UTTAR PRADESH
RAJARSHI TANDON OPEN UNIVERSITY

UGZY/BY - L_1
Laboratory Course-I

List of Experiments

LABORATORY COURSE-I

Laboratory Course I is the first course in laboratory in Life Sciences. In this four credit course you will be introduced to a set of simple, do it yourself experiments in Biology, from areas of Cell Biology, Genetics and Ecology. These experiments are intended to develop your skill to work with hands as well as skills of observation, analysis and interpretation.

Laboratory Course I is an intensive course and is completed over a period of 12 days. A four credit laboratory course requires 120 hours of time-input by the students. Essentially you have to work 10 hours each day with two laboratory sessions of 3 hours each (some sessions could be of 4 hours duration), an hour of video viewing or demonstration by the counsellors and 3 hours for preparing your record note book and viva-voce conducted by the counsellors.

The first twelve experiments in this course are oriented towards the study of cells and tissues. We begin with a study of microscope and micrometric measurements. Besides observations on animal and plant cells, you will make squash preparations for the mitotic and meiotic stages as well as polytene chromosomes. Experiments to explain the transport across cell membrane by physical phenomena such as diffusion and osmosis are included. You will perform simple biochemical tests to identify cellular constituents such as carbohydrates, proteins and lipids, and to study the action of enzymes. Experiments 13 to 20 are related to Genetics. By simple experiments you will verify Mendel's laws of inheritance, study the mendelian traits in humans and comprehend the inheritance of human blood group alleles. Exercises have been included to learn to construct and interpret human pedigree charts, to calculate allelic and genotypic frequencies in populations and to analyse human karyotypes. The last eight experiments are related to the study of Ecology. You will learn to estimate certain of the abiotic factors such as salinity, pH and oxygen content in water samples. Simple methods to study community structure and population estimates have also been introduced. With the help of museum specimens and prepared slides you will learn about adaptations of animals and plants to their habitat and different types of animal associations.

You will be assessed for your performance in the laboratory course during the entire two week period. Your counsellor will be providing you the necessary guidance for carrying out the experiments and simultaneously assessing your performance. At the end of the completion of the course, on the final day, there will be an examination. The examination will be on assigned experiments that you have already performed during the two week period. The assessment on guided experiments will be done for 70% and on assigned experiments for 30%.

Study Guide

1. Before you enter the laboratory you should have read each experiment thoroughly. While reading, spend considerable time on the methodology to be followed and on tables and figures.

2. Underline the important steps in the lab manual itself.

3. Do not forget to carry a dissection kit consisting of a pair of scissors, needles, scalpel and forceps to the laboratory. Keep your working place in the lab. as clean as possible. Be careful and cautious while handling the chemicals and the instruments in the laboratory.

4. **It is very important that you have an observation notebook wherein you record all your observations, results, doubts if any and difficulties experienced in carrying out the experiments. A good worker in the laboratory always makes extensive and detailed notes on the observations and results of his/her experiments.**

5. **Besides the observations notebook, you are expected to prepare a record notebook which you will submit to the counsellor at the time of examination for valuation. You record your experimental data and write a brief discussion on each experiment. You should also make scientific illustrations wherever necessary.** For this purpose purchase a 200 pages biology record notebook with one side of the page ruled and the other side blank. You may do most of the writing in the time specified for it and the rest, if any, you may complete at the end of each day at home.

6. More importantly you need to interact closely with your counsellor during your two week stay at the study centre. Try to get the best out of your counsellor and at the same time give your best so that the two week period spent on this lab. course proves to be useful and meaningful.

EXPERIMENT 1 MICROSCOPY

Structure

1.1 INTRODUCTION

We begin this laboratory course-I with exercises on handling a compound microscope and a dissection microscope. By now, you must be familiar with the principle behind the working of a light microscope. You may refer to Unit 2 of LSE-01 (Cell Biology) where light microscopy is discussed in detail. Some of you may have worked with a compound microscope in your Higher Secondary course. Although some studies require the use of more sophisticated and powerful electron microscope which magnifies the objects upto 500,000 times, a light microscope is sufficient for the study of cells and tissues.

Objectives

This lab exercise should help you:

- to handle a compound microscope freely and carefully;
- to become familiar with the various parts of the microscope;
- to describe the resolution or resolving power of a microscope;
- to learn to draw the pathway of light through various lens systems; and
- to appreciate the usefulness of a dissection microscope for studying larger and thicker objects although at a lower magnification.

1.2 MATERIALS REQUIRED

1. A compound microscope, with low and high power objective lens,
2. A dissection microscope,
3. Prepared slides of *Paramaecium* and
4. Some small insects.

1.3 PARTS OF A COMPOUND MICROSCOPE

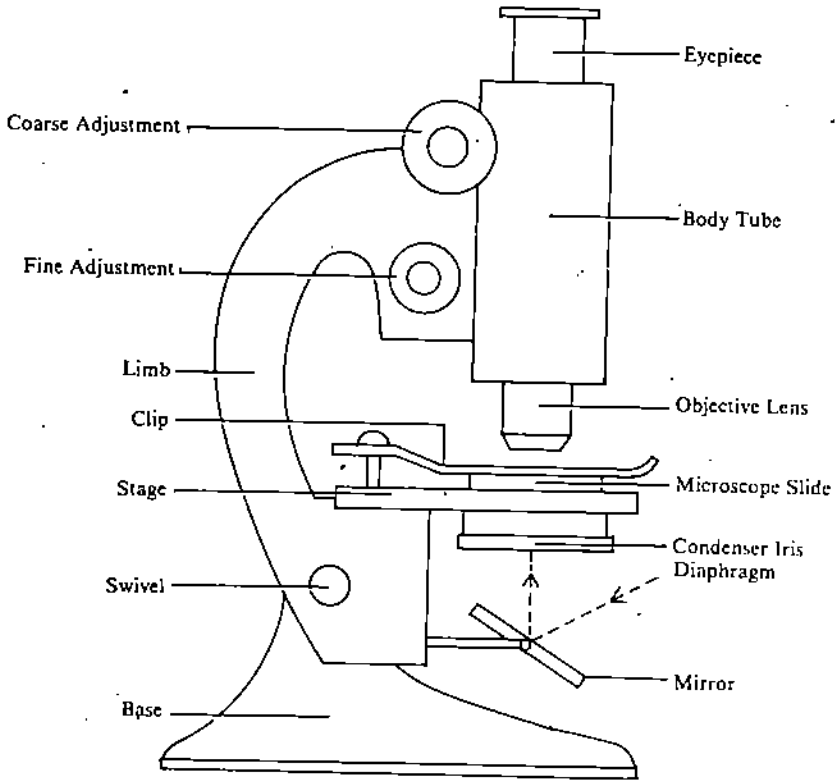


Fig. 1.1 : A compound microscope

The various parts of a compound microscope are seen in Fig. 1.1. A microscope has a **tubular body** the top end of which houses a lens system known as **ocular lens.** The other end of the tube which is close to the specimen has another lens system the **objective lens.** Each lens system is constructed with a series of lenses, usually 8 to 10 in objectives and 2 to 3 in oculars. The closely placed elements, both in objective and ocular lens, act as a single lens and correct any aberration that may occur in the image. The objectives are fixed to a revolving **nose-piece.** The nose-piece has provision for fixing 2 or 3 objective lenses, each one of which has a different **magnifying power.** Usually there will be a **10x** and a **45x** objectives.

You may observe that behind the body tube, there is provision for two types of adjustments. There is a large knob which is a **coarse control** for the vertical movement of lens tube (in some microscopes the movement of specimen stage could be controlled by additional knobs) and a **fine control** to bring the image of the specimen to a sharp focus. The two adjustments are positioned on the **arm** of the microscope. The **specimen stage** is attached to the lower part of the arm of the microscope and is found below the objectives. A **condenser lens** provided just below the specimen stage can focus the light on the specimen from any illuminated source. The condenser lens is also formed of several lenses. The amount of light let into the condenser lens can be controlled by an **iris diaphragm** placed beneath it. Attached to the base of the microscope is a **mirror** which can be adjusted to reflect the light upwards. The mirror is **concave** on one side and **plain** on the other side.

1.4 PATHWAY OF LIGHT

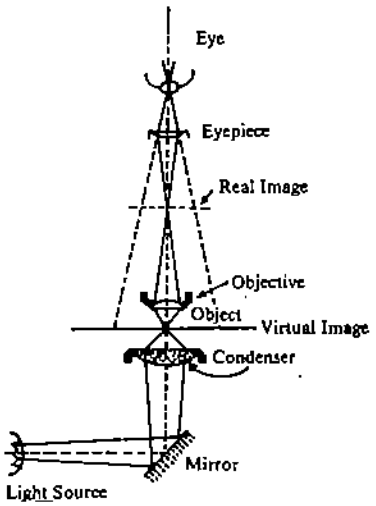


Fig. 1.2 : The pathway of light in a light microscope

The pathway of light from the condenser to the objective lens is shown in Fig. 1.2. The condenser lens can be adjusted in such a way that the light which passes through the condenser lens converges on the specimen and then spreads to a cone of light completely filling the objective.

1.5 RESOLVING POWER OF MICROSCOPE

Assuming you place two objects close together on the stage of microscope and if you can distinguish them as two distinct objects, then the microscope can be said to have a high **resolving power.** In microscopes with low resolving power, the image you see will appear to be that of a single object. Let us quickly recall the meaning of resolving power of a microscope.

$$\text{Resolving power} = d = \frac{0.61\,\lambda}{n \sin \alpha}$$

where λ is the **wavelength,** n is the refractive index of the medium surrounding the specimen and α is the **half angle of light** entering the objective lens from the specimen. n sin α is also referred to as **numerical aperture** of lens **(NA).** The resolving power is highest when λ is smaller and α has a higher value. In other words, resolving power is inversely proportional to wavelength. In a light microscope, from the visible spectrum one can obtain a wavelength of 0.5 µm (0.5×10^{-6}m). With the best resolution in a light microscope one can distinguish two points which are 0.2 µm (0.2×10^{-6}m) apart whereas by way of comparison we can say that in an electron microscope it is easily possible to resolve two points which are about 1×10^{-10}m apart.

1.6 MAGNIFICATION

We earlier referred to the objective lenses of different magnifications. Similarly the ocular lens can also be of varying magnifications. Ocular lenses will usually have 5x, 10x and 15x magnifications. Higher the magnification of ocular lens, greater will be the **field diameter.** You can verify this fact by changing the ocular lens of different magnifications in the microscope.

The number of times a specimen has been magnified is obtained by multiplying the magnifying power of ocular and objective lenses. For instance, with an ocular lens of 10x magnification and the objective of 10x, the specimen will be magnified 100 times. By rotating the nose piece of the microscope you can bring the desired objective in the pathway of light.

1.7 OPERATION OF A COMPOUND MICROSCOPE

Once you are familiar with the various parts of a microscope, you may start using it. The following steps will help you to use a microscope.

1. It is important that the ocular and objective lens are clean. So prior to using the microscope, using a lens cleaning paper and lens cleaning fluid, gently clean both the lenses.

2. Next rotate the revolving nose-piece and bring the low power objective (10x) to the path of light.

3. Open the diaphragm completely.

4. While looking through the ocular of the microscope, adjust the mirror or light source, so that the circular field that you observe gets maximum uniform amount of light and is bright.

5. You may place any prepared slide on the specimen stage.

6. Turn the coarse adjustment knob until the objects comes in view (approximately 0.5 cm above the slide).

7. If necessary use the fine adjustment control and bring the image to sharp focus. You may also slowly open and close the diaphragm to assess the brightness of the field and to obtain a better contrast for the image.

Parfocal: The microscope is said to be parfocal, if an image brought into sharp focus under low power remains in focus when the high power objective is brought into position.

8. If you need to magnify the image further, rotate the revolving nose-piece and bring the high power objective to the path of the light. If the microscope is **parfocal** you should be able to see the object clearly and magnified. Otherwise, you may use the fine adjustment knob to bring the image into sharp focus.

9. Finally, after using the microscope, turn the nose-piece to bring the low power objective to position, remove the slide from the stage, clean the lens if necessary and return the microscope to its box.

1.8 PRECAUTIONS

1. Always keep your microscope clean and under a dust-free cover.

2. Use only lens cleaning tissue papers for cleaning the lenses and never use a coarse paper or cloth as it may cause scratches in the lens.

3. When you lift a microscope, let one hand hold the arm of the microscope and the other support the base. Do not turn the microscope upside down or swing it.

1.9 DISSECTION MICROSCOPE

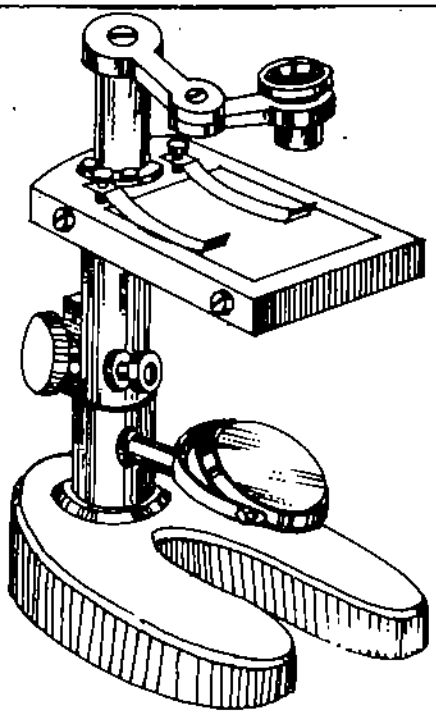


Fig. 1.3 : Dissection microscope

A dissection microscope (Fig. 1.3) is an useful tool to a biologist for making studies on entire small organisms. Even parts of a large organism could be observed in detail under this microscope. The microscope is also used to aid dissections of small organisms and other such experiments. Dissection microscope differs significantly from a compound microscope. The magnification in a dissection microscope is very low and may not exceed 20 to 60 times that of the object. But this lower magnification has certain advantages. It results in a greater depth of focus and therefore, larger and thicker objects could be studied at one time. In a dissection microscope, unlike a compound microscope, light can not be transmitted through the object. This is possible in a compound microscope because the object to be studied is thin enough to allow the light to pass through. In a dissection microscope, the light is directed on the object from above as well as from the sides. Another difference between compound microscope and dissection microscope is that in the latter there is no inverted image formed. The object is seen as placed on the stage of the dissection microscope.

For reasons mentioned above, you could see from the Fig. 1.3 that a dissection microscope is not provided with a condenser. The objective lenses have the magnification powers of 2x and 4x and the ocular lens could be either 10x or 15x. There is only one knob provided for bringing the object to focus.

You may observe a small insect such as an housefly or a mosquito under the dissection microscope and become familiar with the use of the microscope. You may place the slide containing the insect on the stage and use the adjustment knob provided to bring the object under focus.

1.10 SELF ASSESSMENT QUESTIONS

1) To observe the following, would you use a dissection microscope or a compound microscope or an electron microscope?

a) Sections of gut of an earthworm .....

b) A chloroplast to look at its membrane .....

c) An aphid from a cotton field .....

d) The section of a cartilage .....

e) Live *Paramaecium* .....

2) Indicate which of the following statements are true.

a) The greater the magnification of a microscope, higher its resolving power.

[.....]

b) Resolving power refers to the finer focussing property of the mieroscope.

[.....]

c) A higher resolving power would mean that when two small objects are placed close together under the microscope, one should continue to see them as two separate objects.

[.....]

d) A microscope with higher resolving power shows single object as two distinct images.

[.....]

EXPERIMENT 2 MICROMETRY

Structure

2.1 INTRODUCTION

Micrometry is a technique to measure the size of objects under a microscope. You may measure the diameter of the red blood cells or the height of the columnar epithelial cells or the diameter of the nuclei of living cells in a well-fed and starving rat or the diameter of the pollen grains of two closely related malvaceous plants or length and breadth of *Paramaecium* cultured under different environmental conditions, so on and so forth. In this exercise, you will be explained the principle behind micrometry and how to make use of a micrometer to measure the size of microscopic objects.

Objectives

On completing this exercise, you should be able to:

- distinguish between ocular micrometer and stage micrometer,
- calibrate an ocular micrometer with reference to a stage micrometer,
- measure the size of the objects using the ocular micrometer.

2.2 MATERIALS REQUIRED

1. A compound microscope,
2. A 15 cm transparent plastic ruler (with millimeter divisions),
3. An ocular micrometer,
4. A stage micrometer,
5. A few prepared protozoan slides such as of *Euglena* and *Paramaecium*,
6. Sections of tissues showing cells and their nuclei distinctly,
7. Blood smear preparations of frog and man.

2.3 MEASURING THE FIELD DIAMETER WITH PLASTIC RULER

Prior to the commencement of the experiment, record in your notebook, the name and number, if any, of the microscope and the magnification power of the ocular and objective lens with which you are measuring. For instance,

Make : Olympus No. : ..... (if provided)

Ocular : 10x Objective : 10x

This information is necessary since any calibration and measurement you make is valid and specific for only that particular microscope and for the specific magnification. Change in microscope or magnification would require recalibration of the microscope. In other words, there will be differences in calibrated values between any two microscopes. This is also true when the magnifications are changed.

Before calibrating the microscope with micrometer you may use a thin plastic ruler to find out the approximate field diameter of the microscope. Place the ruler on the stage of the microscope and position it in such a way that you could measure the maximum distance across the circular field. First bring the 10x objective lens to position, focus the ruler and record the measurement in your observation note book. Now, find out the field diameter with the high power objective (45x) in position. Record your observation. Is there any difference in the measurements made under different magnifications? If the answer is yes, then how do you account for this difference?

Microscope Make

No. :

S. No.	Ocular	Objective	Field Diameter (mm)
1.	10x	10x	.....
2.	10x	45x	.....

.....

.....

.....

2.4 MICROMETRIC MEASUREMENTS

In order to measure an object under a microscope, two types of micrometers are required:

1. Ocular micrometer (OM) 2. Stage micrometer (SM)

Ocular Micrometer: Ocular micrometer is a small glass disc in the centre of which there is a line etched and divided into 100 divisions. The value of each division is not known and has to be calibrated in relation to stage micrometer. The ocular micrometer can be fitted conveniently into the ocular lens of the microscope. Ocular lens etched with ocular micrometer is also available.

Stage Micrometer: A stage micrometer is a glass microslide in the centre of which a one millimeter line is etched and divided into 100 divisions. Each division in a stage micrometer corresponds to 10 microns (μ).

2.5 PROCEDURE

1. Turn the nosepiece of the microscope to bring the 10x objective to position and place the stage micrometer under it. Fit the ocular micrometer into the ocular lens.

2. Bring into focus the 1 mm line of the stage micrometer.

3. Now; when you observe under the microscope, you should be able to see two sets of lines, one of stage micrometer and the other of ocular micrometer.

4. Looking through the microscope adjust the ocular lens and the stage micrometer appropriately so that the zero of the two scales coincide as shown in Fig. 2.1.

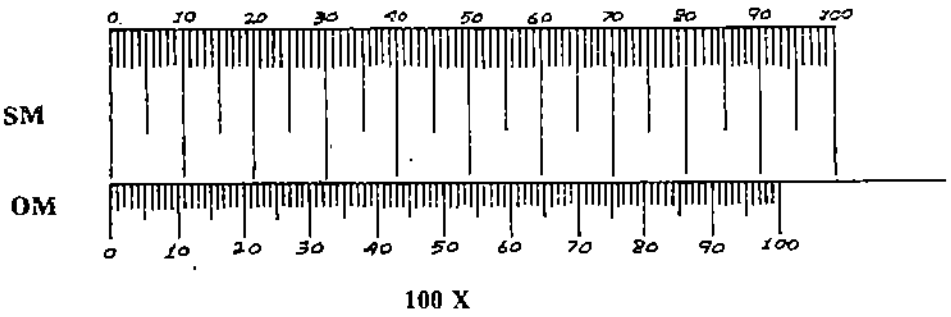


Fig. 2.1 : Ocular and stage micrometer scales with the zero of each of them coinciding with the other

5. Starting from zero position move your eyes towards the right, look for and read lines of coincidence across the field of the microscope. For instance, the 20th division in the stage micrometer may coincide with 18th division in the ocular micrometer. You may record such lines of coincidence in the table provided. Record at least five readings from left to the right of the field. You may now remove the stage micrometer and carefully replace it in the box. Do not remove the ocular micrometer.

Calculate as follows and determine the value of one OM division.

$$\text{Value of one OM division (microns)} = \frac{\text{SM reading}}{\text{OM reading}} \times 10$$

where SM and OM readings refer to numbers relating to the lines of coincidence between the stage and the ocular micrometers and 10 refers to the value of 1 SM division. If you have recorded five readings, you may have calculated 5 different OM values. Obtain the mean of the five values. This mean value is the value of one OM division at the magnification of 10 x 10x for the given microscope. As pointed out earlier, with the change in magnification, the value of one OM division also changes.

You may check this by calibrating the microscope with a 45x objective. Record your results and calculate the value of one OM division.

Ocular : 10x
Objective : 10x

S. No.	OM Reading	SM Reading	$\frac{SM}{OM}$ x 10
1			
2			
3			
4			
5			

Ocular : 10x
Objective : 45x

S. No.	OM Reading	SM Reading	$\frac{SM}{OM}$ x 10
1			
2			
3			
4			
5			

2.6 MEASURING THE MICROSCOPIC OBJECTS

Having calibrated the microscope, you may measure the sizes of various preparations. You may measure the length and breadth of *Paramaecium* as well as the diameter of its nuclei. You may also obtain prepared slides of frog and human blood smear and measure the size of red blood cells. All these measurements are made with the ocular micrometer and denote the number of OM divisions. The number of OM divisions that you have obtained for each measurement has to be multiplied by the value on one OM division you have obtained by calibrating the microscope.

For example, the length of *Paramaecium*

= Value of one OM division x No. of OM divisions

S. No.	Magnification	Parameter measured	No. of OM Divisions	Value of one OM Division	Actual Value of measurement
1.	10 x 10x	Length of *Paramaecium*	10	10.3 microns	103 microns
2.					
3.					
4.					
5.	10 x 45x	Diameter of the nucleus	5	2.5 microns	12.5 microns

2.7 SELF ASSESSMENT QUESTIONS

1. Describe a stage micrometer and an ocular micrometer.

2. Calibrations of a microscope using a 10 x 10x magnification showed that one ocular division is equal to 9.8 microns. Measurements of the length of onion root tip cells showed that they are an average eight ocular micrometer divisions long. What is the actual length of the onion root tip cells?

EXPERIMENT 3 STUDY OF CELLS

Structure

3.1 INTRODUCTION

In the earlier laboratory exercises you have learnt about the parts of compound microscope and to observe objects under it. You have also learnt micrometry and to measure the size of microscopic objects. In this experiment, you will be introduced to the technique of preparation of temporary mounts of animal and plant cells and to study them under a compound microscope.

Cell is the basic unit of life and all living organisms are composed of one or more cells. This exercise will familiarise you with the general structure of plant and animal cells and acquaint you with the basic similarities and differences between these cells. You may refer to Unit 1 of LSE-01 (Cell Biology) Course for the detailed structure of plant and animal cells.

Objectives

At the end of the this lab exercise you will be able to:

- make temporary mounts of animal and plant cells,
- describe the structure of animal and plant cells and
- list the differences between animal and plant cells.

3.2 MATERIALS REQUIRED

1. Slides and coverslips
2. Dissecting needles
3. Pasteur pipettes or droppers
4. Stain—Methylene blue
5. Glycerine

6. Filter paper
7. Microscope
8. Tooth picks
9. Onion
10. Scalpel or blade
11. Brush (No. 6)

Before you learn the procedure for making temporary slides, let us briefly learn about vital staining.

3.3 VITAL STAINING

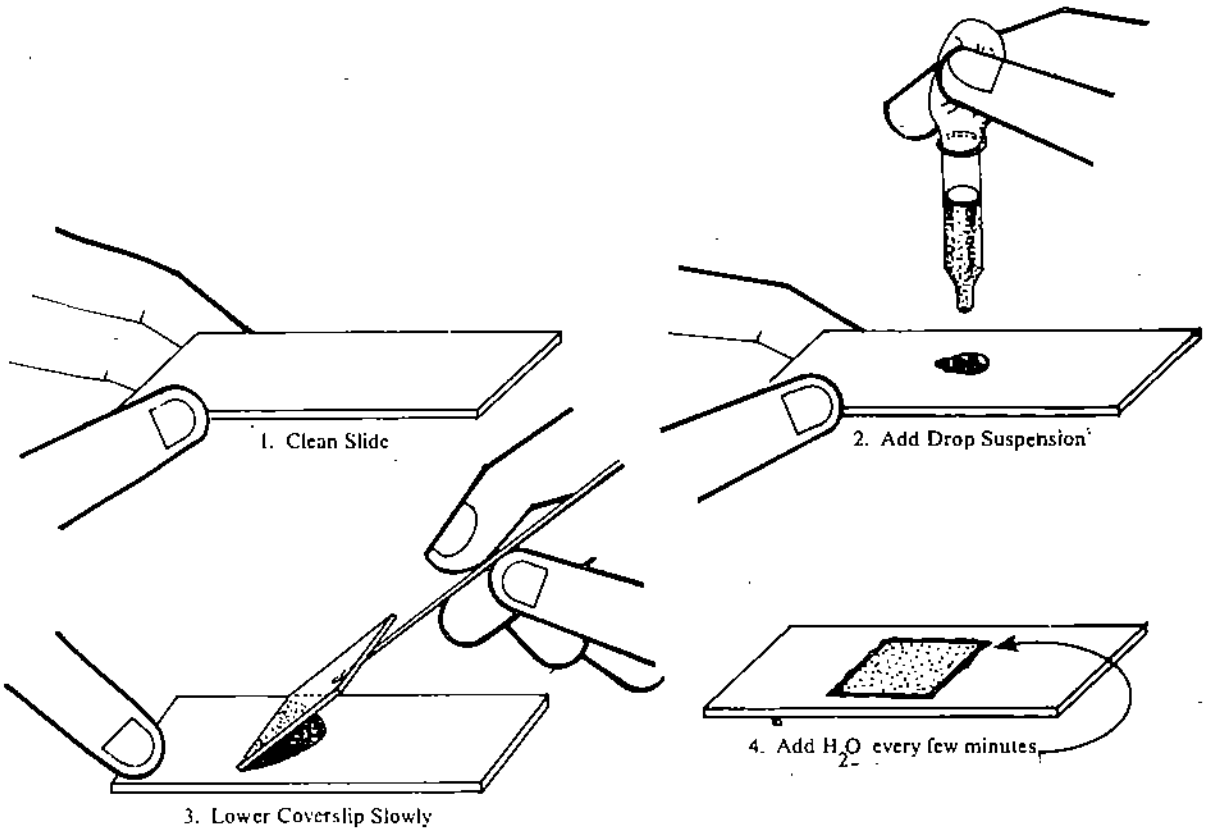
When we examine live unstained cells under the microscope some cytoplasmic structures can be seen because of the differences in their densities. Stains are the dyes that accentuate the differences among the various components of the cells and tissues. The reason is that different cellular components may stain with varying intensity or with different colours with a single dye. Such an examination can be regarded as the vital examination.

Vital staining is a method for the demonstration of structural details of live tissues and cells using certain stains. In other words, vital staining is applied to demonstrate live cells and organelles more or less specifically by using suitable stains. Such a process is also helpful for exploring the cell and tissue permeability phenomenon related to absorption, transport, accumulation and excretion of substances.

Vital stains either belong to acidic type or basic type. Basic stains such as methylene blue stain the nuclei of the cells. The acidic stains such as trypan blue and congo red are useful for the study of excretion and examining lymphatic vessels. Alizarin is an acidic vital stain routinely used for staining bones.

