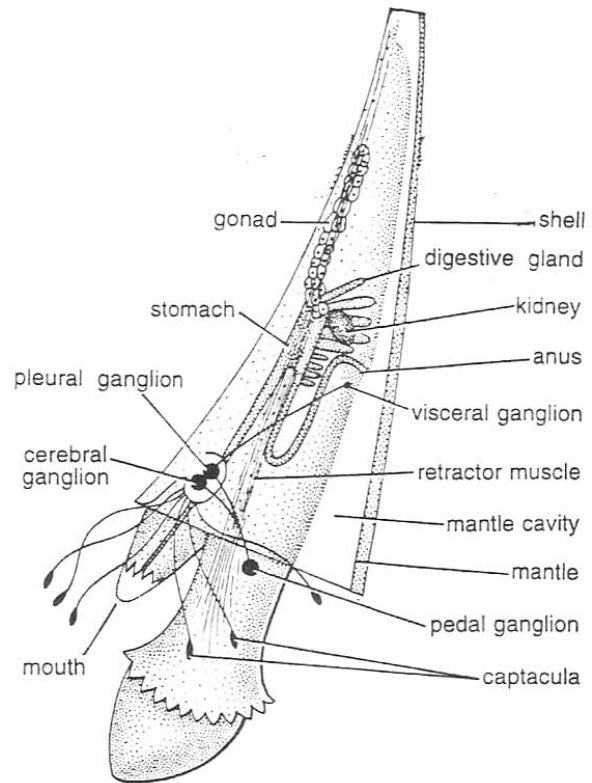


Fig. 36. *Dentalium*.

6. MYTILUS

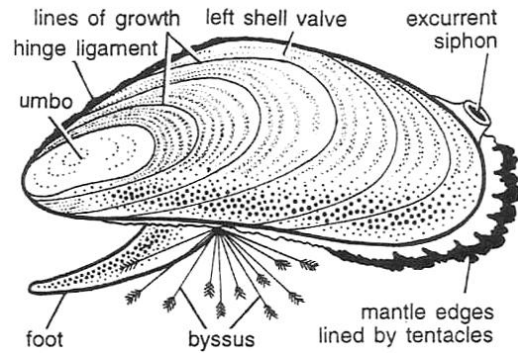
Classification

1. Phylum....: Mollusca: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....: Pelecypoda.....: Bivalved shell; body laterally compressed, head not distinct.
3. Order: Filibranchiata.....: Single pair of plate-like gills; foot small or poor poorly developed
4. Genus: *Mytilus*

Salient features

1. *Mytilus* (Fig. 37) is commonly called **sea mussel**.
1. Body is enclosed in a wedge-shaped shell of two equal valves, which is pointed in front and rounded behind.
2. After removing the shell are seen mantle lobes enclosing internal structures such as gills, foot, kidney, heart and alimentary canal.
3. Byssus threads protrude from between two shell valves ventrally by which it is attached to stones and rocks.
4. Mantle is bilobed and forms an exhalant siphon posteriorly.
5. Foot is cylindrical, elongated with a ventral groove continuous with a byssus pit.
6. Anterior adductor muscle is weaker, while posterior adductor muscle is strongly developed.

7. A pair of gills is present, each gill is provided with gill filaments.
8. A pair of simple eyes is present.
9. Sexes separate. Gonads extend into the mantle.
12. Habit and habitat. *Mytilus* is marine, sedentary, cosmopolitan and found attached to the rocks between tidemarks.
13. Distribution. *Mytilus* is cosmopolitan in distribution. It is commonly found in India, Europe and U.S.A.

Fig. 37. *Mytilus*.

7. SEPIA

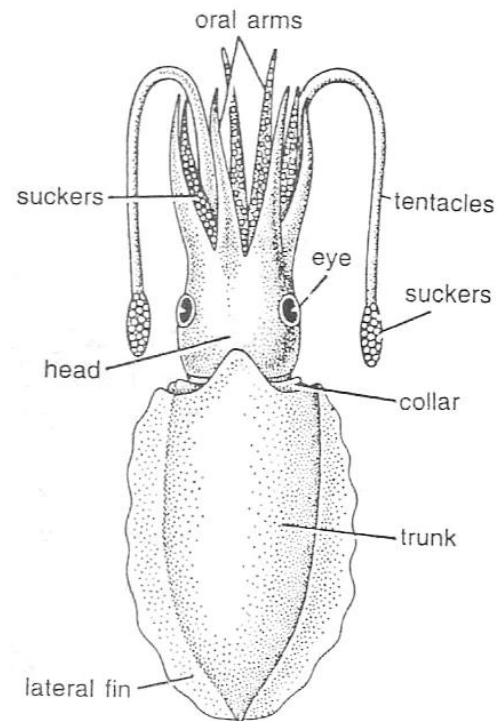
Classification

1. Phylum....:Mollusca.....: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....:Cephalopoda....: Head bears large eyes and mouth; foot modified into arms and tentacles encircling the mouth.
3. Subclass.: Dibranchiata...: Shell internal and reduced; foot modified into 8 or 10 sucker bearing arms; ink gland present; gills, kidneys, auricles and branchial hearts are two in number.
4. Order.: Decapoda.....: Arms 10 of which 8 short and 2 long, 8 smaller arms bear stalked suckers; shell internal and well-developed; nidamental glands usually present.

Genus...: *Sepia*.

Salient features

1. *Sepia* (Fig. 38) is commonly known as **cuttle-fish**.
2. Body is bilaterally symmetrical, dorso-ventrally flattened and is divisible into head, collar (neck) and trunk.
3. Head bears a pair of large eyes and five pairs of arms surrounding the mouth.
4. Of the five pair of arms, four pairs are short and stout bearing four longitudinal rows of suckers on the inner flat surface.
5. The fifth pair of arms is known as tentacles, which are comparatively long and narrow and provided with suckers only towards their free ends.
6. Collar (neck) is constricted and connects the head with the trunk.
7. Trunk is elongated and shield-shaped, bordered by narrow lateral fin on either side.
8. Mantle is thick and muscular, enclosing a large mantle cavity on the ventral side which contains the viscera.
9. Funnel is tubular opening into the mantle cavity.
10. Shell is internal and enclosed in a shell sac in the mantle on the upper surface.
11. Chromatophores are present in the deeper layers of integument over the entire surface.
12. A pair of large plume-shaped ctenidia or gills, one on each side of the mantle cavity, performs the respiratory function.

Fig. 38. *Sepia*.

13. Single kidney is excretory organ.
14. A pear-shaped ink-sac lies over the posterior ventral surface.
15. Sexes are separate. The males are usually smaller. In male, the left fourth arm is hectocotylised. It serves as an intermittent organ.
17. Habit and habitat: *Sepia* is a marine form, found in the shallow waters. It is a good swimmer. It usually swims at night and rest flat on the bottom during daytime. It is carnivorous, feeds on small fishes, crustaceans and other animals.
15. Distribution: *Sepia* is cosmopolitan in distribution. It is commonly found in India, Europe and Mediterranean region.

8. LOLIGO

Classification

1. Phylum....:Mollusca.....: Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class.....:Cephalopoda....: Head bears large eyes and mouth; foot modified into arms and tentacles encircling the mouth.
3. Subclass.: Dibranchiata...: Shell internal and reduced; foot modified into 8 or 10 sucker bearing arms; ink gland present; gills, kidneys, auricles and branchial hearts are two in number.
1. Order.: Decapoda....: Arms 10 of which 8 short and 2 long, 8 smaller arms bear stalked suckers; shell internal and well-developed; nidamental glands usually present.
4. Genus.:*Loligo*.

Salient features

1. *Loligo* (Fig. 39) is commonly known as **squid**.
2. Body is spindle or torpedo-shaped and is divisible into head, foot and visceral hump,
3. Head is short and bears a pair of large eyes and a central mouth surrounded by ten arms.
4. Foot is modified into the funnel and the ten arms.
5. Eight arms are short, stumpy and non-retractile, while two are long, slender
6. and retractile tentacles used for capturing the prey.
7. Inner surface of both arms and tenacles is provided with two rows of suckers.
8. Funnel is muscular tube extending out beyond the edge of the collar beneath the head.
9. Visceral hump is long and pointed, bears two dorso-lateral triangular
10. lateral fins. The animal swims with the help of lateral fins.
11. Mantle is thick and muscular encloses the visceral mass and mantle cavity.
12. Shell is internal, feather-shaped plate concealed beneath the mantle on the anterior surface.
13. Two elongated gills are present in the mantle cavity.
14. Two nephridia or kidneys are also present.
15. An ink sac is present and serves for defence.

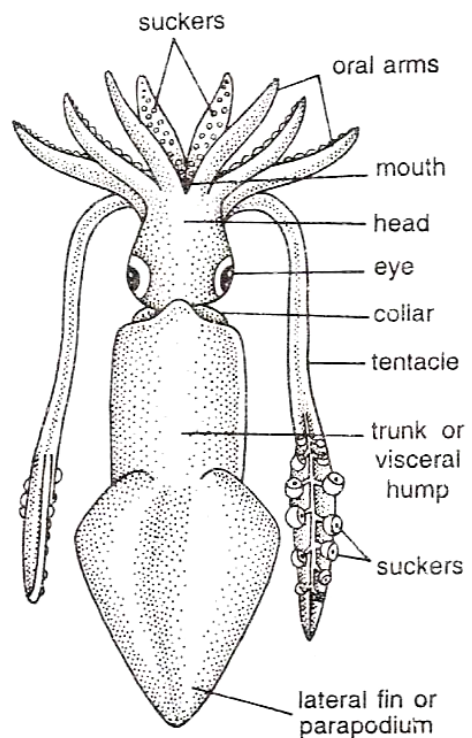


Fig. 39. *Loligo*.

16. Stxes are separate.
17. *Loligo* is used as food by Chinese and Italians and also as bait for marine fishing.
18. Habit and habitat: *Loligo* is a marine form, found in warin seas and coastal shallow or deep waters. it is a fast swimmer in the open sea water.
19. Distribution: *Loligo* is cosmopolitan in distribution, found along the entire Pacific and Atlantic Coast, Incha, China, and U.S.A.

9. OCTOPUS

Classification

1. Phylum..... Mollusca..... Body unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
2. Class..... Cephalopoda... Head bears large eyes and mouth; foot modified into arms and tentacles encircling the mouth.
3. Subclass....Dibranchiata....Shell internal and reduced; foot modified into 8 or 10 sucker bearing arms; ink gland present; gills, kidneys, auricles and branchial hearts are two in number.
4. Class.....Octopoda.....Eight arms with sessile suckers; usually shell absent; nidamental glands absent.
5. Genus*Octopus*.

Salient features

1. *Octopus* (Fig. 40) is popularly known as **devil-fish**.
2. Body is globose and bag-like with large head and trunk region.
3. Head bears a pair of eyes and eight elongated equal arms webbed at the base which surrounded the month.
4. Each arm bears suckers arranged in two rows. Suckers are sessile and large.
5. Third right arm in male is modified or hectocotylized into a spoon-shaped structure which serves for transferring the spermatophores into the mantle cavity of the female for fertilizing the ova.
6. Shell is absent.
7. Mantle encloses the mantle cavity and the visceral mass.
8. *Octopus* produces inky-fluid, which diffuses in water and forms a smoky screen for defence from the enemies.
9. The favourite food of *Octopus* consists of the crabs, bivalves, snails and fish which are seized by the quick movements of cruel snake-like arms and broken into pieces by radula and a pair of powerful sharp, beaklike horny jaws.
10. Habit and habitat: *Octopus* is a marine, bottom dwelling nocturnal cephalopod spending daylight hours in rocky crevice, shady under-water caverns and in coral reefs.

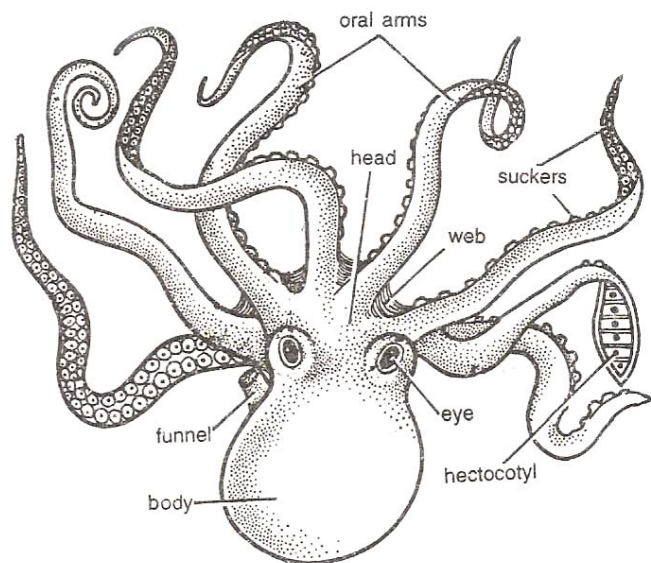


Fig. 40. Octopus.

11. Distribution: *Octopus* is cosmopolitan in distribution. It is commonly found in Europe and India, Atlantic and Pacific Coasts, Alaska to lower California and Cape.

PHYLUM: ECHINODERMATA

1. FEATHER STAR (ANTEDON)

Classification

1. Phylum.....: Echinoderma...: Radially symmetrical; pentaradiate coelomate with spiny skin and water vascular system.
2. Subphylum....: Pelmatozoa.....: Stalked and fixed forms; mouth and anus both dorsal; tube feet absent.
3. Class.....: Crinoidea: Body cup- shaped; arms five and bifurcated at the base.
4. Order.....: Articulata.....: Mouth and ambulacral grooves exposed.
5. Genus.....: *Antedon*.

Salient features

1. *Antedon* (Fig. 41) is commonly known as feather star.
2. Body consists of a central disc or calyx and a series of five radiating arms.
3. Central disc is differentiated into an upper oral surface and lower aboral surface.
4. Oral surface is covered with a soft and leathery skin, the tegmen bearing the central mouth and the anus on a papilla in an inter-radius.
5. Aboral surface bears several slender curved jointed cirri supported by small ossicles which serve for attachment.
6. Each arm is divided at its base into two, so that there are ten long slender flexible arms, bearing lateral pinnules.
7. Five ambulacral ciliated grooves radiate from the mouth towards the arms, where each divide into two and the branches extend along the oral surface of the arms.
Antedon.
8. Tube feet or podia without suckers present along the edges of ambulacral grooves.
9. Sexes are separate, gonads contained in the dilated bases of pinnules.
9. Development includes a pentacrinoid larva with jointed stalk.
10. Habit and habitat: *Antedon* is marine, found at moderate depths attached to the rocks and stones. They are gregarious forms and feed on microscopic living organisms.
11. Distribution: *Antedon* is worldwide in distribution, found in all seas. It is commonly found along Atlantic Coast. The members are known from lower Cambrian to Recent.

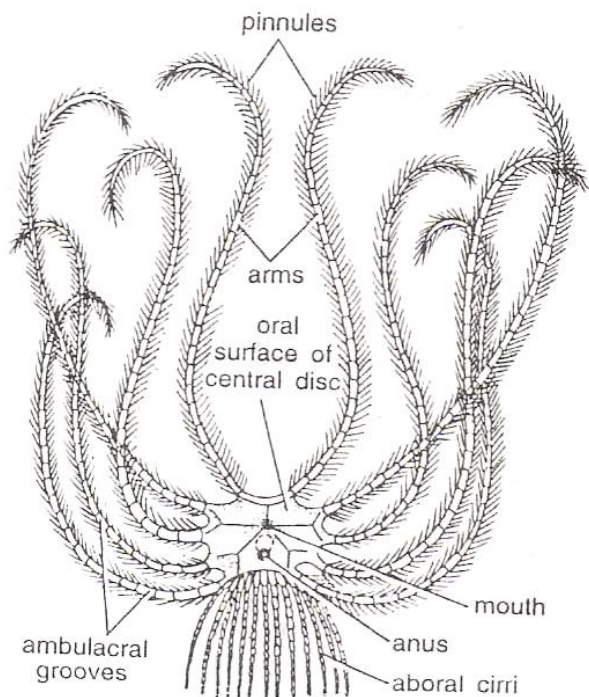


Fig. 41. *Antedon*.

2. SEA CUCUMBER (CUCUMARIA)

Classification

1. Phylum.....: Echinodermata...: Radially symmetrical; pentaradiate coelomate with spiny skin and water vascular system.
2. Subphylum....: Eleutherozoa.....: Stalkless and tree-living; tube feet with suckers; mouth on the oral surface and anus on the aboral surface.
3. Class.....: Holothuroidea.....: Body cylindrical, elongated in oral-aboral axis; anus absent.

4. Order.....: Dendrochirota.....: Oral tentacles dendritic or branched; respiratory trees present

5. Genus.....: *Cucumaria*.

Salient features

1. *Cucumaria* is commonly known as sea cucumber (Fig. 42).
2. Body is elongated, cylindrical with mouth and anus at opposite ends.
3. The body is five-sided bearing five longitudinal ambulacra each provided with double rows of podia or tube feet.
4. Oral end bears the large mouth surrounded by a circular lip and a thin peristomial membrane.
5. Mouth bears ten highly dendritic or branched tentacles.
6. The smooth, thin and collar-like region at the base of tentacles is known as introvert.
7. Anus is terminal placed at the aboral end.
8. Respiratory trees are present.
9. Cuvierian tubules are absent.
10. Sexes are separate; gonads are arranged in two tufts. Development includes an **auricularia larva**.
11. Habit and habitat. *Cucumaria* is marine. They are found at all depths from shallow to very deep water, and are primarily of benthonic and sluggish habit. Some live in rocky crevices and crannies and among corals and sea weeds, but the majority are found on sandy bottoms, either fully exposed or wholly or partially buried in mud.
12. Distribution: *Cucumaria* is cosmopolitan in distribution. This is commonly found in Europe,

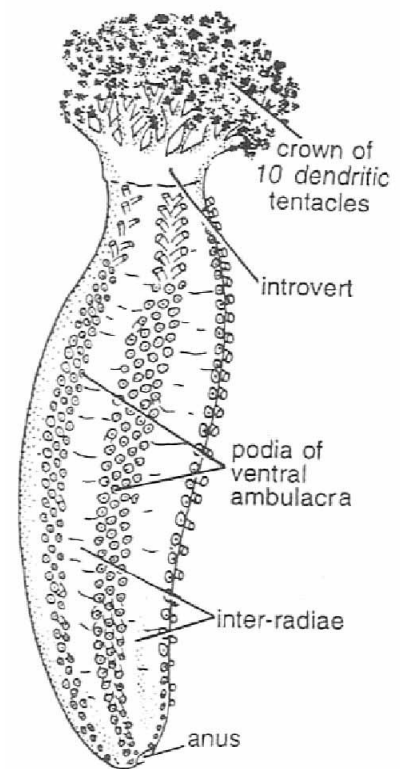


Fig. 42. *Cucumaria*.

3. SEA URCHIN (ECHINUS)

Classification

1. Phylum.....: Echinodermata.....: Radially symmetrical; pentaradiate coelomate with spiny skin and water vascular system.
2. Subphylum....: Eleutherozoa.....: Stalkless and tree-living; tube feet with suckers; mouth on the oral surface and anus on the aboral surface.
3. Class.....: Echinoidea.....: Body spherical, enclosed in a shell or test; ambulacral grooves and anus absent; pedicellariae stalked and three-jawed.
4. Subclass.....: Regularia.....: Corona globular and pentamerous; mouth central placed at the oral surface and anus central at the aboral pole; Aristotle's lantern well-developed; madreporite ambulacral.
5. Order.....: Camarodonta.....: Test rigid; epiphyses of lantern enlarged and meeting above the pyramids; teeth keeled, four types of pedicellariae present.
6. Genus.....: *Echinus*.

Salient features

1. *Echinus* is commonly known as Sea urchin (Fig. 43).
2. Body is globular in shape, somewhat flattened at the two poles forming distinct oral and aboral -poles.
3. Body is enclosed in a rigid globular shell or corona formed of closely fitted calcareous plates.
4. Entire surface of the animal except the peristome and periproct is covered with spines

articulated to the shell.

5. Pedicellariae with three jaws and sphaeridia are present among the spines.
6. Mouth lies in the centre of oral pole and is surrounded by soft membrane known as peristome, through the mouth project the five teeth of Aristotle's lantern.
7. At the aboral pole is a much smaller aperture, the anus surrounded by periproct.
8. The surface of the shell is divided into alternating ambulacral and inter-ambulacral areas.
9. Numerous podia or tube feet project from the surface among the spines. These are arranged in five double rows in ambulacral areas.
10. Sexes are separate. Gonads are five large masses.
11. Development includes a free swimming **echinopluteus larva**.
Habit and habitat. *Echinus* is marine, found in the sea in the rocky places.
12. Distribution. *Echinus* is widely distributed in the Atlantic, Mediterranean and Pacific Oceans.

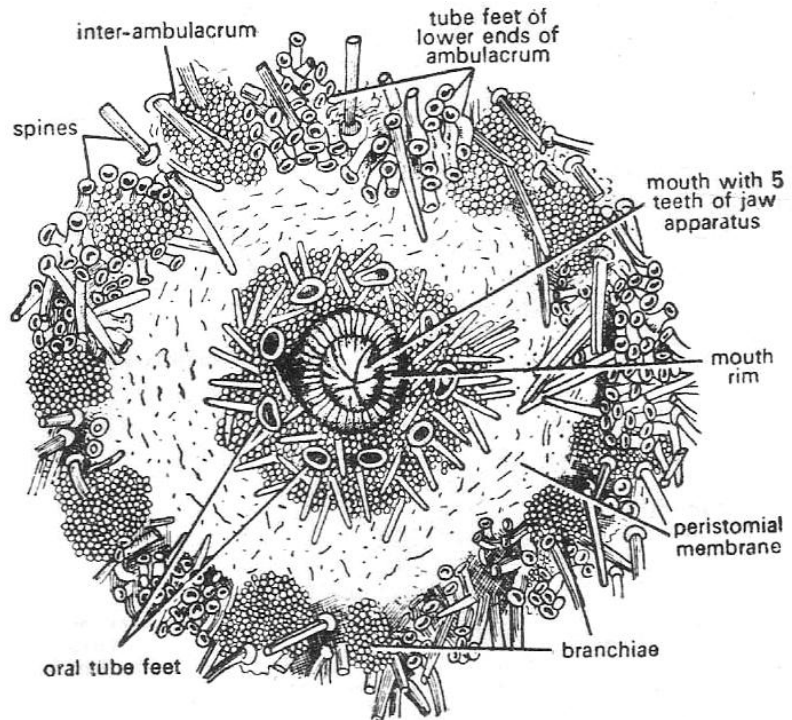


Fig. 43. Sea urchin.

4. STARFISH (ASTERIAS)

Classification

1. Phylum.....: Echinodermata....: Radially symmetrical; pentaradiate coelomate with spiny skin and water vascular system.
2. Subphylum....: Eleutherozoa.....: Stalkless and tree-living; tube feet with suckers; mouth on the oral surface and anus on the aboral surface.
3. Class.....: Asteroidea: Body star-shaped, bases of anus not distinctly marked off from the disc; oral surface directed downwards and aboral surface upwards; ambulacra form prominent grooves provided with tube feet.
5. Order.....: Phanerozonia: Arms with two rows of marginal plates; pedicellariae alveolar or sessile type, podia or tube feet arranged in two rows; mouth frame well developed and adambulacral type.
6. Genus.....: *Asterias*.

Salient features

1. *Asterias* is commonly known as star fish (Fig. 44) or **sea star**.
2. The body is star shaped, consisting of a central disc with five radiating arms which are broad at their base and tapering towards their extremities.
3. Body surfaces are distinguishable into an oral surface directed downwards and an aboral surface directed upwards.
4. Mouth is pentagonal and lies in the centre of the disc on oral surface.

5. Five narrow ambulacral grooves arise from the five corners of the mouth and extend along the middle of the oral surface of five arms up to their margin.
6. Each ambulacral groove is bordered laterally by two or three rows of movable calcareous spines, the ambulacral spines.
7. Each ambulacral groove contains two double rows of podia or tube feet, which serve as organ of locomotion.
8. Aboral surface bears a number of short, stout spines arranged in irregular rows, numerous dermal branchiae among spines and a smaller aperture, the anus which is situated near the centre.
9. Madreporite is a thick circular plate, situated in between the bases of two arms terminal tentacle on aboral surface.
10. Pedicellariae are very small, microscopic bodies scattered all over the body.
11. Water vascular system is well developed.
12. Sexes are separate. Fertilization is external. Development includes a free swimming **bipinnaria larva**.
13. Habit and habitat: *Asterias* is a marine form and found in the sandy and rocky parts of the sea.
14. Distribution: *Asterias* is found in shallow water in North temperate seas and found abundantly on North-Atlantic coast. It is found in abundance in India and U.S.A.

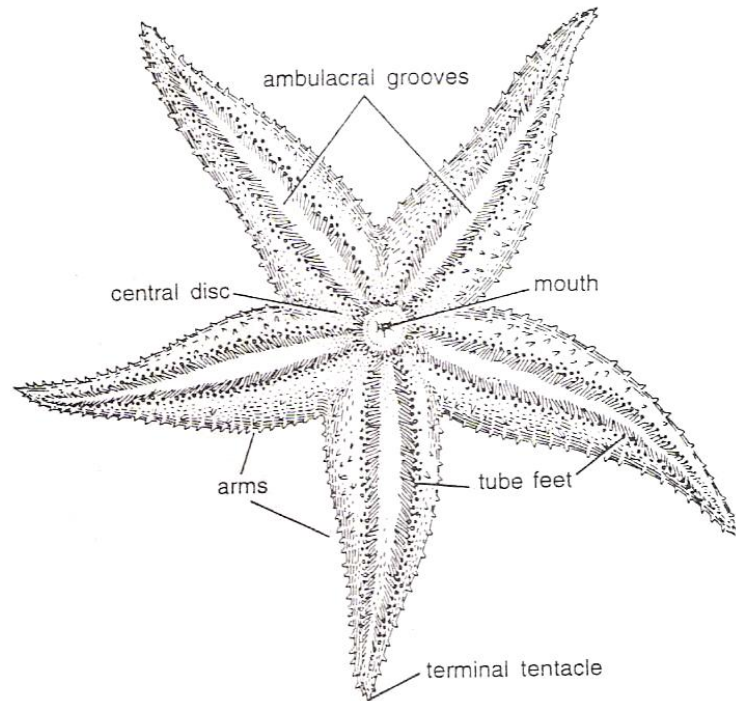


Fig. 44. Starfish.

5. BRITTLE STAR (OPHIOLEPIS)

Classification

1. Phylum.....: Echinodermata.....: Radially symmetrical; pentaradiate coelomate with spiny skin and water vascular system.
2. Subphylum....: Eleutherozoa.....: Stalkless and tree-living; tube feet with suckers; mouth on the oral surface and anus on the aboral surface.
3. Class.....: Ophiuroidea: Oral and aboral surfaces distinct; bases of the arms distinctly marked off from the disc; ambulacral grooves, anus and intestine absent; madreporite on the oral surface; bursa usually ten.
5. Order.....: Ophiurae.....: Arms simple; arm ossicles articulated by pits and projections; arm spines borne laterally, single madreporite.
6. Genus.....: *Ophiolepis*.

Salient features

1. *Ophiolepis* is commonly known as brittle star (Fig. 45).
2. Body is radially symmetrical.
3. Exoskeleton is in the form of calcareous ossicles.
4. Central disc is provided with large plates surrounded by little plates.
5. There are five arms which are sharply marked off from the central disc.
6. Ambulacral grooves, branchiae or dermal papillae and pedicellariae are absent. *Ophiolepis*.
7. Brittle star moves by means of its tube feet.
8. Habit and habitat: *Ophiolepis* is marine and free-swimming.

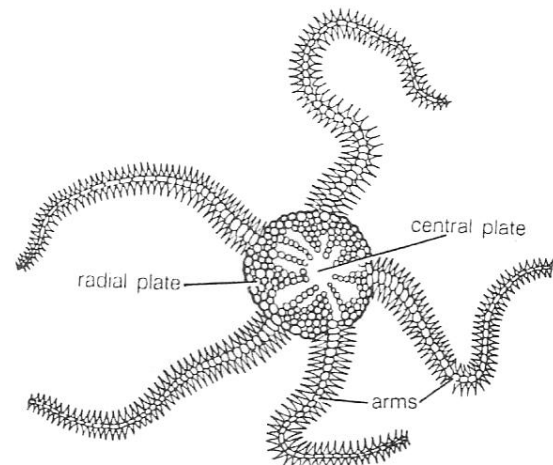
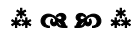


Fig. 45. Brittle star



B. TAXONOMY OF CHORDATES

1. AMPHIOXUS

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Acraniata: No head, cranium or brain.
3. Subphylum: Cephalochordata: Notochord along entire body length and persistent.
4. Class: Leptocardii: Numerous pharyngeal gill-slits.
5. Genus:.....: *Amphioxus*
6. Species.....: *lanceolatus*.

Salient features

1. *Amphioxus* (Fig. 46) is commonly called as lancelet.
2. It was first discovered by Pallas (1778).
3. Adult is less than 5 cm long and has superficially fish-like structural make up.
4. Body is elongated, flattened, non-pigmented and pointed at both ends as the name implies. Anterior end projects forwards as the rostrum.
5. Dorsal, ventral and caudal fins are low and continuous. There are 2 lateral fins or metapleural folds.
6. Ventral mouth is guarded by oral hood containing oral cirri. Pharynx is voluminous having gill slits, endostyle, epipharyngeal groove and peripharyngeal bands.
7. Atriopore is median and ventral. Anus on left side.
8. Myotomes are arranged on both sides of the body as metamerical blocks of striated muscle fibres separated by V-shaped partitions called as myosepta or myocommata.
9. Notochord is an axial skeletal rod extending from anterior to posterior end. Nerve cord lies just above the notochord.

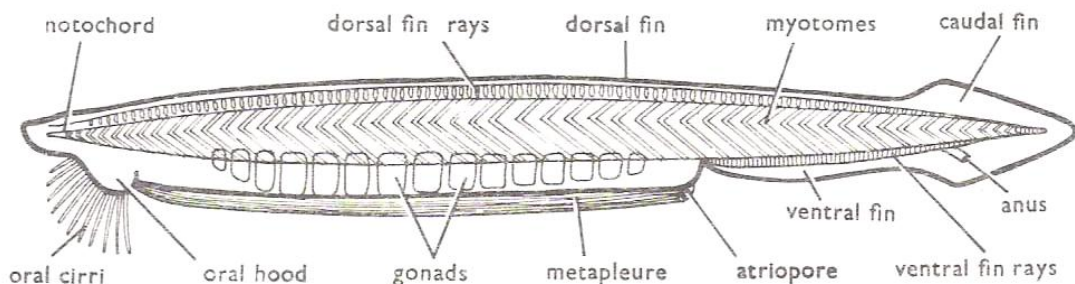


Fig. 46. *Amphioxus*.

10. Gonads 26 pairs. metamerically arranged on pharynx. The two sexes are separate but without sexual dimorphism.
11. Special features. Although eyes, nose, ears, jaws and appendages are completely absent, yet *Amphioxus* is of special zoological interest, because it shows three distinctive characters of the phylum Chordata in simple form i.e., presence of notochord, nerve cord and gill-slits. It is considered to resemble some ancient ancestor of the phylum Chordata. Further *Amphioxus* shows combination of primitive, specialized and degenerate features. Primitive features include notochord extending into snout, segmented myotomes, straight intestine, absence of jaws and paired fins, ciliary feeding and no specialized heart. The specialized features comprise of elaborate velum and oral hood and several gill-slits. The reduced brain and sense organs are degenerate features.
12. Identification: Since the animal contains oral hood, spindle-shaped body and myotomes and above features, hence it is *Amphioxus*.
13. Geographical distribution: Widely distributed in temperate and tropical seacoasts.

14. Habit and habitat: *Amphioxus* burrows in clean shifting and shallow shore waters leaving only its anterior end protruded. For most of the times, it remains buried in the sand but in darkness it swims very rapidly by lashing movements of the tail. It is a ciliary feeder.

2. BALANOGLOSSUS

Classification

1. Phylum : Hemichordata: Solitary, soft-bodied, coelomate with numerous gill-slits
2. Class.....: Enteropneusta: Alimentary canal straight, two rows of caeca.
3. Genus....: *Balanoglossus*.

Salient features

1. *Balanoglossus* (Fig. 47) is commonly known as **acorn worm**.
2. Body is soft and cylindrical having ciliated surface reaching a length of 10-50 cm.
3. The body is divisible into short conical **proboscis**, **collar** and a long **trunk**.
4. **Proboscis** has thick muscular walls and its cavity-proboscis coelom opens to the exteriorly a proboscis pore.
5. Collar is short, muscular, cylinder-like, enclosing a pair of coelomic cavities (collar coelom) opening by a pair of collar pores on the dorsal surface.
6. Trunk is superficially ringed (devoid of segmentation). It is divisible into anterior branchio genital region, a middle hepatic region and a posterior abdominal region.
7. In the branchio-genital region are present a pair of genital wings formed by internal gonads and a branchial groove having numerous paired gill-slits arranged into two rows.
8. In the hepatic region are present double rows of hepatic caeca.
9. Alimentary canal is straight and anus is present on the posterior end of the body.
10. Sexes are separate and fertilization is external.
11. Development includes a free-swimming pelagic larva, the **tornaria**.
12. Habit and habitat: *Balanoglossus* is a burrowing and exclusively marine animal. It is found in shallow waters between tide marks along the coasts of warm and temperate oceans.

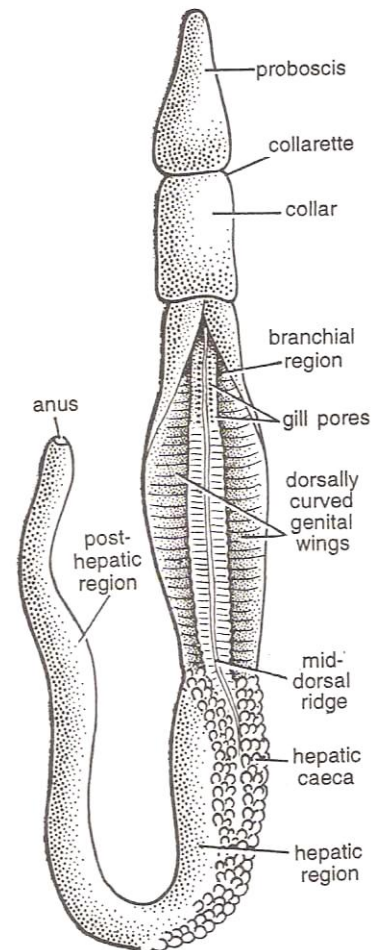


Fig. 47. *Balanoglossus*.

3. ASCIDIAN

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, gill-slits and notochord .
2. Group.....: Acrania.....: No head, cranium or brain.
3. Subphylum.: Urochordata....: Marino- Body covered by a thick test. No notochord present only in larva tail.
4. Class.....: Ascidiacea.....: Numerous pharyngeal gill-slits.
5. Order.....: Enitcrogonia....: Neural gland ventral to nerve ganglion. Gonad one.
6. Genus.....: *Ascidia*

Salient features

1. Ascidians (Fig. 48) are commonly called as sea squirts.
2. Shape of body short and cylindrical with a broad base attached to rocky substratum.
3. Test translucent, wrinkled and brownish in colour.
4. Anteriorly body contains eight-lobed terminal branchial siphon and six-lobed sub-terminal atrial siphon having mouth and anus, respectively. Mouth is surrounded by 50-100 tentacles.
5. Branchial siphon leads into pharynx which is perforated by stigmata.
6. Dorsal tubercle separated from the nerve ganglion and membranous dorsal lamina.
7. Stomach with smooth wall and left side of pharynx is occupied by bent pharynx.
8. Excretory organs are renal vesicles. Anus double lipped.
Monoecious. Ovary, testes in the same animal:
Reproduction sexual. Oviparous. Metamorphosis retrogressive.
9. Geographical distribution: *Ascidia* is commonly found in cold temperate regions. Habit and habitat. *Ascidia* is solitary, marine and sedentary urochordate inhabiting shallow water but some occur down to 2,900 fathoms.
10. Special features: There are some compound ascidians in which individuals are buried in a common test. The development includes tailed larva exhibiting typical chordate characters having notochord, nerve cord, etc. Kowalewsky (1886) carefully studied the development of larval ascidians and brilliantly demonstrated true position of the group among chordates. The tunicates are best understood by studying first free-living larva of an ascidian, than the adult. The larva shows chordate characters but some are absent in adult and others are modified to sessile mode of life. Compound ascidians reproduce asexually by germination and also produce eggs and sperms for sexual reproduction.
11. Identification: Since the animal contains lobed siphons and above features, hence it dorsal lamina.

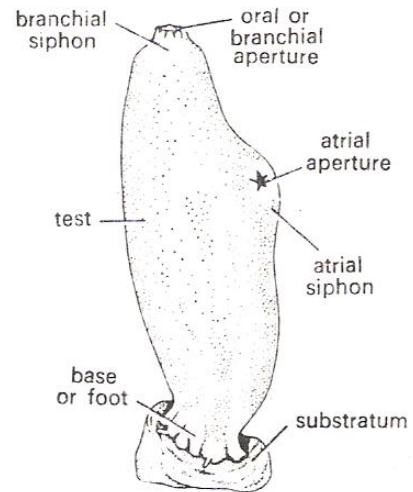


Fig. 48. *Ascidia*.

4. PETROMYZON

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata: Vertebral column present.
4. Division.....: Agnatha.....: Jaws and paired appendages absent.
5. Class: Cyclostomata.....: Mouth circular, suctorial, without jaws.
6. Order: Petromyzontia....: Mouth with funnel, without tentacles. Nasopharyngeal pouch blind. Gills pairs, open independently to exterior. Branchial basket well developed.
7. Genus.....: *Petromyzon*
8. Speceis.....: *marrinus* (= *Lampetra fluviatilis*).

Salient features

1. *Petromyzon* (Fig. 49) is commonly called as lamprey.
2. The body is eel-like, measuring about 90 cm and differentiated into head, trunk and tail, with 2 dorsal and 1 caudal fin.
3. Skin is without scales, slimy, green, brown and with strong metallic lusture.

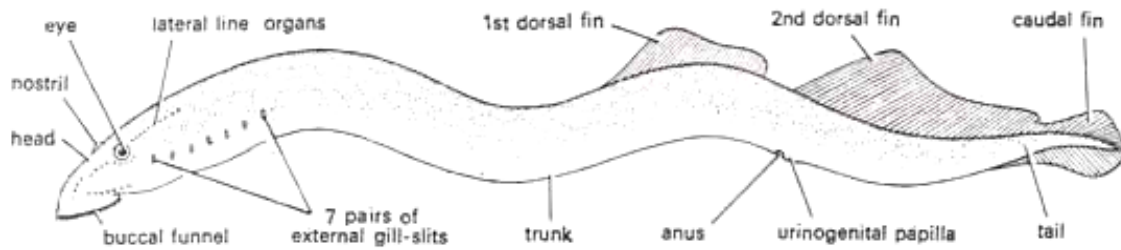


Fig. 49. *Petromyzon*.

4. Head contains mouth but no jaws. Mouth is surrounded by a large, ventral, suctorial funnel with numerous horny teeth. The 'tongue' is toothed and piston-like.
6. Dorsal nasal sac and mouth are unconnected. Pineal body is present behind nasal opening. Paired eyes are large and functional. 2 small median eyes, namely pineal and parietal, are also present.
5. Gill-slits are 7 pairs and branchial basket is well developed.
6. Sexes are separate. Female with large anal fin. Male with urinogenital or copulatory papilla. The development includes ammocoete larva which is very important phylogenetically as it is regarded a connecting link between *Amphioxus* and cyclostomes.
7. Economic importance: Lampreys have very little food value.
8. They injure and destroy fishes by sucking blood and causing secondary infection.
9. Larval lampreys are used as bait for sport fishing and commercial fishing.
10. Special features: Lampreys are the lowest jawless vertebrates and their nearest allies are the ancient ostracoderms of Silurian and Devonian periods. There are no fossil representatives of this group to indicate their course of evolution.
11. Identification: Since the animal has 7 pairs of gill pores and is without jaws and has above features, hence it is *Petromyzon*.
12. Geographical distribution. *Petromyzon marinus* is found in world-wide sea waters, coastal regions, streams and lakes of North America. Europe, West Africa, Australia, Chile, Japan, New Zealand, Tasmania.
13. Habit and habitat: *Petromyzon* is found both in salt and fresh water. They lead an ectoparasitic life on other fishes, attaching to the body of host by buccal funnel and secreting an anticoagulant for continuous flow of blood. They are also anadromous i.e., ascending river for spawning. Carnivorous and predators.

PISCES

1. SHARK (DOG FISH)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
(= Elasmobranchi) Spiral valve in intestine. Opercula absent.
6. Subclass: Selachi: Gills in separate clefts. Cloaca present.
7. Order: Pleurotremata: (Pleuro, sides; trema, opening). Gill-slits lateral, pectoral
(= Squali) fins small.
8. Genus.....: *Scoliodon*.

Salient features

1. *Scoliodon* is commonly called dogfish or dog shark (Fig. 50).

2. Spindle-shaped body, about 60 cm long, is regionated into head, trunk and tail.
3. Dorsal and lateral sides of body are pigmented dark grey or slaty grey, while the ventral side is white.

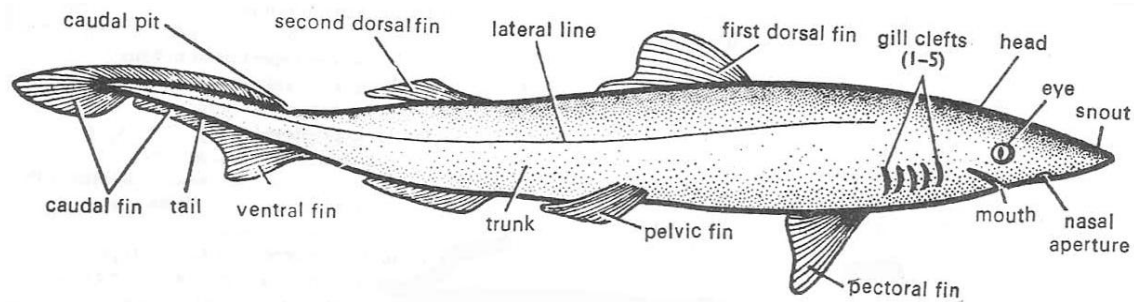


Fig. 50. Shark.

4. Head is dorso-ventrally compressed and flattened into snout. It contains ventrally situated slit-like mouth, obliquely situated nostrils and laterally situated protuberant eyes. A little behind eyes there are five pairs of lateral gill-clefts.
5. Trunk bears (i) median unpaired and (ii) lateral paired fins. Median unpaired fins are (a) large first dorsal fin, (b) small second dorsal fin, and (c) ventral fin. Paired fins include a pair of anterior pectoral fins and a pair of posterior pelvic fins.
6. Heterocercal tail containing musculature and vertebral column is turned upwards.
7. A pair of pigmented lateral lines extends from head to tail.
8. *Scoliodon* exhibits sexual dimorphism. Males are easily recognized by having a pair of organs, called as claspers. Cloaca is found between 2 pelvic fins.
9. Economic importance: *Scoliodon* has great educational and experimental value because of its availability and size and is a favourite dissection fish for undergraduate students.
10. Special features: Sharks are used as human food in many countries. Shark's fins are dried and then boiled to yield a gelatinous substance favoured for soups. The tanned shark skin shagreen is used to case fine books, jewel boxes and sword handles, etc. Sharks are nuisance to fishermen, because they tear nets, steal captured fishes and take bait or fish from hooks.
11. Identification: Since the animal has raised tail, pointed snout, and above features, hence it is *Scoliodon*.
12. Geographical distribution: *Scoliodon* has wide distribution. About 4 species are found all along the Indian sea coast. Its presence has been reported from Zanzibar, Ceylon to Malay Archipelago, East Indies, Philippine Islands, Mexico to Panama, Cuba, West Indies and South America. Lower Carboniferous to Recent.
13. Habit and habitat: The natural home of *Scoliodon* is the sea, but some live in estuaries and even ascend the rivers. They are predacious and voracious feeders attacking their prey with powerful jaws. They are active swimmers.

2. NARCINE

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Subphylum.....: Vertebrata: Vertebral column present.
3. Division.....: Gnathostomata....: Jaws and paired appendages present.
4. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
(= Elasmobranchi) Spiral valve in intestine. Opercula absent.
5. Class.....: Chondrichthyes
6. Genus.....: *Narcine*.

Salient features

1. *Narcine* (Fig. 51) is commonly called electric ray. It is a cartilaginous fish. It is marine.
2. This body consists of a head, a trunk and a tail.
3. The 'head is disc-shaped and dorso ventrally flattened. The pectoral fin is extended toward to form the disc.
4. Skin is smooth without scales. Mouth is ventral.
5. Eyes are dorsal. Two spiracles are present behind the eyes. Gill slits are ventral.
6. The trunk has a pair of pelvic fins and two dorsal flins.
7. The caudal fin is rounded.

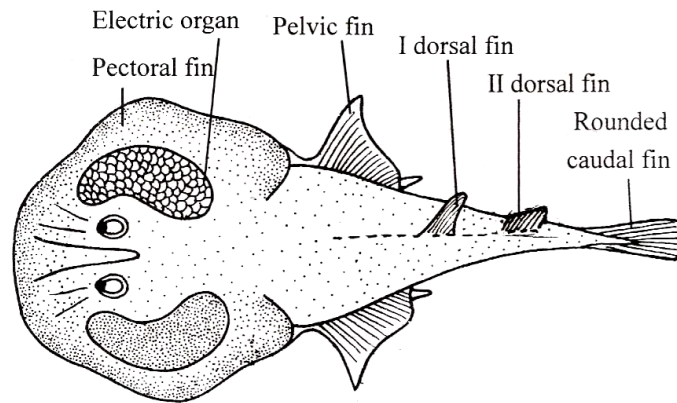


Fig. 51. *Narcine*

8. *Narcine* has two large electric organs on the disc. They are kidney-shaped. Each electric organ is formed of muscle fibres arranged in blocks and serve as batteries.
9. The muscles are arranged into several rows of hexagonal celled electroplexes which represent the electric plates. The dorsal surface of the electric plates is positive and the ventral surface is negative. The electric current passes from dorsal to ventral surface.
10. The electric organs are used for offense and defence.

3. TRYGON

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
(= Elasmobranchi) Spiral valve in intestine. Opercula absent.
6. Subclass: Selachi: Gills in separate clefts. Cloaca present.
7. Order: Pleurotremata: (Pleuro, sides; trema, opening). Gill-slits lateral, pectoral
(= Squali) fins small.
8. Genus.....: *Trygon* = *Dasyatis*.

Salient features

1. *Trygon* (Fig. 52) is commonly called as sting ray or whip-tailed ray because of the presence of a serrated sting at the base of the tail.
2. It consists of huge kite-shaped fleshy body and long whip-like tail. Head and body dorso-ventrally compressed. Outer anterior margin of pectorals continuous along side of head upto end of snout forming sub-rhombic disc-shaped body. Disc less than 1.3 times as broad as long.

3. Pectoral fins being confluent with the sides of the head, their pre-axial endoskeleton radiate to meet in front of the skull along the lateral margins of prenasal rostral cartilage. Pelvic fins small.
4. Skin is smooth or spiny.
5. Mouth is ventral and rectangular. Nasofrontal flap is present in front of the mouth.
6. Head contains a pair of dorsal eyes.
7. Spiracles present behind the eyes.
8. Gill-slits 5 pairs, ventral in position.
9. Viviparous: Long glandular filaments are formed in the uterine wall. During development of the embryo, the uterine filaments secrete a nutritive milky fluid which serves as food for embryo.
10. Economic importance. The fish is edible and its liver gives oil.
11. Special features: Sexually dimorphic. Male contains claspers near the pelvic fins. The tail is especially elongated, whip like and contains a large mid-dorsal poisonous spine which is a modified dorsal fin. The poisonous spine is about 20-35 cm. long and acts as organ of offence and defence. Caudal arm is small and single lobed. By poisonous sting it inflicts wound on the victim. The sting with poison gland produces ugly, slow-healing wound, sometimes complicated by gangrene or tetanus on bathers and fishermen.
12. Identification: Since this fish has whip-like tail and above features, hence it is *Trygon*.
13. Geographical distribution: *Trygon* is abundantly distributed in tropical regions of the Atlantic and Pacific oceans. It has been reported from India, Japan, China, North America, Australia and South America. Upper Jurassic to Recent.
14. Habit and habitat: *Trygon* is found lying quietly on the sea bottom. It occasionally swims to change the place in search of prey or moves in self-defence. It is carnivorous feeding on small fish, Crustaceans and mollusks: It also shows adaptive or protective colouration to conceal itself from the enemies.

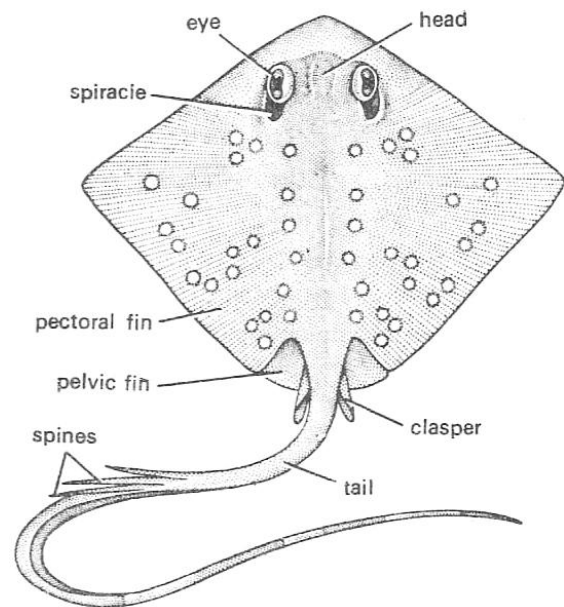


Fig. 52. Trygon.

4. MULLET (MUGIL)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Subphylum.....: Vertebrata: Vertebral column present.
3. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine, Opercula absent.
4. Class: Osteichthyes: Bony fishes.
5. Superorder.....: Teleostei.....: Bony fishes proper.
6. Genus: *Mugil*
7. Species: *cephalus*

Salient features

1. Mullet (Fig. 53) is a bony fish.
2. It is commonly found in the seas and estuaries of India and in brackish waters.

3. The general shape of the body is more or less oblong, and the head is slightly depressed.
4. It is covered by ctenoid scales. The ctenoid scales are marked by concentric lines crossed by a few radiating ridges and the free borders are produced into spines.
5. The body consists of a head, a trunk and a tail.

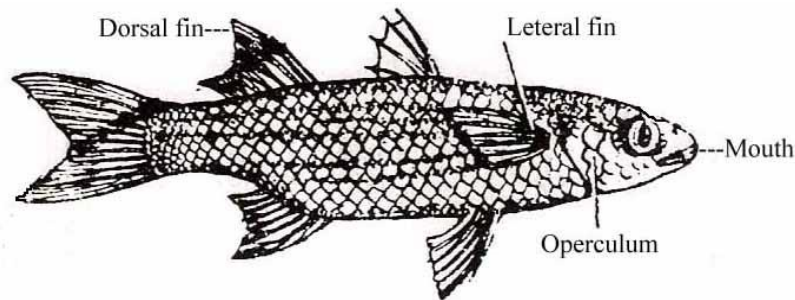


Fig. 53. Mullet.

6. The head contains a terminal mouth, a pair of lateral eyes, a pair of nostrils and a pair of operculum.
7. The trunk has a pair of pectoral fins, a pair of pelvic fins, two dorsal fins and an anal fin.
8. The tail has a caudal fin which is homocercal.
9. They feed on the vegetation contained in the mud at the bottom and many ascend rivers for breeding.
10. It is an edible fish. It can ascend rivers for breeding.

5. SUCKER FISH (ECHENEIS)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine. Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Order: Discocephali: Dorsal fin modified into an adhesive disk. Other fins normal.
8. Genus.....: *Echeneis*
9. Genus.....: *remora*.
1. *Echeneis* (Fig. 54) is commonly called as sucker-fish. Sucking disk is found on head. The disk develops from a transformed spinous dorsal fin, the spines of which are splited to form 10-28 transverse movable laminae inside a fleshy margin. Fish applies the disk against other fish and creates a partial vacuum by operating the sucking action which permits it to obtain rides or larger animals.
2. Body is elongated measuring about 1 metre in length and covered with small scales. Pectoral fin inserted high up.
3. Head is depressed and produced into snout. Eyes are lateral in position. Mouth cleft is wide and deep.
4. Second dorsal and anal fins are elongated, opposed to each other and without spines. Ventral fin is with one spine and 5 rays. Other fins normal and used for swimming. Pre-caudal vertebrae with parapophyses.
5. Supra-clavicle is much reduced.
6. Air bladder absent. Tail bomocercal.
7. Supra-clavicle is much reduced.

8. Air bladder absent. Tail homocercal.
9. Special features: The sucker-fishes are employed to catch turtles on the east coast of India by the natives. It is a lazy fish usually remains attached to sharks, turtles and ships. The attachment is quite firm and not easy to detach. The fish shows commensalism. For instance, *Echeneis remora* attaches the sharks by its modified sucker-like dorsal fin. It feeds on leftovers of the shark. Neither it harms nor benefits the shark.
10. Identification: Since this fish has modified dorsal fin as sucker and above features, hence it is *Echeneis*.
11. Geographical distribution: The fish (*Echeneis*) is distributed all over the tropical and warm seas but specially found on the south coast of England, Indian, Atlantic and Pacific seas.
12. Habit and habitat: *Echeneis* is a common marine fish. It swims in water feeding on small fishes. It attaches itself by means of its adhesive disk to boats, sharks, bony fishes, sea turtles and marine mammals.

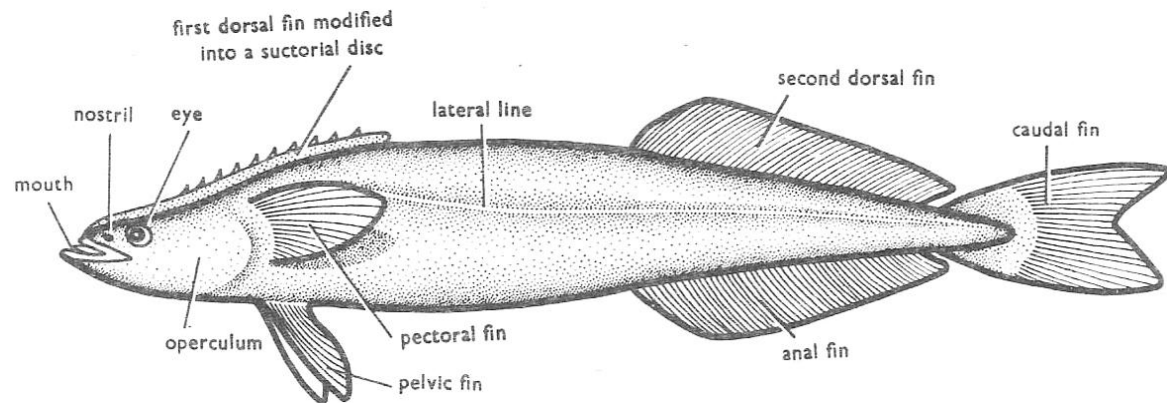


Fig. 54. *Echeneis*.

6. CAT FISH (CLARIUS)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine. Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Subclass.....: Actinopterygii.....: Ray-finned fishes
8. Order: Ostariophysi.....: Anterior vertebrae fused. Weberian ossicles present
between air bladder and ear.
9. Genus.....: *Clarius*
10. Species.....: *batrachus*.

Salient features

1. *Clarius* (Fig. 55) is commonly called as cat-fish or magur. Body is divided into head, trunk and tail.
2. It is characterized by its spikeless dorsal fin, which extends all along the body; pectoral fin is inserted very low; anal fin is not confluent with caudal.
3. Head is flat with four pairs of non-contractile and sensory barbels. Head bones are superficially exposed.
4. Body is covered by scaleless and naked skin.
5. Dendritic accessory branchial apparatus supplements gill respiration and hence fish can live

for a very long period outside water.

6. Air bladder is physostomous.
7. Eyes reduced and spiracles absent
8. Parietals, symplectics and sub-operculum absent.
9. Tail is laterally compressed, diphycercal and having rounded caudal fin.
10. The pectoral fins cause painful wounds. They are placed very low along ventro-lateral angles of abdomen. Weberian ossicles connecting internal ear and air bladder present.
11. Economic importance: Magurs are much valued for food.
12. Special features: Magur supplements gill respiration with accessory organs or respiration. It can remain away out of water for long time. It can also travel a distance of 1/2 kilometer on its paired fins and is called as walking fish. On land respiration by accessory respiratory organs.
13. Identification: Since this fish has peculiar dorsal fin, barbels and above features, hence it is *Clarius*.
14. Geographical distribution: *Clarius* is distributed in India. Burma. Sri Lanka and Malaya Archipelago. Eocene to Recent.

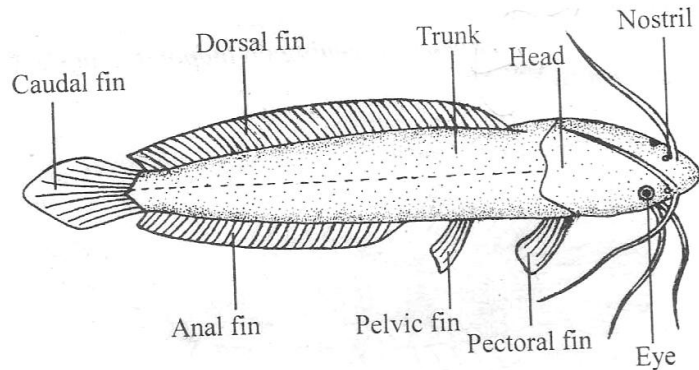


Fig. 55. *Clarius*.

7. OPHIOCEPHALUS

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Pisces:Endoskeleton cartilaginous. Scales usually placoid. Spiral valve in intestine. Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Subclass.....: Actinopterygii.....: Ray-finned fishes.
8. Super order....: Teleostei.....: Bony fishes proper
9. Order: Ophicephaliformes: Teeth present on jaws and palate.
10. Genus.....: *Ophiocephalus*
11. Species.....: *punctatus*.

Salient features

1. Commonly referred to as snake-headed fish (Fig. 56). Colour of the fish varies with water, with greenish back, yellowish sides and striped abdomen. Some specimens possess scattered dots on the head.
2. Body is elongated and cylindrical and differentiated into head, trunk and tail. Head and body covered with cycloid scales.
3. Head triangular, tapers into a pointed snout. Teeth present on jaws and palate. Maxillae excluded from border of upper jaw. Lower jaw protruding beyond upper jaw.
4. Suprabranchial organ present for breathing.
5. Dorsal and anal fins are long. Pectoral fins nearer to pelvic fins.
6. Caudal fin is rounded.

7. Lateral line is slightly curved. Air bladder long.
8. Economic importance: *Ophiocephalus* is eaten as food. Its raw flesh is used to cure ulcers.
9. Special features: It can breathe atmospheric air due to the presence of a supra branchial cavity. The fish acts as host for camallanid nematodes.
10. Identification: Since this fish has characteristic anal and dorsal fin and above features, hence it is *Ophiocephalus*.
11. Habit and habitat: *Ophiocephalus* is commonly found in freshwater ponds and rarely in flowing waters. They are able to survive drought in semifluid or beneath dry mud and have an accessory branchial cavity for aerial breathing.

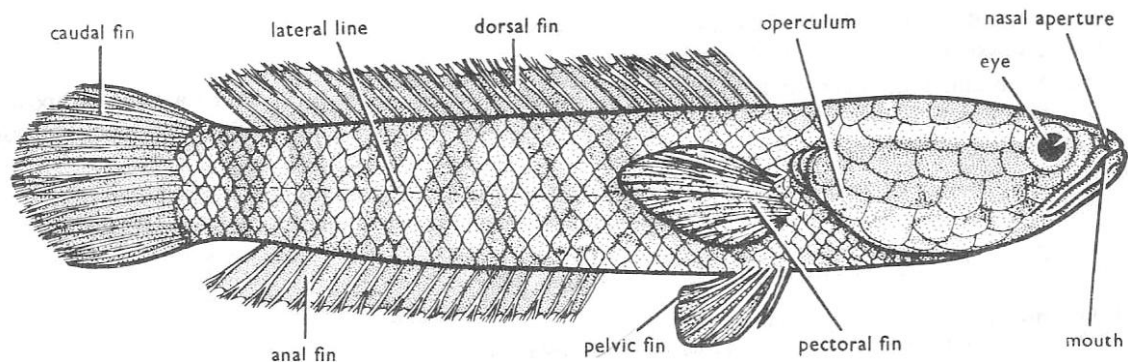


Fig. 56. *Ophiocephalus*.

8. EEL (ANGUILLA)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Super class: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine, Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Sub class.....: Actinopterygii.....: Ray-finned fishes.
8. Super order....: Teleostei.....: Bony fishes proper.
9. Order: Anguilliformes: Body long and slender. Air bladder with ducts. Gill openings small Scales minute or absent
10. Genus.....: Anguilla
11. Species.....: *vulgaris*.

Salient features

1. *Anguilla* (Fig. 57) is commonly known as eel, measuring 1.2 metres in length.
2. Body is slender, elongated and snake like.
3. On each side operculum covers the gill slits,
4. Dorsal fin, anal fin and caudal fin are joined together forming a continuous fin. Pelvic fins are absent.
5. Fins are supported by fin rays.
6. Body is covered by minute scales embedded in the skin and arranged obliquely at right angles to one another forming a curious pattern.
7. Maxillaries and palatop-terygoid present; gill cleft separate and vertebrae greatly enlarged.
8. Spines absent, gill openings small, air bladder has ductus pneumaticus. Oviducts absent.
9. Gills displaced posteriorly with 6-22 branchiostegal rays. There are no special accessory

organs for breathing air. When on land, probably air is taken through skin.

10. Special features: Eels have peculiar breeding habits and life-histories. Both the American and European eels, when about 60 cm long, put on breeding colours. The green European eel travels about 3,000 miles to spawn in hot waters of West Indies. Upon reaching the coastal waters, green colour changes to silver, eyes are enlarged and gonads mature. The fish lays about 10 million eggs, which hatch into pelagic larvae called as Leptocephali. These larvae take homeward journey. On the contrary to present known work, Aristotle thought that they come from "entrails of earth".

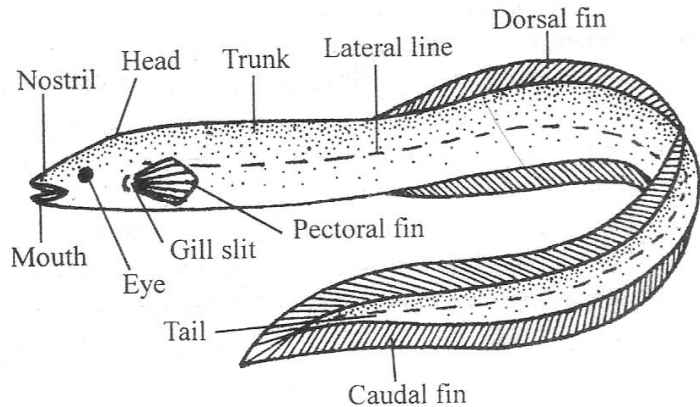


Fig. 57. *Anguilla*.

11. Identification: Since this fish has continuous caudal, anal and dorsal fins and above features, hence it is *Anguilla*.
12. Geographical distribution. *Anguilla vulgaris* is widely distributed in Europe, North Africa, temperate Asia, North America, Mexico, West Indies, Australia and New Zealand. They are not found in Eastern Pacific and South Atlantic. Cretaceous to Recent.
13. Habit and habitat. *Anguilla* is a freshwater fish. It is a voracious feeder and **catadromous** fish and it can live for several hours out of water. The adult eels live in ponds, estuaries, rivers and coastal areas of the sea and damp grass or moss outside water.

9. FLYING FISH (EXOCOETUS)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Super class: Pisces: Endoskeleton cartilaginous. Scales usually placoid. Spiral valve in intestine. Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Sub class.....: Actinopterygii.....: Ray-finned fishes.
8. Super order....: Teleostei.....: Bony fishes proper.
9. Order: Synentognathi: Flying fish, Dorsal fin above anal. Pectorals high on body.
10. Genus.....: *Exocoetus*

Salient features

1. *Exocoetus* (Fig. 58) is commonly known as **flying fish**.
2. Elongated body with silvery white sides measures 30-45 cm in length, divided into head, trunk and tail and covered with overlapping cycloid scales (usually 38-60 in lateral line).
3. Head contains large eyes. The upper part of snout is produced into a process.
4. Mouth opening is small but teeth in both jaws.
5. Preoperculum and symplectic absent.
6. Lower pharyngeals unite as a single bone.

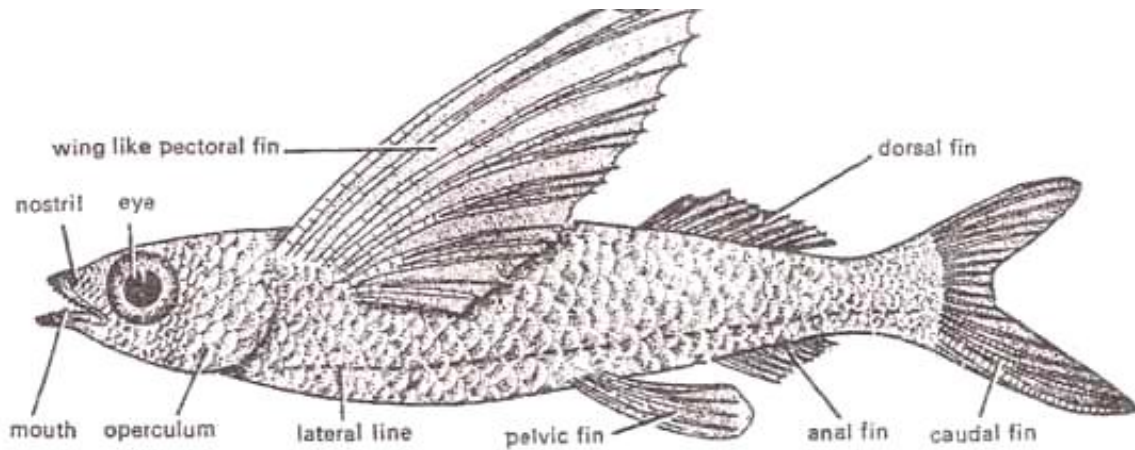


Fig. 58. *Exocoetus*.

7. Dorsal and anal fins are short and supported by 8-16 soft fin rays each.
8. Pectoral fins are exceptionally large, spread like wings and make gliding flights. In some the lower lobe of caudal fin is larger and stronger, and by its powerful strokes the fish is able to leave water with force.
9. Pelvic fins are also developed and adapted for lifting the body.
10. Tail is hypoblastic. Ventral lobe of the tail fin larger.
11. Oviparous.
12. Economic importance. The flying fish also serves as food.
13. Special features: *Exocoetus* is not a true flying fish. Especially in warm seas, it emerges to glide over the water. Pectorals act as wings. It also leaves water to escape from larger fishes, such as Tunas and Mackerels. The fish can glide over the surface of the water for about 400 metres.
14. Identification: Since this fish has large pectoral fins and above features, hence it is *Exocoetus*.
15. Geographical distribution: Distributed in tropical and warmer Atlantic, Pacific and Indian oceans.
16. Habit and habitat: *Exocoetus* is found in sea often skittering near the boats. It is pelagic and feeding on prawns and young fishes and their eggs. Small fishes live in sandy shoal-places near the coast.

10. SYNGNATHUS

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Super class: Pisces.....: Endoskeleton cartilaginous. Scales usually placoid. Spiral valve in intestine. Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Subclass.....: Actinopterygii.....: Ray-finned fishes.
8. Super order....: Teleostei.....: Bony fishes proper.
9. Order: Solenichthyes: Pipe-fish and sea hognoses. Mouth at the end of a tubular snout. Male with brood pouch. Fins minute
10. Genus.....: *Syngnathus*.

Salient features

1. *Syngnathus* (Fig. 60) is commonly known as **pipe-fish**.
2. Compressed and elongated fish covered by ring-like exoskeletal bands is divided into head,

trunk and tail.

3. Anteriorly half of the head is produced into a suctorial snout having mouth and eyes. A fleshy barbel is present at the tip of lower jaw.
4. Mouth is suctorial, toothless and lies at the end of Snout.
5. Gills reduced. Gill openings very small, near the upper posterior angle of gill cover.
6. Dorsal fin is present having 16-20 rays. Caudal fin present.

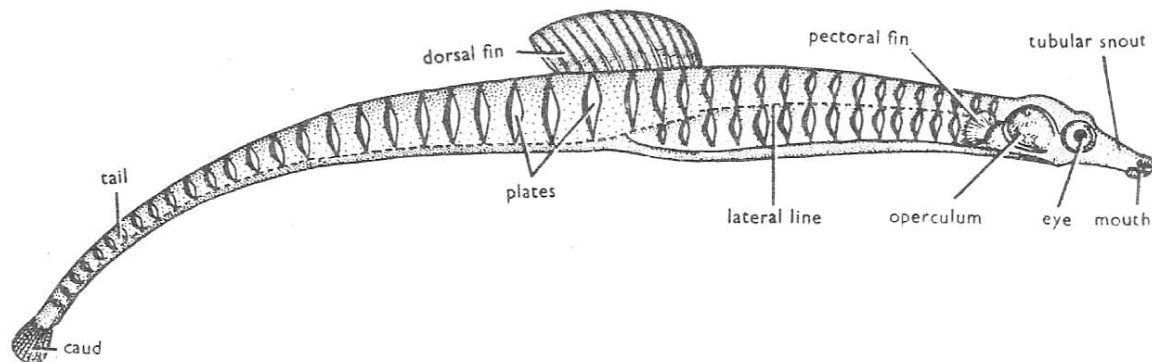


Fig. 60. *Syngnathus*.

7. Tail is long and not prehensile, with a poorly developed fin.
8. Fish swims in vertical-position.
9. Oviparous. Fertilization is external.
10. Males are provided with brood pouch on the ventral side of the abdomen formed by the fold of skin.
11. Special features: *Syngnathus* shows parental care. The young ones develop in the brood pouch till they hatch.
12. Identification: Since this fish has elongated body and above features, hence it is *Syngnathus*.
13. Geographical distribution: *Syngnathus* is distributed in Atlantic, India and Pacific oceans.
14. Habit and habitat: *Syngnathus* is marine found in brackish water and some in fresh water.

11. ANABAS (CLIMBING PERCH)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine, Opercula absent.
6. Class: Osteichthyes: Bony fishes.
7. Subclass.....: Actinopterygii.....: Ray-finned fishes.
8. Super order....: Teleostei.....: Bony fishes proper.
9. Order: Percomorphi: Perches. Dorsal and anal fins with spiny and soft fin rays.
Pectoral arch attached to skull by forked post-temporal.
10. Genus.....: *Anabas*.

Salient features

1. *Anabas* (Fig. 61) is commonly known as climbing perch.
2. Fish measuring about 30 cm is olive green in colour.
3. Body of the fish covered by cycloid scales and divided into head, trunk and tail.

4. Head is conical containing large eyes, nostrils and mouth. Jaws, prevomer and parasphenoid with fixed conical teeth.
5. In front of eyes is a pre-orbital bone containing spines. Small spines also occur along the edge of operculum.
6. Dorsal and anal fins are elongated. They are divided into anterior and posterior parts, supported by stiff spines and soft rays respectively.

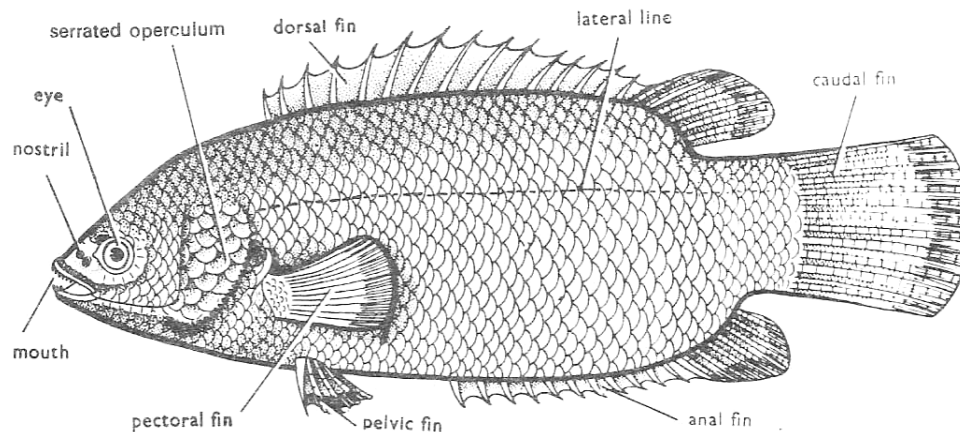


Fig. 61. *Anabas*.

7. Pelvic fins are anteriorly situated almost below pectorals.
8. Accessory respiratory super-branchial organ is well developed, having thin and folded bony laminae covered with mucous membrane. Air bladder physoclistous
9. Tail is perfectly symmetrical.
10. Special features: *Anabas* is able to walk on land by spines in search of earthworms. The crows and kites attack them and take their bodies over trees, and thus the fish is called as climbing perch, as the fish might have climbed trees. But the fish cannot climb the tree. *Anabas* is so much dependent on atmospheric oxygen that it is asphyxiated if kept in water with no access to air. Fish can live out of water for a long period.
11. Identification: Since this fish has dorsal and anal fins rays and above features, hence it is *Anabas*.
12. Geographical distribution: *Anabas* is distributed in Burma, India, Africa, Philippines, Sri Lanka and Malaya, Archipelago. Upper Cretaceous to Recent.
13. Habit and habitat: *Anabas* is a common South Indian freshwater fish. It can live out of water for a long period.
14. It is predator on shrimps, ostracods, gastropod shells and young fishes. Male exhibits parental care.

12. PROTOPTERUS

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Pisces: Endoskeleton cartilaginous. Scales usually placoid.
Spiral valve in intestine, Opercula absent.
- 6... Class: Osteichthyes: Bony fishes.
7. Subclass.....: Choanichthyes: Nostrils connected to mouth cavity. Paired fins with larger median lobe.
- 8.
- 9... Superorder....: Dipnoi.....: Lung fish. Body long and slender. Premaxilla or

maxilla absent. Air bladder lung like.

10. Order: Lepidosireniformes: Body eel-like, scales small, burrowing fish.

11. Genus.....: *Protopterus*

12. Species.....: *annectens*.

Salient features

1. *Protopterus* (Fig. 62) is commonly known as African lung Fish.
2. Body is elongated, cylindrical, eel like and is completely enclosed by small cycloid scales.
3. Head contains small eyes, nostrils and mouth.
4. The dorsal and caudal fins are continuous.
5. Pectorals and pelvic fins are reduced to slender appendages and without fin rays.
6. There are six branchial arches and five clefts.
7. Larval gills are retained as vestigial organ throughout life.
8. There are two lungs (air bladders) extending throughout body cavity.
9. Lateral line well developed. Kidneys not so elongated.
10. Larva contains four pairs of apparent external gill. In some species vestiges of these may be found in the adult just above the opening of the operculum.

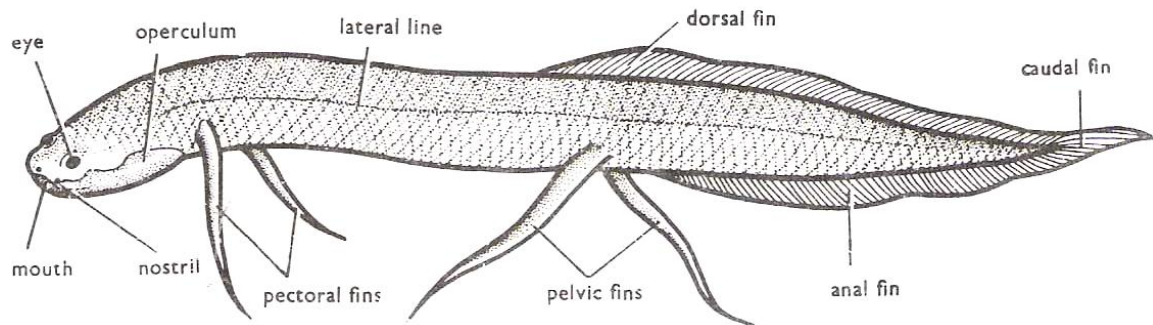


Fig. 62. *Protopterus*.

11. Special features: *Protopterus* is an air-breather fish. *Protopterus annectens* and 2 other species of Central Africa retire to burrows in mud, where mucus dries up to form "cocoon" with lid and a tube leads to mouth of the fish for breathing. It spawns after return of water. These lung fishes show combination of primitive and specialized characters. Presence of spiral valve in the intestine, cloaca, conus and unconstricted notochord are primitive characters. While lack of ossification in the cartilaginous cranium, absence of premaxillae and maxillae and presence of dental plates on jaws are specialized characters. Presence of internal nostrils, lung respiration and autostylic suspensorium show similarity with Amphibians.
12. Identification: Since this fish has slender modified appendages and above features, hence it is *Protopterus*.
13. Geographical distribution: *Protopterus* has wide distribution. It is found in the swamps of great African continent, the Nile, Congo basin, Uke Tanganyika. Devonian to Recent.
14. Habit and habitat: The fishes are adapted for burrowing life. They live in burrows made in muddy water. In dry season, during aestivation, they retire to vertical burrows (nests) in mud lined with mucus. It comes to surface to engulf the air.

AMPHIBIA

1. ICHTHYOPHIS

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Amphibia: Cold blooded. Scaleless glandular skin. Can live in water and land two occipital condyles. Heart three-chambered.
Heart three- chambered.
7. Order.....: Gymnophiona.....: Vermiform Amphibia without limbs or limb girdles.
or Apoda
8. Genus.....: *Ichthyophis*
8. Species.....: *melonosticus*.

Salient features

1. *Ichthyophis* (Fig. 63) is commonly called as caecilian.
2. Animal is worm-like and slender, measuring about 30 cm in length.
3. Body is covered with a smooth, slimy and transversely ringed skin consisting of small calcified scales arranged transverse rows. Squirt glands in skin discharge, an initiating fluid.
4. Head contains eyes, nostrils and a pair of sensory tentacles. Tympanic membrane, tympanic cavity and columella absent.

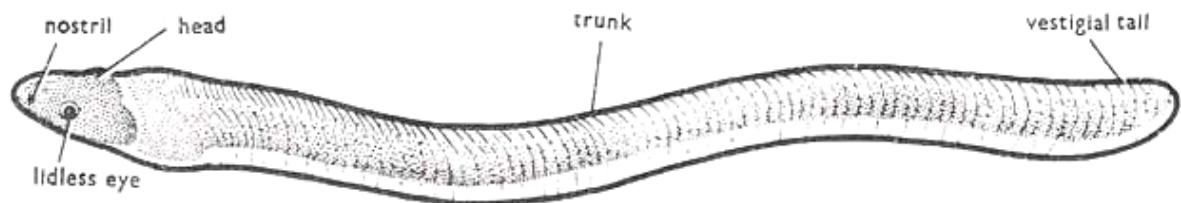


Fig. 63. *Ichthyophis*.

5. Eyes small, functionless and covered by skin. Though reduced but contain all the parts. A small protrusible.
6. Geographical distribution: *Ichthyophis* is distributed in tropical regions and found in Sri Lanka. Philippines. Borneo, Java, Seychelles, Mexico to Argentina and India (Mysore) and is the only representative of Gymnophiona living today in tropical countries.
7. Habit and habitat: *Ichthyophis* lives in burrows and leads a fossorial life in moist ground. The animal is blind and adapted for burrowing life. It feeds on invertebrates.

2. SALAMANDRA

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Amphibia: Cold blooded. Scaleless glandular skin. Can live in water and land Two occipital condyles. Heart three-chambered.
7. Order.....: Salamandroidea...: Body coloured brilliantly black with irregular patches of yellow on back and limbs.

8. Genus.....: *Salamandra*

9. Species.....: *maculosa*.

Salient features

1. *Salamandra* (Fig. 62) is commonly known as fire salamander.
2. Animal lizard like. Males measuring 12-15 cm in length. Females longer than males.
3. Body coloured brilliantly black with irregular patches of yellow on back and limbs.
4. Fore and hind limbs well developed and lift the body well above ground.
5. Head contains prominent eyes and nostrils. Eyes are provided with movable eyelids. The large paratoid glands are present behind the head.
6. Tooth-bearing extensions of prevomers along parasphenoids present.
7. Lungs present. Gills and gill clefts are absent in adults.
8. Vertebrae opisthocoelus. Inter-auricular septum perforated.
9. viviparous. The eggs develop in oviducts. Larva has gills which are shed before hatching.
10. Identification: Since this urodela has brilliancy, coloured body and above the features, hence it is fire salamander.
11. Geographical distribution: *Salamandra* is distributed in Europe, Eastern Asia and North America. Eocene to Recent.
12. Habit and habitat: Terrestrial *Salamandra* commonly inhabits under logs, stones, cracks and crevices of the old walls.

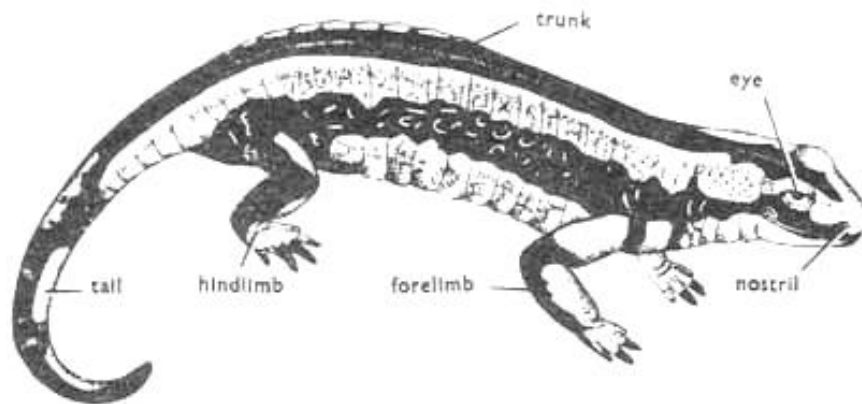


Fig. 62. *Salamandra*.

3. BUFO

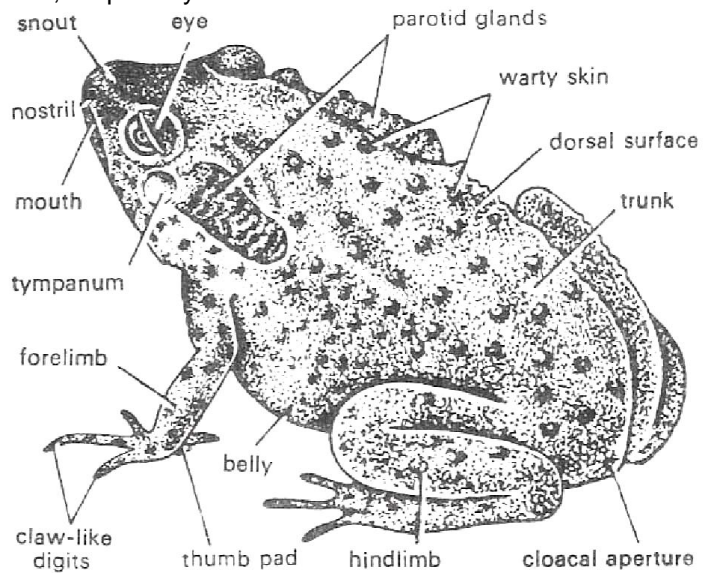
Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Amphibia: Cold blooded. Scaleless glandular skin. Can live in water and land. Two occipital condyles. Heart three-chambered.
7. Order.....: Anura.....: Scaleless Amphibia. Tail, external gills and gill-slits absent. Both hind limbs and forelimbs well developed.
8. Genus.....: *Bufo*.
9. Species.....: *melonosticus*.

Salient features

1. *Bufo* (Fig. 63) is commonly called as true **toad**.

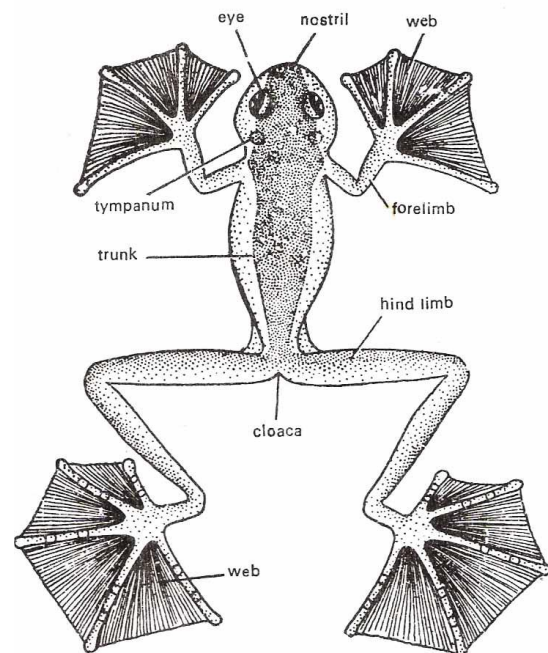
2. It differs from frog in having rough, dry and warty skin with more poison glands than mucous glands. The skin is more or less of protective nature than respiratory.
3. Body divided into head and trunk. Head contains large eyes, nostrils and tympanum.
3. Behind eyes there is a pair of large parotid poison glands.
Hind-limbs are short. Toes provided with horny tips and poorly developed webs.
4. Maxillary teeth, sternum absent and ventral parts of pectoral girdle overlap (arciferous). Sacral vertebra has dilated transverse processes. Vertebrae procoelus. Urostyle with double condyle.
5. Liver is bilobed. Glands of swammerdams absent.
6. Eggs are pigmented and laid in gelatinous string. Young toads mature in many years.
7. Special features: The parotid glands of the toad secrete two toxic substances, bufotalus and bufogus. These toxins cause nausea, respiratory and muscular disturbances and also effect heart functioning, if swallowed by man. *Bufo melanostictus* is found upto 3000 meters in the Himalayas.
8. Identification: Since this Anura contains parotid glands and above features, hence it is *Bufo*.
9. Geographical distribution: *Bufo* has world-wide distribution. They are abundantly found in India, United States and Pacific state of Alaska. Miocene.
10. Habit and habitat: *Bufo* is terrestrial, nocturnal, hiding under logs and stones or in burrows in day. It enters water only to breed and spawn.

Fig. 63. *Bufo*.

4. RHACOPHORUS (Fig. 64)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Amphibia: Cold blooded. Scaleless glandular skin. Can live in water and land. Two occipital condyles. Heart three chambered.
7. Order.....: Anura.....: Scaleless Amphibia. Tail, external gills and gill-slits absent. Both hind limbs and forelimbs well developed.

Fig. 64. *Rhacophorus*.

8. Genus.....: *Bufo*.
9. Species.....: *melonosticus*.

Salient features

1. *Rhacophoras* is commonly known as **flying frog or tree frog**. Body slender, divided into head and trunk. Belly narrows posteriorly. Females larger than males.
2. Head broad and somewhat conical.
3. Eyelids well developed. Tympanum behind eyes.
4. Limbs elongated and contain well-developed webs in digits which also bear adhesive cushions at tips. Digits of hind limbs also contain intercalary cartilage.
5. The flying frogs climb on trees and walls and occasionally glide and while alighting on ground, the webs are spread like a parachute.
6. Geographical distribution. The tree frogs or flying frogs are found in Africa, South-eastern Asia, Japan and Madagascar. Miocene.
7. Habit and habitat. *Rhacophorus* is a tree-living frog. It remains calm and quiet under stones or on trees and comes out during twilight. It has power of rapid colour changing.

REPTILIA

1. CHELONE (GREEN TURTLE)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass....: Tetrapoda.....: Paired limbs, lungs, cornified skin and bony skeleton.
6. Class.....: Reptilia.....: Scaly vertebrates. Right and left aortic arches. Single condyle.
7. Sub class.....: Anapsida.....: Primitive reptiles. Skull completely roofed over. Pulmonary respiration. Embryo with amnion and allantois.
8. Order.....: Chelonia.....: Thoracic vertebrae and ribs fused to carapace. Toes not Webbed.
9. Genus.....: *Chelone*
8. Species.....: *mydas*

Salient features

1. *Chelone mydas* (Fig. 65) is commonly called as green turtle. It measures about 110 cms.
2. Body case is rigid. Carapace flat, heart-shaped and covered with smooth bony shields.
3. Plastron is joined to carapace by ligament and osteoderms of plastron have large unossified space in the middle.
4. Dorsal shields are juxtaposed fitting closely into each other. Costal shields 4 pairs.

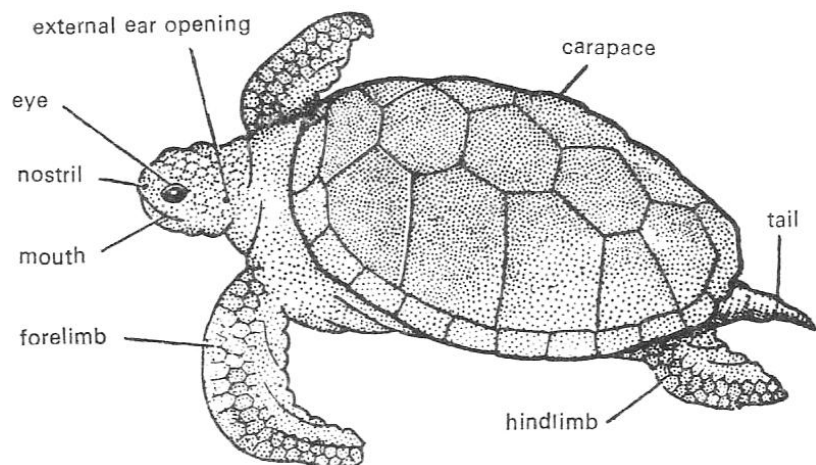


Fig. 65. *Chelone*.

5. Identification. Since this turtle has flipper-like limbs and above features, hence it is *Chelone*.
6. Head is covered by single pair of prefrontal shields. Jaws contain denticulate edges. Head is partially retractile into shell.
7. Eyes well developed, provided with eyelids and nictitating membrane.
8. Limbs adapted for swimming and flipper like. The forelimbs form wing-like paddles. Only flipper like digit is clawed while hind limbs clawed.
9. Special features: The sea turtles are economically important, because their armour is utilized for various purposes and their flesh is edible, being very delicious. *Chelone* weighs about 200 lbs. and is much valued for food.
10. Geographical distribution: *Chelone* is distributed in tropical and subtropical regions and chiefly found in the Indian. Pacific and Atlantic oceans and coasts of the United States. Upper Cretaceous to Recent.
11. Habit and habitat: *Chelone mydas* is a marine reptile. They come ashore only to lay eggs.

2. CARAPACE AND PLASTRON

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia.....: Scaly vertebrates. Right and left aortic arches.
Single condyle.
7. Sub class.....: Anapsida.....: Primitive reptiles. Skull completely roofed over. Pulmonary respiration. Embryo with amnion and allantois.
8. Order.....: Chelonia.....: Thoracic vertebrae and ribs fused to carapace. Toes not Webbed.
- . Genus.....: *Chelone*
8. Species.....: *mydas*

Salient features

Carapace

1. The upper shell of the turtle, under which the head, limbs, and tail can be more or less completely withdrawn, is called the **carapace** (Fig. 66).
2. The carapaces of tortoises are high and domed. The two-part shell is connected to the vertebrae and ribs.
3. The structure and size of the carapace and plastron vary from species to species, along with adaptive changes in behaviour and mode of life.

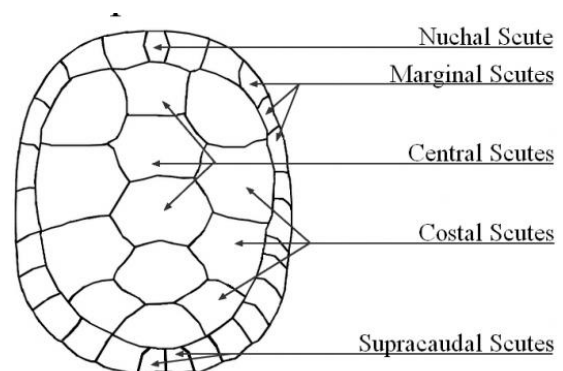
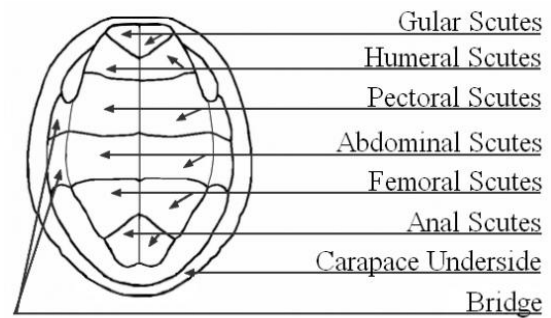


Fig. 66. Carapace.

Plastron

1. The lower shell, characteristically flat, is called the **plastron** (Fig. 67).
2. In an American family of snapping turtles, the plastron consists merely of two narrow, crossed bands is Chelydridae.
3. It is hinged, so that the front and rear parts can be brought up against the high-domed upper shell, or carapace, and closed tight.

4. The shell typically consists of two layers: an inner bony layer, the sections of which are called plates; and an overlapping, horny layer, made up of so-called shields.
5. Although it is hard, and in some species quite thick, the shell is a surprisingly sensitive structure because of the many nerves embedded in it.



3. SPHENODON (TUATARA)

67. Plastran.

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Super class....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Sub class.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Rhynchocephalia: Living fossil, lizard-like, Ribs single headed and with uncinat processes. Vertebrae amphicentrous.
9. Genus.....: *Sphenodon*
10. Genus.....: *punctatum*.

Salient features

1. *Sphenodon* (Fig. 68) is commonly called as Tuatara.
2. Animal is lizard-like having dull olive-green colour with white and yellow spots and measuring about-75 cm. Body divided into head, trunk and tail.

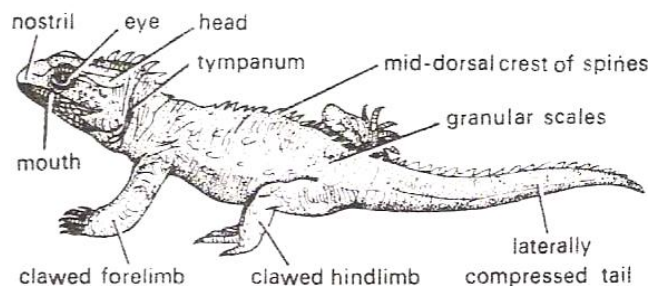


Fig. 68. *Sphenodon*.

3. It contains scaly skin, long tail and four pentadactyl limbs adapted for walking. Several crest-like, spiny scales extend along mid-dorsalline.
4. Skull contains two complete fossae, quadrate is fixed, post frontals are separate (only in *Sphenodon*) and upper jaw has beaks. Teeth acrodont. Mandibles joined by ligament. Between skull and atlas is proatlas. Sternum present and vertebrae amphicoelous. Caudal vertebrae have chevron bones.
5. There is a prominent parietal eye with retina, lens and nervous connection to brain. It is photosensitive.
6. Viscera like lizard. Abdominal ribs present.
7. Anal opening transverse. Male without copulatory organ. About 10 eggs with hard white shell are laid in holes in the ground. Eggs require 13 months to hatch.
8. Special features: *Sphenodon punctatus* is an important living fossil. It has survived from pennian and is fast approaching towards extinction. It is protected by law. The tuatara contains several primitive features, such as two temporal fossae, amphicentrous vertebrae, pineal eye, uncinat processes in the rib, vomerine teeth in young, horny beak on upper jaw and absence of copulatory apparatus in males. Tuatara is close to the type from where all diapsid reptiles might have originated.
9. Identification: Since this reptile has rows of spines on the back and above features, hence is

Sphenodon.

10. Geographical distribution: *Sphenodon* is found in New Zealand and especially in the islets of Bay of Plenty. Permian to Eocene and Recent.
11. Habit and habitat: *Sphenodon* lives in burrows, leads semiaquatic life. It is nocturnal and eats insects, molluscs or small invertebrates. It also produces frog-like croak. Breeding season November to February.

4. DRACO (FLYING LIZARD)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Subclass.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Squamata: Lizards and snakes with horny epidermal scales or shields. Quadrate bone movable. Vertebrae procoelous. Anal opening transverse.
9. Genus.....: Draco
10. Species.....: *maculalus*.

Salient features

1. *Draco* (Fig. 69) is commonly known as flying dragon or flying lizard.
2. Body is dorso-ventrally compressed, measuring 15-22 cm in length and divided into head, neck, trunk and tail.
3. Head is more or less triangular and contains eyes, tympanum behind eyes and nostril. Eyes are small with eyelids. Teeth heterodont and attached to the edges of the jaws.
4. Tongue is thick and short. Some animals have thoracic sac or dorsal spine.
5. Neck contains three hooks. Below the neck there are sac-like structures known as gular pouches, which are larger in males than females and they help in copulation.
6. Limbs are normal. Vertebrae procoelous.

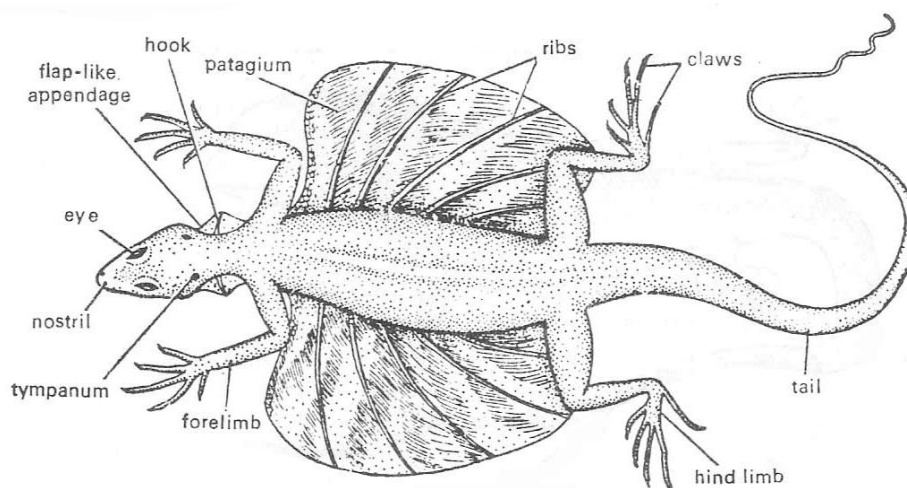


Fig. 69. *Draco*.

7. Tail long, slender and whip-like.
8. Special features: *Draco* shows extreme adaptation for flying lizards and thus avoids its enemies on the ground. Most significant structures are membranous wings or patagia, which are formed by lateral ribs used to volplane from a height. Flying lizard is adapted for climbing and gliding from higher to lower branches. *Draco* is brilliantly and beautifully coloured like flowers of trees in which it lives and thus it shows camouflage (mimicry). Identification. Since this lizard contains patagium and above features, hence it is *Draco*.
9. Geographical distribution: *Draco* is commonly distributed in Burma, India, Malaysia, Europe, Africa, Asia and Australia.
10. Habit and habitat: *Draco* is arboreal, living on trees. It feeds on small insects.

5. NAJA (COBRA)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Subclass.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Squamata: Lizards and snakes with horny epidermal scales or shields. Quadrate bone movable. Vertebrae procoelous. Anal opening transverse.
9. Suborder.....: Ophidia: Snakes. Limbs, feet, ear openings, sternum and urinary bladder are absent. Tongue is bifid and protrusible.
10. Genus.....: *Naja*
11. Species.....: *naja*.

Salient features

1. *Naja* (Fig. 70) is commonly known as cobra.
2. Body measures 2-3 metres in length and is wheatish (gehuwa) in colour. During hibernation the colour becomes golden but on exposure to light it changes to brown.
3. Head is not well differentiated from neck.
4. Neck region is dilatible with elongated ribs. It expands to form hood, which contains binocellate mark on dorsal surface. Some persons call it figure of ten. There is a white band around mark.
5. Third shield of upper labia or lip large and extending from ocular to nasal shield. Ocular shield bears eye and nasal shield bears opening of nostril. Eyes with narrow pupils.
6. A tiny wedge-shield on the undersurface of the 4th and 5th lower labials.
7. Tail shields on the undersurface of the tail in a double row. Body is covered by smooth oblique scales.
8. Maxillary bone extends beyond palatine. Poison fangs are followed by 1-3 small teeth. Loreal absent. Nostrils large and vertically elliptical. Frontal shield truncated.
9. Special features: Cobras are deadly poisonous snakes. They rise their hood when

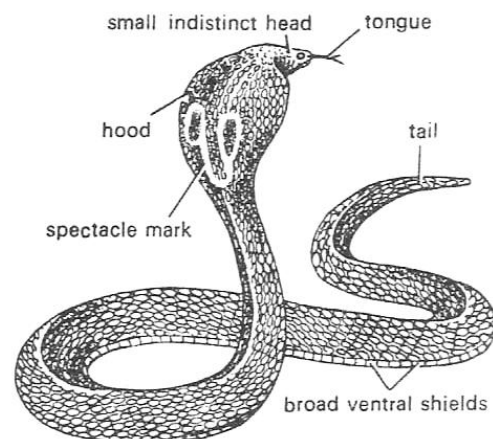


Fig. 70. *Naja*.

alarmed and the hood sways back and forth for striking the object. During this period it produces hissing sound. It will not strike if intruder becomes standstill. The snake-bite cases should be immediately attended by medical persons. The snake (*Naja naja*) is very common in India. It is worshipped on Nagpanchami day. The cobra bite is cured sometimes villages by snake charmers by Mantra or sometimes by sucking poison out of the wound. The presence of a jewel in the head is not correct. *Naja bungarus* is called as King cobra because it eats other cobras.

10. Identification: Since this snake has characteristic hood and its mark and above features, hence it is *Naja*.
11. Geographical distribution: *Naja* has wide distribution, found in India, Africa, China, Philippines, Tasmania, Australia, New Guinea and Egypt.
12. Habit and habitat: Cobra is diurnal, shy, living in holes, under stones, mud walls and in thick vegetation. It is oviparous, carnivorous and feeds on frogs, rats, lizards and other snakes. It hibernates. Three varieties of cobras are found in India:
 - a. Binocellate form having spectacle-like mark connected by U, found in Maharashtra.
 - b. Monocellate with single oval mark surrounded by ellipses found in Bengal.
 - c. Non-cellate without mark found in Rajasthan, Gujrat and Madhya Pradesh.

6. KRAIT (*BUNGARUS*)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Subclass.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Squamata: Lizards and snakes with horny epidermal scales or shields. Quadrate bone movable. Vertebrae procoelous. Anal opening transverse.
9. Suborder.....: Ophidia: Snakes. Limbs, feet, ear openings, sternum and urinary bladder are absent. Tongue is bifid and protrusible.
10. Genus.....: *Bungarus*.

Salient features

1. *Bungarus* is commonly called as Krait (Fig. 71).
2. Body is elongated and cylindrical, measuring one metre in length.
3. Colour of body steel-blue and dark-blue. Dark-blue patches alternate with white cross bars.
4. Head is not differentiated from the neck. Loreal absent. Post-ocular, preocular and supra-labial 2, 1 and 7 in number respectively. Fangs small.
5. Eyes are of moderate size with round pupils. Tongue bifid and protrusible.
6. Scales are smooth forming 13-17 rows. Ventrals are 194-234 and caudals 42-52.
7. Enlarged chain of hexagonal scales is present on mid-dorsal side. Ventral scales beyond the anal region are in a single row.
8. Oviparous. Female shows parental care.
9. Special features: *Bungarus* is a deadly poisonous snake, its venom being more poisonous than that of cobra. Its venom is neurotoxic affecting brain. After an hour of the bite, the victim feels sleepy and if immediate antivenom is not given, the patient may die.

10. Identification: Since this snake contains hexagonal scales on body on dorsal side and above features, hence it is *Bungarus*.
11. Geographical distribution: *Bungarus* is found in South-east Asia, all over India and Malaya.
12. Habit and habitat: It is a common snake, found in the crevices of walls, under the logs and stones. It is nocturnal and feeds on smaller snakes, toads and mice. It is shy and considerate. It attacks only when disturbed or trodden with foot.

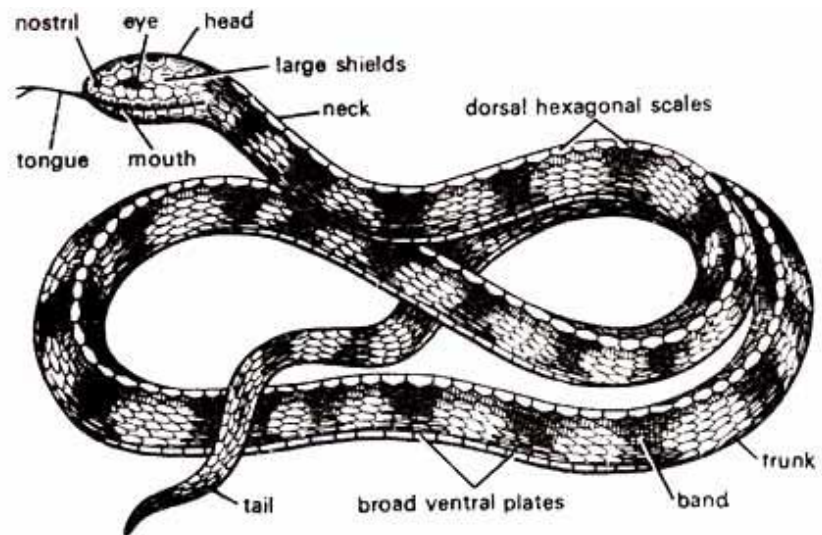


Fig. 71. Krait.

7. VIPER

Salient features

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Subclass.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Squamata: Lizards and snakes with horny epidermal scales or shields. Quadrate bone movable. Vertebrae procoelous. Anal opening transverse.
9. Suborder.....: Ophidia: Snakes. Limbs, feet, ear openings, sternum and urinary bladder are absent. Tongue is bifid and protrusible.
10. Genus.....: *Vipera*
11. Species.....: *ruselli*

Salient features

1. *Viper* (Fig. 72) is commonly called in Hindi as Kadar or Dobia.
2. *Vipera ruselli* is also a common poisonous snake and is often called as pitless viper.
3. Body measures 2 meters in length. Head large, flat, triangular and covered with small scales. A 'V' mark placed overhead. Shields on undersurface of tail subdivided.
4. Colour is brownish but it varies according to its environment. Body is covered with keeled scales. Large black spots arranged on the back.
5. Facial bones movable, maxilla is small and contains long and movable poison fangs with canals.
6. Paired erectile fangs in front of upper jaw, one on each maxillary bone and folded backward when not in use (solenoglypha).
7. Maxillaries short, thick and movable in vertical plane.

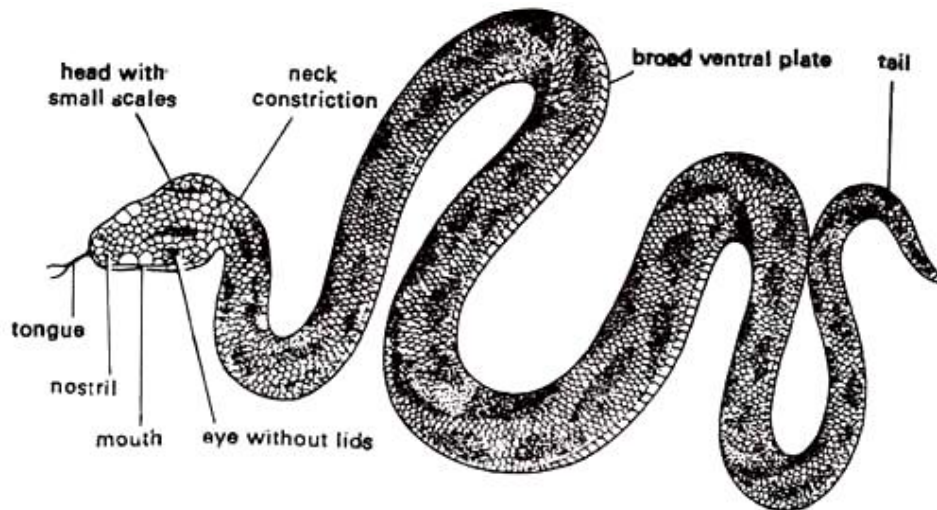


Fig. 72. Viper.

8. No pit between nostril and eye.
9. Supra-Labials are 10- 12. 4Lh supralabial is largest and it does not reach up to eye. Ventral plates large.
10. Snout is angulate, nasal opening prominent and largest and eyes have white margin with golden iris and elliptical pupil.
11. Special features. The snake remains coiled with the head in the centre of the coil and with least provocation or disturbance, tongue is protruded, body rhythmically swells and hissing sound is produced. Its bite is fierce and
12. it strikes to one side with a sudden and forceful spring. Before striking it hisses loudly. Its bite is fatal to man.
13. Its native name is Sus-Karna symbolizing its powerful hissing.
14. Identification. Since the snake contains characteristic ventral shields and above features, hence it is *Vipera*.
15. Geographical distribution. Vipers are Old World snakes except Madagascar. Viper has been reported from Europe, Asia, Sri Lanka, Burma and India.
16. Habit and habitat: It is found in rocky and bushy regions. It feeds on mice, rats, lizards and birds.

8. CROCODILE (MUGGER)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Reptilia: Scaly vertebrates. Right and left aortic arches. Single condyle. Pulmonary respiration. Embryo with amnion and allantois.
7. Subclass.....: Diapsida: Paired limbs, lungs cornified skin and bony skeleton.
8. Order.....: Crocodylia.....: Body and head large. Jaws powerful. Skin thick and leathery. Tongue non-protrusible. Heart 4-chambered.
9. Genus.....: *Crocodylus* (*Magannach*).

Salient features

1. *Crocodylus* is commonly known as Crocodile (Fig. 73) or Mugger.

2. Body is stout, elongated, 4-6 metres in length and divided into head, trunk and tail.
3. Surface covered by leathery armour of osteoscutes arranged in transverse rows.
4. Upper part of the body is dark olive brown with black spots or bends.
5. Its head long and triangular and narrows towards snout which is not differentiated from the rest of the skull. Jaws long, powerful, rimmed with numerous bluntly conical and unequal teeth, dental formula 16-19/14-15. The first tooth fits into a pit and fifth mandibular tooth into a notch on the outer side of upper jaw.
6. Ear opening small and protected by a small flap of skin.
7. Tongue not protrusible.
8. Tail long, heavy and laterally compressed.
9. Fore and hind limbs short and pentadactyle, with 5 fingers and 4 toes, ending in claws and with webs.
10. Heart 4-chambered with separate ventricles. Bladder absent
11. Special features Crocodile is dangerous to mankind. It can eat the man.
12. Identifications: Since this reptile has long and pointed snout with conical teeth and above features, hence it is *Crocodylus*.
13. Geographical distribution: *Crocodylus* is found in Southern Asia. Africa, Australia, Central America and India. Triassic to Recent
14. Habit and habitat: It is found in rivers and lakes. The animal makes 10-13 metres long tunnel below the level of water. The opening of the tunnel is used as entrance and the other side is used to deposit the eggs.

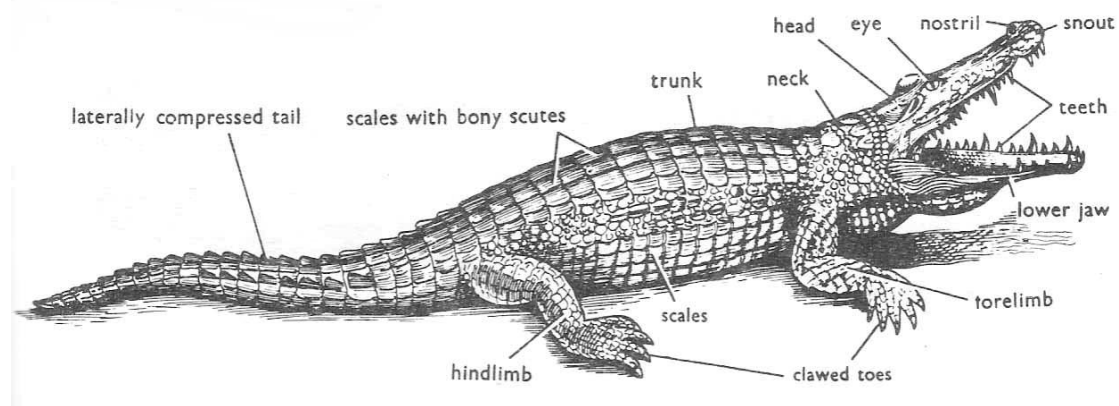


Fig. 73. Crocodile.

AVES

1. PIGEON (COLUMBA)

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Aves: Biped and feathered vertebrates.
7. Subclass.....: Neornithes: True birds. Metacarpals fused.
8. Order.....: Columbiformes: Modern birds, no teeth, sternum keeled.
9. Genus.....: *Columba*
10. Species.....: *livia*.

Salient features

1. *Columba* is commonly called as blue-rock pigeon (Fig. 74) and Kabutar in Hindi.
2. Body is divisible into head, neck, trunk and tail.
3. Plumage is grey with glistening metallic green and purple on breast and neck.
4. Head contains large eyes and slit-like nostrils. It is produced into a short and slender bill or beak. Upper and lower beaks are covered by the horny sheath, called rhamphotheca. At the base of the upper beaks there is a patch of skin called cere.
5. Eyes are large, rounded, with a well developed nictitating membrane and a rounded pupil.
6. Forelimbs are modified into Wings which contain besides skeleton flight feathers called as remiges. Feet are covered with epidermal scutes formed by the fusion of several reptilian epidermal scales.
7. Hind limbs are modified for bipedal locomotion. Tarsus usually shorter than toes.
8. Eggs white and unmarked.
9. Special features: Pigeons are the most common domesticated birds, which were in olden times used as messenger. Man also eats them. Their call notes are very familiar to man as *gootr-goon, gootr-goon*. Pigeons serve as an excellent example for artificial selection of Darwins theory or evolution as various varieties have been produced by man. Crop large, producing "pigeon milk" to feed small young.
10. Geographical distribution: *Columba* is commonly found in India, forested zone of the Pacific coast and United States. Eocene to Recent.
11. Habit and habitat: *Columba livia* is the most common and familiar bird around man, nesting in buildings, old houses, warehouses, sheds and railway stations. Their flight is swift and strong. Breeding continues throughout the year.

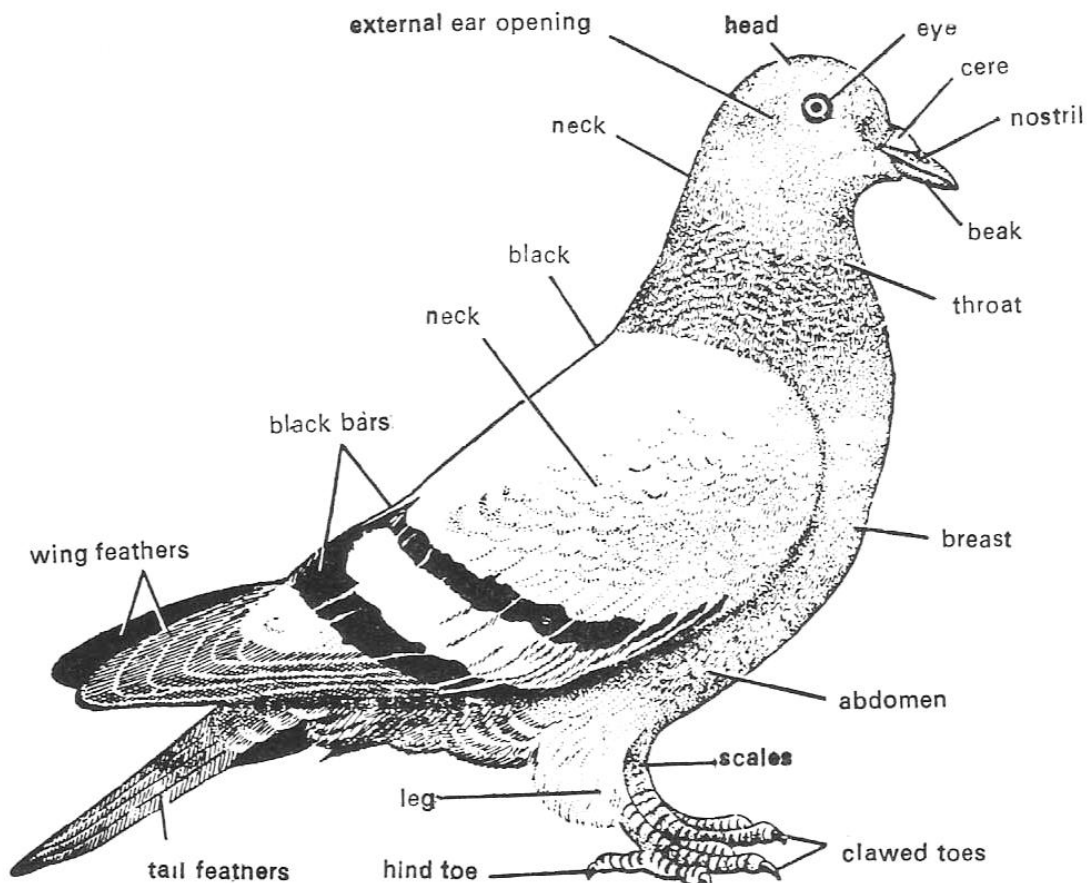


Fig. 74. Pigeon.

2. PARROT

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Aves: Biped and feathered vertebrates.
7. Subclass....: Neornithes: True birds. Metacarpals fused.
8. Order.....: Psittaciformes: Blue-green plumage with massive, deeply-hooked red bill.
9. Genus.....: *Psittacula*
10. Species.....: *eupatria*.

Salient features

1. *Psittacula* the Indian parakeet or parrot (Fig. 75). Its Hindi name is Hiranmanjaya.
2. It has brilliant blue-green plumage with massive, deeply hooked red bill and a distinct maroon patch on each shoulder. *P. krameri* has no shoulder patches, while *P. cyanocephala* has a bluish-red head and maroon shoulder patches.
3. Beak stout narrow sharp edged and hooked at the tip.
4. Upper mandible movable on frontal bone of skull. It is so articulated that its lowering automatically raises the upper beak, which is curved at the tip.
5. Feet adapted for grasping holding and climbing. Foot zygodactylous in which I and IV digits are directed backwards and II and III forward to provide a firm grip on the branch of the tree.
6. Tail feathers elongated.
7. Flight is graceful and voice powerful.
8. Female is green all over, but the male has a rose pink collar and a black throat.
9. Nesting season December to April.
10. Special features: Parrot is a popular domesticated cage bird found almost in every home and it copies and speaks some words like man. It is a serious agricultural pest to the cultivators and food growers. It causes enormous.
11. Habit and habitat: It lives in flocks in the bustling precincts of a city as much as in the countryside. It is commonly found in the fruit trees ripe crops and in jungles. Gregarious with loud voices. Feeds on fruits and crops.
12. Geographical distribution: *Psittacula eupatria* is found in India, Pakistan, Burma, Sri Lanka and the United States. Eocene to Recent.

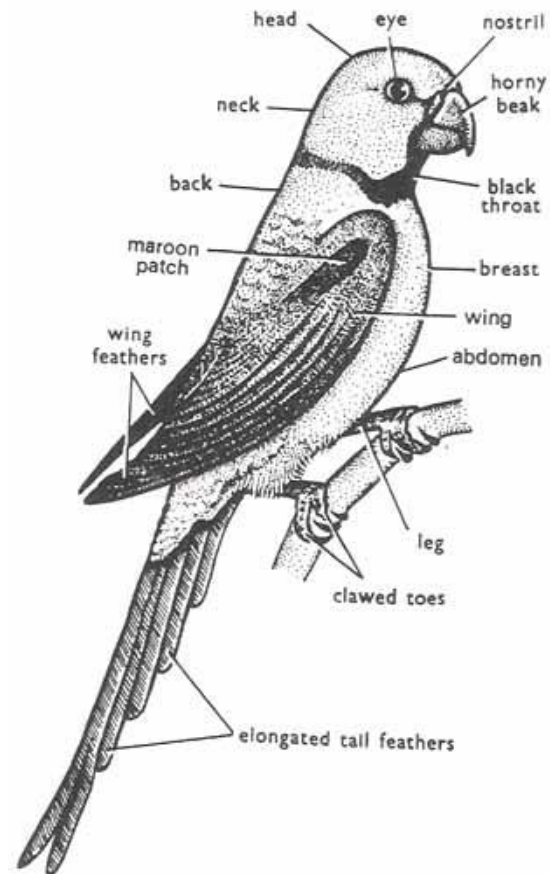


Fig. 75. Parrot.

3. OWL

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.

2. Group.....: Craniata :Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata :Vertebral column present.
4. Division.....:Gnathostomata..... :Jaws and paired appendages present.
5. Superclass.....:Tetrapoda..... :Paired limbs, lungs cornified skin and bony skeleton.
6. Class: Aves..... :Biped and feathered vertebrates.
7. Subclass.....:Neornithes.....:True birds. Metacarpals fused.
8. Superorder.....:Neognathae..... :Modern birds, no teeth. sternum keeled.
9. Order:Strigiformes.....: Large rounded head, eyes and long horns.
10. Genus.....: *Bubo*.
11. Species.....: *bubo*.

Salient features

1. *Bubo* (Fig. 76) is commonly called the great horned owl. In Hindi it is known as Ghughu.
2. It is a fierce looking large owl with large rounded head, huge orange gold eyes and long horns or ears. Plumage soft textured.
3. Bird is heavily built with dark brown back mottled and spotted with buff. The dark brown underside is streaked.
4. Beak short. Eyes are large, yellow and forwardly directed, each in a disk of radial feathers.
5. Ear opening large, often with flap-like cover, sometimes asymmetrical.
6. Legs are fully feathered. Feet adapted for grasping; claws sharp.
7. Nesting season November to April.
8. Special features: *Bubo bubo* is of great economic value to mankind by destroying the harmful animals like rats and mice and these birds need careful protection. Soon after sunset, they produce deep soothing prolonged voice *bubo*.

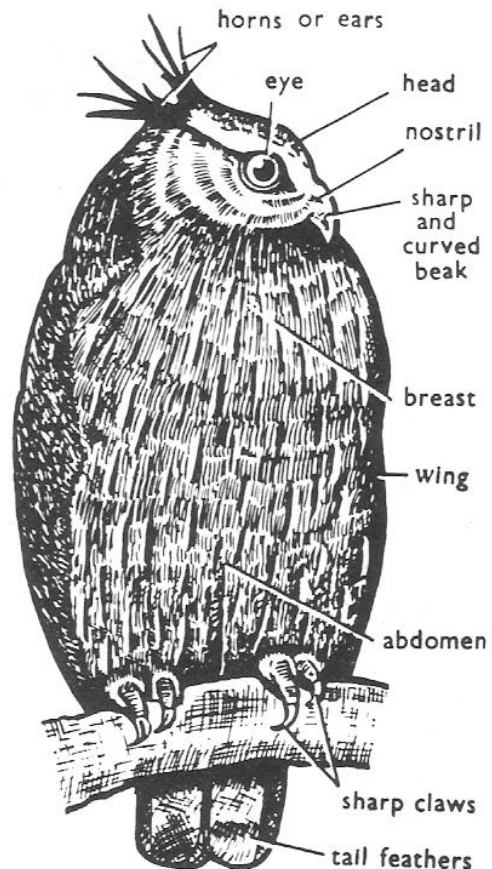


Fig. 76. Owl.

9. Identification: Since this bird has large forwardly directed eyes and above features, hence it is *Bubo*.
10. Geographical distribution: *Bubo bubo* has worldwide distribution, specially found in India, Pakistan and Burma.
11. Habit and habitat: *Bubo bubo* is a nocturnal bird, living in woody places and avoids heavy forests. It feeds on small mammals, rodents, birds, lizards and other animals. It hides in retreat in day.

4. SPARROW

Classification

1. Phylum.....: Chordata :Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata.....: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata.....: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Subclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class.....: Aves: Biped and feathered vertebrates.

7. Subclass.....: Neognathae: True birds. Metacarpals fused.
8. Order.....: Passeriformes: Beak is short and conical head.
9. Genus.....: *Passer*.
10. Species.....: *domesticus*.

Salient features

1. It is the common house sparrow (Fig. 77). Its Hindi name is Gauriyya.
2. It is a small bird measuring 10-16 cm in length.
3. Sexual dimorphism is distinct. Female is ash white, while male is earthy brown with blackish throat and breast and white abdomen.
4. Eyes small and the beak is short and conical head.
5. Feet adapted for perching. Toes 3 in front and 1 behind.
6. Nesting practically throughout the year. Some make elaborate nests; lay 3-8 eggs.
7. Young naked and blind at hatching, require feeding and care by parents before becoming independent.
8. Special features: The sparrows are both useful and harmful to mankind. They destroy several agricultural pests. They destroy vegetable and flower buds.
9. Identification: Since this bird has earthy brown body and above features, hence it is male *Passer domesticus*.
10. Habit and habitat: Sparrow is the most familiar companion bird freely moving and nesting in the houses. They act as commensal to man. In winter they feed on cultivated areas in flocks. They are gregarious.
11. Geographical distribution: Sparrow is abundantly found in Sri Lanka. Burma, Pakistan and India, and has been introduced to N. America, South America, Australia and New Zea land. Eocene to Recent.

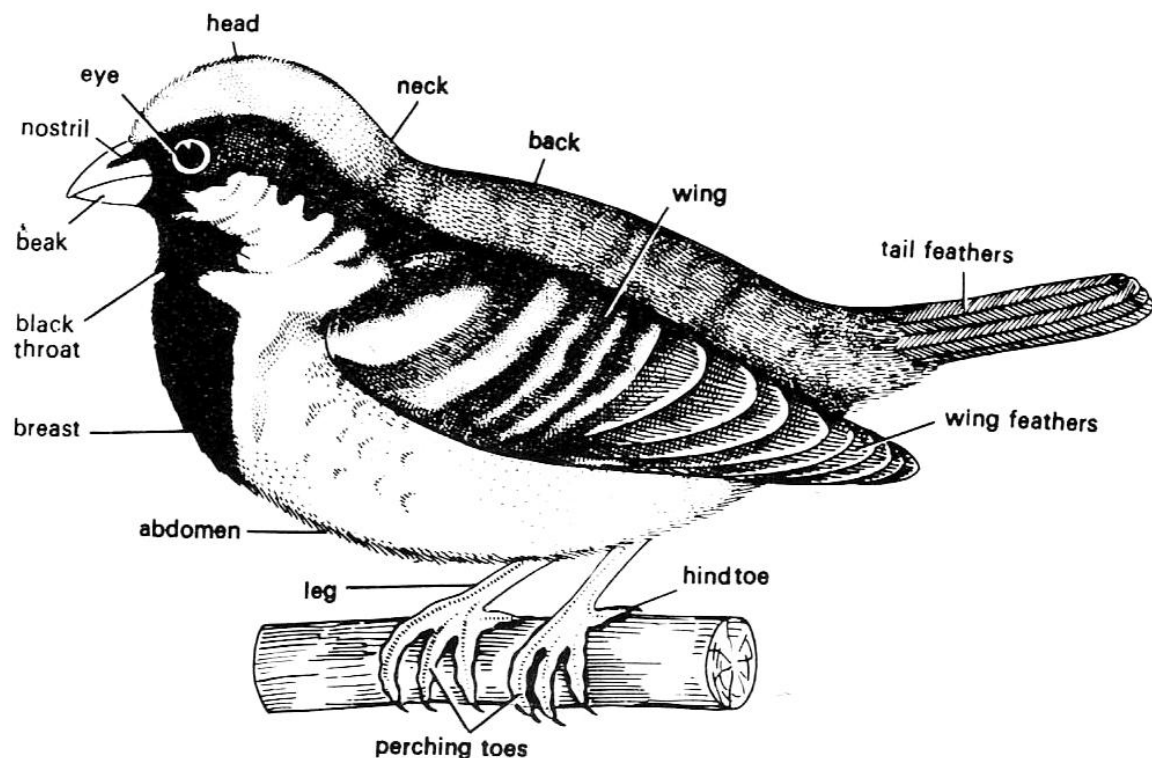


Fig. 77. Sparrow.

MAMMALS

1. BAT

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata.....: Jaws and paired appendages present.
5. Superclass.....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class: Mammalia.....: Biped and feathered vertebrates. Body covered with hair.

Females have mammary glands.

7. Subclass.....: Theria: Viviparous mammals.
8. Superorder.....: Eutheria: Placental mammals. Vagina single.
9. Order: Chiroptera.....: Skin modified into wing.
10. Species.....: *pteropus*

Salient features

1. *Pteropus* is commonly called **Fruit bat** (Fig. 78) or **Flying fox** and in Hindi Chamgadar.
2. Body is dark-brown coloured and shoulders are golden yellow.
3. It is capable of true flight. The forelimbs are modified into wings.
4. Each wing formed by a fold of skin or patagium. supported by elongated forelimb and 2nd to 5th fingers. Only 1st and 2nd fingers bear claws.

Hindlimb and tail also included in patagium. Hind feet small with sharp and curved claws. Tail small and stumpy.

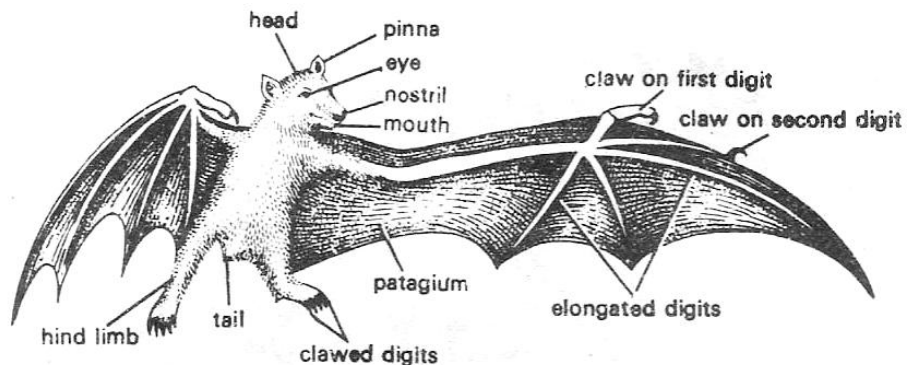


Fig. 78. Bat.

5. Head small having small external ears, large eyes, snout and small teeth.
6. During sleep, head hangs downwards with wings folded clock-like around body.
7. Special features: Bats are important due to five reasons:
 - (i) They have phylogenetic significance with insectivores.
 - (ii) They are the only flying mammals.
 - (iii) They are used for experimental purposes.
 - (iv) Faeces of bats are used as fertilizer.
 - (v) Bats have highly developed Sonar or Echo apparatus, a kind of radar. While flying, they constantly send out ultrasonic sound waves consisting of periodic clicks, which strike on objects or wire and are reflected back to bat. Rate of click increases 50-150 seconds as the object is approached. Ultrasonic sounds are produced from the vocal cords.
8. Identification: By Patagium.
9. Geographical distribution: Found in South-eastern Asia, especially in India. Policene to Recent.
10. Habit and habitat: Adapted for arboreal and aerial mode of life. Live in groups and feed on fruits (frugivorous) and often damage.

2. RABBIT

Classification

1. Phylum.....: Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata: Definite head. Cranium with brain present.
3. Subphylum....: Vertebrata: Vertebral column present.
4. Division.....: Gnathostomata....: Jaws and paired appendages present.
5. Superclass....: Tetrapoda.....: Paired limbs, lungs cornified skin and bony skeleton.
6. Class: Mammalia.....: Body covered with hair. Females have mammary glands.
7. Subclass.....: Theria: Viviparous mammals.
8. Infraclass: Eutherla: Placental mammals. Vagina single.
9. Order: Lagomorpha.....: Upper incisors 2 pair.
10. Genus.....: *Oryctolagus*
11. Species.....: *cuniculus*

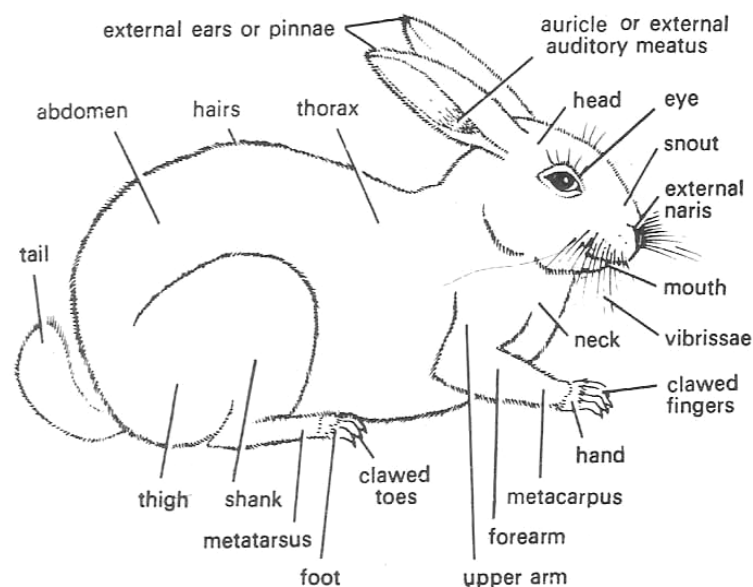


Fig. 79. Rabbit.

Salient features

1. Commonly called as Rabbit (Fig. 79).
2. Body cat-like and divisible into head, neck, trunk and tail
3. Head contains long tactile vibrissae or whiskers, external nares, usually shorter eyes and mouth. External ears drooping.
4. Length 40 cm from mouth to anus.
5. Fur colour white, black, brown or spotted.
6. Males have muscular skin-covered penis.
7. Females have clitoris.
8. Forelimbs used for digging and hind limbs for leaping. Fast runner (30-40 km per hour).
9. Economic value: Fur of rabbit is used to make purses, gloves and caps. Some varieties used as food. They also form important experiment animal for study and research. Most of chordate anatomy is based on rabbit.
10. Geographical distribution: Cosmopolitan.
11. Habit and habitat: Inhabiting fields, grasslands and woodlands. Gregarious, crepuscular (coming out of burrows for feeding in twilight), coprophagous (eating again their soft stool for maximum nourishment and polygamous).

3. RATTUS (BLACK RAT)

Classification

1. Phylum.....:Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata : Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata : Vertebral column present.
4. Division.....:Gnathostomata..... : Jaws and paired appendages present.
5. Superclass.....:Tetrapoda..... : Paired limbs, lungs cornified skin and bony skeleton.
6. Class: Mammalia..... : Body covered with hair. Females have mammary glands.
7. Subclass.....:Theria: Viviparous mammals.
8. Infraclass:Eutherla : Placental mammals. Vagina single.
9. Order: Rodentia: Having one pair of upper incisors.
10. Genus.....:*Rattus*
11. Species.....:*rattus*.

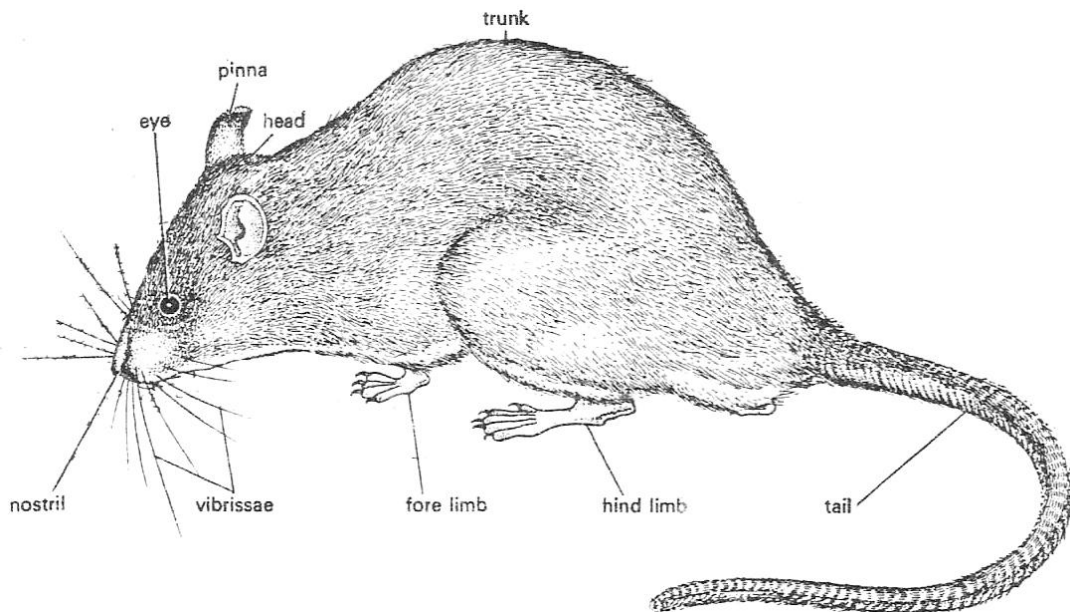


Fig. 80. Rat.

Salient features

1. *Rattus rattus* is commonly called as black rat (Fig. 80). It is considered a parasite of man.
2. Body covered with black hairs.
3. Pinnae well developed. Eyes sharp.
4. Tail is elongated and scaly.
5. Incisor teeth chisel-like, open-rooted, used for gnawing. Canines absent.
6. Viviparous.
7. Special features: Rat destroys the crop and stored grains. It also spreads typhus fever and plague. It acts as carrier of these diseases. Rat has great experimental value. It is largely used in various biophysical and biochemical studies.
8. Geographical distribution: *Rattus rattus* is found in all parts of the world. It prefers warmer and drier conditions. Eocene to Recent.
9. Habit and habitat: It is a common rat inhabiting holes and burrows in houses and in cultivated fields. It feeds on stored grains.

4. LORIS

Classification

1. Phylum.....:Chordata: Dorsal tubular nerve cord, notochord, and gill-slits present.
2. Group.....: Craniata : Definite head. Cranium with brain present.
3. Subphylum.....: Vertebrata : Vertebral column present.
4. Division.....:Gnathostomata..... : Jaws and paired appendages present.
5. Super class.....:Tetrapoda..... ..: Paired limbs, lungs cornified skin and bony skeleton.
6. Class:Mammalia..... : Body covered with hair. Females have mammary glands.
7. Sub class.....: Theria: Viviparous mammals.
8. Super order.....: Eutheria: Placental mammals. Vagina single.
9. Order: Primates: Bipedal. Head turn easily on neck
10. Genus:.....: *Loris*
11. Species.....: *tardigradus*.

Salient features

1. Body of *Loris* (Fig. 81).is covered with brownish fur with silver look. Fur is thick and woolly.
2. Head small and produced into snout.
3. Eyes are closely placed. They are very distinct and bulging.
4. External ear or pinna is conical.
5. Nostrils in the form of small apertures.
6. Teeth thecodont and heterodont.
7. Tail long but not prehensile.
8. Limbs elongated. Some toes clawed, others with flat nails. Locomotion remarkably slow. It is often found hanging upside down.
9. They seem to be survival of an earlier stock.
10. Special features: In Lorises traces of very early features remain, including a transverse fold of skin on abdomen of the female, which is considered by some to represent a marsupium. They show some features that recall the higher primates. For example, the tympanic ring is fused "to the petrosal bulla. The face in some is shorter and brain rounder than in true lemurs.
11. Geographical distribution: Lorises are found outside Madagascar and especially in India and Sri Lanka. Pliocene to Recent.
12. Habit and habitat: *Loris* is solitary, nocturnal and arboreal primate.

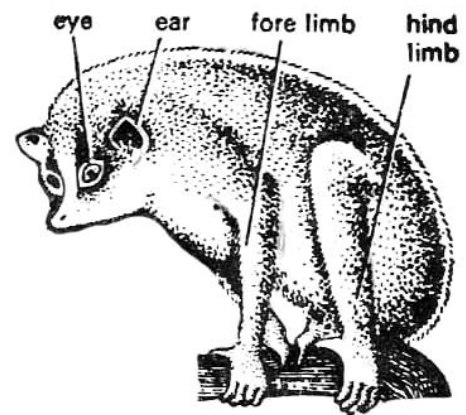


Fig. 81. *Loris*.

II. MOUNTING

1. PARAPODIUM OF NEREIS

1. Parapodium (Fig. 82) is lateral fleshy-lobed outgrowth of body wall of *Nereis*.

2. Each parapodium is a biramous organ consisting of basal portion bearing a dorsal blade, the **notopodium** and a ventral portion, the **neuropodium**.

3. Notopodium has a thin vascular dorsal lobe, respiratory in function and bears dorsal cirrus and a bundle of long setae.

1. Neuropodium also bears a ventral cirrus and similar bundle of setae.

2. The notopodial and neuropodial setae are embedded in the setigerous sacs.

3. Notopodium and neuropodium are supported by dark coloured thick chitinous internal rod called **aciculum**.

4. Parapodia serve as **locomotory and respiratory organs**.

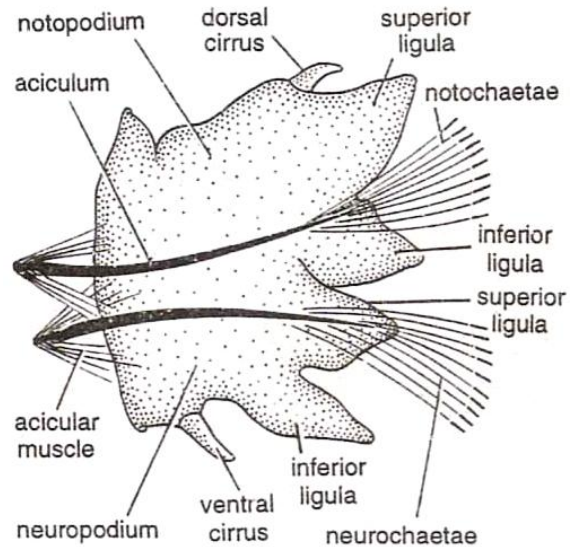


Fig. 82 Parapodium of *Nereis*.

2. MOUTHPARTS OF LEPAS

1. **Mandibles:** The mandibles (Fig. 83) lie on either side of the mouth. Each mandible has a protopodite and the protopodite has calcified teeth. The endopodite is a double segmented process known as palp. There is no exopodite. The mandibles are **mastigatory in function**. While biting, the mandibles move from side to side and not up and down.

2. **First maxillae or maxillulae:** These are small appendages. Each maxilla consists of a protopodite of two flattened leaf-like lobes called gnathobases (Gnathos = jaws). The gnathobases are covered with minute setose hairs. The endopodite of each maxilla is slender and is made up of two segments. The exopodite is absent. The first maxilla is **used to pass food to the mouth**.

4. **Second maxillae:** The protopodite of the second maxilla, is flat and cut into four leaf-like lobes. The leaf-like lobes point towards the mouth, forming the gnathobases or jaws. The exopodite is modified into a broad lobe-like structure called scaphognathite. The endopodite is small and unsegmented. The second, maxilla has two functions, namely **feeding and respiration**.

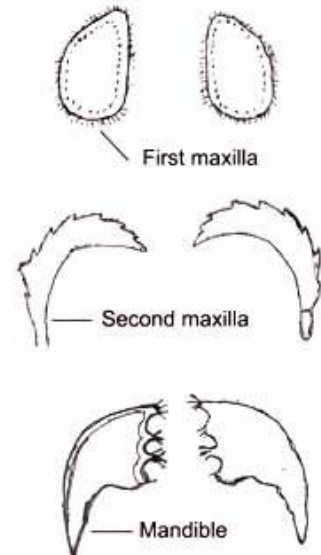


Fig. 83. Mouth parts of *Lepas*.

3. STING OF HONEYBEE

1. Sting (Fig. 84) of honey bee is a complicated structure.

2. It consists of two stylets articulated along their length to the hollow stylet sheath by a groove and rail arrangement.

3. The stylets are held in place by this arrangement and they can move only up and down.

4. The stylets and their sheaths bear barbs at the tips to make a wound.

5. Proximally the stylet sheaths have a dilated bulb.
6. Attached to the stylets proximally is median poison sac into which opens two acid glands and one alkaline gland.
7. The sting has a set of three chitinous plates on either side to serve as levers. The plates are (i) oblong plate, (ii) triangular plate and (iii) quadrangular plate.
8. In stinging, the muscles of plates drive the stylets and stylet sheath into a victim, the secretions of two types of glands mix and pass down the poison canal into the wound.
9. The sheath serves to guide the darts to open up the wound and to aid in conducting the poison.
10. The poison is secreted in acid and alkaline glands and stored in a poison sac.
11. The sting is a modified ovipositor, hence, found in queen and female worker bees only; absent in drones.
12. Queen bee uses it many times for stinging rival queens, while worker bees use their sting as a last resort because this always results in their death.

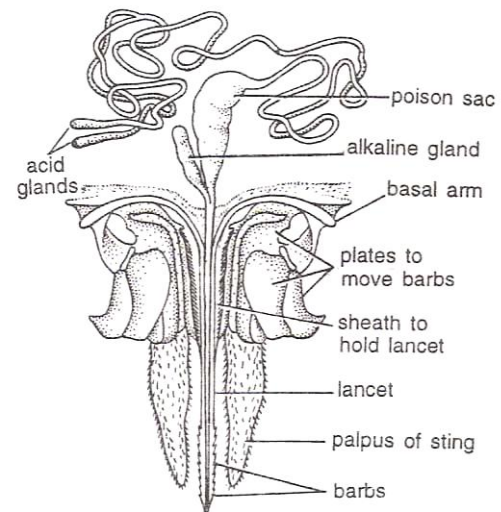


Fig. 84. Sting of Honey bee.

4. PEDICELLARIAE OF SEA URCHIN

1. **Pedicellariae** (Fig. 85) are minute structures found in abundance between the bases of spines and on the peristome.
2. Each pedicellaria consists of head composed of three movable jaws, mounted on a **stalk**.

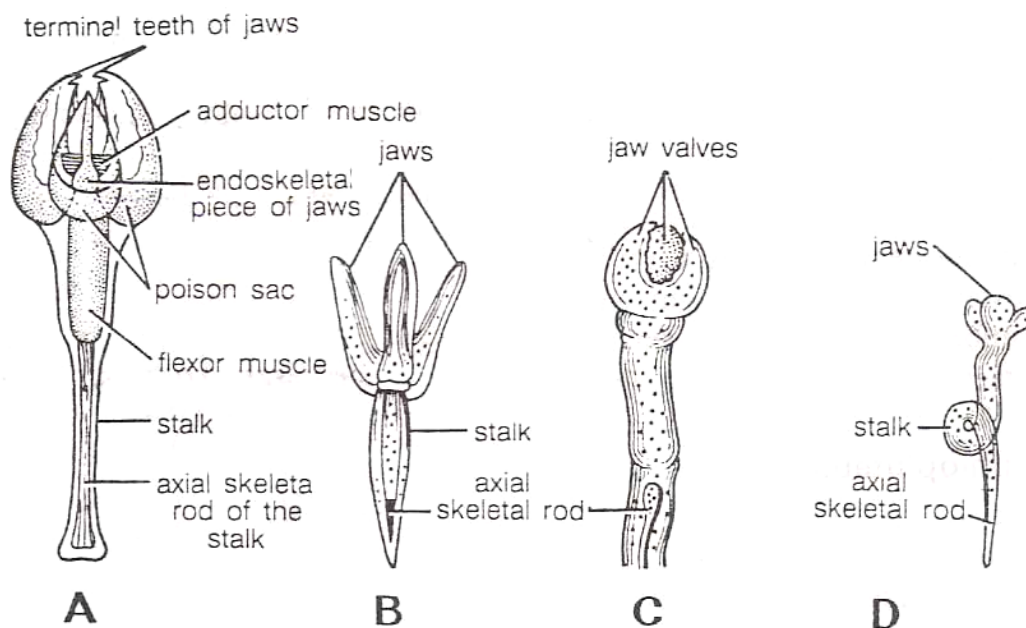


Fig. 85. Pedicellariae of sea-urchin.

(A - Gemmiform pedicellaria, B - Tridactyle pedicellaria, C-Ophiocephalouspedicellaria, D-Trifoliatepedicellaria).

3. Following types of pedicellariae are found in sea urchins:
 - a. **Gemmiform pedicellariae** are composed of stiff stalks and globular heads with a poison sac in each jaw.
 - b. **Tridactyle pedicellariae** are the largest and very common type. These have flexible stalks and elongated, tapering and serrated jaws.
 - c. **Ophiocephalous pedicellariae** are smaller. Each consists of a flexible stalk and short broad and toothed jaws having blunt tips.
 - d. **Trifoliate pedicellariae** are the smallest. Each consists of a flexible stalk and short.
4. **The function of pedicellariae** is to remove debris, bite intruders and keep the body clean.

5. SCALES OF TELEOST

A. Cycloid Scales

1. Cycloid scales (Fig. 86) are found in carps (teleost). They are located in the dermal pockets and possess concentric lines of growth.
2. Each cycloid scale is roughly circular in outline without pulp cavity and with free and smooth border.
3. Scales covering lateral line are frequently perforated penning the passage of small connectives of lateral line to outside.
4. Cycloid scales are derived from of ganoid scales in which ganoin, cosmine and bone cells are lost.
5. Scales are soft, arranged lengthwise in diagonal rows.

Identification: The above scale contains concentric lines of growth. Hence it is cycloid scales.

B. Ctenoid Scales

1. Ctenoid scales (Fig. 87) are commonly found in most teleost and actinopterygian fishes.
2. They are thin, soft and dermal translucent plates.
3. They are composed of underlying fibrous layer covered by bone-resembling layer.

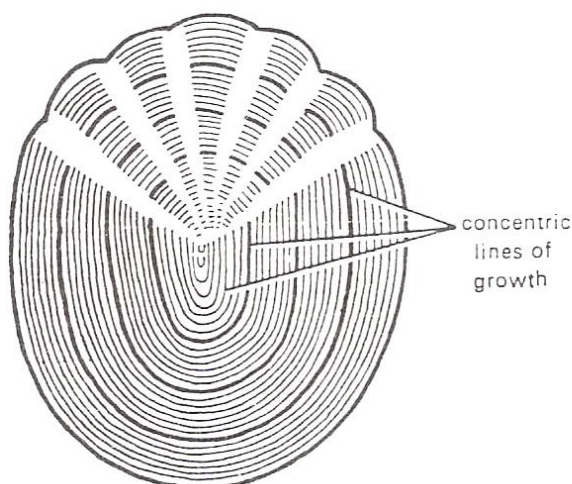


Fig.86. Cycloid scale.

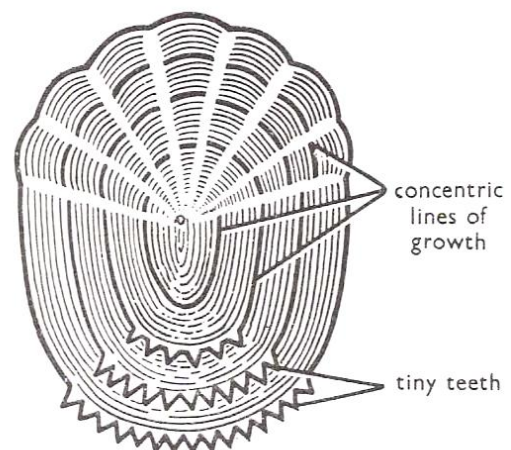
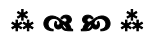


Fig. 87. Ctenoid scale.

4. They contain concentric rings, representing lines of growth which vary in different specimens.
5. Ctenoid scales do not contain ganoin.
6. Each scales is embedded in a small dermal pocket.
7. Scales are obliquely arranged so that posterior end of one overlaps the anterior end of the following scale.
8. Basal end is scalloped and free edge bears numerous comb-like projections.
9. Ctenoid scales are derivatives of ganoid scales in which ganoin, cosmine layers and bone cells are lost. Pulp cavity and dentine are entirely absent.

Identification: Since the above scale has concentric lines of growth with teeth like projections posteriorly, hence it is ctenoid scale.



III. SPOTTERS

A. LARVAL FORMS

1. AMPHIBLASTULA LARVA

1. Amphiblastula larva (Fig. 88) occurs in the development of most of the Calcarea.
2. The stomoblastula stage undergoes a process of inversion. During this process, the flagellar ends of micromeres come outside and then it is called amphiblastula larva.
3. It is more or less oval in shape and consists of one half of small narrow flagellated micromeres and the other half of large rounded granular cells or non-flagellated macromeres.
4. A fully developed amphiblastula first comes in radial canal and then passes to exterior through osculum and then leads a free-swimming life.
5. This larva leads to a free-swimming life for some time during which gastrulation takes place by the invagination of the flagellated cells.
6. Gastrula soon attaches itself to some rock or sea-weed by its blastoporal end and develops a central spongocoel and an osculum.
7. Non-flagellated cells form the dermal covering and the flagellated cells become the choanocytes lining the spongocoel.
8. After the above changes the larva develops into a young sponge.

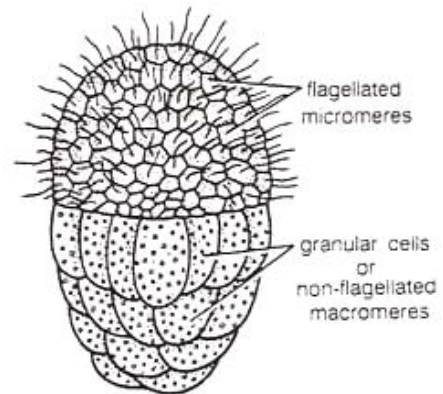


Fig. 88. Amphiblastula larva.

2. PARENCHYMULA LARVA

1. Coeloblastula larva (Fig. 89) modified into a stage corresponding to the planula larva of coelenterates. It is termed stereogastrula or parenchymula.
2. It is larva of Leucosolenia.
3. It consists of an external layer of flagellated cells and an inner mass of amoeboid cells. It has no mouth opening.
4. Parenchymula swims freely for some hours. Then it becomes fixed by its anterior pole and develops into a flat plate with an irregular outline.
5. Most of the amoeboid cells migrate to external surface, passing between flagellated cells and form the pinacoderm and mesenchyme.
6. Flagellated cells, thus enclosed, become the choanocytes.
7. A central cavity or spongocoel appears which increases in size, becomes lined by choanocytes and opens to outside by an osculum.
8. Certain non-flagellated cells in the wall sponge or porocytes, become pedoreated to form incurrent pores or ostia.
9. Monaxon and triradiate sicules are secreted by the scleroblasts or modified amoeboid cells. Within a few days of its attachment, the larva is converted into the adult asconoid sponge.

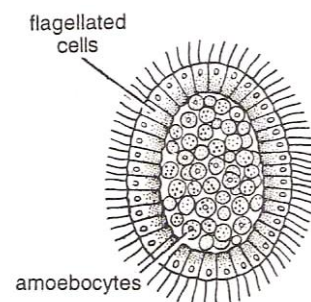


Fig. 89. Parenchymula larva.

3. EPHYRA LARVA

1. Ephyra larva (Fig. 90) is the larva of *Aurelia*.
2. It is a small medusoid form which develops from the scyphistoma larva as a result of transverse fission.
3. Body is saucer-shaped or umbrella-like and having tetramerous symmetry.
4. The umbrella is divided into eight long forked arms.
5. The distal ends of arms are deeply notched and form marginal lappets.
6. Eight prominent tentaculocysts are present in the notches between the marginal lappets.
7. Manubrium with the mouth is present in the middle on the sub-umbrellar surface.
8. Gastric filaments, per-radial and inter-radial canals are also seen.
9. Ephyra larva swims actively in the water and metamorphoses into adult *Aurelia*.

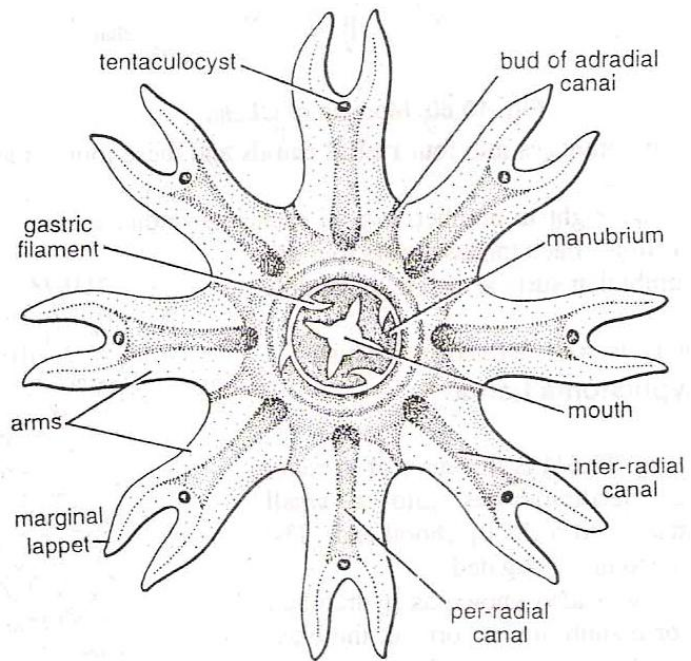


Fig. 90. Ephyra larva.

4. REDIA LARVA

1. Redia larva (Fig. 91) is the third intra-molluscan parasitic larva of liver fluke, *Fasciola hepatica*. It is found in digestive glands of snail.
2. Unique feature of larva is that it develops from germ balls present in sporocyst. Germ balls multiply and give rise to redia.
3. Body is elongated, cylindrical and complex in structure. It measures 1.3 to 1.6 mm in length.
4. Anteriorly, there is a muscular ring-like swelling, called as collar. Just beneath the collar is birth pore.
5. Posteriorly, larva contains two processes near foot, called lappets, which anchor in the tissue of snail.
6. Body wall is composed of tegument, epithelial layer and delicate mesenchyme.
7. Alimentation consists of mouth, pharynx and elongated gut. Pharynx contains pharyngeal glands.
7. Flame cells increase in number forming several twigs. There are still 2 excretory pores.
8. Germ balls occupy major portion of body. They differentiate into next larval stage is called cercaria.
9. In *F. hepatica* redia larva with rich nourishment gives rise to secondary generation of rediae by germ balls.
10. Daughter redia and cercariae come out from mother redia through birth pore.

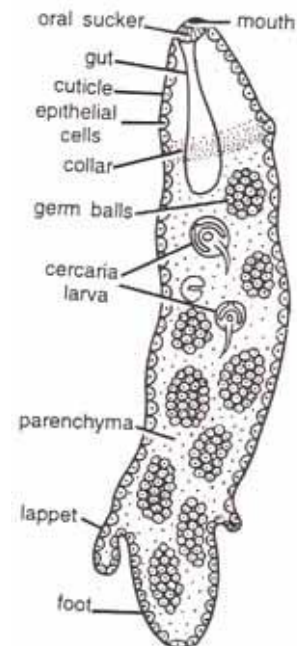


Fig. 91. Redia larva.

5. CERCARIA LARVA

1. Cercaria larva (Fig. 92) is a free-swimming larva of *Fasciola* comes out from redia as well as from snail by the pressure of perivisceral space and between 9-26°C temperature.
2. It has higher grade of organization and considerably resembles the young fluke.
3. Body is oval in shape with a long simple tail, measuring 0.25-0.35 mm in length.
4. Body and tail are covered with tegumental spines. Tail and spines are purely larval structures meant for locomotion.
5. Body wall is composed of tegument, circular, longitudinal and diagonal muscle fibres and mesenchyme.
6. Beneath the muscle layer are numerous unicellular cystogenous glands which will form cyst of future larva.
7. Digestive system consists of mouth, oral sucker, pharynx, oesophagus and forked intestine on trematode pattern, Acetabulum is present just below the fork of intestine.
8. Flame cells increase in number, Nephridial tubes unite to form excretory vesicle, which opens on tail by a single nephridiopore.
9. Germ balls represent genital rudiments.
- 8) After a brief free-life cercaria larva undergoes encystment on water plants. Tail and cuticular spines are shed. Cystogenous glands form cyst and disappear. Encysted cercaria is called metacercaria.

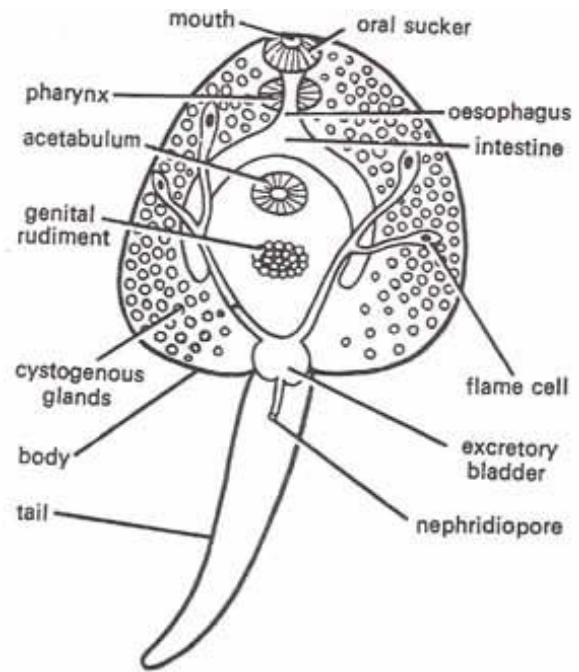


Fig. 92. Cercaria larva.

6. METACERCARIA

1. As many as a thousand metacercaria larva (Fig. 93) may be found attached to a single grass blade.
2. They have a rounded form with a diameter of about 0.2 mm.
3. Metacercaria is in fact the Juvenile fluke, also called Juvenile fluke.
4. It differs from cercaria in that it has a rounded form, a thick hard cyst and large number of flame cells. it lacks a tail and cystogenous gland cells and its excretory bladder opens cut directly through a single pore.
5. Germ cells or the genital rudiments are present as such. Cyst provides protection against short period of desiccation.
6. **Infection of primary host:** Metacercaria develops into adult fluke only inside its definitive host or sheep. The latter gets infection by grazing on leaves and grass blades to which the cysts are attached. Metacercaria survives of host's gastric juice

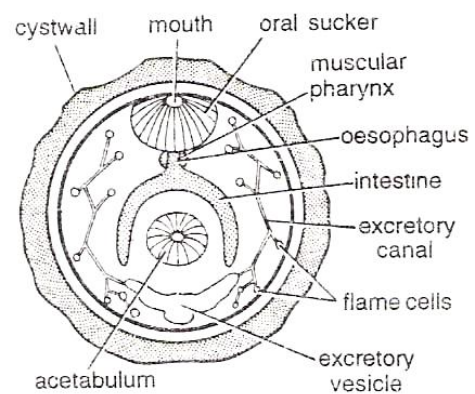


Fig. 93. Metacercaria.

as its cyst is insoluble. Cyst wall finally dissolves in proximal wall of intestine and gets in to coelomic cavity.

7. Now it infects the liver, feeds on its tissue, and grows in size in five to Six weeks. Then it takes up its position in bile duct, where it finally attains sexual maturity. In 11 to 13 weeks, after entering the body of host, it starts laying eggs (capsules).

7. TROCHOPHORE LARVA

1. In the development of *Nereis*, trochophore larva (Fig. 94) is formed from gastrula (*i.e.*, after gastrulation).
2. Trochophore is top-like and transparent.
3. It has a thin external ectodermal epithelium which is thickened at the two ends and along an equatorial ring.
4. There is a curved gut with a mouth, ectodermal oesophagus or stomodaeum, an endodermal stomach, and an ectodermal hindgut opening by an anus.
5. On the thickened parts of the ectoderm, is an anterior ciliated apical sensory plate or sensory apical organ with an apical ganglion below which is an eye.
6. At the posterior end are some large cilia and on the equatorial ring is a preoral ciliated band or prototroch, passing just above the mouth.
7. Between the ectoderm and gut is a large cavity, the blastocoel having mesenchyme cells, larval mesoderm and a pair of larval nephridia.
8. There is a statocyst near the nephridia. Trochophore feeds on microorganisms.
9. The apical organ forms the prostomium with brain, tentacles and eyes.
10. The part immediately behind the apical organ forms the peristomium.
11. The larva grows from the anal end as an elongated cylinder which forms segments of the body by metameric segmentation.
12. The larval nephridia are replaced by permanent nephridia, larval setae are dropped, tentacles, palps and parapodia are formed.
13. The advanced larva consists of the adult head and body segments separated by the body of the larval trochophore, this larval region shrivels up, and the head and body segments are drawn together and joined to metamorphose the larva into a young worm.
14. The young worm, thus, resulted, settles at the bottom of the sea and starts forming its burrow and gradually attains adulthood.

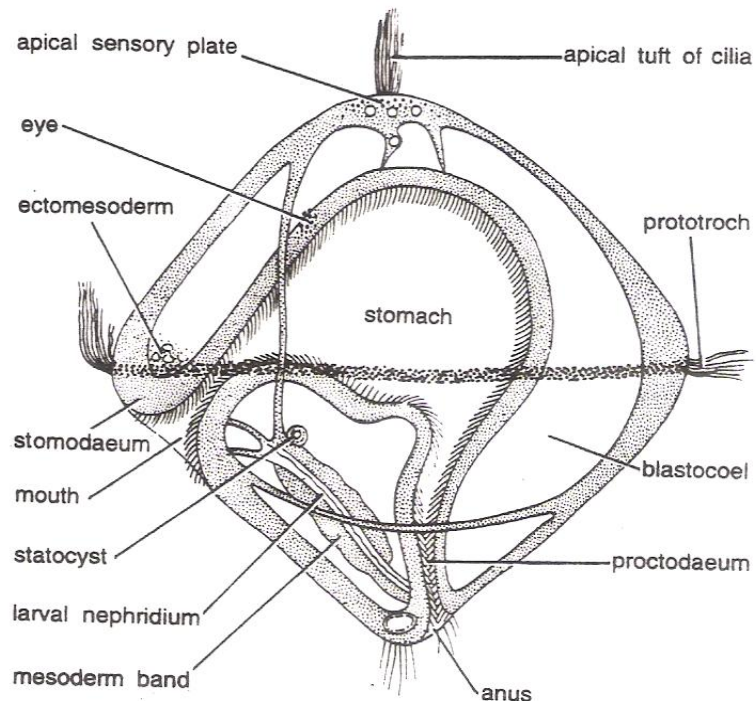


Fig. 94. Trochopore larva.

8. NAUPLIUS LARVA

1. Nauplius larva (Fig. 95) is the first and the simplest and commonest type of larva, found in most marine crustaceans and a few malacostracans.
2. When development proceeds through many larval- forms, the nauplius is the earliest and the basic larva
3. It is minute, oval having unsegmented body.
4. It has a broad anterior head region, middle trunk region and bilobed anal region.
5. Head bears a median eye and a pair of antennules bearing terminal setae.
6. Trunk has two pairs of biramous appendages, *i.e.*, antennae and mandibles.
7. Mouth opens at the anterior end, while anus lies on the posterior extremity.
8. Principal locomotor organs are *mandibles*, along with antennae may share for food collection.
9. In branchiopods, the nauplius develops straight away into the adult stage, but in mostly other crustaceans it may give rise, to other intermediate larval forms, such as metanauplius, protozoaea, zoea, mysis, etc.

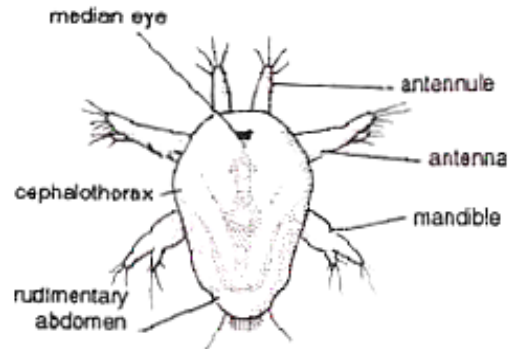


Fig. 95. Nauplius larva.

9. METANAUPLIUS

1. The nauplius larva undergoes successive moults and passes through several stages known as the metanauplius (Fig. 96), the protozoaea, the zoea and the mysis stage before it metamorphoses into the adult.
2. The metanauplius is succeeded by the protozoaea stage, when the seven pairs of limbs, which have appeared are well developed, and the carapace covers the dorsal surface anteriorly.
3. Paired eyes appear, six thorack segments are marked off but the abdomen is unsegmented.
4. In older protozoaea stage, the third pair of thoracic limbs (which become the third maxillipeds) develop, and the five anterior abdominal segments are marked off.
5. Other changes take place leading to the adult form and the metamorphosis is completed

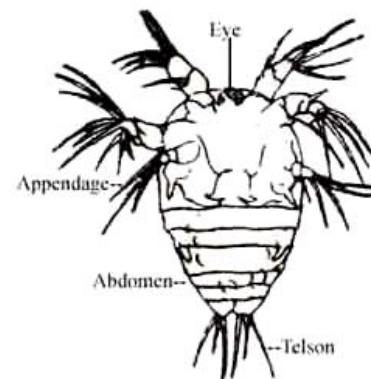


Fig. 96. Metanauplius.

10. PROTOZOEAE

1. The nauplius undergoes successive moults and passes through several stages known as the metanauplius, the protozoaea (Fig. 97), the zoea and the mysis stage before it metamorphoses into the adult.

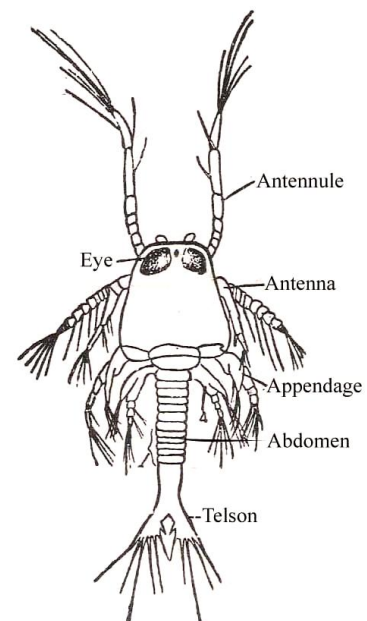


Fig.97. Protozoaea larva.

2. In the metanauplius stage that proceeds the nauplius stage, the mandibular appendage develops a masticatory process; and rudiments of four pairs of limbs develop behind it.
3. A pair of papillae, the frontal organs, develops at the anterior end, and persists throughout the larval stages.
4. These four pairs of limbs become the two pairs of maxillae and the first two maxillipeds of the adult.
5. Other changes take place leading to the adult form and the metamorphosis is completed.

8. ZOEAL LARVA

1. Zoea larva (Fig. 98) is the fourth larval stage of the Crustacea. Nauplius changes into metanauplius, metanauplius into protozoea and the latter changes into zoea larva.
2. In almost all marine decapods, except peneids and sergestids, hatching takes place at the zoea stage (as in true crabs).
3. Body of zoea comprises large unsegmented cephalothorax and long segmented abdomen.
4. A pair of large stalked movable compound eyes is present.
5. The long segmented abdomen which assists in swimming, is provided with a forked telson.
6. Carapace of cephalothorax is produced into long spines, of which one is rostrum, a median dorsal and two lateral.
7. Two pairs of maxillipeds are well developed and six pairs of thoracic appendages develop as buds.
8. Abdomen consists of six segments and devoid of appendages but the last segment bears caudal fork.
9. Biramous maxillipeds are used for swimming.

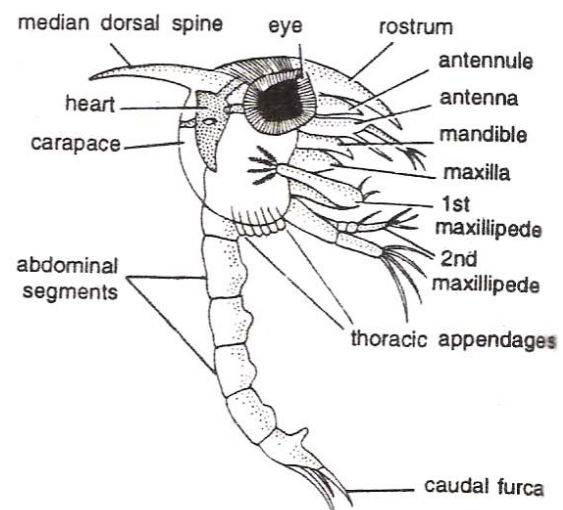


Fig. 98. Zoea larva.

9. MEGALOPA LARVA

1. Megalopa larva (Fig. 99) is the larva of crab (Brachyuran decapods) and it develops from zoea larva through successive moults.
2. It resembles, to some extent, the adult crab and possesses all 13 pairs of appendages.
3. It has a broad and crab-like cephalo thorax bearing an anterior median spine.
4. Eyes are large and stalked.
5. Antennules are small, while antennae are large.
6. Abdomen bears 6 pairs of pleopods and is placed straight in line with cephalothorax.

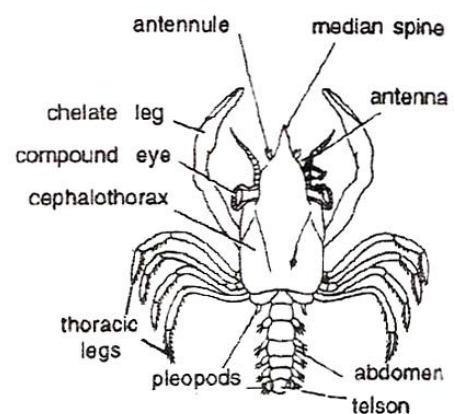


Fig.99. Megalopa larva.

7. Thoracic appendages are well developed.
8. Abdomen is six segmented bearing biramous pleopods and a telson.
9. Megalopa leads a pelagic life for sometime and later on sinks to the bottom and transforms into adult.