3.4 METHOD OF MAKING TEMPORARY MOUNTS

To examine a material under microscope it is necessary to process the material. In many cases you have to view the object as a wet mount. A wet mount or a temporary mount is prepared by placing a drop of liquid containing the material to be studied on a slide or if the material is dry by placing it directly on the slide and adding a drop of water or stain or glycerine to it. The mount is then covered by a coverslip as illustrated in Fig. 3.1. As shown in the figure, while placing the coverslip, place a needle under the coverslip and slowly let it down on the drop of the liquid. If you allow the coverslip to fall on the slide, it will trap air bubbles along with the specimen which will interfere with your observation.



In the following sections, we will discuss the steps involved in the temporary mounting of animal and plant cells on slides for studying them under the microscope.

3.5 HUMAN BUCCAL EPITHELIAL CELLS

Let us learn the procedure for making a temporary mount of buccal (cheek) squamous epithelial cells.

Procedure

1. Rinse your mouth well with water.
2. Gently scrap the inside of your cheek with the broad end of a clean toothpick. Discard this material.
3. Scrape again and spread these cells gently on a clean slide. Add a drop of 0.9% NaCl or physiological saline and a drop of methylene blue with the help of a dropper.
4. After two minutes, remove the excess stain and saline using the edge of a filter paper and add a drop of glycerine on the cells.
5. Mount with a coverslip. Gently press the coverslip with the back of a pencil to spread the cells.
6. Finally examine the slide under the microscope.

You can also make the wet mount of human epithelial cells without using glycerine. In such a case spread the cells on a clean, dry slide. Add a drop of 0.9% NaCl solution and mount a coverslip. Stain the cells by irrigation, i.e. add a drop of the stain at the periphery of the coverslip. The excess saline and stain can be dried from the slide by using the edge of a filter paper. You can also directly stain the specimen on the slide before covering it with the coverslip.

3.6 PLANT CELLS

You can make a temporary mount of plant cells with epidermal cells obtained from onion by the following procedure.

1. Cut an onion into quarters and separate the thick modified leaves which make up the bulb. Note that inner and outer surfaces of each leaf are covered by a delicate membrane. The membrane on the inner or concave surface can be stripped off readily.
2. With a scalpel or blade strip off a thin layer from inside of the onion leaf.
3. Gently place it flat on the slide, cut a small suitable square and discard the rest.
4. Quickly add a drop of water and also a drop of the stain with the help of pasteur pipette.
5. Remove the excess stain using the edge of filter paper and add a drop of glycerine
6. Place a coverslip and press it gently with the back of a pencil to spread the peal and remove the air bubbles if any.
7. Examine the slide under the microscope.

You may also use methylene green or iodine to make the above slides.

3.7 OBSERVATIONS

In the **wet mount** of check epithelial cells you can see irregular shaped cells with a thin cell membrane. The nucleus stained dark blue is seen as a central round body in each cell (see Fig. 3.2).

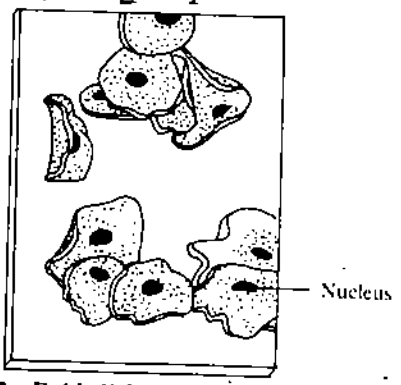


Fig. 3.2 : Epithelial cells from inner lining of the cheek

In the wet mount of onion epidermal cells you can see rectangular shaped cells lying side by side with distinct cell walls. Vacuoles are present in the cell cytoplasm. These vacuoles are so large that they occupy almost the entire cell volume. You can see the nucleus near the cell wall in every cell (see Fig. 3.3).

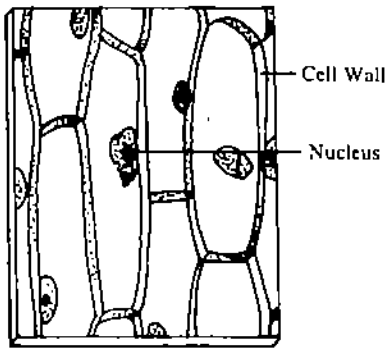


Fig. 3.3 : Onion epidermal cells

Try to examine these wet mounts under higher magnification and record your observations.

3.8 PRECAUTIONS

You should take the following precautions while making temporary preparations.

1. Slides and coverslips to be used should be clean and free from dust and fingerprints.
2. The material and fluid should be placed centrally on the slide.
3. The amount of fluid should be appropriate. With too much of fluid the coverslip will move around and with too little of fluid you will have air bubbles trapped inside.
4. The excess mounting fluid should be removed carefully from the sides of the coverslip using the edge of a filter paper. Filter paper moistened with water will help remove excess glycerine.
5. Place the coverslip gently to prevent trapping of air bubbles.
6. Do not let the specimen dry out. Use a drop of water to keep it moist.

3.9 SELF ASSESSMENT QUESTIONS

1. What is the advantage of using stains while preparing the temporary mounts?

.....

.....

.....

.....

2. Refer to your experiment on micrometry and measure the size of the cell and nucleus of both animal and plant cells. Record your results in your observation notebook in the following format.

	Measurement	
Cell	**Cell size**	**Nucleus size**
Animal Cell		
Plant Cell		

EXPERIMENT 4 STUDY OF ANIMAL TISSUES

Structure

4.1 INTRODUCTION

In previous lab exercises you have learnt about cells and methods to prepare them for microscopic observations. In this exercise you will study about different groups of cells which form different types of tissues. A tissue can be defined as a group of structurally similar cells performing a specific function or functions. The study of the structure and function of the tissues is known as histology. You are aware that an organ is a structural and functional unit of a living body composed of different tissues. Essentially the various organs of plants and animals are composed of different types of tissues. In this lab exercise we will be studying the structure of different types of animal tissues from the prepared slides and know about their function. The study of the plant tissues follows in the next exercise.

Objectives

At the end of this exercise you should be able to :

- categorise the different types of animal tissues,
- describe the structure of each one of them,
- identify the tissues under the microscope, and
- describe the functions of each of the tissues.

4.2 MATERIALS REQUIRED

1. Compound microscope
2. Dissection microscope
3. Prepared slides of the various animal tissues.

4.3 ANIMAL TISSUES

Animal tissues may be as simple as the epithelial tissues or they could be as complex as blood or the bone tissue. All tissues have a matrix in which the cells are found. The cells in a tissue may bear a single nucleus or the tissue may be syncitial as in the case of skeletal muscle. There are five major types of animal tissues:

1. Epithelial tissues
2. Connective tissues
3. Muscular tissues
4. Nerve tissues
5. Vascular tissues

In the following sections we will describe the structure of each one of these tissues in detail. **Observe the permanent preparations of these tissues under the compound microscope and make neat and labelled sketches of the same in your observation notebook.**

4.4 EPITHELIAL TISSUES

Epithelial tissues are usually arranged in a single or multilayered sheets. They cover the internal and external surfaces of the body of an organism. The lower ends of the epithelial cells are attached to a basement membrane. The main function of the epithelial tissues is to cover other tissues and protect them from injury through abrasion, pressure or infection. The free surfaces of the epithelial cells are highly modified to perform specific functions such as absorption, secretion and excretion. Depending upon their shape and functions, epithelial cells are classified into five categories.

i) Squamous epithelial cells

ii) Cuboidal epithelial cells

iii) Columnar epithelial cells

iv) Ciliated epithelial cells

v) Glandular epithelial cells

4.4.1 Squamous Epithelial Cells

Squamous epithelial cells (Fig. 4.1) are thin and flat cells with a little cytoplasm enclosing a round nucleus. The cells are more or less irregular in shape and when viewed from the surface present a mosaic appearance.

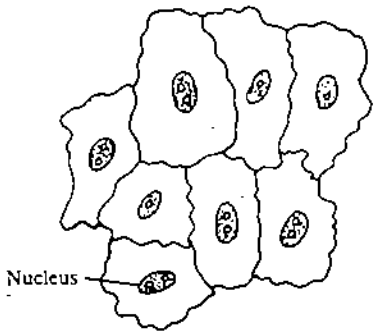


Fig. 4.1 : Squamous epithelial cells

Some of the organs where these cells are found are lining of the nephrons, the alveoli of the lungs and walls of the blood capillaries. These cells also provide smooth lining to hollow structures such as blood vessels and chambers of the heart. Squamous epithelial cells because of their thinness permit diffusion of materials. In blood vessels and heart the cells allow a friction free flow of blood.

4.4.2 Cuboidal Epithelial Cells

The cube shaped cells (Fig. 4.2) are provided with the spherical nucleus. When viewed from the surface, the cells are hexagonal in outline. These cells are found mostly lining the ducts of different glands such as salivary glands and pancreas. They are also found along the collecting ducts of the kidney tubules. In most places where they are found, the cuboidal epithelial cells are secretory. In places such as collecting ducts of kidney these cells are non-secretory.

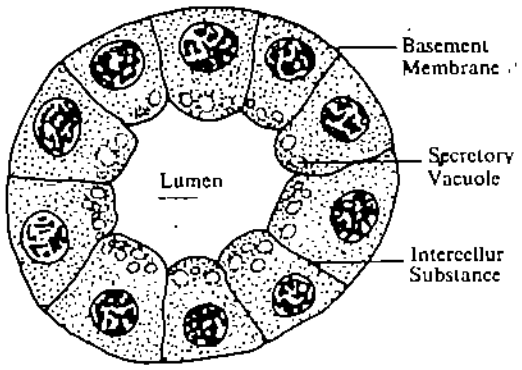


Fig. 4.2 : Cuboidal epithelial cells

4.4.3 Columnar Epithelial Cells

These are long narrow cells having more cytoplasm (Fig. 4.3). The nucleus is often found at the base of the cell. The surface of the cells have a striated border usually formed of microvilli (visible only under electron microscope) which increases the surface area of the cells. Columnar epithelial cells are found lining the stomach, intestine, ducts of the kidney, thyroid gland and gall bladder. The cells are both secretory and absorptive in function.

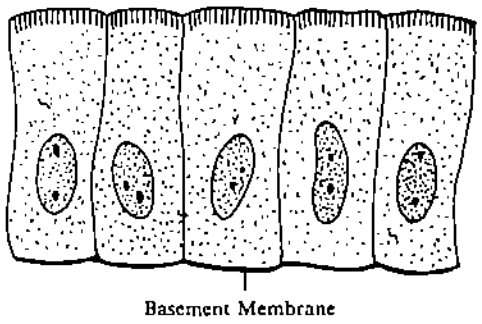


Fig. 4.3 : Columnar epithelial cells

4.4.4 Ciliated Epithelial Cells

These cells are more or less like columnar epithelial cells but bear numerous cilia at their free surfaces (Fig. 4.4). The cells are found lining the oviducts, ventricles of brain, respiratory passages and spinal canal. The cilia usually set up currents which are useful for moving materials from one location to the another.

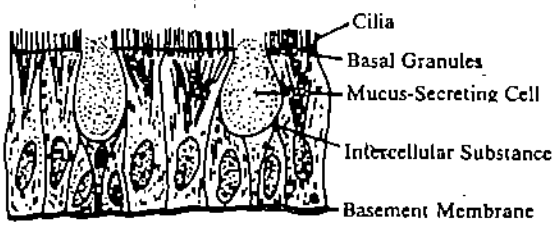


Fig. 4.4 : Ciliated epithelial cells

4.4.5 Glandular Epithelial Cells

Glandular cells are epithelial cells which are exclusively secretory in function. They may be individual unicellular glandular cells known as goblet cells or aggregates of glandular cells forming multicellular glands (Fig. 4.5a, b). Both exocrine and endocrine glands are made up of these cells. If the glandular cells produce a viscous secretion they are called mucus cells or mucocytes. If the secretion is a clear watery fluid the cells are called serocytes.

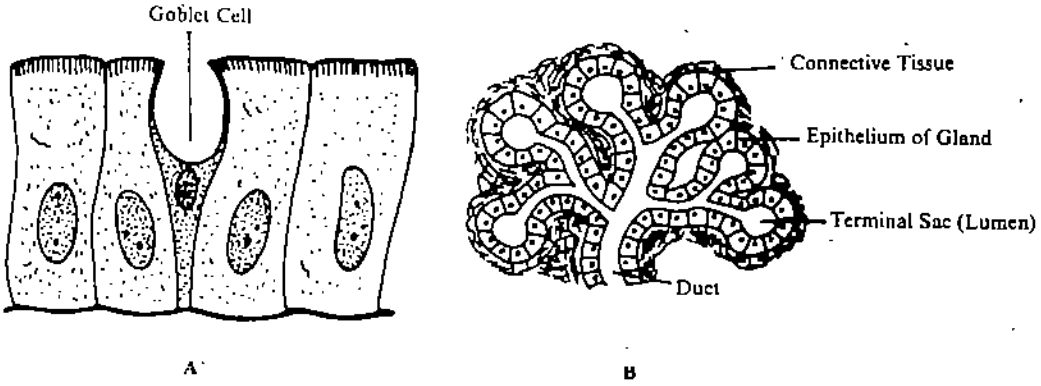


Fig. 4.5 : a) Goblet cell, b) Multicellular glandular cells

4.5 CONNECTIVE TISSUE

Connective tissue is the major supporting tissue of the body. It is composed of a fluid/semi-fluid matrix containing a variety of cells and fibres. The two major types of connective tissue are :

- Skeletal connective tissue
- Binding connective tissue

4.5.1 Skeletal Connective Tissue

The skeletal tissue is of two types: a) cartilage, b) bone

a) **Cartilage** is a supporting connective tissue, the cells of which are embedded in elastic matrix called **chondrin.** The matrix is secreted by cells known as **chondroblasts** or **chondrocytes.** The chondrocytes are found enclosed in spaces called **lacunae.** The matrix contains many fibrils made of collagen. The margin of the cartilage is bordered by a dense layer of cells and fibrils called **perichondrium.** Perichondrium is the site of production of **chondroblasts.** The newly produced chondroblasts are continuously added to the matrix of the cartilage.

Cartilage is a resilient and flexible tissue and can resist any stress placed on it. Because of its compressible and elastic nature the matrix can absorb any mechanical shock that occurs at the articular surfaces of the bones. There are three types of cartilages.

i) Hyaline cartilage

ii) Yellow elastic cartilage

iii) White fibrous cartilage

i) **Hyaline cartilage** is located at the ends of the bones, in the nose and in the air passages of the respiratory system as well as in parts of the ear such as in pinna. The cartilagenous fishes like shark have only this material as supporting tissue. The matrix of hyaline cartilage is made of chondroitin sulphate and collagen fibrils. As shown in Fig. 4.6, the lacunae may enclose one, two, four or eight chondrocytes. The chondrocytes at periphery are flattened while those situated towards the centre are angular.

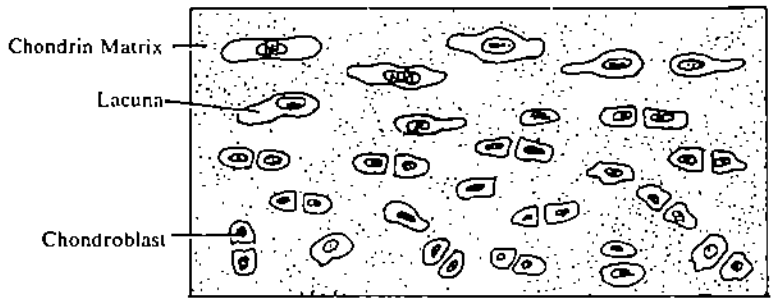


Fig. 4.6 : Hyaline cartilage

ii) **Yellow elastic cartilage** (Fig. 4.7) is located in the external ear, eustachian tube, epiglottis and the pharynx. The matrix is formed of yellow elastic fibres. This type of cartilage is more elastic and flexible. Once the shape of any structure is distorted, the original shape is regained because of this elasticity.

iii) **White fibrous cartilage** (Fig. 4.8) is located in intervertebral discs, and in the ligaments of joints. The tissue consists of a matrix which is embedded with large number of bundles of white collagen fibres. White fibrous cartilage has a great tensile strength and a low degree of flexibility.

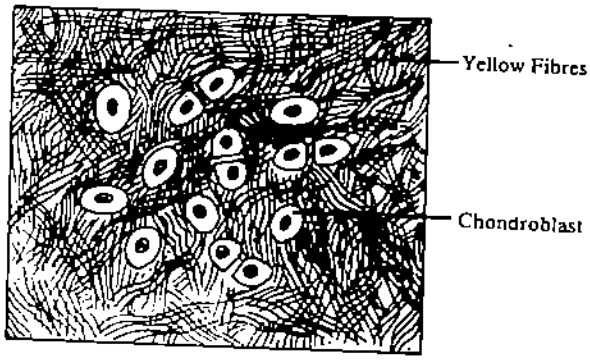


Fig 4.7 : Yellow elastic cartilage

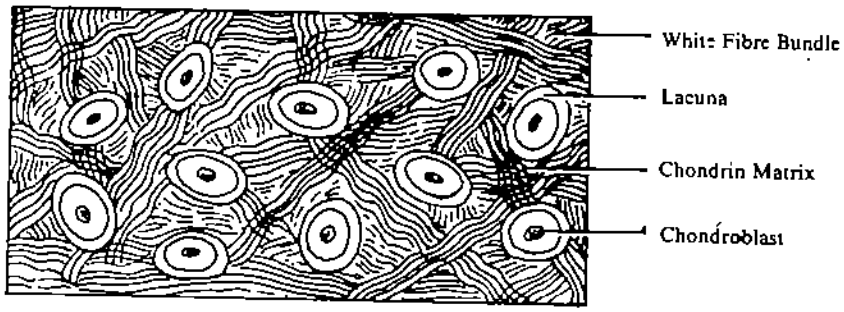


Fig. 4.8 : White fibrous cartilage

b) **Bone** is the supportive tissue that has both metabolic and protective functions. Bone is calcified connective tissue with cells embedded in the firm matrix. 70% of bone matrix is formed of inorganic salts and 30% collagen fibres. The major inorganic component of the bone is known as **hydroxyapatite** formed of calcium phosphate and calcium hydroxide. Other inorganic constituents are sodium, magnesium, calcium, chloride, fluoride, bicarbonate and citrate ions. The cells are known as **osteoblasts**. These cells are found in lacunae which are distributed throughout the matrix. Bone cells secrete the inorganic components of the bone. In transverse section as shown in Fig. 4.9 the bone shows numerous concentric rings called **lamellae** which surround a centrally placed **Haversian canal**. The osteoblasts are found interspersed between lamellae. From each lacuna fine channels containing cytoplasm radiate and link up with Haversian canal. These channels are known as **canaliculi**. The Haversian canal shows an artery and a vein traversing through it. These blood vessels carry the nutrients, metabolic wastes and respiratory gases towards and away from osteoblasts. Covering the bone is a layer of dense connective tissue called **periosteum**.

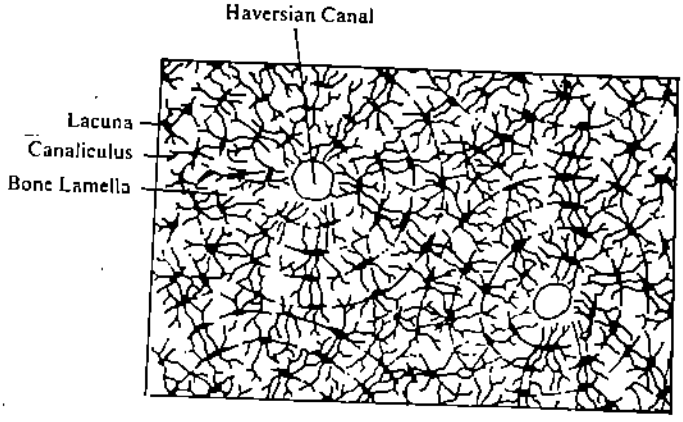


Fig. 4.9 : Transverse section of the bone tissue

4.5.2 Binding Connective Tissues

Binding connective tissue are of two types:

i) Loose connective tissues

ii) Dense fibrous connective tissues

i) Loose connective tissue contain cells which are dispersed in intercellular matrix. The matrix also has loosely arranged fibres. **Areolar tissue** is an example of the loose connective tissue. In this tissue the matrix is semifluid and transparent. It is composed of a mixture of mucin, hyaluronic acid and chondroitin phosphate. The matrix also contains numerous wavy bundles of collagen fibres and the loose network of thin straight fibres of elastin. The two types of fibres provide tensile strength and resilience to the tissue. The fibres are produced by cells called fibroblasts. These cells are flattened and spindle shaped containing an oval nucleus. The matrix also contains other types of cells such as macrophages, plasma cells, mast cells, chromatophores and fat cells. Areolar tissue is found in all organs of the body. It connects the skin to the structures found below it. It binds sheets of epithelia forming mesenteries. It is also found surrounding the blood vessles and nerves when they enter or leave the organs (Fig. 4.10).

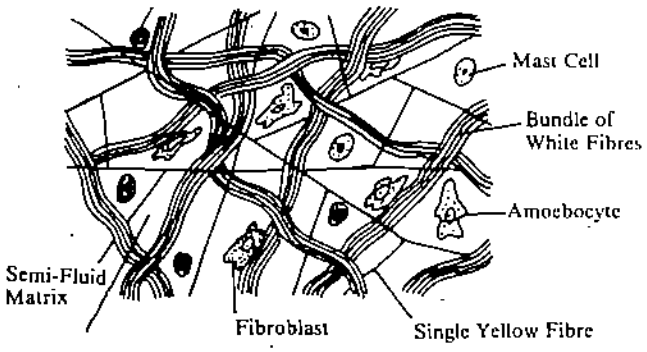


Fig. 4.10 : Areolar tissue

ii) Dense fibrous connective tissue has more fibres located in the matrix than cells. The fibres may be irregularly arranged or the individual fibres may be arranged more or less parallel to each other. There are two types of dense fibrous connective tissues: white and yellow fibrous connective tissues.

White fibrous connective tissues are found abundantly in tendons and ligaments, sclerotic and corneal layer of the eye and in kidney capsule. They are tough tissues formed of regularly arranged bundles of collagen fibres. The fibres are densely packed and run parallel to each other. Fibroblasts, the cells which secrete the fibres are found interspersed among the bundles. The bundles are bound by areolar connective tissue. The strong and flexible fibres owe their tensile strength to collagen (Fig. 4.11).

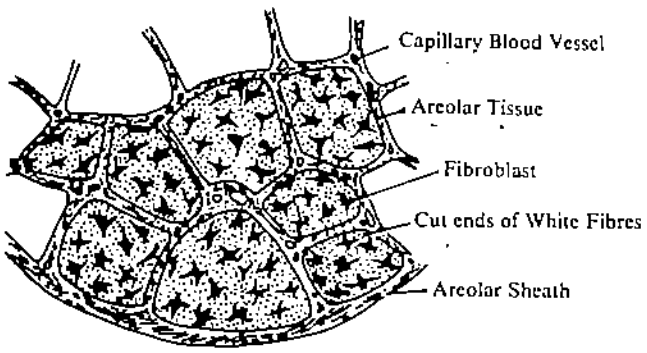


Fig. 4.11 : White fibrous connective tissue

Yellow fibrous connective tissue, is different from white fibrous ones in that the fibres are loosely and irregularly arranged. The yellow elastic fibres form a branched network. The fibroblasts which secrete the yellow elastic fibres are

scattered randomly throughout the matrix and very few collagen fibres are found. The yellow fibres provide the tissues elasticity and flexibility and the collagen fibres the tensile strength. The tissue is located in ligaments, walls of arteries, lungs, and air passages (Fig. 4.12).

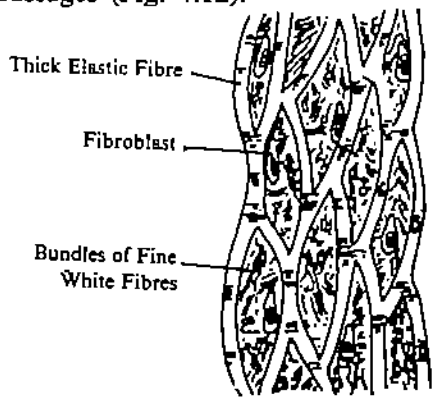


Fig. 4.12 : Yellow fibrous connective tissue

4.6 MUSCULAR TISSUE

Muscular or contractile tissue cells are of three types namely skeletal muscles, smooth muscles and cardiac muscles. Contractility is the characteristic property of all muscle cells. They produce the mechanical force by their contraction. Also, these muscles are responsible for locomotion and the movement of the internal organs. The three types of muscles differ in their structure.

4.6.1 Striated Muscle

These muscles are also referred to as voluntary or striated muscles (Fig. 4.13). Each muscle is a bundle of muscle fibres each of which has a very large multinucleate cell. Usually these cells are very long and cylindrical enclosed by a thin membrane called **sarcolemma**. Each fibre has several longitudinal myofibrils embedded in cytoplasm called **sarcoplasm.** Each myofibril is made of alternating **light and dark bands** or cross striations. Skeletal muscles help in the movement of joints by their strong and rapid contraction. Since the contractions are under the control of our will they are also called as voluntary muscles.

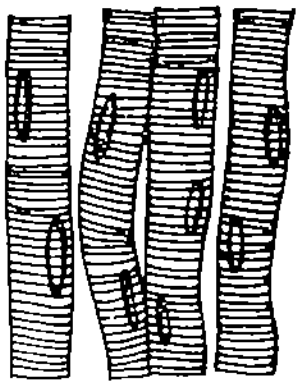


Fig. 4.13 : Striated muscles

4.6.2 Smooth Muscles

Smooth muscles (Fig. 4.14) are present in the digestive tract, urinary bladder, arteries and veins. Each muscle fibre is composed of spindle shaped elongated cells and contain only one centrally located nucleus surrounded by a small amount of sarcoplasm. The remainder of the fibres are delicate contractile threads, the myofibrils running longitudinally. Several such muscle fibres are joined together in bundles by loose connective tissue. Because of lack of

striations, they are called unstriped muscles. These smooth muscles are not under the control of our will, hence the name involuntary muscles.

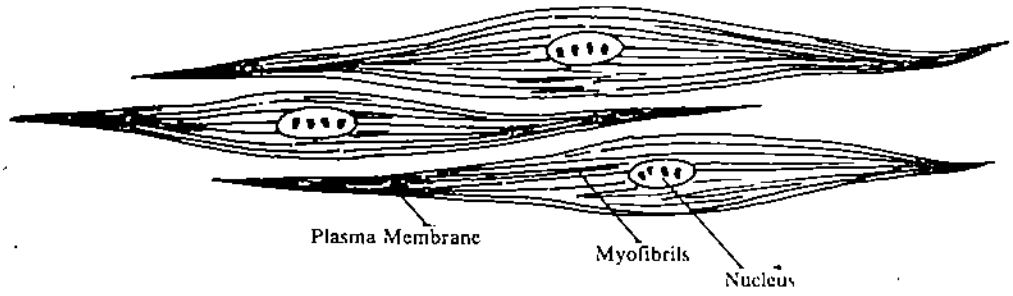


Fig. 4.14 : Smooth muscles

4.6.3 Cardiac Muscle

Structurally the cardiac muscles are intermediate between skeletal and smooth muscles (Fig. 4.15). Heart is made up of cardiac muscles. Like the striated muscles they are branched and the branches are connected to each other. There is a sarcolemma surrounding the muscle fibres and sarcoplasm with the longitudinal myofibrils of alternating light and dark bands. There is only a single nucleus in each fibre.