10. PHYLLOSOMA

1. Larva of *Palinurus*, the spiny crab or rock lobster, is called Phyllosoma larva (Fig. 100) or glass crab. It is a modified mysis stage.
2. It is remarkably large, flattened, leaf like, delicate and glassy. Body is distinguished into head, a transparent thorax and abdomen.
3. Eyes are compound and stalked. Out of six pairs of thoracic appendages, the first or maxillipeds are rudimentary, second are uniramous, third well formed biramous succeeded by rest 3 (4th, 5th and 6th) pair of long biramous legs.
4. A segmented but limbless abdomen is present, before reaching an adult stage, it undergoes several moultings.

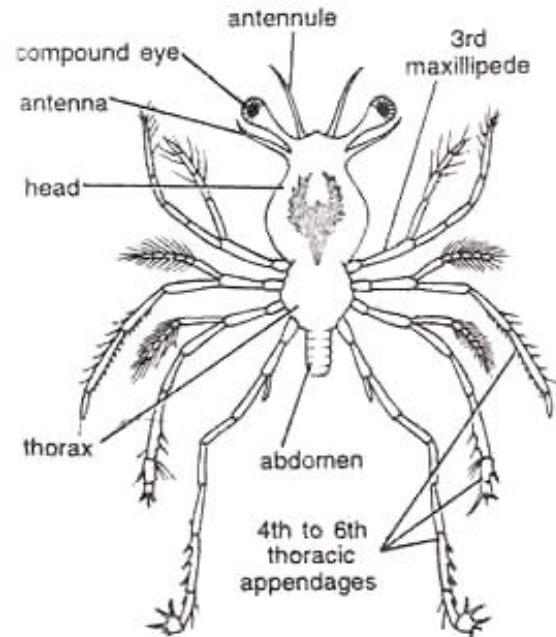


Fig. 100. Phyllosoma larva.

11. ALIMA LARVA

1. Alima larva (Fig. 101) is modified form of zoea found in some malacostracan (e.g. *Squilla*) which hatches from egg.
2. It is a pelagic form with glassy transparency having a slender body. It bears short and broad carapace.
3. It has all the cephalic appendages but only first two thoracic ones.
4. In segmented abdomen with 4 or 5 pairs of pleopods, is present.

It differs from zoea in having well formed second maxillipeds and the armature of the telson.

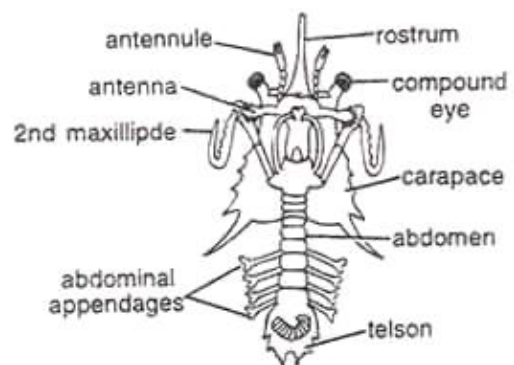


Fig. 101. Alima larva.

12. GLOCHIDIUM LARVA

1. Glochidium larva (Fig. 102) is found in the development of Pelecypoda or Bivalvia.
2. It is a minute larva measuring 0.1 to 0.4 mm, comprises a shell and mantle.
3. Shell consists of two trinagar valves united dorsally and free ventrally.
4. Ventral free end of each valve of the shell is produced into a curved hook bearing spines.
5. Mantle lobes are small and bear brush-like sensory organs.
6. Adductor muscle is well-developed extending between the two valves at the base.

7. The closure of the valves is effected by the large adductor muscle.
8. Byssus gland is situated above the adductor muscle which gives rise to a long sticky thread called provisional byssus.
9. Glochidium larva attaches itself to the skin or gills or fins of a fish and leads a parasitic life for about 10 weeks and metamorphoses into adult.

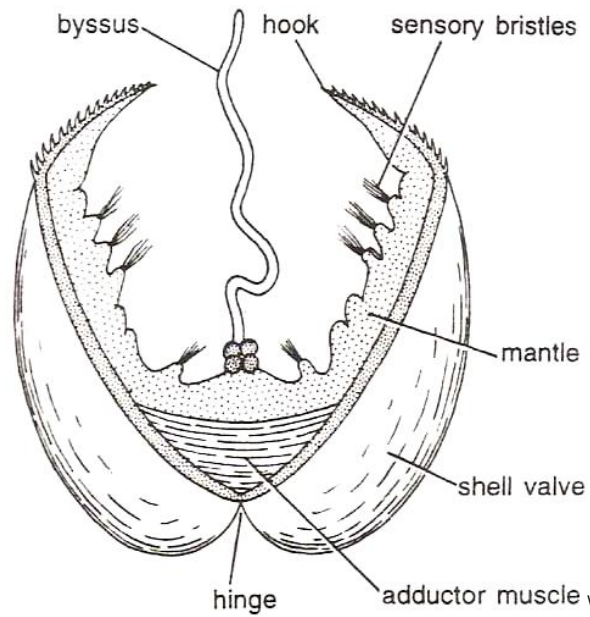


Fig. 102. Glochidium larva.

13. BIPINNARIA LARVA

1. Bipinnaria larva (Fig. 103) is the characteristic free-swimming larva of Asteroidea (starfish).
2. After gastrulation the egg hatches into a larva which develops cilia and begins a free-swimming life.
3. The free-swimming larva within 2-7 days also develops three lateral lobes on each side and gives rise to bipinnaria larva.
4. At the anterior end dorso-median arm, and pre-oral arms are present.
5. On lateral sides antero- dorsal arms, postero- dorsal arms, post-oral arms and postero- lateral arms are present. Alimentary canal consists of mouth, oesophagus, stomach, intestine and anus.

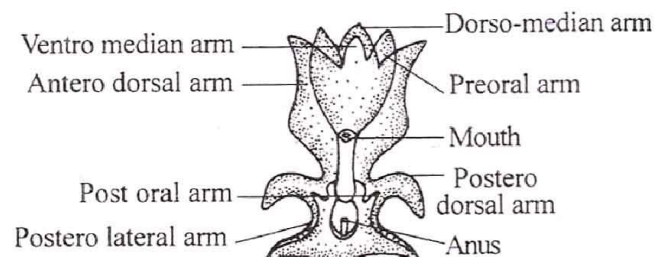


Fig. 103. Bipinnaria larva.

14. ECHINOPLUTEUS

1. Echinopluteus (Fig. 104) is the larva of Echinoidea.
2. It has a small pre-oral lobe and a single ciliary band.

- The arms are supported by calcareous rods.

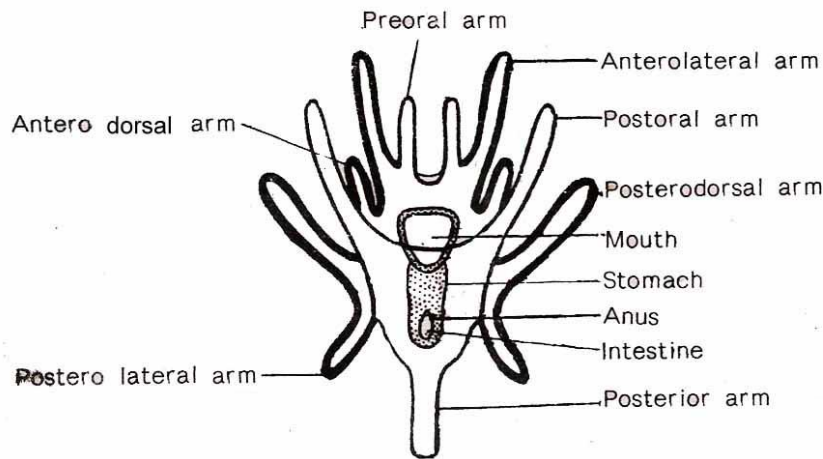


Fig. 104. Echinopluteus larva.

- The larva is provided with a pair of preoral arms, a pair of post oral arms, a pair of antero lateral arms, a pair of antero dorsal arms, a pair of postero dorsal arms, a pair of postero lateral arms and a median posterior arm.
- The postero lateral arms are very short and directed backwards.

15. TORNARIA LARVA

- Tornaria (Fig. 105) is the larva of Balanoglossus.
- This larva was first discovered by Johannes Muller in 1850. He considered it as the larva of Echinoderm. Metschnikoff discovered that tornaria is the larva of Balanoglossus.
- Tornaria is a pelagic larva living on the surface of the water. It is clear and glossy.
- It is microscopic and is about 3 m.m in size.
- It is oval in shape.
- The larva is covered by an ectoderm.
- The larva has two regions, namely an anterior preoral lobe lying in front of the mouth and a post-oral lobe lying behind the mouth.

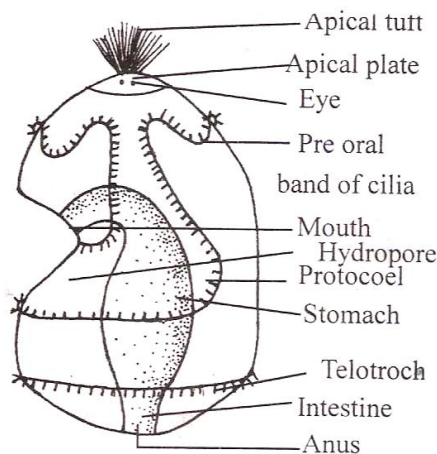


Fig. 105. Balanoglossus.

B. INVERTEBRATE FOSSILS

1. AMMONOID FOSSIL

1. Ammonoids (Fig. 106) were a group of extinct mollusc included in the class Cephalopoda and order Ammonoidea.
2. They originated in the Devonian period of Palaeozoic era about 325 million years ago and died out along with the dinosaur lineages at the end of the Cretaceous period 65 million years ago.
3. They flourished well during the Mesozoic era and all of them became extinct by the end of Mesozoic era.
4. Ammonoids originated from Nautiloids. They were marine. They had spirally coiled shell. The shell had many chambers. The chambers were separated by septa. The septa were perforated by a siphon. The outer chamber was larger than the inner chamber.
5. The junction between the chambers is known as suture.
6. The suture was not smooth. But it was ornamented with sharp folding and it had a wavy or loop or saddle like appearance.
7. The animal lived in the last and largest chamber. Some kinds of ammonites, however, did not have a tightly coiled shell; Baculites, for example, had a straight shell.
8. Most of the Ammonoids had a trap door arrangement (Operculum).
9. The Mesozoic era saw an outburst of ammonoids. About 6000 species of fossils are collected.
10. Some Ammonoids reached a gigantic size with a shell diameter of about 10 feet.

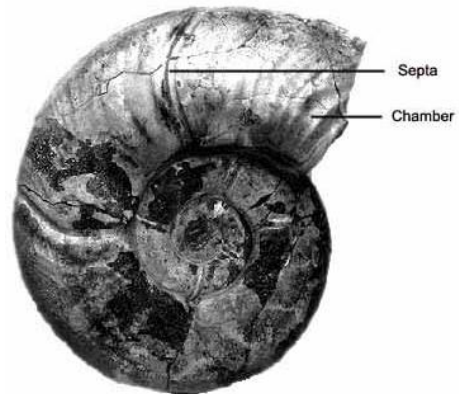


Fig. 106. Ammonoid fossil.

2. BELEMNOID FOSSIL

1. Belemnoids (Fig. 107) were a group of extinct mollusc included in the class Cephalopoda.
1. They were closely resembling *Sepia*, *Loligo* and *octopus*. It is believed that they were the immediate ancestors for modern *Sepia*, *Loligo* and *Octopus*.
2. They originated in the beginning of Mesozoic era about 200 million years ago. They flourished well in the mesozoic era and all of them became extinct by the beginning of Cenozoic era.
3. Belemnites lived in the sea, where they swam in shoals, feeding on any animals small enough to catch and overpower.
4. They originated from Nautiloids. Morphologically they were resembling the *Sepia* and *Loligo*.
5. A belemnite had a torpedo-shaped body, a large head, and strong arms equipped with hooks for grasping its prey. Like all cephalopods, belemnites swam by jet propulsion.
6. They had 6 tentacles with hooks (in place of suckers of modern forms). They had ink glands like those of modern squids.

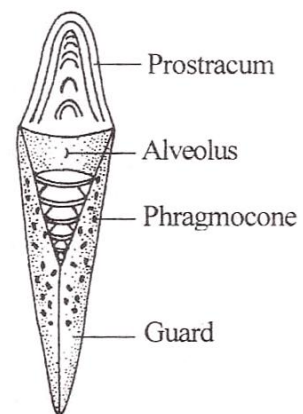


Fig. 107. Belemnoid fossil.

7. The belemnite possessed a gas-filled chambered shell known as a phragmocone. This allowed the belemnite to float in mid-water, resembling the ammonites with which it shared the ancient seas. However, the belemnite's shell differed from that of an ammonite in being an internal structure, completely covered by skin and muscle.
8. The *phragmocone* resembles the external shell of *Orthoceros* a straight shelled Nautiloid. It had a series of 20 chambers separated by concave *septa*. The septa were perforated by a *siphon*. The phragmocone had a shoe-horn shaped extension at the anterior end called *prostracum*.
9. They had internal skeleton like those of modern squids.
10. The internal skeleton was cigar shaped and was called *guard*. The guard was pointed at one end and at the other end there was a deep conical cavity *alveolus*.

3. NAUTILOID FOSSIL

1. Nautiloid (Fig. 108) is a group of Mollusc included in the class Cephalopoda and the order Nautiloidea.
2. This group includes extinct as well as extant animals. The extant animal is represented by the *Nautilus*.
3. The nautiloids are the primitive cephalopods. They originated in the Cambrian period of Palaeozoic era about 500 million years ago. Most of the Nautiloids except *Nautilus* became extinct during the Ordovician period about 425 million years ago.
 1. *Nautilus* is marine. It has a spirally coiled shell formed of many chambers. The outermost shell is large and the innermost shell is small. The chambers are separated by septa, which are perforated in the middle by a siphon. The chambers are filled with air. It helps the animal to float in the water. The junction between the chambers is known as suture. It is simple, curved and smooth in *Nautilus*.
 2. Animals live in the outermost chamber of the shell.. The body has two regions, known as head and trunk. The head has two eyes and ten tentacles; arms are absent. A funnel is present but ink glands are absent. It lives in Indian and Pacific oceans. As the *Nautilus* resembles the extinct nautiloids in many respects it is called a living fossil.
 3. The main difference between the shells of modern *Nautilus* and extinct Nautiloids is the coiling of the shell.
 4. Some of the nautiloids had straight shell. Eg. *Endoceros*. *Cystoceros* had a slightly curved shell. *Gyroceros* had a shell in which the coils were loose. Still others had a coiled shell like that of modern *Nautilus*. This fact shows that the coiled shell of present day *Nautilus* was originated from straight shells.
 5. 15. Some of the extinct nautiloids attained a gigantic size. Some straight shell had a length of 30 feet.

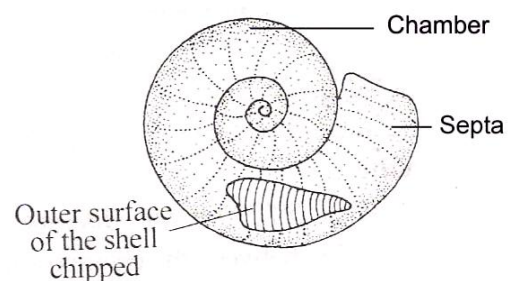


Fig. 108. Nautiloid fossil.

4. ECHINODERM FOSSILS

1. The earliest record of echinoderm fossils were Crenoids (Fig. 109). They were from the lower Ordovician period of Paleozoic era.
2. The Crenoids became abundant as fossils in Silurian rocks and they remain numerous in the Devonian and Carboniferous periods.

3. They occur most typically in calcareous rocks. Since echinoderms possess hard skeletal parts, fossilization was possible and so fossils of echinoderms were abundantly available to the scientists.
4. They were stem forms. Most have rigid calyx and show modification of arms. The dorsal cup consists of three basals free radials and brachioles. There is simple interradiial plate. A small number of inter brachial is present between the brachials.
5. Two opposable evolutionary trends can be seen among the echinoderms. One trend was towards free living (group Eleutherozoa) and the other trend was towards stalked and sessile life (Group Pelmatozoa).
6. Since fossils of echinoderms were available abundantly during the early Palaeozoic era, it was thought that this group had flourished during this period.

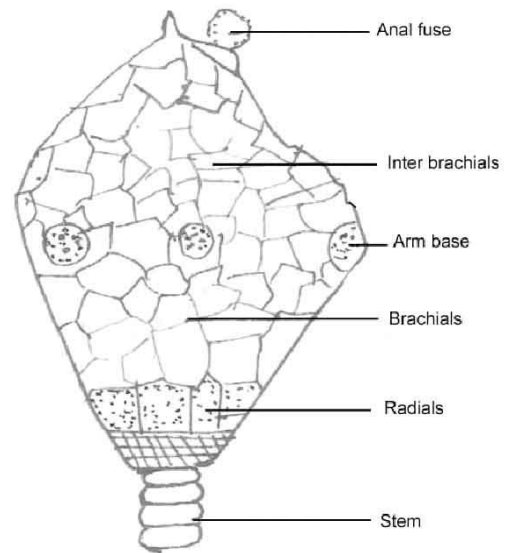


Fig. 109. Crenoid fossil of Echinoderm.

C. MINOR PHYLA

1. CHAETOGNATHA

1. The chaetognatha are minute, transparent, bilaterally symmetrical, coelomate, marine and pelagic animals.
2. They are commonly known as the **arrow worms**. Sagitta (Fig. 110) is a common example.
3. They are cosmopolitan and pelagic animals. of them are epiplanktonic and mesoplanktonic.
4. The body is straight, slender and torpedo shaped. elongated body is divided into head, trunk and tail.
5. The anterior end forms a well-delimited head bearing a pair of eyes. Mouth is a slit-like opening located on the ventral side of the head on either side of the mouth are present sickle-shaped chitinous hooks called grasping spines or seizing jaws.
6. These spines are used in seizing prey and operated by a complex musculature inside the head. The head is also armed with arcs of small spines anterior to mouth.
7. A unique feature of the chaetognatha is the hood, a fold of body wall, containing a coelomic space, that can be drawn over the head.
8. The trunk is slender and fusiform broadening toward the middle or toward the tail region.
9. There are one or two pairs of lateral fins in the trunk region and a caudal fin in the tail region. These fins are supported by double set of fin rays and serve in floating and balancing.
10. The anus lies between the trunk and the tail ventrally.
11. They show affinities to mollusca, nematode, annelida, arachnida, crustacean, hemichordate and even to chordate.

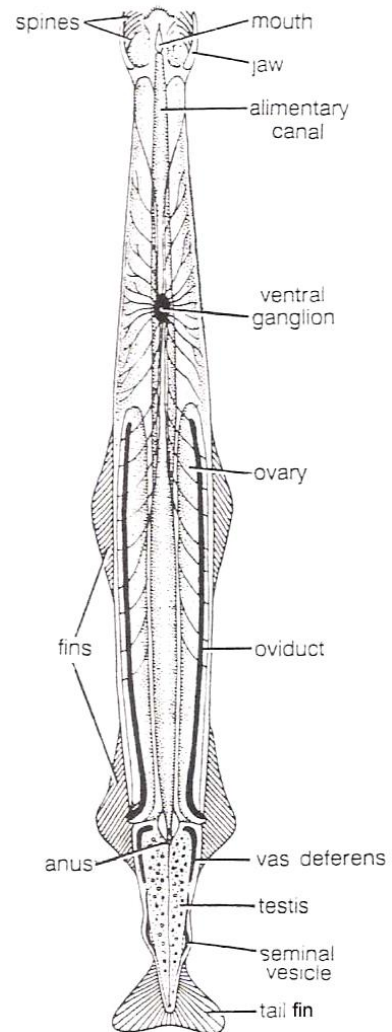


Fig. 110. Sagitta.

2. ROTIFERA

1. Rotifers (Fig. 111) or wheel animalcules are microscopic animals. They live in fresh water in abundance in ponds and ditches. The example is *Brachionus*.
2. The body is divisible into two distinct parts a broad anterior region, the trunk and a slender movable tail.
3. The trunk is enclosed in a glassy cuirass or lorica formed by the thickening of the cuticle.
4. The tail is wrinkled superficially and ends in two slender processes, the toes.
5. Dorsal surface of the trunk is convex, while the ventral surface is flattened and bears the mouth.

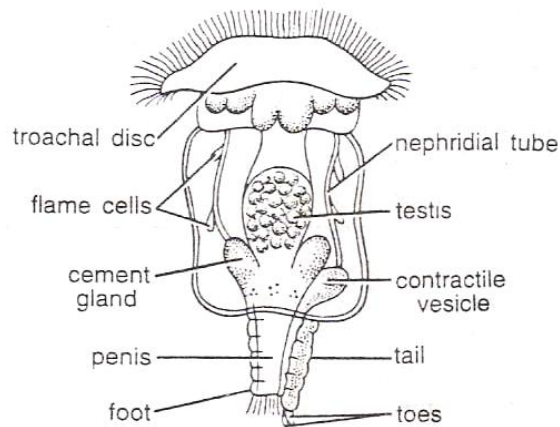


Fig. 111. Rotifer.

6. The anterior portion of the body projects from the lorica in the form of a transverse disc, the trochal disc with a prominent edge fringed with cilia.
7. Three lobes are present at the anterior end.
8. The anus is dorsal in position and is placed at the junction of the tail with the trunk.
9. Sexes are separate.
10. The animal is propelled in water by the action of cilia present at the trochal disc.
11. Rotifers show affinities to almost every invertebrate group especially the arthropods and annelids.

3. PHORONIDA

1. Phoronida are tubicolous, vermiform, coelomate and bilaterally symmetrical animals. There are two genera, the *Phoronis* and *Phoronopsis*.
2. The phoronids are exclusively marine. They have a world wide distribution. They are sedentary animals living in tubes at the bottom of shallow seas.
3. Each phoronid (Fig. 112) occupies a membranous or leather tube secreted by itself.
4. The body of phoronids is elongated cylindrical and unsegmented. It is transparent and colourless, but sometimes yellowish or greenish.
5. The body is divided into an anterior lophophore and a posterior trunk.
6. The lophophore is a horse-shoe shaped tentacular crown. It consists of two prominent ridges, outer and inner, between which is a groove, the lophophoral groove leading into the median mouth.
7. There are a number of hollow ciliated tentacles present on the lophophore.
8. The mouth lies between the two rows of tentacles and is continuous with the lophophoral groove on each side.
9. The anus lies outside the lophophore. Two ciliated nephridial tubes open on either side of the anus.
10. The trunk is narrow cylindrical and is without any appendages.

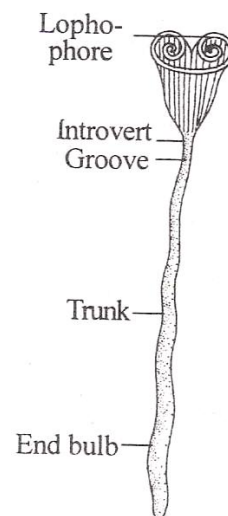


Fig. 112. Phoronid.

11. Phoronids show affinities to annelids, gephyrea, ascidiacea, hemichordate, brachiopoda and ectoprocta.

SIPUNCULIDA

- C. *Sipunculus* (Fig. 113) is commonly called peanut worm. It is found in burrows in sand or crevices in rocks at moderate depth off the coast in most countries outside the tropics.
2. Body is elongated and cylindrical, worm-like, pale brown in colour, without segmentation.
 3. It is about 40 cm long and covered by thick chitinous, transparent and iridescent cuticle.
 4. Anterior part of the body is slightly narrower than the posterior part. The narrow anterior part can be drawn into larger posterior portion and is, therefore, called proboscis or introvert.
 5. The introvert is covered with numerous small and scale-like chitinous papillae and bears terminal mouth surrounded by tentacular folds.
 6. Posterior part is marked by a series of squarish area due to crossing of transverse and longitudinal muscles and devoid of papillae.
 7. Parapodia and setae are absent.
 8. Anus is placed anterodorsally at the base of introvert.
 9. Coelom is spacious traversed by strands of connective tissue and muscle fibres.
 10. Single pair of nephridia lying in the anterior region of the body. Nephridia are called brown tubes.
 11. Sexes are separate. No sexual dimorphism. Development is indirect having a trochophore.
 12. Sipunculids show affinities to annelids.

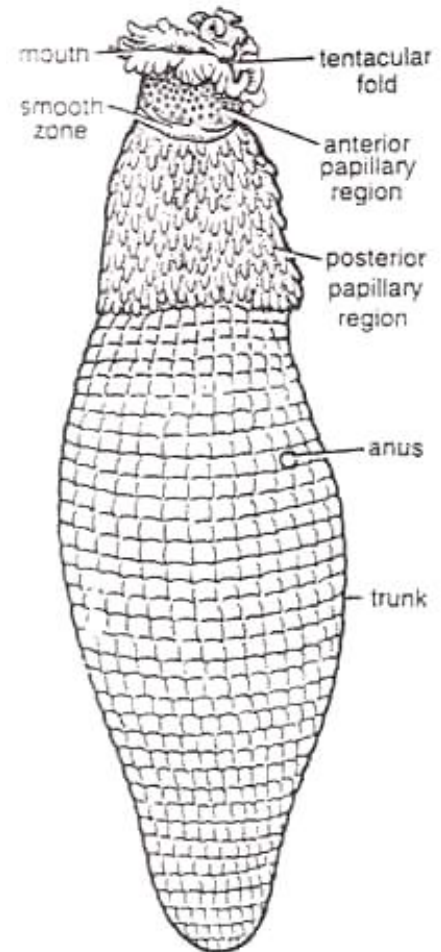


Fig. 113. *Sipunculus*.

IV. CULTURING OF ANIMALS

1. VERMICULTURE

Vermiculture is the rearing of worms. The aim of vermiculture is not to produce worms but to produce super **quality compost**. The compost produced in vermiculture is called **vermicompost**.

In vermiculture, soft organic wastes are converted into valuable fertilizer. Vermiculture helps in the **recycling of wastes** to produce nutrient rich fertilizers which enhance the growth of plants.

Vermicomposting is the easiest way to recycle food wastes. Composting with worms avoids the needless disposal of vegetative food wastes.

The earthworm is one of the **natures pinnacle**. It is a **soil scientist**. It turns common soil into **superior quality compost**. Worms facilitate the percolation of air and water into the soil. In one acre of land, there can be more than one million earthworms. Worms tunnel deeply in the soil and bring the soil to the top. This process helps the mixing of subsoil with the top soil. **Slime**, a secretion of earthworm, contains nitrogen. *Nitrogen* is an important nutrient for plants.

Biology of earthworm

Worms are **metamerically segmented** animals. They are characterised by the presence of *setae* in the skin. They have no head, arms, legs or eyes. Even though they don't have eyes, they can sense light. Worms live where there is food, moisture, oxygen and a favourable temperature.

The earthworm breathes through its skin. Food is ingested through the mouth into a stomach (crop). Later the food passes through the gizzard, where it is ground up by ingested stones. Worms process food quickly and transform food wastes into nutrient-rich **castings**. The food is passed into the intestine, which is almost as long as the worm itself. At the end of the intestine is the anus, for passing out the castings. Worm castings have nutrients needed by plants and compounds to control plant pathogen.

Reproduction

Earthworms are hermaphrodites, which mean they have both male and female sex organs, but they require another earthworm to mate. The wide band (clitellum) that surrounds a mature breeding earthworm secretes mucus (albumin) after mating. Sperm from another worm is stored in sacs. As the mucus slides over the worm, it encases the sperm and eggs inside. After slipping free from the worm, both ends seal, forming a lemon-shape cocoon approximately 1/8 inch long.

Two or more baby worms will hatch from one end of the cocoon in approximately 3 weeks. Baby worms are whitish to almost transparent and are 1/2 to 1 inch long. Redworms take 4 to 6 weeks to become sexually mature.

Each earthworm can produce eggs and fertilize the eggs produced by another worm. Under perfect conditions a mature breeder will produce a cocoon every 7 to 10 days. During mating, any two adult worms can join together to fertilize each other's eggs. Then a mucous tube secreted by the clitellum (the band 1/4 of the way down the worm's body) slips over its head into the soil as an egg case or cocoon (Fig. 114). These cocoons are about the size of a match head and change colour as the baby worms develop, starting out as pale yellow and when the hatchlings are ready to emerge, cocoons are a reddish-brown. It is possible by observing with a good lens to not only see

a baby worm. Total cocoon productivity trend in the life span of *Eudrilus eugeniae* is given in the table 1.

Table 1. Total cocoon productivity trend in the life span of *Eudrilus eugeniae*.

No. Individuals	Life span	Total no. cocoons produced
12	Less than one year	Nil
12	one year	63+ 16
36	two years	266 ± 95
40	three years	594 ± 89

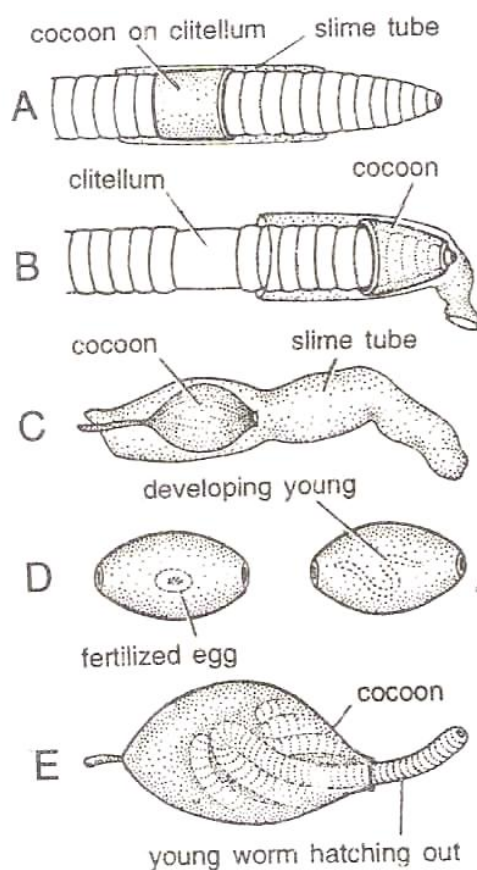


Fig. 114. Stages of cocoon formation in Earthworm.

A-Slime tube and cocoon formed.

B- Earthworm withdrawing posteriorly.

C-Cocoon left in its slime tube.

D-Cocoons with fertilized egg and developing young.

E-Young worm hatching out of the cocoon.

It takes about three weeks development in the cocoon for one to several baby worms to hatch. These newly emerged worms look just like the grown-ups, only lighter in colour and much smaller. They will mature to breeding age in approximately 60 to 90 days.

Culturable Species

Different types of worms are living in our soil. Though there are around 3,000 species of worms, only very few are used in vermiculture. The most commonly used species of earthworms to make compost from waste are

Eisenia foetida (redwiggler's or manure worm).

Lumbricus rubellus.

Pheretima elongata.

Pheretima asiatica.

Perionys excavatus (Indian blue worm).

Eutrillus sps.

Megascolex sps.

Types of Culture

Two methods are followed for vermicomposting.

1. Bin method.
2. Windrow method.

1. Bin Method

Rearing worms in a container or waste bin is called **bin method**.

The container is filled half with **bedding material**. The bedding materials include shredded newspaper, cardboard, shredded fall leaves, chopped straw, wood chips, sawdust, rice hulls, coir and other dead plants, sea weed and aged manure.

This should be 40% **composted manure** and 60% carbon materials. Two hand full of soil is added to provide necessary grit for the worm's digestion of food. The bedding mixture should be in light and fluffy consistency which create good ventilation, control odours and give free movement to the worms.

The bedding is moistured until it reaches 70% moisture content. The worms are placed gently on the top of the bedding, spreading them evenly. Worms prefer darkness. The bin is uncovered initially. So that within a few minutes, the worm will move down into the bedding to avoid light. To conserve moisture, to provide darkness for the worms and to keep out pests, the bin is covered with news paper or coconut leaves.

They can be **fed with** all type of fruit and vegetable wastes, coffee and tea bags, grass chippings, kitchen wastes, egg shells, etc. Once the feed has been eaten down feed them again. Food can be added daily, weekly and monthly. Worm numbers will increase according to the amount of food they are fed. Once a balance is reached, their number will stabilise.

After 60 days, the bin is splitted. Half of the content is placed in another bin that has fresh bedding and fresh bedding is added to the 1st bin.

2. Windrow Method

Rearing worms on a bed prepared on the open ground is called **windrow method**. The bed is prepared in rows exposing to wind. A bedding of 5 inches height is prepared on the ground. The bed is made of a mixture of **domestic wastes and composted manure**. The bedding is moistured until it reaches 70% moisture content.

The worms are gently placed on the top of the bedding. About 1-2 inches of manure is fed on the top. When the **feed** has been eaten down, feed should be given

again. When the windrow reaches about 3 feet height, split the row length wise. A fresh bedding is added to the 151 half and a new wind-row is made with the other half. The windrow is fed up with wet feed materials. The bedding is moistured when the top is dried out.

Harvesting

After a period of three to six months the wastes are completely transformed into **worm castings**. The worm castings are nutrient rich waste materials that worms excrete. The harvesting is done as follows.

The worms are fed 10 days before harvest and left them to starve. A laundry bag with holes is taken and is filled with sweet food items like apple, mango, melon, etc. The bag with food is buried into a corner of the bin. After two days, the worms will migrate into the bag. The bag is removed and the **compost is harvested from the bin**.

After harvesting, the bed is rebuilt with moistened leaves, newspaper and two hand full of sand. The worms from the bag is buried into the bed. After a week the worms are fed with kitchen wastes, fruits, vegetables, etc. The harvested **worms castings or 'compost' is a nutrient-rich super fertilizer**. The compost can be added to garden plants for a wonderful soil amendment.

Advantages of Vermicompost

Vermicompost makes the soil more fertile. It provides vitamins, hormones and enzymes to plants for increasing the growth, vigour and yield. It provides disease resistance to plants.

It prevents the leaching of mineral nutrients from the soil.. It increases the microbial wealth on the soil. It is used to enrich minerals in fish culture ponds. It is used for mushroom cultivation. Mixing compost with soil also contributes to erosion control, soil fertility, proper pH balance and healthy root development in plants. Widespread use of vermicultural biotechnology could result an increased employment opportunity.

The most beneficial feature of vermicomposting is that it eliminates foul smell of decaying organic wastes. Many industrial units covering paper, pulp and tanning make used of vermiculture technology for waste treatment. The vermiculture technology promises for waste disposal and manure generation.

2. APICULTURE

Honey bees are reared in artificial hives for the production of honey and wax. They help in pollination. The practice of rearing of bee is called apiculture or **Beekeeping**. The honey bee are reared in artificial hive. This practice is still very common in the hills but their methods are very cruel, crude and unscientific. At night when the hive is full and inactive, burning torches are brought very close to the hive and a large number of individuals are unnecessarily and barbarously killed. The combs are then removed and cut into pieces and squeezed. The honey, thus, extracted, is hardly pure. This method has now, been given up and replaced by, the introduction of different kinds of artificial hives with movable frames, in which facilities for comb making by the bees, are provided.

An artificial hive has a large brood chamber placed on a wooden platform with slit for the entry and exit of the bees at the bottom. The chamber has a number of frames in each of which a wax sheet bearing hexagonal imprints is held up in a vertical position by a couple of wires. Along the margins of these hexagonal marks, the bees start making walls and ultimately cells. Each wax sheet called **comb foundation**, attracts the bees by providing the foundation for preparing combs on both of its sides. The frames are kept hanging vertically in the brood chamber, which is covered over by another frame, having a wire meshing through which worker can easily pass. Over the brood chamber is placed another chamber called **super** which also contains similar frames containing comb foundations to provide additional space for the expansion of hive. The wire meshing mentioned above lies between the brood chamber and super. A cover having some holes for ventilation, light and safety closes from above the super.

Such artificial hives are kept in kitchen gardens, fields and orchards and a queen is introduced artificially. It soon lays its first brood of eggs from which workers develop and start enhancing the colony when the hive has been active for sufficient time, the combs are removed from the frames and centrifuged as a result of which the honey is collected without disturbing the nest. The same comb can be used over and over again because it remains intact and if need arises, fresh comb foundations can be placed. The individuals are brushed aside while removing the comb for extracting honey. They again start activity on re-introduced combs. The larval stages remain uninjured. In addition to the hive, other appliances required are a smoker, a net veil, a bee-net, a pair of gloves, a knife, a brush and a centrifuge for extracting honey.

Bees must be kept in an area where nectar-producing plants, such as clover, are plentiful. As a rule, the apiaries of major honey producers are established where intensive agriculture occurs, because it is not practical to grow plants for honey production alone. For a commercially successful operation, a location should support 30 to 50 colonies in an apiary.

Biology

Like any other insects, bees also pass through four stages in their life cycle viz. **egg, larvae, pupa and adult**. It is their evolution of life from egg. The Queen is the mother of colony. The queen bee lays the egg at the bottom of each cell. It daily lays about 1,000 to 1,560 eggs. The **egg** lies across at the bottom of the cell for about 24 hours. In the second day the egg turns about 45, to its axis and on third day the formation of **larvae** begins (Fig. 115).

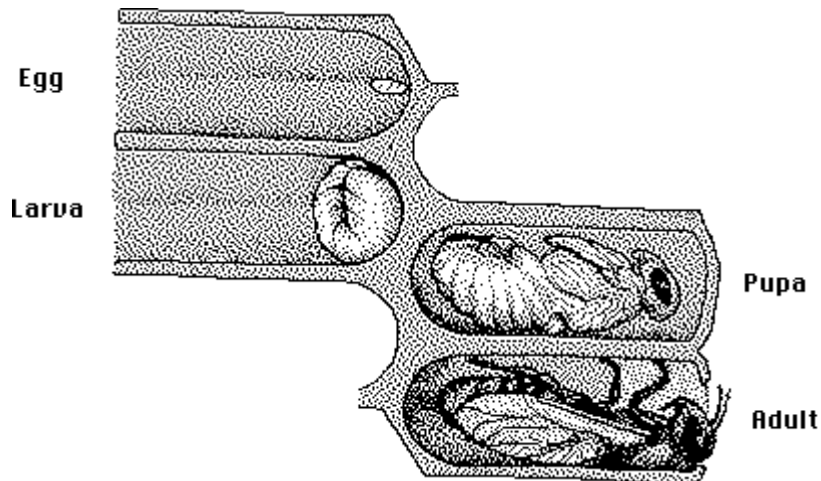


Fig. 115. The four stages in honey bee life cycle.

The nurse bee keeps watch on the development of larvae and feeds them with food according to their need. The infant food is produced by worker bees from their brood food gland. This food is a liquid known as **Royal jelly**. As they are fed with royal jelly, the larvae takes the shape of a semi circle and floats on their food, as the nurse bee continues to feed them not allowing the cell to dry. As the larvae continues to drink it also moves stirring the liquid appropriately. It is said, during larval period, the nurse bees visits the cell on an averap 1300 times.

3. SERICULTURE

The rearing of silkworms for the production of raw silk is known as **sericulture**. Sericulture is a regular industry in India and it has silk-producing centres in Assam, Bengal, Madras, Punjab, Kashmir and Mysore. Healthy eggs of high yielding strains are procured from sericulture research stations. The hatching of the eggs can be controlled (accelerated or postponed) artificially by proper conditions of refrigeration. The eggs are placed in paper-lined trays made of split bamboo. The trays are kept on stools, the legs of which rest in dishes containing water to them anti-proof. A feather periodically stirs the eggs. Larvae are given chopped leaves 5 to 9 times per day during the larval period which lasts for 3 to 5 weeks in which larvae moult four times. The pupae are not allowed to become adult. To procure silk, the cocoons, before the emergence of the silk moth, i.e., 8 to 10 days after the cocoon formation, are dropped in hot water or subjected to steam or dry heat or fumigation. Sometimes they are killed by sunning for 4-5 days called stifling.

The cocoons are first heated in boiling water to dissolve the gummy substance that holds the cocoon filament in place. After this heating, the filaments from four to eight cocoons are joined and twisted and are then combined with a number of other similarly twisted filaments to make a thread that is wound on a **reel**. When each cocoon is unwound, it is replaced with another cocoon. The resulting thread, called raw silk or reeled silk. The raw silk is again boiled, stretched and purified by acids or by fermentation. It is then carefully washed several times to bring about the well-known silk lustre on the thread. It is then spun and woven into fabrics, which we most proudly prize.

The waste outer layers or superficial threads and damaged cocoons, etc., are combed, teased and then the filaments are spun. This product is known as **spun silk**.

The next step in the processing of silk is the twisting of one or more threads of the raw silk into a strand sufficiently strong for weaving or knitting. This procedure is called throwing. Four different types of silk thread may thus be produced: organzine, crepe, tram, and thrown singles.

One cocoon yields about 300 metres of silk thread. It requires about 25,000 cocoons to a half a kilogram of finished silk. An idea of the total number of cocoons sacrificed every year for the benefit of human race can be had from the fact that about 40 to 50 million kilograms of silk is consumed annually in the world.

Biology of Silkworms

After mating (fertilization), each female moth lays about 300-400 eggs. The raising of silkworms, involves the incubation of the tiny eggs of the silkworm moth until they hatch and become worms (Fig. 116). After hatching, the worms are placed under a layer of gauze. For six weeks, the worms eat finely chopped mulberry leaves almost continuously. The larval life lasts for 2-3 weeks.

At the end of this period, they are ready to spin their cocoons, and branches of trees or shrubs are placed in their rearing houses. The worms climb these branches and make their cocoons in one continuous thread. The thread becomes wrapped around the body of the **caterpillar** larva forming a complete covering or pupal case called the cocoon. The cocoon formation takes about 3-4 days. The cocoon serves a comfortable house for the protection of the caterpillar larva for further development.

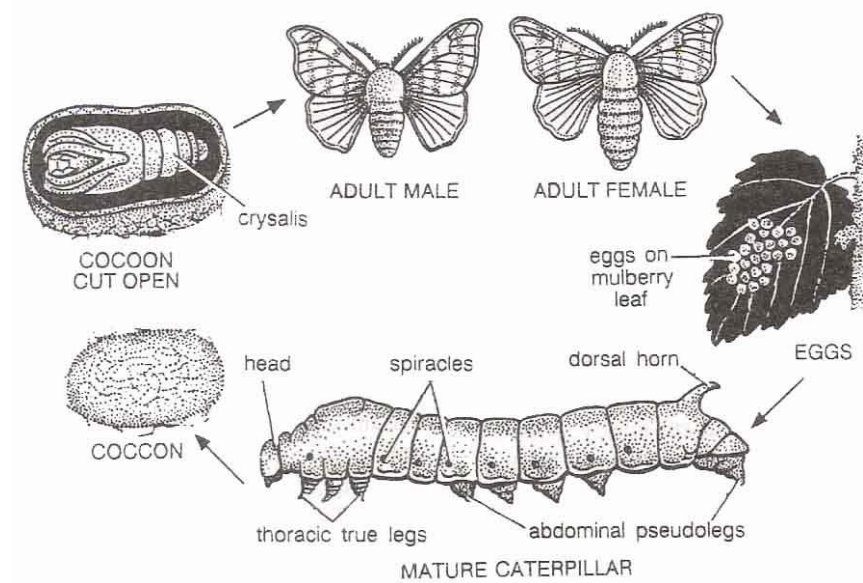


Fig. 116. Stages of life history of silkworm.

The pupa lies dormant, but undergoes very important active changes which are referred to as metamorphosis. The larval organs such as abdominal prolegs, anal horn and mouth parts are lost. The adult organs such as antennae, wings and copulatory apparatus develop. The pupa finally metamorphoses into the imago or adult in about 2-3 weeks time.

The adult moth emerges out through an opening at the end of the cocoon in about 2 to 3 weeks time, if allowed to live. Immediately before emergence, the pupa secretes an alkaline fluid, that softens one end of the cocoon and after breaking its silk strands, a feeble crumpled adult squeezes its way out. Soon after emergence, the adult silk moths mate, lay eggs and die.

4. DAIRY FARM

The dairy farm covering the production, processing and distribution of milk and milk products is unique in its importance as it is concerned with valuable food stuffs universally consumed by man. Milk is the fresh lacteal secretion of animals naturally intended for the nourishment of the offspring, but exploited as an article of food by human-beings.

The young ones of other mammals survive only upon their own mother's milk but man uses milk of other mammals as additional source of nutrients for his offspring as well as for himself. Man also uses mammalian milk for a variety of preparations like curd, butter, cheese, sweet etc. For the proper and regular supply of milk, man has domesticated a number of mammals. The only mammals which have received attention worth the name are cows, goats and buffalos. The other animals like sheep, camels, asses and mares are milked in certain confined localities but as **producers of milk** they are of little importance.

The **dairy cattle** (Fig. 117) thrive best in areas where pasturage and other green forage are grown in abundance. Extremely cold climates are not suitable because of the lack of green forage and much expense for protecting the animals from the weather. The modern dairy industry is some what recent in origin. In earlier phase cows were kept to furnish milk for the farm family. The marketing of milk began when customers called at the farm or farmers to distribute their milk directly to users. Within the past 100 years advances along five different lines have caused milk and milk products to become important articles of commerce.



Fig. 117. The dairy cattle.

1. The processing of milk by factory system was started in the middle of 19th century, resulting in greater uniformity of product.
2. A number of technological advances like concentrating milk and sealing it in container in sterile conditions, distribution of milk in bottles and other advanced processing of milk began.
3. The first milk sold in towns and cities came from nearby farms. The knowledge of refrigeration of milk not only aided in keeping milk fresh for a longer period but also made possible the shipping of dairy products to all parts of the world.
4. In earlier days the method of transporting milk to large cities was to ship it on railways. The motor vehicles and paved roads made possible the supplying of fresh milk to markets hundreds of kilometres distant from farms, where the milk is produced.

5. The adoption of pasteurization and the enforcement of laws requiring proper food value in dairy products greatly benefited the entire dairy industry.

Breeds of Dairy Animals

The number of well-recognised cow breeds in India is about fifty. In addition, a large number of other types, which do not confirm to any definite breed characteristics exist, and are treated as non descripts. The non descripts are very poor producer of milk.

The principal breeds of cows are Haryana, Kankrej, Ongole, Rath, Deoni, Gir and Kangayam. Deoni is found in the locality of North-western and western parts of Hyderabad and are good milkers in the region. Gaolao is common in Wardha and Chindwara districts. Gir is good milker and found in Kathiawar, Rajputana and Baroda regions. Haryana is very good milker found in the vicinity of Rohtak, Hissar, Kamal, Delhi and Uttar Pradesh. Kankrej is common in South east of Rann of Kutch and Ahmedabad region and is fair milker. Ongole is also a good milker and found in Guntur districts. Rath is fairly good milker which is found from Rajasthan to North-western part. Shahiwal and Sindhi are very good milker found in Punjab, Haryana and Uttar Pradesh.

The feeding stuff for **dairy cattle** may be broadly classified into roughages and concentrates. The roughages consist of succulent feeds (natural grazing, cultivated grasses, cultivated fodders and root crops) and dry fodders (hay, straw, chaff). The concentrates consist of carbohydrates-rich materials (oil seed, seed cake and meals). In addition to roughages and concentrates, dairy animals also require a certain amount of common salt to keep them in good condition.

Milk

Milk is produced by the mammary glands, which are specialized skin glands. The actual secretion of milk by the mother is stimulated at birth by a lactogenic hormone (galactogen or prolactin), which comes from pituitary gland located at the bottom of brain, adrenal hormones also are essential for lactation. During gestation, the production of lactogenic hormone from pituitary is inhibited by the presence of another hormone, estrogen that disappears at birth. In some mammals the stimulus of sucking offspring serves, through the nervous system, to stimulate the secretion of lactogen.

Dairy Farming Dairy farmers milk their cows every 12 hours, usually by machine. A glass tube takes the milk directly from the cow's teat to a separate milk house. This arrangement protects the milk from organisms in the air and preserves its taste and cleanliness. Since the 1950s dairy farms in the UK have become more concentrated, with fewer farms producing increased volumes of milk.

Processing of milk

In advanced countries, a major part of the milk is processed and rendered safe before distributing to consumers. Among the methods employed, pasteurization is the most important. This consists of applying heat for the minimum period necessary to destroy microbial contaminations, the temperature being high enough to kill them but not so high as to affect the chemical and physical characteristics or the nutritive value of milk.

Two methods of pasteurization are in use i.e. the holding tind flash High Temperature Short Time (H.T.S.T.). In the holding method, the milk is heated from 140 to 150°F, held at that temperature for 30 minutes are rapidly cooled to 50°F. In the HTST method, the milk is heated from 160 to 162°F, held at that temperature for 10 to 20 seconds and cooled immediately to 50°F. Holding pasteurization gives a safe and satisfactory product. Pasteurization does not affect the concentrations of vitamin A and D or riboflavin. About 25% of thiamine and 50% of ascorbic acid are destroyed. Among the other milk processing methods the irradiation by ultra-violet light, developed in Germany during world war II, is of interest. Irradiation increases the vitamin D content of the milk.

Marketing and distribution

In urban areas 60 to 70 percent of the total milk requirements is produced within the municipal limits, the rest is obtained from adjoining rural areas. Only 6 to 8 per cent of the total milk produced in **the country** is transported from rural to urban centres for consumption as **milk and milk products**. Nearly 2/3rd of milk received from outside the municipal limits comes from within 8 to 15 kilometres of the towns and the remaining 1/3rd from beyond this distance. A part of the milk consumed in large cities, like Calcutta. Mumbai. Chennai, Delhi and others, it is obtained from localities situated at a distance of even 75 kilometres. Some successful efforts have been made to organize the production and marketing of milk on a cooperative basis.

Prices

The price of milk varies to a great extent from place to place. In rural areas there is practically no good market facility for fluid milk. The milk left over after meeting the demands of the producers family is converted into butter, ghee or khoa. These products are sold at weekly markets at prices largely determined by the distance of the market from the village. As a rule, cow milk is cheaper than buffalo milk but it may not be true for all the time and for all the places.

Milk Products

A variety of milk products is known in India and some of them figure in interstate trade. Dairy products such as cheese, butter, condensed milk, milk powder, curd etc. make the dairy farming a highly attractive industry.

Dahi (Curd)

It is prepared by souring milk with a lactic acid starter which is usually curd of the previous day. For the preparation of curd, milk is first boiled and cooled from 60 to 70°F. Further, starter is added and is kept untouched. Thus, curd becomes ready for consumption in 10 to 12 hours. Curd contains 0.6 to 1.0 per cent titratable acid expressed as lactic acid. It has a lactic flavour and a compact smooth texture. Its composition varies with the quality of milk used, the types-of organisms present in the starter and the time allowed for souring. The organisms present in the curd are mainly streptococci (*Streptococcus theriophillus* type) and *Lactobacillus casei*. Curd is normally consumed at the place of production. Curd contains 84.79% water, 7.7% fat, 3.4% protein, 4.6% lactose, 0.7 to 0.8% lactic acid, 0.7% minerals, 0.12% calcium and 0.95% phosphorus.

Cream

Cream is a fat containing fraction separated from milk by centrifuging the liquid milk. The separator, commonly employed, consists of bowl with a large number of conical discs arranged one above the other with intervening spaces. Milk enters through an opening in the centre and as the bowl is rotated, 3,000 to 20,000 rpm, the lighter fraction which is cream, is driven towards the tropical countries for common people. Ghee is produced in India according to the traditional process involving sour curdling of milk, recovering butter and heating the butter to remove water. Desi butter is preferred to creamy butter as ghee obtained from the former is melted into ghee at once or after storage upto 10 days. It may be partially dehydrated and later converted into ghee according to the demand of market. The temperature employed for clarifying butter varies from 80 to 125°C. Short exposure to 120°C does not interfere with the formation of grain nor does it diminish the carotene and vitamin A contents of the ghee. To ensure proper grain formation, the tins to which ghee is transferred should be kept undisturbed and cooling should be allowed to take place gradually. The appearance of colour and grain structure influences its market value. The ghee of cow is yellow and that of buffalo is whitish. The grain in buffalo ghee is bigger than that of cow ghee. The composition of ghee varies according to the composition of the milk from which it is derived. Ghee is

mainly used for cooking, frying and taking directly with the food materials. Ghee is subjected to extensive adulteration in the trade.

Malai: When milk is heated, a layer of fat and coagulated proteins, malai, is formed on the surface. Slow heating helps to increase the thickness of the layer. The volume of malai can be increased by boiling the milk until a voluminous froth is formed and cooling slowly over a dying fire. Malai is either consumed directly, or used in the preparation of sweets. Its composition is moisture 60 to 70%, fat 25 to 30%, proteins 3 to 3.5%, lactose 3.3 to 3.8% and ash 0.4 to 0.5%.

Condensed milk: It is obtained by evaporating milk at 130 to 135°C in a vacuum pan to the required concentration. The concentrate is homogenized to prevent the separation of fat, cooled and fortified if necessary. Stabilizers such as disodium hydrogen phosphate or calcium chloride are added to prevent coagulation during the sterilization. The condensed product is cooled rapidly from 80-86°F and held at that temperature for 15 to 20 minutes. The cooling is so controlled that the crystals are of small dimensions and remain in suspension in the viscous liquid.

Khoa: It is prepared by the rapid evaporation of water from the milk. It is usually prepared from buffalo milk by heating with brisk stirring in flat bottomed shallow steel pots. Until the volume is reduced to about one-fifth. The product is gathered in a compact mass, cooled, and packed for markets. Alum is sometimes added to the milk during the boiling to give a smooth texture to the product. Khoa is consumed directly or used as an ingredient of sweets. It can be kept for 3 to 4 days without deterioration. The common adulterants of khoa are cereal flours.

Cheese: Cheese is the product made from the curd, obtained from whole skimmed milk with or without added cream by coagulating the casein, and then further the separated curd is treated by ripening ferments.

Soft cheese, known as 'Paneer' is prepared by using coagulants as the source. A coagulating enzyme for clotting milk. Milk is warmed to about 100°F and the crushed coagulants are tied in cloth and dipped in it. The milk curdles in 30 to 40 minutes. The coagulum is placed on a muslin cloth and the whey is drained out. The process of cheese manufacture varies very much but not to such an extent as may be with different characters of the final product.



V. GENETICS

CULTURE OF *DROSOPHILA*

Ex. No. 1

Date:

Aim

To prepare and maintain a culture of *Drosophila* in the laboratory.

Materials required

A wide mouthed transparent glass bottle, a piece of white cloth over-ripped fruit and water tray.

Procedure

A wide mouthed bottle was taken with a few pieces of over ripped (rotten) banana. It was kept in the laboratory near the window in a tray of water. Due to keen sense of smell the fruit flies flow towards the bottle and accumulated over the ripped fruits. More flies were found on the fruit within two days. When a considerable number of flies were collected, the mouth of the bottle was covered by a piece of cloth and tied with a thread. The bottle with flies was kept for a few days, so that they can mate and produce a number of eggs.

To collect adult flies, another bottle of the same size was taken and placed over the culture bottle in such a way that the neck of the two bottles were Jointed together simultaneously. The cloth covering the mouth of the culture bottle was removed the flies flew towards the upper bottle. The adult fruit were collected in the new bottle and used for the production of 2nd generation of *Drosophila*. Thus the *Drosophila* culture was maintained in the laboratory.

Result

It was noticed that the *Drosophila* can be raised in any fermentation medium, the yeast that are responsible for the fermentation constitute the bulk of the diet of the fly.

While culture, *Drosophila* laid eggs and embryonic development (Fig. 118) of the fertilized egg occurred very rapidly. The first larval stage hatched within about one day after fertilization. Pupation occurred at about five days post fertilization, and metamorphosis of the adult fly occurred at about nine days post fertilization.

Discussion

Drosophila melanogaster is widely cultured in the laboratory and used as experimental organisms in the field of genetics. Many investigators choose these flies as experimental organisms for the convenient characteristics found in *Drosophila*.

Genetic Importance of *Drosophila*

Easy of culturing, it can be raised on a variety of simple culture media. Generation period is short (short life cycle 9 to 10 days at 25°C). Prodigality of production is high (several hundred off- spring from a single mating pairs). They are smaller in size (easy for handling and storage). Identifications of sex and mutant forms (vestigial wing, yellow body high degree mutant, white eye, etc.) are easier. The number of

chromosomes is few. There are 6 autosomes and 2 allosomes (XX and XY). There are sex linked chromosomes in *Drosophila* (e.g. Eye colour).

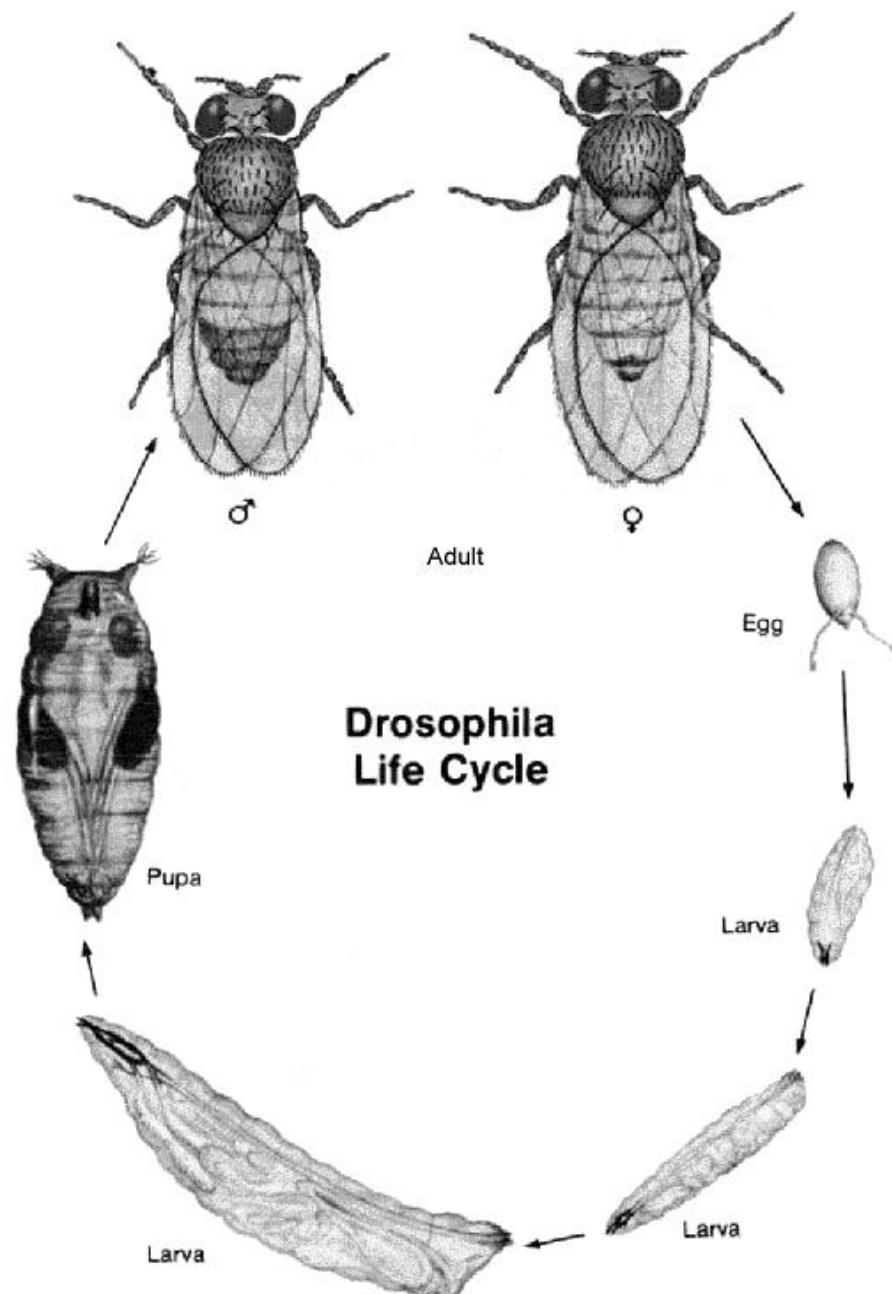


Fig. 118. Stages of development in *Drosophila*'s culture.

IDENTIFICATION OF MUTANT FORMS IN DROSOPHILA

Ex. No. 2

Date:

Aim

To identify the various mutant forms in *Drosophila*.

Materials required

Fruit flies, ether, cotton, etc.

Procedure

A piece of cotton soaked in ether was used to etherize the flies in the bottle. The insects were taken on a clean glass slide and observed under dissection microscope.

Observation

1. Red eye (wild type): The *Drosophila* has normal red eye compared to mutant form like ebony, yellow, vestigial, and white eye (Fig. 119).

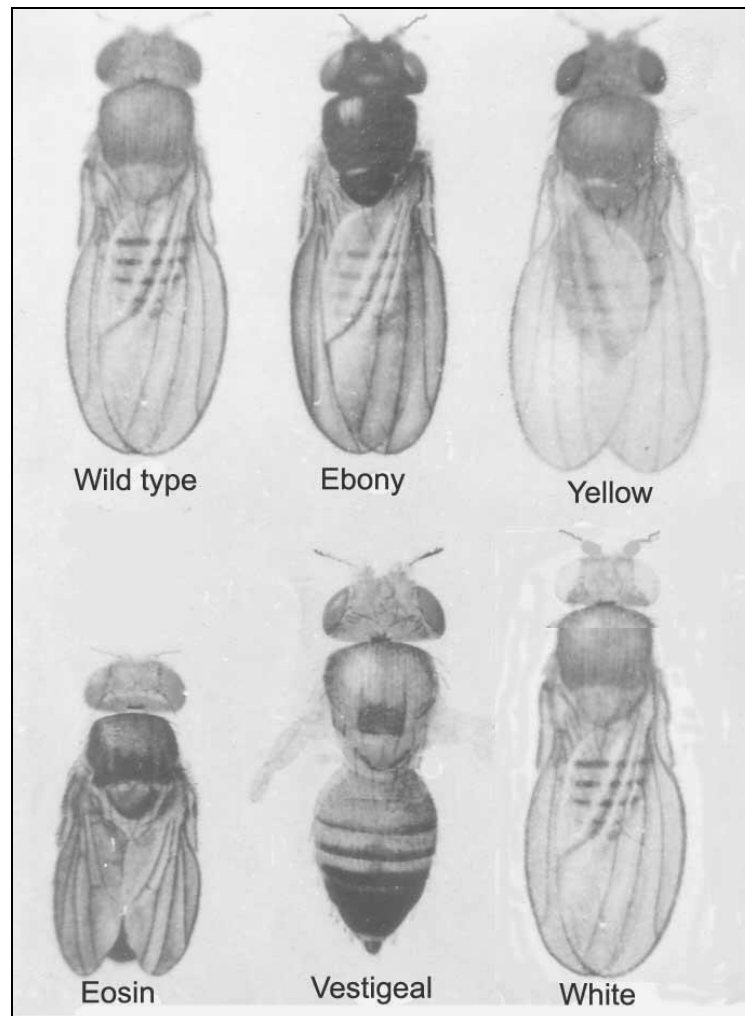


Fig. 119. Mutant forms of *Drosophila*.

2. Ebony body: The colour of the body is darker than the wild type. It is a recessive somatic mutation. The gene for ebony body is located in the third chromosome of the gene loci 70.7.

3. Yellow body: The body is yellow in colour in contrast to the normal colour (black bands, hairs and bristles are brown, wing are yellow). This is a sex linked recessive mutant. The mutant gene locates in the chromosome. This was discovered by Wallace.

4. Eosin eye: The colour of the eye is eosin like. It is due to a sex linked recessive mutation. Normal eye colour in *Drosophila* is red. White eyed *Drosophila* was one of the first mutations known in *Drosophila*. When any two recessive alleles were brought together, intermediate type, eosin eye was obtained. Red eye and white eye showed simple dominant recessive relationship over all others.

5. Vestigial wing: These wings are reduced and are held at right angles to the body. Wing veins are still visible. Vestigial wing (Fig. 120) is a recessive mutation and the gene located in the second chromosome at the gene loci 67.0 (2.67.0). The flies cannot fly. The vestigial wing mutant was discovered by T. H. Morghan.

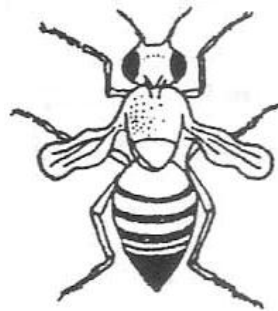


Fig. 120. Vestigial winged *Drosophila*.

6. White eye (W): White eyed *Drosophila* (Fig. 121) was one of the first mutations known in *Drosophila*. Normal eye colour in *Drosophila* is red. The white eye is due to **sex linked recessive mutation** present in the x-chromosome at the gene loci 1.5 (x-15). The eyes are pure white. This mutant was first discovered by T. H. Morghan.

In *Drosophila melanogaster*, white eye colour is recessive to normal red eye colour. If *white eyed* female individual is crossed to *red eyed* male individual, all female individuals in F1 generation are red eyed and all male individuals are white eyed. When these red eyed female individuals and white eyed male individuals from F1 generation are intercrossed, female population in F2 generation will consist of 50% red eyed and 50% white eyed individuals. Similarly, in the male population in this generation, 50% offspring would be red eyed and 50% white eyed. This shows that white eyed condition is a recessive male sex-linked mutation. The red eye and white eye showed simple dominant recessive relationship.

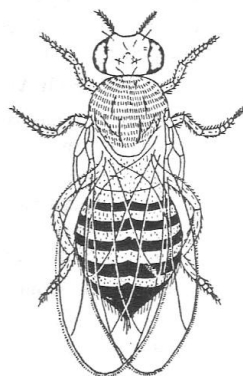


Fig. 121. White eyed *Drosophila*.

IDENTIFICATION OF SEXES IN *DROSOPHILA*

Ex. No. 3

Date:

Aim

To identify the sexes of *Drosophila* from a culture

Materials required

Binocular stereo dissection microscope with lamp, forceps, fine hair brush, pointed needle porcelain tile, ether, cotton, glass slides, etc.

Procedure

Etherisation of flies: A bit of cotton soaked in ether was placed into the bottle containing the fruit flies. After etherisation of flies, they stopped walking around. The flies were poured out on the porcelain plate. Normally the flies remain in anesthetic conditions for 5 to 10 minutes. They begin to awake on the plate they have to etherized. At the same time over etherisation (killing) causes death of the flies. In died flies, the wings are extended at right angles to their bodies and are inconvenient to study them under the microscope.

Examination of flies: Fruit flies were then transferred to glass slide by pushing them with a fine brush or picking the wings with a fine forceps. Then they were observed under the dissection microscope by picking the wings with a fine forceps.

Observation

Adult male (Fig. 122a) fruit fly was identified by the following morphological features:

1. Presence of a sex comb on the upper tarsal joint of each leg of the first pair (the sex comb is formed of a row of about 10 stout black bristles).
2. Presence of visible segments in the abdomen.
3. Presence of a blunt ended abdomen.
4. Presence of fused dark bands of the last few segment of the abdomen forming a blob.
5. Presence of anal plates and darkly pigmented genital arch and penis.

Adult female (Fig. 122b) fruit fly was identified by the following morphological features:

1. Absence of sex comb on the legs. Presence of seven visible segments in the abdomen.
2. Presence of separate dark bands at the very tip of the abdomen.
3. Presence of pointed abdomen.
4. Presence of anal plates and light coloured ovipositor plates in genital region.

Discussion

Drosophila is widely used in breeding experiment of sexes is important. Gyndromorphs can also be noticed in *Drosophila* culture. *Drosophila* has short life span, so that the mechanism of inheritance of sex linked traits can be studied in a number of generation. For such study identification of sexes is important.

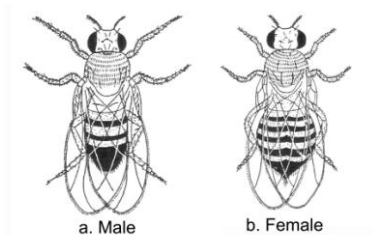


Fig. 122. a. Male and b. Female *Drosophila*.

ABO BLOOD GROUPS AND THEIR SIGNIFICANCE

Ex. No. 4

Date:

Aim

To found out my blood group by agglutination test.

Materials required

Glass slide, Sterilized needle, Antiserum 'A' and Antiserum 'B'.

Principle involved

In the human blood, two types of substances are found namely antigens and antibodies. The antigens are present in RBC. Antibodies are present in plasma. Landsteiner (1900) classified four different blood group in human beings according to the type of antigens present or antigens absent. They are A, B, AB and O groups. The blood group has antigen A and antibody B. The blood group B has antigen B and antibody A. The AB blood group has both the antigens (A and B), but no antibodies. The blood group O has no antigen, but with both antibodies (A and B).

Agglutination (clumping) takes place between the corresponding antigens and antibodies. For e.g. clumping takes place between antigen A and antibody B. Similarly clumping takes place between antigen B and antibodies A. Based on this agglutination the blood group is referred as A and B group. Agglutination takes place between both antigen and antibodies, the blood group is AB, if agglutination does not take place, it is O blood group. Thus the blood group is determined by based on the principle.

Procedure

My left hand ring finger was sterilized and pricked with sterilized needle. A drop of blood was placed at one end of the glass slide and another drop at the other end. The antiserum A and B was added to the blood drops separately and stirred. Then the blood group was identified by observing the agglutination.

Result

According to the agglutination test (Fig. 123). my blood group was identified as.....group because there was..... Agglutination with.....antiserum.

Significance of blood group

The grouping of blood is of great importance in blood transfusion if unmatched blood is transfused to the patient he/she will die due to agglutination. So the blood of the donor and recipient are tested before transfusion.

1. Thus AB group person can receive blood from all the 'four groups, because it does not have any antibody to agglutinate. Therefore AB group persons are called 'universal recipients'.
2. Group 'O' person cannot receive blood from any group except 'O' group, because 'O' group blood has both antibodies A and B for agglutination. At the same time since both antigens are absent. This group person is called "universal donor".
3. Group A person can receive blood from A group and O group and not from B and AB group.

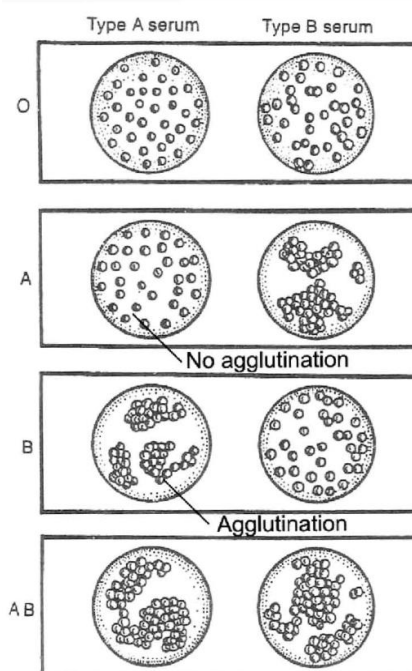


Fig. 123. Agglutination test for blood grouping.

4. Group B person can receive blood from B group and O group and not from A and AB group.

The blood grouping is used to establish identify in disputed cases of parent and children relationship.

GENETIC BASIS OF ABO BLOOD SYSTEM

Multiple allelism

More than two pair of genes located on the same locus controlling a particular character is called multiple allelism.

The blood groups are inherited and remain constant throughout life. The human blood groups are controlled by 3 genes (multiple alleles). They are I^A , I^B and I^O . Among these I^A and I^B are co dominant alleles is a recessive alleles. The A blood group individuals are having the genotype alleles. The A blood group individuals are having the genotype alleles $I^B I^B$ or $I^B I^O$.

The AB blood group persons have the genotype $I^A I^B$. The 'O' blood group persons have the genotype $I^O I^O$.

Blood group dispute cases

Example 1: Human of blood group 'AB' was presented before the judge. A baby of group 'O' which she claimed as her baby what bearing might the blood in formation have on the case.

The genotype of the blood group of women is $I^A I^B$. Both are dominant alleles the genotype of baby is and baby is $I^O I^O$. Both are recessive alleles. So even if the man is 'O' blood group baby by a woman of 'AB' group is not possible.

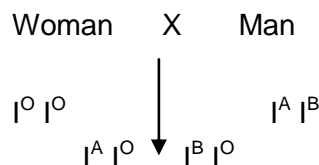
So it is clear that the baby of 'O' group does not belong to the baby who presented the case in the court claims is false.

Example 2: A case has brought before in which a women of blood group 'O' which she claimed as her child and brought suit against a man of group 'AB' whom she claimed was the father.

Of the child what bearing might be the blood type information have on the case.

The blood group of women is 'O' genotype of her blood group is $I^O I^O$. The blood group of man is 'AB' and the genotype of his blood group is $I^A I^B$.

When this 'O' blood group of woman is 'O' blood group lady is married to a man of 'AB' group the child would be either A or B. Above all A type of blood groups (AB, A, B, and O group) is possible. So the man is question in this case could be the father of 'O' group child.



$I^A I^O$ = 'A' blood group child

$I^B I^O$ = 'B' blood group child

Because I^O recessive allele.

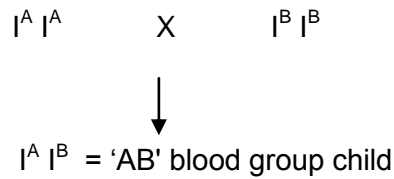
So in this 'AB' blood group man is not the father of 'O' blood group baby. The claim of 'O' blood group woman is false beyond doubt. He is telling lie.

Q.1. A woman brought a case against a man for the support of her child. She has 'O' type blood and her child with 'B' type could be the man father? Explain.

Woman is of 'A' type and the genotype of her blood group is either $I^A I^A$ or $I^A I^O$. The man blood group is 'B' type of his blood group is either $I^B I^B$ or $I^B I^O$.

So there are 2 possible crosses as shown below:

Cross – I



Cross – II



$I^A I^O$ = 'A' blood group child

$I^B I^O$ = 'O' blood group child

$I^A I^B$ = 'B' blood group child

$I^O I^O$ = 'O' blood group child

4. A child with blood group genotype $I^A I^B$ is born to a woman with genotype $I^B I^B$ what type of genotypes the father can be determined.

The genotype of the blood group woman is $I^B I^B$. are obtained from this the genotype of the father can be determined.

Woman	Man	Child
$I^B I^B$	$I^B I^B$	$I^B I^B$
$I^B I^B$	$I^A I^B$	$I^A I^B$
$I^B I^B$	$I^B I^O$	$I^A I^B$ or $I^B I^O$
$I^B I^B$	$I^O I^O$	$I^B I^O$
$I^B I^B$	$I^B I^O$	$I^B I^B$ or $I^B I^O$

Since child is of $I^A I^B$ the genotype of the father may be $I^A I^B$ or $I^A I^O$. So the father could not have the genotypes of $I^B I^B$, $I^O I^O$ and $I^B I^O$.

RH BLOOD GROUPS AND THEIR GENETIC SIGNIFICANCE

Ex. No. 5

Date:

Aim

To determine the Rh blood group in human.

Principle

Rh factor is an antigen present in the erythrocytes of Rhesus monkey and some people. About 93% of the European carry this factor and are therefore called Rh positive, while the rest 7% do not carry it and are Rh negative. Normally the Rh (-) person does not carry any antibody in his blood, but he can produce if his blood comes across Rh (+) factor (antigen).

Apart from ABO system, Rh system is clinically important. The Rh factor named for the Rhesus monkey. Rhesus monkey is a system composed of many antigens. D is a system composed of many antigenic among them and Rh positive means the individual has anti D agglutination. Over 99% of Asians are Rh positive D negative individuals who have received D positive transfusion develop appreciable time of anti D agglutinin and may develop transfusion reaction when again transfused with D positive blood.

Requirements

Glass slide, spirit, cotton, applicator stick, rust free sharp pin, anti Rh serum and microscope.

Procedure

A glass slide was thoroughly cleaned. A drop of antiserum was placed on the centre of slide. The tip of the index finger was pricked with a sharp sterilized needle. A drop of blood was oozed out and was placed near the drop of anti Rh serum. Then blood was thoroughly mixed with anti Rh serum with the help of applicator stick. The slide was observed for agglutination.

Result

Agglutination was observed/not observed. This result indicates that the blood contains Rh antigens. Therefore, the blood is Rh positive/negative.

Discussion

If a Rh⁺ ve person receives a blood transfusion from a Rh⁺ ve person, at first there will be no reaction. But, this contact with Rh⁺ ve factor sensitizes the stem of Rh⁻ ve person and evokes antibody formation against Rh factor. Thus if this person receives a second transfusion of Rh⁺ ve blood, it will be disastrous and lead to serious consequences (Fig. 124).

If a Rh⁻ ve woman marries a Rh⁺ ve person marries a Rh⁺ ve man and conceives a Rh⁺ ve foetus in her uterus, Rh⁺ ve foetus will pass into the circulatory system of the mother through the placenta and evoke Rh antibody formation by sensitizing the system of the mother. Rh antibodies formed during the first conception are little, the first Rh⁺ ve child will be normal. But if the sensitized mother again becomes pregnant with Rh⁺ ve foetus the result and some of these will cross through

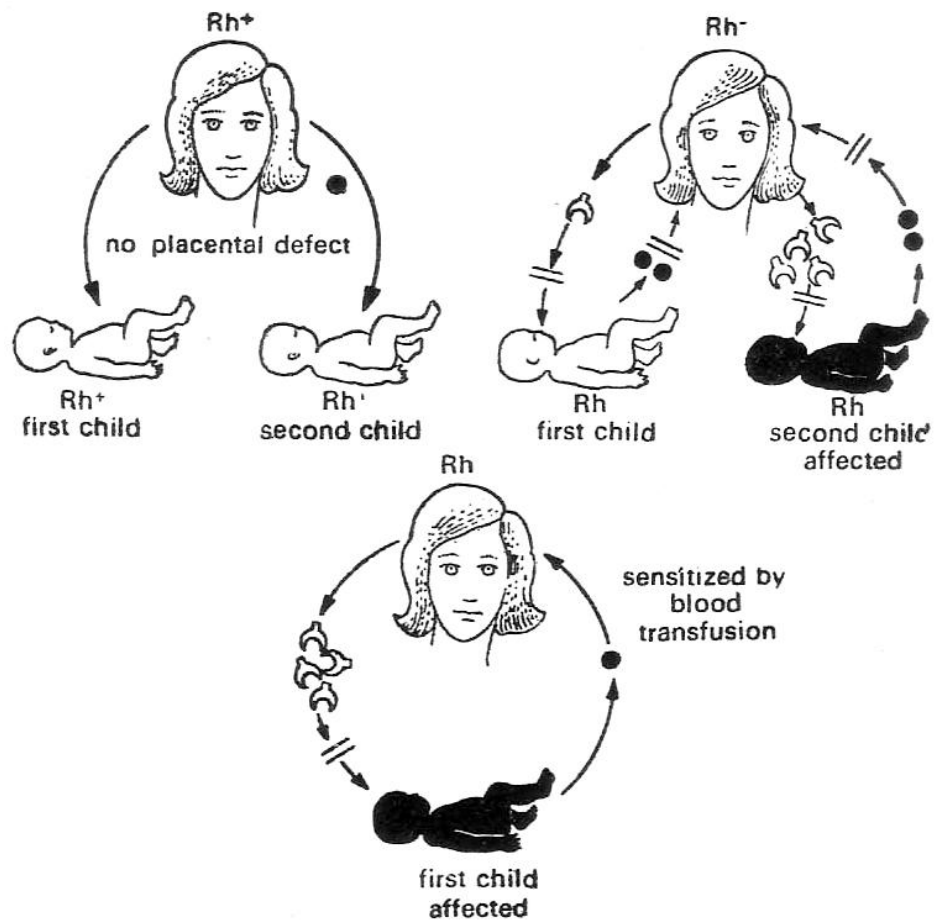


Fig. 124. Erythroblastosis foetalis.

the placenta and reach the foetus blood. Rh antibodies will react with the Rhesus antigen present in the foetal erythrocytes, thereby causing agglutination of red blood. Such child will be born with blood defect called erythroblastosis foetalis.

PEDIGREE ANALYSIS

Ex. No. 6

Date:

Aim

To analysis the Given Pedigree charts.

Materials required

Pedigree charts.

Procedure

Pedigree refers to the study of characters in a family line for several past generations.

The pedigree of a family is a chart. The females are represented by circles and the males by squares. Individual showing the trait under study are indicated by black in symbols. Closing half of the circle represents heterozygous individuals or a connecting line between the individuals indicates a mating between them. All members of the same generation are placed in the same row.

Pedigree chart of haemophilia

This is a family pedigree for haemophilia (Fig. 125). Haemophilia is a hereditary blood disease. It is characterized by delayed blood clotting because of the absence of anti-haemophilic factors. Haemophilia is a sex linked recessive character. It is caused by a recessive gene.

In the pedigree chart mother is normal and the father is haemophilic. Son is normal and the daughters are carrier.

In the F_2 generation one of the grand sons and the grand daughters are normal. One of the grand sons is haemophilic. Since X chromosome is carrying recessive gene males hence than female due to the presence one X chromosomes. On the other hand two recessive genes are necessary for a female to be haemophilic.

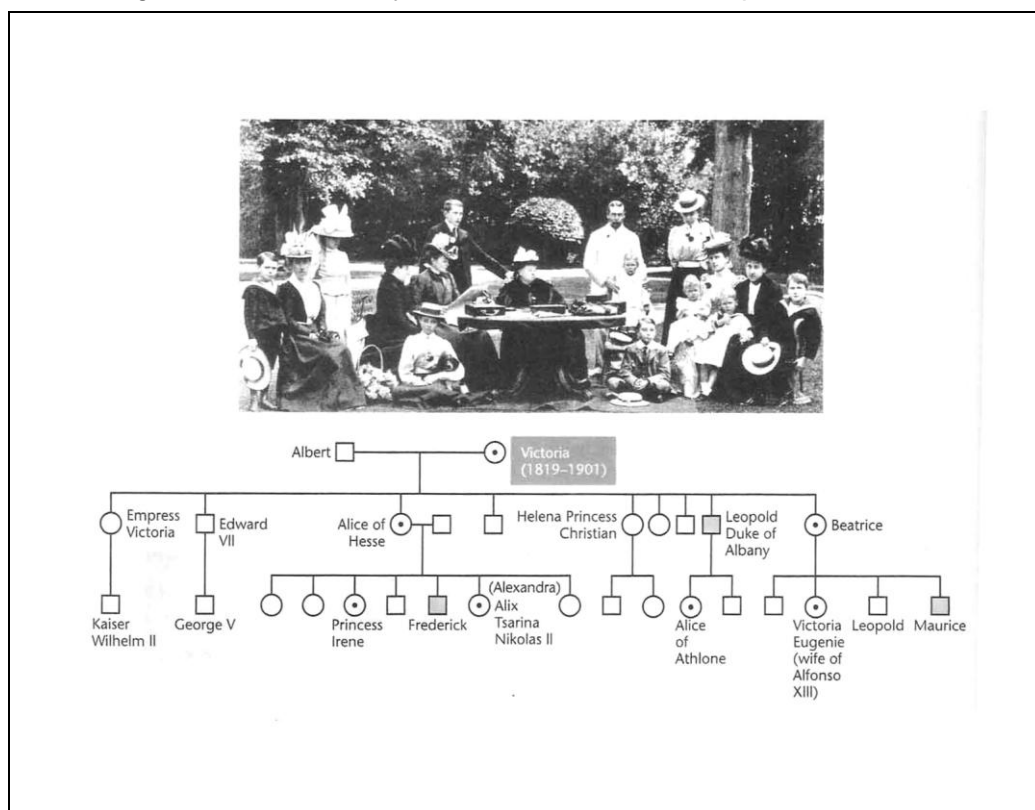


Fig. 125. Pedigree chart of Vitoria family.

HUMAN KARYOTYPING

Ex. No. 7

Date:

Aim

To make an observation of human karyotype.

Principle

Karyotype is a composite picture of a set of chromosomes in the mitotic metaphase of an individual made by taking a photo micrograph of specially prepared cells and then cutting out the chromosomes, matching them and arranging them in the descending order of length.

Materials required

A normal female photomicrograph of chromosome and scissors.

Procedure

To prepare human karyotype from the culture of blood cells treated with colchine were photomicrographed. The application of colchine was to permanently arrest the cell division at the metaphase. A photomicrograph spread out was a normal female. The chromosomes in the nucleus of blood cells were taken to make karyotype. The chromosome were cut out of 'the photomicrograph and paired morphologically arranged in series of decreasing in the length. The chromosomes were arranged according to size and position of the centromere. The karyotype was prepared and it is shown in the sketch.

Observation

According to the position of the centromere, three distinct types of chromosomes are observed. They are given below.

Metacentric chromosome

The position of the centromere is medium. This gives the chromosomes a 'V' shaped configuration.

Sub metacentric chromosome

The position of the centromere is terminal and is very close to one end of the chromosome. So that one arm is minute and the other is much a longer.

A normal human karyotype includes 22 matching pairs of chromosomes and is called as autosomes. The autosomes are numbered from 1 to 22 in the descending order of length. Apart from autosome the female has 2 X chromosome of equal size where as male has one 'X' chromosome and unequal sized 'Y' chromosome. These sex chromosomes are not numbered. The haploid set found in sperm and egg cell consists of 22 autosomes plus one of the sex chromosomes (a total of 23).

When the chromosomes are morphologically paired and are arranged based on their size and shape 7 distinct groups are reorganized. The groups have been denoted by letters A, B, C, D, E, F and G.

Characters of different groups of chromosomes in human karyotypes

Group A

It includes 3 pairs of chromosomes. They are the longest pairs of chromosomes. The 1st pair is metacentric having secondary constriction. 2nd pair is submetacentric and the 3rd pair is metacentric. They are closely distinguished from one another on the basis of the length and position of centromere.

Group B

It includes 4th and 5th pair of chromosomes. They are long but shorter than group A chromosomes. The centromeres are sub medium. They are distinguished from one another the chromosome 4 is slightly longer than chromosome 5th.

Group C

This group has more number of chromosomes. It includes 6 to 12 pairs of chromosomes. The chromosomes of this group are submetacentric. The 10th chromosome has a secondary constriction.

Group D

The chromosomes 13 to 15 are included in this group. The chromosomes are acrocentric because the position of centromere is terminal. All are denoted as SAT chromosomes. Since *satellite* is present at the shorter of all the chromosomes. The 13th chromosome has a secondary constriction also.

Group E

This group includes chromosomes 16 to 18. 16th is metacentric, 17th and 18th chromosomes are submetacentric. The 16th pair has a secondary constriction. These chromosomes are shorter than group D.

Group F

This group includes the chromosomes 19 to 20. They are shorter. The centromeres are nearly terminal and the chromosomes are metacentric. A satellite is present at the short arm of each chromosome. They have nucleolar organizer also.

Group G

'X' and 'Y' chromosomes are included in this group and can be distinguished from 21 and 22 due to its greater variability in appearance.

Conclusion

A human karyotype shows metacentric sub metacentric and accrocentric chromosomes. When they are arranged descending order, the normal human karyotype includes 22 matching pairs of autosome plus 2 X chromosomes in female (Fig. 126) or one X and one Y chromosome in male (Fig. 127).