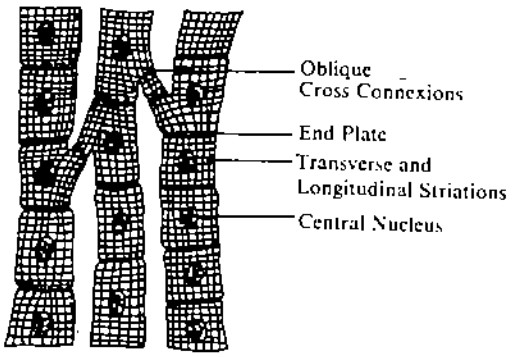


Fig. 4.15 : Cardiac muscles

4.7 NERVE TISSUE

Nerve cells or neurons are specialised cells with properties of responding to stimuli, conductivity and communication. The brain and spinal cord for example, are composed of a network of nervous tissue. The neurons or nerve cells are the units of the nerve tissue. From the neurons arise nerve fibres. Typically each neurons has 3 parts (Fig. 4.16a).

1. **cyton**, the cell body of the neuron

2. **dendron**, one or more short processes arising out of the cyton.

3. **axon**, a single long process forming fine terminal branches ending in an effector organ or another neuron.

A cyton has a centrally placed nucleus and the cytoplasm has fine granules called **Nissl bodies**. A cyton may have a single dendron in which case the neuron is known as the **unipolar neuron**. Presence of a dendron and an axon makes a neuron **bipolar** one and presence of several dendrons make it a **multipolar** neuron (Fig. 4.16b).

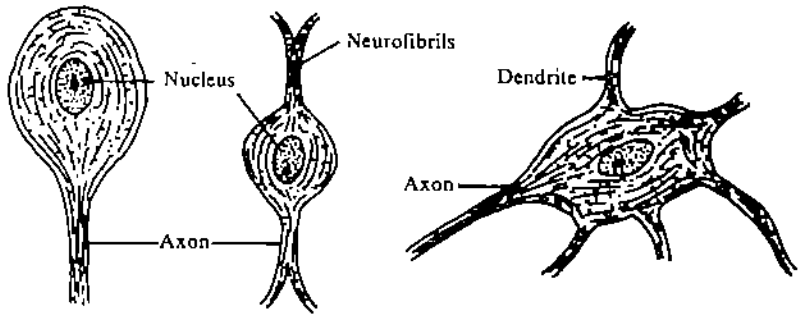


Fig. 4.16 : Uni-, bi- and multipolar neurons

An axon may be surrounded by a myelin sheath and come to be known as a medulary fibre and in the absence of the myelin sheath it is a non-medullary fibre. Both medullary and non-medullary axons are surrounded by a membrane the **neurilemma.** The axons conduct the electrical signals away from the cyton. These signals are a result of a flux of ions across the nerve cell membrane.

4.8 VASCULAR TISSUES

The vascular tissue are the fluid tissue with several functions. The blood and the lymph constitute the vascular tissues. Blood is composed of 60% of a straw coloured fluid, the plasma and 40% of formed elements, the living cells. The plasma itself is 90% water and 10% of organic substances and inorganic ions. Proteins constitute the bulk of the organic constituents of the plasma.

The blood cells are basically of two types: RBC and WBC. The red blood corpuscles (RBC) in mammals are **spherical, biconcave** and **enucleate.** In non mammalian vertebrates they may be **oval, biconvex** and **nucleated.** The RBCs are packed with the respiratory pigment the haemoglobin which imparts the red colour to the blood.

The white blood corpuscles (WBC) are of 3 types : **eosinophils, basophils** and **neutrophils.** These cells are so termed based on their staining properties. They are also termed as granulocytes because of the presence of the granular cytoplasm and polymorphonuclear leucocytes because of the presence of several lobed nucleus. WBCs also include agranulocytes such as lymphocytes and monocytes. In mammals, the blood plasma also contains structures known as blood platelets which are helpful in blood clotting process (see Fig. 6.3).

EXPERIMENT 5 STUDY OF PLANT TISSUES

Structure

5.1 INTRODUCTION

In the last exercise you had observed under the microscope, the structure of different types of animal tissues. Plant organs are also made of different types of tissues. These tissues perform two important functions i) They provide the mechanical strength to the plants and ii) conduct water, minerals and nutrients to the various parts of the plant body. Plant tissues like animal tissues are composed of groups of cells similar in origin, size and shape, performing a specific function. On the basis of their development plant tissues fall into two categories, the meristematic tissue and permanent tissues. The former are immature undifferentiated tissues and the cells are capable of division. Permanent tissues are derived from the meristem by gradual differentiation and they are mature. Their cells do not divide. Permanent tissues fall into two categories i) simple permanent tissues and ii) complex permanent tissues. The first type is composed of similar homogenous cells. The second type is composed of heterogenous cells and are made of different types of cell elements. In the lab exercise, you will be observing the preparations of different types of tissues under the microscope. **Also you will sketch the structure of the tissues in your observation note book and become familiar with their functions as well.**

Objective

At the end of this exercise, you shall be able to

- identify and describe the structure of different types of plant tissues and make sketches of them.
- describe the functions of simple and complex plant tissues.

5.2 MATERIALS REQUIRED

Permanent slides of various types of plant tissues, compound microscope.

Observations on Simple Permanent Tissues

Simple permanent tissues are generally of three types i) Parenchyma ii) Collenchyma iii) Sclerenchyma.

i) Parenchyma

Place a parenchyma slide preparation under the microscope and foucs it. Observe closely the structure of the parenchyma cells and make a neat diagram in your observation note book. Record the structural details of the tissues in the space provided below:

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You will find:

i) The cells are circular and isodiametric

ii) They have intercellular spaces (Fig. 5.1)

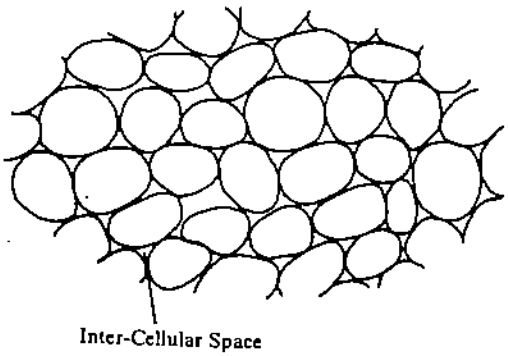

Fig. 5.1: Parenchyma

Occurrence

Most of the lower plants are formed of parenchymatous cells. Meristems are also parenchymatous. Epidermis, cortex, pith, mesophyll of leaves, pulp of fleshy fruits and embryonic tissues are composed of parenchyma cells.

Functions

The cells have an active protoplast. Parenchyma cells have functions of photosynthesis, storage of food material, secretion and excretion. They occur as a part of xylem and phloem and conduct water and nutrients in solution.

In leaves, parenchyma cells have chloroplasts in them, hence called **chlorenchyma**. In aquatic plants, they acquire large air spaces to enable the plants to float in water, hence called **aerenchyma** cells.

ii) Collenchyma

Observe a permanent preparation of collenchyma tissue under a microscope. Make a neat sketch of the cells in your observation note book. Record the details of the structure in the space provided below:

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You will find that:

a) Collenchyma cells are circular and isodiametric. Some of the cells may also be polygonal in shape.

b) The cell wall is thick, more so in corners of the cells. Therefore, there are no intercellular spaces. (Fig. 5.2).

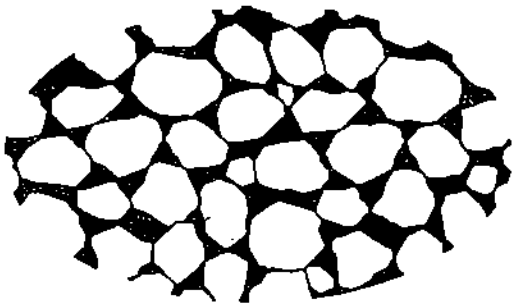

Fig. 5.2: Collenchyma

Occurrence

Young stem, petioles of leaves, and stalk of flowers, ribbed stems and petioles as well as the square stems of certain plants have collenchyma cells.

Function

Collenchyma is a mechanical tissue providing strength and elasticity to the stem and leaves of plants.

iii) Sclerenchyma

Observe a preparation of sclerenchyma cells under a compound microscope. Make a neat sketch of the tissue as observed in your record note book. Write down the structural details of sclerenchyma tissues.

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Were you provided with slides of more than one type of sclerenchyma cells? Yes, the size and shape of sclerenchyma cells are variable and atleast two types could be distinguished.

a) Elongate cells called sclerenchyma fibres (Fig. 5.3).

b) Short cells either isodiametric or irregular in shape called sclereids (Fig. 5.4).

Make drawings of both types of cells in your note book. You will find that sclerenchyma fibres are:

i) long cells with pointed needle like ends.

ii) dead cells with no protoplast in them.

iii) highly thick walled, the thickening due to a substance called lignin.

Occurrence

Found in cortex, pericycle, xylem and phloem.

Function

Provide mechanical strength to plants.
The sclereids are circular or irregular shaped cells with lignified cell walls.

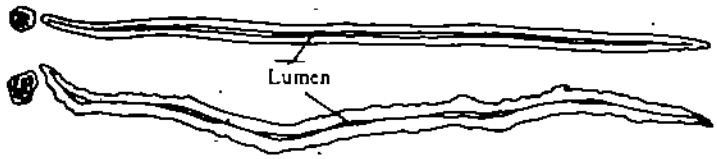

Fig. 5.3: Sclerenchyma fibres

The sclereids are also dead cells with no protoplast in them.

Occurrence

Found in cortex, phloem, pith, seed coats and fruit wall.

Function

Provide mechanical strength to plant parts.

Fig. 5.4: Sclereids

5.4 OBSERVATIONS ON COMPLEX PERMANENT TISSUES

Complex tissues, you have learnt earlier, are composed of different types of cell elements. These different cellular elements form an integral part of a structure of plant, and carry out a specific function. By way of analogy with animal tissues, you may recall that blood is a complex tissue formed of different types of cellular elements. Among plants xylem and phloem are examples of complex tissues. These tissues are concerned with transport of water and nutrients in plants, hence called vascular tissues. The vascular tissues perform a function similar to the ones performed by the blood vascular system in animals.

5.4.1. Xylem

Xylem is a complex tissue forming a part of vascular bundle. It's major function is conduction of water and solutes. It also provides mechanical support to plants. As a complex tissue it consists of different types of cells and elements. The tissues which go to form xylem are a) tracheids b) vessels c) xylem fibres and d) xylem parenchyma. Place a permanent slide preparation of xylem under a microscope and observe the various elements:

a) Tracheids (Fig. 5.5)

A tracheid is a much elogate cell. The cells are devoid of any protoplast, hence they are dead cells. The tracheids have a lumen without any contents in them. The walls of tracheids are thicker and lignified. Depending on the type of thickness, tracheids are classified into annular (ring like thickenings), spiral, reticulate (the walls present a network like appearance), scalariform (ladder like) and pitted (with holes).

b) Vessels

These are long tube like bodies which conduct water and solutes. A trachea is a vessel formed from a row of cylinderical cells arranged in longitudinal series. The partition walls between the cells are perforated so that the entire structure is a long continuous vessel.

c) Xylem Fibres

These are the dead cells which provide mechanical support to the plant. They are long cells with lignified walls.

d) Xylem Parenchyma (Fig. 5.5)

These are the only living component of the xylem of most plants. Parenchyma is abundant in the secondary xylem of most plants. The cells may be thin walled or thick walled. The cells have a storage function, mostly starch and fatty substances are stored.

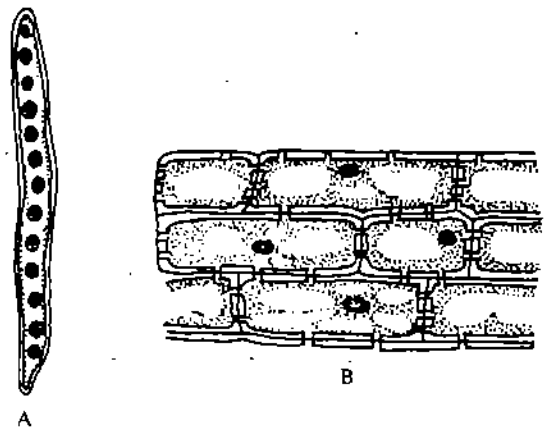

Fig. 5.5: Xylem tissue a) tracheid b) parenchyma

5.4.2 Phloem

Observe a permanent slide preparation of phloem under the microscope. A phloem is a complex tissue composed of again four elements. a) sieve elements b) companion cells c) parenchyma d) fibres.

a) Sieve elements (Fig. 5.6)

These are the mot important elements of phloem. Sieve elements consist of sieve tubes. Sieve tubes are cells arranged in a longitudinal series with perforations in the cell wall, called sieve plate. Therefore, in the sieve plate cytoplasmic connections are established between neighbouring cells. The cytoplasmic connections as called plasmodesmata. The sieve plate is formed by the primary cell walls of two adjacent cells with a middle lamella between them. Sieve tubes have conducting function.

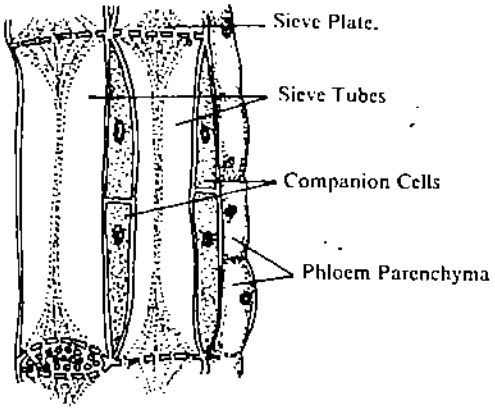

Fig. 5.6: Phloem tissue

b) Companion Cells (Fig. 5.6)

Companion cells as their name suggests are closely associated with sieve tubes of flowering plants both during development as well as during functioning. They are small elongate cells with dense cytoplasm and prominent nuclei. They occur on the lateral walls of sieve tubes. Companion cells accompanying a sieve tube may be a single cell of equal length or a mother cell may be divided transversely forming a series of companion cells. Sieve tubes and companion cells originate from the same mother cell. The companion cells function as long as sieve tubes are functional. The companion cells are firmly attached to sieve tubes.

c) Phloem Parenchyma (Fig. 5.6)

These living cells are also associated with sieve elements. They are living cells with protoplast in them. They are concerned with storage of organic food materials.

d) Phloem Fibres

These are sclerenchymatous cells. These are dead elogated cells with lignified walls having single pits. The fibres are of commercial importance as they are used for the manufacture of ropes and cords.

EXPERIMENT 6 PREPARATION OF BLOOD SMEARS OF FROG AND MAN

Structure

6.1 INTRODUCTION

Smear technique, like the squash technique is one of the simple and useful techniques for the study of cells. In smear technique as you did earlier, a fluid or a semifluid tissue is spread over the surface of the slide and stained subsequently. Smear technique is routinely used in clinical laboratories for the study of blood cells. In this lab exercise you will prepare the blood smears of man and that of frog, and compare the different types of blood cells in these two systems.

Objectives

At the end of this exercise you should be able to:

- prepare a thin blood smear, and
- identify and distinguish between the red blood corpuscles as well as the different types of white blood corpuscles of man and frog in a microscopic slide or a photograph.

6.2 MATERIALS REQUIRED

1. Slides,
2. Coverslips,
3. 70% alcohol,
4. Sterilised needles,
5. Giemsa or Wright's stain,
6. Distilled water,
7. Pasteur pipette.

6.3 PROCEDURE

1. Clean the slides and let them be free from grease, finger prints, etc.

2. Clean the tip of your middle finger with 70% alcohol and prick with a sterilised needle. The sterilization can be done by heating the needle over a spirit lamp flame for a few seconds and then cooling or the needle may be wiped clean with cotton soaked in 70% alcohol.

3. When a drop of blood appears on the finger tip, wipe it away with cotton dipped in alcohol.

4. Press the finger tip to get the next drop of blood and touch it with the clean surface of slide about 1 cm. from the right narrow edge (see Fig. 6.1).

5. Place the narrow edge of a 2nd slide at about 45° angle to the 1st slide and to the left of the drop of blood.

6. Pull to the right until the 2nd slide touches the blood. Along the line of contact, the blood spreads. Now push the 2nd slide towards the left in a steady but brisk movement. Take care to keep the edge pressed uniformly against the surface of the 1st slide. Keep pushing until the other end of the slide is reached. This method spreads the blood thinly over the surface of the slide but does not run over the cells and crush them. You may make 3 or 4 such preparations.

7. Once the smear is made, air dry the slides for about 10 minutes.

8. Mark with a wax pencil the region of the smear that is to be stained on the underside of the slide. This can be approximately the length of a 40 mm. cover slip.

The blood smear of frog is prepared similarly. For obtaining the frog's blood cut open the frog, puncture its heart and place a drop of blood on a microslide with the help of a pasteur pipette.

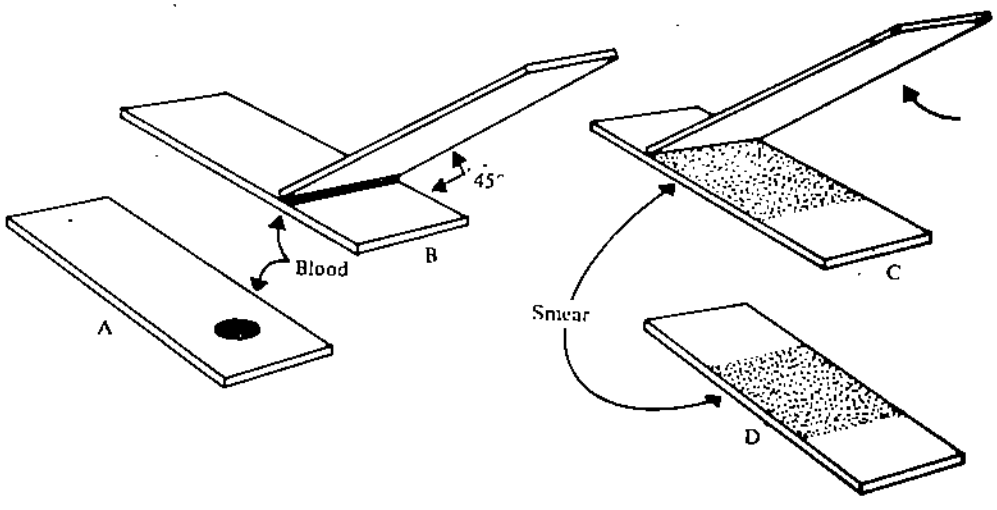

Fig . 6.1

9. **Staining of the slides**

Cover the region marked with wax pencil with 10-12 drops of Giemsa or Wright's stain. Leave the stain over the smear for 2 to 3 minutes. After this period add an equal amount of distilled water and leave for 2 to 4 minutes. Drain off the stain and rinse the slide in distilled water once or twice. Blot with 2 sheets of filter paper. Take care only to press with filter paper and not rub. Air dry the slide thoroughly and mount with a cover slip, using DPX mountant.

Observe the slides under a compound microscope first under low magnification and then at higher magnification.

6.4 OBSERVATIONS AND RESULTS

Your observations under the microscope should enable you to draw the red blood corpuscles of human and frog blood. Figure 6.2 shows the RBCs in frog blood smear. Note that the cells are oval shaped and nucleated. In human blood smear the RBCs are biconcave, spherical and non-nucleated (Fig. 6.3). Observe carefully the various types of white blood corpuscles, the eosinophils, the basophils and neutrophils. Also, observe the blood platelets. Generally with Wright or Giemsa stain the different cells and their nuclei give the following colours.

Erythrocytes — pink
Nuclei — deep blue
Basophils — deep purple
Eosinophils — bright red
Neutrophils — reddish brown
Platelets — violet to purple

6.5 SELF ASSESSMENT QUESTIONS

1. How do the red blood corpuscles of frog and man differ from each other?

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2. What is the shape of the nucleus of the WBC of human blood?

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3. Are there different types of WBCs in frog? Or is there only one type? Give the structure of WBC of frog blood.

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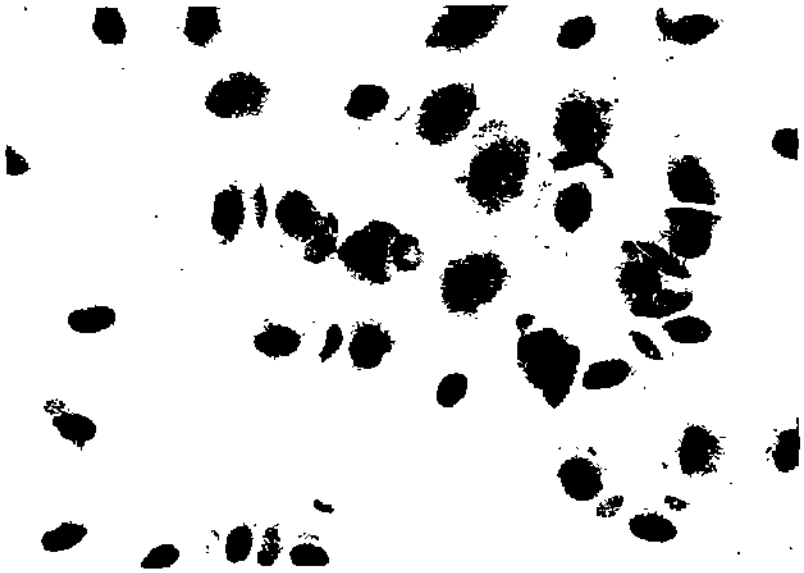

Fig. 6.2 : Frog blood smear

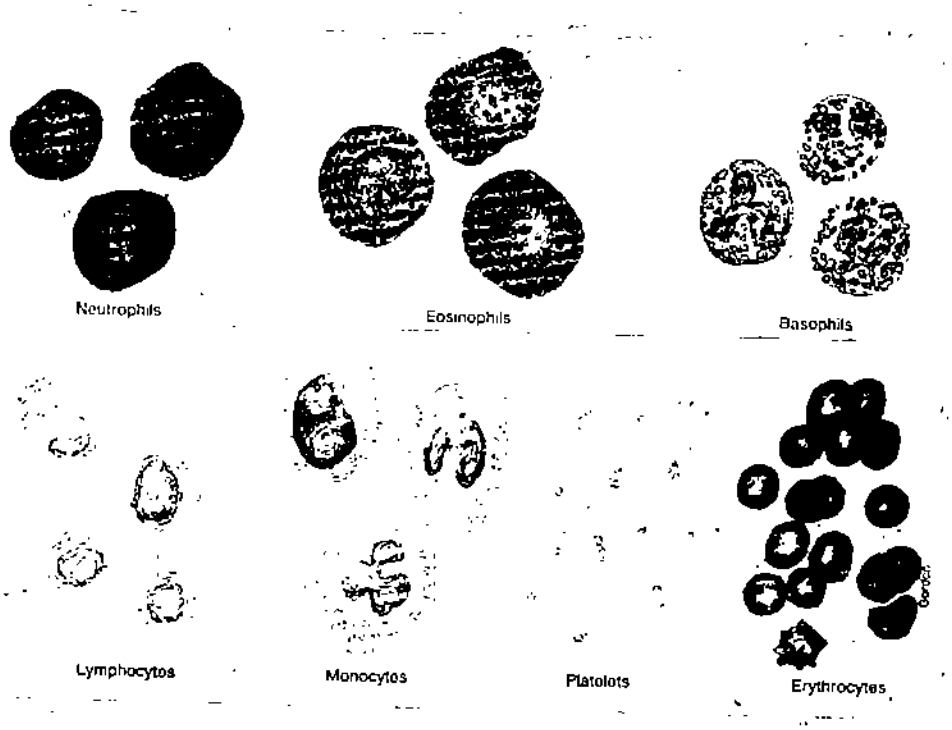

Fig. 6.3 : Cellular components of human blood

EXPERIMENT 7 SQUASH TECHNIQUE FOR THE STUDY OF MITOSIS AND MEIOSIS

Structure

7.1 INTRODUCTION

Squash technique is one of the simple techniques widely used for the study of chromosomes. The technique consists of applying a gentle pressure on a small piece of previously stained tissue to flatten the cells and spread the chromosomes. The technique can be performed rapidly and is an efficient one for the study of dividing cells. The method helps to study a single layer of large cells in their entirety. Before performing this lab exercise you may revise the units on mitosis and meiosis in Block 4 of LSE-01 (Cell Biology) Course. Your counsellor will screen for you the video programme on "Squash and Smear Techniques in Cell Biology" Parts I and II.

Objectives

This lab exercise should enable you to:

- make squash preparations of plant material such as onion root tips and to observe and interpret mitotic stages,
- make squash preparation of both plant and animal reproductive tissues for the study of meiotic stages.
- compare the processes of mitosis and meiosis.

7.2 MATERIALS REQUIRED

1. Onion root tips,
2. Onion flower buds,
3. Testis of grasshopper,
4. Acetic alcohol,

5. Aceto-carmine or acetoorcein,
6. 2N hydrochloric acid,
7. Slides,
8. Pasteur pipettes,
9. Coverslips,
10. Spirit lamp,
11. Watch glass,
12. Filter paper, dissection kit,
13. Nail polish or DPX mountant, and
14. Compound microscope.

7.3 PROCEDURE FOR THE SQUASH PREPARATION OF ONION ROOT TIPS FOR THE STUDY OF MITOSIS

Onion root tip is an ideal material for the preparation of mitotic stages because of its easy availability. It is also easy to study since the chromosomes are relatively large and few in number. The onion roots can be grown by placing onion bulbs with their root side down in contact with water in a beaker or a conical flask. After 3 days you can see the roots grow to a length of 2 to 3 cms. Remove the onion bulbs, cut the root tips and transfer them to a solution of acetic alcohol (acetic acid 1 part and ethyl alcohol 3 parts v/v). The root tips should be kept in the acetic alcohol solution for 12 to 24 hours. At the end of the fixation period the material can be transferred to and stored in 70% alcohol until it is used for squash preparation. Now, follow the procedure given below for squash preparation.

Cells removed from their natural environment will change shape and decompose over a period of time unless the shape and size is preserved. This preservation, prior to their study is known as 'fixation', which can be achieved by heat, or freezing or chemically. A fixative is chosen according to the structural or chemical components of the tissue that are to be demonstrated.

1. Transfer the root from the fixative (acetic alcohol) or storage solution (70% alcohol) onto a watch glass and wash extensively with water.
2. Drain off the water with a pasteur pipette and add a few drops of 2N HCl to the watch glass. The tissues can by hydrolysed in 2N HCl for 10 minutes at room temperature or for a minute over a spirit lamp flame. While hydrolysing over the flame make sure that you move the watch glass over the flame. This would prevent damage to the tissue by over heating.
3. After hydrolysis drain off the HCl and wash the root tips in water.
4. Remove the water and add 1% acetocarmine or acetoorcein to the root tips for staining. The staining requires 10 to 15 minutes.
5. At the end of the staining process, transfer 2-3 root tips onto a slide, cut and retain only the meristematic region and remove the debris from the slide.

6. Place a drop of 45% acetic acid on the material and carefully place a coverslip over it.

7. Remove the excess acetic acid from the sides of coverslip by using the edge of a filter paper.

8. Place the slide between the filter paper and gently press the coverslip down. Using the flat bottom of a pencil slowly tap on the filter paper to obtain a uniform spread of the material. The tapping flattens the cells and spreads the chromosomes.

9. Seal the edges of the coverslip by applying nail polish so that the fluid evaporation is minimised. Observe the slide under the compound microscope.

7.4 OBSERVATION AND RESULTS

You are already familiar with the concept of cell cycle and know that cells can be observed in either dividing or non-dividing phase of the cycle. In a squash preparation you will find that majority of the cells are in interphase (non-dividing cells). The interphase nucleus will appear as a darkly stained body with acetocarmine or acetoorcein. The nuclear membrane is intact and the chromatin is in the form of a network.

You are also aware of the various stages in the process of cell division Unit 16 of the Cell Biology Course (LSE-01). We now provide you a brief description of each stage and you should sketch the stages you observe under the microscope in the record notebook.

Stages of ..iitosis

1. Prophase

Prophase, the first stage in the division process is characterised by the unwinding of the chromatin reticulum. Each chromosome begins to appear as **two chromatids** with a **single centromere.** The nuclear membrane slowly disappears. See Fig. 7.1 for stages of mitosis.

2. Metaphase

In metaphase the chromosomes tend to arrange themselves in the middle of the cell called the equatorial plate. It is possible to count the number of chromosomes at the metaphase stage. When observed carefully, the sister chromatids of the chromosomes can be seen.

3. Anaphase

Anaphase is marked by the movement of the chromosomes to the opposite poles of the cell. Essentially the **centromere splits** and a single chromosome with two chromatids becomes **two independent chromosomes** each with a centromere. One can observe the anaphase in various stages such as early anaphase, middle anaphase and late anaphase depending on the position of the chromosomes during their movement. The spindle fibres which are attatched to the centromere of the chromosomes may not be distinctly visible with the stain you have used.

4. Telophase

In telophase two daughter nuclei each with the same number of chromosomes as the parent nucleus are formed. The chromosomes undergo coiling and form the chromatin reticulum. The nuclear membrane reappears and the nuclear division is completed.

Cytokinesis

Once the nuclear division is complete, cytokinesis that is the division of a cell into two daughter cells occurs. Look for those daughter cells formed at the end of mitosis and try to locate the cell wall between them.

SAQ 1

Why are most cells seen in interphase?

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7.5 PROCEDURE FOR THE SQUASH PREPARATION OF GRASSHOPPER TESTIS OR ANTHERS FROM ONION BUDS FOR THE STUDY OF MEIOSIS

Meiosis, as you are aware, occurs in reproductive tissues. In meiosis the chromosomal number is halved and the cells which are diploid (2n) divide to form cells which are haploid (n). At the end of the meiosis the reproductive cells namely the sperms and the eggs in the case of animals, and the pollens and ovules in the case of plants are formed. The procedure for performing the squash technique is slightly different for plant and animal tissues. Since plant cells have cell walls, the polysaccharides of the cell wall need to be hydrolysed so that the stain can enter into the cells. The hydrolysis procedure is not required for the animal cells. The animal tissues can be transferred directly to the stain after fixation. First, we will describe the steps for the preparation of plant cells.

1. Remove the anthers from the onion buds stored in 70% alcohol. Wash well with water in a watch glass.

2. Transfer the anthers to a watch glass with a few drops of 2N HCl and hydrolyse over the flame of a spirit lamp for about 5 minutes.

3. Wash the anthers well with water and transfer them to 1% acetocarmine or acetoorcein stain for 10 minutes.

4. When the anthers are stained transfer them onto a slide and add a drop of 45% acetic acid.

5. Carefully place a coverslip over the stained tissue and drain off excess acetic acid using the edge of a filter paper.

6. Place the slide between two sheets of filter paper and using the flat end of a pencil gently tap on the coverslip.

7. The cells of the anther flatten and the chromosomes spread. Seal the edges of the coverslip with nail polish before observing under the compound microscope.

The meiotic stages can also be studied from the testis of the grasshopper.

1. Obtain grasshopper testis previously fixed in acetic alcohol (ethanol: acetic acid 3:1 v/v) and stored in 70% alcohol.

2. Wash the testis in water and then release the tubules by teasing with a dissection needle.

3. Transfer the tubules onto a watch glass containing 1% acetocarmine or acetoorcein and stain for 10 to 15 minutes.

4. Transfer one or two tubules onto a slide and add a drop of 45% acetic acid. Place a coverslip over it and remove the excess of acetic acid using the edge of a filter paper.

5. Place the slide between 2 sheets of filter paper and using the flat end of a pencil gently tap on the coverslip or apply pressure with your thumb.

6. The tubules flattened thereby spreading cells and chromosomes. Seal the edges of the coverslip with nail polish and observe under a compound microscope.

7.6 OBSERVATIONS AND RESULTS

Meiosis actually consists of two division processes: meiosis I and meiosis II. Meiosis I constitutes the reduction division where the chromosomal number is reduced by half. Meiosis II resembles mitosis. At the end of the two divisions four cells are formed, each with half the number of chromosomes as the parent cell. The following is a brief description of the various stages of meiosis, You may get a detailed account of meiosis in Block 4 of LSE-01 Course.

Meiosis I

1. Prophase-I

The prophase-I consist of 5 different stages. They are leptotene, zygotene, pachytene, diplotene and diakinesis.

During these stages the uncoiling of the chromatin reticulum, condensation of the chromosomes, synapsing of the homologous chromosomes, crossing over and chiasma formation and recombination occur. If observed carefully the different prophase-I stages can be identified in the squash preparation of the grasshopper testis. On the contrary, in anther preparation you may identify only one or two meiotic stages since the various meiotic events are highly synchronised in plant tissues. Draw your observations in your notebook and compare them with the photographs provided. (Fig. 7.2)

List out the differences between prophase of mitosis and prophase-1 of meiosis in your notebook.

2. Metaphase-I

Metaphase 1 shows the arrangement of the chromosomes at the equatorial plane. Metaphase-I is the most suitable stage for counting the number of chromosomes. Since the number of chromosomes differ in different species of grasshopper, count and record the number of chromosome in the species you have used for your studies. In the anthers of the onion buds (*Allium cepa*) the number of chromosomes present is 7.

How is metaphase-I of meiosis different from metaphase of mitosis? Write down the differences in your note book.

3. Anaphase-I

Anaphase-I is a stage at which the chromosomal number is reduced by half and unlike the mitotic anaphase the centromere does split and the entire chromosome moves to one pole. As a result each nucleus receives **only half** the **number of chromosomes**. Anaphase-I shows the chromosomes moving to the opposite poles of the cell.

4. Telophase-I

In telophase-I two nuclei are formed.

Meiosis II

Meiosis II depicts the different division stages similar to those of mitosis.

You will note that the cells which undergo meiosis II are relatively smaller than the cells which undergo meiosis I. Also, they carry only half the number of chromosomes like the meiosis-I cells. This fact can be verified by actually counting the number of chromosomes during meiosis II.

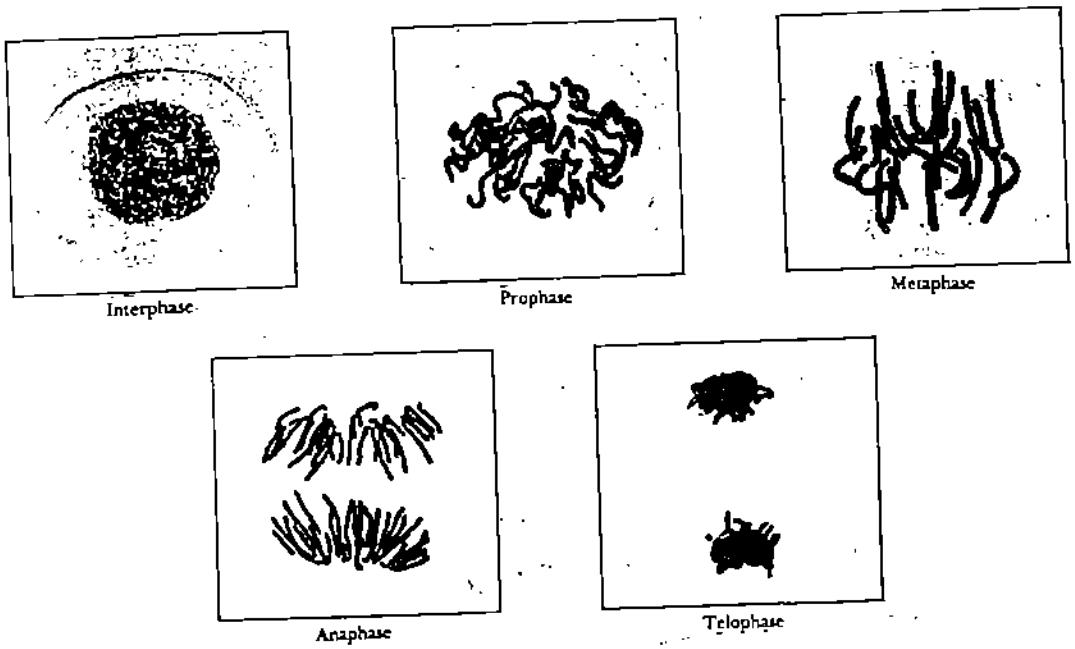

Fig. 7.1 : Stages in mitosis

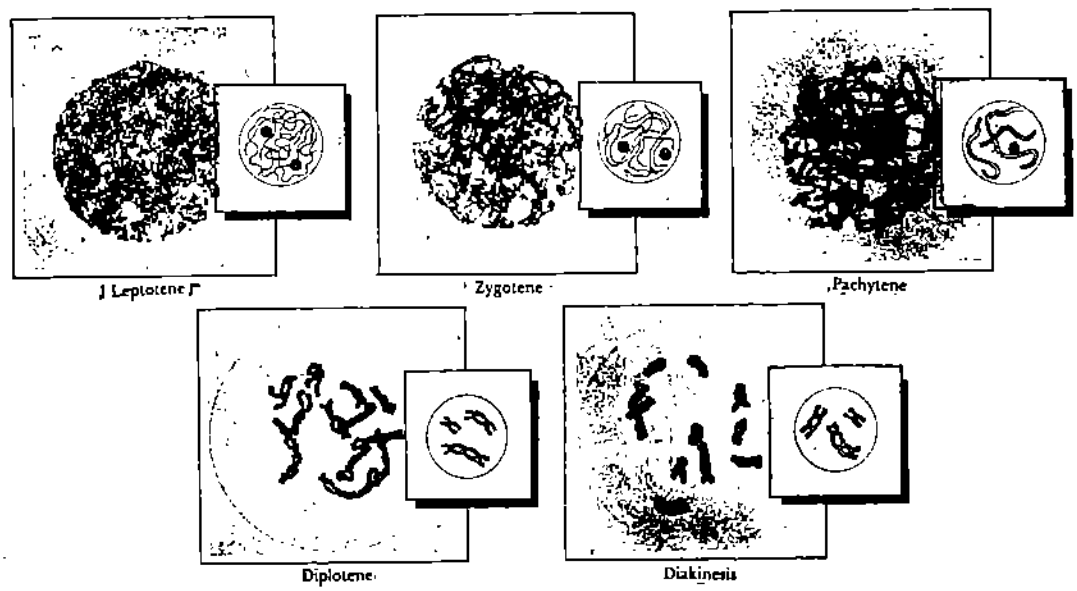

Fig. 7.2 : Stages in meiosis (Prophase I)

EXPERIMENT 8 PREPARATION OF POLYTENE CHROMOSOMES FROM SALIVARY GLANDS OF *Drosophila* LARVAE

Structure

8.1 INTRODUCTION

The salivary glands of Dipteran insect larvae have nuclei which are in perpetual interphase. They were described by Balbiani in 1881. These nuclei have chromosomes which are abnormally large as compared to the chromosomes of the other body cells. Because of their size they come to be known as **giant chromosomes**. The giant chromosomes when fully extended are about 100 times longer than those normally found at the metaphase of mitosis. The DNA of the giant chromosome repeatedly replicates but the daughter strands do not separate. The replication occurs nearly 10 times resulting in 1024 parallel strands held together. The fully developed banded chromosomes are nearly 0.25 to 0.55 mm long. The chromosomes show alternating dark and light bands and each band represents a gene. The amplification of DNA of salivary gland chromosomes in the insect larvae is also known as *polytenisation* and the chromosomes are referred to as polytene chromosomes. In this lab exercise you will learn to prepare the giant polytene chromosomes of the larvae of the fruit fly *Drosophila melanogaster* using the simple squash technique and to study its morphology.

Objectives

At the end of this exercise you should be able to:

- dissect out the salivary glands of *Drosophila* larvae,
- make a squash preparation of salivary glands,
- describe the salient features of polytene chromosomes.

8.2 MATERIALS REQUIRED

1. 3rd instar *Drosophila* larvae,

EXPERIMENT 9 SMEAR TECHNIQUE TO OBSERVE SEX-CHROMATIN IN THE BUCCAL EPITHELIAL CELLS OF HUMAN FEMALES

Structure

9.1 INTRODUCTION

Human beings have 46 chromosomes in their somatic cells. In human males there are 44 autosomes and 2 sex chromosomes termed X and Y.

Human females besides the 44 autosomes have two X chromosomes. This means that human females have the potential to produce twice as much X-chromosome gene products as human males. But this does not happen. Of the two X-chromosome in somatic cells of all mammalian females one X-chromosome is inactivated and generally remains attached to the nuclear membrane in interphase cells. The inactive X-chromosome was first observed by Barr and Bertram (1949) in the nuclei of the nerve cells of female cats. The inactive X-chromosome also known as **Barr body** represents a mechanism by which the dosage compensation for X-lined gene products is achieved. In other words, the females also produce only as much X-chromosome gene products as males, despite having two X-chromosomes. It is possible to demonstrate the inactive X-chromosome or the sex chromatin in the buccal epithelial cells of human females by the smear technique. The smear technique consists of spreading a semifluid or fluid tissue over the surface of a slide and staining it subsequently.

Objectives

At the end of this exercise you should be able to:

- prepare thin smears of semifluid and fluid tissues,
- demonstrate the sex chromatin or Barr body in the somatic cells of mammalian females.

9.2 MATERIAL REQUIRED

1. Spatula/Tooth picks

2. Slides
3. Coverslips
4. Giemsa stain
5. Xylene
6. 90% alcohol and absoute alcohol
7. 6N HCl
8. Compound Microscope

9.3 PROCEDURE

1. Using a spatula or the broad side of a tooth pick scrap the inner side of your cheek and discard the first scraping.
2. Repeat the step and gently spread the scraping on a clean slide. Air dry the preparation.
3. Fix the cells by dipping the slide in a Coplin jar containing 90% alcohol for a minute and air dry the slide again.
4. Transfer the slide to a Coplin jar with 6N HCl for 10 minutes at room temperature. This step of hydrolysis removes the debris from the smear.
5. Wash the slide well with distilled water and stain with phosphate buffered 4% Giemsa stain for 10 minutes.
6. At the end of 10 minutes transfer the slide to water briefly to differentiate and air dry.
7. Clear the slide in xylene and mount with DPX. Observe the slide under the microscope for the presence of Barr body.

Note: The barr body can be observed only in preparations made from the squamous epithelial cells of females. Make a similar preparation from the buccal epithelial cells of males for comparison.

Fig. 9.1 : Barr body in human female

9.4 OBSERVATIONS AND RESULTS

You should be able to observe a distinct darkly staining body attached to the nuclear membrane in the epithelial cells obtained from females, as shown in Fig. 9.1. It is preferable to observe the preparation under the oil immersion lens of a compound microscope which magnifies the cells 1000 times. Otherwise, you may observe under the high power lens which magnifies the objects around 400 times.

EXPERIMENT 10 QUALITATIVE BIOCHEMICAL TESTS FOR ORGANIC CONSTITUENTS OF CELLS

Structure

10.1 INTRODUCTION

You have learnt in Cell Biology Course (LSE-01) about cell and its macromolecules—namely carbohydrates, proteins and lipids. These macromolecules are made of smaller basic molecules joined in set sequences. Proteins are made up of amino acids, carbohydrates of sugars and a neutral fat, of glycerol and fatty acids. In this laboratory exercise, you will learn to perform certain simple tests for the identification of the organic constituents of cells. Various chemicals and reagents will be used for the detection of these molecules which will produce characteristic reactions and specific colour. If a change in colour as specificed for the reaction is observed the test is said to be positive. Non-appearance of the specific colour would indicate a negative result suggesting that the particular molecule is not present.

Objectives

After doing the experiment you should be able to:

- describe the major organic constituents of cells and tissues and recognise their chemical structures,
- name and group tests used for carbohydrates, proteins and lipids,
- perform the simple tests for the identification of amino acids, proteins, carbohydrates and lipids,
- distinguish between positive and negative tests.

10.2 MATERIALS

Test tubes, Test tube rack, Bunsen burner, Pasteur pipettes, spatula, 2 ml syringes, 25 ml measuring cylinders, 5 and 10 ml graduated pipettes, litmus papers.

10.3 CARBOHYDRATES

Carbohydrates consist of simple molecules such as various mono and disaccharides and large complex molecules like starch and dextrin in plants, and glycogen in animals. Glucose, galactose and fructose are examples of monosaccharides (Fig. 10.1) Sugars containing two units of monosaccharides are called disaccharides e.g. sucrose, maltose and lactose (Fig. 10.2). Starch contains hundreds of such units. Sugar, starch and glycogen serve as fuels for cells and cellulose is the structural component of plants. Glucose is the most abundant and extremely important monosaccharide for life processes.

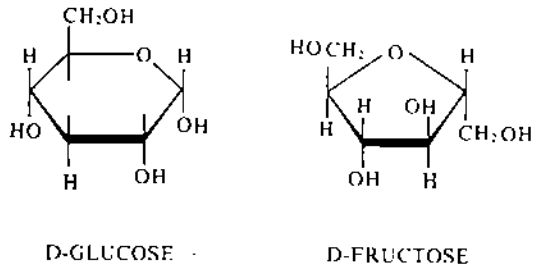

Fig. 10.1 : Monosaccharides

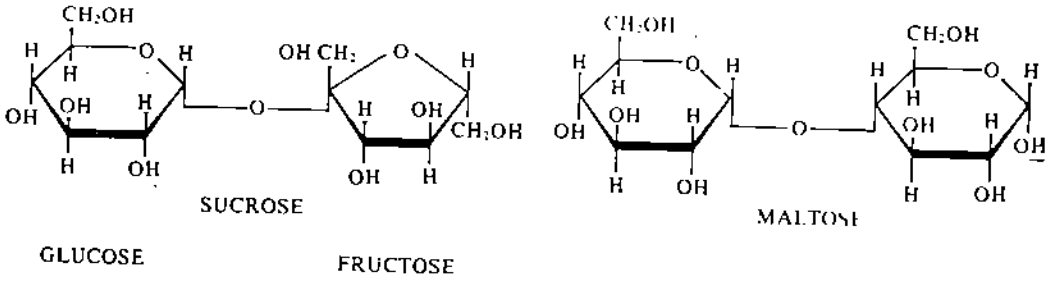

Fig. 10.2 : Disaccharides

10.3.1 General Tests for Carbohydrates

Reagents and Test Solutions

1% alpha naphthal reagent, sulphuric acid, Benedict's reagent, concentrated HNO_3, phenyl hydrazine hydrochloride, sodium acetate, Seliwanoff's reagent, iodine solution, starch solution, 0.5% glucose, 1% sucrose solution, onion juice, potato extract, frog thigh muscle extract, 2% fructose solution, grapes.

Exp 1: HEATING TEST—Heat a small quantity of carbohydrates provided and see that it chars and gives the smell of burnt sugar (smell of caramel). If the sample is in solution form, take about 1.0 ml and heat it to dryness. You will get the same smell of burnt sugar.

Exp 2: MOLISCH TEST—Take 2.0 ml solution of carbohydrates provided and add two drops of 1% alpha naphthol solution. Carefully run about 2.0 ml of concentrated sulphuric acid along the side of the inclined test tube. Write down your observation.

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This reaction is due to the formation of furfural and furfural derivates by the acid acting on carbohydrates. The furfural on combining with alpha naphthol produces a purple colour complex.

10.3.2 Tests for Monosaccharides

Exp 1: SOLUBILITY AND pH TEST—Monosaccharides are soluble in water. To determine the acidic or basic nature of monosachharides dip litmus papers (red and blue litmus one by one) into 1% glucose solution. Note if there is any change in the colour of the litmus paper. Monosaccharides are neutral and do not change the colour of the two types of litmus papers.

Exp 2: BENEDICT'S TEST—This test is by far the most conclusive test for reducing sugars and is semi-quantitative (a rough estimation of the amount of reducing sugar is possible based on the intensity of colour reaction). When any reducing sugar is heated with Benedict's reagent, a fine precipitate is formed and you will observe coloured precipiatate green, yellow or red depending upon the concentration of the sugar. The reactions occuring during the test can be summarised as follows:

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

$$2Cu(OH)_2 + C_6H_{12}O_6 \longrightarrow Cu_2O + C_6H_{12}O_7 \text{ (Gluconic acid)} + 2H_2O$$

(Cuprous oxide—yellow, green or red precipitate).

Non-reducing sugars such as sucrose do not give a positive result with this test due to non-availability of reducing groups.

Take 4 tubes, add 2 ml of water in tube (1), 2 ml of onion juice in tube (2), 2 ml of an extract of frog thigh muscle in tube (3) and 2 ml. of 1% glucose solution into tube (4). Heat the tubes simultaneously in a water bath for about 3-5 minutes. Note if there is any colour change and record such changes according to tube number.

Add about 3.0 ml. Benedict's reagent to each test tube and heat for about 3-5 minutes. Record the colour changes in the table given below:

Prepare onion juice by macerating a small piece of onion in a mortar with pestle with a few drops of water. Filter and use the filtrate for the test.

Frog thigh muscle extract is obtained by grinding small pieces of thigh muscle tissue in a mortar with pestle in a small quantity of distilled water. Filter and use the filtrate for the test.

EXP. 2 : BIURET TEST—Peptide bonds (CO–NH) in proteins give positive test with biuret reagent. Biuret reagent contains a strong solution of sodium or potassium hydroxide and a very dilute solution of copper sulphate. Addition of alkaline copper sulphate solution to protein solutions forms a purple or pink colour depending upon the nature of proteins. Glycoproteins containing peptide linkages also show positive colour reactions. Biuret is the compound derived from urea which also contains the—CONH group and gives a positive result.

To 6 ml of 5% sodium hydroxide solution add 2 drops of 1% copper sulphate solution and mix well. Divide this reagent into two equal portions. To one portion add 3 ml. of protein solution and to another add 3 ml. of water to serve as control. Mix well by shaking the test tubes. Write down your observations.

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The test solution changes the colour of copper sulphate into violet or pink depending upon whether it is a protein or a polypeptide. In case of polypeptide the colour changes to pink.

Exp. 3: XANTHOPROTEIC TEST—Amino acids which have an aromatic ring in their R group give a positive result with xanthoproteic test. Amino acids such as phenylalanine, tyrosine and tryptophan belong to this category.

To 3 ml of protein solution in a test tube add 1 ml of concentrated nitric acid. Dissolve the white precipitate that is formed by boiling over a flame. The aromatic ring present in the R ring of certain amino acids undergoes nitration and the solution turns yellow. Cool and add 10% NaOH solution along the sides of the test tube. The colour becomes deep yellow and to orange owing to the formation of coloured ions. Simultaneously run a control experiment with 3 ml of distilled water.

Exp. 4: MILLON'S TEST—Procedure: To 1 ml of test solution add about 1 ml of Millon's reagent. An yellowish white precipitate is formed. Add about five drops of sodium nitrite solution. On gentle warming, the precipitate turns salmon pink to brick red. Perform a control test simultaneously with 1 ml of water.

Millon's reagent contains mercuric sulphate. The protein is precipitated by mercury which then combines with the amino acid tyrosine. This compound becomes red on treatment with nitrous acid.

10.5 LIPIDS

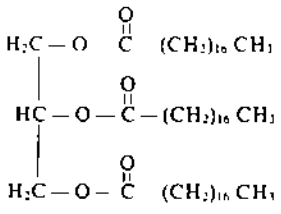

Fig. 10.3 : A triglyceride molecule

Lipids are low melting point compounds of diverse chemical nature. They have a greasy consistency and are soluble in fat solvents like ether and chloroform but insoluble in water. Typically a fat in the form of triglyceride is composed of one molecule of glycerol and three molecules of fatty acids (Fig. 10.3).

Reagents and Test Solutions

Gingili or groundnut oil, cholesterol, ether, potassium bisulphate, Sudan III solution, conc. sulphuric acid, chloroform, acetic anhydride.

Test for Solubility

Exp 1: Take few drops of oil in a dry test tube containing ether and shake. A clear solution is obtained. Repeat the experiment with water and tabulate the results.

	Observation	Conclusion
Oil		
Water		

Exp. 2: Identification of Glycerol—Place one ml of test material in a dry test tube. Add about 100 mg. of powdered potassium bisulphate. Warm gently at first and later increase the heat. Glycerol is liberated and converted into the pungent, lachrymatory unsaturated aldehyde called acrolein.

Exp. 3: Sudan III Test—Take equal quantity of oil (0.5 ml.) and water in a test tube. Add 2-3 drops of Sudan III stain and shake it. Keep it for 5 minutes the two layers of water and oil separate out, and the fat layer becomes red.

Exp. 4: Test for Cholesterol—Dissolve a little cholesterol powder in about 4.0 ml of chloroform and divide this into two tubes. To one tube add 2.0 ml strong sulphuric acid and shake gently. The upper layer of chloroform will become red. The lower layer of sulfuric acid will change from yellow to green. To the other half in a separate tube add about 10 drops of acetic anhydride, and 2 drops of strong sulphuric acid and shake. The colour of the solution becomes deep blue.

Precautions

- All chemicals should be considered as potentially dangerous and handled carefully. Contact of chemicals with skin and clothing should be avoided.
- Organic solvents should be kept tightly closed on a working bench.
- No organic solvent such as ether, chloroform should be heated on naked burner flame. They are highly inflammable.
- No water should be added in vessel containing acids which will react violently and splutter. Addition of acids to a solution should be done very slowly by inclining the test tube.
- Never heat a solution by keeping the open end of the test tube directed towards you; keep it away from you.
- Never fill the test tube with a solution more than one third (1/3) of the tube length while heating on a burner.
- If a reagent gets into the eye or face immediately wash several times with water.

EXPERIMENT 11 MOVEMENT OF MATERIALS IN CELL—DIFFUSION AND OSMOSIS

Structure

11.1 INTRODUCTION

The exchange of gases, water, food material and wastes between cell and its environment involves phenomena of diffusion and osmosis. You may recall that CO_2 and O_2 diffuse in and out from mesophyll cells into the stomatal cavity. Oxygen from air diffuses into the moist cells lining the inner respiratory surfaces, be it gill or lungs and passes from these cells into the capillaries. Water and small uncharged molecules diffuse in and out of the cell. For example, much of the water within the blood plasma diffuses out during the passage through blood capillaries and then diffuses into the open-ended lymph capillaries. Often changes in solute concentration occur within a single cell or within a tissue. Through the phenomena of osmosis and diffusion cells adjust constantly to maintain a steady state (homeostasis) in the midest of an everchanging environment. In this laboratory exercise you will perform experiments to study the phenomena of diffusion and osmosis.

Objectives

After performing the experiments in this laboratory, you should be able to:

- demonstrate the phenomena of diffusion and osmosis,
- demonstrate differences in plasmolysis, deplasmolysis and haemolysis.

11.2 DIFFUSION

The tendency for molecules and ions in solution or gases in the atmosphere to diffuse until uniform distribution results is called **diffusion.** All molecules and ions are in a state of constant random motion due to inherent kinetic energy.

The molecular motion is slight in solids but much greater in liquids and gases. This random motion causes a net movement of molecules towards zones where the concentration of the molecules is lower (Fig. 11.1). We may say that molecules always explore in the space around them diffusing out until they fill it uniformly. Diffusion is a passive process. It does not require stirring or other types of convective disturbances.