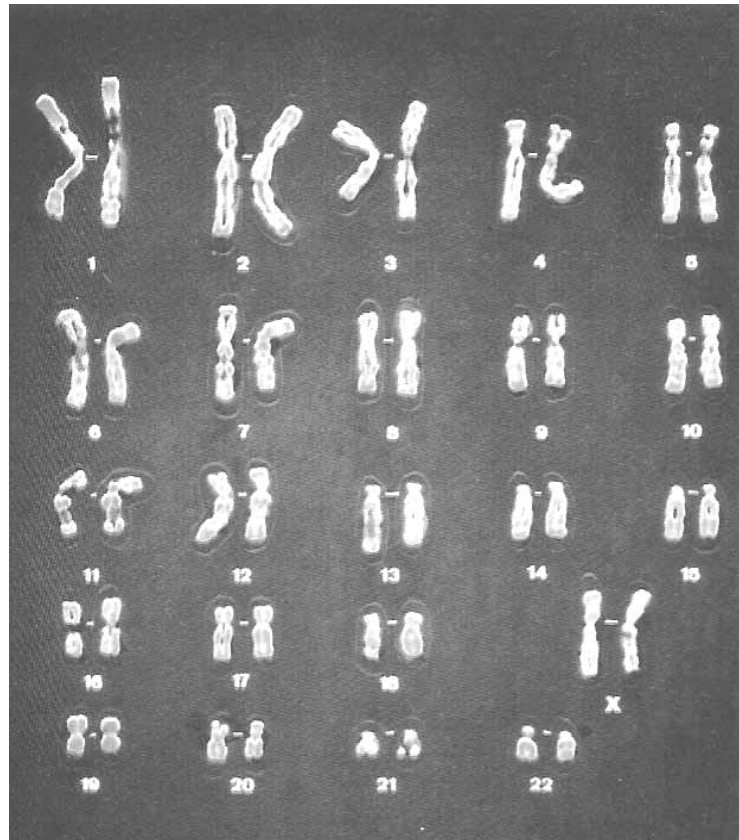


Fig. 126. Female karyotype.

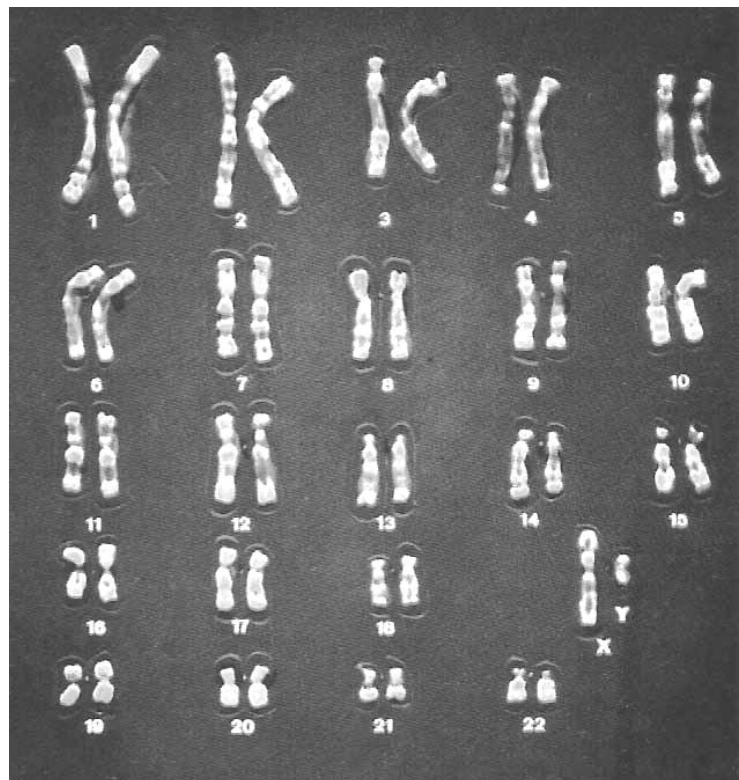


Fig. 127. Male karyotype.

8. HUMAN CHROMOSOMAL ABNORMALITIES

Ex. No.

Date:

Aim

To make an observation of karyotype of the human sex chromosomal syndrosomes.

Principle

Any change on the chromosome number will lead to abnormality called syndrome. This is due to addition or deletion of sex chromosomes, which is known to be a kind of aneuploidy. There are many sex chromosomal abnormalities and out of them Klinefelter's syndrome and Turner's syndrome are more common.

Klinefelter's syndrome

Klinefelter syndrome (Fig. 128) is due to sex chromosomal abnormality in male. It occurs as high as one in 506 births. It is named by Hardy F. Klinefelter in 1941. The individual with this syndrome is phenotypically male. But their testes are under developed. The breasts are larger and legs are also longer than the average body hair in soars. They have highly pitched voice. Most patients are sterile mentally defective. The cells of males with Klinefelter's syndrome have 47 chromosomes (Fig. 129) instead of 46. Thus this sterile male possesses XXY sex chromosomes constitution. Despite of two X chromosome the possession of Y enables the patient to have masculine characters. As in mongolism affected one is born more after to older women. This abnormality is due to non disjunction under abnormal condition older women produces an XX egg on an egg devoid of X chromosome. If the XX egg is fertilized by Y bearing sperm Klinefelter's syndrome results.

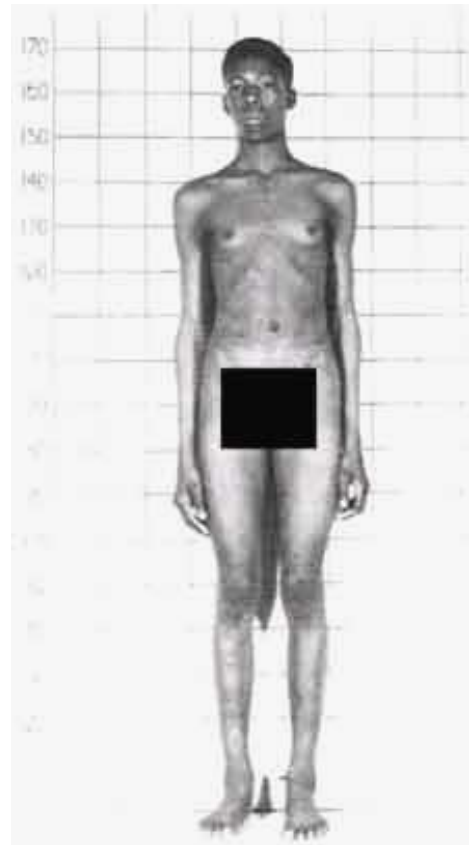


Fig. 128. Male with Klinefelter syndrome.

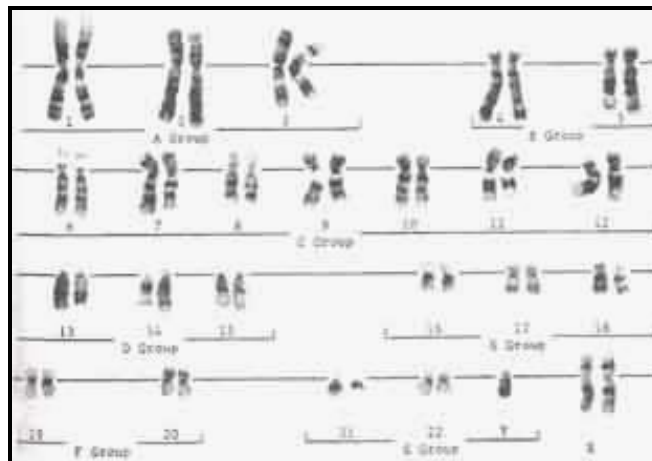


Fig. 129 Karyotype of the male with Klinefelter syndrome.

Turner's syndrome

The Turner's syndrome (Fig. 130) is a sex chromosomal abnormality in female. It was first noticed by Dr. Hendry and H. Turner (1938). Women with Turner's syndrome have rudimentary ovaries and under development anatomically and physiologically they are female although unable to menstruate and ovulate. Instead of normal ovaries only ridges of whitish tissue occur (streak gonad) i.e. why many authors use the term "gonadal dysgenesis" in place of Turner's syndrome. The affected women are short. They are subnormal intelligence (mentally defective).

The cells of female with Turner's syndrome have 45 chromosomes (Fig. 131) instead of 46. Here there is only one X chromosome instead of two. The absence of one X is due to non-disjunction of the sex chromosome during the formation of egg by the mother.

The mother produces either an XX egg or an egg devoid of X chromosomes. If the egg which has no chromosomes is fertilized by normal X bearing sperm an individual with Turner's syndrome is produced. This single X chromosome woman is sterile and she is an under developed female. The incidence of Turner's syndrome is one in 25,000 births.

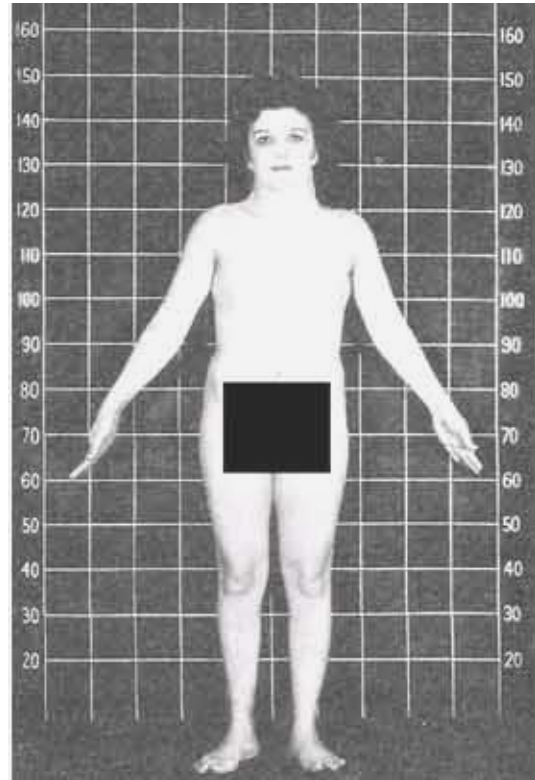


Fig. 130. Female with Turner's syndrome.

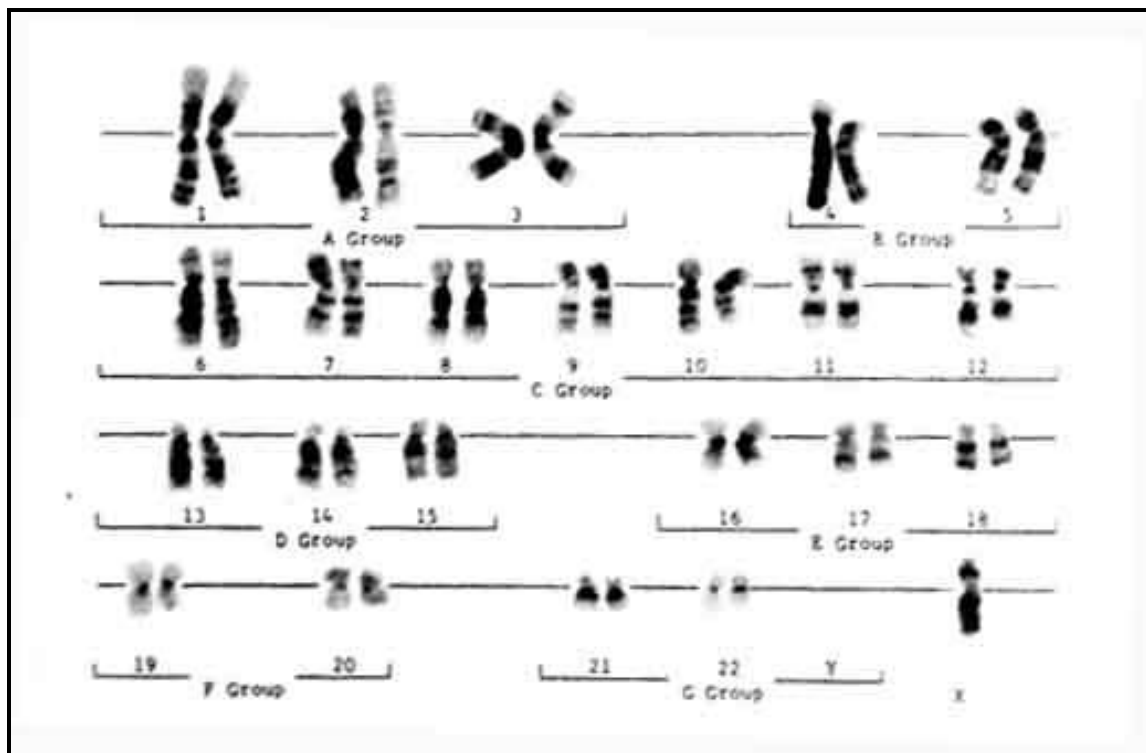


Fig. 131. Karyotype of the female with Turner's syndrome.

9. HARDY-WEINBERG LAW AND CALCULATION OF GENE FREQUENCIES FOR DOMINANT, RECESSIVE AND CODOMINANT TRAITS AND MULTIPLE ALLELES

Date:

Aim

To calculate the gene and genotype frequency in a population (I M. Sc. Zoology Students) by applying Hardy-Weinberg law.

Hardy-Weinberg law

English mathematician C. H. Hardy and a German physician W. Weinberg in 1908 discovered a principle concerning the gene frequencies in a population and this principle came to be known as Hardy-Weinberg law.

Hardy and Weinberg showed that there is equilibrium between frequencies of genes in a population. The relation frequency of each allele tends to remain constant generation after generation. The mathematical relation now is called Hardy-Weinberg theory or law. The law states that the relative frequencies of different genes in a large panictic population after random mating tend to remain constant generation after generation in the absence of mutation. Selection genetic drift and gene flow (panictic) the mating pattern that tends to random union of gametes from the individuals.

Procedure

1. PTC test: Stripes of filter paper dipped in PTC solution can be tasted by students and based upon it the group of students are divided into tasters and non tasters (if no bitter taste is dated that individuals is classified as non tasters) percentage tasters and non tasters in a percentage population is calculate is calculate. This is a typical Mendelian character where tasters are dominant. This is a typical character where tasters are dominant.

Calculation

Total number of strength = 24

Number of taster = 11

Number of non taster = 13

Let us assume T and t.

Calculation of gene frequency

$$(T + t)^2 = T^2 + 2Tt + t^2$$

$$t^2 = 13/24$$

$$t = \sqrt{13/24}$$

$$= 0.735$$

$$T + t = 1$$

$$T = 1 - t$$

$$= 1 - 0.735 = 0.265$$

$$(T + t)^2 = T^2 + 2Tt + t^2$$

$$2Tt = 1 - (0.0702 + 0.541)$$

$$= 1 - 0.6112$$

$$= 0.385$$

Calculation of genotype frequency

$$TT = 0.070$$

$$Tt = 0.0388$$

$$t t = 0.541$$

Phenotype frequency

$$\text{Taster (TT, Tt)} = 0.458$$

$$\text{Non taster (tt)} = 0.541$$

2. Tongue roller: In a population of I M. Sc. Zoology the following characters of rollers and non rollers were observed and were used for calculation of gene frequencies and genotype frequencies by applying Hardy-Weinberg law.

Total number of strength = 24

Number of roller = 18

Number of non roller = 6

Calculation of gene frequency

$$(R + r)^2 = R^2 + 2Rr + r^2$$

$$r^2 = 6/24$$

$$r = \sqrt{6/24}$$

$$= 0.5$$

$$R + r = 1$$

$$R = 1 - r$$

$$= 1 - 0.5 = 0.5$$

Genotype frequency

$$(R + R)^2 = R^2 + r^2$$

$$1 = 0.25 + 2Rr + 0.25$$

$$2Rr = 1 - (0.25 + 0.25)$$

$$= 1 - 0.50$$

$$= 0.50$$

Genotype frequency

$$RR = 0.25$$

$$Rr = 0.50$$

$$rr = 0.25$$

Phenotype frequency

$$\text{Roller (RR, Rr)} = 0.75$$

$$\text{Non roller (rr)} = 0.25.$$

Hardy Weinberg law and calculation of gene and genotype Frequency of multiple allele

In a population of I M. Sc. Zoology the following blood groups were observed among the students and was used for the calculation of gene frequencies and genotype frequencies by applying Hardy-Weinberg law.

Calculation

Total number of strength = 24

Number of A -group = 2

Number of B -group = 9

Number of A B -group = 1

Number of O-group = 12

Calculation of gene frequency

Let us assume

$$A = p, B = q, O = r$$

$$(p + q + r)^2 = p^2 + pq^2 + q^2 + 2qr + 2pr + r^2$$

$$r^2 = 12/24$$

$$r = \sqrt{12/24}$$

$$\begin{aligned}
 r &= 0.707 \\
 (O) \ r &= 0.707 \\
 2pr &= 2 + 12 \\
 &= \sqrt{2 + 12/24} = 0.763
 \end{aligned}$$

$$\begin{aligned}
 P &= 2pr - r \\
 &= 0.763 - 0.707
 \end{aligned}$$

$$\begin{aligned}
 (A) \ p &= 0.056 \\
 p + q + r &= 1
 \end{aligned}$$

$$\begin{aligned}
 q &= 1 - pr \\
 &= 1 - (0.056 + 0.707) \\
 &= 1 - 0.763
 \end{aligned}$$

$$\begin{aligned}
 (B) \ q &= 0.237 \\
 I^A (p) &= 0.056, \\
 I^B (q) &= 0.237, \\
 I^O (r) &= 0.056
 \end{aligned}$$

Calculation of genotype frequency

$$\begin{aligned}
 I^A I^A &= 0.0031 \\
 I^A I^O &= 0.0791 \\
 I^B I^B &= 0.0561 \\
 I^A I^O &= 0.3350 \\
 I^A I^B &= 0.0265 \\
 I^O I^O &= \underline{0.4998} \\
 &\underline{0.9996}
 \end{aligned}$$

Genotype frequency

$$\begin{aligned}
 A \text{ group} &= 0.0822 \\
 B \text{ group} &= 0.3911 \\
 A B \text{ group} &= 0.0265 \\
 O \text{ group} &= \underline{0.4998}
 \end{aligned}$$

*** ❧ ***

VI. MICROBIOLOGY

CULTURE OF BACTERIA

Ex. No.: 1

Date:

Aim

To prepare a culture of bacteria in the given sample (polluted water).

Principle

Sterilized petri plates contain nutrient agar. The bacterial nutrient agar includes the essential nutrients for the bacteria such as minerals, vitamins, amino acids, etc. The incubator provides the optimum temperature for the growth of the bacteria.

Materials required

Autoclave, laminar flow, test tubes, petridishes, autoclave, culture of bacteria, inoculation loop and spirit lamp.

Nutrient agar

Peptone	: 5.0 g
Sodium chloride	: 5.0 g
Beef extract	: 1.5 g
Yeast extract	: 1.5 g
Agar	: 15.0 g

Distilled water : 1000 ml (final pH = 7.4).

(A-Flaming the needle, B. Cotton plug or cap removal, C-Flaming themouth of the tube, D-Culture removal, E-Transferring the culture to a petriplate and F- Inoculation needle).

Procedure

Culture of bacteria or Microbial culture

Nutrient agar was suitably diluted with distilled water (25 g in 1000 ml) which was taken in an Erlenmeyer flask. Aseptic method for the culture of bacteria (Fig. 132) was followed. Culture medium was sterilized in the autoclave. Then the culture medium was cooled up to 55°C and then it was poured into the petri dish in the chambers of laminar flow. The prepared media (petridish) were stored at 4°C in the refrigerator. A drop of polluted water sample was aseptically transferred to the agar plate with sterilized **inoculation loop** (Fig. 133). The agar plate was incubated at 30°C for 48 hours. Then the agar plates were examined for the growth of microbes. Appearance of any colony (Fig. 134) indicates the presence of microbes in this sample.

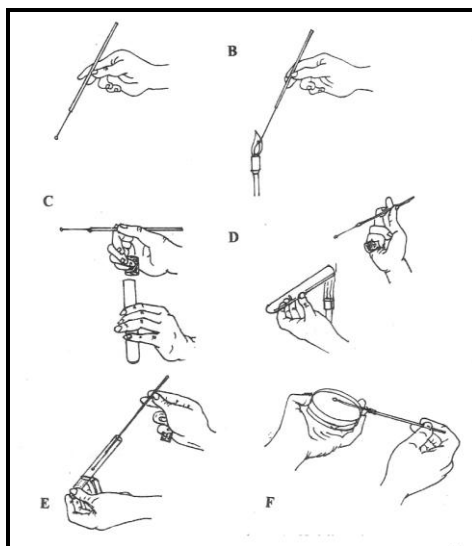


Fig.132. Aseptic method for the culture of bacteria.

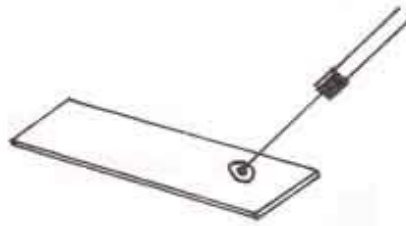


Fig.133. Placing the culture with inoculation loop.

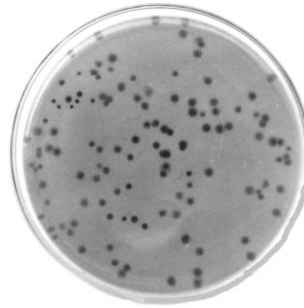


Fig. 134. Microbial colony in the culture.

Culture of pure bacteria

Pure **culture of bacterium** was obtained by the method of Streak Plate Technique. The nutrient agar plates were prepared as earlier. The inoculation needle was sterilized by flaming it red hot and allowed it to cool for 30 seconds. The culture tubes were held in left hand and took the tube near flame and removed the cotton with right hand and flamed the mouth of the tube for a few seconds. The culture was touched with the needle loop and took the culture. Place it on the agar plate and streak it on the plate once. The loops were resterilized and cooled it as above and took the culture from one end of the plate to other end and complete the streak as indicated in the (Fig.135).

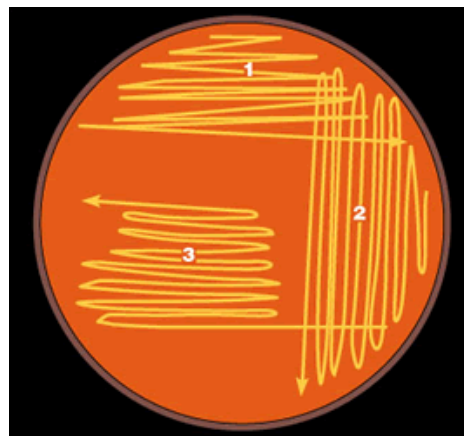


Fig.135. Method for inoculating the pure culture.

- 1 - Primary streaking.
- 2 - Cross streaking from primary streak.
- 3 - Cross streaking from area 2.

Result

Microbial colonies (Fig. 136) were observed in the culture media indicate the presence of microbes in the sample (polluted water).

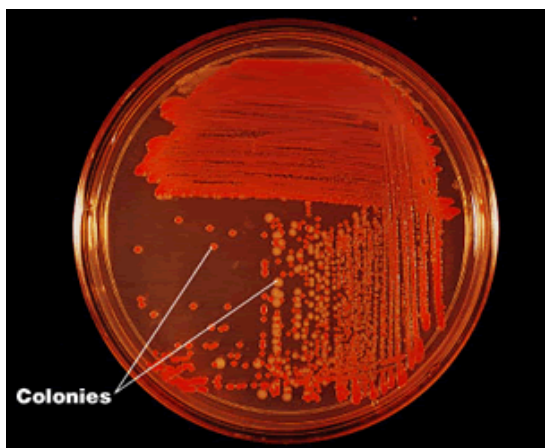


Fig. 136. Microbial colonies in the culture medium.

AUTOCCLAVE

Autoclave is an apparatus used for sterilization by steam pressure.

1. The **basic design and principle and working mechanisms** of these types of autoclaves is similar to that applied in the designing of a kitchen pressure cooker. The heat produces steam to a pressure higher than the atmospheric pressure and so the temperature of boiling water is also raised. The chamber of autoclave is partially filled with water and particles to be autoclaved are placed in a tray inside the chamber. The articles being autoclaved are known as load.
2. The autoclave (Fig. 137) has a valve that releases excess steam beyond a certain pressure and the pressure inside the chamber can be read on a dial or gauge attached with the autoclave and the temperature display device is also there.
3. The outer wall or container jackets the inner container. Autoclave includes two **principles** of controlling microorganisms. They are 1. Temperature (150°) and pressure (15 lb) The water is filled in the outer jacket while load is placed in the inner container. First the outer container is filled with required quantity of water, which is then heated to boil. The steam generated first enters the jacket and then through this path goes into the sterilizing chamber. Then the operator has to wait till all air contained in the chamber is pushed out and the space is fully filled with the steam to saturation. The steam must be saturated not superheated as in latter case it acts more as dry air and not as moist heat. Now the autoclave is locked i.e. out lets are closed. Then the heating is continued to let the chamber be further filled with steam till the desired pressure and temperature.
4. **Uses:** The autoclave is used to sterilize the media, broths and vessels.



Fig. 137. Autoclave.

BACTERIAL GROWTH CURVE

Ex. No. 2

Date:

Aim

To enumerate the bacterial population by using spectrophotometer.

Principle

Bacterial population or rate of growth can be determine by measuring the turbidity as optical density i.e. turbidness of a suspension of a culture. The more turbid is a suspension, less light will be the transmitted through it. Since turbidity can be measured by the optical density. The absorbance is directly proportional to the bacterial cell. The absorbance is a logarithmic value used to plot the bacterial growth in the graph against time factor.

Materials required

Test tube containing 5 ml of nutrient broth, spirit lamp, 24 hours nutrient both and culture of *Bacillus cereus*.

Procedure

Using micropipette, the labeled tubes such as (0, 2, 4, 6, 8, 12, 24) 0.1 ml or 100m of bacterial broth of *Bacillus cereus* culture.

Remaining 7 inoculated tubes served as blanks for adjusting the spectrophotometer. All the 14 tubes were incubated at 37°C for 24 hours.

Result

The optical density of inoculated culture was observed after an interval of 0, 2, 4, 6, 8, 12, 24 hours in a spectrophotometer at 440 nm (Table 1).

Absorbance of optical density for all the inoculated tubes were noted and used for plotting the growth curve of bacteria (Fig. 138).

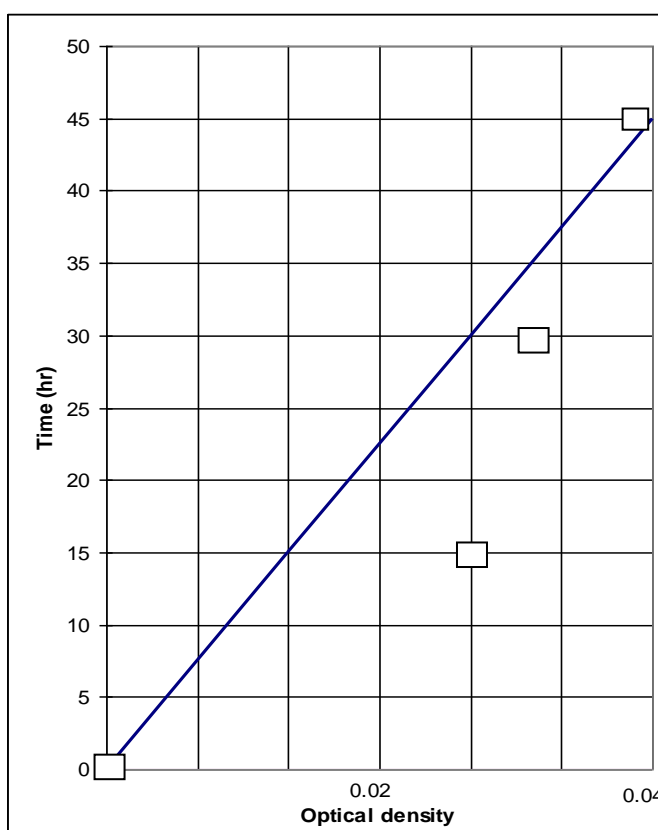


Fig. 138. Bacterial growth curve.

Table: 1. The absorbance of optical density in different time hours.

S. No.	Time (hr)	Optical density
1	0	0.000
2	15	0.029
3	30	0.033
4	45	0.039

COUNTING TEST

Ex. No. 3.

Date:

Aim

To enumerate the number of bacterial cells present in the sample or culture.

Principle

The number of cells in the population is measured directly by counting under the microscope. This method is called direct microscopic count. Since most of the microbial cells are smaller in size and their population is much higher in the sample, it is necessary to use special chambers such as haemocytometer chamber to count the number of cells in the sample. However, this method is not sensitive because at least 10^6 cells per ml must be present for reliable counting using this method. This method of microscopic counting cannot distinguish living cells from dead cells.

In order to count the number of viable cells present in the samples, the plate count method can be used. The two versions of this plate count methods are spread plate and pour plate method (Exercise 3). In the spread plate method, usually the samples are diluted serially 10^{-1} , 10^{-2} , 10^{-3} and so on, then the samples (0.1 ml) from each dilution are spread over the nutrient agar plate. In the pour plate method, the serially diluted sample (1 ml) is mixed with the molten agar and the mixture is poured into the sterile plate. The number of colonies appeared after appropriate incubation conditions represent the number of viable cells present in the aliquote of the diluted sample.

For some reasons if the plate count method cannot be used, viable cell count can be determined with liquid media using most probable number (MPN) method. The sample to be counted is diluted until the small volume would contain one viable cell. A series of small dilutions are inoculated into a number of identical tubes containing growth media. Under suitably diluted conditions some aliquotes will contain a viable cell but not in others so that after incubation, growth of the cells will occur in some tubes but not in others. By counting number of positive and negative tubes for growth at each dilution and referring to a standard statistical table (MPN Table), the most probable number of cells can be calculated.

Materials Required

Haemocytometer, Cover slip, Test tubes, Cultures, Pipettes, Nutrient broth, Cotton, a bacterial culture (*E. coli* or *Pseudomonas*), a yeast culture (*S. cerevisiae*).

1. Procedure for Serial dilution technique

Sterile test tubes (Fig. 139) were arranged (cotton plugged) in a test tube stand and labeled the tubes as 10^{-1} , 10^{-2} , 10^{-3} till 10^{-7} dilutions (Fig. 1). 4.5 ml sterile saline solution (0.85% NaCl) was distributed to each tube aseptically. 0.5 ml of the given bacterial culture (*E. coli*) was transferred to the first tube and mixed by vortexing (Dilution 10^{-1}). 0.5 ml from the first tube was taken using a sterile pipette and transferred to the 2nd tube and mixed by vortexing (Dilution 10^{-2}). This kind of serial dilution from one to next tube was repeated till the last tube (Dilution 10^{-7}). The nutrient agar plates were arranged and labeled them as control, undiluted, 10^{-1} , 10^{-2} , 10^{-3} till 10^{-7} (make-duplicate plates for each dilutions).

Note: The dilutions were in the order of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} till 10^{-7} and the number of cells present in these tubes comparing the original culture were in the order of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-7} .

1. Using sterile pipette transferred 0.1 ml of the diluted sample to the corresponding nutrient agar plate. [Fresh sterile pipette was used each time].

2. The samples on the agar plate were spread using the L. rod. The plates were incubated at 37°C for overnight.
3. The plates that contain the number of colonies between 30 and 300 per plate were selected and count them.
4. The average of number of colonies was calculated at each dilution. The number of cells present in the culture (cells/ml) was calculated from the colonies at a dilution multiplied by 10 (if the sample volume is 0.1 ml) and dilution factor.

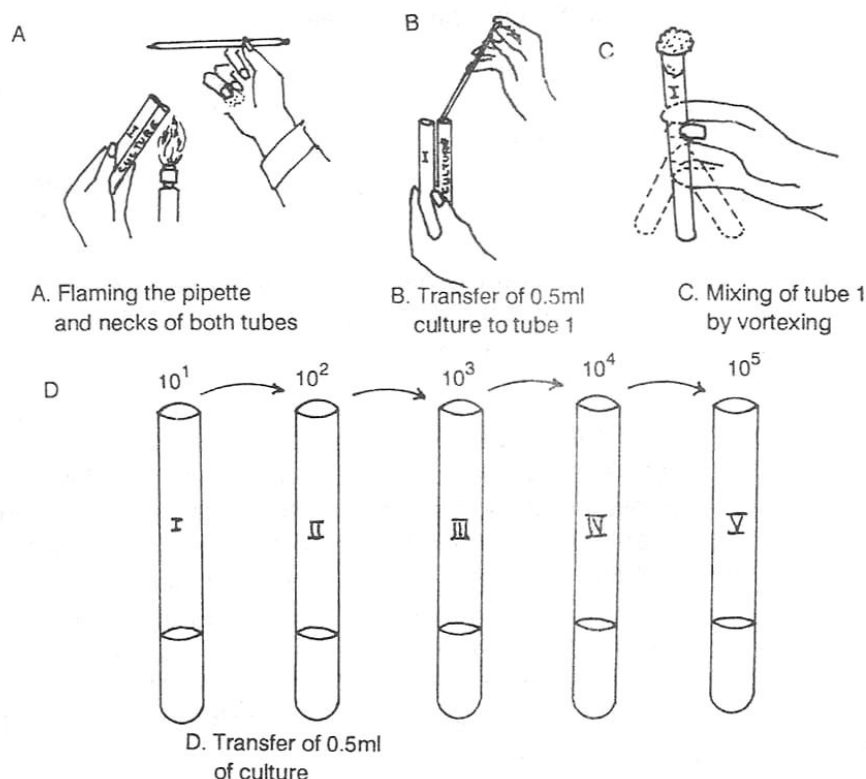


Fig. 139. Serial dilution technique.

2. Procedure for Cell counting using haemocytometer Serial dilution technique

The counting chamber was first examined (Fig. 140) to familiarize the haemocytometer counting. The ruling area (9 large squares) was focused in haemocytometer with 10X and the 40X objective.

Note: The haemocytometer has ruling in 9 mm^2 of which one square can be seen under 100X objective of microscope. The dimension of the counting chamber ruling Noted. Each of the large squares was 1 mm on each side or $1 \text{ mm} \times 1 \text{ mm} = 1 \text{ square mm}$ in area. The depth of the chamber is 0.1 mm. Each of the large squares has volume of $1 \text{ mm} \times 1 \text{ mm} \times 0.1 \text{ cubic mm}$ or 0.1 mm^3 . 1 cubic millimeter was equivalent to $1/1000$ cubic centimeter (cc or ml) or 10^{-3} ml . Therefore $0.1 \text{ cubic millimeter} = 1/10,000 \text{ cc}$ or 0.0001 cc or 10^{-4} ml . Therefore the number of cells counted in this one large square should be multiplied by 10^4 to calculate the cell number per ml of sample used for counting.

Focus the centre large square of the haemocytometer under the microscope before applying sample on the field.

Note: The large square has been divided into 25 medium sized squares. The dimension of the each medium square is 0.2 mm length, 0.2 mm breath, and 0.1 mm depth. Therefore the area of a medium square is $0.2 \times 0.2 = 0.04 \text{ mm}^2$. The volume of the one

medium square was $0.2 \times 0.2 \times 0.1 = 0.004 \text{ mm}^3$. This was equivalent to the $1/25$ of the one large square i.e. $1/25$ of 0.1 mm^3 .

Further each medium sized square in the centre was divided in to 16 small squares. The area of one small square was $= 0.0025 \text{ mm}^2$ and its volume was applied the sample on the grid and cover with the special cover slip (use yeast cells for easy viewing and counting)

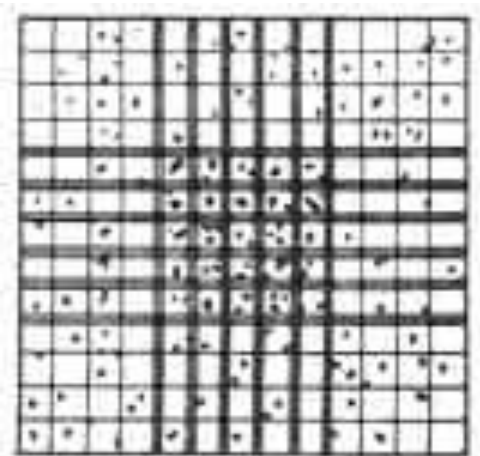


Fig. 140. Counting using haemocytometer.

The cells were focused under microscope and started counting the cells on the large square. The cells were initially counted in the medium sized square (0.2 mm). Counting the cells was started from the top row of these medium squares and continued to the bottom row.

Note: Some cells are present at the lines were forming the squares. In such case, only the cells that were on the line forming top or right side of the square was counted. This avoided the chances of counting the cells twice.

Alternatively for quick counting, four corner squares and one centre square could be counted. Thus obtained cell number could be multiplied with 5 to get the total number of cells present in all 25 squares. To obtain the number of the cells present per ml, the number of cells counted in all 25 squares should be multiplied by 10^4 .

Note: Some of the counting chamber, large square has 25 medium sized squares with the dimension as indicated ($1 \text{ mm} \times 1 \text{ mm}$) but the depth was 0.02 mm . Therefore, the total area of the one large square was 1 mm^2 but the total volume was 0.02 mm^3 . This was $1/5$ of the volume described in this exercise with Neubauer haemocytometer chamber. Therefore, to obtain total number of cells per ml, the number of cells was counted in 25 medium squares of such counting chamber should be multiplied by 5×10^4 .

If the cell density in the sample was high (more than 250 cells in the large square) the sample should be suitably diluted before applied on to the haemocytometer. In such cases the dilution factor was used to multiply the total cell number obtained per millilitre.

Result

1. Serial dilution technique

..... number of cells present in the culture (cells/ml).

2. Counting using haemocytometer

.....total cell number obtained per millilitre.

ANTIBIOTIC SUSCEPTIBILITY TEST

Ex. No. 4

Date:

Aim

To determine antibiotic sensitivity of bacterial strains and minimal inhibitory concentration of antibiotic to bacterial strain.

Principle

Antibiotics are commonly used for the treatment of infectious diseases. Once the causative organism is identified for specific disease and is isolated, it is important to test the sensitivity of the organism to the effective antibiotics. In the clinical laboratories antibiotic impregnated discs are commonly used to identify the antibiotic sensitivity of the causative organism. The effectiveness of the antibiotic in this test is based on the size of the zone of inhibition. The zone of inhibition also depends on the diffusability of antibiotic, the size of the inoculums, type of medium and other factors. The concentration of the antibiotic at the edge of zone of inhibition represents Minimal Inhibitory Concentration (MIC) of antibiotic. Alternatively the antibiotic disc with different concentration of antibiotic could be employed in the test. The minimal inhibitory concentration is the lowest concentration of antibiotic that exhibits the zone of inhibition on the assay plate.

Materials required

Antibiotic discs, Nutrient agar, Bacterial cultures, petriplates.

Procedure

A. Antibiotic sensitivity test

The cultures were selected and labeled that were used for antibiotic sensitivity assay (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*). The nutrient agar plates were prepared (atleast 3 plates). A swab of the test culture was aseptically taken and inoculated the surface of the nutrient agar plate completely so as to make a lawn. 5 minutes was allowed at least for the agar surface to dry before applying disc. A forceps was taken and sterilized the tip by dipping in alcohol and then flaming. Allow to cool. The antibiotic disc was carefully taken and placed over the agar plate at least 15 mm from the edge of the plate. The disc was gently pressed to give a better contact with the agar. Place at least six different antibiotic discs at the same distance apart from each other in the agar plates. The plates were incubated in inverted position for 16 to 18 hrs at 37° C. The zone of inhibition around the antibiotic discs (Fig. 141.) was observed. Whether test organisms was resistant (no zone of inhibition) or sensitive (clear zone of inhibition) to the antibiotic was indicated.

Results

.....Antibiotic(s) is/are highly sensitive.

.....Antibiotic(s) is/are sensitive.

.....Antibiotic(s) is/are resistant.

Few Antibiotic discs and their codes:

Aureomycin	: A	Ampicillin	: Am
Bacitracin	: B	Chloromycetin	: C
Carbenicillin	: CB	Cloxacillin	: CX
Erythromycin	: E	Gentamycin	: G
Kanamycin	: K	Methycillin	: Me
Neomycin	: N	Novobiocin	: NB
Nafcillin	: NF	Penicillin	: P
Polymyxin	: PB	Rifampicin	: RA
Streptomycin	: S	Terramycin	: T
Tetracyclin	: TC	Vancomycin	: VA

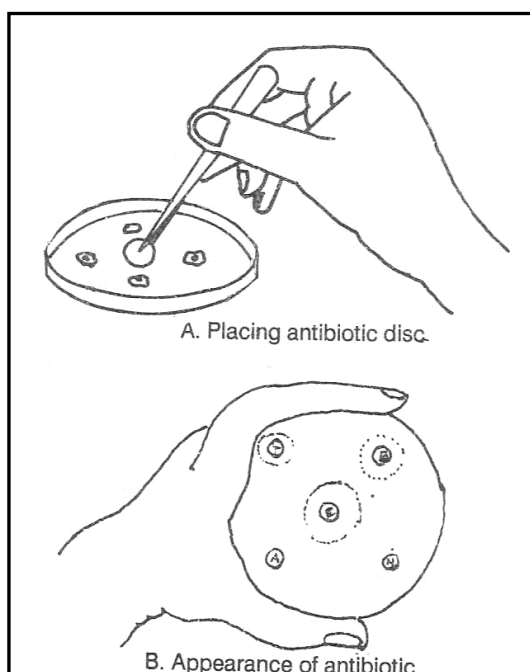


Fig. 141. Antibiotic sensitive test.

PREPARATION OF SMEARS AND SIMPLE STAINING

Ex. No. 5.

Date:

Aim

To prepare a smear with simple stain to study the morphology of Bacterial cells.

Principle

In simple staining, bacterial smear is stained with a positively charged chromogen. Since the bacterial nucleic acid and cell wall components carry negative charges. They strongly attract and bind with cations of the chromogen. Simple staining elucidate the morphology of bacterial cells. Most commonly used strains are methylene blue, crystal violet 20 to 60 second and methylene blue 40 to 60 seconds.

Materials required

Methylene blue, Crystal violet, Distilled water and carbol fuchsin.

Preparation of reagent

1. **Methylene blue:** 3 gm of methylene blue powder in 100 ml of distilled water.
2. **Crystal violet:** 1 gram of crystal violet in 100 ml of distilled water.
3. **Distilled water and carbol fuchsin.**

Solution A: 300 mg basic fushin was dissolved in 100 ml of 90% ethanol.

Solution B: Phenol 5 g in 100 ml of distilled water solution A and solution B was mixed and used as stain.

Procedure

A drop of distilled water was placed on the center of a clean glass slide with the help of the inoculation loop. The bacterial colony from the culture was spread on the glass slide. The smear was stained with violet strain for 1 minute. The stained smear was rinsed in tap water to remove the excess stain. The smear was dried out by using paper. The stained bacterial smear was examined under the compound microscope to observe the bacterial colony from the culture.

Result

The rod shaped gram positive bacteria appeared in violet colour (Fig. 142. a) and the transparent rod shaped Gram negative bacteria appeared in red background (Fig. 142. b) were observed from the bacterial culture.



Fig. 142. a. Gram positive bacteria.



Fig. 142. b. Gram negative bacteria.

SPECIFIC STAINING – GRAM STAINING

Ex. No. 6

Date:

Aim

To identify the gram positive and gram negative bacteria by gram staining.

Principle

Christian gram in 1884 developed a staining procedure which is more valuable. This procedure enables us to differentiate between kinds of bacteria that are morphologically distinguishable. It is therefore called as differential staining.

Gram staining serves to differentiate bacteria into two distinctly separate groups called gram positive and gram negative.

Crystal violet stained cells are subsequently treated in alcohol and acetone to take away the colour. This treatment decolourize the some bacteria which are designed as gram negative on the layer gram positive bacteria will retain the colour following decolouration. Counter staining with safranin stain which stains the gram negative bacteria red while the gram positive bacteria retain the original crystal violet stain.

Materials required

Bacterial culture, spirit lamp, inoculation loop glass slide, crystal violet stain, gram iodine stain, decolourizer and distilled water.

Preparation of reagents

Crystal violet stain

Solution A: 800 g of ammonium oxalate has dissolved in 20 ml of 95% ethonal.

Solution B: 2 g of crystal violet was dissolved in 20 ml of 95% ethonal.

Gram iodine stain

1 g of iodine and potassium iodine were dissolved in 100 ml of distilled water.

Decolourizer

100 ml of 95% alcohol and 100 ml of acetone were mixed.

Stock solution

2.5 mg of safranin was dissolved in 100 ml of 95% ethanol.

Working solution

20 ml of the stock solution was diluted to 100 ml with distilled water.

Procedure

A clear glass slide was taken and an inoculation loop for a drop of water was placed on the glass slide. Smear of the given bacterial cells into the smear were fixed by passing the glass slide gently over the flame. The smear was stained with crystal violet solution for about 2 minutes and washed with tap water a few drops of iodine solution was added to the slide and kept for 2 minutes.

Then the slide was washed in tap water and then with decolourized for 30 seconds. After decolourization the slide was washed in tap water and counter stained with safranin for 1 minute. The stained slide was washed in tap water and air-dried. Then the slide was observed high power of the microscope and then through oil immersion objective.

Results

Gram-positive bacteria was appeared in violet colour (Fig. 143).

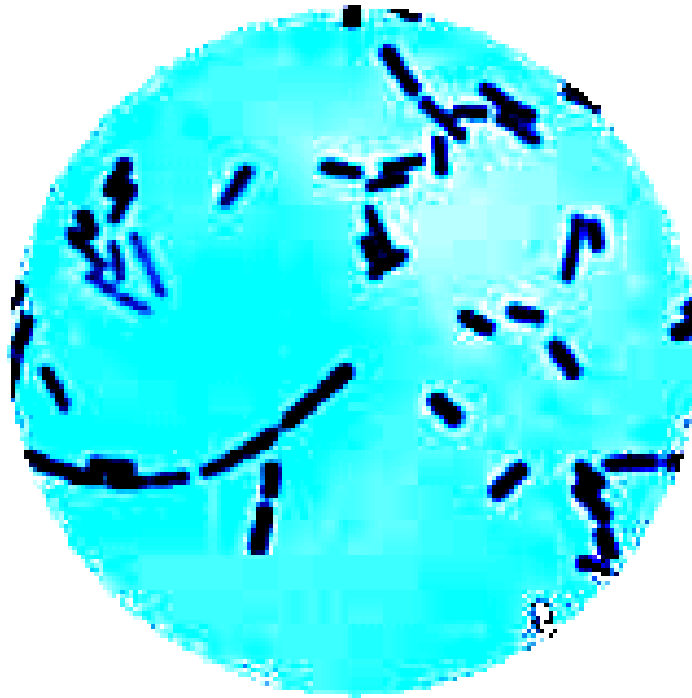


Fig. 143. Gram-positive bacteria appeared in violet colour.

NEGATIVE STAINING

Ex. No. 6

Date:

Aim

To prepare a smear of the given bacteria for negative staining.

Principle

Negative staining is a technique by which bacterial cells are not stained, but are made to be visible against a dark background. Acidic stains like eosin, Congo red are used for this method. Acidic stains have a negative charge. Therefore, they do not combine with the negatively charged bacterial surface. The stain forms a deposit around the cells, resulting in the appearance of bacterial cells as colourless against a dark background.

Materials required

Bacterial culture, inoculation loop, glass slide, microscope and spirit lamp.

Reagents

2 % Congo red stain: 2 gm of Congo red was dissolved in 100 ml of distilled water.

Procedure

A loop full of bacterial suspension was taken from the culture and placed on a clean glass slide. 2 drops of 2% Congo stain were added and mixed thoroughly with the help of the inoculation loop. The mixture was spread as a thin film across the slide by the use of another slide. The smear was allowed to dry in the air. Then the slide was examined under a compound microscope.

Results

The bacteria appear colourless (or) transparent in the red background (Fig. 144).

Discussion

Congo red is an acidic dye and has negative charges. It will not react with the negatively charged bacterial surface. Congo red will not stain the bacteria but will accumulate on the surface of the bacteria. In the red background, the bacteria look transparent. Negative staining will be useful in studying the morphology of bacteria.

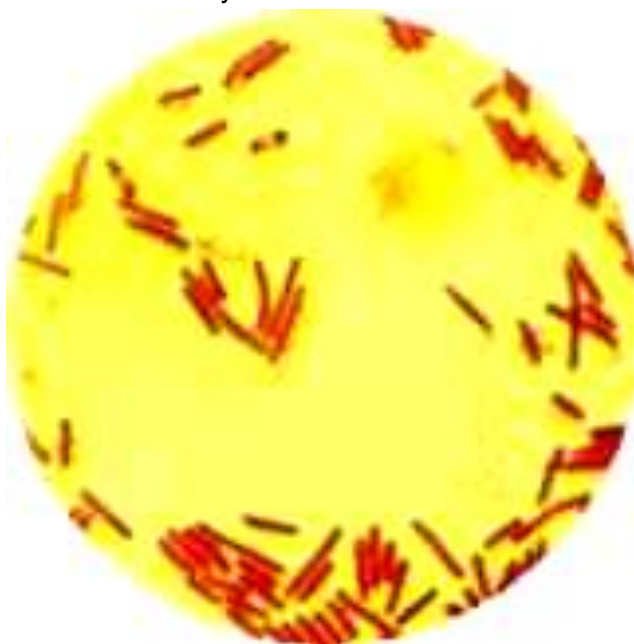


Fig. 144. Gram-negative bacteria appeared in red colour.

VII. CELL AND MOLECULAR BIOLOGY

Ex. No.: 1

MICROMETRY

Date:.....

Aim: To measure the microscopic cells.

Materials required

Stage micrometer, ocular micrometer, microscope and any cell types.

Ocular micrometer

It is a circular glass disc looks like a cover slip. It is fitted with ocular lens or eyepiece. The ocular micrometer (Fig. 153) has a small line at its center. It is divided into 100 divisions whose value is not known. The value of each division is to be calibrated with the stage micrometer.

Stage micrometer

It is in the form of a glass slide, which is placed on the stage of the microscope. The stage micrometer (Fig. 145) has at its center a line that measures 1 mm in length. This line is divided into 100 small divisions. So each division is 0.01 mm in length.

Procedure

Before the micro slide is placed under the microscope, ocular micrometer is fitted with eyepiece and then the stage micrometer is placed on the stage of the microscope. The value of the one ocular micrometer division is to be calibrated. The stage micrometer was adjusted for calibration. The stage micrometer was adjusted so as to coincide the zero of the stage micrometer with 1 division of the ocular micrometer. The division of the stage coincide with the division of the ocular is noted. The values of the stage micrometer are divided by the values of the ocular. The value is multiplied by 10 to express the value in micron ($1\mu = 1/1000$ mm). Thus the value of one division of ocular micrometer is obtained. Then the stage micrometer is removed and the micro slide containing the cells to be measured is noted which is multiplied by the value of one division of ocular micrometer. This gives the measurement of the cell.

Results

1. Value of one ocular division in low power = _____ μ .

2. Value of one ocular division in high power = _____ μ .

Calibration in Low Power (10X)

S. No.	No. of ocular divisions	No. of stage divisions	Value of one Ocular division	Mean Value
1				
2				
3				
4				
5				

Value of one ocular division in low power = _____ μ .

Calibration in High Power (45X)

S. No.	No. of ocular divisions	No. of stage divisions	Value of one Ocular division	Mean Value
1				
2				
3				
4				
5				

Value of one ocular division in high power ____ μ .

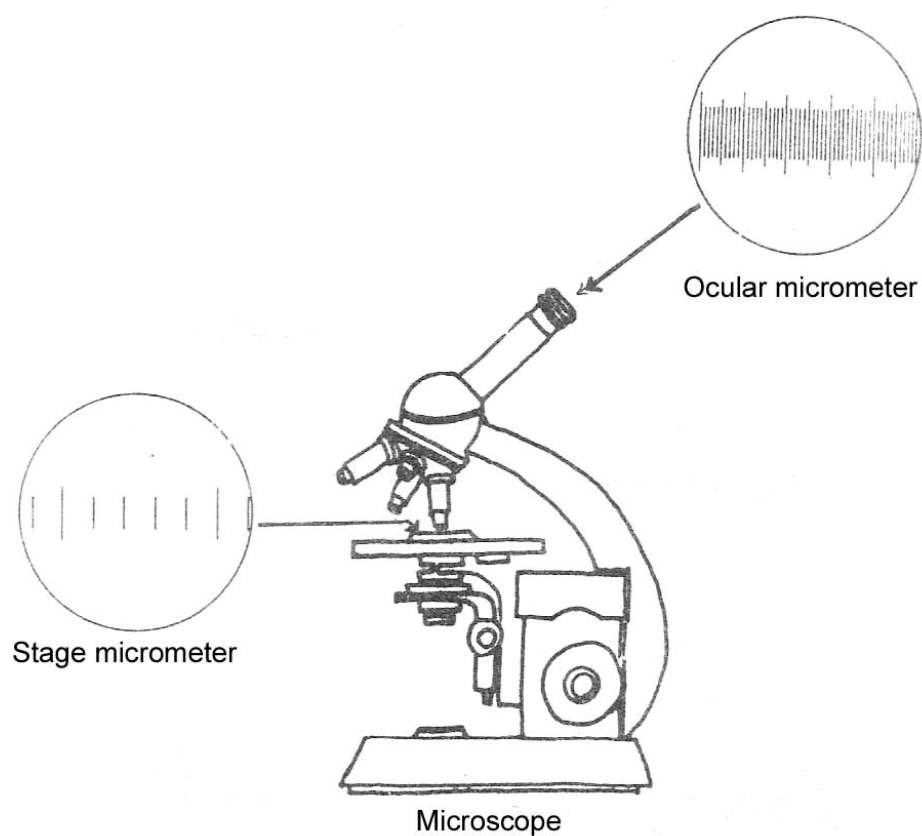


Fig. 145. Ocular and micrometer in microscope.

CAMERA LUCIDA DRAWINGS

Ex. No.: 2

Date:.....

Aim: To draw the microscopic organisms or cells.

Materials required

Camera Lucida, adjustable ring, microscopic cell to be drawn and microscope.

Procedure

The Camera Lucida was fitted on to the microscope. The slide bearing the object was placed, focused, and viewing through the prism of the camera Lucida. Artificial light has to be used and other lights of the room should be dim and fallen away. The light was formally adjusted. A white paper was placed on the right hand side of the microscope on the table. A lead pencil was hold in right hand. The microscopic preparation as well as the white paper and pencil should be visible to eye. It was some times necessary to raise the paper by a few inches from the surface of the table for fine focusing. It could be done easily by placing any lab record under the paper.

Observation

Once the object as well as the paper and the pencil points were clearly focused on the paper and the pencil point is actually moving on the image of the object reflected down on the paper. Such a sketch was later made permanent using Indian ink. Then various parts should be labelled.

Discussion

The Camera Lucida (Fig.146) essentially consists of the following parts.

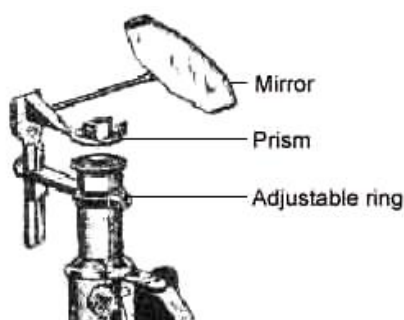


Fig.146. The Camera Lucida fitted with microscope.

Adjustable ring: It can be fitted to the body of any microscope. This is done by removing the eyepiece fitted with ring and than re-inserting the eyepiece in to the body of the microscope.

Reflecting mirror: It is attached to a long lever and screwed the body holding the prism. The image, which is reflected by prism, falls on the mirror, and which is kept in such a manner that the back is upper most and reflecting surface faces table.

Prism head: It consists of a prism fitted in such a manner that it comes to lie exactly over the eyepiece and the structure and the slide can be viewed through this prism and eyepiece.

Uses: It is used to draw the diagram, which is focused under the objective lens of the microscope. The image of the object is reflected by the prism or reflecting mirror on a paper placed by the side of the paper. The person can draw the diagram of object by drawing the pencil over the image on the paper. Camera lucida was used in an olden days. Now it is replaced by photomicrography in which the image can be photographed with a camera fitted in a microscope.

HUMAN BUCCAL SMEAR

Ex. No.: 3

Date:.....

Aim

To observe the human buccal epithelial cells

Materials required

Glass slide, cover glass, physiological saline, Methylene blue stain, cotton and Glycerin.

Procedure: Buccal epithelial cells were collected from the inner portion of the cheek by scraping with the help of a cotton bud. It was smeared on a clean glass slide. A few drop of 0.9% physiological saline was added. After 2 minutes, a few drop of Methylene blue stain was added. After 5 minutes, they were washed in distilled water. The smear was mounted with cover slip using Glycerin. Then the smear was observed and the buccal epithelial cell was measured using micrometry procedure under a compound microscope.

Observation

Rectangular or polygonal shaped nucleated buccal epithelial cells (Fig.147) were observed.

The diameter of one buccal epithelial cell in low power is ____ μ .

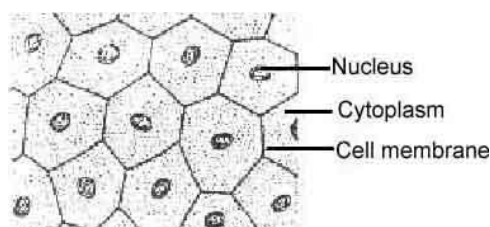


Fig. 47. Buccal epithelial cells.

Micrometry Calibration in Low Power (10X)

S. No.	No. of ocular divisions	No. of stage divisions	Value of one Ocular division	Mean Value
1				
2				
3				
4				
5				

Value of one ocular division in low power = ____ μ .

The diameter of one buccal epithelial cell in low power is ____ μ

(One division = 14.3 μ X 4 division).

BLOOD SMEAR OF COCKROACH

Ex. No.: 4

Date:.....

Aim

To observe different types of blood cells in the smear of cockroach blood.

Materials required

A live cockroach, scissors, glass slide, Leshman's stain, microscope, Glycerin and cover slip.

Procedure

A live cockroach was taken and its antenna was cut at the base. Blood (haemolymph) oozing out from the antenna was spread over the slide. Then this slide is smeared with the help of another slide. The slide was kept in Leshman's stain for 3 minutes. After staining the slide was washed in running water. The slide was mounted with cover glass using Glycerin and observed under the compound microscope. The cells were measured using micrometry.

Observation

The following cells (Fig. 148) were observed in the blood smear of cockroach.

1. **Spherula cells:** These are round or oval in shapes. Acidophilic substances are present in the cytoplasm.
2. **Pro-heamocytes:** These cells are round in shape. They have basophilic substances in the cytoplasm.
3. **Plasmocytes:** These cells are irregular in shape. They have phagocytic nuclei and basophilic cytoplasm. These cells are abundant in haemolymph.
4. **Granulocytes:** They have acidophilic cytoplasm. They are phagocytic in nature.

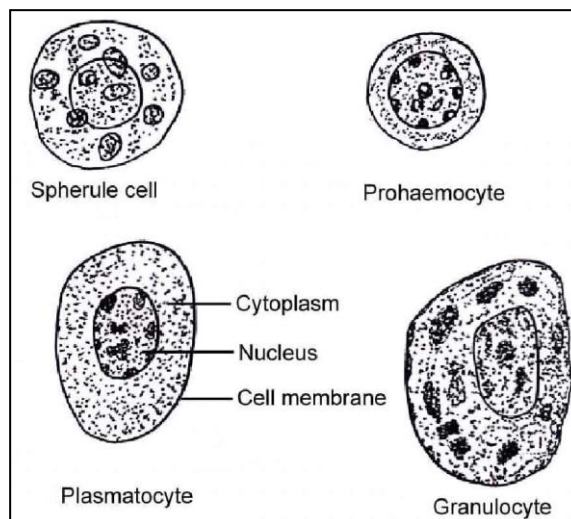


Fig. 148. Blood cells of Cockroach.

Micrometry measurements:

The diameter of one spherule cell in low power is _____ μ .

Micrometry Calibration in Low Power (10X)

S. No.	No. of ocular divisions	No. of stage divisions	Value of one Ocular division	Mean Value
1				
2				
3				
4				
5				

Value of one ocular division in low power = _____ μ .

One division = μ X 4 division =

The diameter of one buccal epithelial cell in low power is _____ μ

BLOOD SMEAR OF MAN

Ex. No.: 5

Date:.....

Aim

To observe different types of blood cells in the smear of human blood.

Materials Required

Human blood, glass slide, Alcohol, Leishman's stain, microscope, Glycerin, sterilized pin and cover slip.

Procedure

A drop of blood was obtained from the index finger by pricking with the sterilized needle. The blood was placed on one end of the slide. Then this slide is smeared with the help of another slide. The slide was kept in Alcohol for 1 minute and in Leishman's stain for 3 minutes. After staining, the slide was washed in running water and mounted with cover glass using Glycerine. The mounted slide was observed under the compound microscope. The cells were measured using micrometers.

Observation

The following Red blood cells and white blood cells (Fig. 149) were observed.

1. Red blood cells: These cells are biconcave shaped without nucleus. They are abundant in blood. Each red blood cell consists of 30 mg respiratory pigment.

2. White blood cells: White blood cells are known by presence of their nuclei. In normal human blood five types of white cells can be differentiated. They are as follows:

1. **Eosinophils:** They have granular cytoplasm and lobed nuclei. They constitute 2.8% of the total leucocytes. They destroy and detoxify the toxicants.
2. **Basophils:** They have condensed granules. They have lobed nuclei. They constitute 0.4% of the total leucocytes.
3. **Neutrophils:** These cells have lobed nuclei. They constitute 65% of the total leucocytes. The important functions are to destroy and detoxify the toxicants.
4. **Lymphocytes:** They have no granules in the cytoplasm. They constitute 26% of the total leucocytes and have diameter of 25 microns.
5. **Monocyte:** They have no granules in the cytoplasm. They constitute 6% of the total leucocytes and have diameter of 25 microns.

Micrometry measurements

6. 1. The diameter of one Red blood cell in low power is ____ μ .
7. 2 The diameter of one White blood cell in low power is ____ μ .

Discussion

The white blood cells are important defensive mechanism. The number of white blood cells in man is much lower than that of the red blood cells. The average number of leucocytes is approximately 7000 per cubic millimeter of blood, with a normal range of from 5000 to 10, 000 per cubic millimeter. The white blood may cells increase rapidly in numbers during infection or during certain abnormal physiological states, sometimes reaching a concentration of 50,000 or more per cubic millimeter. These cells can leave the blood stream through the capillary walls and may aggregate about foreign matter. The granulocytes in particular are able to ingest foreign bodies such as bacteria, and this phagocytic action is comparable with the amoebocyte action observed elsewhere in the animal kingdom. The leucocytes contain several different enzymes, and when these

cells disintegrate at the site of an infection the enzymes are released to aid in the digestion of necrotic tissues.

8. Micrometry Calibration in Low Power (10X)

S. No.	No. of ocular divisions	No. of stage divisions	Value of one Ocular division	Mean Value
1				
2				
3				
4				
5				

Value of one ocular division in low power = ____ μ .

The diameter of one RBC in low power is = ____ μ .

The diameter of one WBC in low power is = ____ μ .

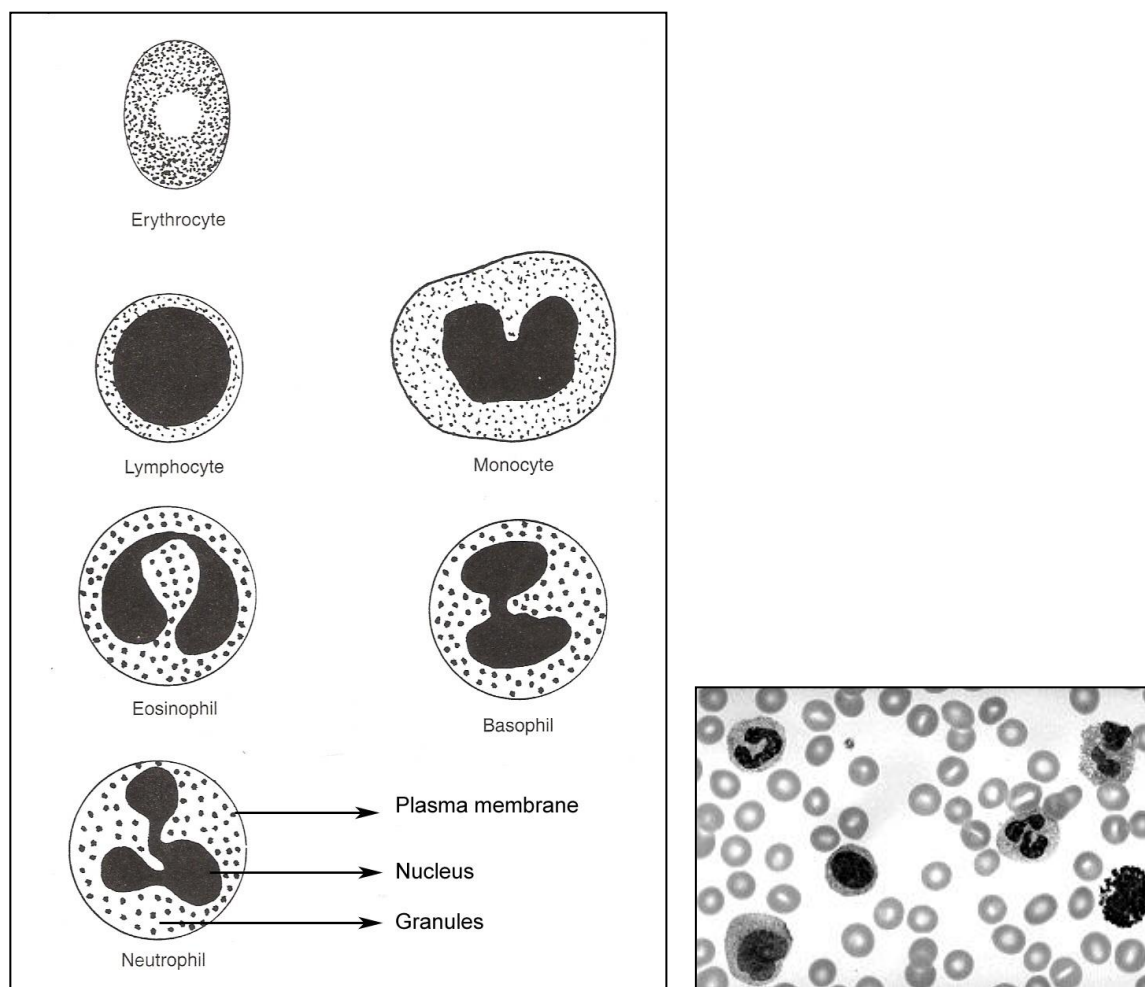


Fig.149. Diagram of human blood cells.

BLOOD CELLS AS OSMOMETERS

Ex. No.: 6

Date:.....

Aim

To determine the role of blood cells as Osmometers.

Principle

The movement of water molecules from the region of higher concentration to the region of lower concentration through a semi-permeable membrane is termed osmosis. The pressure with which the water molecules move from the higher concentration to lower concentration is called osmotic pressure. This phenomenon of osmosis be well demonstrated. When two solution of different osmotic concentration are separated by a semi-permeable membrane, higher the difference in concentration of solution greater will be the osmotic pressure. This phenomenon can be proved by placing the RBC's in the solution of different concentration where RBC behaves as osmometers. When RBS's are placed in isotonic medium the cells behave normally. There is no shrinkage or bulging. When RBS's are placed in hypotonic medium the cells undergo swelling due to the entry of water molecules through the RBC membrane. When RBS's are placed in hypertonic medium the cells shrink due to the loss of water molecules from the cells.

Materials required

Sheep's blood, test tubes, slides and cover slip and microscope.

Two different solutions at different concentration.

- 1) NaCl solution at different concentrations such as 0.4%, 0.6%, 0.8%, 0.9%, 1%, 2% & 3%.
- 2) Sucrose solution with concentration of 2%, 3%, 5% and 6%,

Procedure

One ml of sheep's blood (mixed with sodium citrate to prevent clotting) was diluted with 9 ml of test solution (Nacl solution and sucrose solution at different concentration in separate test tubes). A drop of mixture from each test tubes was placed on a slide to study the response of RBC to the test tube solution under the microscope. A control was maintained where 1 ml of blood was diluted with 9 ml of 0.9% saline and the result were compared.

Result

The tests were tabulated.

Discussion

From the results it is clear that when RBC's are placed in 0.9% Nacl solution they are normal. This indicates that in the isotonic to the contents of RBC's. But they are placed above and below the level of 0.9% Nacl the RBC's shrink and swell respectively. So it is clear that the gradation below 0.9% Nacl solution are hypotonic to the RBC;s and the gradation above 0.9% Nacl solution are hypertonic.

In sucrose solution at 3% no change occurs in the cells showing that the solution is isotonic to RBC's. But gradations of sucrose solutions above and below 3% behave hypotonic and hypertonic to the RBC's respectively. From this experimental analysis it is evident that the blood cells are act as Osmometer.

The recorded results for NaCl Solution.

Conc. of solution	Result	Reaction	Tonicity of the medium
0.4%	Swollen	Entry of water	Hypotonic
0.9%	Normal	No entry or exit of water	Isotonic
3%	Shrunked	Loss of water	Hypertonic

The recorded results for sucrose solution.

Conc. of solution	Result	Reaction	Tonicity of the medium
2%	Swollen	Entry of water	Hypotonic
3%	Normal	No entry or exit of water	Isotonic
4%	Shrunked	Loss of water	Hypertonic

CYTOCHEMICAL DETECTION OF CARBOHYDRATE

Ex. No.: 7

Date :

Aim

To identify the presence of carbohydrate in the given tissue sections.

Principle

Periodic acid oxidizes the 1-2 glycol group of polysaccharides and releases the aldehyde groups, which reacts with Schiff's reagent and gives deep magenta colour.

Materials Required

The slide with tissue sections, xylene, Alcohol series in coupling jars, Periodic acid, Schiff's reagent, cover glass and Glycerin.

Schiff's reagent

1 gram of Basic fuchsin was dissolved in 200 ml of boiling distilled water. After bigorous shaking it was kept heat 50° C and the filtrate 1 N HCl was added. After cooling to 25° C and 1 gm of Sodium metabisulphite was added. The solution was kept in the refrigerator for 14-24 hours. 2 gm of activated Charcoal was added and the filtrate was kept in the dark at 4° C.

Periodic acid (4%): It is prepared by dissolving 4 gm of Periodic acid in 100 ml of Distilled water.

Periodic acid-Schiffs technique: Periodic acid is an oxidant. It breaks the C-C bonds (Fig. 150) where these are available as 1, 2 glycols, convert them into dialdehydes but does not oxidise the aldehydes. Schiff's reagent reacts with free aldehydes to give Mejanta colour.

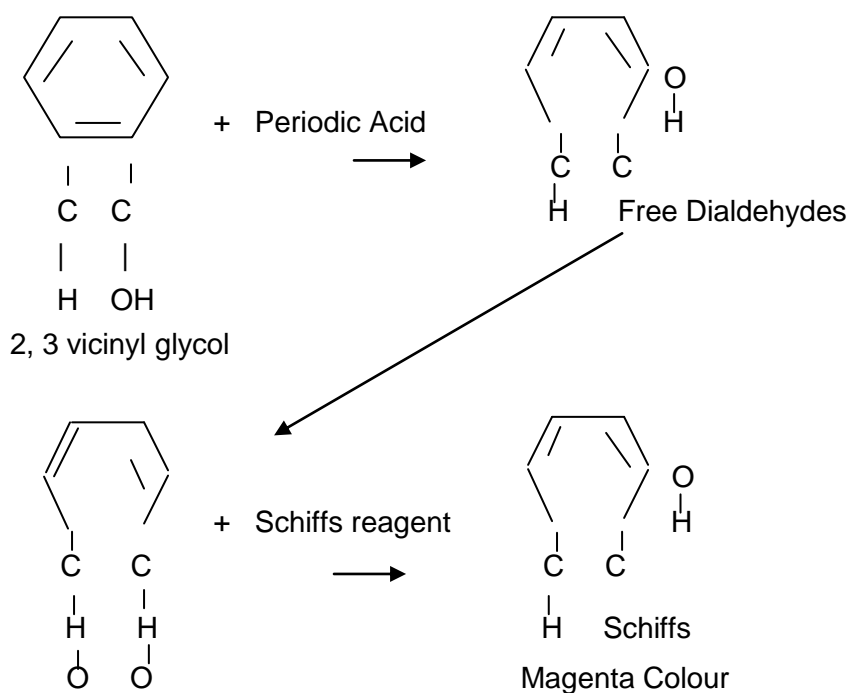


Fig.150. Histochemical reaction of carbohydrate.

Procedure

The sections were deparafinized in xylene and hydrated in descending (100%, 90%, 80%, 70%, 50%, and 30%) series of alcohol and placed in distilled water. Then the sections were oxidized in 1% solution of Periodic acid for 5 minutes at room temperature, washed in water for 5 minutes and stained in Schiff's reagent for 15 minutes. The stained sections were washed in distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted using Glycerin. The stained sections were observed under the compound microscope.

Result

Appearance of deep magenta colour indicates the presence of carbohydrate in the sections.

CYTOCHEMICAL DETECTION OF PROTEIN

Ex. No.: 8

Date :.....

Aim

To identify the presence of protein in the given tissue sections.

Principle

Bromophenol blue is an acidic dye, which reacts with basic groups of the protein and gives blue colour.

Materials Required

The slide with tissue sections, xylene, Alcohol series in coupling jars, 0.1% Bromophenol Blue (100 mg dissolved in 100 ml of distilled water), cover glass and Glycerin.

Bromophenol Blue

There are two types of bromophenol blue are used in tissues staining.

1. Aqueous Bromophenol Blue: It is an acidic dye. In aqueous medium it is capable of reacting with the basic reactive groups (Amino groups). The acidic group of the dye react with basic groups of the proteins to give blue colour.

Reagent: 100 mg of Bromophenol Blue powder is dissolved in 100 ml of distilled water.

2 Mercuric Bromophenol Blue: Mercuric ions of the bromophenol blue solution react with acidic sulphahydryl and aromatic residues of the protein to give blue colour.

Reagent: 1 gm of Mercuric chloride and 0.05 gm of Bromophenol blue are dissolved in 100 ml of 2% aqueous acetic acid.

The bromophenol blue is used to identify the acidic or basic proteins in the cells.

Procedure

The sections were deparafinized in xylene and hydrated in descending (100%, 90%, 80%, 70%, 50%, 30% and 0%) series of alcohol. The hydrated sections were stained were 0.1% Bromophenol blue solution for 5 minutes. The stained sections were washed in distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted using Glycerin. The stained sections were observed under the compound microscope.

Result

Appearance of dark blue colour indicates the presence of protein in the sections.

CYTOCHEMICAL DETECTION OF LIPID

Ex. No.: 9

Date:.....

Aim

To identify the lipid in the given tissue sections.

Principle

Sudan Black-B is a basic Diazole dye, which reacts with acidic group of lipids and gives black colour.

Materials Required

The tissue sections, xylene, Alcohol series in coupling jars, Sudan black-s (saturated solution of Sudan black-s in 70% alcohol), cover glass and Glycerin.

Procedure

The tissue sections were deparafinized in xylene, and hydrated in descending (100%, 90%, and 80%) series and brought to 70% alcohol. Then the tissue sections were stained in saturated Sudan black-B for 15 minutes. After 15 minutes the obtained slide was dried. Then the sections were mounted with cover glass using Glycerin and observed under the compound microscope.

Result:

Appearance of black colour indicates the presence of lipid in the cell.

CYTOCHEMICAL DETECTION OF DNA

Ex. No.: 10

Date:.....

Aim

To identify the DNA in the given tissue sections.

Principle

Methyl green pyronin stain reacts with DNA and gives blue colour.

Materials Required

The slide with tissue sections, xylene, Alcohol series in coupling jars, Methyl green pyronin stain, cover glass and Glycerin.

Procedure

The sections were deparafinized in xylene and hydrated in descending (100%, 90%, 80%, 70%, 50%, 30% and 0%) series of alcohol. The hydrated sections were stained in Methyl green pyronin stain for 5-10 minutes. The stained sections were washed in distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted using Glycerin. The stained sections were observed under the compound microscope.

Result:

Appearance of green colour indicates the presence of DNA in the sections.

CYTOCHEMICAL DETECTION OF RNA

Ex. No.: 11

Date:.....

Aim

To identify the RNA in the given tissue sections.

Principle

Methyl green pyronin stain reacts with RNA and bright red colour.

Materials Required

The slide with tissue sections, xylene, Alcohol series in coupling jars, Methyl green pyronin stain, cover glass and Glycerin.

Procedure

The sections were deparafinized in xylene and hydrated in descending (100%, 90%, 80%, 70%, 50%, 30% and 0%) series of alcohol. The hydrated sections were stained in Methyl green pyronin stain for 5 -10 minutes. The stained sections were washed in distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted using Glycerin. The stained sections were observed under the compound microscope.

Result

Appearance of bright red colour indicates the presence of protein in the sections.



MODEL QUESTIONS ZOOLOGY – MAJOR
PRACTICAL I -FUNCTIONAL MORPHOLOGY & PALAENTOLOGY OF
INVERTEBRATES & CHORDATES, GENETICS, MICROBIOLOGY AND
CELL AND MOLECULAR BIOLOGY

Time: 3 Hours

Maximum: 60 Marks

1. Identify the specimens A, B, C and D and assign them to their respective systematic position up to order giving proper reasons.
10 Marks
2. Mount at least 2 parapodia of the given specimen (E) on clean slide and focus them under microscope. Draw and describe the functional morphology.
10 Marks
3. Identify and comment on the
 F - Identify and comment on the
 G - Write on its biology and culture
 H - Write on its Genetic importance
 I - Its application in Microbiology
10 Marks
4. Determine your blood group. Write down the procedure and discuss the genetic basis of the inheritance of the blood system .
10 Marks
5. Find out whether the organism in the culture (J) is Gram positive (+) or gram negative (-). Write down the principle and procedure.
 Or
 Stain the given tissue (A) to identify protein reactive sites using cytochemical stains.
10 Marks
6. Record
10 Marks

Total 60 Marks

Key:

Q1. Specimens:

- A. Paramecium
- B. Peripatus
- C. Rhacophorus
- D. Krait

Q 2. Specimen E- Nereis

Q3. Spotters:

- F. Identify and comment on the- ammonoid
- G. Write on its biology and culture-Silkworm
- H. Write on its Genetic importance- Turner syndrome or Karyotype of male
- I. Its application in Microbiology-Autoclave

Q5 J.Bacterial culture

MODEL QUESTIONS
ZOOLOGY – MAJOR
PRACTICAL I -FUNCTIONAL MORPHOLOGY & PALAENTOLOGY OF
INVERTEBRATES & CHORDATES, GENETICS, MICROBIOLOGY AND
CELL AND MOLECULAR BIOLOGY

Time: 3 Hours

Maximum:60 Marks

1. Classify the given specimens A. B, C, D up to order giving reasons. 10 Marks
 2. Mount at least 5 scales of the specimen given (E) on clean slide using appropriate stain. Focus it under a microscope. Draw and describe the functional morphology 10 Marks
 - 3 Identify and comment on
 - F - Identify and comment on the
 - G - Write on its biology and culture
 - H - Write on its Genetic importance
 - I - Its application in Microbiology10 Marks
 - 4 Identify the syndrome (J) and comment on its chromosomal basis. 10 Marks
 - 5 Using differential staining procedure differentiate the microbes in the culture provided (K). Indicate the staining reaction. Write down the principle and procedure.
 (Or)
 Prepare a human buccal smear, write down the procedure. 10 Marks
 6. Record 10 Marks
- Total 60 Marks**

Key

Q1. Specimens:

- A. Sea Anemone
- B. Loligo
- C. Sucker fish
- D. Carapace

Q 2. Specimen E- Teleost scale

Q3. Spotters:

- F - Identify and comment on - Nautiloid fossil
- G - Write on its biology and culture- Earthworm-Vermiculture
- H - Write on its Genetic importance – Down syndrome or Drosophila
- I - Its application in Microbiology-Agar plate

Q4. J. Turner Syndrome

Q5. K.Bacterial culture

MODEL QUESTIONS
ZOOLOGY – MAJOR
PRACTICAL I -FUNCTIONAL MORPHOLOGY & PALAENTOLOGY OF
INVERTEBRATES & CHORDATES, GENETICS, MICROBIOLOGY AND
CELL AND MOLECULAR BIOLOGY

Time: 3 Hours

Maximum: 60 Marks

1. Identify the specimens A, B, C and D and assign them to their respective systematic position up to order giving proper reasons.
10 Marks
 2. Mount the mouth parts of the given specimen E on clean slide. Draw and label the parts.
10 Marks

Or

Mount the sting of the given specimens (E) on a clean glass slide using glycerine.

Or

Mount at least two types of pedicellaria of the given specimen E. Draw and label the parts.

10 Marks
 3. Identify and comment on the F, G, H and I.
10 Marks
 4. Determine your blood group. Write down the procedure and discuss the genetic basis of the inheritance of the blood system.

Or

With help of virtual laboratory, dissect and identify the 5th cranial nerves of frog.

10 Marks
 5. Plot the growth curve from the given bacterial culture. Write down the procedure and interpret your results. Stain the given culture and comment on your significance.
10 Marks
 6. Record
10 Marks
- Total 60 Marks**

Key

Q1. Specimens:

- A. Amoeba
- B. Sepia
- C. Flying fish
- D. Loris

2. Specimen E- Lepas mouth parts

3. Spotters:

- F - Identify and comment on - Nautiloid fossil
- G - Write on its biology and culture- Earthworm-Vermiculture
- H - Write on its Genetic importance – Down syndrome or Drosophila
- I - Its application in Microbiology-Agar plate

BIOCHEMISTRY

BIOPHYSICS

BIOTECHNOLOGY

BIOSTATISTICS

COMPUTER APPLICATIONS

PART – II: A. BIOCHEMISTRY

QUANTITATIVE ESTIMATION OF FREE AMINO ACIDS

Ex. No. 1

Date:

Aim

To estimate the amount of free amino acids present in tissue samples.

Principle

Amino acids react with ninhydrin to form a highly conjugated aromatic derivative which absorbs light in the visible portion of the spectrum (Fig. 151). The ninhydrin amino acid derivative is purple and easily visible. One molecule of ninhydrin and one molecule of amino acid combine with the one molecule of amino acid to form a purple coloured compound. The intensity of the colour is proportional to the amount of amino acid present in the sample.

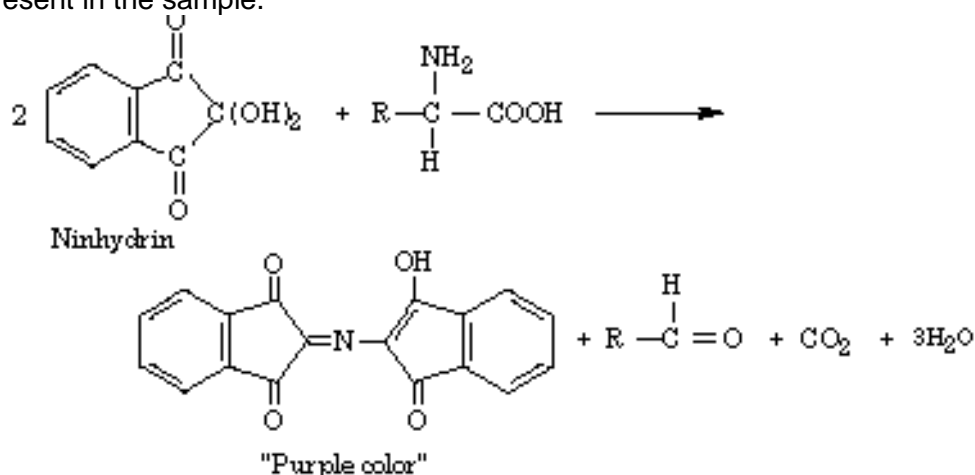


Fig. 151. Amino acids react with ninhydrin.

Materials required

Test tubes, centrifuge, tissue homogenizer, spectrophotometer and hot water bath.

Reagents

1. **Trichloro acetic acid (TCA)Solvents:** 10 g of TCA is dissolved in 100 ml of distilled water.
2. **1 N Sodium hydroxide:** 4 g of Sodium hydroxide is dissolved in 100 ml of distilled water
3. **Ninhydrin reagent:** 200 mg of Ninhydrin reagent dissolved in 25 ml of 0.2 Molar acetate buffer (5.5 pH). It should be freshly prepared.
4. **Standard amino acid:** 5 mg of Leucine dissolved in 10 ml of 1 N HCl.

Procedure

50 mg of the give tissues was homogenized with 2 ml of 10% TCA. The homogenate was centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and 2 ml of 1 N NaoH was added to the residue. Then it was heated in a boiling water bath for 5 minutes to dissolve the residue. The contents were centrifuged at 2,500 rpm for 10 minutes. Three test tubes were taken. The supernatant was taken in the first test tube. 1 ml of standard Amino acid was taken in the second test tubes. 1 ml of distilled water was taken in the third test tubes.

To all the test tubes, 2 ml of Ninhydrin reagent was added. They were boiled in hot water bath for 15 minutes. Then the test tubes were cooled in running tap water. After cooling, the OD was measured at 570 nm.

Result

The amount of free amino acids present in the given tissue was _____
mg/100 mg wet weight.

Calculation

OD of the sample =
 OD of the standard =
 Concentration of the standard = 0.05
 Weight of the tissue = 50

Formula

$$\begin{aligned}
 & \frac{\text{OD of the Sample}}{\text{OD of the Standard}} \times \frac{\text{Concentration of the standard}}{\text{Weight of the tissue}} \times 100 \\
 = & \frac{\text{_____}}{\text{_____}} \times \frac{0.05}{50} \times 100 \\
 = & \text{_____ mg/100 mg wet weight.}
 \end{aligned}$$

QUANTITATIVE ESTIMATION OF PROTEINS

Ex. No. 2

Date:

Aim

To estimate the amount of proteins present in the given sample.

Principle

Carbamyl group of the protein molecules react with copper and potassium of the Biurete reagent and produces violet colour. The intensity of colour is proportional to the amount of protein present in the tissue.

Materials required

Test tubes, centrifuge, measuring cylinder, pipettes, tissue homogenizer, spectrophotometer and hot water bath.

Reagent

1. **Solution A:** 750 mg of copper sulphate and 3 g of sodium, potassium tartarate were dissolved in 250 ml of distilled water.
2. **Solution B:** 50 g of sodium hydroxide was dissolved in 150 ml of distilled water.
3. **Solution C (Biurete reagent):** Solution A was mixed with Solution B and made up to 500 ml of distilled water.
4. **Standard protein solution:** 100 mg of bovine serum albumin was dissolved in 100 ml of distilled water.