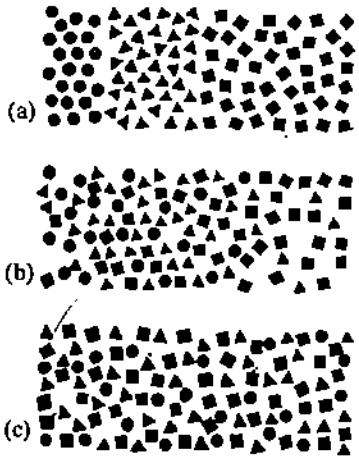

Fig. 11.1 : Diffusion: a) three concentrated substances (b) random and independent diffusion (c) even distribution throughout the system

Two molecules with identical kinetic energies but different masses, at a constant temperature have different velocities. The smaller molecules (low mol. wt.) have higher velocities than larger (high mol. wt.) ones. In other words, relative diffusion of two substances depends on their respective molecular weights.

11.2.1 Demonstration of diffusion

Materials

1) Crystals of $KMNO_4$,

2) NH_4OH,

3) Conc HCl

Method

Perform the following experiments:

i) Carefully open a bottle of ammonia and leave it open for 30 sec. What do you observe?

ii) Bring a glass rod dipped in conc HCl above this bottle. What happens?

iii) Drop a crystal of $KMNO_4$ in a tall cylinder filled with water. Do not disturb it. Observe changes after 5 min, 1 hour, 6 hours. Record the observations for each of the above experiments

11.2.2 Study of rate of diffusion

Perform either of the following two (A or B) experiments.

Experiment A

Materials

Glass tube 30 cm in length and 2 cm in diameter

Two corks of the size to fit in the glass tubes

Cotton

Pins (you may try to fit Johnson cotton swab if they fit in the tube)

Burette clamp stand

Conc HCl

Conc NH_4OH

Cylinder

Glass rod.

Forceps

Method

Fix a glass tube of about 30 cm length and 2 cm diameter horizontally in a burette clamp (Fig. 11.2). Attach a small piece of cotton to each cork with a pin. Holding the cork by a forceps dip cotton of one cork in concentrated HCl and of the other in concentrated NH_4OH. Make sure that the two corks are far apart. Remove excess of HCl or NH_4OH by a filter paper and insert them at the opposite end of the tube at the same time.

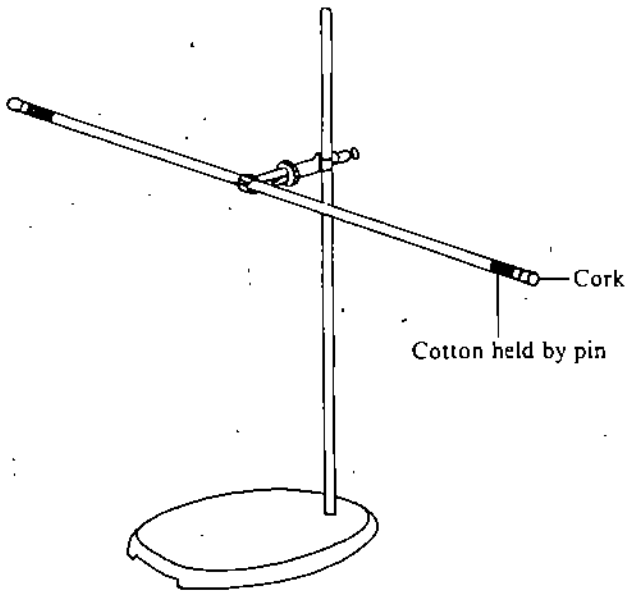

Fig. 11.2 : Apparatus for studying the rate of diffusion

Record the observations.

SAQ 1

a) What does happen when the two gases meet in the tube?

.....

.....

b) Name the substance visible in the tube.

.....

c) When does it appear?

.....

d) Where does it appear?

.....

e) Relate the position of the white ring to the molecular weight of HCl and NH_3.

.....

.....

.....

f) Which of the two has travelled faster?

.....

Experiment B

Materials

1M sodium chloride (NaCl) solution,

1M potassium bromide (KBr) solution,

1M potassium ferricyanide [$K_3Fe(CN)_6$], or silver nitrate ($AgNO_3$) solution.

Method

Solidify gelatin in a petridish. Using the broad side of a pasteur pipette or some other device make four wells of equal size, approximately 1 cm diameter, at equidistance and mark them 1 to 4 in gelatin. Fill one compound in each of the wells. The migrating groups and their molecular weights are also listed below..

Well No.	Compound	Migrating group	Approximate mol. wt. of migrating group
1	1M NaCl	Cl^-	35
2	1M KBr	Br^-	80
3	1M $[K_3Fe(CN)_6]$	$(Fe(CN)_6)^{3-}$	212
4	1M $AgNO_3$	NO_3^-	62

Measure the diameter of each area immediately after pouring the substance, and later after 1 hour and 2 hours.

SAQ 2

a) Which of the groups does move fastest?

.....

b) Which of the groups does move slowest ?

.....

c) What is the relationship between the molecular weights of the migrating groups and their rates of diffusion?

.....

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.....

.....

11.3 OSMOSIS

Most of the molecules can diffuse through fine muslin or cheese cloth. But through plasma membrane only certain molecules can diffuse into the cell and diffuse out in the environment; because it is a semipermeable membrane. The phenomena of osmosis is a special case of diffusion. It is the movement of solvent molecules across a selectively permeable (semi-permeable) membrane from region of higher concentration to a region of lower concentration. Since in living systems water is a ubiquitous solvent, osmosis in living cells refers to diffusion of water only.

The fluid content of a cell may be higher, lower or equal in concentration with respect to its surrounding. If a cell is surrounded by a solution that contains the same concentration of the solution like that of the cell (isotonic solution), there will be *no net* movement of water. If a cell is surrounded by a solution that contains lower concentration of solute particles (hypotonic solution), than the cell, there will be a net flow of water into the cell. This results in swelling and

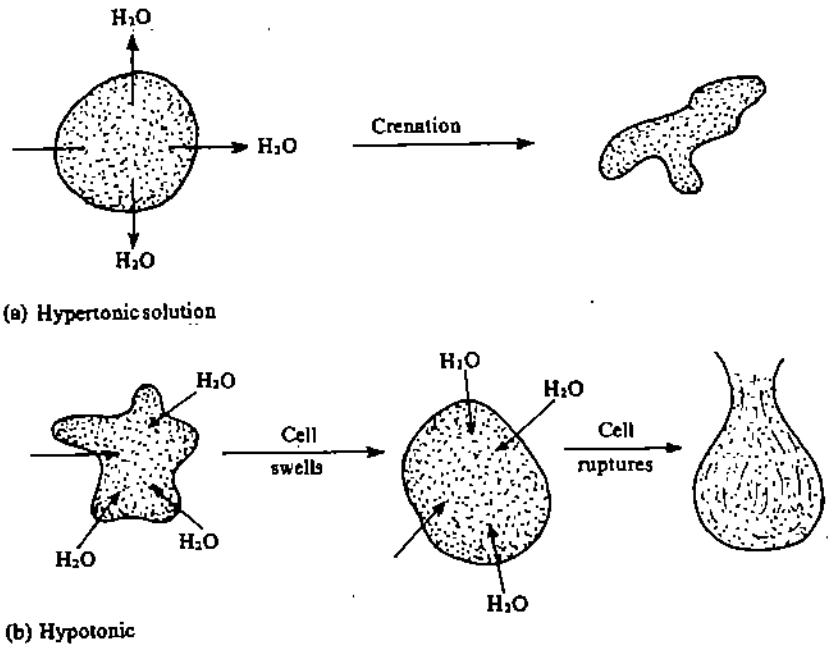

Fig. 11.3 : Osmosis in cell a) If a cell is surrounded by hypotonic solution the cell ruptures. b) If a cell is surrounded by hypertonic solution crenation occurs.

11.3.3 Haemolysis

Materials

Microscope

Slides

Coverslips

5 Test tubes

0.9% NaCl

Sample of blood

Method

Take five test tubes and label them 1 to 5. Add the reagents to each as follows:

No.	Reagents	NaCl concentration as percentage of normal plasma concentration
1	5.0 ml distilled water	0%
2	0.5 ml NaCl (0.9%) + 4.5 ml distilled water	10%
3	1.0 ml NaCl (0.9%) + 4 ml distilled water	20%
4	3.0 ml NaCl (0.9%) + 2 ml distilled water	60%
5	5 ml of 0.9% NaCl	100%

Now, add 3 drops of blood in test tube 1 and immediately place your thumb on the mouth of test tube and gently invert. Place it against a background of a printed page. Observe immediately and after 10 minutes.

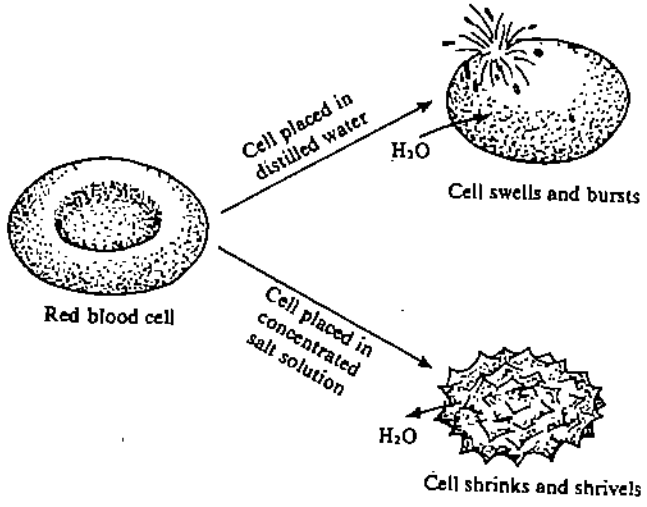

Fig. 11.5 : Haemolysis and Crenation

a) Is the print legible immediately?

.....

b) Is the print legible after 10 minutes? If so, why?

.....

.....

.....

Repeat the procedure for all the test solutions and answer the following questions.

c) Which of the test solutions was hypotonic, hypertonic or isotonic to the cell plasma?

.....

d) Predict what would be the state of cells in each tube if examined under a light microscope.

.....

.....

e) Which of the following solutions should be used for injecting a drug to a patient?

a) hypotonic

b) hypertonic

c) isotonic

f) Choose the correct alternative in the following statements.

a) Marine organisms tend to lose (ions/water) and gain (ions/water) from their environment.

b) In fresh water the organisms tend to (gain/lose) water.

11.3.4 Dialysis

It is the movement of small molecules and ions in addition to water across the membrane. Such a membrane is called dialysing membrane. Cell membranes are essentially dialysing membranes.

Note: Your counsellor might demonstrate experiment on dialysis.

All kinds of enzyme cannot be studied by simple laboratory techniques. Therefore, in this laboratory course you are going to study the action of oxidoreductases and hydrolases only that are easy to study.

Objectives

After doing the experiments on enzyme action, you should be able to:

- demonstrate the action of xanthine oxidase, phenolase complex, catalase, anaerobic dehydrogenase and amylase,
- explain the chemical reactions involved in the enzymes studied.

12.2 OXIDOREDUCTASES

They catalyse addition of O_2 or removal of electrons from substrate and thus effect oxidation or reduction. Probably you are aware that oxidation reactions are common in catabolism and reduction reactions in anabolism in the cell. Oxidoreductases are grouped according to their mode of action.

1) Enzymes that directly involve oxygen.

2) Enzymes that do not directly involve oxygen, but transfer electrons through a series of carriers like various cytochromes in respiratory electron transfer chain to oxygen forming water. These are called **anaerobic dehydrogenases.**

1. **Enzymes that directly involve oxygen**

   There are three types of enzymes in this group.

   i) Enzymes that transfer 2 to 4 electrons from substrate to oxygen as shown below. These are called **oxidases.**

   $$2H^+ + 2e^- + O_2 \longrightarrow H_2O_2$$

   $$4H^+ + 4e^- + O_2 \longrightarrow 2H_2O$$

   Examples are cytochrome oxidases which catalyse the oxidation of cytochromes (Fe^{2+}) in respiratory electron transfer chain.

   Some enzymes of this category can transfer electrons to dyes such as methylene blue as well.

   ii) These enzymes incorporate one atom of oxygen in the substrate and the other oxygen atom reacts with 2 electrons donated by electron donors like $NADH_2$ or $NADPH_2$ to form water. Therefore these enzymes are called **mixed function oxidases.** Examples are phenolase complex, catalase and peroxidase.

   iii) Enzymes that transfer both the atoms of oxygen to the substrate. These are called **oxygenases.** For example lipoxidase, tryptophane pyrrolase.

   $$X + O_2 \longrightarrow XO_2$$

   substrate

Certain dyes can accept electrons from the substrate during enzymatic redox reaction. The dye changes colour when reduced and thus indicates the occurrence of reaction (redox indicator). Such artificial electron acceptors are commonly used to study the action of oxidoreductases. For example, methylene blue (MB) and dichlorophenol indophenol (DCPIP) are blue in the oxidised state and colourless when reduced.

$$\underset{\substack{\text{Methylene blue}\\ \text{oxidised}\\ \text{(blue)}}}{MB} + 2e^- + 2H^+ \longrightarrow \underset{\substack{\text{Methylene blue}\\ \text{reduced}\\ \text{(colourless)}}}{MB(H)_2}$$

12.2.1 Xanthine Oxidase

This enzyme is present in milk. It can transfer electrons (and H^+) from hydrated aldehyde such as formaldehyde to O_2 or to dye like methylene blue.

$$\underset{\text{Formaldehyde}}{HCHO} + H_2O \longrightarrow \underset{\text{Hydrated formaldehyde}}{HCH(OH)_2} + \underset{\text{Methylene blue}}{MB} \xrightarrow[\text{oxidase}]{\text{Xanthine}} \underset{\text{Formic acid}}{HCOOH} + \underset{\text{(colourless)}}{MBH_2}$$

Materials

1. Unpasteurised milk
2. 0.02% Methylene blue solution
3. 0.4% Formaldehyde solution
4. Paraffin oil.

Procedure

Take three test tubes and number them 1 to 3. Pour in each 5 ml of unpasteurised milk. Boil tube 1 for 2 minutes and cool. Add 1 ml of 0.02% methylene blue solution in each tube. Then add 0.4% formaldehyde to tube 1 and 2 but not in tube 3. Mix the contents of the tube by gentle rotation and add 1-2 ml of paraffin oil and place at 40°C. Record observations.

Tube No.	Contents in the Tube	Change in the colour of dye
1.	Boiled (cooled) milk + methylene blue + formaldehyde	
2.	Unboiled milk + methylene blue + formaldehyde	
3.	Unboiled milk + methylene blue	

SAQ 3

a) Is there any difference between the reactions carried out with cut pieces of tissue and macerated tissue?

.....

.....

.....

.....

b) Why are the results of tube 2 different from that of tube 3?

.....

.....

12.2.4 Dehydrogenases

They form the most important group of enzymes in respiratory metabolism. A few examples are succinic acid dehydrogenase (Kreb cycle), alcohol dehydrogenase (fermentation) and NADH dehydrogenase (electron transfer chain). You will study dehydrogenases of yeast.

Materials

1. Four test tubes (15 ml)
2. Four clean rubber stoppers
3. Four pipettes
4. 5% solution of glucose
5. 2% solution of starch
6. Methylene blue .05% solution
7. Formaldehyde 4%

Procedure

Arrange four test tubes in a rack and number them 1 to 4.
Introduce the various material in the test tubes as listed in the table.

Tube No.	Distilled Water	Glucose	Starch	Formal-dehyde	Active yeast	Methylene blue
1.	10 ml	x	x	x	5 ml	√
2.	5 ml	5 ml	x	x	5 ml	√
3.	5 ml	x	5 ml	x	x	√
4.	x	5 ml	x	5 ml	5 ml	√

Introduce yeast at last. Fill all the tubes to the brim by adding distilled water, if necessary. Now, add 2 drops of methylene blue in each tube. Mix the contents of the tubes by placing your thumb on the tube and inverting it. Make sure the initial intensity of the colour is same in all the tubes. Stopper each tube and place them in rack. Let the liquid overflow. Record your observations after 20, 40, 60 min and 2 hours. Grade the intensity of colour as follows. Note the evolution of gas also and interpret the results.

Colour	Intensity grade
Colourless	O
Pale	+
Light-blue	++
Moderate light blue	+++
Deep blue	++++

SAQ 4

a) Interpret the results (intensity grade) keeping in view the contents in each of the tube.

.....

.....

b) What gas is evolved during the reaction?

.....

12.3 HYDROLASES

These enzymes breakdown larger molecules into smaller units. The most common enzymes in this category are digestive enzymes—amylases, lipases and proteinases that break down starch, lipids and proteins respectively.

12.3.1 Salivary Amylase

Salivary amylase is present in the saliva of most mammals. It initiates the hydrolysis of starch.

Materials

1. Starch (Cracker)
2. Benedicts solution
3. Iodine solution
4. 3 ml of saliva

Procedure

Collect 3 ml of saliva in a test tube by chewing a piece of paraffin wax. Take four test tubes and number them 1-4. Add the reagents as shown in the table.

Test tube	Starch (cracker)	Water	Iodine	Benedicts Solution	Saliva	Obser-vation
1.	x	10 ml	10 drops	5 ml	x	
2.	√	10 ml	10 drops	x	5 ml	
3.	√	10 ml	x	5 ml	x	
4.	√	10 ml	x	5 ml	5 ml	

After adding Benedicts solution heat over a flame until it boils gently. Benedicts reagent gives positive reaction if reducing sugars are present

SAQ 5

a) Explain the results of your experiment.

.....

.....

EXPERIMENT 13 VERIFICATION OF MONOHYBRID MENDELIAN RATIO AND CHI-SQUARE ANALYSIS

Structure

13.1 INTRODUCTION

In Unit 1 of LSE-03, you have studied Mendel's laws of Inheritance, i.e., the law of segregation, and the law of independent assortment. This and the following laboratory exercise pertain to these two laws respectively. Before beginning this exercise, let us, recapitulate briefly the law of segregation. You may recall that Mendel used varieties or lines of garden pea (*Pisum sativum*), that exhibited pairs of contrasting characters, e.g., the long stem lines (or the *tall* plants) and the short stem lines (or the *short* plants) as parents (P generation) in his crosses. Each line was pure breeding, so that plants from that line always bred true for the character being studied, i.e., tall x tall produced only tall, progeny. When two lines with contrasting characters were crossed (e.g., tall x short) to produce a first filial generation, all the progeny were of one phenotype, which was the same as one of the parents (e.g., tall). This character was said to be *dominant*, and the character that did not appear in the F_1 (e.g., short) was called *recessive*. There had been no blending of the two characters. When the F_1 was allowed to self, the progeny in the next generation (F_2) were in the ratio of approximately 3 dominant to 1 recessive phenotypes (e.g., 3 tall : 1 short). What were his conclusions from the above experiments? (i) Each parent contained two unit factors (genes) of which one was contributed to each member of the F_1 progeny. (ii) Each gene could exist in two alternate forms or *alleles*, one of which was for the dominant character (i.e., T) and determined the phenotype of the F_1 with the other being for the recessive character (i.e., t). (iii) Each member of the F_1 contained one of each allele (Tt) and was heterozygous, whereas each parent contained two identical alleles (TT in the tall parent; tt in the short parent) and was homozygous. (iv) Thus, the dominant phenotype (tall) resulted from two different genotypes, the homozygous one (TT) or the heterozygous one (Tt), whereas the recessive phenotype was only determined by one homozygous genotype (tt). (v) When the F_1 plants produced pollen and eggs, they were clearly of two types, occurring with equal frequency and containing either one allele for the dominant character (T) or one allele for the recessive character (t), i.e., the two alleles in the F_1 segregated clearly from each other when gametes were formed. (vi) The pollen and egg nuclei of the two genotypes T and t fuse

at random to produce the F_2 zygotes. Thus, the F_2 genotypes would be of three types in the ratio of 1 homozygous for alleles for the dominant character : 2 heterozygous : 1 homozygous for alleles for the recessive character (i.e., 1TT : 2Tt : 1tt). This gives the F_2 phenotypic ratio of 3 dominant : 1 recessive (3 tall : 1 short). Let us now proceed to do the exercise.

Objectives

After doing this laboratory exercise you should be able to:

- demonstrate Mendel's law of segregation in a monohybrid cross;
- correlate the experimental steps with the natural processes occurring;
- analyse the ratio obtained with regard to its goodness of fit, by using Chi-Square test.

Prior Reading for this Experiment

1. Unit 1, Genetics (LSE-03)
2. Unit 16, Mathematical Methods (MTE-03)

13.2 MATERIALS REQUIRED

1) 3 containers/250 ml plastic beakers

2) 50 red beads

3) 50 yellow beads

13.3 PROCEDURE

You will have to work in pairs for this investigation.

Step 1 : Place 50 red beads in one container to represent the gametes of a tall parent (T). Place 50 yellow beads in the other container to represent the gametes of a dwarf parent (t). We assume that both the gametes are from parents that breed true for the chracteristic, namely, stem height, whose inheritance is being studied in this experiment.

See Fig. 13.1 (a) p. 100

Step 2 : Withdraw a bead from each container. Each bead withdrawn represents a gamete containing a single allele of a pair. Place the beads together. This represents the process of fertilisation, by which the paired alleles of gene in the offspring are re-established.

See Fig. 13.1 (b)

Step 3 : Just as in Step 2, continue to withdraw pairs of beads as above, and arrange on the table.
What would be the genotype of the individuals of the F_1 generation? .....

See Fig. 13.1 (c)

Step 4 : To simulate the gametes of this F_1 generation, place 50 beads (25 red and 25 yellow) in each container. One container represents the female

See Fig. 13.1 (d)

gametes, and the other represents the male gametes produced by the F_1 generation.

Step 5 : Shake each container vigourously for 30 seconds, taking care the beads do not fall off.

Step 6 : To produce the F_2 generation, withdraw one bead from each container **with your eyes closed** and place them together. Your partner should note the combination of genes obtained.
This represents the genotype of an F_2 individual.

See Fig. 13.1 (e, f)

Step 7 : After noting the genotype of each pair, discard the pair of beads into a spare container.

Step 8 : Repeat steps 6 and 7 until all beads have been paired and their combinations noted.

Step 9 : Calculate the ratio of the **phenotypes** of the F_2 individuals.

Step 10: Record the ratios obtained by other groups in your batch and calculate the average ratio.

13.4 OBSERVATIONS AND RESULTS

A. F_1 Generation

a) Total number of F_1 individuals .....

b) Phenotypes of F_1 individuals .....

c) Genotypes of F_1 individuals .....

B. F_2 Generation

a) Total number of F_2 individuals .....

b) Phenotypes of F_2 individuals .....

c) Number of individuals in each phenotypic class .....

d) The phenotypic ratio .....

e) Genotypes of F_2 individuals .....

f) Number of individuals in each genotypic class .....

g) The genotypic ratio .....

Group Average	Phenotypic classes	
	T–	tt
Group 1	.....	.....
Group 2	.....	.....
Group 3	.....	.....
.....	.....	.....
.....	.....	.....
Group n	.....	.....
Total		
Average	.....	.....

13.5 DISCUSSION OF RESULTS

1) In the above exercise which character was dominant?

.....

.....

2) Was the F_1 homozygous or heterozygous?

.....

.....

3) When F_1 plants were self-pollinated how many different types of pollen and egg nuclei were produced? What were their genotypes and what were the frequencies of the genotypes?

.....

.....

4) Why were the beads shaken in step 5, and withdrawn with closed eyes in step 6?

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5) How does your ratio, and the group average ratio compare with Mendel's prediction? Explain any differences?

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6) Make a Punnett square to show the outcome of F_1 x F_1 cross.

7) Why is it called a monohybrid cross?

.....

.....

.....

.....

8) What would be the monohybrid test cross of the example taken in this experiment?

9) What would be the back cross for the example taken in this experiment?

10) Can you record and present the results in another manner?

11) Explain how this practical acts as a model for breeding and inheritance in pea?

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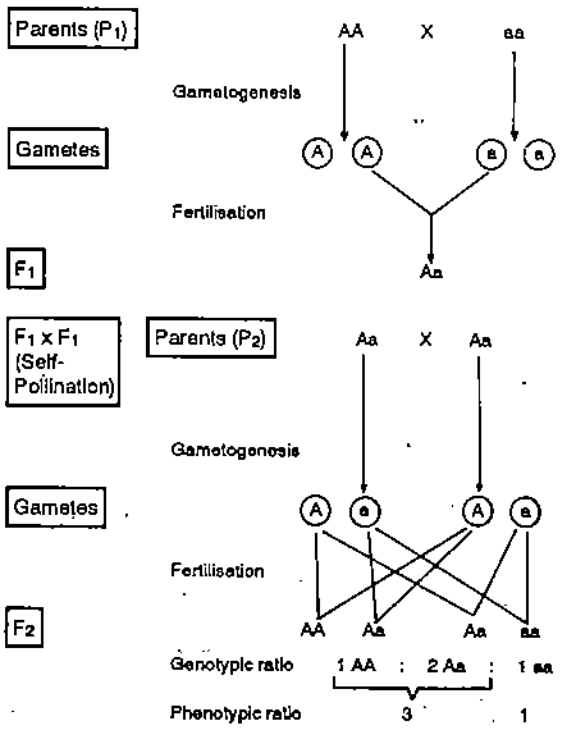
12) In the diagram given below, write as to which step (described in experimental procedure above) corresponds to the indicated stage.



(i) .....?.....

(ii) .....?.....

(iii) .....?.....

(iv) .....?.....

(v) .....?.....

13) Supposing a random sample of F_2 seeds obtained were sown and their mature plants were allowed to self. What proportion of plants would produce (i) only tall plants (homozygous), (ii) only dwarf plants, (homozygous) and (iii) a mixture of tall and dwarf (heterozygous) plants?

.....

13.6 χ^2 ANALYSIS

By using the χ^2 (Greek letter, chi pronounced as 'kye squared') test, you can evaluate your results of this experiment. This is a statistical test that is frequently used to determine whether the data obtained experimentally provides a 'good fit', or approximation to the expected or theoretical data. Basically this test can be used to determine whether any deviations from the expected values are due to chance. Chance alone can cause the actual observed ratio to vary from the calculated ratio for a genetic cross. For example, for a monohybrid cross you would get very rarely an exact ratio of 3 : 1. The observed results differ, but there comes a point when the difference is so great that the observed data is not in conformity with the expected one. The chi-square test indicates this point. In this exercise, for example, the hypothesis is that the F_2 data do not differ significantly from a 3 : 1 ratio. This type of formulation is called **null hypothesis,** because we will test that nothing has happened to disturb the expected ratio significantly. Consider an alternative hypothesis, such as the F_2 data does differ significantly from a 3 : 1 ratio because gametes with different genotypes are produced in unequal numbers. Such a hypothesis would be imprecise and would not lead to an exact numerical prediction. From this example it should be clear that the null hypothesis being tested should always be stated for the test.

The formula for this test is:

$$\chi^2 = \sum \frac{(o - e)^2}{e} \text{ or } \sum \frac{d^2}{e}$$

χ^2 = chi-square

Σ = sum of

d = difference between the expected and observed results, often termed as deviation (o – e)

e = expected results

o = observed results

Let us see how we can apply this formula. As you know, in a monohybrid cross a 3 : 1 ratio is expected. Supposing you count a total of 160 plants, out of which 120 are tall and 40 are dwarf. But another student counts 116 tall plants and 44 dwarfs. Then the value for the chi-square test would be calculated as shown in Table 13.1.

Table 13.1: Calculation of the Chi-square Value

Phenotype	Observed number (o)	Expected number (e)	difference (d = o – e)	d^2	Partial Chi-square d^2/e
Tall	116	120	4	16	16/120 = .133
Dwarf	44	40	4	16	16/40 = .400
					$\frac{\Sigma d^2}{e}$ = .533
					χ^2 = .533

The next step is to look up this Chi-square value (χ^2) in the Table 13.2, that indicates whether the probability (P) is that the differences noted are due only to chance in the form of random sampling error or whether it would be better to explain the results on the basis of a different prediction or hypothesis.

Table 13.2: Critical Values of Chi-square for 1-30 degrees of freedom that are equalled with or exceeded with particular probabilities (P). Figures at the top of the table indicate levels of significance.

df	.99	.98	.95	.90	.80	.70	.50	.30	.20	.10	.05	.02	.01	.001
1	.00016	.00063	.0039	.016	.046	.15	.46	1.07	1.64	2.71	3.84	5.41	6.64	10.83
2	.02	.04	.10	.21	.45	.71	1.39	2.41	3.22	4.60	5.99	7.82	9.21	13.82
3	.12	.18	.35	.58	1.00	1.42	2.37	3.66	4.64	6.25	7.82	9.84	11.34	16.27
4	.30	.43	.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	11.67	13.28	18.46
5	.55	.75	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	13.39	15.09	20.52
6	.87	1.13	1.64	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	15.03	16.81	22.46
7	1.24	1.56	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	16.62	18.48	24.32
8	1.65	2.03	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	18.17	20.09	26.12
9	2.09	2.53	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	19.68	21.67	27.88
10	2.56	3.06	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	21.16	23.21	29.59
11	3.05	3.61	4.58	5.58	6.99	8.15	10.34	12.90	14.63	17.28	19.68	22.62	24.72	31.26
12	3.57	4.18	5.23	6.30	7.81	9.03	11.34	14.01	15.81	18.55	21.03	24.05	26.22	32.91
13	4.11	4.76	5.89	7.04	8.63	9.93	12.34	15.12	16.89	19.81	22.36	25.47	29.69	34.53
14	4.66	5.37	6.57	7.79	9.47	10.82	13.34	14.22	18.15	21.06	23.68	26.87	29.14	36.12
15	5.23	5.98	7.26	8.55	10.31	11.72	14.34	17.32	19.31	22.31	25.00	28.26	30.58	37.70
16	5.81	6.61	7.96	9.31	11.15	12.62	15.34	18.42	20.46	23.54	26.30	29.63	32.00	39.29
17	6.41	7.26	8.67	10.08	12.00	13.63	16.34	19.51	21.62	24.37	27.59	31.00	33.41	40.75
18	7.02	7.91	9.39	10.86	12.86	14.44	17.34	20.60	22.76	25.99	28.87	32.35	34.80	42.31
19	7.63	8.57	10.12	11.65	13.72	15.35	18.34	21.69	23.90	27.20	30.14	33.69	36.19	43.82
20	8.26	9.24	10.85	12.44	14.58	16.27	19.34	22.78	25.04	28.41	31.41	35.02	37.57	45.32
21	8.90	9.92	11.59	13.24	15.44	17.18	20.34	23.86	26.17	29.62	32.67	36.34	38.93	46.80
22	9.54	10.60	12.34	10.04	16.31	18.10	21.34	24.94	27.30	31.81	33.92	37.66	40.29	48.27
23	10.20	11.29	13.09	14.85	17.19	19.02	22.34	26.02	28.43	32.01	35.17	38.97	41.64	49.73
24	10.86	11.99	13.85	15.66	18.06	19.94	23.34	27.10	29.55	33.20	36.42	40.27	42.38	51.18
25	11.52	12.70	14.61	16.47	18.94	20.87	24.34	28.17	30.68	34.38	37.65	41.57	44.21	52.62
26	12.20	13.41	15.38	17.29	19.82	21.79	25.34	29.25	31.80	35.56	38.88	42.86	45.64	54.06
27	12.88	14.12	16.15	18.11	20.70	22.72	26.34	30.32	32.91	36.74	40.11	44.14	46.96	55.48
28	13.56	14.85	16.93	18.94	21.59	23.65	27.34	31.39	34.03	37.93	41.34	45.42	48.28	56.89
29	14.26	15.57	17.71	19.77	22.48	24.58	28.34	32.46	35.14	39.09	42.56	46.69	49.53	58.30
30	14.95	16.31	18.49	20.60	23.36	25.51	29.34	33.53	36.25	40.26	43.77	47.96	50.89	59.70

In Table 13.2, the notation df refers to the degree of freedom, which in this experiment would be determined by the number of phenotypic traits studied. In our example, we have two classes, tall and dwarf plants. As indicated in the table for the value of df, i.e., we need to know the value of C–1. You might have followed that the degree of freedom (df) is calculated by using the formula C–1, where C is the total number of classes. In this case C is 2 therefore, df is equal to 1 (i.e., 2 – 1 = 1). Therefore, you should look for χ^2 value in the first row (i.e., in 1), of the Table 13.2. The value .533 lies between .50 and .30 probability values. This means that by random chance, this difference between

he actual count and the expected count would occur between 30 and 50% of the time. In biology, it is generally accepted that a P value greater than 0.05 is acceptable, while a P value lower than 0.05 would indicate that the results cannot be due to random sampling and, therefore, do not fit the original prediction (hypothesis).

Do a Chi-square analysis of your results by filling in Table 13.3.

Table 13.3 Chi-Square Analysis of Results

Phenotype	Observed number (o)	Expected number (e)	difference (d = o – e)	d^2	Partial Chi-square d^2/e
					$\Sigma \frac{(d)^2}{e} =$

χ^2 = ...

C–1 = ...

P (from Table 13.2) = ...

Do your results support Mendel's prediction? .....

If no, can you account for this? .....

B) Do Chi-square analysis for your entire batch?

Phenotype	Observed Numbers					
	Group 1	Group 2	Group 3	------	Group n	Total
Tall						
Dwarf						

Total no. of Individuals .....

Chi-square analysis

Phenotype	Observed number (o)	Expected number (e)	difference (d = o – e)	d^2	Partial Chi-square d^2/e
Tall					
Dwarf					
					$\Sigma \frac{(d)^2}{e} =$

χ^2 = ...

C–I = ...

P (from Table 13.2) = ...

Do these results support Mendel's prediction? .....

.....

.....

Comment on the above results? .....

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What are the advantages of taking a bigger sample?.....

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EXPERIMENT 14 VERIFICATION OF DIHYBRID MENDELIAN RATIO AND CHI-SQUARE ANALYSIS

Structure

14.1 INTRODUCTION

This experiment pertains to the Second law of inheritance, that is, the law of independent assortment. Mendel, by choosing plants with two pairs of contrasting characters provided evidence that the alternate forms of factors assort independently and recombine to give a dihybrid ratio of 9 : 3 : 3 : 1. Alternatively, segregation of one gene pair is independent of the segregation of the other gene pair, and will give a similar ratio (3 : 1 x 3 : 1). In this experiment, you will learn to demonstrate a Mendelian dihybrid ratio using coloured beads and test the significance with the statistical procedure — the Chi-square test.

Prior Reading for this Experiment

1. Unit 1, Genetics (LSE-03)
2. Unit 16, Mathematical Methods (MTE-03)
3. Experiment 13 of this course

Objectives

After doing this laboratory exercise you should be able to:

- demonstrate Mendel's law of independent assortment in a dihybrid cross;
- correlate the experimental steps with the natural processes occurring;
- analyse the ratio obtained for its goodness of fit, using Chi-square test.

14.2 MATERIALS REQUIRED

1) 5 Containers

2) 48 Green beads

3) 48 Yellow beads

4) 48 Black beads

5) 48 White beads

6) 1 Packet Modelling clay

14.3 PROCEDURE

You will have to work in pairs for this experiment

Step 1 : Place 48 beads of red, yellow, black and white colours separately in four containers.

See Fig. 14.1 (a) p. 100

Black beads represent the dominant trait, smooth seed coat (S); and

White beads represent the recessive trait, wrinkled seed coat (s).

Yellow beads denote the dominant character, yellow colour of seeds (Y); and

Green beads denote the recessive character, green colour of seeds (y).

Step 2 : Make a cross between parents with phenotypes smooth, yellow and wrinkled, green.

Fig. 14.2 (b)

Step 3 : What kind of gametes would be produced by each parent?

.....

.....

Check from Fig. 14.2 (c)

Step 4 : Place one bead each of white, green, black and yellow colour together. This process represents fertilisation. Note the phenotype and genotype of F_1 individual?

Fig. 14.2 (d)

.....

.....

Step 5 : Next step is to make a cross between the F_1 individuals. From the F_1 individual obtained above what kind of gametes can be formed?

Check from Fig. 14.2 (e)

.....

To simulate the gametes of this F_1 generation, place 24 beads (24 white, 24 black, 24 green and 24 yellow) in each of two containers. One container represents the female gametes and the other represents male gametes produced by the F_1 parents.

Step 6 : In order to make the identification of the four kinds of gametes clear, use a small ball of modelling clay to join the respective beads. For example, take one white and one green bead and join them firmly with a small amount of modelling clay. Similarly make pairs of one white, one yellow bead; a black and a green bead and a black and a yellow bead. Thus, make pairs of all the beads of both the containers.

Fig. 14.1 (f, g)

Step 7 : Shake both the containers (each having four kinds of gametes) for 30 seconds.

Step 8 : To produce the F_2 generation, withdraw a bead pair from each container **with your eyes closed,** and place them together. Your partner should note the combination of genes obtained. This represents the phenotypes and genotypes of an F_2 individual.

Fig. 14.1 (h)

Step 9 : After noting the phenotype and genotype of each pair, discard the pair of beads into a spare container.

Step 10: Repeat steps 8 and 9 till all the pairs of beads of both the containers have been utilised and their combinations noted.

Step 11: Calculate the ratio of the phenotypes of the F_2 individuals.

Step 12: Record the ratios obtained by other groups in your batch and calculate the average ratio.

Step 13: Analyse your results as well as the group average ratio separately for their goodness of fit by using Chi-square method that you have used in the previous experiment.

14.4 OBSERVATIONS AND RESULTS

I. Individual Obervations

F_1 Generation

a) total number of individuals .....

b) phenotypes .....

c) genotypes .....

F_2 Generation

a) total number of individuals .....

b) phenotypes .....

c) number of individuals in each phenotypic class:

....

....

....

....

d) the phenotypic ratio .....

e) genotypes .....

.....

f) number of individuals in each genotypic class:

....

....

....

....

g) the genotypic ratio .....

II. Group Average

	Number of individuals in the phenotypic classes			
Group 1	.....	.....	.....	.....
Group 2	.....	.....	.....	.....
Group 3	.....	.....	.....	.....
Group 4	.....	.....	.....	.....
Total				
Average	.....	.....	.....	.....

14.5 DISCUSSION OF RESULTS

1) Why is it called a dihybrid ratio?

.....

.....

2) Compare your ratio and the group average ratio with Mendel's prediction. Explain any differences.

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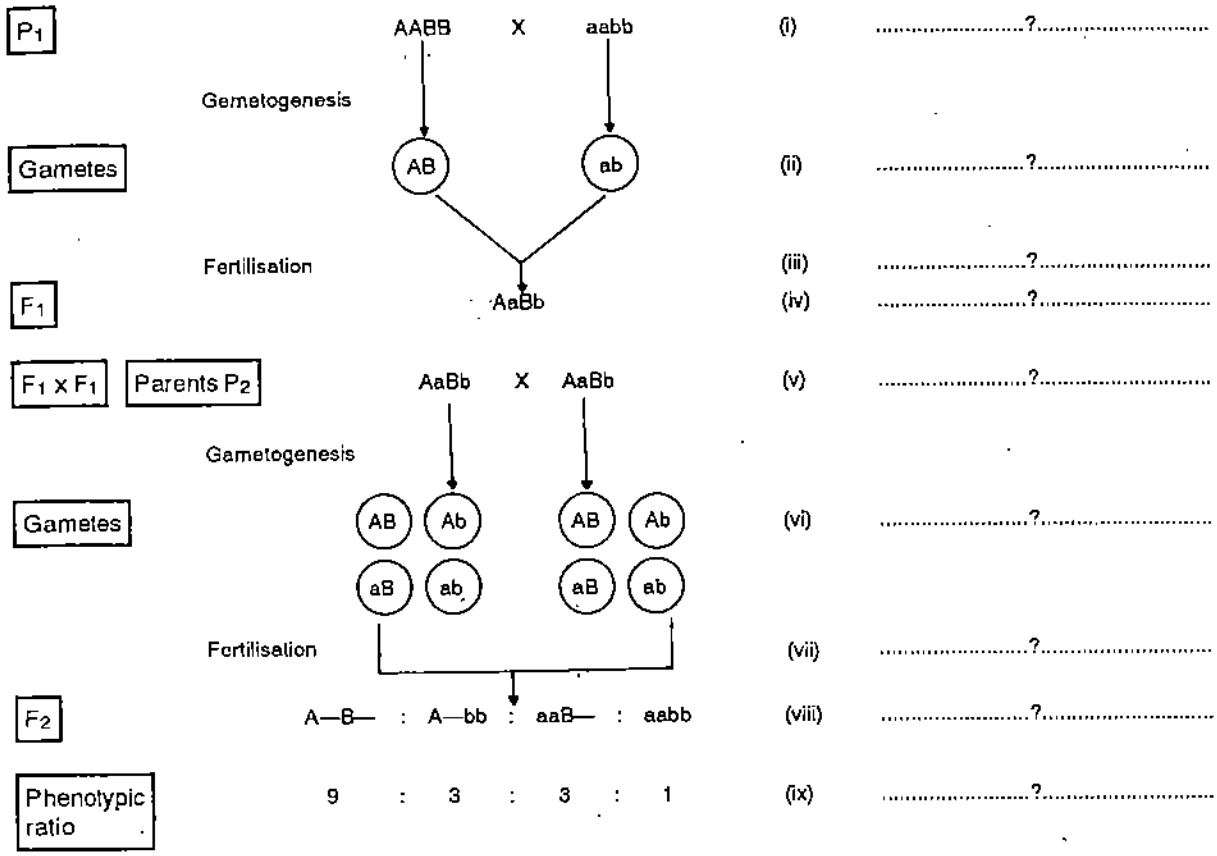
3) Make Punnett square or Branch diagram to show the outcome of $F_1 \times F_2$ cross.

4) What would be the dihybrid test cross of the example taken in this experiment?

5) Can you record and present your results in another manner?

6) In the diagram given below, write as to which step (described in experimental procedure above) corresponds to the indicated stage?



7) Do Chi-square analysis of your results?

Phenotype	Observed number (o)	Expected number (e)	difference (d = o – e)	d^2	Partial Chi-square d^2/e
					$\Sigma \frac{(d)^2}{e} =$ $\chi^2 =$

8) Do Chi-square analysis of your group using the average values?

Phenotype	Observed number (o)	Expected number (e)	difference (d = o – e)	d^2	Partial Chi-square d^2/e
					$\Sigma \frac{(d)^2}{e} =$ $\chi^2 =$

FIG. 13.1

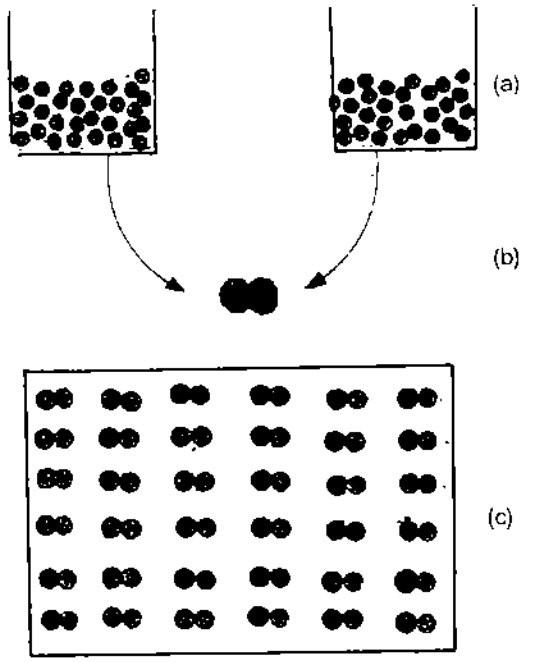
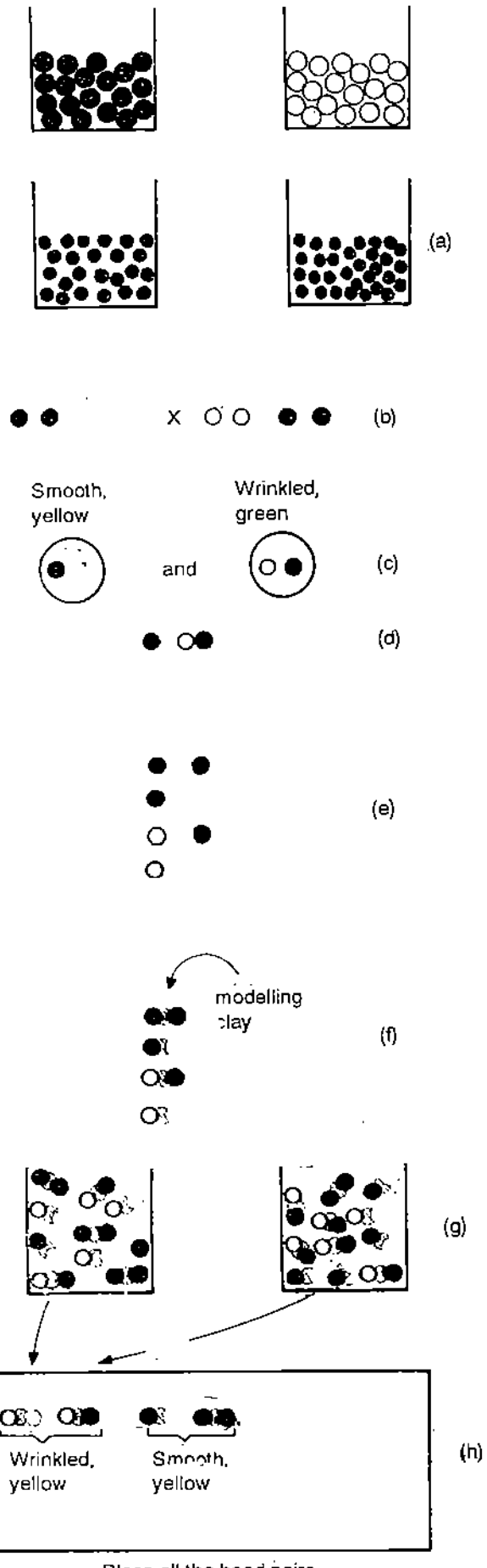


FIG. 14.1



Place all the bead pairs and note their genotypes and phenotypes

EXPERIMENT 15 A STUDY OF MENDELIAN TRAITS IN HUMAN

Structure

15.1 INTRODUCTION

In this exercise you will learn some human traits which exhibit dominant-recessive relationships and follow a mode of inheritance as formulated by Mendel. We have chosen for the study five easily identifiable traits, namely, (i) tongue rolling, (ii) attached ear lobe, (iii) widow's peak, (iv) Hitchhiker's thumb, and (v) PTC tasting.

i) **Tongue rolling:** The ability to roll the tongue into a U-shape is controlled by a dominant allele (R). Non-rolling (r) is a recessive trait (See Fig. 15.1).

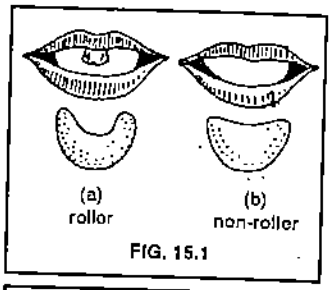


FIG. 15.1

ii) **Attached ear-lobe:** Ear lobes that hang below the point of attachment to the head are said to be free. This is a dominant condition and is represented by allele F. The attached lobe is recessive (f) see Fig. 15.2.

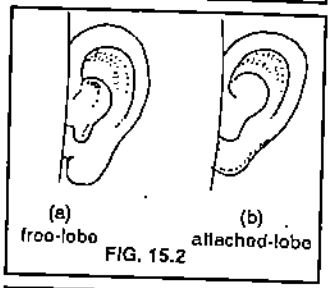


FIG. 15.2

iii) **Widow's peak:** A hairline that forms a distinct peak as it crosses the forehead is said to be a widow's peak (see Fig. 15.3a). This trait is dominant (W) to a straight hairline (w) as shown in Fig. 15.3b.

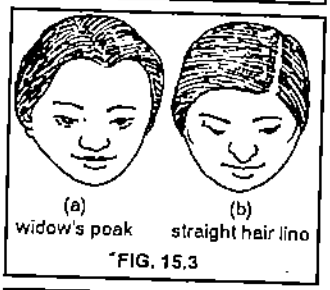


FIG. 15.3

iv) **Hitchhiker's thumb:** The ability to hyperextend the thumb (extend it backwards at the last joint) is due to a recessive allele (n). A straight thumb (N) is dominant to hitchhiker's thumb (see Fig. 15.4).

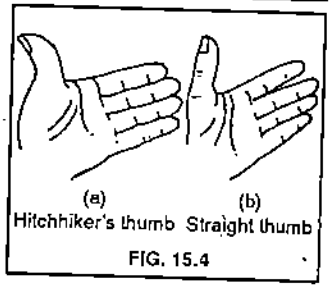


FIG. 15.4

v) **PTC tasting:** The ability to taste phenyl thiocarbamide (PTC) is dominant (T) to the inability to taste it (t). Your counsellor will provide you with small pieces of paper that have been previously soaked in this harmless chemical. If you still wonder whether you are a taster after chewing one of these papers, then you are not.

Objectives

After doing this exercise you should be able to:

- appreciate the variation in human population that contribute to the diversity of the species;
- identify the different types of human traits that could possibly be classified as Mendelian traits exhibiting simple dominant-recessive relationships.

15.2 MATERIALS REQUIRED

PTC paper

15.3 PROCEDURE

Step 1 : Have you read the above descriptions carefully? Now, determine your phenotype and genotype and record it in Table 15.1. If you exhibit the dominant phenotype, represent it by only one symbol for the genotype since you cannot know the specific genotype. For example, if you are PTC taster, write only F–.

Step 2 : Given below is a chart (Fig. 15.5). Use this for designating your combination of traits. It begins with tongue rolling, and see whether it places you in the box-marked R– or rr. Put a tick (√) mark in the appropriate box.

Step 3 : Move to the next pair of boxes to the right of the box where you have put a tick mark. Determine whether you belong to the box F– (free ear lobes) or the one marked ff (attached lobes). Again put a tick mark in the appropriate box. Continue with this process until you reach the fifth trait.

Step 4 : The number representing your combination of traits is next to the last box you marked with a √. Circle this number.

Step 5 : Collect the numbers that your classmates determined for themselves, and see how many have common numbers and which ones are different. Record your observations in Table 15.2.

15.4 OBSERVATIONS AND RESULTS

Record your observations in following table.

Table 15.1: Your genotype and phenotype on the basis of the above five Mendelian Traits.

Trait	Your phenotype	Your genotype
Tongue-rolling		
Ear-lobes		
Widow's peak		
Hitchhiker's Thumb		
PTC tasting		

In which class you are classified? .....

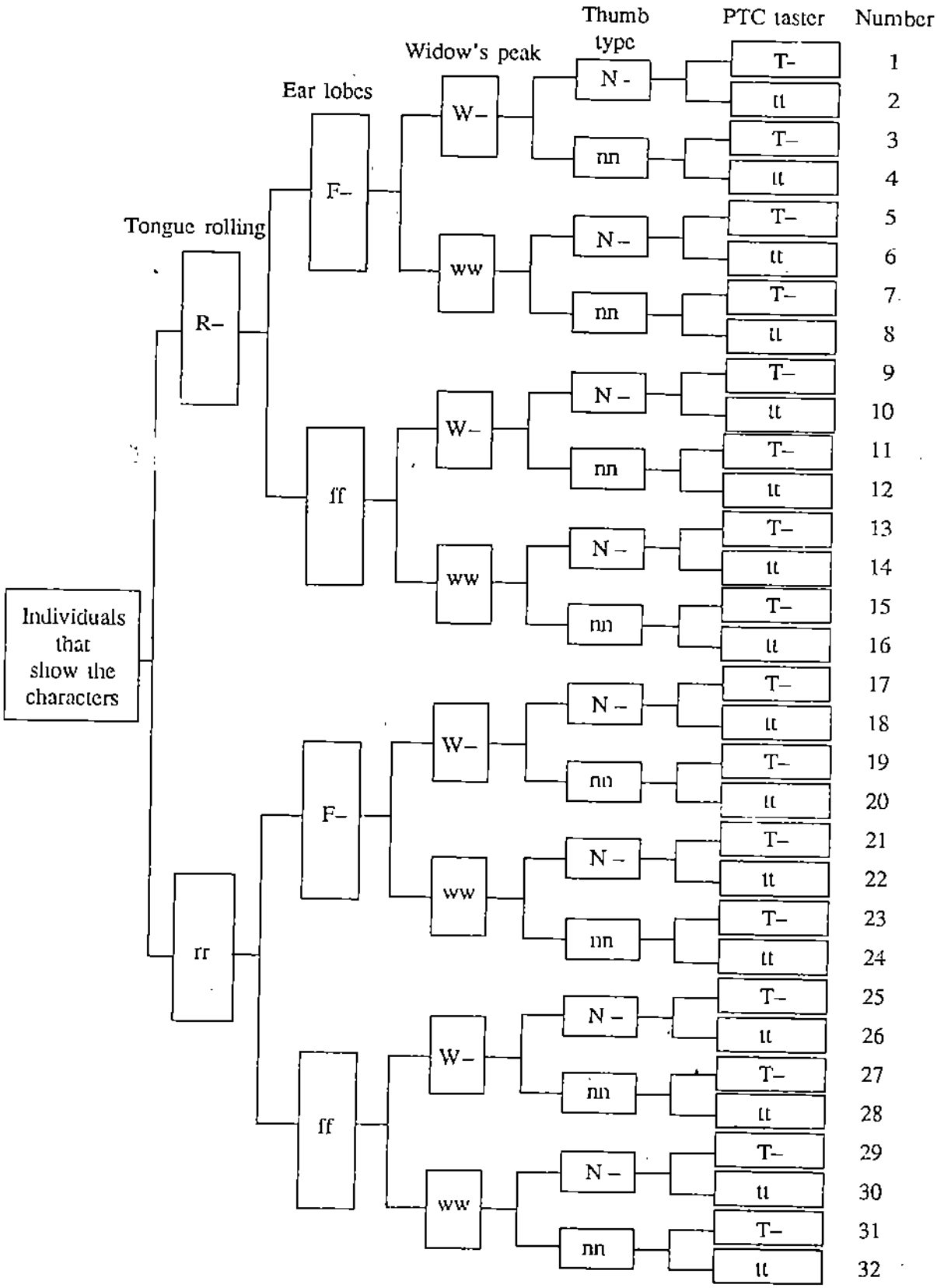

Thumb type
PTC taster
Number
Widow's peak
Ear lobes
Tongue rolling
Individuals that show the characters
R–
rr
F–
ff
W–
ww
N –
nn
T–
tt
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

Fig. 15.5

Table 15.2: A Population Sample (Your Group) for the above five Mendelian Traits.

Trait	Number with dominant phenotypes	Number with recessive phenotype	Total	Frequency of dominant phenotype
Tongue-rolling				
Ear-lobes				
Widow's peak				
Hitchhiker's thumb				
PTC-tasting				

15.5 DISCUSSION OF RESULTS

1) Are there other students in your class who have the same phenotype and therefore belong to the same class as shown in Fig. 15.5 (extreme right)? If so, how would you show that they are different?