Procedure

30 mg of the given tissue was homogenized with 1 ml of distilled water. It was taken in a clean centrifuge tube. Simultaneously two test tubes were taken. To one test tube 1 ml of BSA (standard protein solution) was taken and in another test tube 1 ml of distilled water was taken. 4 ml of biurete reagent was added to the all the three tubes. They were allowed to stand at room temperature for 30 minutes. Then the sample test tube alone centrifuged at 3000 rpm for 10 minutes. The colour developed in the supernatant and the standard were measured at 540 nm in Spectrophotometer against a reagent blank.

Result

The amount of proteins present in the given tissue was _____ mg/100 mg wet weight.

Calculation

OD of the sample =

OD of the standard =

Concentration of the standard = 0.1

Weight of the tissue = 30

Formula

$$\begin{aligned}
 & \frac{\text{OD of the Sample}}{\text{OD of the Standard}} \times \frac{\text{Concentration of the standard}}{\text{Weight of the tissue}} \times 100 \\
 & = \frac{\text{OD of the Sample}}{\text{OD of the Standard}} \times \frac{0.1}{30} \times 100 \\
 & = \text{_____ mg/100 mg wet weight.}
 \end{aligned}$$

QUANTITATIVE ESTIMATION OF CARBOHYDRATES

Ex. No. 3

Date:

Aim

To estimate the amount of carbohydrates present in the given sample.

Principle

Sulphuric acid in the anthrone reagent hydrolyses di and oligosaccharides into monosaccharides and dehydrates them into monosaccharides and then into furfural derivatives. Then the anthrone reagent reacts with these derivatives to produce green coloured compounds. The intensity of the colour thus formed is proportional to the amount of total carbohydrate present in the given sample.

Materials required

Test tubes, centrifuge, tissue homogenizer, colorimeter and hot water bath.

Colorimeter

Colorimeter, device used to compare or measure colours and their intensities. A simple colorimeter uses an optical system to place an unknown colour, such as of a chemical sample, next to a well-established colour. In more advanced devices this comparison field can be adjusted in various quantifiable ways. In some, photoelectric cells may be used to measure the transmitted light. Colorimeters are used in chemical research and in various industries, such as the manufacture of dyes and paints.

Reagent

1. **Anthrone Reagent:** 50 ml of Anthrone was dissolved in 66% H_2SO_4 .
2. **5% Trichloro acetic acid (TCA):** 5 g of TCA was dissolved in 100 ml of distilled water.
3. **Glucose standard:** 100 mg of D. glucose dissolved in 100 ml of distilled water.

Procedure

50 mg of the given tissue was homogenized with 5 ml of 5% TCA. The homogenate was centrifuged at 2,500 rpm for 5 minutes. 1 ml of supernatant was taken in a clean test tube and was labelled as sample. 1 ml of distilled water and 1 ml of standard glucose solution were taken in 2 separate test tubes. They were labeled as blank and standard respectively. To all the test tubes 15 ml of anthrone reagent was added. Then the test tubes were kept in a boiling water bath for 50 minutes. After cooling in the dark place for 30 minutes, the OD of the standard and sample were measured at 620 nm using the blank in a Spectrophotometer.

Result

The amount of carbohydrates present in the given tissue was _____ mg/100 mg wet weight.

Calculation

OD of the sample =
 OD of the standard =
 Concentration of the standard =
 Weight of the tissue = 50

Formula

$$\begin{aligned}
 & \frac{\text{OD of the Sample}}{\text{OD of the Standard}} \times \frac{\text{Concentration of the standard}}{\text{Weight of the tissue}} \times 100 \\
 = & \frac{\quad}{\quad} \times \frac{0.1}{50} \times 100 \\
 = & \quad \text{mg/100 mg wet weight.}
 \end{aligned}$$

QUANTITATIVE ESTIMATION OF LIPIDS

Ex. No. 4

Date:

Aim

To estimate the amount of lipids present in the given sample.

Principle

The reaction of sulphuric acid, phosphoric acid and vanilline with lipids give a red colour compound. The intensity of colour formed is proportional to the amount of lipid present in the sample.

Materials required

Test tubes, centrifuge, tissue homogenizer, spectrophotometer and hot water bath.

Reagent

1. **Vanilline reagent:** 2 g of vanilline powder in 850 ml of 88% phosphoric acid and 200 ml of distilled water.
2. **Concentrate sulphuric acid:** 100 % sulphuric acid was used.
3. **Chloroform: methanol mixture (2:1):** 200 ml chloroform was mixed with 100 ml of methanol.
4. **0.9% sodium chloride:** 900 mg of sodium chloride was mixed with 100 ml of distilled water.
5. **Standard lipid:** 10 mg of commercially available cholesterol dissolved in 10 ml dissolved water.

Procedure

25 mg of the given tissue was homogenized with 5 ml of chloroform: methanol mixture. To this 0.5 ml of sodium chloride was added and the contents were mixed well and centrifuged at 3000 rpm for 10 minutes to get biphasic condition. Using a syringe the lower phase was separated which was free from fluff. The volume was made to original quantity of 5 ml with chloroform. 0.5 ml of the extract was measured into a clean test tube and left to dry in a water bath. The dried lipid was dissolved in 0.5 ml of concentrated sulphuric acid. Then the test tube was plugged with non absorbent cotton wool. Then it was boiled in the water bath for 10 minutes and cooled to room temperature. Two other test tubes were taken. In one test tube 1 ml of cholesterol standard was taken and in another test tube 1 ml of distilled water was taken. 2.5 ml of vanilline reagent was added to the sample, standard and blank test tubes. These test tubes were allowed to stand at room temperature for 30 minutes. The OD of the standard and sample were measured at 580 nm in a spectrophotometer using blank.

Result

The amount of lipids present in the given tissue was _____ mg/100 mg wet weight.

Calculation

OD of the sample =
 OD of the standard =
 Concentration of the standard =
 Weight of the tissue = 50

Formula

$$\begin{aligned}
 & \frac{\text{OD of the Sample}}{\text{OD of the Standard}} \times \frac{\text{Concentration of the standard}}{\text{Weight of the tissue}} \times 100 \\
 = & \quad \quad \quad \times \quad \quad \quad \times 100 \\
 = & \quad \quad \quad \text{mg/100 mg wet weight.}
 \end{aligned}$$

PREPARATION OF SOLUTIONS: MOLARITY, NORMALITY AND PERCENTAGE

Ex. No. 5

Date:

Aim

To prepare one molar, one normal, one percent solutions of the given salt.

Materials required

Salt, distilled water, measuring jar, standard measuring flask, beaker, top pan digital electronic balance.

Molarity

Molarity of a solution is defined as the molecular weight of the solute (salt) dissolved in one litre of solvent (distilled water).

$$\text{Molarity} = \frac{\text{Molecular weight}}{\text{Litre}}$$

Normality

Normality of solution is defined as the equivalent weight of the solute (salt) dissolved in one litre of solvent (distilled water).

$$\text{Normality} = \frac{\text{Equivalent weight}}{\text{Litre}}$$

Percentage

One percent solution is defined as 1 gram solute dissolved in 100 ml of solvent.

Preparation of 1 M sodium chloride solution

The molecular weight of NaCl is 58.44 gm. 1 Molar NaCl is prepared by dissolving 58.44 gm of NaCl in 1 litre of distilled water.

Preparation of 1 N sodium chloride solution

The equivalent weight of NaCl is 58.44 gm. 1 Normal NaCl is prepared by dissolving 58.44 gm of NaCl in 1 litre of distilled water.

Preparation of 1 % sodium chloride solution

One percent NaCl is prepared by dissolving 1 gm of NaCl in 100 ml of distilled water.

CALCULATION OF MOLES, MILLIMOLES, MICROMOLES AND NONOMOLES**Ex. No. 6****Date:****Aim**

To prepare molar solution, milli molar solution, micro molar and nano molar solution.

Materials required

Salt, measuring jar, beaker and top pan digital electronic balance.

Preparation of Reagents

- 1. Preparation of 1 Molar (M) solution:** 1 M solution was prepared by diluting with molecular weight of the salt (Sodium chloride = 58.44 mg) in 1 litre of distilled water.
- 2. Preparation of 1 Millimolar (mM) solution:** The prepared 1 M solution was taken and made up to 1 litre with distilled water. Each 1 ml contains 1 mM solution of Salt (sodium chloride, 1 Mole is diluted to 1000 ml = 1 mM).
- 3. Preparation of 1 Micromolar (μ M) solution:** 1 ml of mM solution was diluted to 1 litre of distilled water. Each ml contains 1 μ M of salt sodium chloride.
- 4. Preparation of 1 Nanomolar (nM) solution:** The 1 ml of μ M solution of a salt (sodium chloride) was diluted to 1 litre distilled water. Each ml contains 1 nM (1 μ M is diluted to 1000 ml = 1 nM).

BUFFER PREPARATION

Ex. No. 7

Date:

Aim

To prepare the following buffers:

- a. Acetate buffer
- b. Phosphate buffer and
- c. Citrate buffer

1. PREPARATION OF ACETATE BUFFER

Reagents required for Acetate buffer

1. Acetic acid
2. Sodium acetate

Procedure for Acetate buffer preparation

1. **0.2 M Acetic acid:** 60.05 ml of acetic acid was made up to 1 litre with distilled water to prepare 1 M acetic acid. 200 ml 1 Molar acetic acid solution was diluted to 1 litre distilled water to get 0.2 M Acetic acid.
2. **0.2 M Sodium acetate:** 136.08 gm of sodium acetate was dissolved in 1 litre of distilled water to get 1 M sodium acetate solution. 200 ml of 1 Molar sodium acetate solution was diluted to 1 litre of distilled water to get 0.2 M sodium acetate solution.

The desired pH of acetate buffer can be prepared by mixing the appropriate volume of acetic acid with respective volume of sodium acetate by using the table given.

2. PREPARATION OF PHOSPHATE BUFFER

Reagents required for Phosphate buffer

1. Sodium hydroxide
2. Sodium orthophosphate

Procedure for Phosphate buffer preparation

1. **0.2 M NaOH:** 8 gm of NaOH was dissolved in 1 litre of distilled water.
2. **0.2 M NaH_2PO_4 :** 31.2 gm of NaH_2PO_4 was dissolved in 1 litre of distilled water.

The desired pH of phosphate buffer can be prepared by mixing the appropriate volume of 0.2 M NaOH with respective volume of 0.2 M NaH_2PO_4 by using the table given.

3. PREPARATION OF CITRATE BUFFER

Reagents required for Citrate buffer

1. Citrate acid
2. Trisodium citrate

Procedure for Citrate buffer preparation

1. **0.5 M Citrate acid:** 10.507 gm of citric acid was dissolved in 1 litre of distilled water.
2. **0.5 M Trisodium citrate:** 14.705 gm of trisodium citrate was dissolved in 1 litre of distilled water.

The desired pH of phosphate buffer can be prepared by mixing the appropriate volume of 0.5 M Citrate acid with respective volume of 0.5 M Trisodium citrate by using the table given.

Preparation of Acetate buffer

1. **0.2 M Acetic acid:** 12 ml of acetic acid was made up to 1 litre with distilled water.
2. **0.2 M Sodium acetate:** 27.216 gm of sodium acetate was dissolved in 1 litre of distilled water.

The desired pH of acetate buffer can be prepared by mixing the appropriate volume of acetic acid with respective volume of sodium acetate by using the tables (1, 2 and 3 given.

Table 1. Preparation of Acetate Buffer.

pH	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6
0.2 M Acetic acid	92	88	83	76	66	55	43	32	22	17	07
0.2 M Sodium acetate	8	12	17	24	34	45	57	68	78	83	93

Table 2. Preparation of Phosphate Buffer.

pH	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
0.2 M NaOH	3.5	5.8	9.1	13	18	24	30	35	40	43	45	47
0.2 M NaH ₂ PO ₄	96.5	94.2	90.9	87	82	76	70	65	60	57	55	53

Table 2. Preparation of Citrate Buffer.

pH	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
0.5 M Citrate acid	91	86	80	75	70	65	60	55	50	44	39	34	29	24	19
0.5 M Trisodium citrate	9	14	20	25	30	35	40	45	50	56	61	66	71	76	81

DETERMINATION OF pH USING pH METER

Ex. No. 8

Date:

Aim

To determine the pH various samples using pH meter.

Principle

pH is defined as the negative logarithm of hydrogen ion concentration of the liquid. $\text{pH} = -\log [\text{H}^+]$. In the expression pH, p stands for potential and H stands for Hydrogen ion. In any solution, hydrogen ion concentration is in between the 10^{-1} and 10^{-14} . Hence the pH of a solution is between 1 and 14.

Materials required

pH meter, thermometer, beaker, water, sample water, pH 4 and p.2.

Reagent required

pH buffer solution: It was obtained by dissolving pH 4 in 100 ml distilled water and pH 9.2 in 100 ml distilled water or standard buffer having pH 4 and 7 were used for standardization.

Procedure

pH meter was switched on 10 minutes before the pH measurement. The correct temperature of the sample was set up by using temperature knob. Then the needle of the knob was adjusted for 'O' setting. The base of the electrode in buffer solution was immersed. If the sample has pH less than 7, pH 4 buffer solution could be used. When the pH 4 buffer solution was used, the dial should be pH 4. When it was not showing 4, the buffer was adjusted till the dial showing 4. Then the knob was turn to 'O'. The electrodes were removed from the buffer solution and rinsed it in distilled water. Then it was quickly blotted with paper. The electrode was immersed in beaker containing samples.

The reading (pH of sample) was noted down before measuring the pH of second sample. The electrode needle was adjusted to 'O'. Thus pH was determined for 5 samples (Table 1) using the pH meter. Then the readings were plotted and tabulated.

Result

The pH value of various samples were (3.6, 3.8, 4.2, 4.6 and 5.2) determined using pH meter.

Discussion: Human extracellular and intracellular fluids have definite pH, because all the reactions are governed by enzymes. The enzyme required optimum pH for its cavity. pH of the blood is 7.4 due to the transport of carbonate solution in the form of alkaline. Urine is in the form of acid. pH of gastric fluid is 2. It is due to secretion of HCl in the stomach. In this high pH all the microorganisms are killed in the stomach.

Table 1. Determination of various pH samples using pH meter.

S. No.	Buffer solution	pH
1	18.4	3.6
2	13.2	3.8
3	8.6	4.2
4	4.4	4.6
5	1.4	5.2

pH METER

pH is defined as negative logarithm of hydrogen ion (H^+) concentration in gram ions per litre of solution. It expresses the relative acidity of a solution. The amount of hydrogen ions determines the acidity or the alkalinity of solution. If the hydrogen ions are less and hydroxyl ions are more, then the solution will be alkaline. The concentration of H^+ ions per litre of solution ranges between 10^{-1} and 10^{-14} . So, the pH scale ranges between 1 and 14. The pH of 7 is neutral; it is neither acidic nor alkaline. The pH of 7 is acidic and pH above 7 up to 14 is alkaline.

The pH meter is used to determine the pH of a solution. The pH meter consists of electrode and a potentiometer (Fig. 152). The potentiometer has a dial in which pH scale is noted. There is also a needle that moves on the scale. The electrodes are two types namely glass electrode and calomel electrode.

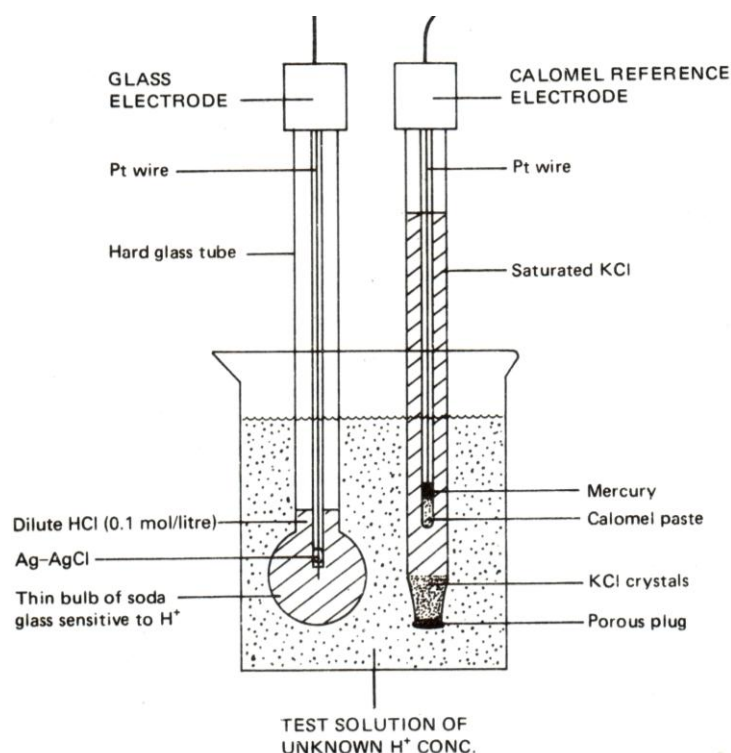


Fig.152. Electrodes of pH meter.

The electrodes are connected to the potentiometer through wire. Now, the potentiometer is switched on. Now, the electrodes are dipped into a solution of unknown solution. Depending upon the hydrogen ion concentration, the glass electrode changes. Now the glass electrode becomes either positive or negative to the calomel electrode. The resulting current flow will be the measure of H^+ concentration, which moves the needle on the pH scale. The dial reading directly gives the accurate value of pH.

B. BIOPHYSICS

COLORIMETER: DETERMINATION OF OPTICAL DENSITY OF SAMPLE USING STANDARD

Ex. No. 1

Date:

Aim

To determine the optical density of the given sample by using standard.

Principle

Sulphuric acid in the anthrone reagent hydrolyses the simple sugar into glucose and then dehydrates it into furfurals. This compound reacts with alnthrone to produce a coloured complex product, the intensity of the coloured complex is proportional to the amount of glucose present in substance.

Reagents

1. **Solution A:** 750 mg of copper sulphate and 3 g of sodium, potassium tartarate were dissolved in 250 ml of distilled water.
2. **Solution B:** 50 g of sodium hydroxide was dissolved in 150 ml of distilled water.
3. **Solution C (Biurete reagent):** Solution A was mixed with Solution B and made up to 500 ml of distilled water.
4. **Standard protein solution:** 100 mg of bovine serum albumin was dissolved in 100 ml of distilled water.

Procedure

Six clean test tubes were taken and were labelled as S1, S2, S3, S4, S5 and blank. In the test tubes S1 to S5, 200, 400, 600, 800 and 1000 micro litres of dextrose solution were taken respectively. All the test tubes except S5 were made up to 1000 μ l with distilled water. 1 ml of distilled water was taken in the blank test tube. To all the test tubes 4 ml of Biurete reagent were added. They were allowed to stand at room temperature for 30 minutes. Then the sample test tube alone centrifuged at 3000 rpm for 10 minutes. The colour developed in the supernatant and the standards were measured at 540 nm in Spectrophotometer against a reagent blank.

Results

The optical density values were given in the table 1.

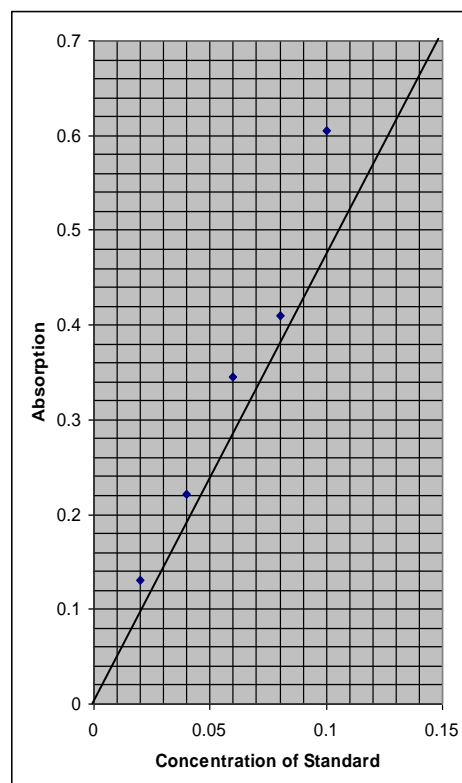


Fig. 153. Optical density graph.

Table 1. Optical density of the given sample using standard.

S. No.	Vol. of std	Vol. of distilled water	Conc. of std	Vol. of Biurete Reagent (ml)	Optical density
1	0.2	0.8	0.02	4	0.06
2	0.4	0.6	0.04	4	0.08
3	0.6	0.4	0.06	4	0.09
4	0.8	0.2	0.08	4	0.10
5	1.0	0.0	0.10	4	0.14

Spectrophotometer

The spectrophotometer is widely used for measuring the intensity of a particular spectrum in comparison to the intensity of light from a standard source. The essential components of a spectrophotometer (Fig. 154) are detector, light source mirrors and lenses, cuvettes, monochromator, prisms and gratings.

The word spectrophotometer means the photons (light) over the whole range of spectrum (continuous wavelengths) that the light source can produce and meter stands for the instrument that measures it. The concentration of the substance that emits or absorbs the spectrum can be determined from this comparison. Based on the principle of Beer's Lambert's law, molecules absorb the light when monochromatic light is passed into a solution. The amount of light is absorbed by the solution is proportional to the concentration of the solutions.

The spectrophotometers are also useful for studying spectra in the nonvisible areas because their detecting elements are photoelectric cells.

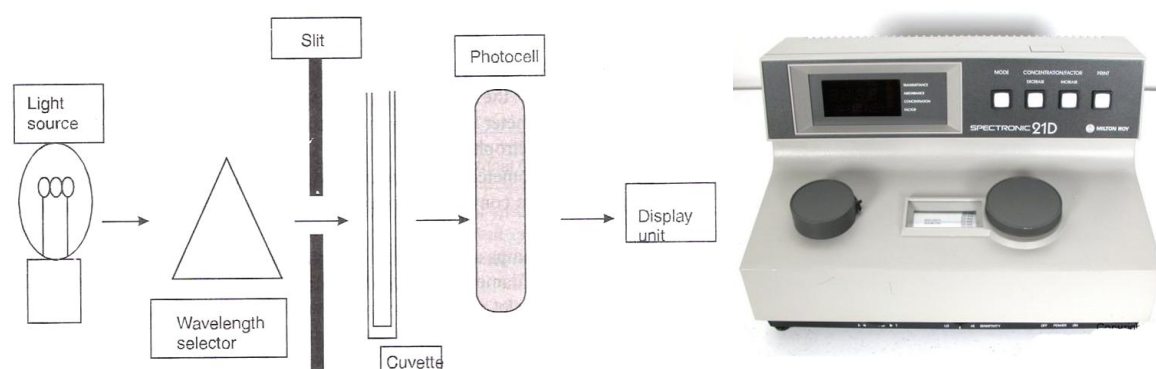


Fig. 154. The essential components of a spectrophotometer.

Photocolorimeter

A photocolorimeter is a device consisting of 1) a light source, which can be as simple as tungsten-filament light bulb; 2) some optics for focusing the light 3) a coloured filter, which passes light of the colour which is absorbed by the treated sample; 4) a sample compartment to hold a transparent tube or cell containing the sample, 5) a light-sensitive detector, like the light meter on a camera, which converts the light intensity into an electric current, and 6) electronics for measuring and displaying the output of the detector (Fig. 155). Some photocolorimeters may be designed to read out directly in concentration units, while others may show the results in units of light absorbance which need to be compared to a calibration curve. (An interesting point is that the filter is not the same colour as the solution being tested, but rather the complementary colour. We want to use a filter which transmits light of the color which the solution absorbs. A yellow solution looks yellow because it absorbs blue light, so a blue filter would be used.). Photocolorimeter works based on the principle of Beer - Lambert's law. Molecules absorb the light when monochromatic light is passed into a solution. The amount of light is absorbed by the solution is proportional to the concentration of the solutions.

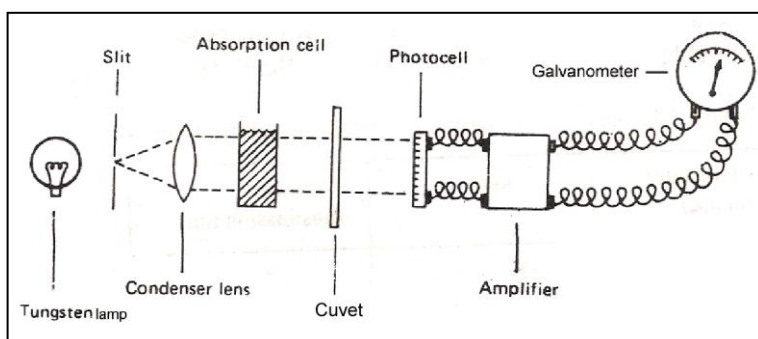


Fig. 155. The essential components of a photocolorimeter.

CENTRIFUGE: PREPARATION OF SAMPLE USING HIGH AND LOW SPEED CENTRIFUGE

Ex. No. 2

Date:

Aim

To separate cell organelles using differential centrifuge.

Principle

Centrifugation is a process in which a solution is rotated in circles around a central axis. The solution subjected to centrifugal force is moving outward from the axis. This force is expressed in term of gravitational or in g units. Faster the speed of rotation, greater is the force. Longer the radius of the rotation, greater is the force. In a centrifugal field the particles of solution sediment to the bottom. The rate of sedimentation of the particle depends on the density of the particle, the size of the particle, the viscosity of the medium and the gravitational pull.

Materials required

Liver, Eppendorff tube, microfuge, high and low speed centrifuge and tissue homogenizer.

Procedure

A piece of liver tissue was homogenized in ice cold 0.25 M sucrose solution. The homogenate was centrifuged at 6000 rpm for 10 minutes. The supernatant was taken in another tube, the pellet includes nuclei. The supernatant was again centrifuged at 8000 rpm for 10 minutes, the pellets include mitochondria. Then the clear supernatant was centrifuged at 15000 rpm for 10 minutes, the residue contains lysosomes.

Results

1. The nuclei were isolated at 6000 rpm for 10 minutes.
2. The mitochondria were isolated at 8000 rpm for 10 minutes.
3. The lysosomes were isolated at 15000 rpm for 10 minutes.

Microfuges

Microfuges are small centrifuge tubes (Fig. 156) used for the separation of smallest molecules cells or organelles of the cells. These are the simplest and least expensive centrifuges and exist in many types of design. They are often used to collect small amounts of material that rapidly sediment (yeast cells, erythrocytes, coarse precipitates), and generally have a maximum speed of 4000 to 6000 rev min⁻¹, with maximum relative centrifugal fields of 3000 to 7000g. Most operate at ambient temperature, the flow of air around the rotor controlling rotor temperature. Some of the latest designs, however, incorporate a refrigeration system to keep rotors cool, thus preventing denaturation of proteins. Small Microfuges are available, providing virtually instant acceleration to maximum speeds of 8000 to 20 000 revolution/minute and developing fields of approximately 10 000 g. These centrifuges have proved extremely useful for sedimenting small volumes (250 MM³ to 1.5 cm³) of material very quickly (1 or 2 min). Typical applications include the rapid sedimentation of blood samples, and of synaptosomes used to study the effect of drugs on the uptake of biogenic amines.

This Micro Centrifuge is exclusively equipped with an aerosol tight rotor, which can be operated without the rotor lid for customer convenience but without an y significant increase in noise level. The Centrifuge is perfect for customers that demand a high quality centrifuge for processing a reduced amount of samples.



Fig. 156. Microfuges.

High speed centrifuges

High speed centrifuge (Fig. 157) instruments are available with maximum rotor speeds in the region of 25 000 rev min⁻¹, generating a relative centrifugal field of about 60 000 g. They generally have a total capacity of up to 1.5 dml, and a range of interchangeable fixed-angle and swinging-bucket rotors. These instruments are most often used to collect microorganisms, cellular debris, larger cellular organelles and proteins precipitated by ammonium sulphate. They cannot generate sufficient centrifugal force to effectively sediment viruses or smaller organelles such as ribosomes.



Fig. 157. High speed centrifuge.

PAPER CHROMATOGRAPHIC SEPARATION OF FREE SUGARS IN DIFFERENT SAMPLES

Ex. No.: 3

Date:.....

Aim

Separation of free sugars in different samples by paper chromatographic method.

Materials required

Sprayer, capillary tube and Whatman No. 1 filter paper (Usually is used because of its known standard texture).

Reagents required

1. Water-saturated phenol + 1 % Ammonia.
2. N-butanol-acetic acid-water (4:1:5 v/v).
3. Isopropanol-pyridine-water- acetic acid (8:8:4:1 v/v).
4. A. Ammoniacal silver nitrate: Add equal volumes of NH_4OH to a saturated solution of AgNO_3 and dilute with methanol to give a final concentration of 0.3M.
5. B. Alkaline permanganate: Prepare an aqueous solution of KMnO_4 (1%) containing 2% Na_2CO_3 .
6. C. Aniline diphenylamine reagent: Mix 5 volumes of 1 % aniline and 5 volumes of 1% diphenylamine in acetone with 1 volume of 85% phosphoric acid.
7. D. Resorcinol reagent: Mix 1% ethanolic solution of resorcinol and 0. 2N HCl (1:1 v/v).

Procedure

The samples of various sugars and standard samples were spotted on Whatman No. 1 filter paper using capillary tube. To separate the different sugars in the samples the following different reagents were sprayed.

After spraying Ammoniacal silver nitrate on the developed chromatograms, they were placed in an oven at 100°C for 5-10 minutes, when the reducing sugars appear as brown spots.

After spraying with Alkaline permanganate mixture, the chromatograms were kept at 100°C for a few minutes, when the sugar spots appear as yellow spots in purple background.

After spraying the dried chromatograms with Aniline diphenylamine reagent, the spots were visualized by heating the paper at 100°C for a few minutes.

After spraying the dried chromatograms with Resorcinol reagent, placed them in an oven for 5-10 minutes and visualized the spots by heating at 90°C until the reducing sugars appeared as brown spots

R_f values of the spots were measured.

Results

The different sugar substances observed in the paper chromatographic (Fig. 158) method were given in the tables 1 and 2.

$$R_f = \frac{\text{Distance traveled by lipid spot from the origin}}{\text{Distance traveled by solvent spot from the origin}}$$

$$R_f = \frac{\quad}{\quad} =$$

Table 1. Detection of Sugars

Sugar	Spray reagent			
	a	b	c	d
Aldohexoses	+	+	+	Pink
Ketohexoses	+	+	+	Red
Aldopentoses green	+	+	+	Blue
Ketopentoses	+	+	+	-
Deoxy <i>sugars</i>	-	+	+	-
Glycosides	+	-	-	-
Amino sugars	+		+	+

Table 2. Rf Values of some sugars in the solvents a, b and c.

Sugar	Solvent a	Solvent b	Solvent c
Glucose	0.39	0.19	0.64
Galactose	0.44	0.16	0.62
Fructose	0.51	0.25	0.68
Ribose	0.59	0.31	0.76
Deoxyribose	0.73	-	-
Lactose	0.38	0.09	0.46
Maltose	0.36	0.11	0.50
Sucrose	0.39	0.14	0.62

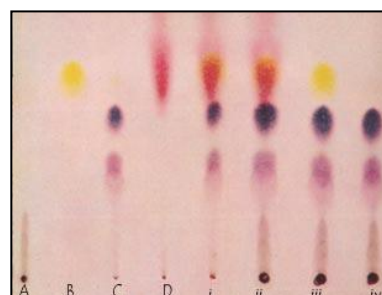
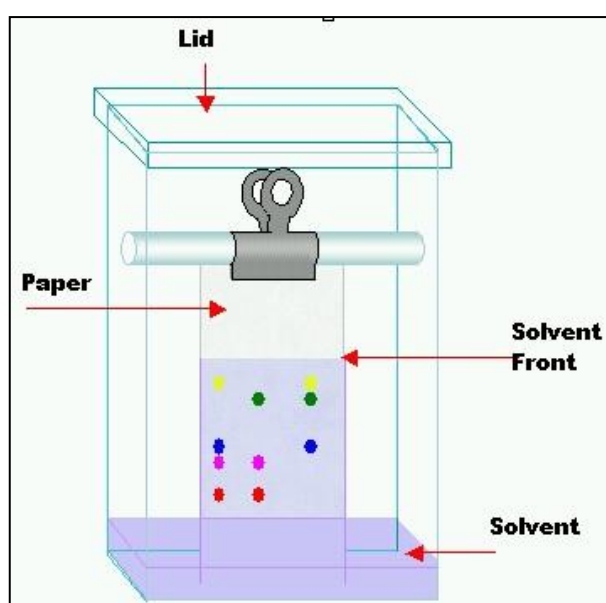


Fig. 158. The different sugar substances in the paper chromatograph.

THIN LAYER CHROMATOGRAPHIC (TLC) SEPARATION OF LIPIDS

Ex. No. 4

Date:

Aim

To separate the lipids using thin layer chromatographic method.

Principle

A chromatographic separation in general is technique in which a mobile phase while over a stationary phase transports different substances with different velocities in the direction of flow. In this case of thin layer chromatography, the stationary phase (an absorb such as silica gel, sialic acid or cellulose) placed into a chromatographic chamber containing a relevant solvent system (mobile phase). As the solvent rises through the absorbent by absorption and capillary action it bends to resolve the compounds of the sample. Electrostatic forces of the stationary phase act to retard the compounds in the sample as the mobile phase rises.

Materials required

TLC plate set.

Reagents required

1. **Chloroform: Methanol (2:1):** 2 Volume of chloroform was mixed with 1 volume of methanol.
2. **Eluting solvent:** Benzene, ether, ethyl, and acetate were mixed in the ratio of 80: 10: 10: 0.2.

Procedure

300-400 mg of hepatopaneas was taken. 15 ml chloroform: methanol mixture was added to it. Then it was homogenated for 15 minutes. It was mixed with water in the ratio of 0.3 volume of water shaking vigorously. The whole sample was allowed to settle in the settled in separating funnel. The choloroform layer was separated and evaporated by the solvent at room temperature. The weight of the lipid was found out. the weight of the lipid. Gravitational force was calculated based on the weight of tissue and amount of lipid extraction. Lipid was dissolved in the minimum quantity of chloroform to make to spotting. The liquid extract was spotted in the TLC plate using a micro pipette. The spots were small base line but concentrated. The plate was kept vertically in eluting chambers which had 150-180 ml of eluting solvent to run the plate till it reached $\frac{1}{2}$ below the top edge of the plate. The plate was air dried and transferred to iodine vapour chamber. After few minutes, the plate was taken. The spots of sub limited iodine were marked to calculate the Rf value. The spots were identified by using known Rf values of standard neutral lipid eluted under identical conditions.

Results

The measured spot of the Rf value was _____.

$$R_f = \frac{\text{Distance traveled by lipid spot from the origin}}{\text{Distance traveled by solvent spot from the origin}}$$

$$R_f = \text{_____} =$$

TLC PLATE

TLC plate is one of the chromatographic techniques (Fig. 159) used to separate the lipids. A thin layer of the stationary phase is formed on a suitable flat surface. Since the layer is so thin, the movement of the mobile phase across the layer, generally by simple capillary action, is rapid, there being little resistance to flow. As the mobile phase moves across the layer from one edge to the opposite, it transfers any analytes placed on the layer at a rate determined by their distribution coefficients, K_d , between the stationary and mobile phases. In practice, the principle of the distribution process may be based on that of adsorption, partition, chiral, ion-exchange or molecular exclusion chromatography. Analyte movement ceases either when the mobile phase (solvent front) reaches the end of the layer and capillary action flow ceases or when the plate is removed from the mobile phase reservoir. One of the biggest advantages of TLC is the speed at which separation is achieved. This is commonly about 30 min and is hardly ever greater than 90 min.

In order to improve the resolution of partition and adsorption separations, the technique of two-dimensional chromatography may be used. The material to be chromatographed is placed towards one corner of the plate as a single spot and the plate developed in one direction and then removed from the tank and allowed to dry. It is then developed by another solvent system, in which the compounds to be separated have different K_d values, in a direction at right angles to the first development.

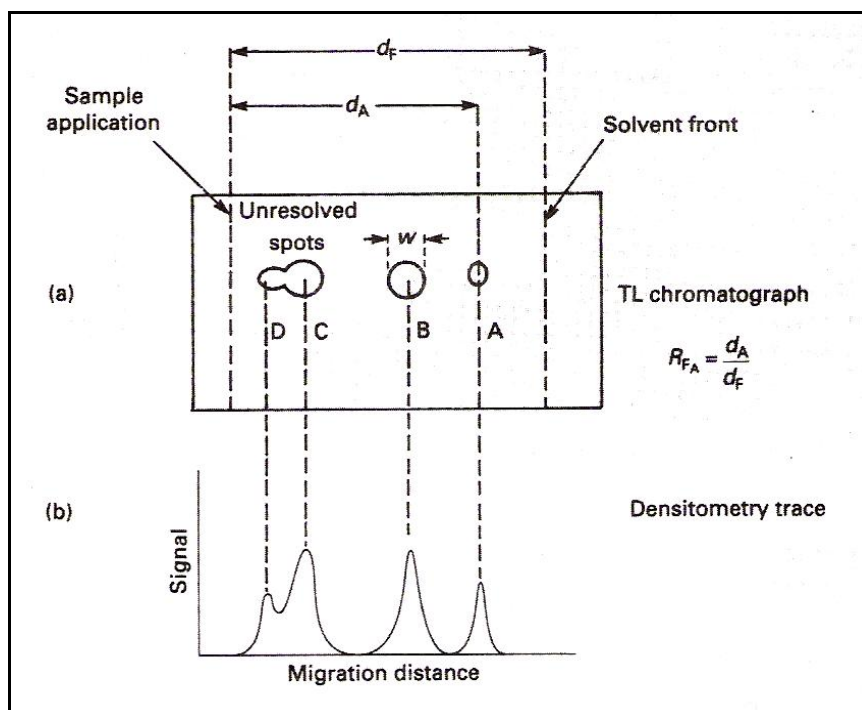
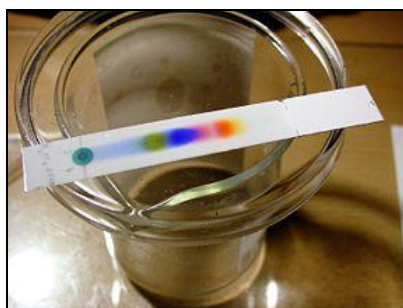


Fig. 159. Thin layer chromatograph.

ELECTROPHORETIC SEPARATION OF HUMAN SERUM PROTEINS (Demonstration only)

Ex. No. 5

Date:

Aim

To separate the serum proteins using thin layer electrophoresis method.

Principle

In polyacrylamide gel electrophoresis the samples are heated with 2% Sodium dodecyl sulphate solution containing 5% Mercapto ethanol and 10% Sucrose or Glucose. SDS denatures proteins by binding with individual polypeptide bonds to keep the peptides apart. In this buffer system, polypeptides stack before start separating in the small pore gel. They migrate according to their size. Comparing the migration of known peptides, size (mw) of unknown peptides can be determined.

Materials for SDS – PAGE

Electrophoresis unit.

Reagents

- 30% Acrylamide (100ml):** It was prepared by mixing 29.2 g Acrylamide, 0.8g N-N methyl bis acrylamide and 50 ml MQ water. Finally they were dissolved and made up to 100 ml, then filtered through Whatmann No. 1 filter paper and stored in Brown bottle at 4°C.
- Separating gel:** It was prepared by mixing 10% (for a single 1.5 mm thick Laemmli gel for SE 250) Acrylamide : Bis stock 3.33 ml, 1.5 M Tris HCl (pH 8.8) 2.5 ml, 10% SDS 100 µl, 10% Ammonium persulfate 50 µl, TEMED 5 µl and Water 4 µl.
- Stacking gel:** : It was prepared by mixing 4% Acrylamide 670 µl, 0.5 M Tris HCl (pH 6.8) 1.25 ml, 10% SDS 50 µl, 10% Ammonium persulfate 25 mg, TEMED 20 µl, and Water 3 ml.
- IX Tank buffer:** It was prepared by mixing Tris (0.02 M, pH 8.3) 12 g, Glycine (*0.192 M), 57.6 g, SDS (0.1 %) 4 g and Distilled water 41 ml.
- 2 X Loading Dye:** It was prepared by mixing 0.125 Tris HCl (pH 6.8) 2.5ml, 10% SDS 4 ml, Glycerol 2 ml, 2 mercapto ethanol 1 ml, Bromophenol Blue (0.15%) 15 mg and Water 10 ml.
- Staining solution:** It was prepared by mixing Coomassie brilliant blue 100 mg, Methanol (50%) 50 ml, Acetic acid (10%) 10 ml, Coomassie brilliant blue was first dissolved in methanol, before adding acetic acid and water.
- Destaining solution:** Methanol (5%) 5ml, Acetic acid (7%) 7ml, and Water 100 ml.

Procedure

A sandwich was made with two glass plates separated by spacer strips width 1.5 cm using Vaseline, 1% Agarose solution was poured into the glass plate to prevent leakage. The separating gel was poured into the glass plates immediately after the addition of TEMED and APS. 400 µl of 0.1% SDS was added over the separating gel to form an even surface and prevent drying. 0.1% SDS was poured off after the polymerization of the separating gel. A Teflon comb of thickness 1.5 mm was inserted between the glass plates immediately after pouring over the stacking gel. The comb was removed after the polymerization of the stacking gel, then the well was rinsed with 0.1% SDS solution. The lower spacer strip was removed after polymerization. The sticking vaselin on the glass plates were wiped out with the help of needle and cotton. The glass plate with the polymerized gel was fixed on the gel tank with help of side clips. 20 µl of

sample was loaded to the sample well in the stacking gel and then the chamber was filled with running buffer.

A potential difference of 50 volts was applied to the electrophoresis until the sample enter the resolving gel. Then the power supply was maintained at 100 volts for 3 hours till the dye reached the bottom the gel.

Staining and destaining

Then the gel was removed from the glass plate and stained with Coomassive brilliant blue stain for 3 hours. After destaining the gel was photographed.

Results

Many protein bands (Fig. 160) were observed in the gel tubes.

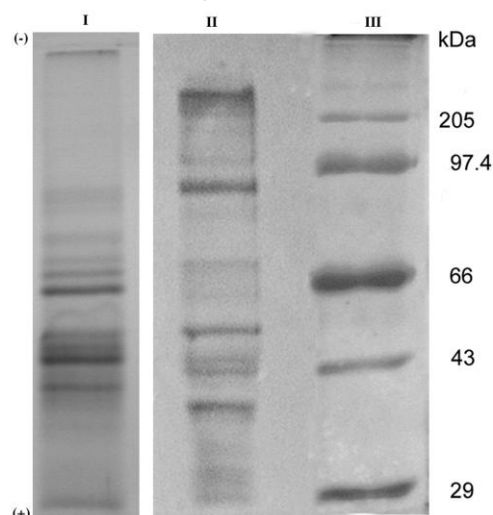


Fig. 160 Protein bands in the gels.

Electrophoresis unit

Electrophoresis unit (Fig. 161) is used to separate the molecules like enzymes, proteins, amino acids, nucleotides and nucleic acids. Various types of instrumental approaches have been used to separate and purify charged molecules using electrophoresis. However, the most common method for purifying enzymes is through electrophoresis on polyacrylamide gel. Polyacrylamide is a polymer of acrylamide and methylene bisacrylamide and when prepared as a gel it is transparent, thermostable, non-ionic and extremely regular in structure. The gel may be taken either in the form of a column or a slab, although the latter is preferred over the former. The protein mixture is loaded in the gel and the components are separated under a direct current of constant voltage (electrode). The migration rate of the various components of the mixture is dependent upon their charge and molecular weight.

A variation of the above polyacrylamide gel electrophoresis is the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which is used to determine the molecular weight of proteins. In this method, the separation is catised by the sieving action of the gel. The proteins migrate through the gel depending on their shapes and mass to charge ratio. Gel electrophoresis is also used to separate various isozymes of a given enzymes perform the same catalytic function but differ in their regulatory and some kinetic aspects.

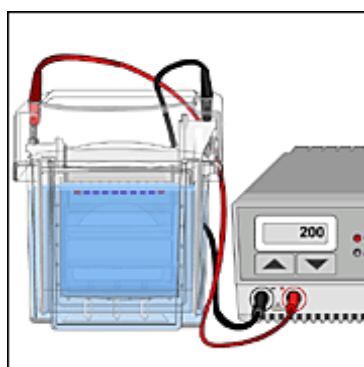


Fig. 161. Electrophoresis unit.

C. BIOTECHNOLOGY

ISOLATION OF GENOMIC DNA

Ex. No. 1

Date:.....

Aim

To isolate the DNA from the Sheep's liver.

Principle

The DNA content of the cell is subjected to mechanical shearing. Necessary pH is maintained by Tris buffer and EDTA. SDS will prevent the DNA from the dehydration during chelation. Phenol and chloroform will coagulate the proteins. Centrifugation will remove the debris and coagulated proteins. Chloroform and alcohol are used to remove the remaining proteins. The DNA is precipitated by ethanol.

Materials required

Sheep's liver, centrifuge, refrigerator, Eppendorff tubes, micropipette, dissection box, conical flask, mortar and pestle.

Reagent

1. **Tris EDTA buffer solution:** 1.21 gm of Tris was dissolved in 70 ml of distilled water. The pH was adjusted to 7.2 by adding HCl. The solution was made up to 100 ml with distilled water.
Tris and EDTA buffer is used in the isolation of DNA. For recombinant DNA technology the isolated DNA molecule should be intact without any damage. A little change in the pH of the isolating medium will cause severe damage to the DNA molecule. Tris EDTA buffer will maintain the pH at 7.5. This pH will keep DNA molecule intact.
2. **Chloroform-isopropanol solution:** It was prepared by mixing 25 ml of chloroform and 1 ml isopropanol.
3. **10% SDS solution:** It was prepared by mixing 10 g of Sodium dodecyl Sulphate in 100 ml of distilled water.
4. **Saturated phenol solution:** Saturated phenol crystals were melted in warm water. Then 100 ml of distilled water was added. The solution was taken in a separating funnel and allowed to settle. The lower phase was collected, which was used as saturated phenol.
5. **1.0 Molar sodium acetate solution:** It was prepared by mixing 40 g of sodium acetate and 40 g sodium hydroxide in 100 ml of distilled water.

All the reagents (except 10% SDS solution) were chilled in refrigerator.

Procedure

1 g of liver tissue was ground in a mortar and pestle using Tris buffer. The contents were centrifuged at 10000 rpm for 20 minutes. It was made into a pulp. Then it was made up to 9 ml with Tris EDTA buffer solution. The filtrate was taken in three test tubes equally. 1 ml of 10% SDS solution was added to each test tube. The test tubes were closed with aluminium foil and were agitated vigorously for thorough mixing. The contents were poured into a test tube and equal volume of saturated phenol was added. The flask was closed with aluminium foil and was shaken well for few minutes. The contents were centrifuged at 10,000 rpm for 20 minutes. The upper aqueous phase was collected in a test tube and equal volume of saturated phenols and chloroform-isopropanol solution were added. The contents were mixed well and centrifuged at 10,000 rpm for 20 minutes. The aqueous phase was transferred to a pre-chilled test tube. Then 0.6 ml of 1 molar sodium acetate solution was added. To this solution, 2 volumes

of ice cold ethanol was added drop by drop. The DNA was precipitated as a white cottonish substance.

Result: The white cottonish precipitate is the isolated DNA (Fig. 162).

Significance: The rapid and efficient method of DNA isolation, storage, and cleavage that provides DNA suitable for amplification in a sequence-based DNA amplification system employing primers that hybridize to the DNA in order to determine the presence of a specific DNA sequence in the fragments. The combination of the isolation and amplification methods can be useful for the detection of parasitic, bacterial, and viral diseases by identification of DNA sequences associated with the organisms causing them.



Fig. 162. The white cottonish precipitate of isolated DNA .

PLASMID ISOLATION

Ex. No. 2

Date:.....

Aim

Isolation of plasmid DNA from *Escherichia coli*.

Principle

In alkaline lysis method, the cells are lysed using EDTA (that chelates metal ions) and an SDS detergent. It weakens the bacterial cell wall and also inactivates the enzymes digesting the DNA (DNases). SDS removes lipid molecules, disrupts the cell membrane and also denatures the bacterial proteins.

After adding NaOH, pH of the solution increases to 11-12. Hence it denatures the bacterial chromosomal DNA and the plasmid DNA. When the pH is reduced after adding potassium or sodium acetate in the solution, the plasmid DNA renatures because of its small size. But the chromosomal DNA strand and bacterial proteins form a precipitate along with SDS. You can remove the precipitate by centrifugation and adding isopropanol and can concentrate the renatured plasmid in solution. You can remove the contaminating RNA by digesting thoroughly RNAase.

Requirements

1. Tris, Sodium Dodecyl Sulphate (SDS), tryptone, yeast extract, sodium chloride, agar, potassium-acetate, Boric acid, glucose, EDTA., sodium hydroxide, Isopropanol, distilled water, high speed refrigerated centrifuge, pH meter micropipettes, water bath, vortex mixer,

2. Agarose gel electrophoresis apparatus, Eppendorf tubes, Tips and glassware.

The following solutions were prepared in advance:

Preparation of reagents

1. **IM Glucose, IM Tris (pH 8.0), 0.5 MEDTA (pH 8) and 5 N sodium hydroxide** (autoclave solutions were prepared and separately stored at room temperature).
2. **10% SDS:** Stored at room temperature.
3. **Ampicillin solution:** 100 mg penicillin dissolved in sterile distilled water to get stock solution of 100 mg/ml stored at -20°C or 4°C. The concentration of ampicillin in any medium was 50-100 µg/ml. Did not autoclave this solution.
4. **Luria Bertani (LB Medium):** 1 g bactotryptone, 0.5 g yeast extract, 1 g sodium chloride were added. The pH adjusted to 7 with 1 N NaOH and made up the volume to 100 ml with water. Autoclaved the medium and stored at room temperature.
5. **Solution I:** 5 ml of IM glucose (50 mM final concentration of glucose), 0.25 ml of IM Tris (pH 8) (25 mM final concentration of Tris, pH 8) and 2 ml of 0.5 MEDTA (pH 8.0) mM final concentration of EDTA) were added. By using water made up the volume to 100 ml. The solution autoclaved and stored at room temperature.
6. **Solution II:** Fresh solution on the day of the experiment prepared by mixing 4 ml of 5N NaOH (0.2 N final concentration of NaOH) and 10 ml of 10% SDS (final concentration of 1% SDS). By using autoclaved water made the volume of solution to 100 ml. Discarded the solution after use.
7. **Solution III:** M CH₃COOK solution prepared 3 of pH 4.8. Autoclaved it and stored at 4°C.
8. **Running buffer (for electrophoresis):** There were two common types of running buffers used in agarose gel electrophoresis : Tris-Borate-EDTA (TBE) and Tris-Acetate-EDTA TAE).

The following stock solutions were prepared: **i) 5X TBE, 1 Litre:** 54 g Tris, 27.5 g boric acid, 20 ml 0.5 MEDTA, pH 8.0. and **2) 50X TAE, 1 litre:** 24.2 g Tris, 5.71 ml of glacial acetic acid, 20 ml of 0.5 M EDTA, pH 8.0.

9. **TE:** 1 ml of 1 M Tris, pH 8 (10 mM 1M Tris, pH 8.0) and 0.2 ml of 0.5 MEDTA, pH 8.0. (1 mM final concentration of EDTA) were added. Using distilled water made the volume to 100 ml. The solution was autoclaved and stored at room temperature.
10. **6X Gel-Loading Buffer:** 0.15% bromo phenol blue, 0.15% xylene cyanol, 30% (v/v) glycerol were prepared in water.
11. **Ethidium bromide solution:** Dissolved 10 mg/ml in water. Stored at 4°C wrapped in an aluminium foil (wore gloves while working with this dye because it is mutagenic).
12. **IX-TBE, 1 litre:** Diluted with water 200 ml 5X stock of TBE so as to get 1 liter.

A single bacterial colony inoculated into 2 ml of autoclaved LB medium containing 2 µl of 100 mg/ml ampicillin solution. The culture incubated at 37°C overnight with shaking (200-250 rpm) condition.

Procedure

1. After 24 hours incubation, took 1.5 ml culture from the 2 ml culture using an eppendorf tube pipette.
2. The cells centrifuged at 6,000 rpm for 5-10 minutes. Discarded the supernatant completely by inverting the eppendorf tube on the blotting paper. The eppendorf tube put on ice.
3. Completely re-suspended the pellet in (0.1 ml) of ice cold Solution I to get a uniform suspension, put on ice for 5 minutes then keep at room temperature.
4. To this suspension, added 0.2 ml of freshly-prepared Solution II. Tube was closed tightly. Properly mixed the contents by inverting the tube five times.
5. 0.15 ml of ice-cold Solution III added. The tube tightly closed and mixed the contents properly by inverting the tube. Kept for 5-7 minutes on ice. Vortexed at 10,000 to 12,000 rpm for 10 minutes at 4°C.
6. Soon transferred the supernatant to a fresh eppendorf tube and added 0.45 ml of isopropanol. Gently mixed by inverting the tube and kept at room temperature for 10 minutes. Centrifuged at 10,000 rpm for 20 minutes at room temperature and took out the supernatant.
7. Thereafter, added 0.1 ml of 70% ethanol in to the pellet and spin at 10,000 rpm for 5 minutes at 4°C. Then discarded the supernatant carefully and dried the tube at 37°C so that any traces of isopropanol could be removed.
8. Then, added 20 µl of IX-TE from the side and gently tap the tube with your fingers. 3.33 µl of 6X gel loading buffer added and run on 1% agarose gel as it has been described below (Note: alternatively, a plasmid-isolation kit of any company can be used).

Preparation of 1% agarose gel and set up of electrophoresis: The following steps were followed:

1. Diluted 50X TAE or 5X TBE buffer with distilled water to get IX TAE or IX TBE.
2. Poured 50 ml of IX TAE or 1X-TBE buffer in a 250 ml conical flask and added 0.5 g of agarose into it. Boiled to get a clear solution and cool to warm a liquid (60°C).
3. In the electrophoresis, set put the combs in such a way that it should be about 2 cm away from the cathode.
4. Ethidium bromide (10 mg/ml stock) was added to make a final concentration of 0.5 g/ml of gel when the temperature of agarose gel was around 60°C.

5. Gently poured the solution into the gel tank. Poured the agarose gel in such a way that it could be 0.5-0.9 cm thick and without air bubbles. Allowed the gel to get solidified.
6. The bacterium sample was loaded into the well very carefully and recorded that which sample is being loaded into which well as Lane 1, Lane 2, and so on. The power connection started and set voltage to 50 V.
7. Until the second dye (blue dye) had reached 3/4 of the gel, run the gel. (it took about 1 hour). Observed under UV transilluminator.

Result

After precipitating with isopropanol and centrifugation, a white precipitate was observed on the sides or at the bottom of the centrifuge tube. A band was observed when the plasmid DNA is run on an agarose gel (Fig. 163).

Discussion

Plasmids are the circular, autonomously, replicating, extra chromosomal double-stranded DNA molecules found in bacteria cyanobacteria, fungi, etc. They are classified according to the phenotypes conferred by them on the host cells i.e. antibiotic resistance, fertility (F) factor, etc. Naturally occurring plasmids also encode for genes that are required for conjugation e. g. F plasmid. There are three

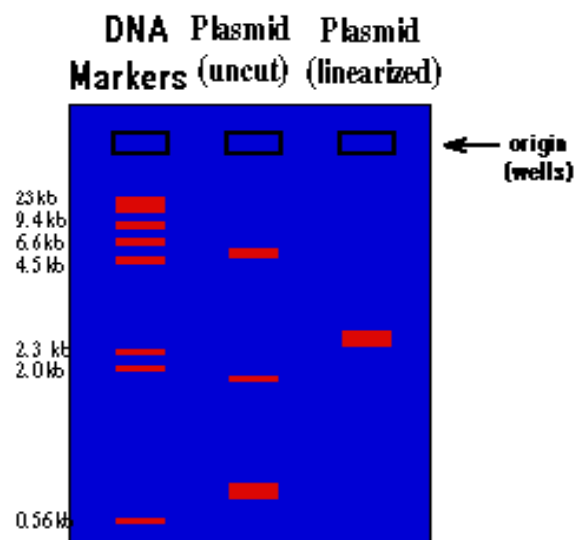


Fig. 163. Plasmid.

forms of plasmids: super-coiled, relaxed and linear. In recombinant DNA technology, plasmids are used as cloning vehicle or vector to introduce foreign genes into a host organism.

Vectors are the cloning vehicle. Vectors are transferred into the host cells for cloning. One of the standard cloning vectors widely used in gene cloning experiment is **plasmid vector PBR 322** (Fig. 164). It is an artificially synthesized vector. It was developed by Boliver and Rodrigus. The derivation of the name PBR 322 is based on P = Plasmid, B = Boliver and R = Rodrigus. The number (322) is given for the strain to distinguish it from other strains. The numbers given for other strains are 322, 325, 327 and 328.

The vector PBR 322 has restriction enzyme sites for a numbers of restriction enzyme such as Pst I, Eco R1, Hind III, Pam H1, Sal G1, etc. It has two marker gene namely ampicillin resistant gene and tetracycline resistant gene. They are useful in screening the recombinant DNA (rDNA).

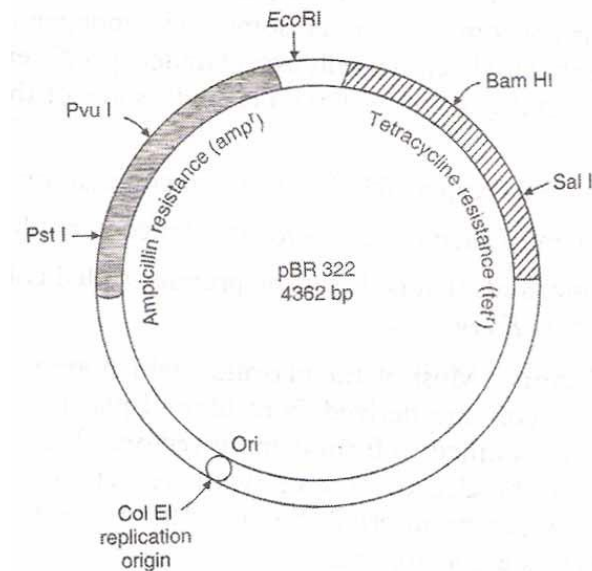


Fig. 164. Plasmid p BR322.

Advantages of pBR 322

The plasmid PBR 322 is an ideal cloning vector. It is the most common plasmid used in gene cloning experiments due to the following reasons:

1. The plasmid is much smaller than a natural plasmid, since this makes it more resistant to damage by shearing, and increases the efficiency of uptake by bacteria during transformation.
2. Since this plasmid is small, foreign DNA upto 6 kb in length can be inserted into this plasmid.
3. It has two antibiotic resistance genes namely Amp^R and Tet^R with unique restriction sites. Insertion of a new gene at a site in any one of these marker genes will inactivate the marker gene. This helps in the selection of *E.coli* cells harbouring recombinant plasmids.
4. PBR 322 has relaxed origin of replication (its multiplication is not tightly linked to cell division). So in presence of chloromphenicol its number may increase to 1000-3000.
5. This plasmid is used as a raw material to construct several other useful plasmids or other cloning vectors.
6. Single restriction sites for various enzymes, is present around the plasmid, which can be used to open the circle at a specific point prior to insertion of foreign DNA.

AGAROSE GEL ELECTROPHORESIS OF DNA

Ex. No. 3

Date:.....

Aim

Principle

The technique of electrophoresis is based on the fact that DNA is negatively charged at neutral pH due to its phosphate backbone. For this reason, when an electrical potential is placed on the DNA it will move toward the positive pole. The rate at which the DNA will move toward the positive pole is slowed by making the DNA move through an agarose gel. This is a buffer solution (which maintains the proper pH and salt concentration) with 0.75% to 2.0% agarose added. The agarose forms a porous lattice in the buffer solution and the DNA must slip through the holes in the lattice in order to move toward the positive pole. This slows the molecule down. Larger molecules will be slowed down more than smaller molecules, since the smaller molecules can fit through the holes easier. As a result, a mixture of large and small fragments of DNA that has been run through an agarose gel will be separated by size. This is a graphic representation of an agarose gel made by "running" DNA molecular weight markers, an isolated plasmid, and the same plasmid after linearization with a restriction enzyme:

These gels are visualized on a U.V. trans-illuminator by staining the DNA with a fluorescent dye (ethidium bromide). The DNA molecular weight marker is a set of DNA fragments of known molecular sizes that are used as a standard to determine the sizes of your unknown fragments.

Materials required

- Buffer solution, usually TBE buffer or TAE 1.0x, pH 8.0
- Agarose
- An ultraviolet-fluorescent dye, ethidium bromide, (5.25 mg/ml in H₂O). The stock solution should be carefully handled. Alternative dyes may be used, such as SYBR Green.
- Nitrile rubber gloves. Latex gloves do not protect well from ethidium bromide
- A color marker dye containing a low molecular weight dye such as "bromophenol blue" (to enable tracking the progress of the electrophoresis) and glycerol (to make the DNA solution denser so it will sink into the wells of the gel).
- A gel rack
- A "comb"

- Power Supply
- UV lamp or UV lightbox or other method to visualize DNA in the gel

Preparation

There are several methods for preparing gels. A common example is shown here. Other methods might differ in the buffering system used, the sample size to be loaded, the total volume of the gel (typically thickness is kept to a constant amount while length and breadth are varied as needed). Most agarose gels used in modern biochemistry and molecular biology are prepared and run horizontally.

1. Make a 1% agarose solution in 100 ml TAE, for typical DNA fragments (see figures). A solution of up to 2-4% can be used if you analyze small DNA molecules, and for large molecules, a solution as low as 0.7% can be used.
2. Carefully bring the solution just to the boil to dissolve the agarose, preferably in a microwave oven.
3. Let the solution cool down to about 60 °C at room temperature, or water bath. Stir or swirl the solution while cooling.

Procedure

After the gel has been prepared, use a micropipette to inject about 2.5 μ l of stained DNA (a DNA ladder is also highly recommended). Close the lid of the electrophoresis chamber and apply current (typically 100 V for 30 minutes with 15 ml of gel). The colored dye in the DNA ladder and DNA samples acts as a "front wave" that runs faster than the DNA itself. When the "front wave" approaches the end of the gel, the current is stopped. The DNA is stained with ethidium bromide, and is then visible under ultraviolet light.

Result

The separated DNA fragments were seen as bands (Fig. 165) in the gel tube.

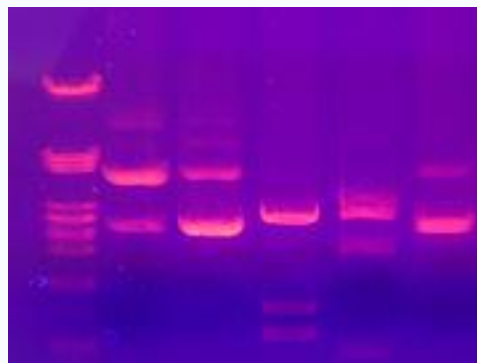


Fig. 165. A pattern of DNA-bands under UV light.

Significance

Agarose gel electrophoresis is an easy way to separate DNA fragments by their sizes and visualize them. It is a common diagnostic procedure used in molecular biological labs.

Agarose Gel Electrophoresis Unit (An electrophoresis chamber and power supply)

- **Gel casting trays**, which are available in a variety of sizes and composed of UV-transparent plastic (Fig. 166). The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
- **Sample combs**, around which molten agarose is poured to form sample wells in the gel.
- **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
- **Ethidium bromide**, a fluorescent dye used for staining nucleic acids. NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.
- **Transilluminator** (an ultraviolet lightbox), which is used to visualize ethidium bromide-stained DNA in gels. NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.

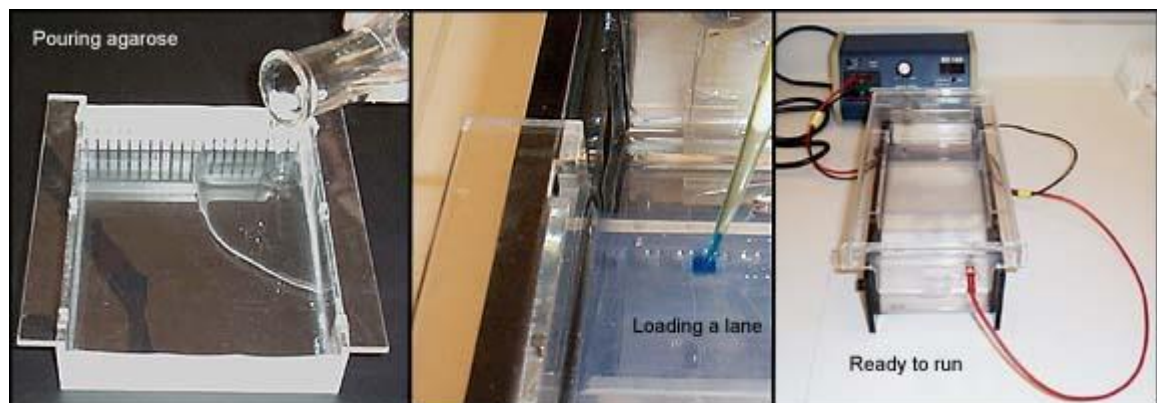


Fig. 166. Agarose Gel Electrophoresis Unit.

DNA FRAGMENTATION USING RESTRICTION ENZYMES (Demonstration only)

Ex. No. 4

Date:.....

Aim

To fragment the DNA using a restriction enzyme of HindIII (lane 1) and EcoRI (lane 2).

Principle

Phage lambda (λ) DNA is a linear double-stranded DNA containing of 48,502 base pairs (bp). Its DNA becomes circularized after release (inside the cell of *E. coli*) at a cohesive site called COS site. It contains five recognition sites for EcoRI and seven recognition sites for HindIII. The complete digestion of lambda DNA with EcoRI results in 21, 226, 7421, 5804, 4878 and 3530 bp long six DNA fragments. Similarly, a complete digestion of lambda DNA with HindIII results in eight DNA fragments viz., 23, 130, 9416, 6557, 4361, 2322, 2027~ 564, 125 bp long fragments.

Requirements

1. Lambda DNA, restriction enzyme such as EcoRI or HindIII.
2. Assay buffer for restriction enzyme, sterilized water, Tips, Eppendorf tubes, micropipettes.
3. Agarose gel electrophoresis apparatus.
4. 5X TBE or 5X TAE.

Procedure

1. Always kept restriction enzyme (EcoRI or HindIII), substrate (λ DNA) and assay buffer in an ice bucket.
2. 2-5 μ l of the lambda DNA took as substrate in an eppendorf tube and dissolved in an appropriate volume of water.
3. 2 μ l of about 10X assay buffer was added (available with the restriction enzyme) to the DNA in the eppendorf tube, followed by respective enzyme (5-12 units of EcoRI or 10-25 units of HindIII depending upon the amount of DNA used in the reaction step (ii).
4. Sterilized water was added to make the final volume of reaction mixture to 20 μ l. Centrifuge gently or mix by tapping with fingers.
5. Incubated the reaction mixture for 1 hour at 37°C in a water bath or incubator.
6. In the mean time, 1 % agarose was prepared gel for loading and electrophoresis.
7. After one hour, the reaction was stopped by addition of 3.33 μ l of 6X gel loading buffer to the eppendorf tube. Labelled the vial as 'A' and put on ice.
8. As described in step (ii) the same amount of DNA was taken in another fresh 1.5 ml eppendorf tube and marked as 'B'.
9. On a 1% agarose gel, the samples A and B were loaded in separate wells. Noted the order of the samples loaded in each well. As described in the plasmid isolation experiment, start electrophoresis.
10. Run it until the bromophenol blue dye has reached 3/4 of the gel (it takes about 1 hour). Observe under UV transilluminator.

Results

6 DNA fragments (Fig. 167 and 168) were observed in lambda DNA by using EcoRI restriction enzyme and 8 DNA fragments are observed after using HindIII).

This reveals the cleavage of sample 'B' by the respective restriction enzymes. The exact number and size of the bands obtained depend on the restriction enzymes used for digesting the lambda DNA (sample 'B').

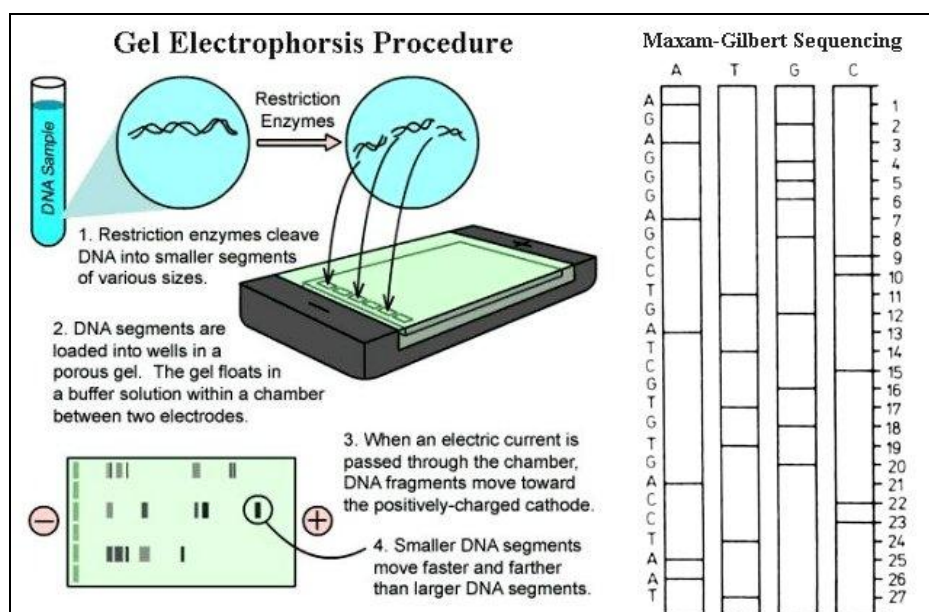


Fig. 167. DNA fragmentation using restriction enzymes and separation using gel electrophoresis.

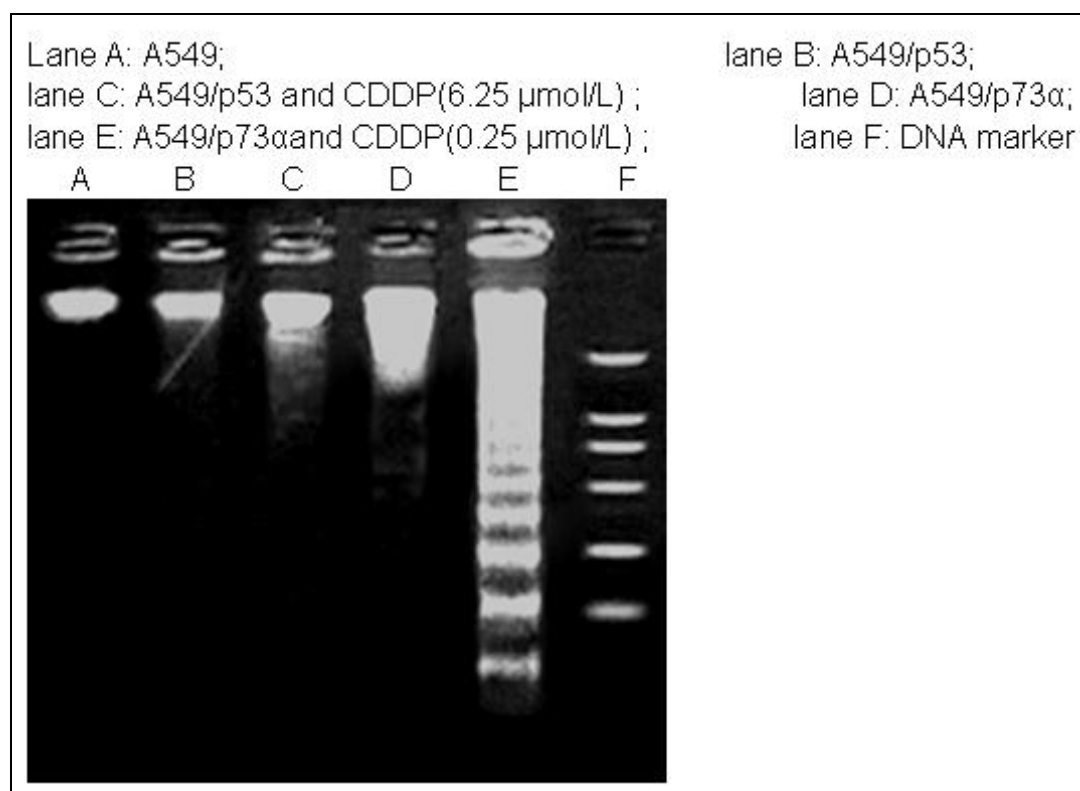


Fig. 168. DNA fragmentation and transferring cell line in agarose gel electrophoresis of DNA.

Discussion

Restriction enzymes are endonucleases which recognize and cleave the specific DNA sequences called restriction sites for example, EcoRI (isolated from *Escherichia coli*) that recognizes and cleaves the sequence 5'-GAATTC-3' to generate cohesive or sticky ends. Similarly, HindIII isolated from *Haemophilus influenzae* recognizes and cleaves the sequences 5'-AAGCTT-3' to generate cohesive or sticky ends.

Enzyme activity is represented as IU (International Unit). One unit of a restriction enzyme is the amount of enzyme required to completely digest one micro-gram of lambda DNA [in a reaction volume of 50 μ l] in one hour under optimal conditions of salt, pH and temperature (about 37.1°C for most restriction enzymes).

SOUTHERN BLOTTING TECHNIQUE (Demonstration only)

Ex. No. 5

Date:.....

Aim

To separate a specific restriction fragment of DNA to a nitrocellulose membrane against a background of many other restriction fragments .

Principle

Southern blotting is a technique that enables a specific restriction fragment to be detected against a background of many other restriction fragments. It involves transfer of DNA fragments from an electrophoresis gel to a nitrocellulose or nylon membrane in such a way that the DNA banding pattern present in the gel is reproduced on the membrane. The restriction fragments of DNA to be separated on the basis of their size prompted the development of techniques for the transfer of separated fragments en masse from gel to nitrocellulose support. Hybridization probing is then used to detect the restriction fragment that is being sought. The procedure described by Southern (1975), involving capillary transfer of DNA from the gel to a nitrocellulose sheet placed on top of it, was simple and effective, and although embellished over the years this original procedure differs only slightly from the routine method still used in many molecular biology laboratories.

Reagents

nitrocellulose membrane or nylon membrane, agarose gel, 0.25 mol/L HCl, 3.0 mol/L NaCl (salt), 0.3 mol/L sodium citrate, electrophoresis unit, UV radiation chamber, restriction fragments of 20 kb and more.

Procedure

Preparation of genomic DNA: The techniques used to prepare DNA for Southern blotting depend on the type of DNA that is being studied. For genomic DNA, the objective is to obtain molecules that have not become extensively fragmented by random shearing during the extraction process, so that specific restriction fragments of 20 kb and more can be obtained. Cells must therefore be broken open under relatively gentle conditions. For tissue culture cells and blood samples incubation in a buffer containing a detergent such as sodium dodecyl sulfate (SDS) is usually sufficient to disrupt the cell membranes and release high-molecular weight DNA. This causes some fragmentation of the DNA but provides a much higher yield. After cell disruption, genomic DNA extraction procedures continue with steps aimed at removing the major biochemicals other than DNA present in the initial extract. A protease such as proteinase K might be included in the buffer used for cell disruption, in order to begin the degradation of proteins in the extract, but deproteinization is routinely carried out by phenol extraction, the addition of phenol or a 1:1 mixture of phenol and chloroform resulting in precipitation of proteins. After centrifugation, the precipitated proteins migrate to the interface between the organic and aqueous phases, whereas the nucleic acids remain in the aqueous phase. Most extraction procedures also include digestion of RNA with ribonuclease and a final treatment with ethanol, which precipitates the remaining nucleic acid polymers, enabling ribonucleotides and other low-molecular weight contaminants to be removed. The precipitated DNA is dried and then redissolved in water or a Tris-EDTA buffer.

Separation of restriction fragments of DNA: An agarose electrophoresis gel, containing the fractionated restriction fragments, is placed on a filter paper wick that forms a connection between the gel and a reservoir of high-salt buffer. The nitrocellulose membrane is placed on top of the gel and covered with a tower of paper towels that are held in place with a weight. Capillary action results in the buffer soaking through the filter paper wick, gel and membrane and into the paper towels. As the buffer passes through the gel the DNA fragments are carried with it into the membrane, where they become bound to the nitrocellulose. Effective transfer of fragments up to 15 kb in length takes approximately 18 h, roughly equivalent to

'overnight'. The only technical complication is the possibility that the buffer bypasses the gel by soaking directly from wick to paper towels, which is unlikely if the setup is assembled carefully.

After the purified DNA has been treated with one or more restriction endonucleases it is fractionated by agarose gel electrophoresis and the gel then pretreated prior to setting up the Southern blot. The pretreatment has two objectives. First, it is desirable to break the DNA molecules in individual bands within the gel into smaller fragments, because smaller fragments transfer more quickly than larger ones. This is achieved by soaking the gel in 0.25 mol/L HCl for 30 min, which results in a small amount of depurination – cleavage of the β -N-glycosidic bond between purine bases (adenine or guanine) and the sugar component of their nucleotides – which is followed by decomposition of the sugar structure and breakage of the polynucleotide chain. The second pretreatment is with an alkaline solution that denatures the double-stranded DNA molecules by breakage of their hydrogen bonds, so the molecules become single-stranded. This aids their transfer and subsequent binding to the membrane, and also ensures that after binding the base-pairing components of the polynucleotides are available for hybridization with the probe. If a nitrocellulose membrane is being used then the alkali pretreatment is followed by neutralization of the gel by soaking in a Tris-salt buffer, this step being essential because DNA does not bind to nitrocellulose at a pH of greater than 9.0. The Southern blot is then set up, as illustrated in Figure 1, with a high-salt transfer buffer, usually the formulation called '20 × SSC', which comprises 3.0 mol/L NaCl (salt) and 0.3 mol/L sodium citrate. The same buffer can be used for transfer to a nylon membrane, but with a positively charged nylon membrane an alkaline transfer buffer (0.4 mol/L NaOH) is used because, as described earlier, this results in immediate covalent attachment of the transferred DNA to the membrane. With this type of transfer the alkali pretreatment is unnecessary. The blot is then left for at least 18 h for a high-salt transfer, or 2 h for an alkaline blot.

After blotting, the transfer setup is dismantled and the membrane rinsed in 2 × SSC and left to dry. If the blot has been made onto a nitrocellulose or uncharged nylon membrane, then the DNA is only loosely bound to the membrane at this stage. More permanent immobilization must therefore be carried out, either by baking at 80°C for 2 h, which results in noncovalent but semipermanent attachment of DNA to a nitrocellulose membrane, or UV irradiation, which results in covalent attachment of DNA to a nylon membrane.

Result

Noncovalent but semipermanent attachment of a specific restriction fragment of DNA to a nitrocellulose membrane against a background of many other restriction fragments was detected and covalent attachment of DNA to a nylon membrane are separated. The separated DNA can be seen under UV irradiation or autoradiogram (Fig. 169).

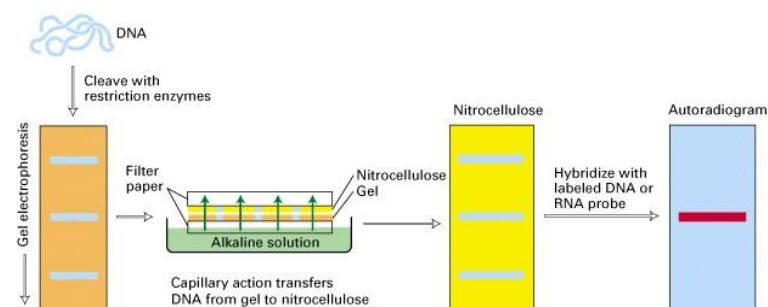


Fig. 169. Southern blotting.

Significance

Southern blotting has many applications in molecular biology, including the identification of one or more restriction fragments that contain a gene or other DNA sequence of interest, and in the detection of RFLPs used in construction of genomic maps.

NORTHERN BLOTTING TECHNIQUE (Demonstration only)

Ex. No. 6

Date:.....

Aim

To detect specific proteins in a given sample of tissue homogenate or extract using a specific antibody in a polyacrylamide gel and immobilized onto a membrane.

Principle

Western blotting is an immunoblotting technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses polyacrylamide gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions).

The SDS PAGE technique is a prerequisite for Western blotting. Proteins are separated based on weight and electrical properties as they migrate through a polyacrylamide gel matrix. Acrylamide gel preparation is a process that involves the crosslinking of acrylamide monomers with the use of catalysts. Once the gel has set, protein samples can be loaded into the gel and separated along an electric field. The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.

Reagents and Equipment

1. 0.3 M TRIZMA base, 20% methanol.
2. 0.025 M TRIZMA base, 20% methanol.
3. 0.02 M TRIZMA base, 20% methanol, 5.25 mg/ml epsilon-amino caproic acid.
4. Tris buffered saline (TBS).
5. Tris buffered saline with 0.05% Tween 20 (TBS/Tween).
6. Bovine serum albumin (BSA).
7. Nitrocellulose (NC) membrane 0.45 5m pore size. 0.2 5m pore size nitrocellulose membrane may be used for low molecular weight molecules.
8. Blotting paper, extra thick.
9. Primary antibody.
10. Alkaline phosphatase-conjugated secondary antibody.
11. Phosphate buffered saline with 0.05% Tween 20 (PBS/Tween).
12. SIGMA FASTTM BCIP/NBT Alkaline Phosphatase Substrate Tablets Liquid BCIP/NBT).
13. Minigel system.
14. 2000 volt power supply.
15. Semi-dry blotter.
16. Orbital shaker.
17. Troughs 2-3 mm depth, 7-10 mm wide.

Procedure

SDS-PAGE: Proteins are separated by gel electrophoresis (Fig.170), usually SDS-PAGE.

1. Carry out SDS-PAGE (cassette size approx. 80 x 80 mm). For analytical runs, load 5-20 micrograms of protein per well; for preparatory purposes, use 600-800 micrograms of total cell or tissue homogenate per gel. Apply constant voltage at 70-140 V or 1-2 hours until the tracking dye migrates 1.0 cm from the gel end.

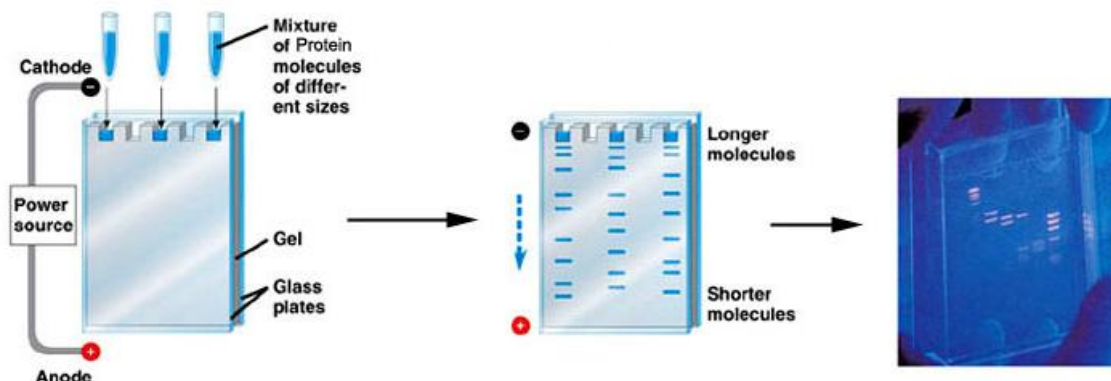


Fig. 170. Proteins are separated by gel electrophoresis.

Protein Blotting

1. Build the transfer "sandwich" onto the anode(+) plate as follows: 2 sheets blotting paper soaked in 0.3 M TRIZMA base, 20% methanol, 1 sheet blotting paper soaked in 0.025 M TRIZMA base, 20% methanol, NC membrane pre-wet with deionized water, Slab gel, 3 sheets blotting paper soaked with 0.02 M TRIZMA base, 20% methanol, 5.25 mg/ml epsilon-aminocaproic acid.
2. Carry out the transfer at 90 mA for 1.5 hours at room temperature.
3. Remove the NC membrane from the apparatus and air dry the NC blot thoroughly.

Note: The dry membrane may be stored at 2-8 °C between two sheets of blotting paper in a plastic sleeve.

Immuno-Detection

1. The following incubation and washing steps are carried out at room temperature on an orbital shaker platform. Primary antibody is diluted in 1% normal serum from the secondary antibody host animal in TBS. Other non-interfering proteins (e.g., BSA, hemoglobin, ovalbumin) may be substituted.
2. Cut the membrane into strips. Place the NC strips, with the side in contact with the gel during the transfer facing up, in the troughs.
3. Block NC blot using 5% w/v BSA in TBS, overnight at 4 °C or 2 hrs. at room temperature. The choice of blocking reagent depends on the type of probe that will be subsequently used in the overlay procedure and should be chosen accordingly.
4. Remove the blocking buffer.
5. Overlay the blot with 5 ml primary antibody at an appropriate dilution (generally 1:50-1:500). Incubate for 1-3 hours at room temperature on shaker.
6. Wash the NC strips four times for 5 minute each, with sufficient TBS/Tween.
7. Incubate the strips for 1 hour in alkaline phosphatase-secondary antibody conjugate at an appropriate dilution. (1:30,000 for upgraded alkaline phosphatase conjugates) in TBS/Tween.

8. Wash the strips as in step 4. Rinse 3 times in TBS for 5 minutes each.
9. Prepare SIGMA FAST™ BCIP/NBT Tablets according to package directions. Liquid BCIP/NBT substrate may be used.
10. Incubate the NC strips in the substrate mixture for 10-30 minutes until color development.
11. Stop the reaction by washing the strips in several changes of distilled water.
12. Air dry the strips and store in the dark in a plastic sleeve.
12. The location of the antibody is revealed by incubating it with a colorless substrate that the attached enzyme converts to a colored product that can be seen and photographed.



Result

The separated protein molecules are detected using the specific antibody (Fig 171).

Fig. 171. An illustration of a developed membrane, showing protein of interest occurring in the tested samples. From left to right, the visualized lanes contain: molecular weight standards markers and samples 1, 2 and 3.

Significance

The goal of Western blotting, or more correctly, immunoblotting, is to identify with a specific antibody a particular antigen within a complex mixture of proteins that has been fractionated in a polyacrylamide gel and immobilized onto a membrane.

Western blotting occurs in six stages: (1) extraction and quantification of protein samples; (2) resolution of the protein sample in sodium dodecyl sulfate polyacrylamide denaturing gel electrophoresis (SDS-PAGE); (3) transfer of the separated polypeptides to a membrane support; (4) blocking nonspecific binding sites on the membrane; (5) addition of antibodies; and (6) detection.