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2) With 5 traits, we could arrive at 32 different phenotypes. For further separation, supposing we consider eight different traits. What would be the possible number of phenotypes? Name the other three traits that you would like to consider.

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3) If two or more people have same number in Fig. 15.5, would they look alike? Explain.

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4) How does the genotype of the number 12 (Fig. 15.5) differ from that of number 27, with reference to the five traits we have considered?

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5) What would be the expected frequency of the dominant phenotype for a mendelian trait? Explain.

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6) Based on the group data, does the observed frequency correspond to expected frequency of traits? .....

Which ones, if any do not? .....

Explain, is it due to any of the following:

a) sampling errors, b) selection, c) linkage?

7) Can you suggest another experimental design to study the variations in human beings?

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EXPERIMENT 16 STUDY OF HUMAN BLOOD GROUPS

Structure

16.1 INTRODUCTION

Landsteiner's studies in the early part of this century led to the discovery of existence of different types of blood groups in man and the mode of their inheritance. Four types of blood groups A, B, AB and O occur among human populations and their expression is controlled by three different alleles I^A, I^B and I^O. Both I^A and I^B are dominant to I^O and I^A and I^B themselves are codominant. I^A, I^B and I^O represent a system of multiple alleles. You may recall that when Mendel studied segregation, he dealt with genes, each of which had only two alternate expressions or alleles. But inheritance of human blood groups represent a situation where more than two alleles represent a single locus. Such loci are multiple allelic loci. In this lab. exercise you will be explained the technique of blood group determination based on antigen-antibody reactions.

You may also recall from your Genetics Course (LSE-03) that blood group A individuals have anti B bodies in their serum and those with blood group B have anti A bodies. Individuals with blood group AB have neither anti A nor anti B body and persons with O group have both the antibodies in the serum. You are also aware that when we say that an individual belongs to a particular blood group we mean that the surface of his red blood corupuscles (RBCs) contain a specific antigen in the form of a polysaccharide. Thus, individuals of A blood group have antigen A in their red cell membranes, B blood group have antigen B, AB blood group have both A and B antigens and O blood group have none. The following table summarises the details outlined so far.

Blood Group	Antigen	Antiserum	Genotype
A	A	B	I^AI^A, I^AI^O
B	B	A	I^BI^B, I^BI^O
AB	A, B	—	I^AI^B
O	—	AB	I^OI^O

Objectives

At the end of the experiment you should be able to:

- perform the blood grouping tests based on antigen-antibody reactions as outlined in the procedure below.
- interpret correctly the results of such tests.

16.2 MATERIAL REQUIRED

Pasteur pipettes, alcohol, anti A serum, anti B serum, microslides, tooth picks, needle, cotton, spirit lamp.

16.3 PROCEDURE

1. Sterilise your index or middle finger with cotton soaked with alcohol.
2. Similarly sterilise a new and non-rusty needle or ball pin either with alcohol or by showing it over a flame of the spirit lamp.
3. Prick your finger once with the sharp end of the needle and wipe out the first drop of blood that oozes out with cotton.
4. Transfer the next drop of blood to a clean slide on one part of it and another drop to the other part as shown in Fig. 16.1. With a marker pencil you may name the drops as 1 and 2.

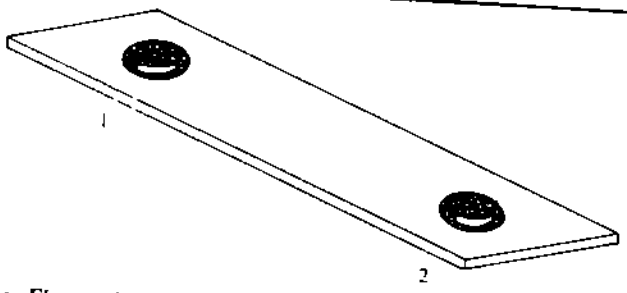

Fig. 16.1 : Figure showing the placement of blood drops on the slide.

5. To drop (1) add a drop of antiserum A and mix well with a tooth pick. Similarly to (2) add a drop of antiserum B and mix well once again. Let stand for 2 to 3 minutes.
6. Observe the preparation carefully. Based on your observation, you have to arrive at the following conclusions:

   a) If there is a precipitation reaction only in 1 then the blood group is A.

   b) If there is a precipitation reaction only in 2 then the blood group is B.

   c) If the reaction occurs both in 1 and 2 then the blood group is AB.

   d) If there is no precipitation formed in 1 and 2 then the blood groups is O.

Compare your slide with Fig. 16.2. Normally, you should be able to distinguish the precipitation reactions from the non-precipitating ones with the naked eye. If you are not able to do so, you may observe the antigen-antiserum mixture under a microscope.

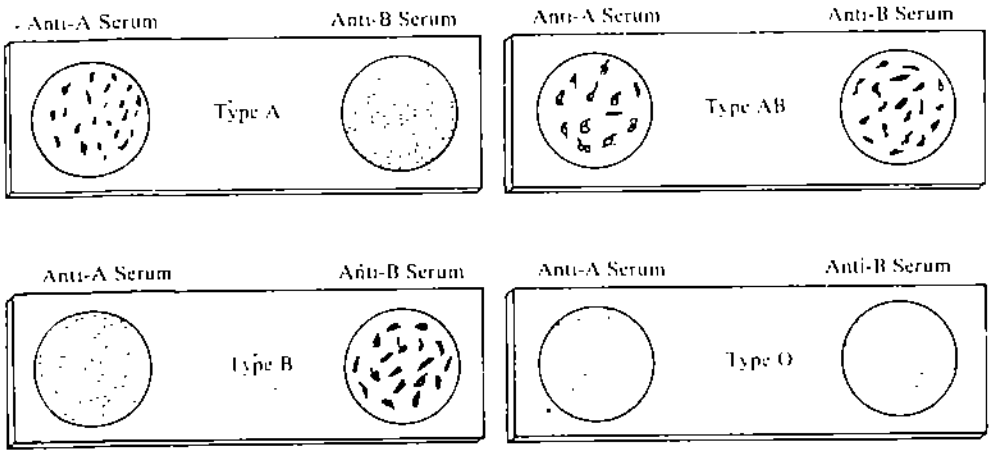

Fig. 16.2 : Antigen-Antiserum reactions in ABO blood group system.

The observations on the precipitation reactions should have helped you to understand as to why there is an insistence on giving the same group of blood as that of recipient at the time of blood transfusion. If a person with group A blood were to be transfused with group B blood, the antibodies found in the serum of the donor would cause the agglutination of the RBCs of the recipient. The agglutinated or clumped cells would occlude the capillaries in the recipient, sometimes resulting in death. This is the precise reason why the blood groups of both the donor and recipient are determined prior to blood transfusion.

SAQs

1. How would you explain the precipitation reactions shown in Fig. 16.2?

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   .....

2. Of the four groups of blood we discussed, one type is called the 'universal donor' and a second type as 'universal recipient' because of their ABO compatibilities. Can you identify them and explain why they are called so?

3. In a paternity dispute, two couples claimed the parentage of a child with blood group O. The two women had AB and B blood group and their husbands had A and B blood group respectively. As a geneticist you are asked to provide evidence in the case. To which couple would probably the child belong? (Refer to Unit 19 Genetics of Blood in Block 4 of LSE-03 Course).

EXPERIMENT 17 DETERMINATION OF ALLELIC AND GENOTYPIC FREQUENCIES

Structure

17.1 INTRODUCTION

In this lab exercise you will learn certain simple calculations relating to the frequencies of alleles and genotypes in populations. The term frequency refers to the number of times an allele or a genotype occurs in a given total population. For instance, in a population consisting of 500 people, if 180 of them carry the genotype AA, then the frequency of AA in the given population is 180/500 or 36% or 0.36. You have learnt that Mendelian monohybrid ratio for a given pair of alleles (A and a) is 3:1 for phenotypes and 1:2:1 for genotypes. Similarly if two pairs of alleles are involved (A and a, B and b) the phenotypic ratio is 9:3:3:1 and the genotypic ratio is 1:2:1:2:4:2:1:2:1. These ratios refer to the number of times a phenotype or a genotype appears relative to other pher. /pes or genotypes in specific crosses. They do not give the picture of frequencies of genotypes occurring in a given population. You will now learn the process of calculating the frequencies of dominant and recessive alleles and those of the genotypes that these alleles produce using some simple algebraic expressions.

Objectives

At the end of this exercise, you should be able to

- calculate the frequencies of dominant and recessive alleles based on the population data available for the phenotypes,
- arrive at the frequencies of genotypes based on allelic frequencies.
- extend the concept to the multiple allelic systems and calculate the frequencies of blood group alleles and the genotypes they produce.

17.2 DETERMINATION OF FREQUENCY OF DOMINANT AND RECESSIVE ALLELES AND GENOTYPES

For purposes of calculation, let us assume that the total frequency of a given pair of alleles, A and a is equal to 1. Let the frequency of allele A be equal to p and that of the recessive allele be equal to q. Then,

Frequency of A + frequency of a = p + q = 1.

In other words, frequency of A = p = (1–q) and that of a = q = (1–p).

The two alleles, as you are aware, form three genotypes, AA, Aa and aa and the three genotypes are in the ratio of 1:2:1

(1AA : 2Aa : 1aa).

The frequencies of the genotypes could then be,

$1AA = p \times p = p^2$

$2Aa = p \times q = pq$

$q \times p = pq$

$1aa = q \times q = q^2$

Assuming the total frequency of the three genotypes = 1,

$p^2 + 2pq + q^2 = 1.$

Let us now look into an example. In a population of 304 students in a college, 214 were able to taste the chemical phenylthiocarbamide (PTC) bitter and the rest 90 did not taste it. PTC tasters have the dominant allele T in their genotype and the non-tasters are recessive homozygotes (tt). Based on the data provided, let us calculate the frequency of alleles T and t in the given student population as well as the frequency of genotypes (TT, Tt and tt).

Total number of students = 304

Tasters = 214

non-tasters = 90

Frequency of taster phenotypes = 214/304 = 0.7

Frequency of non-taster phenotypes = 90/304 =0.3

Frequency of tt = q^2 = 0.3

Frequency of the allele t = $\sqrt{q^2} = \sqrt{0.3} = 0.55$

Frequency of the allele T = (1-q) = (1-0.55) = 0.45

Based on the allelic frequencies (T = 0.45 and t = 0.55),

we can predict the genotype frequencies of the population

Frequency of TT = p^2 = 0.45 x 0.45 = 0.2025

Frequency of Tt = pq = 0.45 x 0.55 = 0.2475

Frequency of tT = qp = 0.55 x 0.45 = 0.2475

Frequency of tt = q^2 = 0.55 x 0.55 = 0.3025

1.0000

Thus, from the frequencies of alleles, those of genotypes are calculated. The data on total population number and the number of persons possessing the recessive trait are sufficient to arrive at the frequencies of alleles and the genotypes, provided that the two alleles have simple dominant—recessive relationship. You attempt the following problem to test your understanding of determining allelic and genotypic frequencies in a population.

A survey conducted in a population of 930 individuals showed that 325 of them are non-tasters of PTC. What is the frequency of T and t alleles in the population? Also, calculate the frequency of TT, Tt and tt genotypes.

17.3 DETERMINATION OF FREQUENCIES OF MULTIPLE ALLELES

In the earlier section you have learnt the application of equations ($p + q = 1$) and $(p+q)^2 = 1$, in which the alleles exhibiting simple dominant-recessive relationship are involved. When more than two alleles occupy a locus, then the frequency of additional alleles also should be included in the equation. For instance, in ABO system of blood group inheritance, there are three alleles involved. The blood groups A, B, AB and O are controlled by a multiple allelic system of I^A, I^B and I^O. I^A and I^B are dominant to I^O but codominant themselves. The genotypes of the four blood groups are

Blood group	Genotype
A	I^AI^A, I^AI^O
B	I^BI^B, I^BI^O
AB	I^AI^B,
O	I^OI^O

Let the frequency of allele $I^A = p$, $I^B = q$ and $I^O = r$

Then $p + q + r = 1$

Let us now analyse the blood group allelic frequencies in a population.
Blood group determination of a population of 1000 individuals revealed that 328 of them belonged to A group, 122 of them to B group, 32 of them to AB group and 518 to O group. Calculate the allelic and genotypic frequencies in the population.

Type A persons — 328
Type B persons — 122
Type AB persons — 32
Type O persons — 518

Assuming the frequency of I^A, I^B and I^O to be p, q and r respectively, the frequency of the genotypes formed by the three alleles would be

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

Let us summarise the frequencies of phenotypes and probable genotypes in the form of a table.

Phenotype	Phenotypic Frequency	Genotype	Genotype Frequency	Sum of the frequencies of genotypes with similar phenotypes
A	$\frac{328}{1000} = 0.328$	I^AI^A I^AI^O	p^2 $2pr$	$p^2 + 2pr$
B	$\frac{122}{1000} = 0.122$	I^BI^B I^BI^O	q^2 $2qr$	$q^2 + 2qr$
AB	$\frac{32}{1000} = 0.032$	I^AI^B	$2pq$	$2pq$
O	$\frac{518}{1000} = 0.518$	I^OI^O	r^2	r^2

$r^2 = 0.518$

$r = \sqrt{0.518} = 0.72$

The frequency of $I^O = r = 0.72$

Frequency of $I^A = p = 1 -$ Frequency of B + frequency of O

Frequency of $B = q^2 + 2qr$

and the frequency of $O = r^2$

Frequency of B + frequency of $O = q^2 + 2qr + r^2 = (q+r)^2$ And the frequency of $I^A = 1 - \sqrt{(q + r)^2}$

$= 1 - \sqrt{(q^2 + 2qr + r^2)}$

$= 1 - \sqrt{0.122 + 0.518}$

$= 1 - \sqrt{0.640}$

$= 1 - 0.8$

$= 0.2$

Frequency of $I^A = 0.2$

Since the frequency of I^A and I^O are known, the frequency or I^B can be calculated using the expression.

Frequency of I^B = 1 – (frequency of I^A + frequency of I^O)

= 1 – (0.72 + 0.2)

= 1 – 0.92 = 0.08

Frequency of I^B = 0.08

The allelic frequencies are : $I^A = 0.200$

$I^B = 0.080$

$I^O = 0.720$

1.000

The genotype frequencies would then be

$I^AI^A = p^2 = 0.2 \times 0.2 = 0.0400$

$I^AI^A = 2pq = 2 \times 0.2 \times 0.72 = 0.2880$

$I^BI^B = q^2 = 0.08 \times 0.08 = 0.0064$

$I^BI^O = 2qr = 2 \times 0.08 \times 0.72 = 0.1152$

$I^AI^B = 2pq = 2 \times 0.2 \times 0.08 = 0.0320$

$I^OI^O = r^2 = 0.72 \times 0.72 = 0.5184$

$(p+q+r)^2 = 1.0000$

The above calculations show that the sum of the genotype frequencies is also equal to 1.

$p + q + r = 1$ and $(p + q + r)^2 = 1$

Since alleles I^A and I^B exhibit codominance and I^O is recessive to both alleles, the distribution of alleles and genotypes differs from the distribution of phenotypes. You will study more about the distribution of allelic and genotypic frequencies and the changes which they undergo in the population from your LSE-03 Genetics course in the Unit titled "Behaviour of Genes in Populations"

EXPERIMENT 18 PEDIGREE ANALYSIS FROM PEDIGREE CHARTS

Structure

18.1 INTRODUCTION

In exercise 15 you have studied certain of human traits which follow the Mendelian concept of dominance-recessive relationship. You are aware that Mendel followed the inheritance of such traits in pea plant through successive generations by controlled crosses. Also, such crosses led to the deduction of principles of segregation and independent assortment. These principles are applicable to all plants and animals including human beings. But the type of crossing experiments done with plants and animals, cannot be performed on humans. Therefore, other methods are used to study the human inheritance patterns. One method that is extensively used by human geneticists and genetic counsellors is the construction and analysis of pedigree charts. The pedigree charts consist of a set of symbols which convey the details regarding the transmission of a trait over a number of successive generations. It is possible, after a careful study of pedigree chart to conclude whether a trait is dominant or recessive and whether it is an autosomal trait or a sex linked one. In this exercise we shall learn to draw pedigree charts and analyse them meaningfully. Make sure that your are familiar with the concepts of autosomal and sex chromosomal inheritance from your studies on LSE-03 course.

Objectives

From this experiment; you should learn:

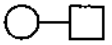
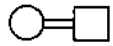
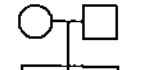
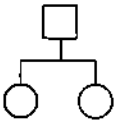
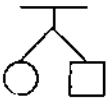
- to explain various symbols that are commonly used in pedigree charts
- to draw a pedigree chart based on the data available or given
- to analyse and interpret pedigree chart and assign the trait to autosome or sex chromosome and say whether the concerned allele is dominant or recessive.

18.2 PROCEDURE FOR CONSTRUCTING PEDIGREE CHARTS

Before we begin constructing or analysing the pedigree charts it is necessary to

become familiar with different symbols that are commonly used in pedigree charts. Table 18.1 provides you the symbols used and their meanings. We shall begin with the construction of a simple pedigree chart and then start analysing the more complex ones.

Table 18.1: Symbols used in Pedigree Charts

1.		Normal female
2.		Normal male
3.		Indicates marriage
4.		Indicates consanguinous mating or mating between close relatives.
5.I		Normal parents with 3 normal children, 2 daughters and one son.
II	1 2 3	Roman numerals denote generation numbers and arabic numerals, the order in which the children are born.
6.		Only a single parent is shown as the other parent is normal and is of no significance in pedigree analysis.
7.		Fraternal twins or twins arising from two different zygotes (dizygotic).
8.		Identical twins or twins arising from a single zygote (monozygotic).
9.		Sex unknown.
10.	3 and 4	Number of children for each sex
11.	and	Shaded circle or square indicates affected daughter or son.
12.	and	An arrow below the affected individuals indicates that the analysis begins from that individual. The individual is an index case. The affected individual is thus a proband or propositus.
13.	or and or	Autosomal heterozygous recessive.
14.		Sex linked carrier individual.
15.	and	Deceased individual.
16.		Aborted foetus or a stillborn child.

Humans fall into two categories depending on their ability to taste a chemical the phenylthiocarbamide (PTC). The tasters taste this chemical bitter and the non-tasters do not taste the chemical at all. The allele T determines the ability to taste PTC and people who are homozygous recessive to the allele (tt) are non-tasters. We have the following data on taste blindness from a family.

The female parent is a non-taster and the couple had five children, three daughters and two sons, of whom a son and a daughter are non-tasters. One of the taster daughters is married to a non-taster man. This couple had eight children, five sons and three daughters, of whom two sons and a daughter are non-tasters.

Based on the data provided, we may construct a pedigree chart to show the inheritance pattern of non-taster allele in the family.

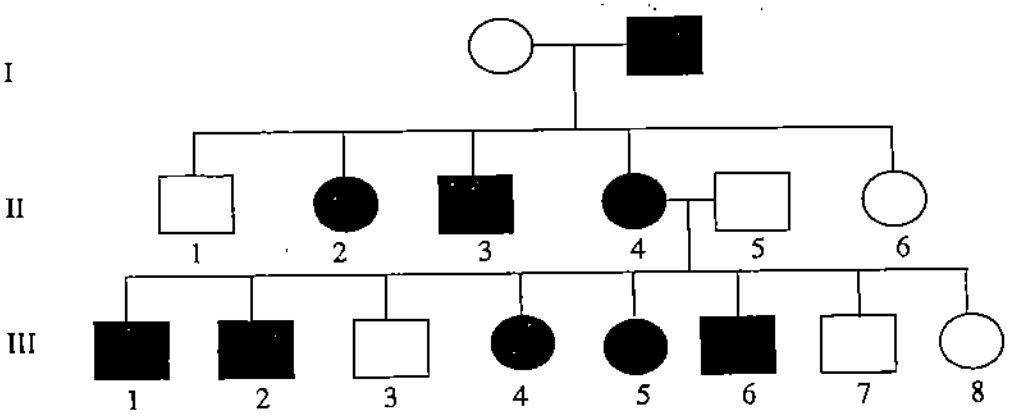


Fig. 18.1

The pedigree (Fig. 18.1) shows that the female parent is a non-taster. Similarly II-1 and II-6 individuals are non-tasters; so also III-3, III-7 and III-8 individuals. It is possible to deduce from the pedigree that the trait is determined by an autosomal allele. The method of deducing is discussed below. Briefly it could be said here that if the allele were to be a sex linked trait, all the males of the second generation would have inherited it. You have learnt from unit 4 of Genetics course that sex linked characters are generally transferred from mother to sons and from father to daughters, a phenomenon known as criss-cross inheritance. From the pedigree chart it is possible to deduce whether the trait is a dominant one or recessive. Assuming the trait is a dominant one and the father is homozygous for it (TT), then the mother will be homozygous recessive (tt). All the children of this couple, then would necessarily be tasters and heterozygous (Tt). However, this is not the case here. Assuming the father is heterozygous dominant, then half their children would be non-tasters and the other half tasters. More or less this appears to be the case in the pedigree cited. The II-4 female, a taster is married to a non-taster. Assuming she is also heterozygous dominant, then half of the children would be non-tasters and the other half tasters. The data suggests that this is so in the III generation. So could the taster allele be dominant?

Assuming the taster allele to be a recessive one, and the female of generation is heterogyzous for non-taster trait, then again results similar to the one shown in the pedigree chart would be obtained. The pedigree chart is thus incomplete in the sense that it does not help us to decide whether the concerned allele is dominant or recessive. Essentially the chart has to be expanded by collecting more data on the family. Let us say that we have additional data. The III-8 female, a non-taster is married to a taster male and has four children all of whom are tasters. Let us now redraw the pedigree chart with the additional information (Fig. 18.2).

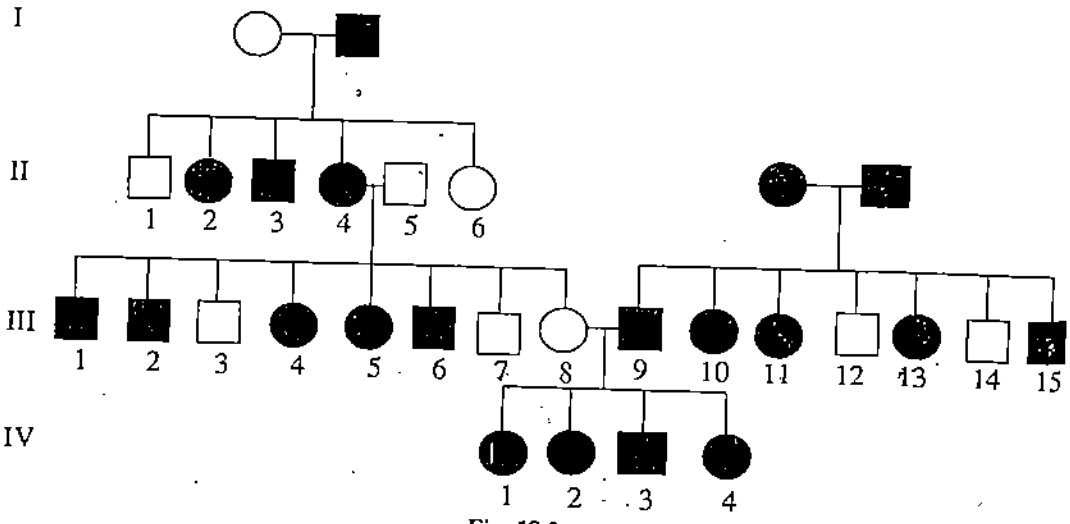


Fig. 18.2

Now, the III-8 and III-9 individuals are married and have four taster children. This part of pedigree chart helps us to determine beyond any doubt the dominance or recessiveness of the allele. The fact that all the four children born to III-8 and III-9 parents are tasters and that only one of the parents is a taster, clearly tells us that the father is homozygous dominant (TT) and the mother is recessive (tt) (Fig. 18.3).

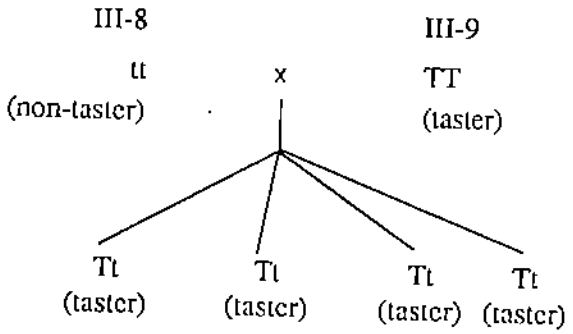


Fig. 18.3

Therefore, the allele in question is a dominant allele. Essentially PTC taster trait is an autosomal dominant trait.

Certain general patterns can be followed to identify whether a trait is autosomal or sex chromosomal and whether it is dominant or a recessive one. Let us categorise these patterns.

18.3 AUTOSOMAL DOMINANTS

a) Autosomal dominant traits make their appearance invariably in all generations. In otherwords, they do not skip generations.

b) An individual carrying the gene for the affected trait (heterozygous) married to a normal individual generally produces normal offspring to affected ones in the ratio of 1:1.

c) There is no discrimination between sexes and the trait is distributed equally in both the sexes.

The pedigree in Fig. 18.4 shows the general pattern of inheritance of an autosomal dominant trait. Study the pedigree carefully and make sure that the pattern of autosomal dominant inheritance conforms to the statements we made above.

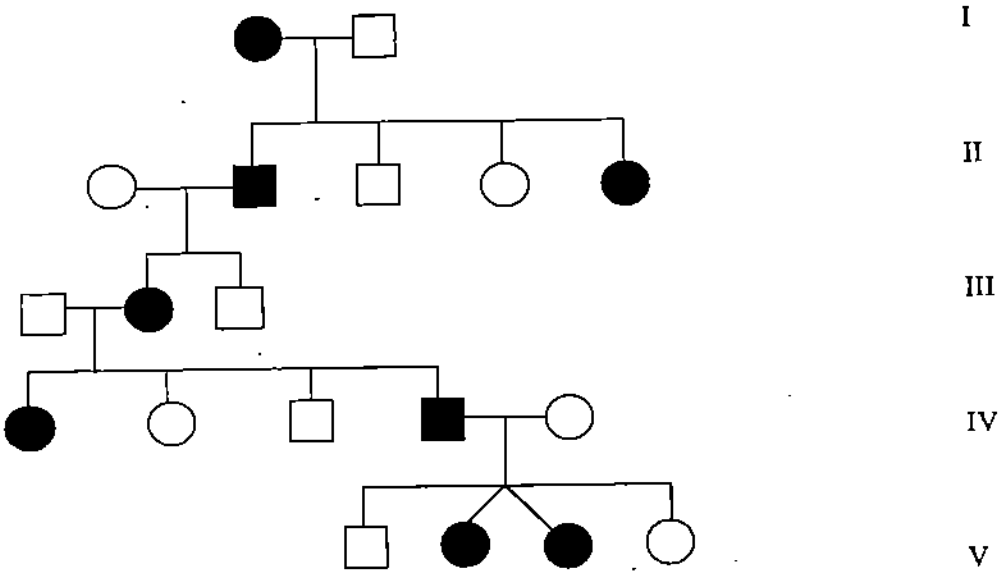


Fig. 18.4

Brachydactyly, Huntington's disease, ability to taste phenylthiocarbamide and polydactyly are some of the autosomal dominant traits in humans.