Immunoblotting can be used to determine a number of important characteristics of protein antigens—the presence and quantity of an antigen, the relative molecular weight of the polypeptide chain, and the efficiency of extraction of the antigen.

The specificity of the antibody-antigen interaction enables a single protein to be identified in the midst of a complex protein mixture. Western blotting is commonly used to positively identify a specific protein in a complex mixture and to obtain qualitative and semiquantitative data about that protein.

This method is used in the fields of molecular biology, biochemistry, immunogenetics and other molecular biology disciplines.

D. BIOSTATISTICS

PROBLEMS RELATED TO CHI-SQUARE TEST

Ex. No.: 1

Date:.....

Aim: To compute the measure the actual divergence of the observed and expected frequencies.

Principle: In Chi-square analysis “O” is the observed frequency and “E” is the expected frequency or theoretical frequency on the basis of some hypothesis to test the goodness of the fit. The summation is to be carried over all the cells or classes in the table. In sampling studies it is expected that there would be perfect coincidence between the expected and the observed frequencies.

Materials required: Data and formula.

Q. In a cross between white rabbit and brown rabbit, 2361 white and 831 brown were obtained. Suggest if a ratio of 3:1 is suitable or not.

Formula

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where O = Observed frequency in a class

E = Expected frequency in a class

Σ = Summation overall class

Solution:

Observed total number = 2361 + 831 = 3192

Expected ratio = 3:1 = 4

Expected white rabbit = 3192 x $\frac{3}{4}$ = 2394

Expected brown rabbit = 3192 x $\frac{1}{4}$ = 798

Observed value	Expected value	(O – E)	(O – E) ²	$\frac{(O - E)^2}{E}$

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Result: The calculated value of the Chi-square =

Table value of Chi-square (Degrees of freedom [2-1] = 1) at 5% level =

Since the calculated value () is less than the table value (), there is no significant difference between the observed and expected frequencies. The two types of plants are in accordance with the mendalian monohybrid ratios of 3:1. They do not deviate from their ratios.

PROBLEMS RELATED TO STUDENT'S T - TEST

Ex. No.: 2

Date:.....

Aim: To test the difference between the significance of the sample means.

Principle: In research often required making decisions or judgments concerning various kinds of differences. It is usually necessary to make these judgments on the basis of samples drawn from populations that too large to measure directly. Decisions are usually made on the differences whether significant or not. Analysis and interpretation of data are called test of significance.

Materials required: Data and formula.

Data (Question): A certain stimulus administered to the each of 10 patients, resulted in the following increases of blood pressures ($\mu = 0$).

9 10 8 6 0 -2 3 4 -1 -2

Can it be concluded this stimulus changes the blood pressures?

Formula:

$$t = \frac{\bar{X} - \mu}{\sigma} \times \sqrt{n}$$

$$\bar{X} = \frac{\sum x}{n} =$$

$$\sigma = \sqrt{\frac{\sum d^2}{n}}$$

$$\mu = 0$$

$$\sigma =$$

$$\bar{X} =$$

$$t =$$

Result: The calculated t value is 2.39.

Tabular value (n-1, 10-1=9) at 95% level is 2.26.

Conclusion: Since the calculated value (2.39) is greater than the table value (2.26), the stimuli significantly change the pressure.

PROBLEMS RELATED TO CORRELATION

Ex. No.: 3

Date:.....

Aim: To compute the correlation (r) of the length and weight of the sample of fishes given.

Principle: Correlation analysis is concerned with measuring the strength (or) degree of relationship between variables.

Materials required: Data and formula.

Q. Compute the correlation for the length and weight of sample fishes given.

Length	16.0	14.2	11.5	17.0	16.5	21.7	21.3	14.7	15.0	18.0
Weight	40	20	10	35	36	90	80	28	25	46

Procedure: Using the following formula the above table measurements that the relationship between fish length and fish body weight were calculated.

$$r = \frac{N \sum xy - (\sum x)(\sum y)}{N \sum x^2 - (\sum x)^2 \quad N \sum y^2 - (\sum y)^2}$$

S. No.	Length x	Weight y	x ²	y ²	xy
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
	Σx	Σy	Σx ²	Σy ²	Σxy

Result: The above value of r =, which indicatesdegree (positive correlation) of correlation coefficient between the fish length and fish weight.

PROBLEMS RELATED TO REGRESSION

Ex. No.: 4

Date:.....

Aim: To compute the regression (r) of pupal weight and number of ovarioles of the sample of insect given.

Principle: Regression analysis is the estimation of or prediction of value of one variable from the value of other given variables. That is the functional relationship of one variable on the other. The variable, which is to be predicted, is called dependant as independent variable concerns with measuring the strength (or) degree of relationship between variable.

Materials required: Data and formula.

Question: Calculate the regression equation for the following data. Based on the equation estimate the weight of the fish expected to be present in a fish length 22.0 cm.

Length cm	16.0	14.2	11.5	17.0	16.5	21.7	21.3	14.7	15.0	18.0
Weight gm	40	20	10	35	36	90	80	28	25	46

Procedure: Using the above data and formula, the regression equation for the above variables were calculated.

Formula

Regression equation = X on Y : $X = a + by$

Y on X : $Y = a + bx$

The value of 'a' and 'b' can calculated with the help of the following normal equations.

Regression equation of X on Y

Regression equation of Y on X

$$\Sigma X = Na + \Sigma Y$$

$$\Sigma Y = Na + \Sigma X$$

$$\Sigma XY = a\Sigma Y + b\Sigma Y^2$$

$$\Sigma XY = a\Sigma X + b\Sigma X^2$$

Steps:

1. Square the value of X series and obtain ΣX^2
2. Square the value of Y series and obtain ΣY^2
3. Multiply X and Y and get ΣXY
4. Calculate the value of 'a' and 'b' by solving following equation.

Result:

The estimated the weight of the fish.....expected to be present in a fish length is 22.0 cm.



E. COMPUTER APPLICATIONS

ANALYSIS OF DATA USING EXCEL SOFTWARE PACKAGES, UNIVARIATE AND MULTIVARIATE ANALYSIS OF DATA.

Ex No : 1

Date:.....

Aim

To generate a graph using the data.

Materials Required

Data and computer.

Principle

Excel is an electronic calculator. It is a product of Microsoft Corporation. An Excel document is called a workbook. Each workbook contains multiple pages, which are called worksheets or spread sheets. The active worksheet is displayed in the document window. A new Excel workbook contains 3 worksheets, we can add as many we want. Worksheet is made up of rows and columns. The rows are identified by numbers, labeled down the left side of the worksheet in the heading area. The columns are identified by letters, labeled across the top of the sheet in the heading area. Each worksheet has a total of 256 columns and 65,536 rows. At the junction of each row and column is a cell. It is in the cells that you enter data, including text, numbers and formulas. Cell is referred by cell address.

The statistical data can be presented by making use of diagrams and graphs. They present the data in convincing, appealing and easily understandable manner. It give a bird's eye view of the whole mass of statistical data.

Procedure

Excel can be opened by clicking Start button -> Program -> MS Excel. It will display a blank worksheet.

a) Using Univariate Data

The table 10 gives the number of eggs laid by lizard in a season. It is entered in Excel as shown (Fig. 172) below:

Table 10. The number of eggs laid by lizard in a season.

Season	1	2	3	4
No of Eggs	33	35	45	34

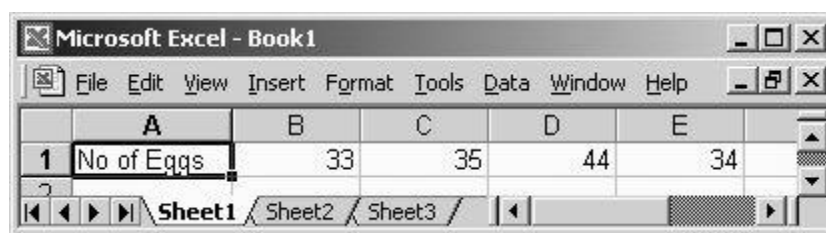


Fig. 172. Excel.

The entire data is selected and the following commands are carried out.

Insert → Chart.

Select chart type as Column

Press Next Button

If needed change the Series in option

Click Finish button (Fig. 173) to draw the bar diagram (Fig. 174) or pie diagram (Fig.175).

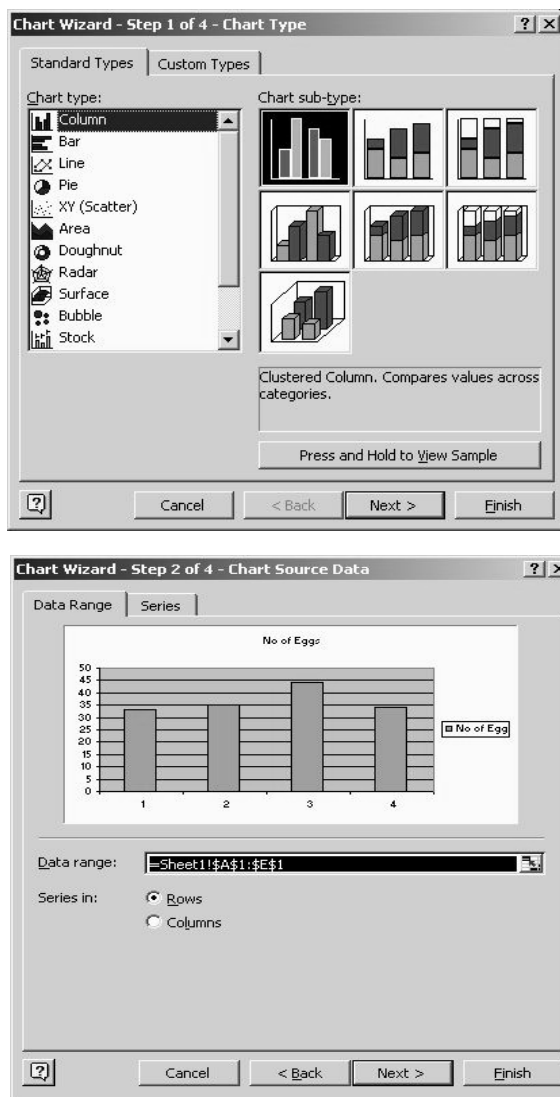


Fig. 173. Selection of bar diagram in excel.

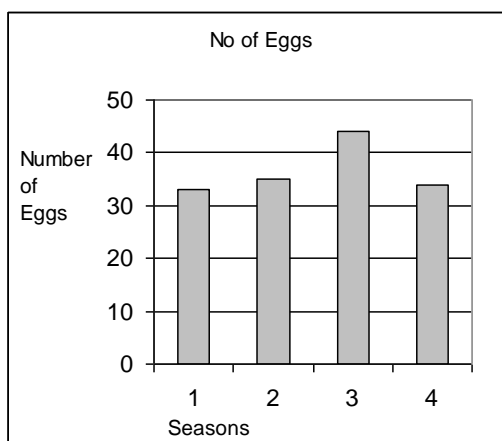


Fig. 174. The bar diagram indicates the number of eggs laid by lizard in a season.

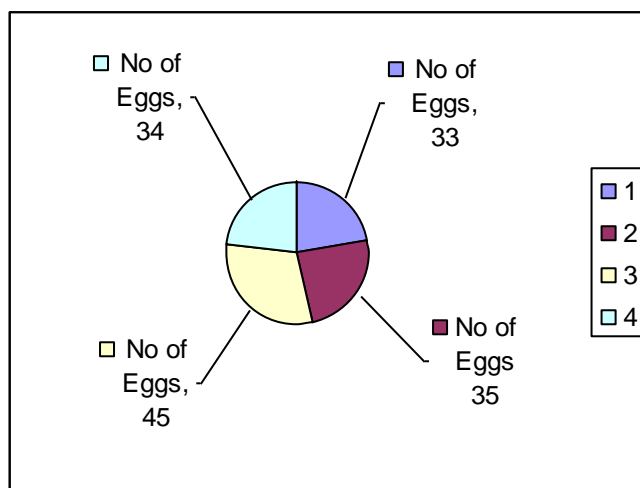


Fig.175. The pie diagram indicates the number of eggs laid by lizard in a season

Using Multivariate Data

The table 11 gives the aqua cultural production over the past 5 years.

Table 11. The aqua cultural production over the past 5 years.

Year	Fish	Prawn
1998	120	130
1999	130	115
2000	110	140
2002	150	160
2003	110	130

The entire data is selected and the following commands are carried out.

Insert → Chart.

Select chart type as Column

Press Next Button

If needed change the Series in option

Click Finish button (Fig.176) to draw bar diagram (Fig.177) or line diagram (Fig.178).

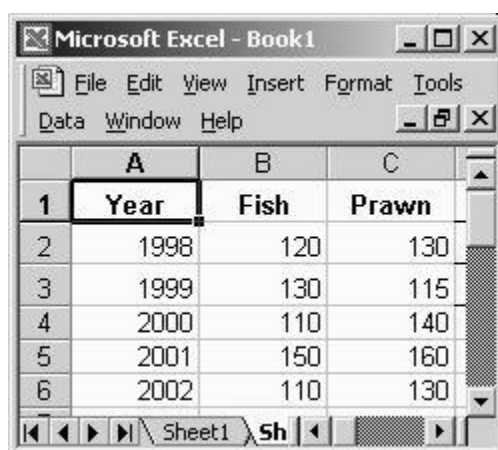


Fig. 176. Selection of double bar diagram in excel.

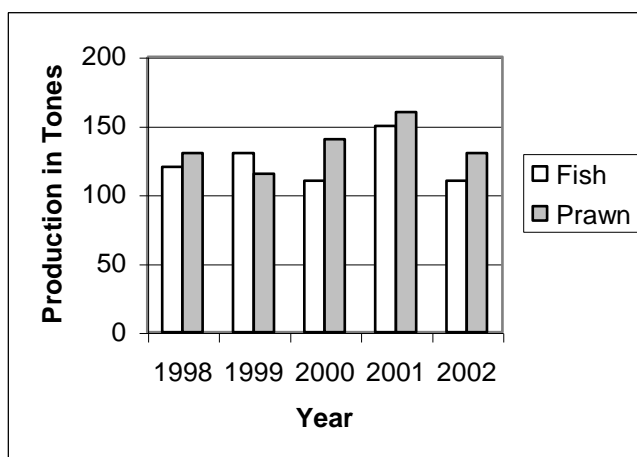


Fig.177. The bar diagram indicates the fish and prawn production over the past 5 years.

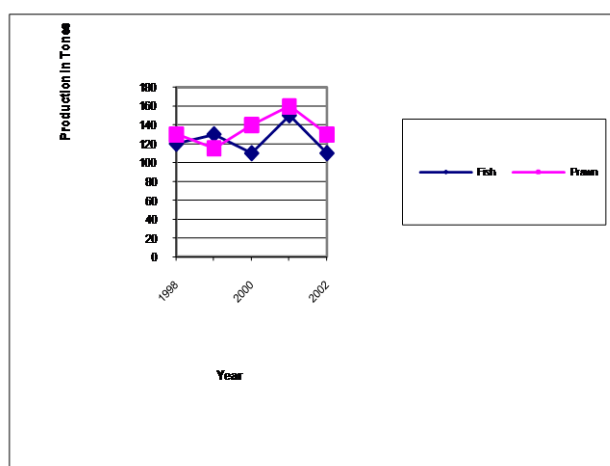


Fig.178. The line diagram indicates the fish and prawn production over the past 5 years.

Discussion on Computer application

Excel includes a variety of tools to use for data analysis and forecasting. The Goal seek tool can look at a formula and then determine the appropriate value of a single variable, given the formula result. Goal seek is good for single-variable formulas. The solver tool is designed for finding multivariable solutions.

We can create a table of data and we can convert the data table into a chart.

The solver tool uses algorithms to solve problems with multiple variables.

Single variable data analysis:

Report of marks obtained in all semesters in all subjects by a single student maintained in a single file (univariable analysis of data).

Multivariable data analysis:

Report of marks obtained in all semesters in all subjects by the entire class (say 40 students) maintained in different files (multivariate analysis of data).

SPOTTERS

Ex No : 2

Date:.....

MOUSE

A device that controls the movement of the cursor or pointer on a display screen. A mouse (Fig. 179) is a small object you can roll along a hard, flat surface. Its name is derived from its shape, which looks a bit like a mouse, its connecting wire that one can imagine to be the mouse's tail, and the fact that one must make it scurry along a surface. As you move the mouse, the pointer on the display screen moves in the same direction. Mice contain at least one button and sometimes as many as three, which have different functions depending on what program is running. Some newer mice also include a *scroll wheel* for scrolling through long documents.

Invented by Douglas Engelbart of Stanford Research Center in 1963, and pioneered by Xerox in the 1970s, the mouse is one of the great breakthroughs in computer ergonomics because it frees the user to a large extent from using the keyboard. In particular, the mouse is important for graphical user interfaces because you can simply point to options and objects and click a mouse button. Such applications are often called *point-and-click* programs. The mouse is also useful for graphics programs that allow you to draw pictures by using the mouse like a pen, pencil, or paintbrush.



Fig. 179. Mouse.

KEY BOARD

The set of typewriter-like keys that enables you to enter data into a computer. Computer keyboards (Fig. 180) are similar to electric-typewriter keyboards but contain additional keys.

The standard layout of letters, numbers, and punctuation is known as a *qwerty* keyboard because the first six keys on the top row of letters spell *qwerty*. The qwerty keyboard was designed in the 1800s for mechanical typewriters and was actually designed to slow typists down to avoid jamming the keys. Another keyboard design, which has letters positioned for speed typing, is the *Dvorak keyboard*.

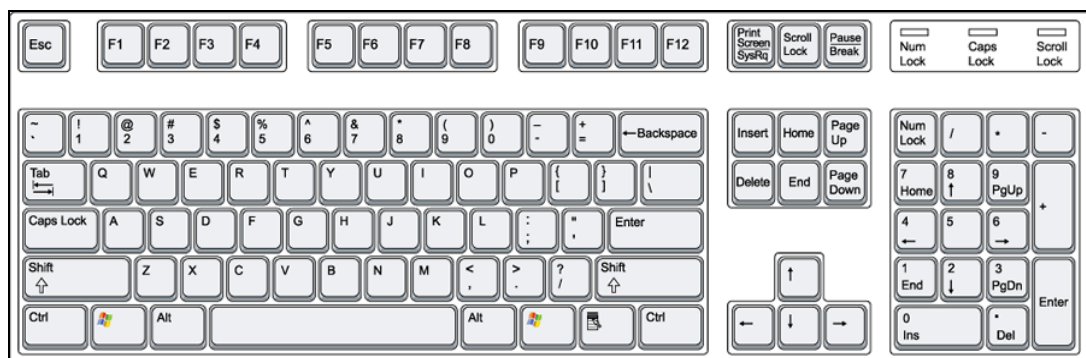


Fig. 180. Keyboard.

There is no standard computer keyboard, although many manufacturers imitate the keyboards of PCs. There are actually three different PC keyboards: the original PC keyboard, with 84 keys; the AT keyboard, also with 84 keys; and the *enhanced*

keyboard, with 101 keys. The three differ somewhat in the placement of function keys, the Control key, the Return key, and the Shift keys.

In addition to these keys, IBM keyboards contain the following keys: Page Up, Page Down, Home, End, Insert, Pause, Num Lock, Scroll Lock, Break, Caps Lock, Print Screen. There are several different types of keyboards for the Apple Macintosh. All of them are called *ADB keyboards* because they connect to the Apple Desktop bus (*ADB*). The two main varieties of Macintosh keyboards are the *standard keyboard* and the *extended keyboard*, which has 15 additional special-function keys.

CENTRAL PROCESSING UNIT (CPU)

Abbreviation for *central processing unit* (Fig. 181), and pronounced as separate letters. The CPU is the brains of the computer. Sometimes referred to simply as the *central processor*, but more commonly called *processor*, the CPU is where most calculations take place. In terms of computing power, the CPU is the most important element of a computer system. It is a small chip which processes all of the information going through the computer.

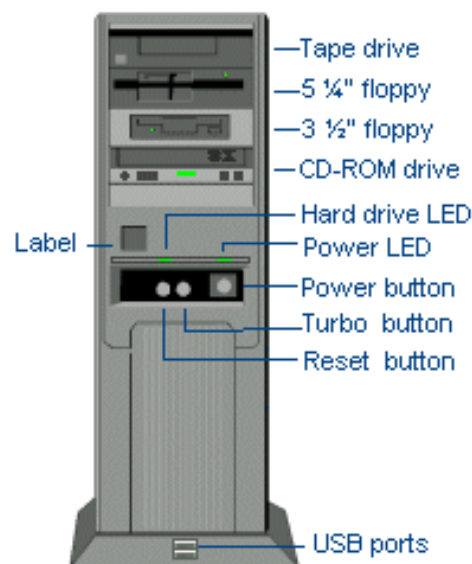


Fig. 181. CPU.

There are two variations of processor, boxed and OEM. Boxed versions come with a heatsink and manufacturers warranty. The motherboard is another crucial part of the system. It is often referred to as the heart of the system. It is what sends the messages around the system telling components what to do and when to do it.

Another aspect of computer is RAM. RAM is one of the most important components in a computer. It determines how fast your system will run when operational. This is where the computer temporarily stores data for quick access. If you run out of RAM, the computer uses hard drive space (virtual memory). The latest DDR2 RAM comes in matched pairs (called kits).

The power supply is another important factor when building a computer. It supplies all the power to your system so this is one component you don't want to skimp on. There are plenty of cheap power supplies out there but usually they aren't very good. The wattage they show (e.g. 430W) is an indication of max wattage rather than true wattage. The Power Supply comes in two main form factors, ATX and BTX, which are meant for their respective Cases which you will see later on.

MODEL QUESTIONS - 1

M.Sc. DEGREE EXAMINATION :ZOOLOGY – CC IX PRACTICAL - II
BIOCHEMISTRY, BIOPHYSICS, BIOTECHNOLOGY, BIOSTATISTICS
AND COMPUTER APPLICATIONS

Time: 3 hours

Maximum: 60 marks

- | | |
|---|----------|
| 1. Determine the phosphate buffer of 6.5 pH. Write the procedure adopted. | 10 Marks |
| 2. Estimate the optical density of samples using standards | 10 Marks |
| 3. Write the procedure for isolation of plasmid. | 10 Marks |
| 4. Draw a Pie-chart for the following population of an ecosystem. Use the computer.
Herbivore = 42
Omnivore = 35
Carnivore = 20
Top-Carnivore = 5 | 8 Marks |
| 5. Identify and write as per directions given
(A) its application in Biochemistry
(B) its application in Biophysics
(C) its application in Biotechnology
(D) its application in Computer | 12 Marks |
| 6. Practical Record | 10 Marks |
| Total | 60 Marks |

Key

- (A) ninhydrin
(B) Photocolorimeter OR High speed centrifuges
(C) Plasmid
(D) Mouse

MODEL QUESTIONS - 2M.Sc. DEGREE EXAMINATION :ZOOLOGY – CC IX **PRACTICAL - II****BIOCHEMISTRY, BIOPHYSICS, BIOTECHNOLOGY, BIOSTATISTICS****AND COMPUTER APPLICATIONS**

Time: 3 hours

Maximum: 60 Marks

1. Estimate the amount of proteins present in the given tissue. Write down the procedure adopted and record your results.
10 Marks
2. Separate the free sugars using paper chromatographic
10 Marks
3. Isolate the DNA from the given tissue (A) by using appropriate reagents.
10 Marks
4. Apply your computer knowledge and draw a bar diagram on the fishery statistics.

Month	January	Feb.	March	April	May	June
Tons	240	29	320	420	300	350

8 Marks
5. Identify and write as per directions given
 (A) its application in Biochemistry
 (B) its application in Biophysics
 (C) its application in Biotechnology
 (D) its application in Computer
 12 Marks
6. Practical Record
10 Marks

 Total 60 Marks

Key

- (A) pH meter
 (B) Electrophoresis unit
 (C) Blotting technique
 (D) Key Board



DEVELOPMENTAL BIOLOGY

IMMUNOLOGY

ANIMAL PHYSIOLOGY

MICROTECHNIQUE

PART – III: A. DEVELOPMENTAL BIOLOGY

PREPARATION OF SPERM SUSPENSION IN BULL AND OBSERVATION OF SPERMATOZOA

Ex. No. 1

Date:

Aim:

To observe the structure of spermatozoa.

Materials required

Bull semen, watch glass, glass slide, cover slip and compound microscope.

Reagent required

Holt freter's solution: 35 mg NaCl, 5 mg KCl, 10 mg CaCl_2 and 2 mg NaHCO_3 were dissolved in 1009 ml of distilled water.

Procedure

2 ml of Holt freter's solution was taken in a watch glass. A drop of bull semen was pipetted out into the watch glass. From the suspension, a drop was taken and placed on a slide. To the slide methylene blue stain was added. After 5 minutes, the slide was washed to remove excess stain. A cover slip was placed on the slide. The slide was then examined under the microscope using low power and high power objectives.

Observation

Many active spermatozoa of bull were observed in high power objectives. The structure of spermatozoa (Fig. 182) was observed. Head, middle piece and tail of sperm were identified. Soon after the preparation of slide, the sperms are very active, but they become less active or immotile when the time passes on.

Discussion

Sperms were the male gametes. They were motile and they could themselves reach the egg. It has half the number of paternal chromosomes (n). These haploid set of chromosomes were due to meiotic or reduction division during oogenesis. The sperm activates the egg at the time of fertilization by donating paternal chromosomes. The head has acrosome on the top. The nucleus of egg has haploid set of chromosomes. The middle piece of sperm is formed by the crowding of mitochondria. Some time the mitochondria spiral or coiled around the axial filament. Mitochondria supply energy for the movement of sperm. The posterior axial filament extended as tail but without flagellar sheath. The tail helps for the movement of sperm and to reach the site of amphimixis. The sperm pronuclei fuse with the egg pronuclei. This process is called as amphimixis. Then the egg undergoes the development.

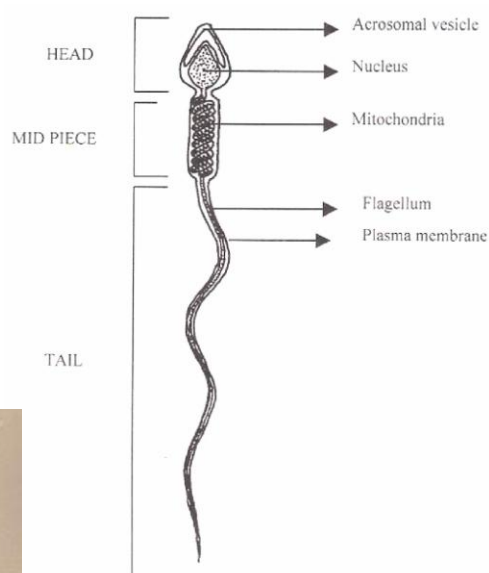


Fig. 182. Structure of spermatozoa.

OBSERVATION OF LIVE SPERMATOZOA AND STUDY OF RATE OF MOTILITY OF SPERM IN BULL SEMEN

Ex. No. 2

Date:

Aim

To observe the motility of live spermatozoa in bull semen.

Materials required

Bull semen, methylene blue, vasaline, watch glass, glass slide, cover slip and compound microscope.

Reagent preparation

Holt freter's solution: 35 mg NaCl, 5 mg KCl, 10 mg CaCl_2 and 2 mg NaHCO_3 were dissolved in 1009 ml of distilled water.

Procedure

A drop of bull semen was placed on a clean glass slide. A cover slip was placed and a drop of methylene blue stain was added through the edge of the cover slip. Vasaline was added on the edge of cover slip to avoid evaporation then the slide was focused under a compound microscope. The movements of the sperm were observed. The rate of motility was calculated by using ocular and stage micrometer.

Study rate of motility: The rate of motility of sperm was measured by applying micrometry. The ocular micrometer is a circular glass. It consists of a straight line at the centre. It is equally divided into 100 divisions. The measurement of each unknown division was calibrated by the help of stage micrometer. The stage micrometer is a rectangular glass slide at the centre of stage micrometer, there is a line which is 1 mm length. The line is divided into 100 equal divisions, so, each division is 100 microns in length.

The ocular micrometer was placed into the eye piece. The stage micrometer was placed on the stage of compound microscope (high power 45X). The number of coincidence was observed and number of divisions between successive coincidences were noted and tabulated (Table 12). The value of one division of ocular meter was obtained as

$$1 \text{ ocular division} = \frac{\text{No. of division of stage micrometer}}{\text{No. of division of ocular micrometer}} \times 10.$$

The stage micrometer was removed from the stage then the glass slide with sperm suspension was placed on the stage microscope. The number of ocular division crossed by a sperm during motility was noted and recorded (Table 13). The rate of motility of sperm was calculated by using the formula.

$$\text{Rate of motility} = \frac{\text{Distance traveled by the sperm}}{\text{Time taken}}$$

Observation

Fast and mass activity and motility of sperm were observed. Their mass activity was assessed by a small drop of freshly collected and diluted semen under the high power objective lens (45X) of the microscope. The mass activity was observed at the centre of the drop. The sluggish movement was exhibited at the edge of the slide due to drying. The mass activity was correlated with sperm concentration and motility. At first, the motility of spermatozoa was quick and vigorous then it decreases gradually.

Result

Rate of motility of sperm = _____ μ /sec.

Discussion

Motility of the sperm is essential for the fertilization of the egg. Slow motility or low motility leads to failure of the fertilization. At least 80% motility is essential for successful fertilization. The slow motility or low motility may be due to deficiency of nutrients in the semen fluid or weak tail filaments. Strong tail filament is needed to reach the egg in the reproductive tract (fallopian tube) for fertilization.

Table 12. Micrometric calibration in High power (45 x).

S. No.	Stage micrometer Reading	Ocular micrometer reading	Value of one ocular division (SM/OM x 10)	Average value of one ocular division
1				
2				
3				
4				
5				

Average value of one ocular division at high power = _____ μ .

Table 13. Rate of motility of sperm.

S. No.	No. of ocular Division Traveled by sperm	Value of one ocular division	Distance Travelled by sperm (μ)	Time Taken (sec)	Rate of motility* μ /sec.	Average of motility μ /sec.
1						
2						
3						
4						
5						

Distance traveled by the sperm

*Rate of motility = -----

Time taken

Rate of motility sperm = _____ μ /sec.

EFFECT OF THYROXIN ON METAMORPHOSIS OF FROG

Ex. No. 3

Date:

Aim

To study the effect of thyroxine on the metamorphosis of frog.

Materials required

Thyroid tablet (10 mg of Thyroxine), tadpoles of equal size, distilled water, 0.1 N sodium chloride solution, 0.1N HCl and petridish.

Preparation of Reagents

5 thyroid (thyroxine) tablets were dissolved in distilled water containing 6 gm NaCl per litre.

Procedure

The healthy frog tadpoles of equal size were selected and their tail lengths were measured and recorded before releasing the tadpoles into the medium. Then experimental tad pole was placed in a petridish containing thyroid tablet solution with the little amount of water. A graph paper was placed beneath the petridish. Simultaneously control tad pole was maintained placed in a petridish without thyroid tablets. They were daily fed with boiled spinach leaves. The length of the tail was measured and recorded up to 1 week.

Results

The tail of the control and thyroxine treated tadpole were gradually increased from 15 mm to 20 mm and 15 mm to 25 mm respectively (Fig. 183). The growth of the tail was more in experimental tadpole than control. There was a growth of hind limbs. The structure of the head and mouth become modified.

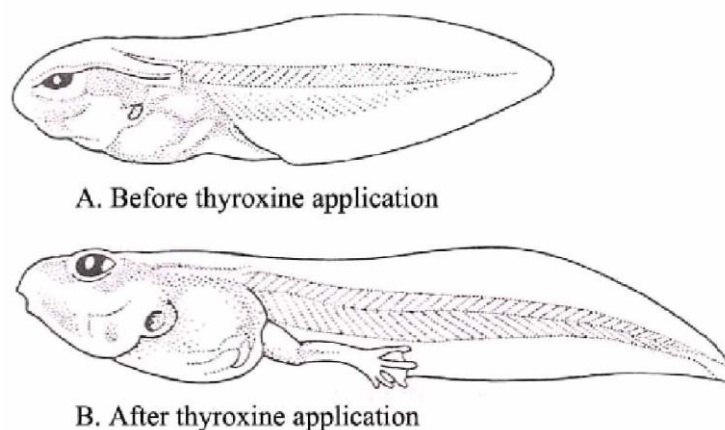


Fig. 183. Effect of thyroxine on metamorphosis of tadpole.

Discussion

From this experiment, it is concluded thyroid tablets are influencing the metamorphosis of frog. The thyroid tablets contain a chemical called thyroxine which is comparable to the thyroxine secreted by thyroid gland of tadpole. The thyroid gland secretes thyroxine which is activated by thyroid stimulating hormone secreted by pituitary gland. The pituitary gland was induced by neurosecretion of hypothalamus of brain. Thyroxine is a type of protein containing globule and iodine. The thyroxine is otherwise called tri iodo thymine if 3 iodine atoms or molecules are present or tetra iodo thyroxine if 4 iodine molecules are present. Many experiments were conducted to prove that the thyroxine is very much influencing the metamorphosis of frog. Even 0.0003833% iodine is enough to influence the metamorphosis of frog.

VAGINAL SMEAR PREPARATION IN MOUSE TO STUDY THE STAGES OF OESTROUS CYCLE

Ex. No. 4

Date:

Aim

It is aimed to study the different stages of oestrous cycle of rat.

Materials required

Rat, slide, methylene blue, glycerine, cover slip, scalpel and microscope.

Procedure

Matured rat was selected using the tip of the scalpel or ear bud, the epithelial cells of vagina of rat was scraped and smeared, on the glass slide. A drop of vital dye, methylene blue was added to the smear. The dye was allowed added to the smear. The dye was allowed to dry. After 5 minutes, the glycerine was added. Then the cover slip was placed over the smear. The mounted slide was focused under the microscope to observe the various stages of oestrous cycle of rat.

Result

The stage of oestrous cycle observed in the given rat was _____ stage.

Discussion

Mammals other than primates do not menstruate and their sexual cycle is oestrous cycle. The oestrous stage was a conspicuous period of oestrous cycle (Fig. 184). When ovulation occurred, the

animal was said to be in heat period. It was a period during which several interest of female was assured. It was accompanied by many physiological and cytological changes. The period from the beginning of one heat to next heat is called as oestrous cycle. The oestrous cycle is under the influence of hormones like estrogen and progesterone.

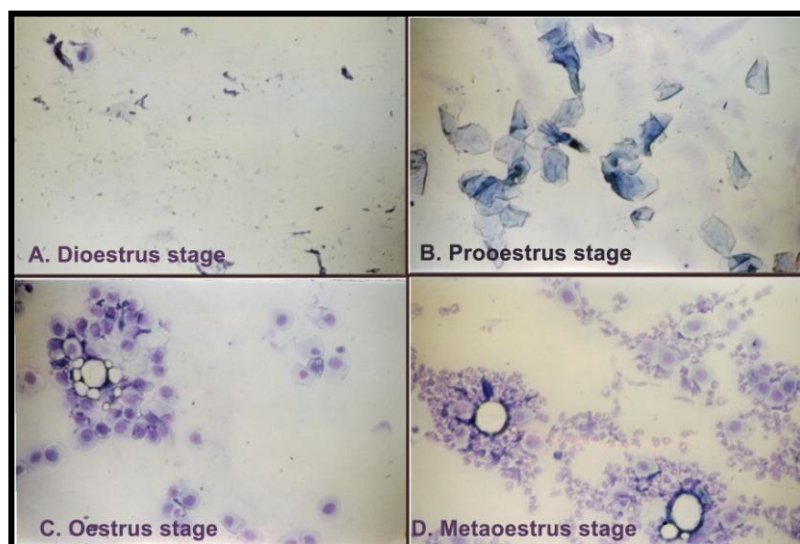
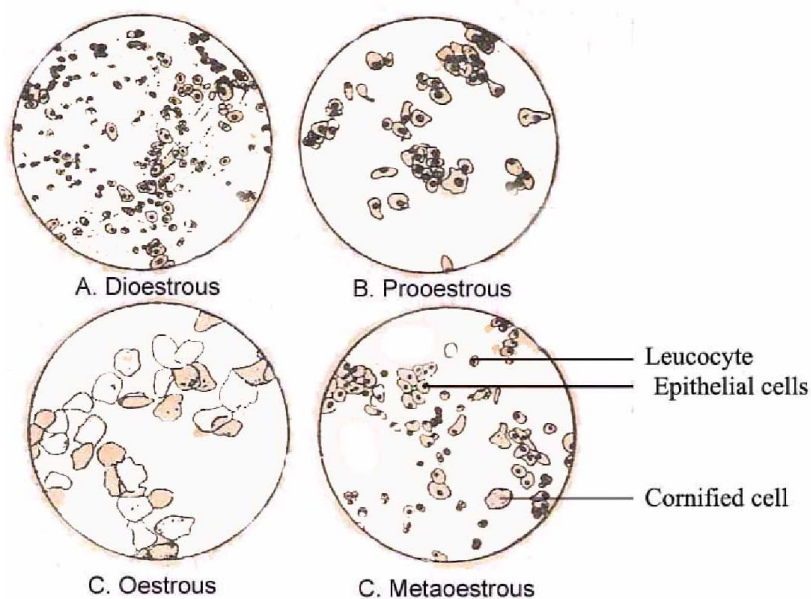


Fig. 184. Different stages of rate oestrous cycle.

The duration of oestrous or heat cycle varies from animal to animal. There are 4 stages in an oestrous cycle. They are Dioestrous stage, Proestrous stage, Oestrous stage and Metaoestrous stage.

The following changes occurred during these stages.

1. **Changes during dioestrous stage:** This phase follows metaoestrous. It is associated with the regression of corpus luteum. Therefore, the estrogen and progesterone hormone levels are decreased. This phase is analogous to menstruation i.e. bleeding as in primates. The duration of this phase varies from animal to animal. It is associated with regression of female genital tract viz. decrease in size of uterus. The following changes may be observed in female genital system during this phase.
 - i. The ovaries and uterus become smaller in size.
 - ii. The follicles may not be present in the ovaries
 - iii. The blood circulation decreases in the uterus
 - iv. The vaginal muscle is thin and leucocytes migrate through it.
2. **Changes during Proestrous stage:** This phase follows dioestrous and proceeds oestrous stage. This is associated with commencement of enhanced ovarian activity under the influence of (FSH) follicle stimulating hormone from adenohypophysis.

This phase is analogous to proliferation phase of menstrual cycle as in primates. This phase is associated with the following changes:

 - i. The ovaries become active and show mature follicle cells
 - ii. The uterus collects the fluids and it becomes contractile
 - iii. The vaginal epithelium grows thicker
 - iv. The vaginal smear is determined by nucleated epithelial cells, which may occur singly or in sheath.
3. **Changes during oestrous stage:** In this stage, graffian follicle ruptures and ovulation commences. The uterine mucosa membrane becomes thickened and gets cornified. The female allows the male for copulation.
4. **Changes during metaoestrous stage:** This stage follows oestrous and occurs shortly after ovulation. This is accompanied by corpus luteum formation. Therefore, this stage is analogous to secretory or luteal phase of menstrual cycle. This phase is under the joint influence of estrogen and progesterone hormones. The duration of metaoestrous varies from animal to animal. It is associated with the following changes.

Formation of corpus luteum may be observed in the ovary

- i. The uterus diminishes in vascularity and contractivity.
- ii. The vaginal and uterine epitheliums grow in thickness and are infiltrated by leucocytes.

Thus the different stages and different structural changes were observed in the stages of oestrous cycle of rat.

B. IMMUNOLOGY

IDENTIFICATION OF LYMPHOID ORGANS IN RAT

Ex. No. 1

Date:

Aim

To study the lymphoid organs found in the rat.

Materials required

Rat, chloroform, cotton, dissecting board with accessories and dissection box.

Procedure

A healthy rat was taken and nailed on a dissection board. On the ventral side, the skin is cut and lymphoid organs are identified.

Observation

Primary lymphoid organ and secondary lymphoid organs (Fig. 185) were observed. In rat, thymus and bone marrow are the primary lymphoid organs. The secondary lymphoid organs are spleen and lymph nodes.

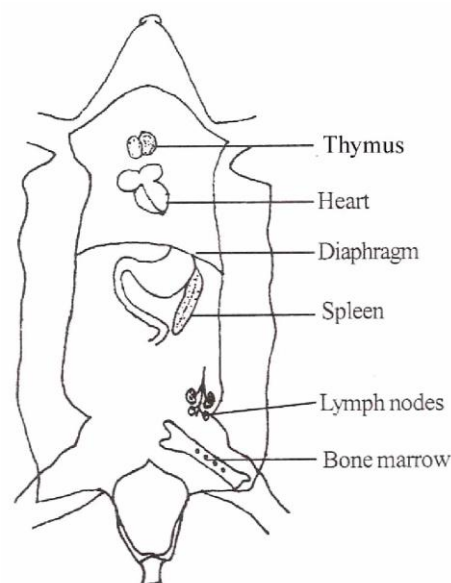


Fig. 185. Lymphoid organs of Rat.

Discussion

Thymus

Thymus (Fig. 186) is a primary lymphoid organ and situated in the chest just above the heart. It resembles a thyme leaf and hence it is termed thymus. It is oval in shape and bilobed structure. It is covered by a capsule. It consists of many small compartments called lobules. The lobules are separated by partitions called trabeculae. Each lobule is made up of two zones, namely an outer cortex and an inner medulla. Thymectomy (removal of thymus) affects cell mediated immunity.

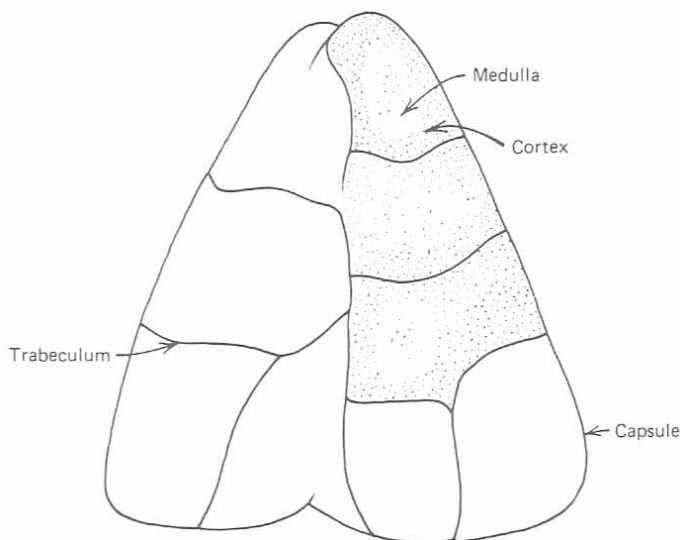


Fig. 186. Thymus.

Bone marrow

Bone marrow (Fig. 187) is a primary lymphoid organ. It is a soft tissue located within the cavities of the bones. It is a major haemopoietic organ producing blood cells. It is also a major site of antibody synthesis. During secondary immune response memory B cells migrate to the bone marrow where they mature into antibody producing plasma cells.



Fig. 187. Bone marrow.

Spleen

Spleen (Fig. 188) is a secondary lymphoid organ. It is located in the abdominal cavity behind the stomach, close to the diaphragm. It is deep red in colour. It has direct

connection with arterial system and filters the blood. It is covered by a capsule. The capsule penetrates into the spleen and forms the septa or trabeculae. It has two regions namely an outer red pulp and an inner white pulp. The red pulp contains large amount of blood containing phagocytes and plasma cells. The white pulp is made up of lymphoid tissue containing T and B lymphocytes. The Spleen does three main functions. It is a graveyard of RBCs where old RBCs are destroyed. During emergency conditions it synthesizes RBCs along with bone marrow. It filters the foreign antigens from the blood and it brings about humoral and cellular immunity.

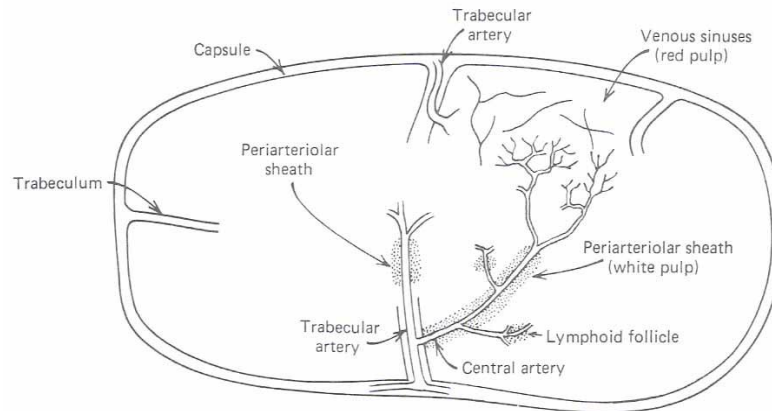


Fig. 188. Spleen.

Lymph nodes

Lymph nodes (Fig. 189) are the secondary lymphoid organs. They are bean shaped bodies present along the lymphatic ducts. They are distributed throughout the body. Each lymph node has a concave surface and a convex surface. The concave surface is called hilus. The lymph nodes receive lymph through afferent lymphatic vessels on the convex surface. The lymph is taken away through the hilus by the efferent lymphatic vessels. An artery and a vein also enter the lymph node through the hilus. Each lymph node is covered by capsule. Here and there the capsule is inserted into the node to form partitions called trabeculae.

The cortex has three zones, namely an outer cortex, a middle para cortex and an inner medulla. The cortex contains B-cells, the paracortex contains T-cells and the medulla contains T and B cells. The lymph nodes are rich in phagocytosis. The lymph nodes are responsible for cell mediated and humoral immunity.

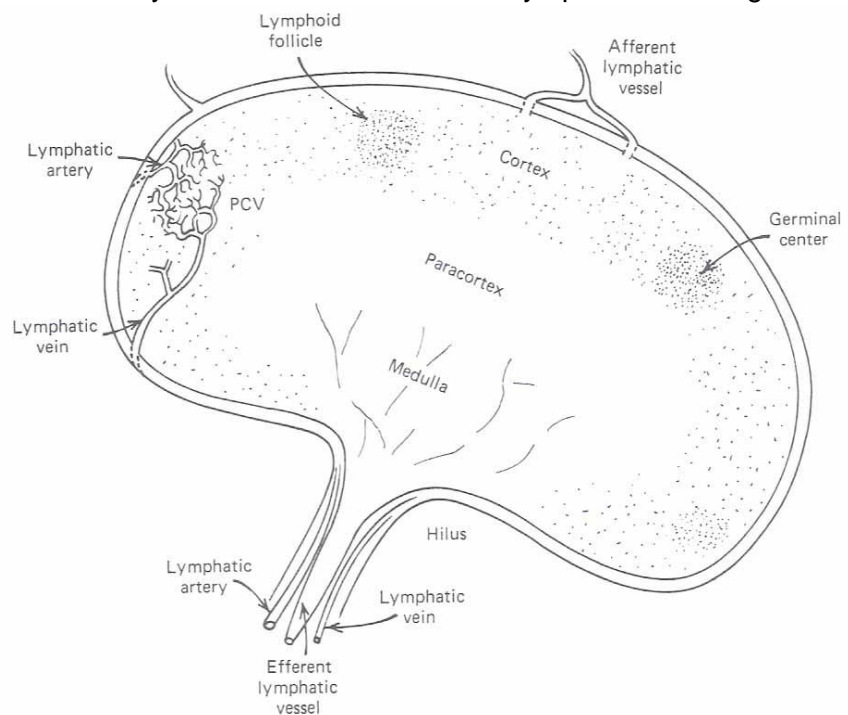


Fig. 189. Lymph nodes.

PREPARATION OF ANTIGEN AND RAISING OF ANTIBODY FROM RBC OF SHEEP BLOOD

Ex. No. 2

Date:

Aim

To prepare the antigen and rise the antibody from RBC of sheep blood.

A. PREPARATION OF ANTIGEN FROM RBC OF SHEEP BLOOD

Materials required

Alsever's solution (anticoagulant), physiological saline, syringe, needle and sheep's blood.

Preparation of reagents

1. Alsever's solution:

Dextrose	-	20.5 g
Tri sodium chloride	-	8.0 g
Sodium chloride	-	4.2 g
Citric acid	-	0.55 g
Distilled water	-	1000 ml

All these salts were dissolved in one litre of distilled water and autoclaved at 121 6S pressure for 10 minutes and stored in a refrigerator.

2. Physiological saline

Sodium chloride	-	0.9 g
Distilled water	-	100 ml

The sodium chloride solution was autoclaved at 151 6S pressure for 10 minutes and stored in a refrigerator.

Procedure

One ml of Sheep's blood was obtained from slaughtered sheep and released into 9 ml of Alsever's solution. Then it was centrifuged for 10 minutes in 3000 rpm. The supernatant (antigen) was collected in a test tube. The required concentration of antigen was prepared using physiological saline given below:

1 ml of sheep's RBC when made upto 100 ml saline gives 1%.

1 ml of packed sheep's RBC made upto 10 ml saline gives 10%.

The concentration of antigens (RBC) in 0.1 ml of 10% sheep's blood serum was checked by counting RBC in Haemocytometer.

Result

Antigens were produced from sheep's RBC. The number of RBC (antigen) in 0.1 ml of 10 % sheep's RBC was about 6×10 cells.

B. RISING ANTIBODY AGAINST RBC'S ANTIGEN IN RABBIT

Materials required

Physiological saline (0.9% Na Cl) test tube, 1 ml syringe, pipette with mouth piece, antibiotic drops (Gentamycin), rabbit, sheep RBC and BSA.

Procedure

Maintenance of animal: Rabbit was maintained in laboratory in cages. It was fed with cabbage and water.

Injection of antigen: The rabbit was immunized with required concentration (10 mg/1 ml) of antigen given by a single injection through its intra peritoneal route. A needle was inserted into the peritoneal cavity about 1 cm above the hind limb, on the lateral side of the body. The entry of needle into the cavity was continued by the free movement of the end of the needle inside 0.1 ml of 25% sheep's RBC in each animal. 1 mg of BSA (Bovine Serum Albumin) in 0.2 ml was injected into another animal. For enhanced responding of BSA in 0.2 ml of saline mixed with 0.2 ml of adjuvant was used. The blood was taken 10 days after injection.

Bleeding: The rabbit was held on its side of the head raised. The tip of the micropipette attached to their suction tube gently at the posterior region of orbit without rupturing the cornea of the side ball. The pipette was rotated clockwise and anticlockwise directions for 5 minutes. The posterior optic vessel was ruptured. The blood rose up to a certain point in their capillary action and the blood pressure of rabbit.

Negative pressure was applied by section to facilitate further flow of blood into the larger segment of the micropipette after necessary quantity of blood was taken. The pipette was removed from the orbit and their blood was transferred to a serological tube 2 or 3 drops of antibiotic were added to the injury of the animal to prevent infection.

The collected blood was kept at room temperature for 24 hour at 4° C. The serum was separated by spinning at 3000 rpm for 20 minutes. Then the serum was kept at 57° C in water bath for 30 minutes to inactivate complement and slowed at 20° C until use.

Observation

The separated serum from rabbit (Fig. 190) blood was kept as antiserum in refrigerator at 20° C until use.

Discussion

A serum antibody product provides immediate treatment or prevention of a specific threat, like bacteria or a toxin capable of causing disease in the animal. This type of immunity is known as passive immunity. Serum antibody products can be used as an immediate, but temporary prevention of a disease or as a treatment of a current disease.



Fig. 190. Rabbit.

DETERMINATION OF HUMAN BLOOD GROUP BY HAEMAGGLUTINATION TEST AND ASSESSMENT OF SPECIFICITY OF ANTIGEN-ANTIBODY REACTION

Ex. No. 4

Date:

Aim

To determination of human blood group by haemagglutination test and assessment of specificity of antigen-antibody reaction.

Materials required

Sterilized needles, glass slide, antiserum-A and antiserum-B.

Principle

In the human blood, there are two types of substances. They are antigen and antibody. The antigens are present in RBC. The antibodies are present in serum. Landsteiner (1900) classify 4 different blood groups in human beings according to the type of antigen present or absent. They are A, B, AB and O. The blood group A has antigen-A on the surface of RBC. The blood group B has antigen-B on the surface of RBC. The blood group AB has both antigens-A and B. The blood group O has no antigen.

Agglutination (clumping) takes place between antigen and antibody. For example in the case of blood group A, antibody A specifically reacts with antigen-A as result antigens A are clumped together. Based on this principle we can identify the blood groups. Similarly in the case of blood group b, antibody B specifically agglutinates with antigen B; in blood group AB, antibody AB specifically agglutinates with antigen AB and in blood group O antibody AB does not agglutinate since O blood has no antigens A and B.

Procedure

A fine sterilized needle was used to prick the tip of my finger. After pricking, the finger was pressed to drop the blood. A drop of blood was placed on either end of the glass slide. Then a drop of antiserum A was placed on one end of the glass slide and a drop of antiserum B was added to the other end of the slide. They were stirred to observe the agglutination.

Result

Observation shows that Antigen-A agglutinate/ does not agglutinate with antiserum A and agglutinate with antiserum-B/ does not agglutinate with antiserum-B (Fig. 191). So this reaction indicates that my blood is belongs to ____ blood group.

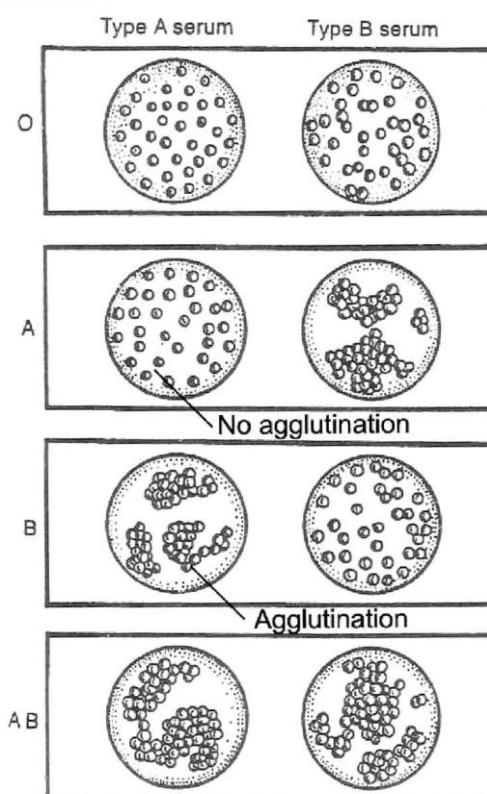


Fig. 191. Agglutination test for blood grouping.

Discussion

Medical significance: The grouping of blood has great importance in blood transfusion. Suppose, unsuitable blood is transfused to a patient, he/she will die due to agglutination. So, the blood of donor and recipient is tested before transfusion. Thus AB group person can receive blood from the entire 4 group, because it does not have any antibodies to cause clumping. Therefore, AB person is called universal acceptor.

O group person cannot receive blood from any group except O group, because O group person has both A and B antibodies to cause clumping. At the same time both antigens are absent in O group person which can give blood to all the groups. Hence O group person is called as universal donor.

A group person can receive blood from A and O group and not from B and AB groups. B group person can receive blood from B and O group and not from A and AB groups. Blood group O, A, B and AB identify can be done in disputed cases of parent and children relationship.

Genetic significance: The blood group is inherited and remains constant throughout life. The human blood group is the example of multiple allelism. The blood groups are controlled by three multiple alleles. They are I^A , I^B and I^O . Among them I^A and I^B are dominant alleles where as I^O is recessive allele. The blood group individual is having the genotype I^A or $I^A I^O$. The B group individual is having the genotype I^B or $I^B I^O$. The AB persons have the genotype $I^A I^B$. O persons have the genotype $I^O I^O$. The dominance hierarchy of this allelic series can be symbolized as $L^A < L^B < L^O$.

Further studies have shown that the antigen A is heterogeneous and may have four uncommon subgroups as A^1 , A^2 , A^3 and A^4 . The B antigen also has three variants. The L^A allele may occur in at least three allelic forms. Pedigree analysis has shown that L^{A1} allele is dominant over $L^{A2} = L^{A3}$. Now the dominance hierarchy $L^A = L^B < L^O$ can be better represented as $[(L^{A1} > L^{A2} > L^{A3} > L^B] > L^O$.

The series of multiple alleles of gens L may produce is genotype and 8 phenotypes. Besides A and B antigen another antigen called Rh factor was reported from erythrocytes of certain persons.



DETECTION OF PRESENCE OF PRECIPITATING ANTIBODY (IgG) USING SOLUBLE ANTIGEN BY PRECIPITATION RING TEST (RADIAL IMMUNO TEST)

Ex. No. 5

Date:

Aim:

To determine the concentrations of antigen by radial immuno diffusion method.

Principle

In radial immuno diffusion method the antiserum is incorporated in the agar and the antigen is placed in wells. The antigen diffuses radially from the well into the agar containing antiserum. The antigen reacts with the antiserum, a ring shaped precipitin band is formed around the well. The concentration of the antigen can be calculated by measuring the diameter of the precipitin ring.

The radial immuno diffusion the antigen alone in the agar plate hence it is called single radial immuno diffusion.

Materials required

Borate buffer, agar, 1% BSA antiserum, micro slide, cork borer, petridish and cotton.

Procedure

Preparation of agar: 1g of agar was dissolved in 100 ml of Borate saline solution (5 ml of Borate buffer and 95 ml of physiological saline form the Borate saline solution). In a conical flask, by boiling in a water bath, 2.5 ml of hot agar solution was transferred to a microslide with the help of a pipette.

The agar solution was poured to a depth of 2 to 15 minutes. The agar coated slide was called **agar plate**. After cooling 1% BSA antiserum was added on the agar plate. Antiserum diffused uniformly in the agar plate kept in the refrigerator for 30 minutes.

With help of a cork borer, a well was cut on the agar plate or in the centre of the slide. Antigen diffused out radially when it was diffused out. It came in contact with the antibody to form a precipitin ring.

Results

The diameter of the ring (Fig. 192) was a measure of the concentration of the antigen. The diameter of the ring depends on the concentration of antigen. The log of the diameter of the ring is proportional to the antigen concentration. The higher the antigens content the longer the ring.

Inference : The diameter of the ring is a measure of tht concentration of the antigen. The diameter of the ring dl-pends on the concentration of antigen. The log of the diameter of the ring is proportional to the antigen concentration The higher the antigen content, the larger the ring.

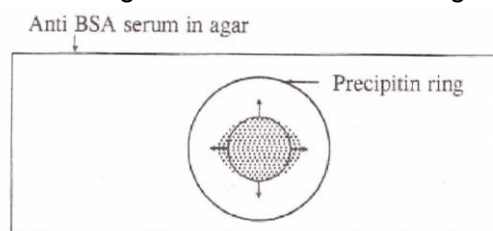


Fig. 192. Redial immunodiffusion ring.

DETECTION OF THE SPECIFIC REACTIVITY OF PRECIPITATING ANTIBODY (IGG) WITH SOLUBLE ANTIGENS BY DOUBLE IMMUNODIFFUSION (OUCHTERLONY) TEST

Ex. No. 6

Date:

Aim

To study the precipitation reaction between antigen and antibody by simple diffusion.

Principle

When antigen and antibody are mixed, they form an antigen antibody complex called precipitin. The reaction between antigen and antibody resulting in the formation of precipitin is called precipitation.

In double immuno diffusion (Fig. 193) technique the antigen and antibody are kept in separate wells in an agar plate. The antigen and antibody diffuse towards each other in the agar plate. In this experiment both antigen and antibody diffuse in the agar and hence it is called double immunodiffusion.

When the antigen and antibody diffuse and meet in the agar in optimal concentration, antigen-antibody complexes are formed. They appear in the form of lines or curves on the agar plate. They are called precipitin bands. They precipitin bands are very useful in detecting antibodies and to identify unknown antigens. This method was devised by Ouchterlony and hence it is called Ouchterlony double immunodiffusion.

Double immunodiffusion can be carried out either in glass slide or rectangular glass plate or Petri dish. The antigen and antibody diffuse in the agar plate. When they meet, they react together to produce precipitin bands. When the antigen and antibody are at optimal concentrations, the precipitin bands appear at equal distances between the wells of antigen and antibody.

Materials Required : Borate buffer, agar, antigen, antiserum, microslide, petridish, cotton, cork borer, etc.

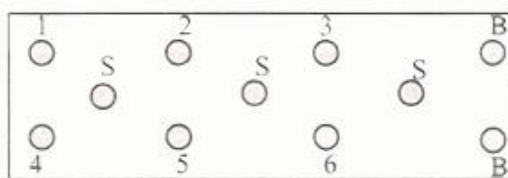
Borate Buffer : Borate buffer was prepared by dissolving 18.55 gms of boric acid and 3.65 grns of sodium hydroxide in one liter of distilled water.

Agar: 1 gram of agar was dissolved in 100 ml of borate-saline solution [5 ml of borate buffer and 95 ml of physiological saline (0.9 gm NaCl dissolved in 100 ml distilled water) from the borate saline solution] in a conical flask by boiling in a water bath. 2.5 ml of hot agar solution was transferred to a microslide with the help of a pipette.

The agar solution was poured to a depth of 2 to 3 mm. The gel was allowed to settle for 10 to 15 minutes. Now, the agar coated slide is called agar plate.

With the help of cork borer wells were cut on the agar plate. It was best to cut wells of 0.6 to 0.7 cm in diameter approximately 2 cm apart (as measured from the centre of the wells).

For convenience 11 wells were made in 3 rows with 3 wells in the middle row and 4 wells each on the upper and lower rows.



1 to 6 Antigen wells containing BSA.
 B - Control wells containing buffer.
 S - Antibody wells containing anti-BSA serum.

Fig. 193. Double immunodiffusion.

After cutting wells, a small amount of agar gel was heated with several drops of water in a test tube. A few drops of this diluted agar solution were added to each well to seal the gaps between the agar gel and the bottom of the glass slide.

Antigen: The commonly used antigen for immunodiffusion was 1% Bovine Serum Albumin (BSA). It is prepared by dissolving 1.0 gm of BSA in 100 ml of distilled water.

Antiserum: The antiserum used was BSA antiserum. It was prepared from rabbit. The rabbit was injected intravenously 2 ml of 1% solution of BSA in saline on alternate days for 9 injections. 7 days after the last injection, the rabbit was bled. The blood was collected in glass vials. It was allowed to clot.

The clot was separated with the help of a spatula. The remaining solution was centrifuged for 2 minutes at 2000 rpm. The supernatant was collected and it was called BSA antiserum.

Procedure : Six test tubes were cleaned and marked serially. 1 ml of distilled water was added to each tube. Then 1 ml of 1 % BSA was added to the first tube and was mixed well. From this 1 ml was transferred to the second tube with the help of a pipette. The content of the second tube was mixed well and from this 1 ml was transferred to the third tube. Like this, the procedure was repeated up to the 6th tube and from the 6th tube 1 ml was discarded. This was the serial dilution of BSA (Fig. 194). This gave two fold dilution of BSA antigen. The first tube contains maximum concentration and the last tube contains minimum concentration.

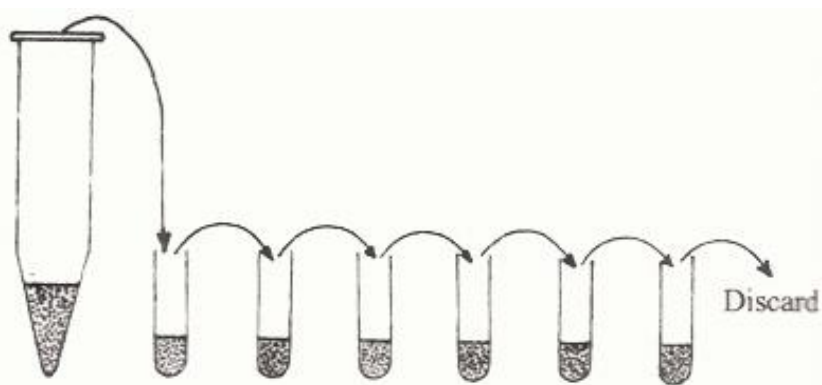


Fig.194. Serial dilution of BSA.

Equal volumes of serially diluted BSA antigens are loaded in six wells with the help of a micropipette. The loading was done beginning with minimum concentration and ending with maximum concentration. That was, the first well was loaded with the antigen of the 6th tube and the last well was loaded with the antigen of the first tube. Hence there was a two fold increase in the concentration of antigen from the first well to the 6th well.

The BSA antiserum was added in the three middle wells. Two wells on one side were loaded with buffer. These two wells served as controls.

The agar plate was kept in a petri dish at a constant temperature between 16°C and 20°C. Humidity was maintained by keeping a cotton soaked in water in the petri dish.

The slide was examined for 7 days for precipitin bands. For observation of bands, the slide was washed several times in saline and stained with 0.5% amidoblack (Amidoblack 0.5 gm, 40 ml methanol, 10 ml acetic acid, 50 ml distilled water). The stained slide was washed in water. It was destained with 7% acetic acid. The slide was then photographed.

Results : The antigen and antibody diffuse in the agar plate. When they meet, they react together to produce precipitin bands (Fig. 195). When the antigen and antibody are at optimal concentrations, the precipitin bands appear at equal distances between the wells of antigen and antibody.

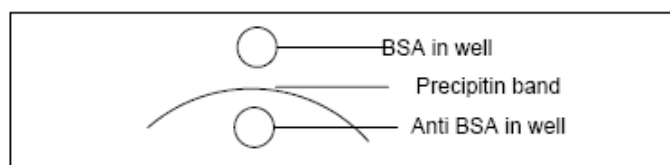


Fig. 195. Agar plate showing a precipitin curve in double immunodiffusion.



DETECTION OF THE SPECIFIC REACTIVITY OF PRECIPITATING ANTIBODY (IgG) WITH FRACTIONATED ANTIGENS BY IMMUNOELECTROPHORESIS

Ex. No. 6

Date:

Aim

To separate protein fractions in serum samples using immuno electrophoresis.

Principle

Immuno electrophoresis consists of two methods that are combined, namely electrophoresis and immune diffusion. The agar plate has a longitudinal trench and a circular well.

First antigen is separated from the serum through electrophoresis by keeping it in the well. The protein components (antigens) separate according to their charge. But unlike normal electrophoresis the bands are not stained for protein. Instead antiserum is pipetted into the longitudinal trench and the agar plate is incubated in a humid chamber. Diffusion of the antiserum and protein occurs and where they meet, precipitin curves are formed.

Materials required

Michaelis diethylbarbiturate acetate buffer, glass slides, incubator, tracking dye, (bromophenol blue 0.1 g/lit), electrophoresis equipment, physiological saline, human serum, antiserum (rabbit antihuman serum), thimerosal, micropipette, 25 needle and syringe, 19 gauge needle, physiological saline.

Agar: The agar used in immuno electrophoresis must be of good quality. It must be transparent and should have a low calcium concentration. Agar specifically prepared for this experiment is available from Behring Diagnostic Hoechst India Ltd., Hoechst House, 3/1, Asaf Ali Road, New Delhi.

Michaelis Diethylbarbiturate Acetate Buffer: 13.38 gm of sodium 5,5-diethyl barbiturate and 8.83 gm of sodium acetate trihydrate are dissolved in distilled water to 8.2 by adding approximately 180 ml of 0.1 N hydrochloric acid. This solution possesses an ionic strength of $\mu = 0.1$ (pH = 8.2).

Physiological saline: 0.9 gm NaCl dissolved in 100 ml distilled water.

Antiserum: Rabbit antihuman serum suitable for immuno electrophoresis with rabbit antiserum to human serum, approximately 30 different human serum proteins can be demonstrated by immuno electrophoresis. This and other types of antiserum are commercially available and can be purchased from Behring diagnostic Hoechst India Ltd.

Human Serum: Human serum is collected from human blood.

Immuno electrophoresis equipment: The equipment for immuno electrophoresis with all needed accessories is commercially available.

Preparation of Agar plate

2 gm of pure agar is dissolved in 50 ml of diethyl barbiturate acetate buffer, pH 8.2, $\mu = 0.1$ and 50 ml of distilled water. This solution is heated for 15 minutes in a water bath of 100° C. Any undissolved particles can be removed from the heated agar by centrifugation at 3000 rpm for 2 to 3 minutes.

10 mg of Thimerosal are added as a preservative. The hot agar solution is applied by pipette to alcohol cleaned glass slides. About 3 ml is poured on each slide. Slides were left for solidification for 15 minutes. The glass slides are now put into a moisture chamber. A simple plastic container with petridish of water could serve as a moisture chamber.

Cut the wells and troughs just before the plates are used with the shandon cutting device to give troughs 5 cm long and 1 mm wide. The agar remaining in the trough must be removed only after electrophoresis.

Punch out an antigen well in the agar plate with a 1 mm diameter needle just below the trough. Agar remaining in the punch is aspirated by a syringe connected to the barrer moisture containing sealed containers preferably at 4°C.

Application of antigen: The human serum is introduced into the antigen well by a 26 gauge needle attached to a tuberculin syringe or by means of an appropriate tuberculin syringe or by means of an appropriate micropipette. Complete filling of the well requires about 0.002 ml of serum.

Electrophoresis: The apparatus used for immuno electrophoresis is similar to that used for paper electrophoresis.

The agar plate loaded with human serum (antigen) is placed on the support of the electrophoretic apparatus. Two filter paper strips soaked in buffer are placed at the two ends of the agar plate. The other ends of the filter paper strips are dipped into the buffer contained in the trough of electrophoretic apparatus. A lid is placed to prevent desiccation.

An electric current of 3 to 6 v/cm is applied. Electrophoretic separation is achieved in 45 minutes. After 45 minutes, the current is stopped and the agar plate is taken out.

Application of antiserum

After electrophoretic separation of human serum, the agar strip from the pre-cut trough is removed with a 19 gauge needle attached to a tuberculin syringe. With the help of a micropipette about 0.4 to 0.6 ml of antiserum is load in the trough. After application of antiserum, the agar plate is placed in a moisture chamber (Petri dish containing cotton soaked in water) for immuno diffusion.

The electrophoretically separated proteins (antigen) and antibody diffuses towards one another. When they meet ag-ab complexes are formed. They are marked by precipitin curves at the points of confluence. It occurs in 20 hrs.

Fixation of precipitin: Immuno electrophoretic patterns can be recorded by photographing unstained or stained precipitin curves. For most purposes direct photography of the slide is sufficient.

Staining of precipitin patterns: The agar plate is washed with physiological saline for one or two days in order to remove the non-precipitated protein from the agar. It is then covered by filter paper and dried completely either in the incubator at 37°C or in air at room temperature. After removal of the filter paper, the agar slides are placed in 2% acetic acid solution for 5 minutes. Then the agar plate is stained by any one of the following techniques.

- 1. Amido black:** 0.5% amidoschwarz 10 B in methanol, glacial acetic acid (9:1) stained for 5 to 10 minutes and washed for 15 minutes.
- 2. Light green:** 0.5% green SF in 5% trichloro acetic acid stained for 60 minutes and washed with 5% trichloro acetic acid.
- 3. Sudan black:** Sudan black 0.1% is dissolved in 60% ethanol at 37°C with occasional stirring is then filtered at 25°C and stored in dark containers. Before use 0.1 ml of a 30% NaOH solution is added to 160 ml of Sudan black solution. After staining for 2 hours, the slides were washed using 50% ethanol.

4. Lipid-Protein double staining: 0.5% oil red solution in 50% ethanol is suitable as a lipid stain. Place the slide in the dye mixture for 2 hours. Then wash with 50% ethyl alcohol. 0.5% light green solution in 5% Trichloroacetic acid is then applied to achieve the specific protein stain. The staining time for protein is approximately 1 hour. Wash with 5% Trichloroacetic acid.

Results

The separated antigens and the antibodies migrate toward one another and form precipitin arcs (Fig. 196) at the region of optimal concentrations.

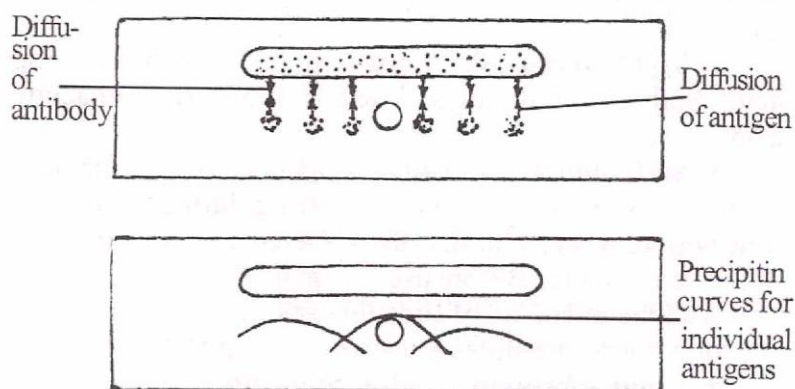


Fig. 196. Immunoelectrophoresis test.

Discussion

The slides must be photographed for keeping a permanent record. Precipitin curves can be copied directly onto photographic paper. For this purpose the surface of the agar plate is carefully rinsed with tap water to remove dust or other undissolved particles in the antigen or antiserum. To obtain an optically uniform surface, the agar is covered by tap water or physiological saline. The agar plate is then placed on a photographic paper in a dark room and illuminated from above with a lamp of 200 watts. When optically exposed, the photographic paper will exhibit fine precipitin lines with great sharpness.

C. ANIMAL PHYSIOLOGY

QUANTITATIVE ESTIMATION OF AMYLASE ACTIVITY

Ex. No. 1

Date:

Aim

To estimate the amount of amylase present in the human saliva.

Principle

Amylase in the saliva hydrolyses the starch into smaller units called dextrin and finally to maltose. 3,5 – Dinitro salicylic acid reagent (DNS) combines with maltose to give orange red coloured compound. The intensity of the colour is proportional to the amount of Maltose formed from the hydrolysis of starch. The activity of salivary amylase can be quantified from this value.

Materials required

Test tube, beaker, water bath and Spectrophotometer.

Reagents

1. **Dinitro salicylic acid reagent:** 1 gm of 3,5 – Dinitro salicylic acid was dissolved in 20 ml of 2 N NaOH (4 gm of NaOH dissolved in 100 ml distilled water) to the above solution. 30 gm of Sodium potassium tartarate was added and then made up to 1000 ml with distilled water.
2. 0.1 mole of citrate buffer.
3. Starch solution: 2 gm dissolved in 100 ml distilled water.
4. Standard maltose: 100 mg dissolved in 100 ml distilled water.

Procedure

Three test tubes were taken and were labelled as sample, standard and blank. 1 ml of saliva, 1 ml of 0.1 mole of citrate buffer, 0.5 ml of 2 % starch solution was taken in the sample test tubes. In the standard test tube 1 ml of standard Maltose solution was taken. 1 ml of distilled water was taken in the blank test tube. The sample test tube alone incubated at 37°C for 10 minutes. 2 ml of Dinitro salicylic acid reagent was added to the sample, standard and blank test tubes. All the three test tubes were kept in boiling water bath for 5 minutes and then allowed to cool. The content in each test tube was made up to 10 ml with distilled water. Then the optical density of the sample, standard was measured at 540 nm using a spectrometer.

Result

Amount of salivary amylase present in human saliva _____µg maltose released/
mg protein/ minutes.

Discussion

Saliva is one of the digestive juices. It is slightly acidic having pH 6.3 to 6.8. Ptyalin (salivary amylase) is found in the saliva. The optimum temperature for salivary amylase is 37°C. Ptyalin acts on starch and converts the starch into maltose. The secretion of saliva is under the reflex action. The physiological stimulus of saliva is the

presence of food in the mouth. The food induces the mucous membrane of the mouth which in turn stimulates the salivary centre of the brain.

Calculation

Amount of amylase present in the human saliva =

$$= \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{\text{Dilution factor}}{\text{Volume of starch}} \times \text{Volume of extract}$$

=

Result

Amylase present in the human saliva = _____ μg maltose released/mg protein/min.



QUANTITATIVE ESTIMATION OF AMMONIA

Ex. No. 2

Date:

Aim

To estimate the amount of ammonia present in the given sample.

Principle

Sodium hypochloride combines with ammonia in the sample to produce chloride and hydroxyl ions. Sodium nitroprusside acts as catalyst for this reaction. Ammonium chloride in the presence of hydroxyl ions combines with phenol to form a complex. This complex combines with another phenol molecule to form yellow phenol, which is a coloured compound. The colour thus formed is directly proportional to the amount of ammonia present in the sample.

Materials required

Test tube, measuring cylinder, pipette, fish tank water, boiling water bath and Spectrophotometer.

Reagents

1. Reagent - A: 2 gm of phenol and 10 mg of sodium Nitroprusside dissolved in 100 ml of water.
2. Reagent - B: 1 gm of sodium hydroxide is added to 2 ml of sodium hypochloride and make up to 100 ml with distilled water.
3. Reagent - C: Ammonium standard solution: Dissolve 38 mg of anhydrous ammonium chloride in 100 ml of distilled water. This was stock solution. From this solution working solution was prepared. 1 ml of stock solution was made up to 100 ml with distilled water (1 ml of solution = 0.00122 mg of ammonia).

Procedure

Three test tubes were taken. In one test tube 1 ml of fish tank water was taken. To it, 2.5 ml of reagent A was added. In the second test tube, 1 ml of distilled water was added and it was used as blank. In the third test tube 1 ml of standard ammonium was taken to it 2.5 ml of reagent A was added. After 5 minutes, 2.5 ml of reagent B was added to all the test tubes viz. sample, standard and blank. Then the test tubes were incubated at 37° C for 20 minutes and allowed to cool for 30 minutes. The O. D. of the sample and the standard were measured at 625 nm by using Spectrometer.

Result

The amount of ammonia present in the 1 ml of given sample = _____mg/100 ml.

Discussion

Ammonia is constantly produced in all organisms by deamination of amino acids. It is highly soluble, toxic and injurious to cells. In aquatic animals it is quickly eliminated into the surrounding water. The ammonia can not be excreted by terrestrial animals as such. Because water forms a valuable commodity. Ammonia is quickly converted into less harmful compounds and excreted. Fishes excrete their chief nitrogenous waste product in the form of ammonia and therefore called ammonotelic animals. Some amount of ammonia is also excreted through the gills of the fishes.

Calculation

$$\text{Amount of ammonia present in the given sample} = \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{\text{Conc. of the standard}}{\text{Volume of the sample}} \times 100$$

=

The amount of ammonia present in 1 ml of the given sample = _____mg/100 ml.

QUANTITATIVE ESTIMATION OF UREA

Ex. No. 3

Date:

Aim

To estimate the amount of urea present in the given sample.

Principle

Urea when heated with Diacetyl monoxein in an acidic solution produce yellow colour, in the presence of Orthophosphoric acid, and Sulphuric acid. The intensity of the colour thus formed is directly proportional to the amount of urea present in the given sample.

Materials required

Test tube, 10 ml measuring cylinder, 1 ml pipette, boiling water bath and Spectrophotometer.

Reagents

1. 2.5% Diacetyl monoxin: 2.5 gm of Diacetyl monoxine is dissolved in 100 ml of distilled water.
2. Acid ferric chloride solution: 1 ml of H_2SO_4 is made up to 100 ml with 5% Ferric chloride solution containing 50 gm in 1 ml of water.
3. Acid reagentL 5 ml of Orthophosphoric acid and 40 ml of Sulphuric chloride solution are made up to 50 ml with distilled water.
4. Colour reagent: 300 ml of acid reagent and 10 ml of 2. 5% Diacetyl monoxine are made up to 500 ml with distilled water.
5. Standard urea: 100 mg of Unhydrous urea crystal is dissolved in 100 ml of distilled water.

Procedure

2 ml of sample, 2 ml of standard urea and 2 ml of distilled water were taken in three separate test tubes and were labelled accordingly. To each test tube, 3 ml of colour reagent was added. All the test tubes were kept in a boiling water for 20 minutes and then cooled to room temperature. The O.D. of the sample and standard were measured at 520 nm in a Specrophotometer by using the blank.

Result

The amount of urea present in the given sample = _____mg/ 100 ml.

Discussion

Urea is the characteristic nitrogenous excretory product of mammals including monotromes. The composition of urine may vary from species to species and within the same species depending upon the diet. From 60-65 % of all fluid taken into the body is excreted as urine. The major constituent of urine is urea. It is noted that high concentration of urea won't create any problem, as it is less toxic. In 1904, the amino acids, arginine was identified as a precursor of urea in mammals and the enzyme arginase was shown to be essential for the conversion of urea in the liver. The

accumulation of urea in the liver is higher than those body tissues. It was also found that the major pathway of urea excretion is only through urine. So the biosynthesis of urea and its elimination from all sources including ornithine cycle is found to be the urine. Thus urea is important in maintaining homeostatic machinery.

Calculation

$$\begin{aligned} \text{Amount of urea present in the given sample} &= \frac{\text{O.D of the sample}}{\text{O.D of the standard}} \times \frac{\text{Conc. of the standard}}{\text{Volume of the sample}} \times 100 \\ &= \end{aligned}$$

The amount of urea present in the given sample = _____ mg/100 ml.



ESTIMATION OF RATE OF SALT LOSS IN FISH

Ex. No. 4

Date:

Aim

To estimate the amount of salt lost by the given animal (estuarine fish).

Principle

When an estuarine fish is placed in a hypotonic medium (fresh water medium), the entry of water and loss of salt will take place. This salt loss may be defined as the amount of salt lost/hour/gm/body weight. Mohr's titration method was used to estimate the amount of salt present in the medium.

Materials required

Fish, distilled water, burette, pipette, conical flask and porcelain.

Reagents

1. **Silver nitrate: (AgNO₃):** 1.698 of AgNO₃ dissolved in 1 lit of D. water.
2. **5% Potassium chromate solution:** 5 g of Potassium Chromate dissolved in 100 ml of D. water.

Procedure

The salinity of the distilled water was estimated. 5 ml of distilled water was taken in a conical flask. 3 drops of 5% potassium chromate was added to it. The addition of Potassium chromate (K₂Cr₂O₈) results in a golden yellow coloured solution. The solution was titrated against 0.01 N AgNO₃ solutions. The appearance of brick red colour was noted as the end point and was recorded. Then the fish was introduced into a beaker containing 500 ml of D. water and allowed to remain in the medium for 30 minutes. After 30 minutes of incubation the fish was removed from the medium and the salinity of the incubated medium was estimated. The difference in the salinity values was calculated. The calculated value was the amount of salt lost by the fish to the medium. Then the weight of the fish was calculated. The rate of salt loss was tabulated (Tables 14 and 15) and calculated by dividing the amount of salt lost into the medium by weight of fish.

Result

The rate of salt loss = _____ %/gm/hr.

Discussion

Due to low salinity of the water and more salt content of the body fluids the water in the external medium tends to enter the body continuously. The salt content of the body fluid is removed along with fresh water into the external medium. That is why many of fresh water animals especially fishes face the problem of osmoregulation. This clearly indicates the loss of salt by the fish when introduced into the hypotonic medium.

TABULATION AND CALCULATION

Formula

$$19.381 \times \text{Volume of AgNO}_3 \text{ consumed}$$

Chlorinity = . -----

17.15

$$\text{Salinity} = 0.03 + (1.805 \times \text{Chlorinity}).$$

$$\text{Salt loss} = \text{Salinity of medium after incubation of fish} - \text{Salinity of medium before incubation of fish}$$

$$\text{The rate of salt loss} = \frac{\text{Amount of salt loss}}{\text{Weight of the fish}}$$

Table 14. Salinity of medium before incubation.

S. No.	Volume of the sample	Burette reading		Volume of AgNO ₃
		Initial	Final	
	5 ml			
	5 ml			

Calculation

$$\text{Chlorinity} = \frac{19.381 \times \text{_____}}{17.15}$$

$$= \text{_____}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____}).$$

$$= \text{_____} \text{‰}$$

Table 15. Salinity of medium after incubation.

S. No.	Volume of the sample	Burette reading		Volume of AgNO ₃
		Initial	Final	
1	5 ml			
2	5 ml			

Calculation

$$\text{Chlorinity} = \frac{19.381 \times \text{_____}}{17.15}$$

$$= \text{_____}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____}).$$

$$= \text{_____} \text{‰}$$

$$\text{Salt loss} = \text{Salinity of medium after incubation of fish} - \text{Salinity of medium before incubation of fish}$$

$$= \text{_____} - \text{_____}$$

$$= \text{_____} \text{‰}.$$

$$\text{Salt loss for an hour} = \text{_____} \times 2$$

$$= \underline{\hspace{2cm}} \text{‰}.$$

Weight of the fish = gm.

The rate of salt loss = $\frac{\text{Amount of salt loss}}{\text{Weight of the fish}}$

$$= \text{-----}$$

$$= \text{-----}$$

Rate of salt loss = ‰ /gm/hr.

Result

The rate of salt loss = ‰/gm/hr.

ESTIMATION OF RATE OF SALT GAIN IN FISH

Ex. No. 5

Date:

Aim

To estimate the amount of salt gained by the given animal (estuarine fish).

Principle

When an estuarine fish is placed in a hypertonic medium there is loss of water and gain of salt from the medium. The amount of salt gained by the fish/hour/gram of body weight can be estimate by Mohr's titration method. 0.1N Silver nitrate solution was used for titration. 5% potassium chromate solution is used as an indicator.

Materials required

Fish, salt water, burette, pipette, conical flask, porcelein tile and salt water.

Reagents

1. **Silver nitrate: (AgNO₃):** 16. 987 of AgNO₃ dissolved in 1 lit of D. water.
2. **5% Potassium chromate solution:** 5 g of Potassium Chromate dissolved in 100 ml of D. water.

Procedure

The salinity of the salt water was first estimated 5 ml of salt water was taken in a conical flask 3 drops of 5% potassium chromate solution was added. The addition of Potassium chromate (K₂ Cr₂ O₈) resulted in a golden yellow coloured solution, which was titrated against 0.1 N AgNO₃ solution. The appearance of brick red colour was noted as the end point and was recorded. Then the fish was introduced into the beaker containing 500 ml of salt water and the fish was allowed to remain in the medium for 30 minutes. After 30 minutes of incubation, the fish was removed from the medium and the salinity of the incubated medium was estimated. The difference in the salinity value was calculated. The calculated value was the amount of salt gained by the fish to the medium. Then the weight of the fish was calculated. The rate of salt gained was tabulated (Tables 16 and 17) and calculated by dividing the amount of salt gained into the medium by the weight of the fish.

Result

The rate of salt gain = _____ %/gm/hr.

Discussion

Due to the high salinity of the water and loss of the more salt content of the body fluids, the water in the external medium tends to enter the body continuously. The salt content of the body fluid is removed along with sea water into the external medium. That is why many of marine animals especially fishes face the problem

Formula

Chlorinity = 19.381 x Volume of AgNO₃ consumed.

Salinity = 0.03 + (1.805 x Chlorinity).

Salt gain = Salinity of medium before incubation of fish – Salinity of medium after incubation of fish.

The rate of salt gain =
$$\frac{\text{Amount of salt gain}}{\text{Weight of the fish}}$$

Result

The rate of salt gain = ___‰/gm/hr.

TABULATION AND CALCULATION**Formula**

$$\text{Chlorinity} = \frac{19.381 \times \text{Volume of AgNO}_3 \text{ consumed}}{17.15}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{Chlorinity}).$$

$$\text{Salt gain} = \text{Salinity of medium before incubation of fish} - \text{Salinity of medium after incubation of fish}$$

$$\text{The rate of salt gain} = \frac{\text{Amount of salt gained}}{\text{Weight of the fish}}$$

Table 16. Salinity of medium before incubation.

S. No.	Volume of the sample	Burette reading		Volume of AgNO ₃
		Initial	Final	
1	5 ml			
2	5 ml			

Calculation

$$\text{Chlorinity} = \frac{19.381 \times \text{_____}}{17.15}$$

$$= \text{_____}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____}).$$

$$= \text{_____} \text{‰}.$$

Table 17. Salinity of medium after incubation

S. No.	Volume of the sample	Burette reading		Volume of AgNO ₃
		Initial	Final	
	5 ml			
	5 ml			

Calculation

$$\text{Chlorinity} = \frac{19.381 \times \text{_____}}{17.15}$$

$$= \text{_____}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____}).$$

$$= \text{_____} \text{‰}.$$

$$\text{Salt gain} = \text{Salinity of medium before incubation of fish} - \text{Salinity of medium after incubation of fish.}$$

$$= \text{_____} - \text{_____}$$

$$= \text{_____} \text{‰}.$$

$$\text{Salt loss for an hour} = \text{_____} \times 2$$

$$= \text{_____} \text{‰}.$$

$$\text{Weight of the fish} = \text{_____} \text{ gm.}$$

$$\text{The rate of salt gain} = \frac{\text{Amount of salt gain}}{\text{Weight of the fish}}$$

$$= \text{_____}$$

$$= \text{_____}$$

$$\text{Rate of salt gain} = \text{_____} \text{‰/gm/hr.}$$

Result

$$\text{The rate of salt gain} = \text{_____} \text{‰/gm/hr.}$$

ESTIMATION OF BLOOD CHLORIDE

Ex. No. 6

Date:

Aim

To estimate the amount of chloride salt present in sheep's blood.

Principle

Mercuric nitrate reacts with chloride to form mercuric chloride. When excess of Mercuric nitrate is added in excess, the mercuric ions become clumped and form a violet blue colour in the presence of an indicator Diphenyl carbazone.

Materials required

Centrifuge, Test tube, 1 ml pipette, conical flask (125 ml) and micropipette.

Reagents

1. Mercuric nitrate: 834 mg of Mercuric nitrate was dissolved in 3 ml of conc. HNO_3 and was made up to 1 l with distilled water.
2. Diphenyl carbazon: 100 mg of Diphenyl carbazone was dissolved in 100 ml of Ethanol. It should be stored in brown bottle.
3. Standard chloride: 585 mg of Sodium chloride was dissolved in 100 ml of distilled water.

Procedure

3 ml of blood was centrifuged at 3000 rpm for 10 minutes. Two test tubes were taken and were labelled as sample and standard. 9.9 ml of distilled water was added to both the test tubes. 0.1 ml of sheep's blood serum (supernatant) was added to the sample conical flasks. 0.1 ml of standard chloride solution was added to the standard conical flasks. Both the conical flasks, 5 ml of diluted serum was taken in conical flask, to it 2 drops of diphenyl carbazone solution was added and mixed well. This solution was titrated against Mercuric nitrate solution taken in a 5 ml pipette. The appearance of violet blue colour was noted as end point. The same procedure was repeated for diluted standard chloride solution.

Calculation

$$\text{Amount of chloride present in the sheep's blood} = \frac{\text{Reading of the sample solution}}{\text{Reading of the standard solution}} \times 100.$$

The amount chloride present in the sheep's blood= _____mEq/lit.

Result

The amount chloride present in the sheep's blood= _____mEq/lit.

Discussion

Chloride forms one of the main constituents of the blood plasma. It is very essential for normal healthy life. Chloride occurs as sodium chloride and Potassium chloride. Concentration of Sodium chloride is higher in plasma and is responsible for "saltish taste" of the blood. The chloride level in whole blood is 250 mg% (70 m Eq/l) in the plasma it is 365 mg% (103 m Eq/l) and in cells it is 190 mg % chloride is essential for water balance, osmotic pressure and acid-base balance. Chloride deficiency in diet produces retardation of growth and failure of reproduction.



D. MICROTECHNIQUE

FIXING, EMBEDDING, SECTIONING, SPREADING, STAINING AND MOUNTING

Ex. No.: 1

Date :

Aim To prepare the serial sections of the tissues.

Principle

Micro technique (or) microtomy is a specialized branch of biology involving the study of animal tissues. Micro technique is one of the processes by which slides are prepared containing animal tissues for microscopical studies. Micro technique is also known as histological techniques because the animal tissues are sectioned and studied for their structure after staining with the help of a microscope.

Micro technique consists of the following steps.

- 1) Fixation
- 2) Washing
- 3) Dehydration
- 4) Clearing
- 5) Embedding
- 6) Blocking
- 7) Sectioning
- 8) Spreading the ribbons on the slides and fixing.

Materials Required

Alive *Pila globosa*, dissection box, specimen tube, labels, 500 ml beaker, 100 ml measuring cylinder, 250 ml reagent bottle, petridish, embedding bath, paraffin wax, L-mould one pair, porcelain tile, spirit lamp, camel hair brush, block holders, microtome, coupling jars, wax, Bouin's fixative, Isopropanol, distilled water, methyl benzoate, xylene and DPX mountant.

Preparation of the fixative

1. **Bouin's fluid:** 75 ml saturated (aqueous) picric acid, 25 ml formalin and 5 ml glacial acid were dissolved in distilled water. Then the fixative was filtered.

2. **Dehydrating reagents for the fixed tissues**

Preparation of 30%, 50%, 70%, 80%, 90% and absolute alcohol in different reagent bottle using distilled water:

30% alcohol = 30 ml Isopropanol and 70 ml distilled water

50% alcohol = 50 ml Isopropanol and 50 ml distilled water

70% alcohol = 70 ml Isopropanol and 30 ml distilled water

80% alcohol = 80 ml Isopropanol and 20 ml distilled water

90% alcohol = 90 ml Isopropanol and 10 ml distilled water

100% alcohol = 100 ml absolute alcohol (Isopropanol)

3. **Formalin:** Most commonly used fixative is formaldehyde. Both its solution and its combination with other reagents are used. The commercially sold one is 30-40% solution of formaldehyde gas in water and is called formalin. This solution is taken to be pure formalin and a 10% solution of the same is applied as fixative i.e. a 10% solution of commercial formalin contains actually 4% of formaldehyde. In formalin there may be certain impurities and if very sensitive study of histochemical nature is

to be done. 10% Formalin is a good general fixative and preservative for both animal and plant depending on size of specimen.

Formalin: Acetic acid: Alcohol (FAA) solution is also used for histological fixation. It is prepared by mixing 15 ml Formalin, 5 ml of acetic acid and 20 ml of Ethanol.

Procedure

1. **Fixing** (Fixation is the preservation of material to keep the tissue in a life like condition) the animal tissue: Pila was collected from the pond and brought to the laboratory. The animal was dissected in physiological saline solution. The organs were removed from the viscera of Pila and with the help of forceps; the organs were washed in distilled water and transferred to a small specimen tube (or) glass vial containing 10 ml of Bouins fixative. The tissue was kept in the fixative for 24 hours or over night.
2. **Washing** (washing removes the excess fixative) the fixed tissue: After the fixation is completed i.e. the tissue has remained in the fixative for the minimum time has not been left in it beyond the maximum time, the fixative should be poured out in other specimen tube and distilled water should be added to the tube containing the tissue. Be careful not to through the tissue along with the fixative. The fixative should be poured out slowly so that the tissue remains in the tube for 10-15 minutes. The tissues should be washed with distilled water by putting it in the specimen tube or in running water for over night. The tissue rinsed many times with distilled water.

Precaution

1. Those tissues fixed in aqueous (i.e. water based) fixative should be washed with distilled water. In case any alcoholic fixative has been used, the tissue should be washed with the appropriate grade of alcohol. If the tissue has fixed in 70% alcohol it should be washed with 70% only.
2. Usually aqueous Bouin's fixative is used and the tissue is fixed in it. 4 or 5 changes in distilled water are required to remove the yellow color in the fixed tissues. Even if the yellow colour is not removed little a bit of lithium carbonate may be added to the tube. It helps to remove the yellow colour (picric acid of the Bouins fixative) at a faster rate.
3. When it is difficult to follow the other steps due to the non-availability of time is better to keep the tissue in 70% alcohol.
3. **Dehydrating the fixed tissues:** The tissue dehydrated finally in absolute alcohol was transferred to a petric plate. Later the tissue was taken by a forceps and put in a small glass vial containing 10 to 15 ml methyl benzoate.
4. **Clearing the dehydrated tissue:** When the tissue is observed through a light source we can see the transference of the tissue even the mussel layer, blood vessel, minute hard parts maybe visible. We should keep the tissues in clearing agent (methyl salicilate) until they sink.
5. **Embedding of dehydrated tissue (The aim of embedding is to standardize the tissue for microscopic examination by sectioning with microtome):** Fine paraffin wax scrapings were collected in a beaker and transferred to embedding bath cups. The uniform temperature was maintained in Embedding bath (60-62°C).
Embedding means dehydrated tissue & soaked with xylene with wax. This is also called infiltration. Infiltration can be done in two ways 1) cold filtration 2) hot filtration. Embryo cups were used for infiltration at least utilizing 5-10 change may be under take cold filtration was followed by hot infiltration. The purpose of embedding is to make dehydrated tissue again soft & transparent tissue.
6. **Fixing the sections to slides (or) mounting and spreading of ribbons:** Sectioning follows after blocking the tissue. It involves the following steps.

- 7. Trimming of the block:** It is the first step in the sectioning of the tissue. The wax block containing the tissue to be sectioned cut should be trimmed in a proper size with the help of good scalpel. It is better to trim the block on the four sides or six sides till the block is rectangular in size. The opposite side of block should be parallel to each other. The tissue should be visible in trimmed the block. The trimming of block may also be done with the block kept on a clean porcelain tile. By this method the excess wax is removed. The block should be packed and the details about the tissue should also be written on a piece of paper, which is enclosed along with the block.
- 8. Mounting the block on the holder:** After trimming the block, the wax block should fix to the block holder for this the following procedure is adapted place a little wax on the upper surface on the block holder on which the block is mounted. Heat spatula, melt the wax placed on the block holder spread the same evenly on the surface. Repeat this till the wax coating on the surface of block holder 1 mm of thick. Now heat this spatula and melt a central cavity into the wax coating and immediately placed block prepared & trimmed into this cavity. So that the front side of the block is upper most the front side of the block the section to be cut.
- 9. Cutting the Section:** Section is cut with the help of microtome.
- 10. Sectioning:** The embedded material is sliced into thin sections on a microtome. On sectioning the paraffin block of a tissue, the sections come out as a ribbon containing the sections of the tissues (Fig. 197). If mounting is not to be done immediately, the ribbons can place in a section box or section tray. Before mounting on a slide, the long ribbon is always cut into pieces of small lengths, which could be conveniently accommodated on a slide with sufficient space on one side of the slide for labeling. The slide on which the ribbons are to be mounted, are first smeared with any adhesive such as Mayer's albumen, so that the sections remain fixed to the slide, while staining subsequently. Mayer's albumen is the most commonly used adhesive and thoroughly mixing equal proportions off egg albumen, and glycerol and adding a few crystals of thymol, which act as a preservative, prepare it. Care should be taken not be to use too much of adhesive substance which otherwise interferes in staining. After smearing the slide with Mayer's albumin, place small pieces of ribbon on a slide and flood it with water by a dropper. Now place the slide on hot place, where the paraffin ribbons beings to stretch also the sections. If the ribbon or section are considerably folded or wrinkled, then carefully stretch the ribbon with the help of the needle. Care should be taken to see that the wax does not begin to melt; otherwise the sections of the tissue lose the contour and become distorted. Soon after the ribbon is completely stretched, remove the slide from the hot place, drain of the water and allow it to dry.

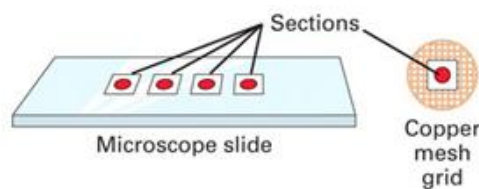


Fig. 197. A ribbon containing the sections of the tissues.

- 11. Labeling:** After the slide is completely dried, label the slide by a diamond pencil. The labeling should include the name in abbreviation of the tissue, the range of section numbers in the ribbon pieces, the date, the number of the slide and the name of the special stain (in short) to be given.
- 12. Storing the slides:** Such labeled slides should be stored in the wooden slide boxes having grooves. Never keep the slides one over the other. Always keep the boxes well closed. Temporarily, the slides can be stored in the card-board slide trays with flaps for closing. The staining should be undertaken 48 hours after the drying of the sections in the ribbon-pieces on the slide.
- 13. Staining of paraffin sections:** The slides are at first deparaffinised by keeping them in the xylene for nearly 30 minutes to one hour (preferably by giving a change

in xylene after 15 to 30 minutes). The deparaffinised slides are now passed through a down graded series of ethyl alcohol, a process often termed as running down slide to water (or hydration), because a series of alcohols of decreasing strengths are used. During this procedure, never at any time the slides should be allowed to get dried. The entire procedure of haematoxylin-eosin staining is carried out in the following order.

1. Hydrate deparaffinised slides by passing through a graded series of ethyl alcohol in descending order of 100%, 90%, 80%, 70%, 50%, and 30% and water.
2. After hydration, stain the slide in haematoxylin by keeping it for nearly 2 to 5 minutes in the stain.
3. Wash the slide in water and observe it behind any white back ground; if over stained (sections turn reddish brown on washing with water) then destains by giving one or two drops of acid water (a coupling jar of distilled water + 1 drop of HCl).
4. Immediately transfer the slides under running tap water for about dipping 3 to 5 minutes, when the sections turn blue in colour.
5. Now dehydrate the slides by passing through a graded series of alcohol in ascending order up to 70% alcohol.
6. Counter stain in eosin by giving one or two quick dips in the stain solution, and then washes it in a fresh grade of 70% alcohol.
7. Dehydrate further by passing through ascending grades of ethyl alcohol, i.e. 80% 90% rectified spirit, absolute alcohol I and absolute alcohol II.
8. Allow the slides to remain in the absolute alcohol for about 5 to 10 minutes, so that they can completely dehydrated.
9. Now transfer slides to Xylene I for clearing, where by, Xylene penetrates the tissue and replaces the tissue alcohol. Give one or two changes of Xylene and mount in DPX mountant or Canada balsam.

Precautions

1. Allow complete deparaffinization in Xylene, otherwise traces of paraffin interfere with staining.
2. Never keep the slide in differentiating solution (acid alcohol) for a long time; otherwise all the stain will be removed.
3. Never use ammonia solution for bluing the solutions, otherwise the solutions get attached from the slides and start floating.
4. Dehydrate the slide thoroughly in absolute alcohol; otherwise on mounting in DPX, a foggy ring appears around the sections, which subsequently spoils the permanent slides.

12. DPX Mountant

This mounting medium is commercially available as DPX and has largely replaced Canada balsam for almost all routine and research work for lower cost and ease of work. It takes a few hours to harden to sufficient consistency required for slide storage while balsam takes much longer time and DPX is also free from any acidic or basic reactions so it does not fade the stains for good length of time (Diestrene 80: 10 g; Dibutyle Pthalate: 05 g and Xylene: 35 ml. It is used to mount the specimen. It is synthetic media used to protect stains.

Observation

The stained slides were focused under the microscope and observed the histology of the tissues.

MICROTOME

Ex. No.: 2

Date :

Principle

Microtomes are mechanical devices for cutting thin uniform slices of tissue (sections). The tissue is supported by hard paraffin wax, or some similar material, and is moved automatically towards the knife, between cuts, by the thickness desired for the section; the latter mechanism is usually graduated in micron (μ) states. When cutting paraffin wax embedded tissue each section sticks to the previous one by its edge, and a ribbon of sections is produced.

The microtomes are named according to the mechanism. Two types of microtomes are commonly used for the sectioning. They are rotary microtome and rocking microtome.

The Rotary Microtome

The rotary microtome (Fig. 198) is so called because a rotary action of the hand-wheel actuates the cutting movement. The block holder is mounted on a steel carriage, which moves up and down in grooves, and is advanced by a micrometer screw; it therefore cuts perfectly flat sections. It has the advantage of being heavier and therefore more stable than the rocking type, and is ideal for cutting serial sections; consequently it is more after used in teaching establishment for cutting large numbers of sections from each block. Larger block of tissue may be cut on this machine, and the cutting angle of the knife (tilt of knife) is adjustable. Since a heavier and larger knife is used with this types of microtome there is less likelihood of vibration when cutting exceptionally hard tissue. The first machine of this type was designed by Professor Minot, and is sometimes known as the Minot Rotary. By using a special holder to set the knife obliquely it may be used for cutting celloidin-embedded sections.

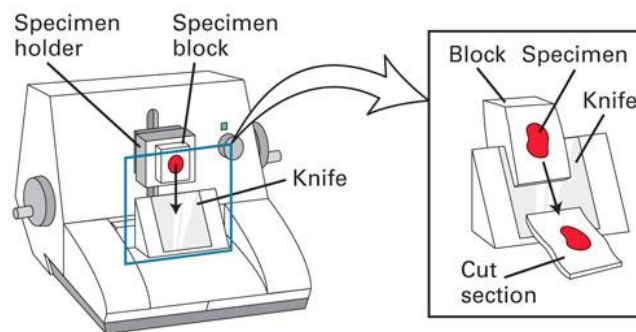


Fig. 198. The rotary microtome.

The Rocking Microtome

In the Cambridge rocking microtome the knife is fixed and the block of tissue moves through an arc strikes against the knife; between strokes the block is moved towards the knife for the required thickness of the sections, by means of a micrometer thread operated by a ratchet. The name of the apparatus comes from the rocking action of the cross arm. It was for many years a familiar sight in almost every histological laboratory for routine work; it is still favoured by some including the writer- for cutting sections from small blocks of tissue of any type. The mechanism is simple and rarely, if ever gives trouble, and the machines liberally last a life time.

The disadvantage is that size of block that can be is limited, although a recent model has been designed to overcome this.

The rocking microtome was designed primarily for cutting paraffin wax sections, but, in a emergency, it may be adapted for frozen sections by inserting in the chuck a simple wooden block, on which the tissue is frozen with an ethyl chloride spray: this method however, is not to be recommended except where no other apparatus is available and a frozen section is required urgently.

In view of the lightness of this type of microtome it is advisable either to fit it into a tray, which is screwed to the bench, or to place it on a damp cloth to avoid movement during cutting. The movement of the cutting arm should depend on the type of tissue to be cut: normally a steady forward and backward movement of the handle will give ribbons of good sections, but with difficult tissue there are two alternative movements worth trying 1) pulling the handle forward and releasing it from this position, allowing the spring to pull it back sharply, or, if this does not produce a good section, 2) pulling the handle forward and letting it back very slowly. One of these methods will usually result in a ribbon of good sections.

STAINING

Ex. No.: 3

Date :

Principle

For routine histological studies, the sections of any tissue are commonly stained with haematoxylin and counter stained with eosin, and the procedure is commonly termed 'as direct haematoxylin staining' the solution used for this technique is always a mixture of both a dye and a mordant.

Principles and need of Staining

For obtaining thin sections of any tissue to be observed under the microscope, the tissue is subjected to various preparatory processes like fixation, dehydration, clearing, embedding, etc. Most of the tissues do not retain sufficient colour after processing to make their components clearly visible when observed under microscope. Moreover many tissues, when become impregnated with mounting media, become much transparent whereby it becomes extremely difficult to observe their structure under the microscope. It is therefore, customary to add colour to tissues, thereby rendering them visible when observed under the microscope and the process is commonly referred to as staining.

In many tissues an assortment of component are present, which may be distinguished by their ability to retain dyes of contrasting colours, when stained in two or three different types of stains during differential staining. Unless specifically desired to study the cells at cytochemical or histochemical levels, the most commonly used dyes basically differentiate the nucleus and the cytoplasm in a cell, which could, make them clearly visible under the microscope.

Through the adhesion of dyes to the cytoplasm is usually direct, the adhesion of dyes to nuclei is rarely direct. Thus in case of nuclear staining, some other compound must first be applied to the nucleus and the nuclear stain is then caused to adhere to this compound. This other compound is referred to as 'Mordant' this process of staining is commonly termed as 'mordant staining'. It involves first the application of the mordant and seconds the application of the dye. However, the more useful method of staining is known as indirect staining where by a single staining solution contains both mordant and dye. In this process, the whole tissue becomes coloured. On transferring, the tissue in a differentiating solution, which is usually a weak acid, the colour taken by the cytoplasm, is removed while leaving it is the nucleus. It is very essential to control the timing and concentration of differentiating solution, because the differentiating solution will otherwise remove all the dye.

Types of stains

Depending on the source of availability, the stains are basically of two main categories, namely

1. Natural strains and
2. Synthetic stains.

1. Natural stains

There are stains, which are obtained from natural sources like that plants and animals. The most common example is "Haematoxylin" which is extracted from the residue of an extract of cameach wood. Staining solutions of Haematoxylin incorporating the mordents, require ripening for several months, which result in complete oxidation of Haematoxylin to haematin, and then used for staining because it is the haematin, which fixes to nucleus

2. Synthetic stains

There are usually the artificial chemical compounds composed of an acid and a base and depending on whether the acid is coloured or the base is coloured, they are commonly classified into three types namely

1. **Acidic stains:** e.g. acid fuchsin, where acid component is coloured and a base is colourless, and usually stains the basic component of a cell such as cytoplasm.
2. **Basic stains:** e.g. basic fuchsin, where the basic component is coloured while the acid component is colourless. These stains usually stain acid component of a cell such as nucleus.
3. **Neutral stains:** The neutral stains are formed due to interchange of ions when the sodium salt of an acid dye and the chloride of a basic are mixed. Such neutral stains, e.g., Romanovsky stains give results, which are difficult from those, obtained with ordinary double staining, using separate acid and basic dyes.

The commonest mordents are either potassium or ammonium alums.

Haematoxylin Stain

1. It is a nuclear stain, which is always desirable to seek basic differentiation between nuclei and cytoplasm. For this purpose, for usual classroom practice haematoxylin is used both for plant and animal tissues. This stain is a powder obtained from *Haematoxylin campechionum* L., a small leguminous plant that contains haematoxyl. This haematoxyl, used for the purpose of staining.
2. Haematoxylin or haematin themselves are useless unless some mordant (e.g. alum) is added and only then they give blue or black or violet staining depending on preparation and process. They are universally used due to high contrast, convenient for photomicrography, easy control of dyeing and the insolubility of color in aqueous or alcoholic media after staining as well as in Canada balsam and synthetic mounting media.
3. Three methods for preparing haematoxylin for routine classroom are being given below that have proved useful to our experience. The choice of method depends on the time available between preparation and use of stain; if longer time lapse is there, slow ripening method is better to be opted and if an immediate requirement is there, the quick oxidation is followed.
4. The Ehrlich's Haematoxylin Staining solution is commonly used for staining. Its compositions are:

1. Haematoxylin	-	2 gms
2. Potassium alum	-	10 gms
3. Alcohol (ethyl)	-	100 ml
4. Glycerine	-	100 ml
5. Distilled water	-	100 ml
6. Glacial acetic acid	-	10 ml

Dissolve haematoxylin in alcohol. Then add the above ingredients in the order given. Place the bottle in sunlight for 6-8 weeks until the solution ripens deep red is colour. The stains needs no filtrations and keep for years.

Eosin

Eosin is a dye of xanthetic group and is derived from flurescein. It stains cytoplasm and connective tissue in different shades and is usfully differentiable stain. Eosin Y (eosin yellowish), readily soluble in water and less so in alcohol. is most commonly used. A 0.2% solution in 951% alcohol is generally used for counter staining the sections in which nuclei are stained blue with haematoxylin. Some people prefer a stock solution prepared in tap water and regard the alkalinity of tap water to give superior results. They add a crystal of thymol or a few drops of formalin to prevent mould growth and if grow, they may be removed by filtration. From the stock solution. 1% eosin is usually prepared as a working solution. It is prepared from the following combinations:

Eosin powder	1.00 gm
70% ethyl alcohol	100 ml.



MODEL QUESTIONS-1

PRACTICAL-III: DEVELOPMENTAL BIOLOGY, IMMUNOLOGY, ANIMAL PHYSIOLOGY AND MICROTECHNIQUE

Time: 3 Hours

Maximum: 60 Marks

1. Prepare a sperm suspension of the given specimen A. Record the duration of motility of at least 5 sperm. 10 Marks

2. a) Write down the procedure adopted for raising an antibody.
 b) Write about the technique involved in the test slide B.
 c) Mention the precipitin reaction in the given slide C. 15 Marks

3. Estimate the amounts of Ammonia present in the given samples D & E and write down procedure. 10 Marks

4. Identify and comment on the uses of F & G. 10 Marks

5. Record with field work report and slide submission, 10 + 5 Marks

Total

60 Marks

Key

1. A – Cattle live Semen

2. B – Manssoni's Ring test
 C – Immunoelectrophoresis

3. D – Fish tank water – I
 E – Fish tank water – II

4. F– Eosin
 G – DPX Mountant

MODEL QUESTIONS-2**PRACTICAL-III: DEVELOPMENTAL BIOLOGY, IMMUNOLOGY, ANIMAL PHYSIOLOGY AND MICROTECHNIQUE****Time: 3 Hours****Maximum: 60 Marks**

1. Prepare a vaginal smear of the specimen provided A. Observe under the microscope and identify the stage of oestrous cycle giving proper reasons.
10 Marks
2. a) Identify and write notes on B.
b) Comment on the procedure adopted for the technique C.
c) Mention the precipitin reaction in the given slide D.
15 Marks
3. Quantify the amount of UREA present in the samples D & E. Write down procedure adopted and comments on the results obtained.
10 Marks
4. Identify and comment on the uses of F & G.
10 Marks
5. Record with field work report and slide submission,
10 + 5 Marks

Total**60 Marks****Key**

1. A – Female Rat
2. B – Haemagglutination test
C – Ouchterlony Double Immunodiffusion Plate
D – Immunoelectrophoresis slide
4. E – Microtome
F – Xylene

MODEL QUESTIONS-3

PRACTICAL-III: DEVELOPMENTAL BIOLOGY, IMMUNOLOGY, ANIMAL PHYSIOLOGY AND MICROTECHNIQUE

Time: 3 Hours

Maximum: 60 Marks

1. Prepare a hanging drop of the sperm suspension of the sample A provided. Find the rate of sperm motility. 10 Marks

2. a) Write down the procedure adopted for the preparation of antigen.
b) Comment on the structure and function of the lymphoid organ B.
c) Write down the technique involved in C. 15 Marks

3. Quantify the amount of UREA present in the samples D & E. Write down procedure adopted and comments on the results obtained. 10 Marks

4. Identify and comment on the uses of F & G. 10 Marks

5. Record with field work report and slide submission, 10 + 5 Marks

Total

60 Marks

Key

1. A – Bull semen

2. B – Spleen of Rat/Mouse
C – Double Immunodiffusion plate

3. D – Urine sample – I
E – Urine sample – II

4. F – Hot infiltration of wax
G – Alcohol grades.

MODEL QUESTIONS-4

PRACTICAL-III: DEVELOPMENTAL BIOLOGY, IMMUNOLOGY, ANIMAL PHYSIOLOGY AND MICROTECHNIQUE

Time: 3 Hours

Maximum: 60 Marks

1. Quantitatively estimate the amount of urea present in the given sample and write the procedure.
15 Marks
2. Make a live smear of spermatozoa of the given animal and draw a labeled sketch of the sperm you have observed.
10 Marks
3. Stain the two-given slides and write the technique adopted by you.
10 Marks
4. Demonstrate the antigen-antibody interaction with the serum provided and, comment on your result.
10 Marks
5. Record with field work report and slide submission.

Total

10 + 5 Marks
60 Marks

Key

1. Urine sample/Blood
2. Cattle live Semen
3. Two unstained sections
5. Anti Serum A and B.

ENVIRONMENTAL BIOLOGY

PART – IV: ENVIRONMENTAL BIOLOGY

REPORT ON SANDY FAUNA AND THEIR ADAPTATIONS

Expt. No. 1

Date:

Aim

To study the characteristics of sandy seashore and adaptations of sandy shore fauna.

Characteristics of inter-tidal sandy shore

The eulittoral sandy shore is formed by sandy substratum. This zone is subjected to high and low tides. Sandy shore offers no surface for attachment like rock. Hence animals of this region lead burrowing mode of life to avoid wave action and desiccation. Seaweeds are absent and the organic detritus forms the food. Hence this biotope is poor in its faunal content.

Faunal composition of sandy shore

Amphioxus, *Balanoglossus*, *Albunea*, *Hippa*, *Chaetopterus*, *Arenicola*, *Terebella*, *Anadara*, *Astropecten*, etc. are found in the sandy shore.

Adaptations of sandy shore fauna

1. Amphioxus

Amphioxus (Fig. 199) is a Cephalochordate living in the intertidal shallow sandy shore bottom. It has several adaptations.

1. It leads a burrowing mode of life to avoid impact of wave action when it is buried, its anterior end is projecting outside. As the anterior end is pointed with stiff notochord, animal is able to burrow efficiently.
2. It is a ciliary mucoid feeder. The cilia in the wheel organ and the gill bars of the pharynx beat to produce feeding current. Velar tentacles prevent the entry of sand particles. Small organisms are filtered and concentrated in the pharynx by the cilia. The mucus secreted by endostyle, entangles food organisms.

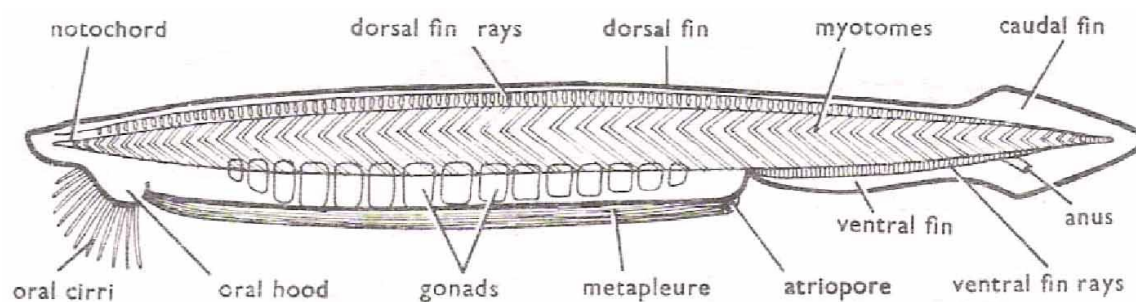


Fig. 199. *Amphioxus*.

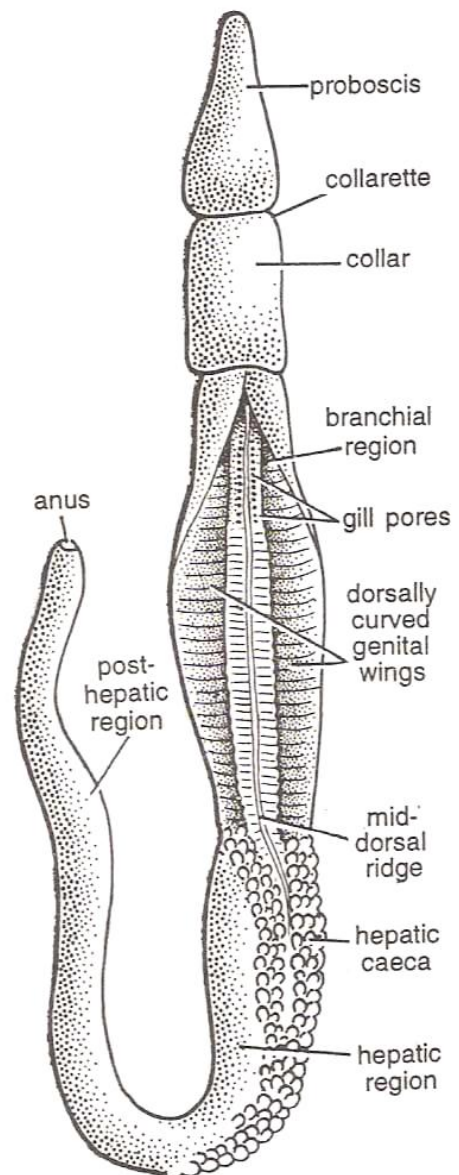
3. The vascularized lining of the gill slits of the pharynx is used for respiration. Food current also serves as a respiratory current.
4. Occasionally *Amphioxus* emerges from the sand and swims rapidly in water by the lateral movements of its body. The alternately arranged 'V' shaped muscle blocks (myotomes) and the continuous fin fold (a dorsal, a caudal and a ventral fin) serve for locomotion.

5. Ocelli in the nerve cord are sensitive to light. They may help in the orientation of the animal as it burrows into sand.

2. Balanoglossus:

Balanoglossus (Fig. 200) is a hemichordate

1. It leads a burrowing mode of life to avoid wave action. It lives in 'U' shaped burrows excavated in the sandy bottom. The walls of the tube are lined with mucus secreted by the animal. They are opened at both ends.
2. The body is long and cylindrical adapted for burrowing mode of life. The pointed muscular proboscis helps in burrowing.
3. It is a mud feeder like an earthworm. But non-burrowing forms are ciliary feeders. While burrowing it takes inorganic food matter along with mud and water. Ciliary currents produced by cilia of gill clefts bring this about. The undigested matters along with sand grains are usually seen at the posterior opening of the burrow as spiral coils of faeces (like the castings of earthworm). In non-burrowing forms, cilia and mucus of proboscis play a role in food collection.
4. Water, which enters the pharynx during feeding current is sent out by gill clefts. The gill clefts are the respiratory organs.
- 2 It has the power of regeneration. The ciliated free swimming tornaria larva of Balanoglossus is advantageous for the propagation of the race.



following ada

Fig. 200. Balanoglossus.

3. Albunea

Albunea (Fig. 201) is a sandy shore burrowing decapod crustacean. It has certain adaptations to lead a life in sandy shore.

1. It burrows into sand to avoid the impact of wave action prevailing in the sandy beach. If it does not get buried it will be washed away by the waves. It uses its powerful thoracic legs to make burrows.
- 3 The thoracic legs are short and stout with claws suitable for walking and burrowing. The first pair of legs is in the form of Chelipods.
- 4 It has a special device for breathing. When the animal is buried, the setose antennules are projecting outside. These antennules are closely held together to form a long tube to draw water for respiration. Thus antennules form a respiratory siphon.

- 5 The antennae act as filters to collect plankton and organic particles. Hence it is a filter feeder. The mouth appendages are so arranged as to prevent the entry of sand grains.
- 6 The colour of the Albunea matches with the colour of the sand and escape from enemies. This is referred to as protective colouration.

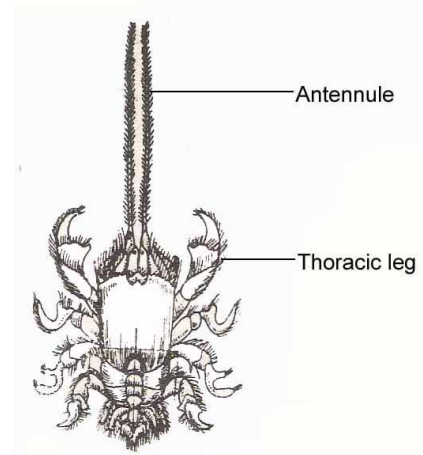


Fig. 201. Albunea.

4. HIPPA (EMERITA)

Hippa (Fig. 202) is a sandy shore burrowing decapod crustacean adapted to live in intertidal zone. It is commonly called **mole crab**.

1. The body is oval in shape with large Carapace, which gives protection and prevents desiccation. The abdomen remains flexed beneath the thorax.
2. Hippa is found buried in sand to prevent itself from being washed away by the waves. Out of 7 pairs of thoracic appendages, first 2 pairs are partially chelate and others are pointed so as to dig holes in the sandy substratum. Thus the animal burrows with the legs.
3. Out of 6 abdominal appendages, first 3 are swimming appendages. They produce water current to bathe the gills. Gills are attached to the thoracic legs. They are respiratory organs.
4. The last three abdominal appendages are called Uropods. They are directed posteriorly. They are used for darting.
5. Hippa is a filter feeder. This crab while buried in sand projects its long densely fringed second antennae above the sand surface and these antennae filter the plankton and detritus from the receding wave current.

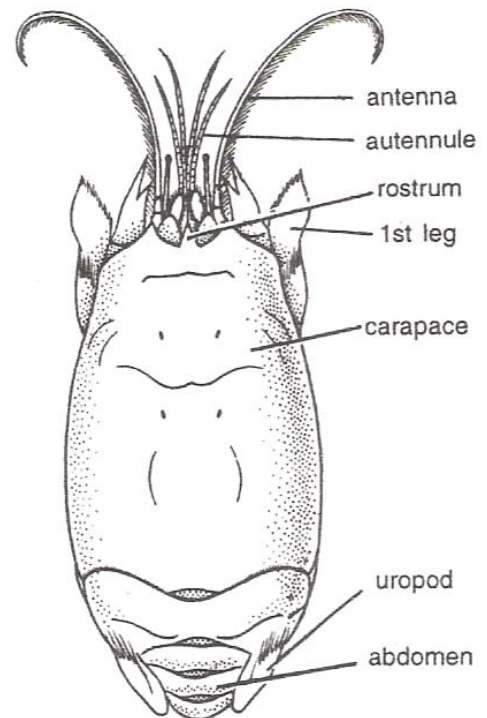


Fig. 202. Hippa.

5. CHAETOPTERUS

Chaetopterus (Fig. 203) is a ploychaete worm found in sandy shore. It is commonly called as paddle worm. It is a tubicolous form having following adaptations.

1. Chaetopterus lives permanently in a U-shaped tube made of sand and mucus. The tube has an incurrent and an excurrent opening. The tube provides a safe place for the worm to escape from the impact of wave action, which is common in sandy shore.
2. The parapodium in the middle region of the body is variously modified for the tubicolous mode of life. They are: (a) a pair of wings (modified notopodia of 10th segment) (b) a pair of suckers and a food cup (modified parapodia of 11th segment) (c) 3 pairs of fans (modified notopodia of 12 to 14th segments).

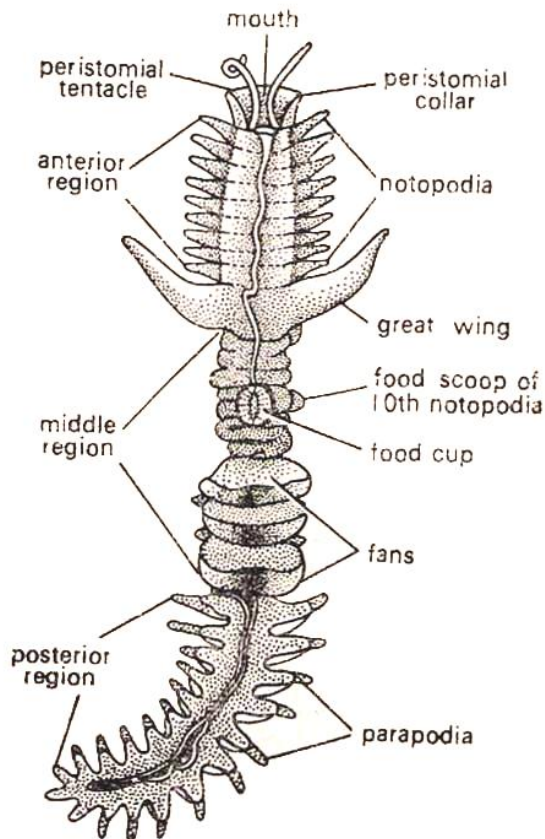


Fig. 203. Chaetopterus.

6. ARENICOLA

Arenicola (Fig. 204) is a tubicolous and burrowing polychaete found in sandy sea bottom. It has following adaptations to lead life in the sandy substratum.

1. This worm lives in J-shaped burrow made of sand and mucus. The burrowing mode of life is necessary to escape from waves prevailing in the intertidal sandy shore.
2. Body is stout elongated and cylindrical suitable for a life in burrows. It is greenish in colour the segments of the body are divided into annuli.
3. At the anterior end there is 'head' formed by Prostomium, peristomium and the next segment. The remaining segments bear parapodia.
4. The buccal mass is everted as proboscis through the mouth. In feeding, the proboscis is forced into the sand then retracted with a load of sand from which organic matter is digested. Thus proboscis is used for feeding.

Proboscis is also used for locomotion.

5. The middle region of the body has 13 segments of which first 11 bear 11 pairs of gills for respiration. The gills are branched and of red colour they modified cirri of notopodia. The worm pumps water through the incurrent opening for respiration there

3. Chaetopterus is a ciliary mucoid feeder. The fans and unmodified parapodia of posterior end produce-feeding current by their rhythmic flapping. Water along with food particles enters through the incurrent opening of the tube. A mucus bag secreted by wing like parapodia immediately collects the food particles. The food-laden mucus cord is then sent into a food cup by the ciliate beat and from there it moves forward to enter the mouth.
4. The sucking discs keep the worm temporarily attached to the tube.
5. Chaetopterus exhibits bioluminescence. It produces bluish green light.

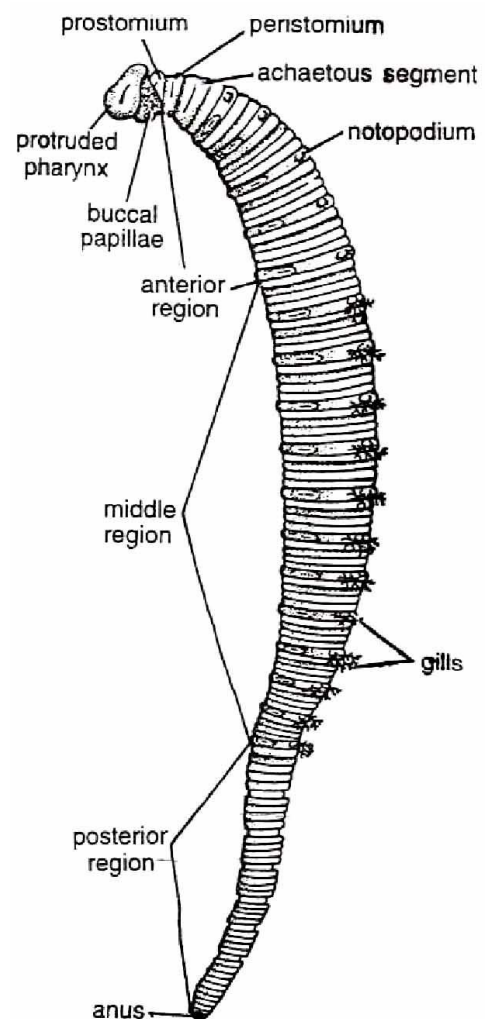


Fig. 204. Arenicola.

are parapodia in the middle region of the body also. They bear tuft of setae. The posterior region is narrow without parapodia. There is a terminal anus through which mud passes out as casting.

6. TEREbella

Terebella (Fig. 205) is a burrowing polychaete adapted to sandy sea bottom.

1. Body is elongated, cylindrical, broad anteriorly and narrower posteriorly. This is most suitable for borrowing mode of life. Animal escapes from wave action by being buried.
2. Head is made of Prostomium that contains several long filiform tentacles. The tentacles have grooves, which lead to the mouth. By ciliary action food particles are sent along the groove into the mouth. Thus tentacles are used for feeding.
3. There are 2 pairs of eyes sensitive to light.
4. The gills are branched found on the dorsal surface of few anterior segments. They are respiratory organs.
5. On the dorsal side of the body there are notopodia with simple setae. On the ventral side there are neuropodia with hooked setae. There is diaphragm dividing the body into thorax and abdomen.
6. The ventral surface of the body has many glands which secrete mucus. It is used to build tubes out of foreign bodies.

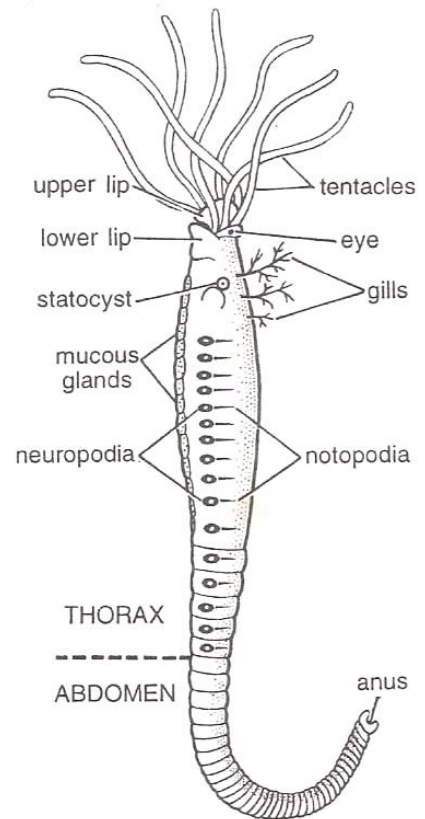


Fig. 205. Terebella.

REPORT ON MUDDY FAUNA AND THEIR ADAPTATIONS

Expt. No: 2

Date :

Aim

To study the characteristics of muddy shore and adaptations of muddy shore fauna.

Characteristics of inter-tidal muddy shore

The inter-tidal Muddy shore is found in quiet bays and estuaries. The substratum is soft formed by the deposition of silt, clay and organic matter brought in by the rivers. There is feeble wave action. The muddy substratum provides rich food supply in the form of detritus and deficiency of oxygen.

Muddy shore fauna

Nereis, Aphrodite, Gelasimus Crab, Asterias, Ascidian, Boleophthahmus, etc.

Adaptations of muddy shore fauna

1. NEREIS

Nereis (Fig. 206) is a burrowing polychaete adapted to muddy sea shore and estuaries. Its adaptations are as follows:

1. As the substratum is soft, Nereis makes holes in the mud easily with the help of prostomium and live in burrows. The body is cylindrical and elongated with about 200 segments. This is suitable for burrowing mode of life.
2. The prostomium carries the prostomial tentacles, palps and eyes to explore the surrounding.
3. Each segment has a pair of parapodia for locomotion and respiration.
4. Nereis is nocturnal in habit and hiding itself during day time.
5. Nereis creates the current of water with the help of tentacles and parapodia which bring in detritus (Decaying organic matter). In the case of deposit feeding, eversible pharynx is applied to the substratum and detritus is ingested.
6. Nereis can tolerate wide range of salinity variations (Euryhaline). The blood and tissue fluids are isotonic with the surrounding environment.
7. It has the power of regeneration. It is a device to increase its population at a faster rate.
8. Reproductive individuals namely epitokes are formed during breeding season. Epitokes have a modified posterior region packed with gametes. They have swimming setae. They swim to the surface and shed their gametes during lunar spring tides (Lunar periodicity).

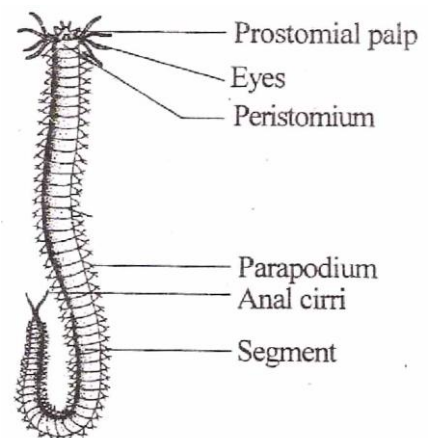


Fig. 206. Nereis.

2. APHRODITE

Aphrodite (Fig. 207) is a polychaete, which is adapted to live in muddy sea shore.

1. It is popularly called "Sea-Mouse", because the setae covering the body give the appearance of mouse. It rolls itself with stiff setae like porcupine to escape from enemies.
2. Parapodia are locomotor organs. They are greatly modified. The dorsal cirri of Notopodia become plate like Elytra on the back of the animal. The notopodia contain 3 kinds of setae: a) stiff setae, b) soft setae and c) iridescent setae.
3. The Elytra are completely covered with iridescent setae. The soft setae form a blanket like protective covering dorsally. During movements colour changes from brown to golden.
4. The dorsal surface is convex while the ventral surface is flat forming a creeping sole.
5. By the pumping action of the dorsal body wall water can be drawn into the space between the "Blanket" and the back. This helps in respiration.

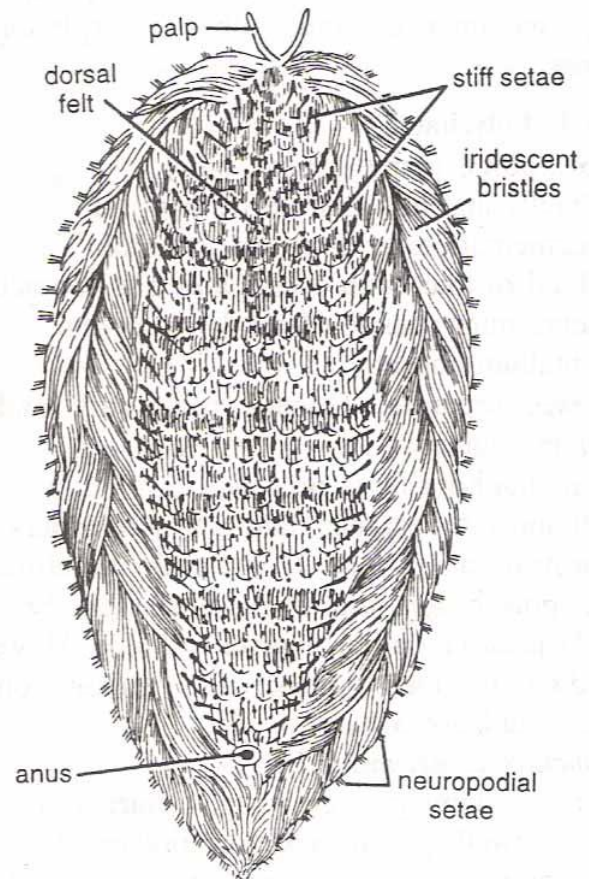


Fig. 207. Aphrodite.

3. GELASIMUS CRAB

1. Gelasimus Crab (Fig. 208) or Uca is a decapod crustacean found in muddy beach or estuary. It has peculiar adaptations to live in muddy substratum.
2. This crab lives in burrows. It excavates burrow in the soft mud with the help of its well developed Chelae of the enlarged left leg.

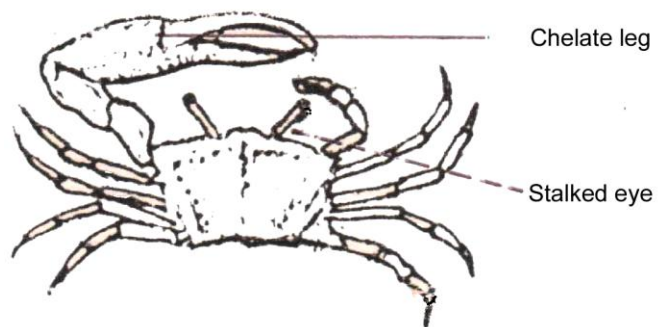


Fig. 208. Gelasimus crab.

3. It comes out of hole very often and runs swiftly here and there. When it waves its large chelate leg beautifully, it looks like a person who plays the fiddle and hence this crab is also called fiddler crab.
4. The crab is partially adapted to air-breathing to overcome the deficiency of oxygen in the muddy substratum. The gill chambers have vascularised wall for air-breathing.
5. It is a detritus feeder. It separates organic detritus particles from non-organic matter in the mud by filtering the substrate through a set of fine hairs around the mouth.
6. The carapace on the dorsal side gives protection from desiccation.

7. Legs are adapted for swift running. The stalked eyes provide an all round vision. This enables the crab to get into the holes immediately on seeing enemies.

4. STARFISH (ASTERIAS)

Starfish (Fig. 209) is an echinoderm adapted to muddy sea-shore.

1. Starfish has a central disc and 5 flexible radiating arms. The body wall consists of calcareous ossicles and spines. They give protection to the body.
2. It swims in water with the help of arms.
3. It can also creep over the substratum by the co-oriented action of the arms and the tube feet. The tube feet are arranged in two rows in the ambulacral groove of each arm. The tube feet are connected with water vascular system. Sea water enters the water vascular system through the madreporite. Finally water is forced into the tube feet by ampullae, as a result of this, tube feet are protruded and suckers are applied to the substratum. Then the longitudinal muscle of the tube feet contract and the animal is drawn forward.
4. Starfish is a voracious animal and feeds on crustaceans worms and bivalves. During feeding starfish bends its arms over the mussel and attaches tube feet with suckers to the shell valves and exerts a pull to separate the valves. As soon as the valves are separated, Starfish everts its stomach through the mouth and swallows the soft body of the mussel. Prey is digested in the stomach outside the body. Then stomach is withdrawn.
5. Respiration is effected by the dermal branchia.
6. Pedicellariae are used from cleaning the surface of the body. They are also defensive in function.
7. Asterias regenerates its injured parts. Power of regeneration is a device to increase the population at a faster rate.

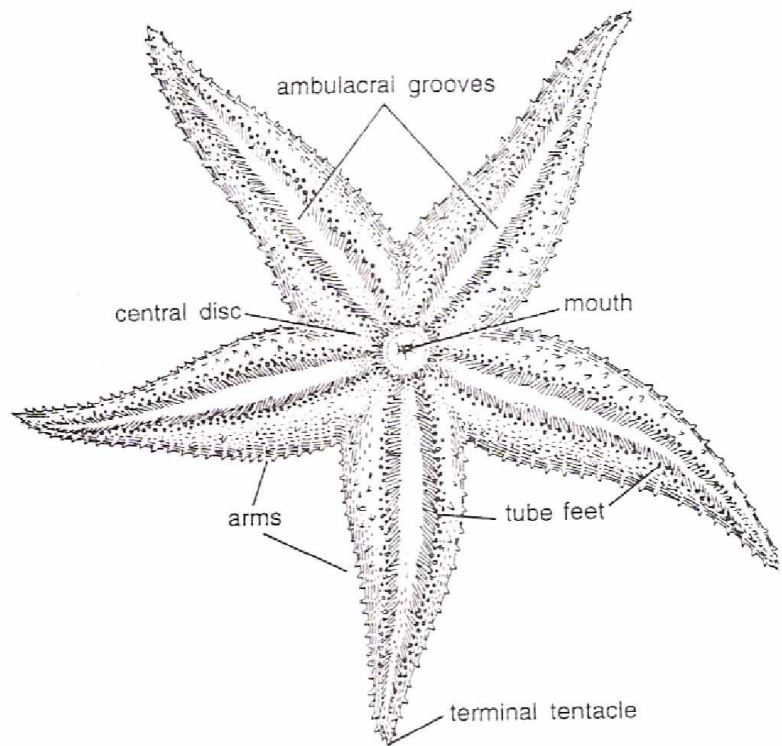


Fig. 209. Star fish

5. ASCIDIAN

Ascidian (Fig. 210) is a prochordate found in muddy shore. It has following adaptations

1. It leads a sedentary mode of life. The bag like body is covered and protected by a test or tunic. Due to its sedentary life mouth (Oral Siphon) and Anus (Atrial Siphon) are brought to the free end at the same level.

2. Ascidian is a ciliary mucoid feeder. The water current is set up by the beating of cilia in the Pharynx. The water current brings in smaller organisms which are entangled by the mucus secreted by the endostyle. The food-laden mucus cord is thus prepared by the Pharynx before sending to the stomach.
3. When the animal is disturbed it squirts out a jet of water through the atrial siphon to terrify the enemies (hence the name Sea-squirt).
4. Ascidian is hermaphrodite. Hermaphroditism is necessary for the sedentary animals like Ascidian, because fertilization is certain in Hermaphrodites.

Though the ascidian is sedentary its larva is free swimming. This is necessary for the dispersal of the species.

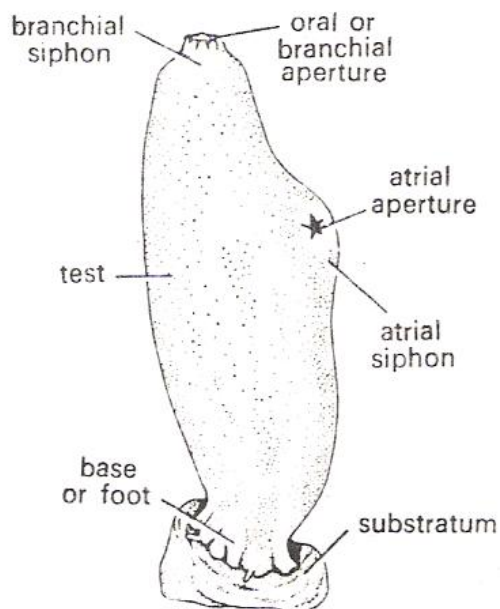


Fig. 210. Ascidian.

6. BOLEOPHTHALMUS

1. Boleophthalmus (Fig. 211) is a fish. It is commonly called Mud skipper. It is found crawling on the muddy beach and estuary.
2. It lives in burrows of the mud. But it comes out of the burrow and spends most of its time out of water, crawling on the mud flat. It uses its burrow for refuge and breeding.
3. It moves fast with the help of strong pectoral fins (Skipping motion). The pectoral fins are broad and well developed for crawling.
4. The eyes are big and set high on the head so as to have a clear all round vision. This enables the fish to get into the hole immediately at our approach.
5. As Oxygen content is poor in muddy sea shore Boleophthalmus uses its accessory respiratory organs for aerial respiration. This is accomplished by vascularized sacs in the mouth cavity and gill chambers. The gills are reduced. The vascularised caudal fin is also used as accessory respiratory organ.
6. It feeds on detritus.
7. The male fish is distinguished from the female by having a long fin ray of the dorsal fin (Sexual dimorphism).

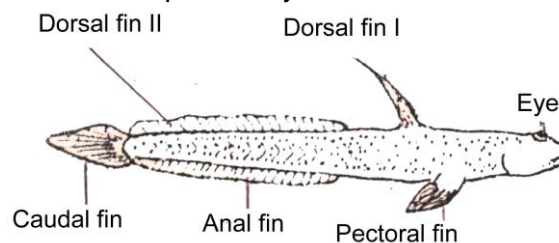


Fig. 211. Boleophthalmus.

REPORT ON ROCKY FAUNA AND THEIR ADAPTATIONS

Expt. No: 3

Date

Aim

To study the characteristics of rocky shore and adaptations of rocky shore fauna.

Characteristics of inter-tidal rocky shore

The eulittoral rocky seashore is characterised by the presence of a firm rocky substratum. Rocky shore is subjected to high tides and low tides. Waves dash over the rocks. Unless the animals have adaptations for firm attachments to rock, they will be washed away. At low tide, rocks are exposed to air. Hence animals should have adaptations to prevent water loss as well as to escape from enemies. Seaweeds are abundant and hence this biotope is richer in faunal content.

Faunal composition of rocky shore:

Sea anemone, Zoanthus, Balanus, Chiton, Mytilus, Patella, Hermit Crab, Sea Urchin, Asterias (Starfish) etc.

1. SEA ANEMONE

Sea anemone (Fig. 212) is a solitary anthozoan (Coelenterate) found attached to rock of eulittoral zone of sea. It has adaptations to face the danger of desiccation and wave action.

1. In the rocky shore waves dash over rocks. So, to escape from the impact of wave action Sea anemone is firmly found attached to rock with the help of a pedal disc.
2. It has several tentacles surrounding the mouth. Tentacles are provided with nematocysts. When the prey comes into contact with tentacles, it is paralysed by the nematocysts and taken to the mouth by the tentacles. Digestion is in the gastrovascular cavity.
3. Mouth leads into a stomodaeum which has ciliated longitudinal grooves called siphonoglyphs. Water enters into the gastrovascular cavity through one groove and passes out through the other. Stomodaeum is partitioned by mesenteries, which strengthens the column.
4. As sea anemone leads sedentary life, it may fall an easy prey to carnivores. So in order to escape from enemies it uses its stinging cells. The stinging cells have poison to kill the enemies. Moreover, acontial threads of mesenteries are defensive in function.
5. Under unfavourable conditions sea-anemone shortens its length and contracts its tentacles. This is carried out by the rapid contraction of longitudinal muscles.
6. Sea anemone forms symbiotic relationship with hermit crab.

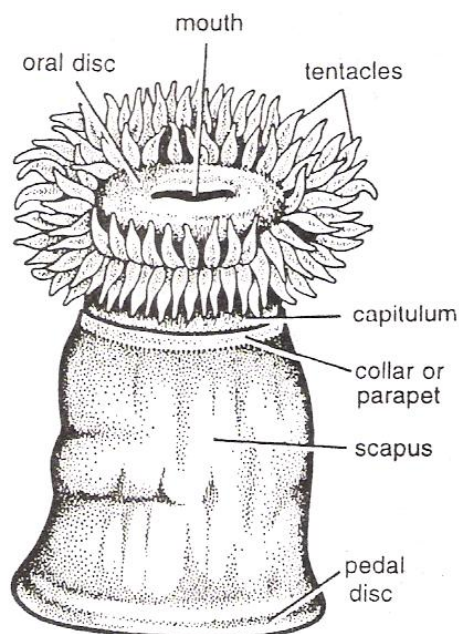


Fig. 212. Sea anemone.

2. ZOANTHUS

Zoanthus (Fig. 213) is a colonial anthozoan resembling sea anemones, found attached to rocks in the inter-tidal rocky shore. It has following adaptations.

1. Zoanthus colony consists of many polyps. They are united by basal stolons and attached firmly to the substratum. This prevents the colony from being washed away by the waves.
2. Body wall is encrusted with sand grains. This strengthens the body.
3. Each polyp of the colony resembles sea anemone in structure consisting of oral disc and column. The oral disc has mouth surrounded by tentacles. The nematocysts of tentacles paralyse the prey.
4. New polyps arise from stolon by budding. This rapid power of reproduction increases the size of the colony.
5. Zoanthus is epizoic i.e. it is found on other animals such as Corals and Sponges.

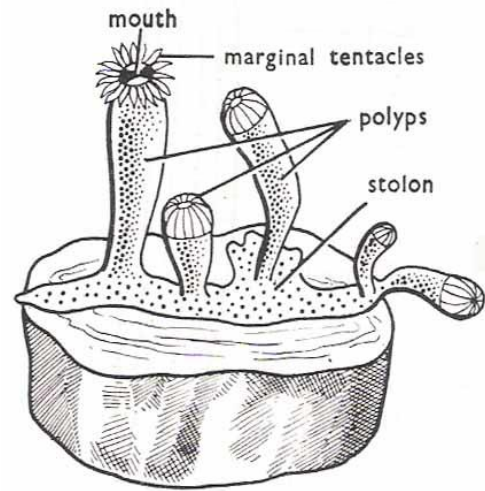


Fig. 213. Zoanthus.

3. BALANUS

Balanus (Fig. 214) is a marine sedentary crustacean. It is found attached to rocks and molluscan shells between the tide marks. It is adapted to rocky substratum.

1. Balanus is attached to rocks in great numbers. The attached end has two vestigial antennules with cement glands. The secretion of cement glands is used for fixing the animal to the substratum. This helps the animal to escape from wave action. Balanus is commonly called acorn barnacle.
2. There are 6 calcareous plates surrounding the body. They not only give protection but also prevent desiccation. The plates are Carina, Rostrum and 2 pairs of Carina lateral plates.
3. The opening of the shell is provided with a lid composed of scuta and 2 terga. Water enters into the mantle cavity through this hole. Whenever Balanus is exposed to air, shell is tightly closed retaining water inside. An air bubble is imprisoned which serves as an additional Oxygen source. If Barnacle is forced to live out of water it can even survive for about 44 days.
4. Thorax has 6 pairs of biramous thoracic legs which are many jointed and beset with cirri or spines (hence the name cirriped). These appendages protrude through the shell opening and collect the food by their constant movements.

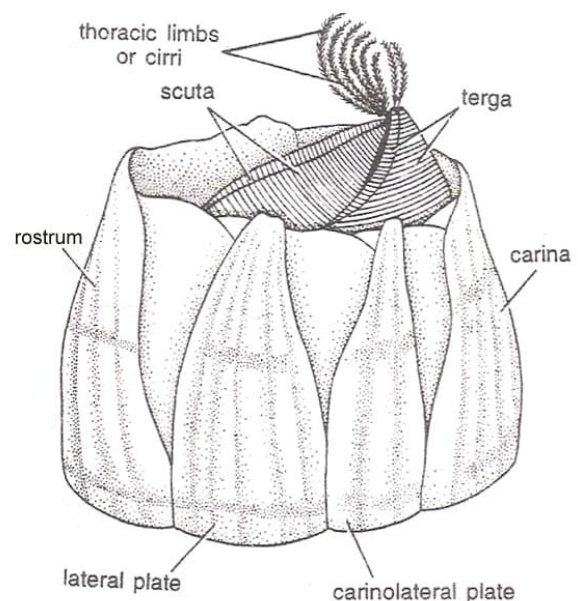


Fig. 214. Balanus.

5. The animal is hermaphrodite. This enhances chances of fertilization. This is necessary for the sessile animal.
6. Life cycle includes a free-swimming nauplius and cypris larva (meroplankton) which is necessary for the dispersal of the race.

4. CHITON

Chiton (Fig. 215) is a marine sluggish slow moving mollusc attached to rock, which is exposed at low tides. It has following adaptations to lead a life in rocky substratum.

1. Body is elliptical and dorso ventrally flattened so as to provide a large surface area for attachment. A flat sole like foot extending along the whole length of the body serves for creeping and adhering to the substratum. This attachment prevents the body from being washed away by the waves dashing over the rocks.
2. A Calcareous shell present on the dorsal side not only prevents water loss (dessiccation) but also protects the body.
3. As the shell is composed of 8 overlapping plates hinged on one another, the animal is able to roll itself like a millipede. Chiton behaves like this when it is disturbed.
4. It is mostly nocturnal and remains concealed under rocks during daytime.
5. It is a vegetable feeder and its food consists of algae and diatoms.

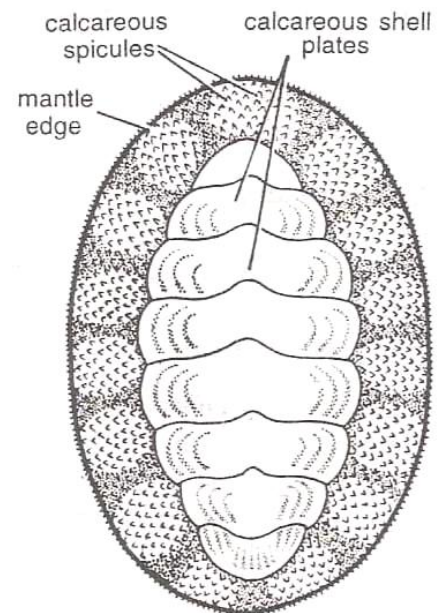


Fig. 215. Chiton.

6. MYTILUS

1. Mytilus (Fig. 216) is a bivalve mollusc found attached to rock in the inter-tidal rocky shore. It has following adaptations to solve two main problems namely impact of wave action and desiccation.
2. Mytilus is found attached firmly to rock by its byssus threads to escape from the impact of wave action. There are byssus glands in the foot region. Their secretion becomes hardened into byssus threads.
3. Even though Mytilus is sedentary, foot is modified to creep.
4. This sedentary bivalve has a shell made of two halves, which are broad at the posterior end and narrow at the anterior end. The adductor muscles are well developed to close the shell. This protects the body and prevents desiccation.

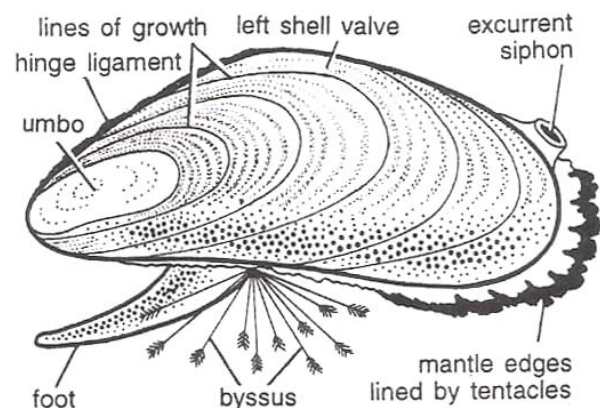


Fig. 216. Mytilus.

5. It is a filter feeder. It filters planktonic organisms from the incurrent waters. Incurrent water enters the mantle cavity through the inhalent siphon. The excrements pass out through the exhalent siphon.
6. Feeding current is used as respiratory current to carry out gill respiration. The gills are lamelliform.

6. PATELLA

Patella is commonly called as limpet (Fig. 217) is a gastropod mollusc found on rocky beaches between tide marks. It is endowed with following adaptations.

1. The dorsal side of the body is enclosed with a roundish shell which is raised into a conical elevation. The shell protects the animal from desiccation as the animal is exposed at low tides.
2. It is firmly attached to rock to face the impact of wave action. A large foot is there for attachment. Since the foot is attached there is no operculum.
3. The large and broad sole of the foot is also used for creeping.
4. The head contains two stout sensory tentacles to explore the surrounding.
5. It feeds on sea-weeds. Teeth are strong and pillar shaped to cut the vegetation into pieces.
6. Ctenidia are absent. But respiration is carried out by the process of the mantle (pallial branchiae) present in a circle between the mantle and foot.

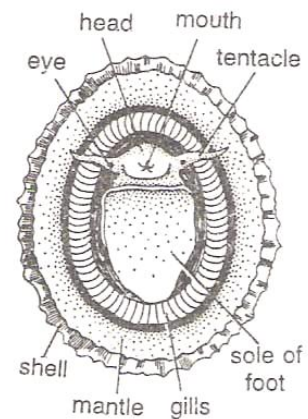
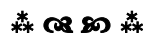


Fig. 217. Patella.



REPORT ON DEEP SEA FAUNA AND THEIR ADAPTATIONS

Expt. No: 4

Date :

Aim

To study the characteristics of deep sea and adaptations of deep sea fauna.

Characteristics of deep sea

The average depth of the ocean is 3800 meters or 12,500 feet. Little or no light (absence of light) penetrates this area of the ocean, and most of its organisms rely on falling organic matter produced in the photic zone for subsistence. Vegetation or photosynthetic organisms are completely absent. Most of the environmental factors are unchanging in deep sea. Tides and waves are completely absent. The water pressure is high in the deep sea. The temperature is low in the deep sea system.

Deep sea fauna

Jelly fish, Sebella, Limulus, Octopus, Crinoid, Angler Fish,

Adaptations of deep sea fauna

1. JELLY-FISH

16. *Aurelia* (Fig. 218) is the commonest jelly-fish.
17. The medusa or umbrella has a slightly convex upper surface known as umbrellar surface and a lower concave, the subumbrellar surface.
18. The margin of the umbrella is divided into eight lobes or lappets by notches. Each notch contains a tentaculocyst or rhopalium enclosed by a pair of marginal lappets.
19. Numerous short, hollow tentacles are present all round along the margin of the umbrella and are known as marginal tentacles.
20. The mouth is four cornered situated on the short manubrium, which hangs down in the centre of subumbrellar surface.
21. Each corner of the mouth is drawn out into a long frilled, tapering process, the oral arm. The four oral arms lie along the four per radii.
22. On the subumbrellar surface lying between the oral arms are four rounded apertures leading into shallow pouches called sub genital pits.
23. Habit and habitat: *Aurelia* is a solitary, marine jelly-fish.
24. Distribution: *Aurelia* is found in coastal waters of all oceans of the world.
25. Jellyfish inhabit every major oceanic area of the world and are capable of withstanding a wide range of temperatures and salinities. Most live in shallow coastal waters, but a few inhabit depths of 12,000 feet.

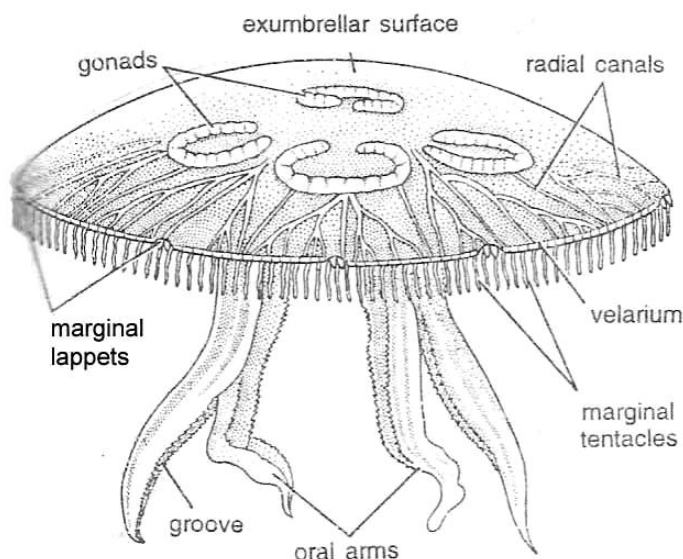


Fig. 218. Jelly-fish.

2. SEBELLA,

1. The fan worm, *Sebella spallanzani* (Fig. 219) is a polychaete adapted to live in deep sea. Its adaptations are as follows:
2. Habitat: The fan worm is generally found in shallow and deep sea. They readily form large, dense mats of individuals and quickly outnumber their neighbors. The worm is enclosed in a hard tube (about 1 cm diameter) that is attached to hard surfaces such as rocks, pilings, shells, and even mats of sea grass. When its feeding tentacles are extended, the worm resembles a feathery palm tree.
3. Cylindrical body
4. The outer layer of tube is comprised of silt or mud and often has organisms growing on it.
5. Only one of the two groups of radioles making up the crown is in a spiral shape. The radioles are webbed for the first 5 mm. Turned down lappets are often orange in colour and the first ventral shield is the widest.

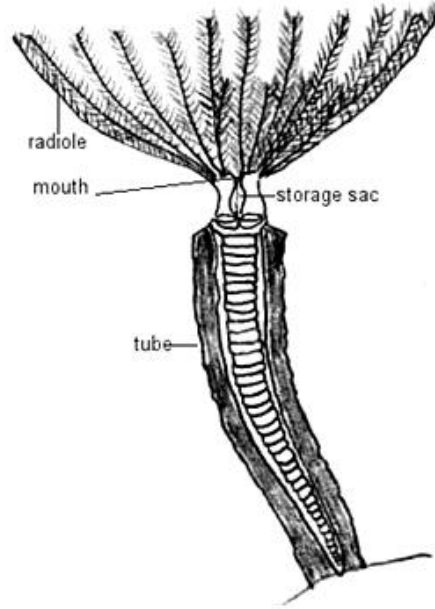


Fig. 219. *Sebella*.

3. LIMULUS

Salient features

18. *Limulus* (Fig. 220) is commonly known as **king-crab**.
19. Body consists of anterior prosoma and a posterior opisthosoma terminating in a long spike-like telson.
20. Prosoma is covered by an unsegmented, semicircular, or horse-shoe-shaped carapace.
21. Prosoma is convex above with sloping sides and bears three longitudinal ridges one median and two lateral.
22. A pair of simple median eyes and a pair of lateral compound eyes are placed on the dorsal surface of prosoma.
23. Prosoma bears six pairs of appendages grouped round the mouth, the first pair of chelate chelicerae, four pairs of chelate legs and a last pair of non-chelate legs.
24. Opisthosoma is hexagonal, movably articulated with prosoma by a transverse hinge. It is composed by the fusion of an anterior large 6-segmented mesosoma and a posterior small 3-segmented metasoma.
25. The mesosoma is covered by a dorsal shield bearing a single median row of three prominent spines and two lateral rows of six small pits.

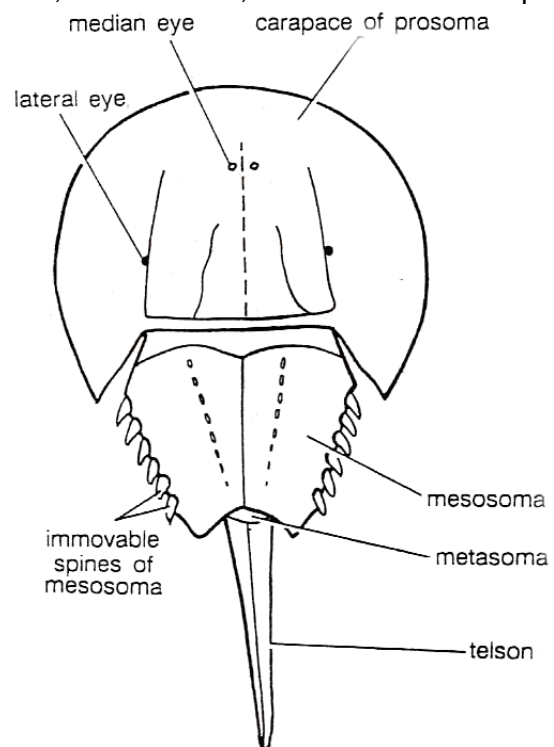


Fig. 220. *Limulus*.

26. Each lateral side of mesosoma is serrated, produced into six short immovable spines and carries six short movable spines.
27. The metasoma is much reduced. It bears the mid-ventral anus.
28. Opisthosoma bears six pairs of appendages, first pair forms genital operculum-4 the remaining five pairs carry book-lungs.
29. Respiration by book-gills.
30. Excretion by coxal or brick red glands.
31. The king-crab is of interest because it is a living member of a very ancient fossil group of animals, hence, sometimes called a living fossil.
32. Habit and habitat: *Limulus* is a marine form, found burrowing in the sand. They live comparatively in shallow waters along sandy and muddy shores of sheltered bays and estuaries. These sluggish creatures spend most of the time burrowing in sand or mud for worms, soft molluscs and small animals on which they feed.
33. Distribution: *Limulus polyphenus*, the American horse-shoe-crab, is restricted to North-western Atlantic coast, Gulf of Mexico and West Indies. All the other members are found along the South-east Asian Pacific coasts, from Japan and Korea through East Indies and Philippines.

4. OCTOPUS

1. *Octopus* (Fig. 221) is popularly known as **devil-fish**.
2. Body is globose and bag-like with large head and trunk region.
3. Head bears a pair of eyes and eight elongated equal arms webbed at the base which surrounded the mouth. Krill – bilobed eye. The Field Of Vision Of A Typical Mesopelagic Fish. Having Photophores Is Adaptive.
4. A dim *deep-red* appearance is the *characteristics* of dark-adaptation.
5. Each arm bears suckers arranged in two rows. Suckers are sessile and large.
6. Third right arm in male is modified or hectocotylized into a spoon-shaped structure which serves for transferring the spermatophores into the mantle cavity of the female for fertilizing the ova.
7. Shell is absent.
8. Mantle encloses the mantle cavity and the visceral mass.
9. *Octopus* produces inky-fluid, which diffuses in water and forms a smoky screen for defence from the enemies.

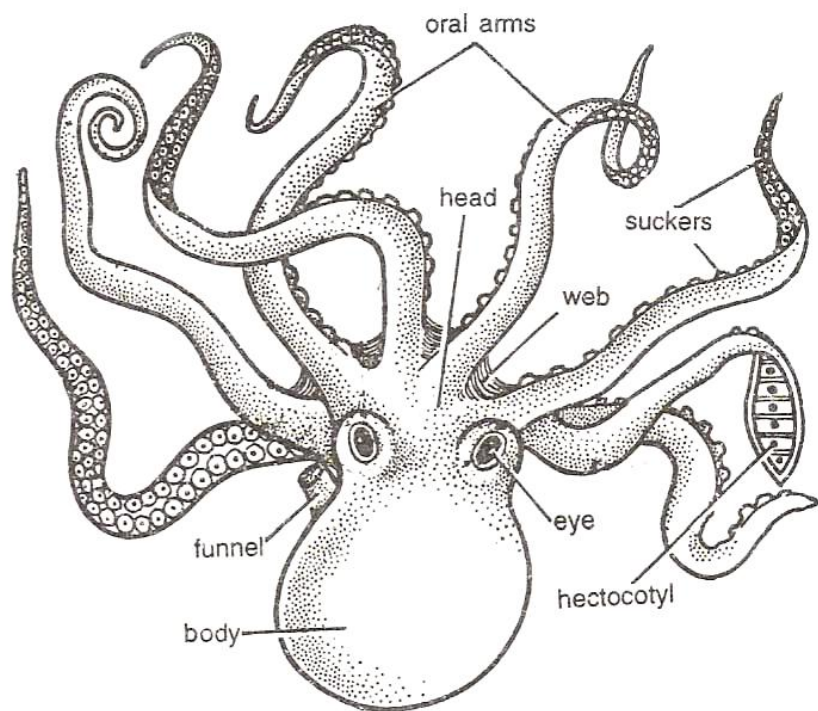


Fig. 221. Octopus.

10. The favourite food of *Octopus* consists of the crabs, bivalves, snails and fish which are seized by the quick movements of cruel snake-like arms and broken into pieces by radula and a pair of powerful sharp, beaklike horny jaws.
11. Habit and habitat: *Octopus* is a marine, bottom dwelling nocturnal cephalopod spending daylight hours in rocky crevice, shady under-water caverns and in coral reefs.
12. Distribution: *Octopus* is cosmopolitan in distribution. It is commonly found in Europe and India, Atlantic and Pacific Coasts, Alaska to lower California and Cape.

5. CRINOID

1. Crinoids (Fig. 222) are either sessile, attached to the substrate by a skeletal column.
2. The skeleton is made up of numerous calcareous plates.
3. During life, these pores are filled with soft tissues, for example, muscle.
4. The stem is segmented and composed of a number of circular (although some may be pentameral or elliptical) ossicles, held together by soft tissues.
5. The crinoid body is composed of three basic structural elements, the stem, the calyx and the arms.
6. In some crinoids, small rootlike projections from the base of the stem are used to root the crinoid into soft substrate or cement it to a hard one.
7. The calyx is built of two rings of interconnected plates forming a five-rayed symmetry. On the upper side of the cup, the mouth is situated in the centre and the anus on the periphery.
8. The arms, or brachials, extend out from the calyx, usually comprising five branching structures. These brachials are also composed of ossicles and have cilia, which gather food and pass it down the arms to the mouth.

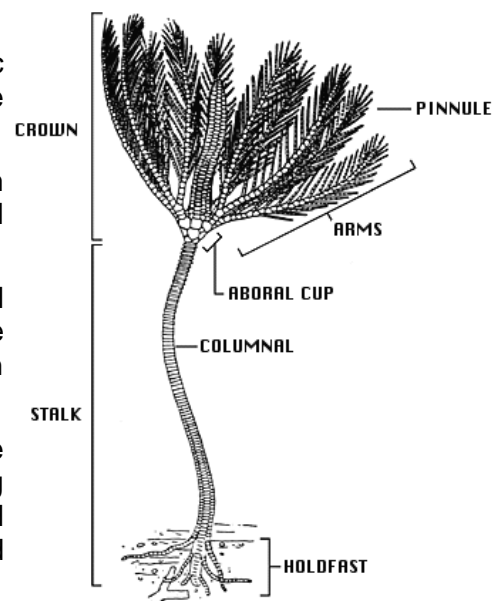


Fig. 222. Crinoid.

Angler Fish

1. The deep sea anglers(Fig. 223) or *Lophius* are found at a depth of 1500 to 6000 feet. It is other wise called as sea devil.
2. Body is depressed, dorsoventrally flattened, ugly and soft.
3. The head and anterior part of the body are very large, and without scales.
4. Mouth is large and wide containing strong cordiform or recurved teeth.
5. deep sea anglerfish is equipped with a long, thin modified dorsal fin on their heads tipped with a photophore lit with biolumenescence used to lure prey.
6. Eyes are large and lateral position; nostrils small.
7. First dorsal fin greatly modified. Its first three spines are not united by fold skin and the first spine becomes rod-like and bears a fleshy mass or bait at its tip called illicium. It is used to attract other fishes and small worms. When any curious and hungry animal comes near bait, it is ferociously attacked and eaten by angler fish.

8. Deep sea anglerfish have an interesting reproductive adaptation. Males are tiny in comparison to females and attach themselves to their mate using hooked teeth establishing a parasitic relationship for life. The blood vessels of the male merges with the female's so that he receives nourishment from her. In exchange, the female is provided with a very reliable sperm source.

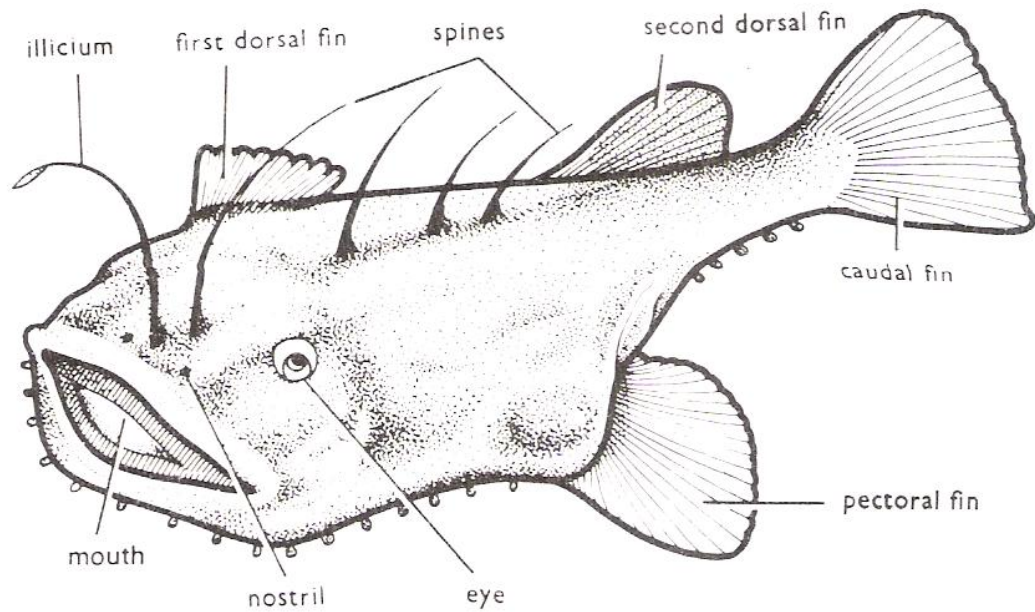
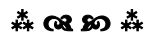


Fig. 223. Angler.



ESTIMATION OF CHLORIDES IN POLLUTED WATER SAMPLES

Expt. No. 5

Date:

Aim

To estimate the salt content of the given water Samples A and B.

Materials required

Burette with a stand, pipette, conical flask, measuring jar and porcelain tile.

Reagents

2.725% Silver Nitrate solution (27.25 grams of AgNO_3 crystals dissolved in 1 litre of distilled water (adding a few drops of dilute nitric acid to dissolve the salt if necessary) stored in black bottle. 5% Potassium Chromate solution (5 grams of $\text{K}_2\text{Cr}_2\text{O}_4$ dissolved in distilled water and made up to 100 ml)

Principle

The salinity of the water sample has a definite and constant relationship with the chlorinity (chloride ions) Chloride ions form 55% of the dissolved solids. Hence Chlorinity is first determined by precipitating the chlorides present in the sample as Silver Chloride by titrating against AgNO_3 solution using Potassium Chromate solution as indicator. The slightest excess of AgNO_3 produces a brick red colour reacting with Potassium Chromate, which is taken as endpoint. From the chlorinity salinity can be calculated by the equation.

$$\text{Salinity } \text{‰} = 0.03 + (1.805 \times \text{Chlorinity})$$

Procedure

1. A burette is cleaned with distilled water and rinsed in AgNO_3 solution. Then it is filled with 2.725% AgNO_3 solution and the initial reading is noted.
2. 5 ml of water sample A is taken in a conical flask and 3 drops of 5% Potassium Chromate solution is added with it as indicator. As a result the colour of the sample changes into yellow. This is titrated against AgNO_3 solution with constant shaking until the yellow colour of the solution turns to brick red colour. The first appearance of brick red colour is the end point. (The constant shaking of the sample while titration prevents the formation of lumps holding uncombined Chlorides). The final reading in the burette is taken. The difference between the final and initial reading gives the required amount of AgNO_3 solution for titration.
2. The experiment is repeated until two concordant values are obtained. The experiment is repeated with Sample B. The readings are tabulated (Table).

Result

Salinity of the Water Sample A = _____ ‰

Salinity of the Water Sample B = _____ ‰

Discussion

Before inferring the results of the samples A and B, the knowledge of salt content in different aquatic habitats is necessary. The amount of salinity in different aquatic bodies is described in the foregoing paragraphs.

Salinity – definition

All types of natural water contains various amounts of different salts (ions) such as Na^+ , K^+ , Mg^{++} , Cl^- , SO_4^{++} , PO_4^{++} , CO_3^- , HCO_3^- , NO_3^- , F^- , Ca^{++} , etc. and all these salts are responsible for the salt content of water.

Salinity has been defined as "the total amount of solid material in grams contained in 1 Kg. of the sample when all the carbonate has been converted into oxide, the bromine and iodine replaced by chlorine and all organic matter completely oxidised".

Salinity variation in different habitats

The salinity varies in different habitats. The salinity of seawater is 35‰ and it is rather constant. The salinity of fresh water is very low and is below 0.5‰. The salinity of estuary is intermediate between seawater and fresh water. The range of salinity in estuary varies between 1 ‰ and 35 ‰ depending on the river discharge rainfall, temperature and the influx of neritic water into the estuary. During rainy season the salinity is very low due to the heavy influx of freshwater to the estuary. But in summer the salinity is high in estuary due to the high influx of seawater and poor river discharge. The salinity may reach 40 ‰ in a bar built or closed estuary due to the evaporation of water. In salt lakes salinity may rise more than 40‰ in dry season.

Types of brackish water

Estuary or brackish water is of three kinds based on the range of salinity. They are Oligohaline, Mesohaline and Polyhaline. Oligohaline brackish waters are those having a salinity 2 ‰. Mesohaline brackish waters are those having a salinity between 2 ‰ and 20‰. Polyhaline brackish waters are those with salinities lying between 20‰ and 34‰.

Inference

Based on the facts describe in the aforesaid paragraphs, the result of the given samples may be inferred as detailed below.

It is inferred from this experiment that sample A may be..... as it contains salinity than the sample B. The sample B may be.....as it contains salinity than the A sample.

Biological importance of salinity

Salinity of water acts as an important limiting factor for the distribution of a number of species of plants and animals. Certain animals such as spider, crab, maia can tolerate only narrow fluctuations in salinity of water and are called stenohaline animals. Some like and Mytilus can withstand wider ranges of salinity and are called euryhaline forms. Generally esturine forms are euryhaline.

TABULATION AND CALCULATION

$$\text{Chlorinity of the sample} = \frac{\text{*Chlorinity of the standard Sea water} \times \text{Vol. of AgNO}_3 \text{ consumed by the sample}}{\text{Vol. of AgNO}_3 \text{ consumed by the standard Sea water}}$$

*Chlorinity of the standard Sea Water = 19.381 grams/Kg. Of water Vol. of AgNO₃ consumed by 5 ml of standard Sea water = 17.15ml

Therefore Salinity = 0.03 + (1.805 x Chlorinity of the sample).

Where 0.03 is a correction factor and 1.805 is a conversion factor.

Table 17 for Water sample A

S. No.	Vol. of Sample	Burette Reading		Vol. of the AgNO ₃ consumed
		Initial	Final	
1	5 ml			
2	5 ml			
3	5 ml			

Calculation of salinity for sample A

$$\text{Chlorinity} = \frac{19.381 \times \text{Vol. of AgNO}_3 \text{ Consumed}}{17.15}$$

$$= \text{_____ gms/kg of water.}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____})$$

$$= \text{_____ } \text{‰} \text{ (Parts per thousand).}$$

Table for Water sample B

S. No.	Vol. of Sample	Burette Reading		Vol. of the AgNO ₃ consumed
		Initial	Final	
1	5 ml			
2	5 ml			
3	5 ml			

Calculation of salinity for sample B

$$\text{Chlorinity} = \frac{19.381 \times \text{Vol. of AgNO}_3 \text{ Consumed}}{17.15}$$

$$= \text{_____ gms/kg of water.}$$

$$\text{Salinity} = 0.03 + (1.805 \times \text{_____})$$

$$= \text{_____ } \text{‰} \text{ (Parts per thousand).}$$

Result

Salinity of the Water Sample A = _____‰.

Salinity of the Water Sample B = _____‰.

ESTIMATION OF SILICATES IN POLLUTED WATER SAMPLES

Expt. No.: 6

Date :

AIM

To estimate the amount of Silicate present in the given water sample A and B.

Principle

The Silicates present in the water samples is estimated by Standard Picric Acid method. The Silicates present in the water samples react with ammonium molybdate which results in the yellow colour of the sample. This colour is compared with the colour of the picric acid.

Reagents

1. Standard picric acid solution (25 mg of dry picric acid is dissolved in 100 ml of the distilled water).
2. 10% ammonium molybdate solution.
3. 1 : 1 Sulphuric acid solution.

Materials required

Pipette, beakers and measuring jar.

Procedure

50 ml of water sample 'A' was taken in two separate 100 ml beakers. In one of the beakers 2 ml of ammonium molybdate solution and 2 ml of 1:1 Sulphuric acid solution were added. Addition of more reagents resulted in the appearance of yellow colour with the Silicate present in the sample. In the other beaker containing the water sample the standard Picric acid was added using a pipette drop by drop until the colour was matched with that of the other beaker. The volume of picric acid consumed is tabulated. The same procedure was repeated for the sample B.

Result

The amount of Silicate present in Sample A =ppm.

The amount of Silicate present in Sample B =ppm.

Discussion

Silicates are inorganic nutrients present in the water. In this experiment the concentration of Silicate isin Sample A than in Sample B. Usually in running water and Pond water the concentration of Silicate is higher than the drinking water. So the Sample may be running water or Pond water.

The Silicate oxide is found mainly as Silicodioxide in water. It may exist in colloidal form in the river and streams. The difference in Silicate contents in various water samples depends upon the habitat in flow of nutrients to the medium content of inorganic, organic salts and the entry of decaying materials. Silico Flagellates, Sponges and diatoms make use of Silicates to buildup their body.

TABULATION AND CALCULATION

Table for Water sample A

S. No.	Vol. of Sample	Pipette Reading		Vol. of the Picric Acid consumed	Concordant value of Picric Acid
		Initial	Final		
1	50 ml				
2	50 ml				
3	50 ml				

Calculation of Silicate for sample A

Silicate present in the sample A = $0.25 \times \text{Vol. of Picric Acid consumed} \times 20$

=

Silicate Present in the sample A =ppm.

Table for Water sample B

S. No.	Vol. of Sample	Pipette Reading		Vol. of the Picric Acid consumed	Concordant Value of Picric Acid
		Initial	Final		
1	50 ml				
2	50 ml				
3	50 ml				

Calculation of Silicate for sample B

Silicate present in the sample B = $0.25 \times \text{Vol. of Picric Acid consumed} \times 20$

=

Silicate Present in the Sample B =ppm.

Result

The amount of Silicate present in sample A = ..ppm.

The amount of Silicate present in sample B = .. ppm.

ESTIMATION OF CALCIUM IN POLLUTED WATER SAMPLES

Expt. No. : 7

Date :

Aim

To estimate the amount of Calcium present in the given water samples A and B.

Principle

The concentration of Calcium is estimated by titrimetry using EDTA (Ethyline Diamine Tetra Acetic-Acid). The amount of Calcium is found out by altering the pH with the ammonium buffer. Muroxide is used as an indicator. Magnesium is removed completely as its hydroxide and hence it answers for Calcium alone.

Reagents

1. EDTA solution

Dissolve 16.825 grams of EDTA in 1 litre of distilled water (1 ml of 0.1 N EDTA contains 1 mg of calcium).

NaOH solution

Dissolve 80-gram of NaOH in 1 litre of distilled water.

Muroxide Solution

1 grain of Muroxide is dissolved in 100 ml distilled water. It is used as an indicator.

Materials required

Pipette, Conical Flask, Measuring Jar and Porcelain tile.

Procedure

1 ml of the sample A is taken in a clean conical flask add 1 ml of NaOH solution and 1 ml. of muroxide to the conical flask. The solution is diluted to 20 ml with distilled water and this is titrated against EDTA until the colour changes from pink to blue. Before titration, a drop of pink coloured solution may be left on a porcelain tile and the end point colour can be compared with this for better judgment. Pipette readings are tabulated. The same procedure is adopted for the sample B.

Result

The concentration of Calcium in sample A = ppm.

The concentration of Calcium in sample B = ppm.

Discussion

In the experiment the concentration of Calcium is _____ in sample A than in sample B. Usually pond water has high Calcium content due to the decomposition of organic substances. Hard water has high concentration of Calcium. Calcium is an important ion in imparting the hardness to the water. At high pH Calcium gets precipitated as CaCO_3 . Drinking water from bore well sources contains lesser amount of Calcium. So from this experiment it may be concluded that the sample A may bewater and sample B may be.....water. Calcium content in water may range from zero to several hundreds mg/lit.

We require 800 mg Calcium daily. It is a major constituent of bones and teeth. CaCO_3 forms calcareous spicules in sponges and shells of molluscs. About 99% of the body Calcium is in the skeleton where it is deposited as Calcium Phosphate. Ionized Calcium has great importance in blood coagulation. It is also required for muscle

contraction and nerve irritability. Calcium deficiency results in poor development of bones, dental disorders, stunted growth rickets in children and osteoporosis in adults. Excess Calcium leads to the formation of stones in the urinary tract.

In plants Calcium is essential for the formation of cell plate and cell wall. Calcium is deposited in the cell wall in the form of Calcium pectate (middle lamella). It gives rigidity to the cell wall. It stimulates the development of root hairs. Absence of Calcium leads to Chlorosis, Calcium is necessary for the translocation of Carbohydrates in Plants.

TABULATION AND CALCULATION

Table for Water sample A

S. No.	Vol. of Sample	Pipette Reading		Vol. of the EDTA consumed	Concordant EDTA value
		Initial	Final		
1	1 ml				
2	1 ml				
3	1 ml				

Calculation of calcium for sample A

$$\text{Calcium present in the sample A} = \frac{2 \times \text{Vol. of EDTA Consumed} \times 1000}{1}$$

$$= \frac{2 \times \text{ } \times 1000}{1}$$

$$\text{Calcium present in the sample A} = \text{ } \text{ppm.}$$

Table for Water sample B

S. No.	Vol. of Sample	Pipette Reading		Vol. of the EDTA consumed	Concordant EDTA value
		Initial	Final		
1	1 ml				
2	1 ml				
3	1 ml				

Calculation of calcium for sample B

$$\text{Calcium present in the sample B} = \frac{2 \times \text{Vol. of EDTA Consumed} \times 1000}{1}$$

$$= \frac{2 \times \text{ } \times 1000}{1}$$

$$\text{Calcium present in the sample B} = \text{ } \text{ppm}$$

Result

The concentration of Calcium in sample A =ppm.

The concentration of Calcium in sample B =ppm.

ESTIMATION OF TOTAL HARDNESS IN POLLUTED WATER SAMPLES

Expt. No: 8

Date :

Aim

To estimate the total hardness present in the given water samples A and B.

Principle

Hardness is generally, caused by the calcium and magnesium ions present in water, Poly valent ions of same other metals like Streontium, Iron, Aluminium, Zinc and Manganese etc. are also capable of precipitating the soap and thus contributing to the hardness. However the concentration of these ions is very low in natural waters, therefore hardness is generally measures as concentration of only calcium and magnesium as calcium carbonate, which are far higher in quantities over other hardness producing ions.

Calcium and Magnesium form a complex of wine red colour with Erio-chrome Black -T at pH 10.0 ± 0.1 . The EDTA has got a stronger affinity towards calcium and magnesium and therefore, by addition of EDTA the former complex is broken down and a new complex of blue colour is formed.

Materials

Burette, Pipette, Conical flask and Measuring Jar.

Reagents

A) EDTA solution 0.01 M (or) 0.02 N: Dissolve 3.723 gms of Disodium salt of EDTA in distilled water to prepare one litre of solution.

B) Ammonia buffer solution: Dissolve 3.38 ammonium chloride (NH_4Cl) in 28.6 ml of concentrated ammonium hydroxide (NH_4OH). Dissolve 0.2358 gms of Disodium EDTA and 0.156 gms of Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) in 10 ml of distilled water, mix these two solutions and dilute to 50 ml with distilled water.

C) Erio-chrome black 't' indicator: Mix 0.25 gms of Erio-Chrome Black 'T' powder with 50 gms of sodium chloride salt and grind to get a homogenous mixture.

Procedure

In a clean conical flask 50 ml of the water sample is taken to that 1 ml of Ammonia buffer solution is added. Then 400 mg of Erio-chrome Black 'T' indicator is added the solution turned wine red color and this is titrated against EDTA within 5 minutes. The end point is the change of wine red colour to dark blue. The same procedure is adopted for the sample B.

Result

The hardness of the water sample A =mg/l

The hardness of the water sample B =mg/l

Discussion

The Total hardness of water is the sum of concentration of alkaline earth metal cations present in it. The calcium and magnesium are the principal cations imparting hardness. Hardness when caused because of bicarbonates and carbonates of these cations is called Temporary hardness since it can be removed by boiling the water. Sulphates and chlorides of these cations cause permanent hardness, which is not removed by simple boiling of water. In General practice the hardness is measured as

concentration of only calcium and magnesium (as CaCO_3) which are far high in concentration over other cations.

In this experiment the hardness of the sample A is..... than sample B. Usually drinking water has lesser amount of CaCO_3 . Hardness of water prevents lather formation with soap. Therefore, hard water is not suitable for bathing and washing. Hard water has high boiling point and so is not good for cooking too. Surface waters are softer than ground water. So from this experiment it may be concluded that the sample A may be.....water and sample B may be.....water.

TABULATION AND CALCULATION

Table for Water sample A

S. No.	Vol. of Sample	Pipette Reading		Vol. of the EDTA consumed	Concordant value
		Initial	Final		
1	50 ml				
2	50 ml				
3	50 ml				

Calculation

$$\begin{aligned} \text{Hardness as Ca CO}_3 &= \frac{\text{Volume of EDTA} \times 1000}{\text{Volume of Sample}} \quad (\text{mg/l}) \\ &= \text{Volume of EDTA} \times 20 \\ &= \\ \text{Hardness as Ca CO}_3 \text{ present in the sample A} &= \dots\dots\dots\text{mg/l.} \end{aligned}$$

Table for Water sample B

S. No.	Vol. of Sample	Pipette Reading		Vol. of the EDTA consumed	Concordant value
		Initial	Final		
1	50 ml				
2	50 ml				
3	50 ml				

Calculation

$$\begin{aligned} \text{Hardness as Ca CO}_3 &= \frac{\text{Volume of EDTA} \times 1000}{\text{Volume of Sample}} \quad (\text{mg/l}) \\ &= \text{Volume of EDTA} \times 20 \\ &= \\ \text{Hardness as Ca CO}_3 \text{ present in the sample B} &= \dots\dots\dots\text{mg/l.} \end{aligned}$$

Result

The hardness of the water sample A =mg/l.

The hardness of the water sample B =mg/l.

ESTIMATION OF PHOSPHATES IN POLLUTED WATER SAMPLES

Expt. No: 9

Date :

Aim

To estimate the amount of Phosphate present in the given water Samples A and B.

Principle

The Phosphate and ammonium molybdate react in acid solution to give phosphomolybdic acid which can be reduced by a number of reagents to molybdenum blue, a colloidal substance of complex composition. The phosphate content should be studied by comparing the optical density of the sample with the standard solution using spectrophotometer.

Materials required

Beaker, Pipette, Measuring Jar and Spectrophotometer.

Reagents required

Acidified Ammonium Molybdate solution: 2.5 grams of ammonium molybdate is dissolved in 25 ml of distilled water. To this 75 ml of 50% Sulphuric acid is added.

Stannous Chloride solution: 2.5 grams of stannous chloride (Tin foils) is dissolved in 100 ml of distilled water and filtered.

Standard Phosphate Solution: 3.1 grams of Potassium Phosphate dissolved in 1 litre of distilled water (1 ml of this solution contains 1 mg of phosphate).

Procedure

50 ml of water sample A and 50 ml of standard phosphate solution were taken in separate beakers. In each beaker 1 ml of acidified ammonium molybdate was added. The solutions were stirred well and after 2 minutes 0.5 ml of stannous chloride was added to each beaker and stirred again. The O.D. of the sample and standard phosphate solution were read by using spectrophotometer at the wavelength of 650 nm. The same procedure was adopted for the sample B.

Result

The concentration of Phosphate in the water sample A =ppm.

The concentration of Phosphate in the water sample B =ppm.

Discussion

Phosphates are important minerals, which are found dissolved in water. They are released to the medium when the dead bodies are decomposed. Plants make use of phosphates for their growth and the photosynthesis. The application of fertilizers to the field increases the phosphate contents of the soil as well as the fresh water bodies.

In this experiment it is understood that the sample contains more.....phosphates than the sample..... The sample may be pond water, because the pond water is enriched with phosphates followed by the decomposition of aquatic organisms. The water sample ... may be drinking water.

TABULATION AND CALCULATION

The concentration of the phosphate present in the given sample =

$$\frac{\text{OD of unknown}}{\text{OD of Standard}} \times \text{Concentration of Standard} \times 20$$

Calculation

Sample A

The concentration of the phosphate present in the given sample A =x 1 x 20
=ppm.

Sample B

The concentration of the phosphate present in the given sample B =x 1 x 20
=ppm.

Result

The concentration of the phosphate present in the water sample Appm.

The concentration of the phosphate present in the water sample Bppm.

ESTIMATION OF NITRATES IN POLLUTED WATER SAMPLES

Expt. No: 10

Date :

Aim

To estimate the amount of Nitrates present in the given water samples A and B.

Principle

The sample containing nitrite is mixed with 6(-naphthalamine and sulphuric acid, phenol sulphuric acid was formed which gives pink colour.

Reagents

1. Sulphuric acid.

2. α -naphthalamine.

5 g of naphthalamine was dissolved in the mixer of 375 ml of distilled water and added 250 ml of glacial acetic acid.

Procedure

100 ml of water sample was taken in a conical flask and added 2 ml of naphthalamine and 2 ml of H_2SO_4 solution. The content was mixed thoroughly. Development of pink colour indicated the presence of nitrite. The colour intensity of the solution was read out in the Spectronic-21 at 530 nm against blank. The amount of nitrite was estimated by comparing the reading of test solution with the reading of known volume standard solution.

Result

The concentration of Nitrates in the water sample A =ppm.

The concentration of Nitrates in the water sample B =ppm.

Discussion

Nitrates are important minerals, which are found dissolved in water. They are released to the medium when the dead bodies are decomposed. Plants make use of phosphates for their growth and the photosynthesis. The application of fertilizers to the field increases the phosphate contents of the soil as well as the fresh water bodies.

In this experiment it is understood that the sample contains more..... Nitrates than the sample..... The sample may be pond water, because the pond water is enriched with Nitrates followed by the decomposition of aquatic organisms. The water sample ... may be drinking water.

Calculation

Concentration of Nitrates in the given sample =

$$\frac{\text{OD of unknown}}{\text{OD of Standard}} \times \text{Concentration of Standard}$$

Calculation

Sample A

The concentration of the Nitrates present in the given sample A = 20

=ppm.

Sample B

The concentration of the Nitrates present in the given sample B =x 1 x 20

=ppm.

Result

The concentration of the Nitrates present in the water sample Appm.

The concentration of the Nitrates present in the water sample Bppm.

ESTIMATION OF pH IN POLLUTED WATER SAMPLES

Expt. No.: 11

Date :

Aim

To estimate the Hydrogen Ion concentration of the given water samples A and B.

pH definition

The term pH was introduced in 1909 by Sorenson who defined pH as the negative logarithm of hydrogen ion concentration (Moles/Litre).

$$\text{pH} = -\log(\text{H}^+)$$

The normal H^+ concentration per litre of solution ranges between 10^{-1} and 10^{-14} and g^- ion/lit. The negative logarithm of 10^{-1} is 1 and the negative logarithm of 10^{-14} is 14. So the pH scale ranges between pH of 1 and pH of 14. Pure distilled water has a pH of 7 at room temperature and is neutral (neither acidic nor alkaline). It has an equal concentration of H^+ and OH^- . The pH values less than 7 (low pH) denote an acidic solution with a high concentration of H^+ . The pH values more than 7 (high pH) denote a basic solution with a low concentration of H^+ . Thus pH scale is logarithmic and inversely related to (H^+).

Principle

Any one of the following methods can determine the pH of a solution.

1. pH Paper, 2. pH Meter and 3. pH indicators. In all these three methods of pH estimation, certain Ionization reactions are involved which are governed by Law of Mass Action and Equilibrium constants.

pH paper method

pH paper is impregnated with various dyes (indicators). The pH paper will develop different shades of colour at different pH values and thus pH of a solution is determined. This colour change is due to the Ionization of a corresponding dye in the paper depending on the pH of the solution.

Materials required

Narrow range and wide range pH papers, watch glass, forceps and pH meter.

Procedure

pH paper method for the estimation of pH: A small quantity of the sample A is taken in the watch glass. A small piece of paper from a narrow range booklet is removed with the help of forceps and dipped in the sample and the colour developed is matched with the colour marked on the narrow range booklet for accurate pH value. The same procedure is repeated for the sample B and pH value is recorded.

Result

pH of the water sample A = ____.

pH of the water sample B = ____.

Discussion

Water sample A has ____ pH value than water sample B. It may be inferred that sample A may be ____ water and sample B may be ____ water. Because pH of fresh/pond water ranges between 6-7 and that of sea water or estuarine water ranges between 7-8.

Interrelationship between pH and salinity

As salinity increases the values of pH also increases. The pH of seawater increases due to the greater concentration of hydroxyl ion than hydrogen ions. Fresh water has pH value between 6 and 7 due to the greater concentration of hydrogen ions than hydroxyl ions.

Importance of pH in biological system

The pH of most parts of the body is maintained close to neutrality. The pH value of blood is between 7.4 and 7.5. This must be maintained for the normal functioning of the cells. Enzyme catalysed reactions require a certain range of pH. Hydrogen ion acts as a biocatalyst. The structure of many biological molecules is pH dependent. For example, in an acidic pH amino acids become cations and behave as acids where as in a basic pH they become anions and behave as bases.

ESTIMATION OF DISSOLVED OXYGEN IN POLLUTED WATER SAMPLES

Expt. No: 12

Date :

Aim

To determine the amount of dissolved oxygen in water samples A & B by Winkler's method.

Materials required

Pipette, burette, conical flask, measuring jar, narrow mouthed sample bottles (of about 125 ml capacity), porcelain tile, etc.

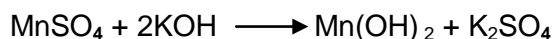
Reagents

1. 48% Manganous Sulphate (480 gms of MnSO_4 dissolved in 1 litre of distilled water).
2. Alkaline Iodide (700 gms of KOH & 150 gms of KI dissolved in 1 litre of distilled water).
3. 0.025 N Sodium thiosulphate solution (6.205 gms of $\text{Na}_2\text{S}_2\text{O}_3$ dissolved in 1 litre of distilled water).
4. Concentrated Sulphuric Acid (commercial Sulphuric Acid used as such).
5. 1% Starch solution (1 gm of Starch dissolved in 100 ml of boiling distilled water, freshly prepared).

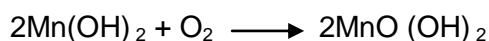
Principle

The principle involved in the estimation of dissolved oxygen in water by Winkler's iodimetric method is as follows: -

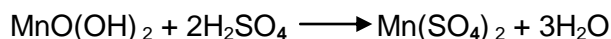
1. When Manganous Sulphate is added to the sample of water followed by alkaline iodide, MnSO_4 reacts with KOH present in the alkaline iodide and a white precipitate of Manganous hydroxide is formed.



2. Manganous Hydroxide combines with oxygen forming Manganic Hydroxide, a brown precipitate.



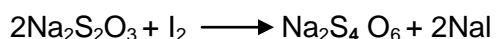
3. On acidification (addition of H_2SO_4) Manganic Hydroxide dissolves forming manganic sulphate .



4. An immediate reaction between Manganic Sulphate and Potassium Iodide leads to the release of Iodine.



5. The amount of Iodine released is proportional to the amount of oxygen trapped by the reagents in the sample. The amount of iodine is determined by titrating against sodium thiosulphate using starch as an indicator.



Procedure

1. A glass stoppered narrow mouthed sample bottle is taken. It is filled with water and stoppered. Then the stopper is removed and the volume of the bottle is noted by measuring the contained water with a measuring jar.

- The sample bottle is completely filled with sample 'A' avoiding air bubbles and is stoppered. Then the stopper is removed and to the water sample 1 ml of Manganous sulphate solution followed by 1 ml of alkaline iodide is added keeping the tip of the pipette below the surface of the water sample.
- The bottle is stoppered displacing the water at the top. Bottle is shaken well for 1 minute and the precipitate is allowed to settle.
- After 10 minutes stopper is removed and 1 ml of Con. H_2SO_4 is added to dissolve the precipitate. The bottle is shaken well till the entire precipitate dissolves. A clear yellow solution is retained in the bottle.
- 25 ml of this yellow coloured sample is measured into a conical flask. To this 5 drops of freshly prepared starch (1%) indicator solution is added. Now the colour of the sample is changed to dark blue due to the presence of iodine.
- A clean burette is taken and filled with 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ and the initial reading is noted. The dark blue coloured sample obtained (25 ml) is titrated against the Sodium thiosulphate solution till the blue colour disappears, which is taken as endpoint. The volume of $\text{Na}_2\text{S}_2\text{O}_3$ consumed (final reading) is noted. The same procedure is carried out for the sample B. The burette readings for samples A and B are tabulated separately.

Result

Oxygen content of Water Sample A = _____ ml/lit.

Oxygen content of Water Sample B = _____ ml/lit.

Discussion

Before inferring the results of sample A and B, the knowledge of oxygen content in different aquatic habitats is essential. The amount of oxygen dissolved in different water bodies depends on temperature, salinity, flow of water, pollutants, aquatic autotrophs and the depth of the water column as described below.

1. Oxygen content in fresh water and salt water

Fresh water can dissolve more oxygen than seawater. Because, solubility of oxygen in water decreases when more salts are dissolved. Like salinity, temperature also influences the solubility of oxygen. The lower the temperature the greater is the oxygen retaining capacity of water. The following table proves this fact.

Water	Temp. $^{\circ}\text{C}$	Saturation Point
Fresh Water	0	10.27 ml/lit.
Salt Water	0	8.08 ml/lit.
Fresh Water	30	5.57 ml/lit.
Salt Water	30	4.52 ml/lit.

2. Oxygen content in surface and bottom water

The surface water of any aquatic body has more oxygen than the bottom layer of water. As the water surface is in immediate contact with the atmosphere, oxygen readily diffuses into it. On the contrary, the deepest layers of water usually have a very low oxygen concentration. Because aerobic bacteria consume more oxygen to decompose the organic matter found at the bottom mud. More over there is no photosynthetic activity in this region to enhance oxygen concentration. Thus the amount of oxygen decreases with increasing depth. Usually anaerobic organisms occupy the bottom mud.

3. Oxygen content in river water

In river water oxygen content is high and uniform due to constant turn over and flow of water. During fluxing more oxygen gets dissolved.

4. Oxygen content in tank or pond water

In tank or pond water oxygen content is lesser due to its stagnancy. Stagnant bottom water has high reduction potential, because it has high percentage of H_2S or CO_2 . When fresh oxygen diffuses into it through the surface, this oxygen will be used in lessening its reduction potential. Aerobic forms cannot use this oxygen. They can use it only when the medium has oxidation potential. Thus availability of oxygen depends on redox potential.

5. Oxygen content in well water

In well water oxygen content is much lesser due to its lesser chance of oxygen contact with atmospheric air.

6. Oxygen content in sea water

In seawater oxygen content is low compared to fresh water due to salinity. Solubility of oxygen decreases with increasing salinity.

7. Oxygen content in polluted water

In polluted water oxygen content is very low. Water polluted by organic wastes accelerates bacterial growth. As aerobic bacteria consume more oxygen from the medium to decompose these organic wastes, the amount of oxygen decreases resulting in oxygen demand. Thus polluted water has high biochemical oxygen demand (High BOD).

8. Oxygen content in water bodies enriched with phytoplankton

Aquatic medium enriched with phytoplankton and other autotrophs contains more oxygen at noon due to the higher rate of photosynthesis. (As we know, oxygen is released during photosynthesis). During night time oxygen content is very low.

Inference

Thus based on the facts described in the aforesaid paragraphs the result of the samples given may be inferred as detailed below: -

It is inferred from this experiment that samplemay beas it contains oxygen than the sample..... The Sample.....may be as it containsoxygen than the sample.....

Biological importance of oxygen

Oxygen is a life support gas. Except for a few anaerobic forms, all living things require oxygen for respiration (Oxidation). As oxygen is the small electron acceptor in the oxidation process, oxygen is essential for the continuous release of energy.

TABULATION AND CALCULATION

The dissolved oxygen content of the water sample is calculated using the following formula: -

$$O_2 \text{ Content} = \frac{K \times 200 \times \text{Vol. of } Na_2S_2O_3 \text{ consumed} \times 0.698}{\text{Volume of the sample titrated}} \text{ ml/lit.}$$

$$\text{Where } K = \frac{\text{Volume of the sample bottle}}{\text{Volume of Sample bottle} - \text{Vol. of reagents added}}$$

0.698 is the conversion factor to convert parts per million (ppm) to ml/lit.

200 is constant which is obtained by multiplying the equivalent weight of oxygen and Normality of $\text{Na}_2\text{S}_2\text{O}_3$ and 1000 ml ($8 \times 0.025 \times 1000 = 200$).

Table for water sample A

S. No.	Vol. of Sample	Burette Reading		Vol. of the $\text{Na}_2\text{S}_2\text{O}_3$ consumed
		Initial	Final	
1	25 ml			
2	25 ml			
3	25 ml			

Calculation

$$\text{O}_2 \text{ Content in sample A} = \frac{\text{.....} \times 200 \times \text{.....} \times 0.698}{25}$$

$$= \text{_____ ml/lit.}$$

Table for water sample B

S. No.	Vol. of Sample	Burette Reading		Vol. of the $\text{Na}_2\text{S}_2\text{O}_3$ consumed
		Initial	Final	
1	25 ml			
2	25 ml			
3	25 ml			

Calculation

$$\text{O}_2 \text{ Content in sample B} = \frac{\text{.....} \times 200 \times \text{.....} \times 0.698}{25}$$

$$= \text{_____ ml/lit.}$$

Result

Oxygen content of Water Sample A = _____ ml/lit.

Oxygen content of Water Sample B = _____ ml/lit.



ESTIMATION OF BOD IN POLLUTED WATER SAMPLES

Expt. No: 13

Date :

Aim

To determine the amount of BOD in water samples of A and B.

Principle

BOD (Biochemical oxygen Demand) is the measure of the degradable organic material present in a water sample, and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions.

The principle of the method involves, measuring the difference of the oxygen concentration between the sample and after incubating it for 5 days at 20°C.

Materials required

BOD bottles, BOD incubator (Fig. 37) having temperature controlled at 20°C.

Reagents

A. Phosphate buffer

Dissolved each 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4 , Na_2HPO_4 , $7\text{H}_2\text{O}$, and 1.7 g NH_4Cl in distilled water to prepare 1 liter solution. Adjusted the pH to 7.2.

B. Magnesium sulphate

Dissolved 82.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water to prepare 1 litre of solution.

C. Calcium chloride

Dissolved 27.5 g of anhydrous CaCl_2 in distilled water to prepare 1 litre of solution.

D. Ferric chloride

Dissolved 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water to prepare 1 litre of solution.

E. Sodium sulphate solution, 0.025 N

Dissolved 1.575 g Na_2SO_4 and diluted to 1000 ml. Solution should be prepared freshly.

Procedure

Dilution water was prepared in a glass container by bubbling compressed air in distilled water for about 30 minutes. Added 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride solutions for each litre of dilution water and mix thoroughly. The sample was neutralized to pH around 7 by using 1 N NaOH or H_2SO_4 . Since the dissolved oxygen (DO) in the sample was likely to be exhausted; it was usually necessary to prepare a suitable dilution of the sample according to the expected BOD range (Dilution of the sample given in the table). Dilution was prepared in a bucket or a large glass trough, mixed the contents thoroughly. 2 sets of the BOD bottles was filled. One set of bottles was kept in BOD incubator (Fig. 224) at 20°C for 5 days, and the DO content was determined in another set immediately. DO was determined in the sample bottles, immediately after the completion of 5 days incubation. Similarly for blank, 2 BOD bottles was taken for dilution water (Table 1). In one, the DO content was determined and the other incubated with the sample to determine after 5 days.

Result

The BOD of the sample is _____mg/l.

Significance

Measurement of BOD has long been the basic means for determining the degree of water pollution. It is the most important measurement made in the operation of a sewage treatment plant. By comparing the BOD of incoming sewage and the BOD of the effluent water leaving the plant, the efficiency and effectiveness of sewage treatment can be judged. For example, in a typical residential city raw sewage has a BOD value of around 300 mg/L.

If the effluent from the sewage treatment plant has a BOD of about 30 mg/L, the plant has removed 90 percent of the BOD. If water of a high BOD value flows into a river, the bacteria in the river will oxidize the organic matter, consuming oxygen from the river faster than it dissolves back in from the air. If this happens, fish will die from lack of oxygen, a consequence known as a fish kill.

Thus, sewage treatment plants must remove as much BOD as possible from the sewage water. To check sewage treatment effectiveness and to study and control organic matter pollution, millions of BOD tests are performed.

Table 1. Preparation of dilutions for various ranges of BOD in the samples

Range of BOD (Mg/IO ₂)	Dilution (%)	Sample volume in 1 litre of mixture
0-6	No dilution	1000
4-12	50	500
10-30	20	200
20-60	10	100
40-120	5	50
100-300	2	20
200-600	1	10
400-1200	0.5	5
1000-3000	0.2	2
2000-6000	0.1	1
Above 6000	0.05	0.5

Calculation

$$\text{BOD} = (D_0 - D_5) \times \text{Dilution factor}$$

Where, D_0 = Initial D_0 in the sample.

D_5 = D_0 after 5 days.

$$\text{BOD} = (\text{.....} - \text{.....}) \times \text{..... mg/l.}$$

The BOD of the sample is _____mg/l.



Fig. 224. BOD incubator.

CALCULATION OF WATER QUALITY INDEX (WQI) USING 9 PARAMETERS

Expt. No: 14

Date :

Aim

To water quality index (using 9 parameters of polluted) water samples.

Principle

Nine factors were chosen and some were judged more important than others, so a weighted mean is used to combine the values. The 9 parameters are pH, Temperature, Turbidity, Conductivity, Total solids, Dissolved Oxygen, BOD, Nitrate and Phosphate. For example, dissolved oxygen has a relatively high weighting factor (.17); because it is more significant in determining water quality than the other tests. The nine resulting values are then added to arrive at an overall water quality index (WQI). The highest score a body of water can receive is 100.

Materials required

Water sample tested result data, computer program.

PROCEDURE

Sampling

It is important to exercise care in the way samples are collected for analysis. A collected sample should be representative of the river or lake being tested. Near-shore samples may not be representative of the river at that location. If possible, water samples should be collected from a bridge spanning the river, from a boat, or off the end of a dock. A rule of thumb for sampling is to sample midway across the river and below the surface.

A simple device can be constructed from a series of metal rods that can be extended and rubber tubing attached that holds the sample bottle. This device might be extended out from shore if no bridges are available and particularly if the river is narrow or shallow. A golf ball retriever can also be adapted very easily for this purpose.

Calculation

After the variables and guidelines are chosen, this information and the appropriate water quality data are inputted into a computer program that calculates the WQI. Some variables also require additional information (i.e. modifying factors) in order for the score to be calculated. For example, the guidelines for several metals are dependant on water hardness, therefore hardness values must also be included for the WQI to be calculated.

Using the table below as an example, the average Q-value of the three tests is 62 - or a fecal coliform count of 20 colonies per 100 ml of water.

Result

The water quality index is _____.

Discussion

The Water Quality Index is used for the statewide assessment of surface waters. Note that the WQI is based only on the protection of aquatic life, not on the protection of water for drinking or agriculture or any other use.

Excellent: (Value 95-100) - water quality is protected with a virtual absence of threat or impairment; conditions very close to natural or pristine levels. These index values can only be obtained if all measurements are within objectives virtually all of the time.

Good: (Value 80-94) - water quality is protected with only a minor degree of threat or impairment; conditions rarely depart from natural or desirable levels.

Fair: (Value 65-79) - water quality is usually protected but occasionally threatened or impaired; conditions sometimes depart from natural or desirable levels.

Marginal: (Value 45-64) - water quality is frequently threatened or impaired; conditions often depart from natural or desirable levels.

Poor: (Value 0-44) - water quality is almost always threatened or impaired; conditions usually depart from natural or desirable levels.

Test results of water samples and their Water Quality Factors and Weights

Test Results for:	Raw Data (Column A)	Q-value (Column B)	Weighting Factor (Column C)	TOTAL (Column D)
1. Dissolved Oxygen	60% sat.	58	0.17	9.86
2. Fecal Coliform	20 colonies/100 ml	62	0.16	9.92 (est)
3. pH	8 units	85	0.11	9.35
4. BOD	6 mg/L	51	0.11	5.61
5. Temperature	0; C	92	0.11	10.12
6. Total Phosphate	.4 mg/L	70	0.10	7.00
7. Nitrates	.8 mg/L	58	0.10	5.80
8. Turbidity	3/feet (100/cm)	76	0.08	6.08
9. Total Solids	709 mg/L	20	0.07	1.40
Overall Water Quality Index				65.14 (est)

The following table to enter the tested data.

Test Results for:	Raw Data (Column A)	Q-value (Column B)	Weighting Factor (Column C)	TOTAL (Column D)
1. Dissolved Oxygen				
2. Fecal Coliform				
3. pH				
4. BOD				
5. Temperature				
6. Total Phosphate				
7. Nitrates				
8. Turbidity				
9. Total Solids				
Overall Water Quality Index				

The 100 point index can be divided into several ranges corresponding to the general descriptive terms shown in the table below.

Water Quality Index Legend	
Range	Quality
90-100	Excellent
70-90	Good
50-70	Medium
25-50	Bad
0-25	Very bad

Result

The water quality index is _____.

QUALITATIVE ESTIMATION OF MARINE PLANKTON

Expt. No: 15

Date :

Aim

To analyse qualitatively the plankton sample collected from the marine environment and to identify the zooplankton.

Material required

Plankton net, Plankton collecting bottles, Stereo binocular, dissecting microscope, Micro-loop or brush, Glass slides, cover slips, eosin stain, Glycerine and formalin.

Procedure

The Plankton sample is collected from the Marine environment for qualitative analysis. The collection of Zooplankton is made by a net made of bolting silk of # 25 meshes with openings between the meshes 0.06 mm square. By this net, micro plankton (0.06 mm to 0.5 mm) can be collected apart from larger forms. Immediately after collection the Plankton are preserved in 10 formalin (1 part of formalin diluted with 3 parts of distilled water).

The preserved Planktonic sample is taken for qualitative analysis of zooplankton. From the Planktonic concentrate preserved in formalin, Planktonic forms are isolated and transferred to the clean glass slides by a brush or a specially designed micro loop. Plankters are washed in water twice or thrice and stained with a drop of aqueous eosin (1 gm in 100 ml of water). After 10 minutes they are mounted using dissecting microscope. Fine needlessly are used to separate the parts of taxonomic importance. Planktonic organisms are identified based on the keys for the identification of Plankton. (Devis, 1955; Hardy, 1956 and Kasthurirangan, 1963).

Result

The Zooplanktonic forms identified are given below. Their special adaptations to planktonic existence are discussed in detail with labeled sketches.

List of Zooplankton identified: 1. Mysis larva of Prawn, 2. Zoea larva of Crab, 3. Lucifer, 4. Paracalanus, 5. Acartia, 6. Acrocalanus, 7. Sagitta, 8. Oithona, 9. Tintinnids and 10. Copepod nauplius.

Adaptations of Plankton

1. Mysis Larva of Prawn

Mysis larva (Fig. 225) of Prawn is a macrozooplankton (size vary from 1 mm to 1cm). It cannot maintain its distribution against water current. It has the following planktonic adaptation.

1. Mysis larva of prawn lives in estuary and is adapted to varying salinity conditions of estuary. After post mysis stage, it is carried to sea by river discharge during monsoon season.

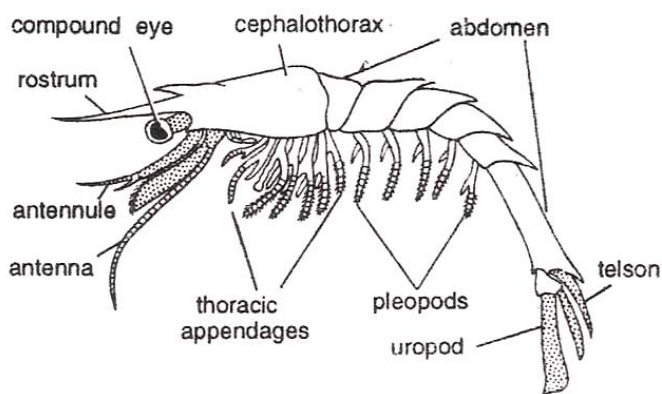


Fig. 225. Mysis larva.

2. The body of this crustacean larva consists of a cephalothorax and a long abdomen. The long curved rostrum, antennae, antennules and thoracic appendages increase

the surface area of the body to prevent the rate of sinking. Thus the larva is able to float.

3. There are 5 pairs of bilobed thoracic appendages on the water surface.
4. The last abdominal segment is long, which is also a floating device. The abdominal appendages begin to develop at this stage.

2. ZOEAL OF CRAB

Zoea larva (Fig. 226) of crab is a microzooplankton (about 1 mm in size). It floats freely in neritic water and it cannot maintain its distribution against water current. Its Planktonic adaptations are discussed below.

1. The body of this crustacean larva is differentiated into a cephalothorax or carapace is produced into a rostrum anteriorly a median spine dorsally. These projections serve to Increase the surface area of the body and there by retard sinking of the body.
2. The head contains large compound eyes for vision. The antennules, antennae, mandibles, maxillae and maxillipeds are used for swimming and to maintain the body.
3. The abdomen is long with six segments and a caudal fork. This elongated structure aid in floatation. Pleopoda are not developed.
4. Zoea undergoes and changes into megalopa larva which later transforms into crab. The casting (moulting) is to help the body to get rid off nitrogenous waste matter which goes into the formation of chitin.

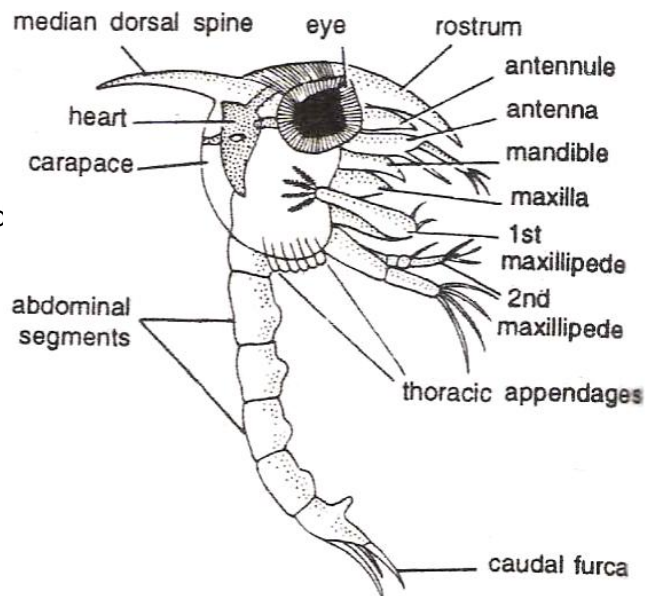


Fig. 226. Zoea larva.

3. LUCIFER

Lucifer (Fig. 227) is a Marine Microzooplankton which cannot maintain its distribution against water current. Its adaptations to lead a planktonic life is discussed hereunder.

1. This pelagic malacostraca has a minute slender and delicate body. Since the body is weightless the animal floats easily in water.
2. Lucifer is extremely transparent. As it is transparent it becomes unnoticed by its enemies. This protective device is necessary for the plankton which exists in an environment where there is no hiding place.

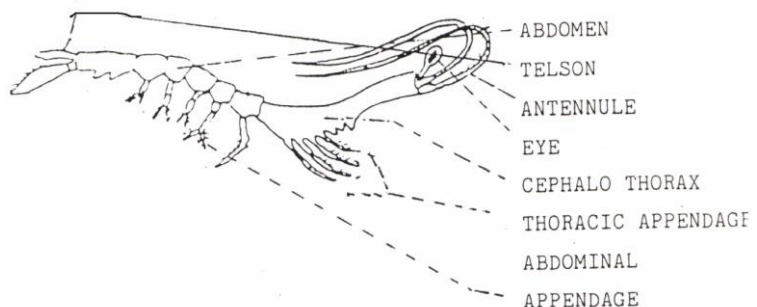


Fig. 227. Lucifer.

3. The body is divisible into cephalothorax, abdomen and telson. The Cephalothorax is smaller than abdomen. The long and thin abdomen prevents sinking of the body.
4. The head is extremely elongated. It bears long antennae and eyes with long stalks. These structures increase the surface area of the body without increase the weight. Such structures give frictional resistance to sinking.
5. The thoracic limbs are non-chelate and whose movements help the plankton to maintain the body in the water surface.

4. PARACALANUS

Paracalanus (Fig. 228) is a Calanoid copepod leading a free floating existence in estuary and sea. Being micro plankton it is unable to maintain its distribution against water current. It has several adaptations to planktonic existence.

1. The body of this copepod consists of an anterior cephalosome, a middle metasome and a posterior urosome. The metasome is broader and larger than the urosome. The urosome includes genital and abdominal segments. Paracalanus is identified by the presence of a spine in the last segment of the exopoditis of II, III and IV legs which separates the segment into larger proximal and a shorter distal segment. The biramous thoracic appendages (5 pairs) help in swimming. The feeble power of locomotion maintains the body on the surface of the water.

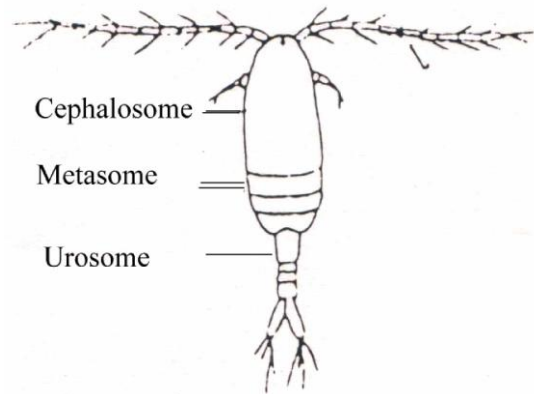


Fig. 228. Paracalanus.

3. The cephalosome has long antennae with spines and short biramous second antennae. There are setae in the caudal rami. These structures increase the surface area of the body to prevent the rate of sinking.
4. Paracalanus swims in water with the help of thoracic legs, antennae and caudal remi with setae. They also keep the plankton poised in water.
5. This copepod is a filter feeder. The antennae, I maxillae and setae of mandibular palps move at faster rate to set up feeding current. The swimming movements of I antennae and thoracic legs are also helpful to collect food organisms. The micro organisms are filtered by the setae of the maxillae.
6. The I antennae of the male is geniculate (hinge formation) for grasping the female during mating.
7. Paracalanus shows vertical diurnal migration.

5. ACARTIA

Acartia (Fig. 229) is a planktonic calanoid Copepod distributed in sea and estuary. This microzooplankton floats freely and cannot maintain its distribution against water current. The planktonic adaptations of Acartia are narrated below.

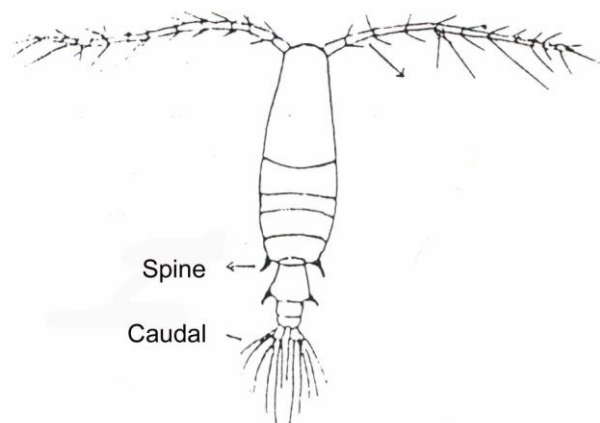


Fig. 229. Acartia.

1. The body of *Acartia* consists of a cephalosome, a long elliptical metasome and a small urosome. There are spines at the posterior margin of the metasome. The exopod of II antenna is less than half as long as endopod. The 5th legs of the female are always uniramous slender and spine like. The urosome has spines laterally. The spiky long I antennae, biramous II antennae, thoracic appendages and caudal rami bearing setae not only help in swimming but also keep the animal poised in water.
2. *Acartia* is a filter feeder. The antennae, the I maxillae and the setae of mandibular palps produce feeding current and micro organisms are filtered by a net formed by the setae of the II maxillae.
3. It is adapted to salinity variations.
4. The body is light blue in colour match with the colour of seawater. This helps the plankton to escape from enemies (Protective colouration). This type of adaptation is essential for the plankton which lives in an environment where there is no hiding place.
5. *Acartia* exhibits vertical diurnal migration. During day time it is distributed in deeper water and at night it migrates to surface water. Again at day-break it begins to migrate to deeper water from the surface.

6. SAGITTA

Sagitta (Fig. 230) is a marine macroplankton, though a few species are found in brackish water. It is adapted to planktonic existence. It cannot maintain its distribution against current.

1. Sagitta is called "arrow worm" because of its shape. The shape facilitates for easy movement.
2. The body is transparent, hence it is called "glass worm". Since the body is transparent it becomes unnoticed by enemies (protective adaptation).
3. The body consists of head, trunk and tail. Head is reduced due to the development of muscles. The function of these muscles is to move a series of lateral capture the prey.
4. There are lateral fins and a caudal fin. They are provided with fin rays. The function of the fin is the stabilization of the animal in water. But the animal moves from place to place by the alternate contraction of the dorsal and ventral bands of longitudinal muscles in the trunk region.
5. Nervous system is well developed consisting of brain ganglion and numerous nerves to coordinate the contraction of muscles.
6. Digestive system is simple. Anus lies ventrally between trunk and tail.
7. Chaetognatha is hermaphroditic. Testes lie in the tail segment. In hermaphroditic forms, reproduction is certain. As survival is questionable in planktonic environment, this type of reproduction is inevitable to perpetuate the race.

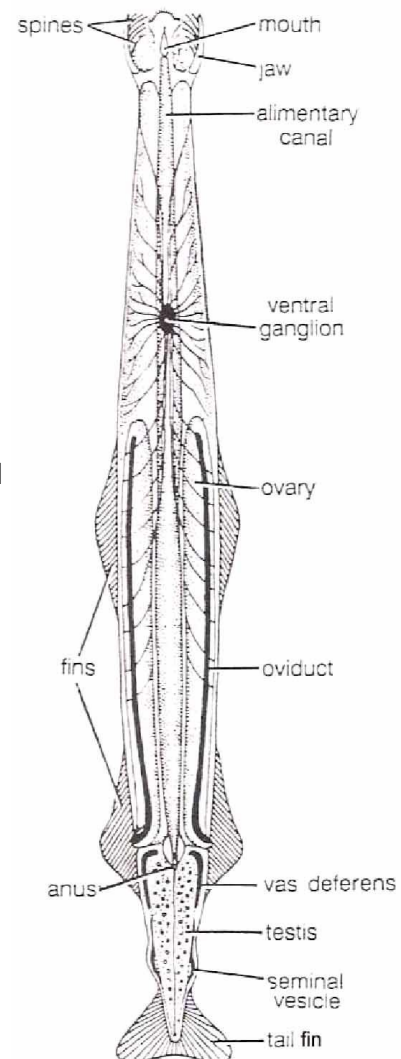


Fig. 230. Sagitta.

ng jaws (hence

8. Chaetognatha exhibits vertical diurnal migration occupying the deeper water at noon and surface water at night.

7. ACROCALANUS

1. Acrocalanus (Fig. 231) is a calanoid copepod seen in coastal water and estuary. It is a microzooplankton which cannot maintain its distribution against water current. It has following planktonic adaptations.

The body consists of a Cephalosome, an oblong metasome and a slightly bent urosome with caudal rami. The metasome has swimming legs. The terminal segment of the exopodite of legs II, III and IV has a spine which separates this segment into a small proximal portion and a long distal portion. Acrocalanus is identified based on this structure. The biramous thoracic legs help in swimming.

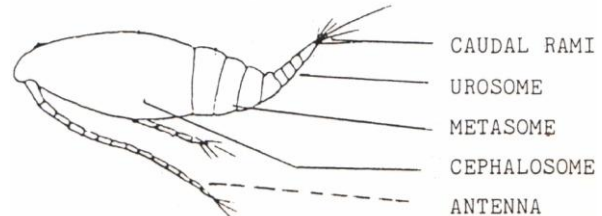


Fig. 231. Acrocalanus.

2. The long I antennae with spines and thoracic appendages give frictional resistance to sinking. This helps the plankton to maintain its distribution near the surface water.
3. It is a filter feeder. Water current is set up by the movements of II antennae, maxillae and palps. The feathery setae of II maxillae form a filter and the food organisms are filtered out by this net and conveyed to mouth. In male the I antenna of right side is geniculate for grasping the female during mating.

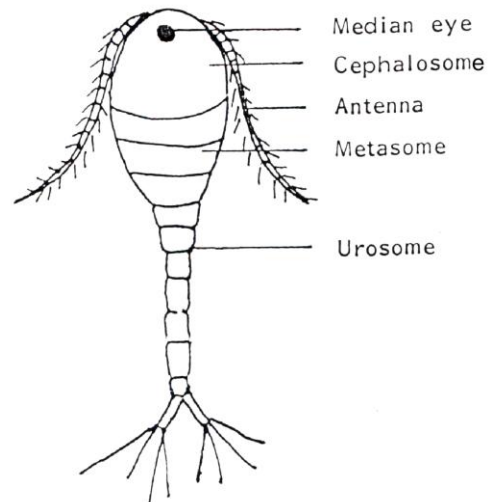


Fig. 232. Oithona.

8. OITHONA

1. Oithona (Fig. 232) is copepod found in large numbers in the estuary.
2. Body is cycloid in form. The metasome was much wider than urosome. It is longer and narrower.
3. The fourth thoracic segment was movable on the fifth. The first antennae were elongate and modified for clasping the female.
4. There are numerous setae on the antennae.
5. The egg sacs were paired and attached to the lateral side of the genital segment.
6. The head terminated in front in a pointed rostrum. Long antennae with setae and thoracic appendages prevent the body from being sinking.

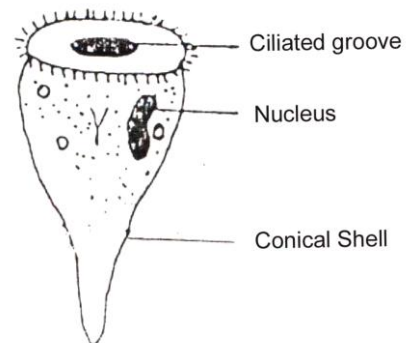


Fig. 233. Tintinnids.

9. TINTINNIDS

1. Tintinnids (Fig. 233) belong to the family tintinnidae of phytoprotozoa. The tintinnids are common in coastal water.
2. The shell is cone shaped.
3. There is a spherical membrane gullet in the broad oval end with cilia.

4. The animals swim with the help of this umbrella like protrusion and conjugation.
5. The reproduction is by means of binary fission and conjugation.
6. The shape of the lorica and its other characteristics are so very constant from one individual to another.

10. COPEPOD NAUPLIUS

1. The copepod nauplius (Fig. 234), like most other crustaceans start their active life with nauplius larva.
2. The body of nauplius larva is kite shaped.
3. It has three pairs of appendages.
4. The first pair is uniramous antennules.
5. The second pair is biramous mandibles.
6. The appendages are used for swimming.
7. They also prevent the body from being sunk.
8. This is an adaptation to lead Planktonic life.
9. The nauplius becomes copepod before reaching adult stage.

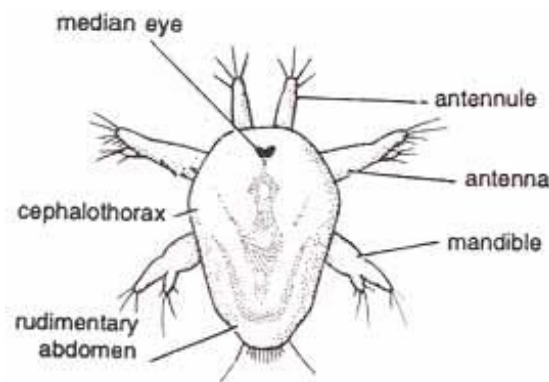


Fig. 234. Nauplius larva.

QUANTITATIVE ESTIMATION OF FRESHWATER PLANKTON

Exp. No: 16

Date :

Aim

To study different types of planktonic organisms and their adaptations.

Materials required

Plankton net, plankton collecting bottles, stereo binocular dissection microscope, micro loop brush glass slides, cover slips, eosin stain glycerine and formalin.

Procedure

The plankton sample is collected from the pond water for qualitative analysis. The collection of zooplankton is made by a net made of bolting silk of # 25 mesh with opening between the meshes 0.06 mm square. By this net, micro plankton (0.06 mm to 0.5 mm) can be collected from larger forms. Immediately after collection the plankton are preserved in 10% formalin (1 part of formalin diluted with 3 parts of distilled water).

The preserved plankton sample is taken for qualitative analysis of zooplankton. From the planktonic concentrate preserved in formalin plankton are isolated and transferred to the clean glass slides by a brush or a specially designed microscope. Plankton are washed in water twice or thrice and stained with a drop of aqueous eosin and mounted using glycerine and cover slip and studied under the stereo binocular dissecting microscope. Fine needles are used to separate the parts of taxonomic importance. Planktonic organisms are identified based on the keys for identification of plankton.

Result

The zooplanktonic forms identified are given below. Their adaptations to planktonic existence are discussed in detail with labelled sketches.

List of zooplanktonic forms identified

- | | |
|---------------------------|------------------------|
| 1. Daphnia species | 4. Mesocyclops species |
| 2. Moina species | 5. Brachionus species |
| 3. Paradiaptomess species | |

Adaptations to planktonic existence

1. Daphnia

Daphnia (Fig. 235) is cladoceran species of the sub class Branchiopoda of Class Crustacea. It is found in fresh water bodies such as ponds and streams. Daphnia is commonly called water flea. Body is soft laterally compressed. Segmentation is very important. In the body except the head is enclosed in a bivalved shell of a large fold of carapace. The carapace ends into a pointed dorsal spine. Head is rounded and bears a pair of large biramous antennae, a pair of small antennules and a compound sessile eye. Thorax bears usually five pairs of leaf like appendages. Large biramous antennae are the chief organ of locomotion. Abdomen is devoid of appendages. Sexes are separate. Female carries eggs and embryos in a large brood pouch situated between abdomen and posterior part of Daphnia, the carapace.

Daphnids are adapted for planktonic life. Long antennae and thoracic appendages prevent the sinking of the body. They give buoyancy to the body during summer season. Water is warm and tends to sink along with daphnia. In order to prevent sinking of the body daphnia develops helmet like projection on the head. This disappears during winter. The setae of the thoracic appendages form a trapping basket

to filter the food particles. The vesicular epipodites of trunk appendages form respiratory organs. It loses haemoglobin when the medium is well aerated. So the presence of haemoglobin depends on the presence of oxygen. The eggs are brooded in brood pouch. Parthenogenesis is common. The parthenogenetic eggs develop into female. But during summer sexual eggs develop into a male or female.

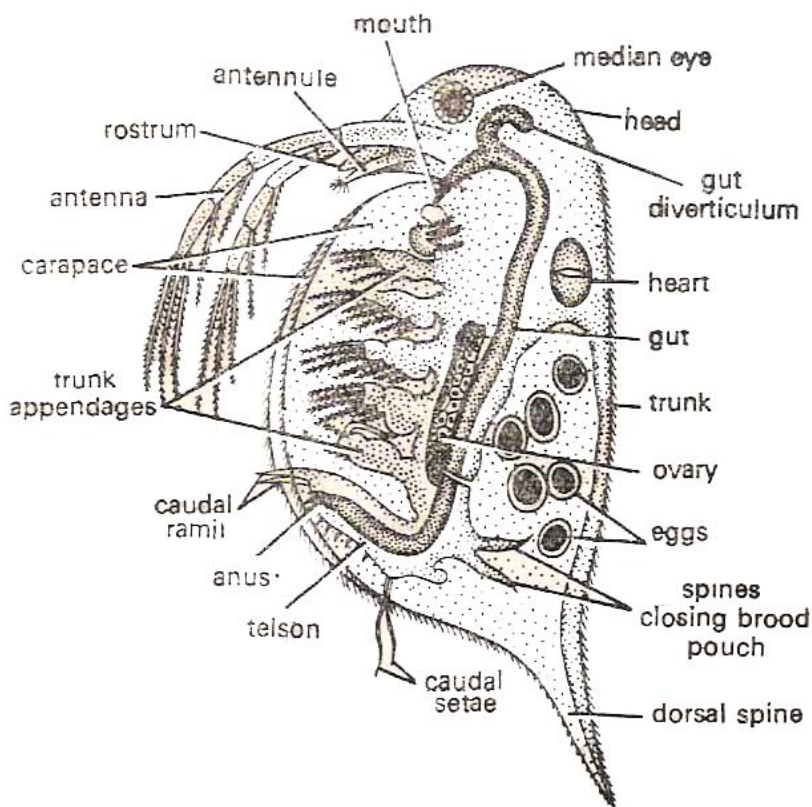


Fig. 235. Daphnia.

2. Moina

Moina (Fig. 236) belongs to the family moinidae and order Cladocera. It is abundant in ponds and freshwater streams. The body is thick and heavy; head is large. Neck rounded in front, sometimes with deep depression above eye, eye is large, with numerous lenses. Antennules are long, more or less spindle shaped, freely movable with terminal and lateral sense hairs. The dorsal and ventral margins of carapace are without spinules, but a few spinules present on the ventral edge: post abdomen is extended into a conical part bearing ciliated spines and a bident abdominal setae are long while abdominal processes present only in older females. Claus small, mostly pectinate or denticulate. Males are smaller than females with long antennule, with hook on first leg. During certain times of the year, swarms of species have been seen. Males are also present in equally good numbers during such periods. Antennules with sensory hairs, ciliated spines and long abdominal setae give frictional resistance to water so that animal is able to float.

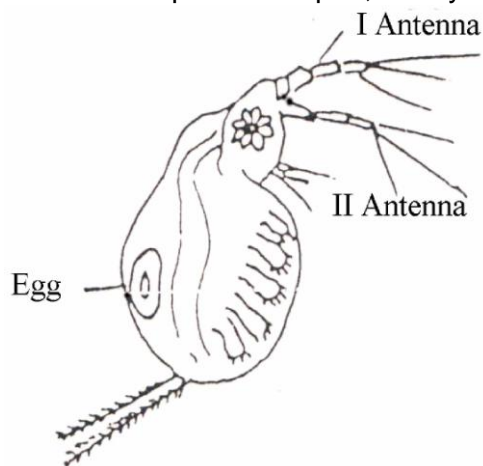


Fig. 236. Moina.

3. Paradiaptomus

Paradiaptomus (Fig. 237) is a fresh water calanoid copepod, belonging to the family Diaptomidae. Paradiaptomus has a cylindrical segmented body. The segmented body is divisible into a six-segmented metasoma and a posterior narrow five segments in male only two or three segments in female. Urosome is separated by major articulation. In male right 5th leg is without exopod spine. In female, the 5th leg has a large endopod bearing two long spines. In male the antennae is geniculate to hold the female during mating. The spiny antennae are used for swimming. The thoracic appendages are also bringing about the movement. The setae of some thoracic appendages form a trapping basket to filter the food particles. The long appendages give buoyancy to the body

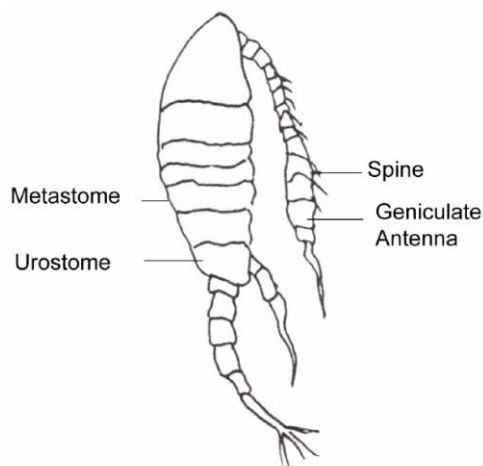


Fig. 237. Paradiaptomus.

4. Mesocyclops

Mesocyclops (Fig. 238) is free swimming freshwater cyclopoid copepods. Body is slender, clearly demarcated into anterior and posterior parts. The first thoracic segment is fused with head forming the Cephalothorax is oval much broader than abdomen. The last segment bears the anus dorsally and a pair of caudal styles produced into plumose setae. Antennules are very large and bears 17 segments reaching slightly beyond the metasome. It serves as principal organ of locomotion. The Antennae are used for swimming. In male they are modified as clasping organs for copulation. Thoracic biramous appendages bring about jerk, movements. Respiration is through the general body surface.

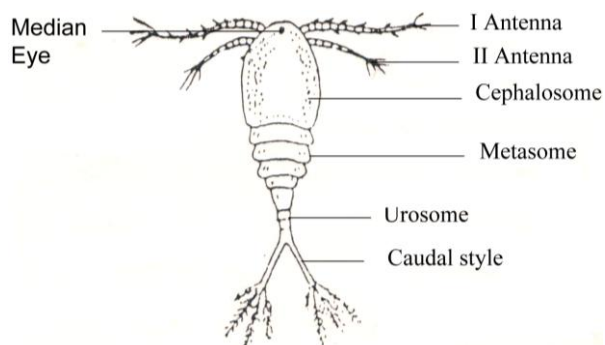


Fig. 238. Mesocyclops.

5. Brachionus

Brachionus (Fig. 239) is fresh water rotifer, found in abundance in ponds. It is commonly known as wheel animalcule. The body is divisible into two distinct parts a broad anterior region; the trunk and a slender movable tail. Dorsal of the trunk is convex, while the ventral surface is flattened and bears the mouth. The truck is enclosed in a glossy lorica formed by the thickening of the cuticle. The tail is wrinkled superficially and ends in two slender processes, the toes. The anterior portion of the body projects from the lorica in the form of a transverse disc the trochial disc with a prominent edge fringed with cilia. Three ciliary lobes are present at the anterior end. The anus is dorsal in position and is placed at the junction of die tail with the trunk. Sexes are separate. There is ciliary mode of feeding in rotifers. Beating of cilia produces feeding current. Long slender processes, the toes give buoyancy to the body. The animal is propelled in water by the action of cilia present at the trochial disc.

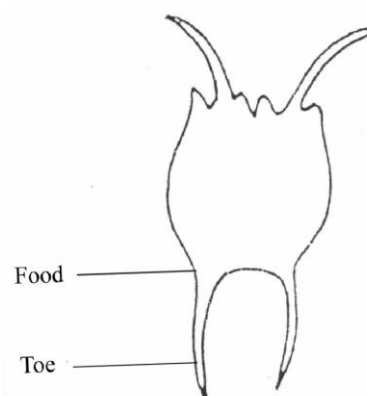


Fig. 239. Brachionus.

QUANTITATIVE ANALYSIS OF MARINE PLANKTON

Expt. No: 17

Date :

Aim

To analyze quantitatively the marine zooplankton.

Materials required

Plankton sample, 100 ml measuring jar, pipette, distilled water, Sedgewick-Rafter, cover slip, glass slide, brush and compound microscope.

Procedure

The preserved plankton sample (collected from 200 litres of sea water) was diluted to 100 ml of distilled water and from this 1 ml of plankton sample was transferred to Sedgewick-Rafter. (The Sedgewick-Rafter has a counting chamber at its centre with 1 ml capacity. This counting chamber is divided into 100 small squares). After transferring 1 ml of sample to the counting chamber a cover slip was placed over it and observed under the low power of the microscope. The different plankton components counted in all the 100 small squares are tabulated (Table 1). The numbers counted in 1 ml of sample are computed for 1000 litres (M^3).

Result

Total number of plankton in 1000 litre = / M^3 .

Discussion

Among the zooplankton components were found in abundant measure. Next to, were numerically abundant. This shows that are found more adapted to that environment, others such as and were lesser in number. Other forms such as Mysis, Sagitta, Lucifer and Zoea were still lesser in number. Plankton plays an important role in food chain, zooplankters are grazing on phytoplankton. The zooplankton forms the food of fishes. The landings of fishes are directly proportional to the quantity of plankton. Thus plankton is considered as an index of fertility. Estuary is a fertile biotope. It is the nursery ground of many of the marine oriented fishes and prawns. The larval stages of these forms are spent in the estuary. The plankters form the food of these larval forms.

Table 1. The numbers counted in 1 ml sample are computed for 1000 litres (m^3).

S. No.	Name of the Plankton	Tally marks	Number in 1 ml	Number per 1000 litre (M^3)
				$\frac{\text{.....} \times 100 \times 1000}{200}$
1				$\frac{\text{.....} \times 100 \times 1000}{200}$
2				$\frac{\text{.....} \times 100 \times 1000}{200}$
3				$\frac{\text{.....} \times 100 \times 1000}{200}$
4				$\frac{\text{.....} \times 100 \times 1000}{200}$
5				$\frac{\text{.....} \times 100 \times 1000}{200}$
6				$\frac{\text{.....} \times 100 \times 1000}{200}$
7				$\frac{\text{.....} \times 100 \times 1000}{200}$
8				$\frac{\text{.....} \times 100 \times 1000}{200}$
9				$\frac{\text{.....} \times 100 \times 1000}{200}$
10				$\frac{\text{.....} \times 100 \times 1000}{200}$
Total				

QUANTITATIVE ESTIMATION OF FRESH WATER PLANKTON

Expt. No: 18

Date :

Aim

To analyze quantitatively the plankton sample collected from the fresh water pond.

Materials required

Plankton sample, 100 ml measuring jar, pipette, distilled water, Sedgewick-Rafter, cover slip, glass slide, brush and compound microscope.

Procedure

The preserved plankton sample (collected from 200 litres of water from pond) was diluted to 100 cc of distilled water and from this 1 ml of plankton sample transfers to plastic slide (Sedgewick-Rafter). The Sedgewick-Rafter has a counting chamber at its centre with 1 ml capacity. This counting chamber is divided into 100 small squares. After transferring 1 ml of sample to the counting chamber cover slip was placed over it and observed under the low power of the microscope. The different plankton components counted in all the 100 small squares are tabulated (Table 1). The numbers counted in 1 ml of sample are computed for 1000 litres (M3).

Result

The Total number of plankton in 1000 litres = M³.

Discussion

Among Zooplankton components of pondwere found in abundant measure. Next to.....,were numerically abundant. This shows that they are found more adapted to the fresh water environment, other plankton such as ,.....and..... were least in number.

Plankton plays an important role in food chain Zooplanktons are grazing on phytoplankton. The Zooplankton form the foods of fishes are directly proportional to the quantity of plankton. Thus plankton is considered as an index of fertility.

Table 1. The numbers counted in 1 ml sample are computed for 1000 litres (m³).

S. No.	Name of the Plankton	Tally marks	Number in 1 ml	Number per 1000 litre (M ³)
				$\frac{\text{.....} \times 100 \times 1000}{200}$
1				$\frac{\text{.....} \times 100 \times 1000}{200}$
2				$\frac{\text{.....} \times 100 \times 1000}{200}$
3				$\frac{\text{.....} \times 100 \times 1000}{200}$
4				$\frac{\text{.....} \times 100 \times 1000}{200}$
5				$\frac{\text{.....} \times 100 \times 1000}{200}$
6				$\frac{\text{.....} \times 100 \times 1000}{200}$
7				$\frac{\text{.....} \times 100 \times 1000}{200}$
8				$\frac{\text{.....} \times 100 \times 1000}{200}$
9				$\frac{\text{.....} \times 100 \times 1000}{200}$
10				$\frac{\text{.....} \times 100 \times 1000}{200}$
Total				

EFFECT OF POLLUTANTS ON PRIMARY PRODUCTIVITY

Expt. No: 19

Date :

Aim

To measure the primary productivity of water sample.

Principle

The increase in dissolved oxygen of water, as a result of photosynthesis is measured in BOD bottles (Biological Oxygen Demand bottles) containing the sample water simultaneously, the decrease in O₂ content in a darkened bottle is measured to estimate the respiration alone in the same sample of water gross, net primary productivity and the respiration of primary producers are calculated from the dates.

Materials required

BOD bottles (125 ml) Dark cotton cloth, Burette, conical flasks, pipette, measuring cylinder, 40% Manganous chloride, Alkaline iodide, 1% Starch solution, Conc. H₂SO₄ 0.025N Sodium thiosulphate.

Oxygen measurement method

This method is known as Light Dark bottle method involves the measurement of oxygen produced which is equal to the production of food in Ecosystem. Pond is the suitable media for this method.

This Winklers's method of determining dissolved oxygen is normally used in the light and dark bottle technique for studying the primary productivity.

Procedure

3 BOD bottles was filled with water sample of the pond. One was initial O₂ bottle (IB), other was light bottle (LB), and the third one was Dark bottle (DB).

Dark bottle was enclosed with a black cloth and tied up. Then the Dark bottle is suspended by means of a rope into the desired location of the pond. At that time oxygen of the initial bottle is fixed. Dark and light bottles are allowed to be suspended in the pond for a suitable period (2 or 3 Hours) so that photosynthesis and respiration is taking place. Then light and Dark bottles were removed from the pond and the O₂ content of both the bottles are fixed.

O₂ content of the 3 bottles (initial, Light, Dark) were estimated by titrating against Sodium thiosulphate using starch as indicator. From the O₂ content of the 3 bottles primary productivity could be calculated.

Result

The primary productivity of water sample.....

Discussion

The technique developed by Gaarder and Gran uses variations in the concentration of oxygen under different experimental conditions to infer gross primary production. As photosynthesis has not taken place in the dark vessel, it provides a measure of ecosystem respiration. The light vessel permits both photosynthesis and respiration, so provides a measure of net photosynthesis (i.e. oxygen production via photosynthesis subtract oxygen consumption by respiration). Gross primary production is then obtained by subtracting oxygen consumption in the dark vessel from net oxygen production in the light vessel.

TABULATION AND CALCULATIONS

Water sample (Control)

1) Initial Bottle (IB)

S. No.	Vol. of Sample	Burette Reading		Vol. of NaOH
		Initial	Final	
1	5 ml			
2	5 ml			
3	5 ml			

$$M \times E \times 1000 \times \frac{1}{50} \times 0.698 \times \text{Vol. of } \text{Na}_2\text{SO}_4$$

Where M = Molarity of the thiosulphate

E = Equivalent wt of O_2

0.698 = Conversion Factor

To convert the O_2 values to the amount of organic carbon

$$\text{O}_2 \times 0.01 \times 1000 \times \frac{1}{50} \times 0.698 \times V = \dots\dots\dots \text{ml O}_2/\text{lit}$$

1 ml of O_2 = 0.536 mg of carbon

1 ml of O_2 = 0.375 mg of carbon

$$\text{Photosynthetic Quotient P Q} = \frac{\text{O}_2 \text{ out put}}{\text{CO}_2 \text{ op take}}$$

$$\frac{\text{O}_2 \text{ ML} \times 0.536}{\text{CO}_2 \text{ op take}} =$$

$$\text{O}_2 \text{ utilised / mg c/ hr} = \frac{\text{O}_2 \text{ ML} \times 1000 \times 0.536}{\text{P Q} \times t}$$

2) Light Bottle (LB)

S. No.	Vol. of Sample	Burette Reading		Vol. of NaOH
		Initial	Final	
1	5 ml			
2	5 ml			
3	5 ml			

$$O_2 \times 0.01 \times 8 \times 1000 \times \frac{1}{50} \times 0.698 \times \text{Vol. of } Na_2SO_4$$

Dark Bottle (DB)

S. No.	Vol. of Sample	Burette Reading		Vol. of NaOH
		Initial	Final	
1	5 ml			
2	5 ml			
3	5 ml			

$$O_2 \times 0.01 \times 8 \times 1000 \times \frac{1}{50} \times 0.698 \times \text{Vol. of } Na_2SO_4$$

$$\text{Respiratory Quotient (RQ)} = \frac{CO_2 \text{ released}}{O_2 \text{ released}} = 1.0$$

To convert the O_2 values to the amount of organic carbon produced, the PQ to be taken into account.

Normally the PQ value is about 1.2 and the conversion value is

$$\frac{O_2 \text{ ml} \times 0.536}{PQ} \quad \text{Or} \quad \frac{O_2 \text{ mg} \times 0.375}{PQ}$$

O_2 content in IB ml/1 = x

O_2 content in DB ml/1 = y

O_2 content in LB ml/1 = z

Respiratory action = IB – DB ml/1/t

Cross production = LB – DB ml/1/t

Net production = LB – IB ml/1/t

Primary productivity = Net productivity \times 0.536

= Net productivity \times 0.536.

DETERMINATION OF LC₅₀

Expt. No: 20

Date :

Aim

To determine the LC₅₀ value of a toxicant on a fish.

Principle

Toxicity studies were conducted to obtain reliable data regarding the effects of the toxicant on the test species. An LC₅₀ value is the concentration of a concentration in water that will kill 50% of the test subjects (animals, typically fish) or permits a 50% survival of the test organisms under the experimental condition during a specific time interval when administered as a single or daily exposure (typically 24 to 96 hours) . Also called the median lethal concentration and lethal concentration 50, this value gives you an idea of the relative acute toxicity of a toxicant based on the standard methods (Standard methods published by APHA, 1975).

Materials required

Mercury, *Catla*, container, Distilled water, measuring jar,

Methods

A wide range of concentrations viz., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 and 20 ppm of mercury solutions were prepared. 2 fishes were introduced in each plastic trough containing 10 litres of water with the required amount of metal. Preliminary observations showed that beyond 6 ppm of mercury all the test fishes were died. Therefore, concentrations of mercury falling within 6 ppm were prepared and test fishes were introduced in a narrow range of concentration of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ppm of mercury solutions. The toxicity data was analyzed following the method of Litchfield and Wilcoxon (1949) to determine LC₅₀ value (theoretical estimate of the concentration, lethal to 50 per cent of the test animals). The LC value as obtained by straight line graphical interpolation method based on observed percentage of test animals, surviving at concentrations lethal to more than half and less than half of the test subjects. The LC₅₀ values were derived by plotting the experimental data (Table 1) on an arithmetic scale and survival percentages on the probability scale. Then a straight line was drawn between the points, representing survival percentages vs concentrations. From the point at which this line intersects the 50 percent survival line, a perpendicular line was drawn to the concentration ordinate which indicates the LC₅₀ concentration of the particular exposure period (Fig. 240).

Result

The 96 hr LC₅₀ value for *Catla* was ppm for mercury.

Discussion

Acute bioassay study can be useful to compare the sensitivity of various species of aquatic animals and potency of toxicants using LC values and to derive the safe environmental concentration for their non lethality and stress to organisms. Application of LC 50 value has gained acceptance and is the most highly related test for assessing the potential adverse effects of aquatic life. The LC 50 value differs from species to species for the same heavy metals as well for different toxicants due to their mode of action on fish.

Table 1: Mortality data for *Catla* exposed to various concentrations of mercury for a period of 96 hours.

Concentration of mercury (ppm)	Mortality
1.0	0
2.0	20
3.0	40
4.0	60
5.0	80
6.0	100

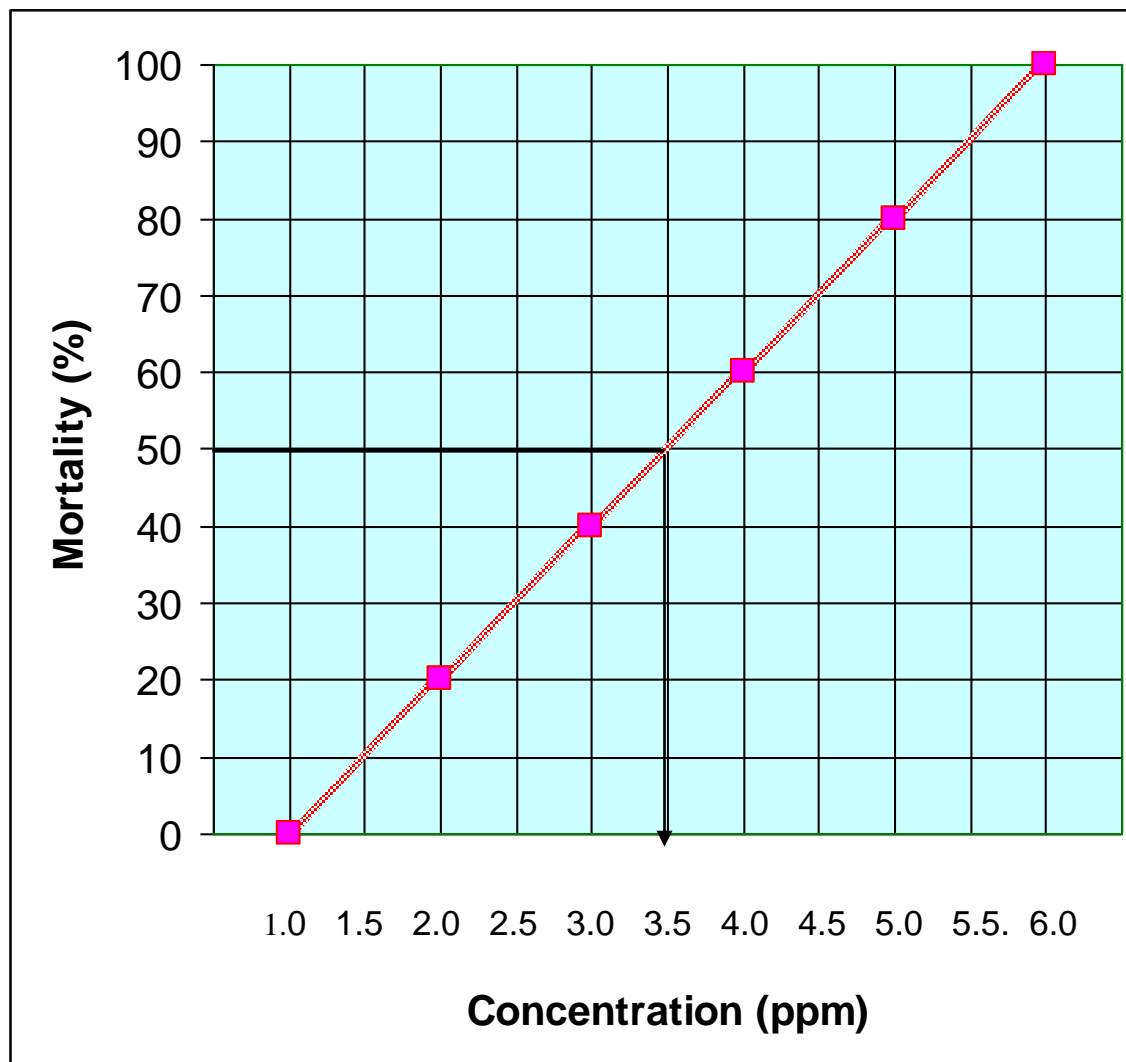


Fig. 240: Mortality data for the Indian major carp *Catla catla* exposed to various concentrations of mercury for a period of 96 hours.

Result: The 96 hr LC₅₀ value for *Catla* was 3.5 ppm for mercury.

MODEL QUESTIONS -1

PRACTICAL-IV: ENVIRONMENTAL BIOLOGY

Time: 3 Hours

Maximum: 60 Marks

- | | |
|--|-----------------|
| 1. Identify the fauna of ecological collection and write their adaptations to the habitat. | 20 Marks |
| 2. Estimate the Calcium present in the samples A and B. Write down the procedure adopted and comment on the results. | 10 Marks |
| 3. Identify and mount the 5 planktons and write their adaptations. | 10Marks |
| 4. Write the procedure to estimate the LC_{50} value and comment on its significance. | 10 Marks |
| 5. Record | 10 Marks |
| Total | 60 Marks |

Key

1. Sandy shore fauna
2. A. Pond water
B. River water
3. Fresh water plankton sample

MODEL QUESTIONS -2

M.Sc. DEGREE EXAMINATION :ZOOLOGY

PRACTICAL-IV: ENVIRONMENTAL BIOLOGY

Time: 3 Hours

Maximum: 60 Marks

- | | |
|---|----------------|
| 1. Identify the fauna of ecological collection and write their adaptations to the habitat. | 20 Marks |
| 2. Estimate the dissolved oxygen present in the samples A and B. Write down the procedure adopted and comment on the results. | 10 Marks |
| 3. Identify and mount the 5 freshwater planktons and write their adaptations. | 10 Marks |
| 4. Calculate the Water quality index(WQI) using 9 parameters and comment on the their significance. | 10 Marks |
| 5. Record | 10 Marks |
| Total | <hr/> 60 Marks |

Key

1. Rocky shore fauna
2. A. Pond water
B. River water
3. Marine water plankton sample
4. Results of 9 water quality parameters