18.4 AUTOSOMAL RECESSIVES

a) Unlike the autosomal dominant traits, the autosomal recessive ones do not make their appearance in every generation. In other words, they may skip certain generations and appear only in certain others.

b) Like autosomal dominants, the distribution is equal between the two sexes.

c) They are more commonly found among children born to consanguinous couples, that is couples who were first cousins before marriage or otherwise closely related.

d) If both the parents are affected, all the children born to them will also be affected.

e) The parents of the affected children may be normal.

f) The affected child born to a normal parents essentially suggests that the parents are heterozygous and are carriers of the allele for the trait.

g) If both the parents are heterozygous, then the chances are that approximately 50% of the children born to them would inherit the recessive trait.

The pedigree given in Fig. 18.5 is an example of the inheritance pattern of albinism in humans. Albinism is a rare autosomal recessive trait and refers to a condition in which a person cannot synthesise the pigment melanin. Study the pedigree carefully and varify that the inheritance of albinism follows the pattern that is characteristic of an autosomal recessive trait.

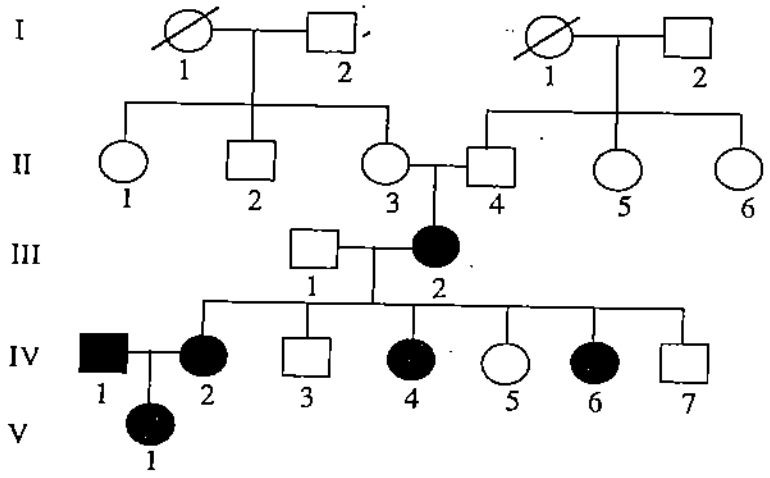
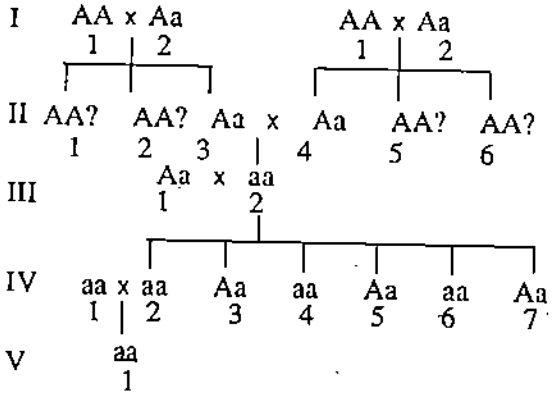


Fig. 18.5

An analysis of the pedigree in Fig. 18.5 shows that III-2 individual is an albino, although both her parents are normal. Essentially they should be heterozyous and the daughter receives the two recessive alleles. Also individuals IV 2, 4 and 6 are also albinos, suggesting that their father is also heterozygous. Since both IV 1 and 2 are albinos their daughter is also an albino. The possible genotypes of the above pedigree can be written as follows (Fig. 18.6).



The question mark suggests that the genotypes of individuals could be either AA or Aa.

Fig. 18.6

18.6 SEX CHROMOSOMAL DOMINANTS

a) As in the case of autosomal dominants, in the inheritance of sex linked dominants also the generations are not skipped.

b) Males always inherit the trait from their mother.

c) Females may receive the dominant allele either from their mother or from their father.

d) If the female is affected, then approximately half of her sons and half of her daughters are affected.

e) If the male is affected then all his daughters will be affected but none of his sons will be affected.

Sex linked dominant traits are very rare. A sex linked dominant trait is oral-facial-digital syndrome which results in cleft tongue, absence of teeth and mental retardation. Other sex linked dominat alleles responsible for certain diseases include the allele for Albright's hereditary osteodystrophy causing seizures, stunted growth and mental retardation, Goltz's syndrome whose symptoms are mental retardation, small eyes and flexed digits and incontinentia pigmenti which causes the non-retention of melanin in melanoblasts. Fig. 18.7 shows a pedigree for one such sex-linked dominant trait.

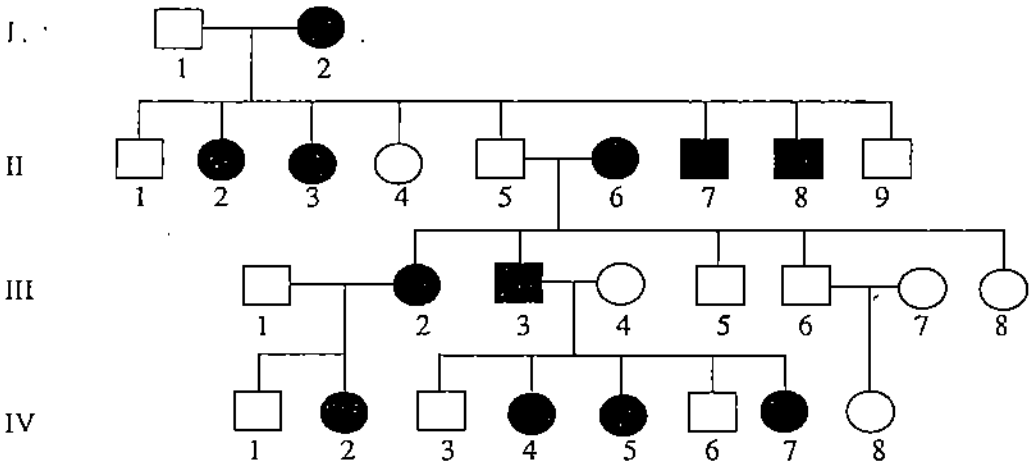


Fig. 18.7

You may observe in the Fig 18.7 that the trait appears in all generations; and the trait always passes from the father to the daughters and not to the sons (Note that III-3 male has passed the trait to all his daughters but the two sons are normal).

18.6 SEX-LINKED RECESSIVE TRAITS

a) Since males have only one X-chromosome and receive the same from their mother, they are the ones most affected by sex linked recessive alleles.

b) Generally, the females are carriers of the recessive allele and thus heterozygous in genotype.

c) The females are known to be heterozygous because of affected brothers, fathers or maternal uncles.

d) Males receive the X-linked recessive trait from the their mother.

e) Females express the trait only when they have a homozygous recessive genotype and the alleles are received from carrier mothers and affected fathers.

f) All the sons of an affected female are affected and 50% of the daughters are carriers.

g) Nearly 50% of the sons of the carrier female would be affected.

Several sex linked recessive traits are known in humans and haemophilia is the most famous case of sex linked recessive inheritance. Colour blindness, the

inability to distinguish red and green colours is another one. Many deficiencies relating to enzymes such as G-6-PD deficiency (glucose 6 phosphate dehydrogenase deficiency) are also sex linked. The pattern of inheritance of a sex linked recessive allele is shown in the Fig. 18.8

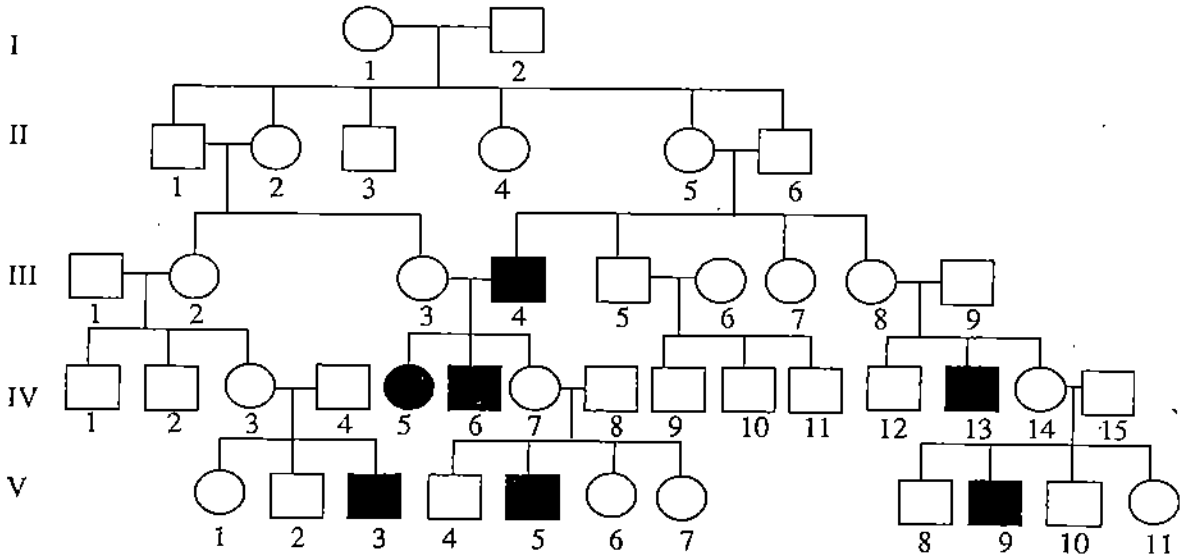


Fig 18.8

In the case of sex linked recessive inheritance, the number of males affected are always more than females. This is essentially due to the fact that the males receive only one X chromosome and that is received from the mother. You can observe from Fig. 18.8 that III-3, a normal female married to III-4, an affected male gives birth to 3 children, 2 daughters and one son. Since the son is affected, it is obvious that the mother is the carrier of the recessive allele and has passed the trait to him.

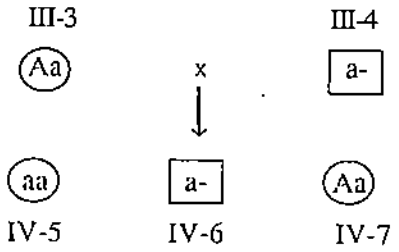


Fig. 18.9

Similarly, III-8, IV-7 and IV-14 are heterozygous mothers who have passed the trait to their son.

We have thus far discussing the analysis of pedigree charts and assign the traits to autosomes or sex chromosome and to identify them as dominant or recessive ones. You may now work on the following problems to test your understanding of pedigree charts.

SAQ

1. Analyse the following pedigree and answer the questions as directed.

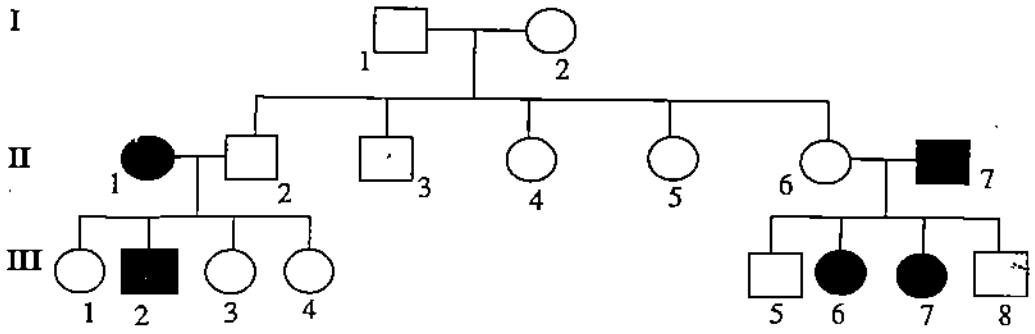


Fig. 18.10

a) Is the trait an autosomal or a sex chromosomal one?

b) Is the gene that causes the trait a dominant or a recessive one?

c) What could be the genotypes of I-1, I-2, II-1, II-6, II-7, III-5 and III-7 individuals, assuming the dominant allele is S and recessive allele is s.

2. Below is given the pedigree of a family, certain individuals of which are affected by an inherited metabolic disorder alkaptonuria. The disease is caused by a defect in the metabolism of the amino acid phenylalanine, Answer the questions given below after a careful analysis of the pedigree.

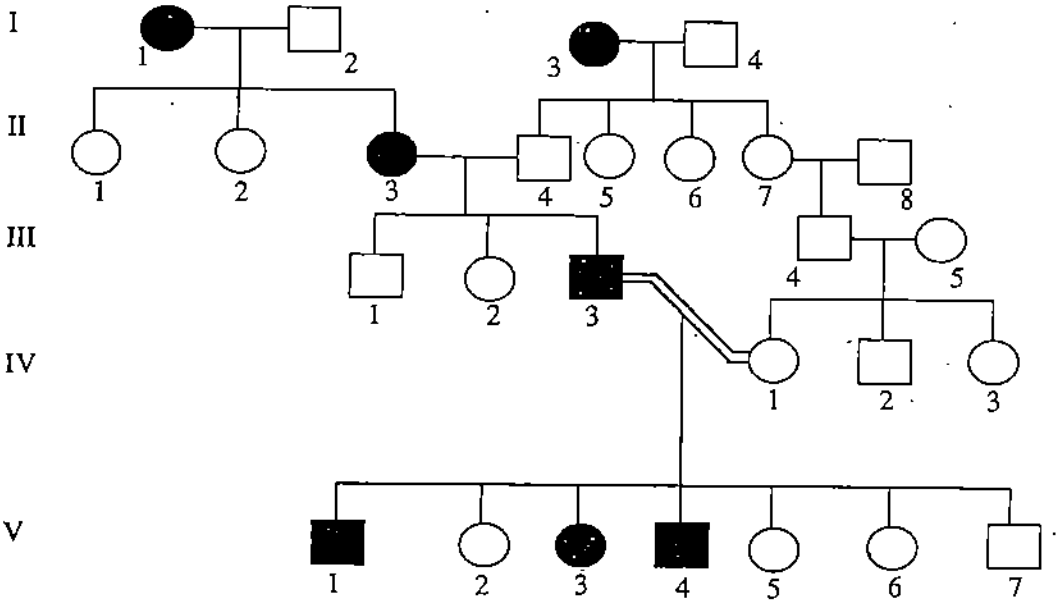


Fig. 18.11

1) Does the above pedigree suggest an autosomal or sex chromosomal inheritance?

2) Does the inheritance pattern suggest the involvement of a dominant allele or a recessive allele?

3) How would you explain the marriage between III-3 and a IV-1 individuals?

4) Assuming the dominant and recessive alleles are designated as A and a, what are the genotypes of I-2, II-3, II-4, III-3 and IV-1 individuals?

EXPERIMENT 19 APPLICATION OF PROBABILITY TO PROBLEMS IN GENETICS

Structure

19.1 INTRODUCTION

One of the effective tools for a geneticist and a genetic counsellor to assess the possible occurrence of a trait in a family is the application of probability theory. A probability is the ratio of the number of times a particular event occurs to the number of trials during which the event could have happened. Assuming a man and his wife seek advice of a genetic counsellor on a genetic problem, the counsellor analyses the pedigree of the couple's family, establishes the genotypes of the couple and then makes calculations relating to the probability of the appearance of the trait in question in the children to be born to them in future. In this lab. exercise, you will learn some of the basic rules of probability theory. You should try to apply them to problems in genetics.

Objectives

At the end of this lab. exercise, you should be able to:

- describe the basic principles of probability,
- make use of the principles of probability to solve genetic problems,
- apply the formula for the binomial expansion to determine the probability of any combination of events,
- comprehend the Pascal's triangle to determine the coefficient of binominal expression that tells how many ways a particular combination may be obtained.

19.2 THE BASIC PRINCIPLES OF PROBABILITY THEORY

The probability theory, it can be said, helps you to make a proper and

meaningful guess of the occurrence of an event. The probability P of the occurrence of an event is the number of favourable cases a divided by the total number of possible cases n.

$$P = \frac{a}{n}$$

In determining the probability of an event, one way is to observe a large number of cases and record the number of times an event occurs or does not occur. But this is an empirical method. A better way of determining the probability is the one that is generally used by the geneticists for making predictions on the occurrence of an event. The probability obtained by this method is *a priori* probability. You look into the following example that explains the calculation of probability before we take up specific examples from genetics.

Take a die (plural = dice) with six faces numbered 1 to 6. When the die is rolled, the probability P of any one face showing up is 1/6.

$$P = \frac{a}{n} = \frac{1}{6} = 0.167$$

The probability of picking up the nine of spades from a deck of 52 cards is

$$P = \frac{1}{52} = 0.0192$$

And the probability of drawing any one club from a deck of cards is

$$P = \frac{13}{52} = \frac{1}{4} = 0.25$$

Now let us cite one or two examples from genetics. The probability of an offspring to be of recessive genotype when a monohybrid is self-fertilised is

$$P = \frac{1}{4} = 0.25$$

and the probability of an offspring to be of dominant phenotype for both traits on self-fertilisation of a dihybrid is

$$P = \frac{9}{16} = 0.5625$$

When we say that the probability of the occurrence of an event is P, the combined probability of occurrence of all the other events is $Q = (1 - P)$. Thus when the P of occurrence of dominant phenotypes = 9/16, the combined probability of occurrence of other phenotypes = $Q = 1 - 9/16 = 7/16$ and $P + Q = 9/16 + 7/16 = 1$. $P + Q$ is always equal to 1. In fact all probabilities must lie between 0 and 1. A probability of 1 means that the event is certain to occur; a probability of zero indicates that the event cannot occur.

Now, let us look into situations where we consider the occurrence of two events. By two events, we mean the occurrence of either one of the two events or both the events simultaneously. Essentially in such cases we will be combining the probability of occurrence of the two events. There are three rules under which the combining of the probabilities can be done.

19.2.1 Addition Rule

When the occurrence of one event precludes the possibility of the occurrence of another event, the two events are said to be **mutually exclusive**. Essentially it means that when one event occurs, the other does not. And the probability of occurrence of one of several mutually exclusive events is the sum of the probabilities of individual events. For instance, when a die is thrown what is the probability that it shows either a two or a three?

P of getting a two = 1/6 = 0.167
P of getting a three = 1/6 = 0.167

Therefore, the two events are mutually exclusive. The probability of getting either a two or a three = 1/6 + 1/6 = 2/6 = 1/3 = 0.33. Since the probability of occurrence of mutually exclusive events is summed up, the rule is called **addition rule.**

19.2.2 Product Rule

Product rule is used when the occurrence of one event is not dependent on the occurrence of another event; in other words we are dealing with independent events. For instance, when two dice are thrown simultaneously the probability of getting a two and a three in that order are

P of getting a two = 1/6 = 0.167
P of getting a three = 1/6 = 0.167

Probability of getting a two and a three = 1/6 x 1/6 = 1/36 = 0.028. Since the probability of occurrence of independent events is the product of their separate probabilities, this rule is called the **product rule.**

Look into this example,

What is the probability of getting a head and a tail, when two coins are tossed simultaneously? This procedure as we are going to demonstrate to you, requires the use of both addition and product rules. For each coin the probability of getting a head H or tail T is

P (H) = 1/2 = 0.5
P (T) = 1/2 = 0.5.

When the coins are tossed one at a time, there are two ways of getting a head or a tail.

First head and then tail (HT)
First tail and then head (TH)

The results of each of the two tosses in a sequence are independent events.

The probability of getting HT and TH = 1/2 x 1/2 = 1/4 = 0.25

At the same time, the two sequences are mutually exclusive. The probability of

getting either of two sequences of a set of mutually exclusive events is

$$\frac{1}{4} + \frac{1}{4} = \frac{1}{2} = 0.5$$

Thus when events are unordered, the probability can be obtained by combining addition and product rules.

19.2.3 The Binomial Theorem

The probability of unordered events can be determined by using binomial theorem. This theorem defines the probability of the occurrence of some arrangement of two mutually exclusive trials where the final order is not specified. According to this theorem the frequencies or the probabilities of the occurrence of various combinations correspond to the terms of the binomial expansion. The first three binomial expressions are as follows.

$$(a + b)^2 = a^2 + 2ab + b^2$$

$$(a + b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$$

$$(a + b)^4 = a^4 + 4a^3b = 6a^2b^2 + 4ab^3 + b^4$$

A simple formula based on binomial theorem would help you to calculate the probability by a short-cut method. According to this formula:

The symbol ! is read as factorial. 5! or factorial 5 = 5 x 4 x 3 x 2 x 1.

$$P = \frac{n!}{s!t!} \times p^s q^t$$

Where n is the total number of events, p is the probability of the occurrence of an event (X), q is the probability of the occurrence of an alternate event (Y), s is the number of times the event X will occur and t is the number of times the event Y will occur out of n number of trials. Here, $s + t = n$ and $p + q = 1$.

Let us look into the previous example. When

$$n = 2$$

Probability of getting a head = $p = \frac{1}{2}$

Probability of getting a tail = $q = \frac{1}{2}$

and $s = t = 1$.

Substituting the above data in the formula,

P = the probability of getting a head and a tail when two coins are tossed simultaneously $= \frac{2}{1!1!} \cdot \left(\frac{1}{2}\right)^1 \cdot \left(\frac{1}{2}\right)^1$

$$= \frac{2 \times 1}{1 \times 1} \cdot \frac{1}{2} \cdot \frac{1}{2} = \frac{1}{2}$$

$$= 0.5$$

Let us now look into more specific examples from genetics. What is the probability that a family with five children will have 3 boys and 2 girls?

The probability of a child to be a boy = $p = \frac{1}{2}$

The probability of a child to be a girl = $q = \frac{1}{2}$

Applying the formula

$$P = \frac{5!}{3!\,2!} \cdot \left(\frac{1}{2}\right)^3 \cdot \left(\frac{1}{2}\right)^2$$

$$= \frac{5 \times 4 \times 3 \times 2 \times 1}{3 \times 2 \times 1 \times 2 \times 1} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = 0.312$$

Assuming the parents want the 5 children to be born in a specific order—say 2 boys, 1 girl 1 boy and a girl. Essentially this would mean that you have to apply the product rule; in which case the probability would be

$$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{32} = 0.312$$

Thus you can see from the two answers, that when an order is specified, the probability is 10 times less than when no order is specified. In other words, while there is only one way of getting 2 boys, 1 girl, 1 boy and 1 girl, there are 10 different ways of getting 3 boys and 2 girls.

1	2	3	4	5	6	7	8	9	10
B	B	B	B	B	G	G	G	G	G
B	B	B	G	G	G	B	B	B	G
B	G	G	G	B	B	G	B	B	B
G	B	G	B	G	B	B	G	B	B
G	G	B	B	B	G	B	B	G	B

[B = boy; G = Girl]

Assuming a couple is heterogygous (Aa) for albinism, what is the probability that 4 children out of 6 born to them are normal?

Let A = allele for normal skin

a = allele for albinism

Aa x Aa

AA Aa Aa aa

normal albino

Since the ratio of normal to albino is 3 : 1, the probability of a normal son being born is $\frac{3}{4}$ and an albino is $\frac{1}{4}$.

The probability of 4 children being normal is

$$\frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} = \frac{81}{256} = 0.316$$

What is the probability of 4 children being normal and 2 children albinos?

In this case,

$$P = \frac{6!}{4!\,2!}$$

$$= \frac{6 \times 5 \times 4 \times 3 \times 2 \times 1}{4 \times 3 \times 2 \times 1 \times 2 \times 1} \times \frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{1}{4} \times \frac{1}{4}$$

$$= \frac{1215}{4096} = 0.297$$

Assuming you specify the order in which the normal and albino children are born say the first 3 children are normal, 1 albino, 1 normal and 1 albino, then the probability would be

$$\frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{1}{4} \times \frac{3}{4} \times \frac{1}{4} = \frac{81}{4096} = 0.0198$$

You can again observe that once the order is specified, the probability assumes a lower value. In other words, if no order is specified, the probability is fifteen times larger than when it is specified.

19.3 PASCAL'S TRIANGLE

We earlier said that in the formula $P = (n!/s!t!)\ p^s q^t$, $(p+q) = 1$ and $(s+t) = n$. This formula essentially separates the binomial equation $(p+q)^n = 1$ and gives the probability of one of the terms. The binomial expansion of $(p+q)^n$ contains $(n+1)$ terms. A device called Pascal's triangle is useful to get the coefficient of the terms. The coefficients tell you the number of ways by which one can obtain a particular combination of events. The following is the Pascal triangle upto $n = 7$.

1.	n	=	0	$(p+q)^0$					1					
2.	n	=	1	$(p+q)^1$				1		1				
3.	n	=	2	$(p+q)^2$			1		2		1			
4.	n	=	3	$(p+q)^3$		1		3		3		1		
5.	n	=	4	$(p+q)^4$	1		4		6		4		1	
6.	n	=	5	$(p+q)^5$	1	5		10		10	5		1	
7.	n	=	6	$(p+q)^6$	1	6	15		20		15	6		1
8.	n	=	7	$(p+q)^7$	1	7	21		35		35	21	7	1

The above triangle is built out of the coefficient of binomial expansion. You may observe that any given row begins with 1, then proceeds by the addition of two adjacent coefficients from the preceeding row and finally ends in 1. For example, take the 7th row, $(p+q)^6$.

$$(p+q)^6 = (1)p^6 + (1+5)p^5q + (5+10)p^4q^2 + (10+10)p^3q^3 + (10+5)p^2q^4 + (5+1)pq^5 + (1)q^6$$

Let us get back to our earlier problems. What is the probability of 4 normal and 2 albino children born to a couple heterozygous for the albino allele?

$$n = 6;\ s = 4\ t = 2\ p = \frac{3}{4} \text{ and } q = \frac{1}{4}$$

The problem is completely described by the expression of $(p+q)^6$. In the equation $(p+q)^6$ (remember $n = 6$), the term that is of interest to us is $15p^4q^2$, since $s = 4$ and $t = 2$.

$$15p^4q^2 = 15\ (3/4)^4\ (1/4)^2$$

$$= 15 \times \frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{3}{4} \times \frac{1}{4} \times \frac{1}{4}$$

$$= \frac{1215}{4096} = 0.297$$

This is the same answer that we obtained using the formula. But when the order of the binomial increases, its expansion to get all the terms becomes difficult. In such cases we can readily use the formula.

19.4 MULTINOMIAL EXPRESSION

The formula has one more advantage. It could be expanded to include more than two events. The multinomial expansion $(p+q+r\ ...)^n$ can be represented as the general formula for calculating the probability.

$$p = \frac{n\ !}{s\ !\ t\ !\ u!} \times p^s q^t r^u .....$$

Where $p+q+r..... = 1$ and $s+t+u.... = n$.

Assuming a couple were told by the genettic counsellor that each of them carry an allele for albino trait. The couple wants to have six children. What is the probability that of the six children, two will be normal daughters, two normal sons, one albino son and one albino daughter?

In this problem, you first apply the product rule to know the probability for each item and then apply the formula to get the probability for the entire event. For example,

Probability of getting a normal son p = (3/4) (1/2) = 3/8

Probability of getting a normal daughter q = (3/4) (1/2) = 3/8

Probability of getting a albino son r = (1/4) (1/2) = 1/8

Probability of getting a albino daughter k = (1/4) (1/2) = 1/8

Probability of getting 2 normal sons (s), 2 normal daughters (t), 1 albino son (u) and 1 albino daughter (v) =

$$p = \frac{n\ !}{s\ !\ t\ !\ u\ !\ v\ !} \cdot p^s q^t r^u k^v$$

$$p = \frac{6\ !}{2\ !\ 2\ !\ 1\ !\ 1\ !} \cdot (3/8)^2 \cdot (3/8)^2 \cdot (1/8)^1 \cdot (1/8)^1$$

$$= \frac{6 \times 5 \times 4 \times 3 \times 2 \times 1}{2 \times 1 \times 2 \times 1 \times 1 \times 1} \cdot 3/8 \times 3/8 \times 3/8 \times 3/8 \times 1/8 \times 1/8$$

$$= \frac{14580}{262144} = 0.0556$$

19.5 SAQ

1. Assuming a sex ratio of 1:1, what is the probability that a family of 4 children will consist of

   i) 3 daughters and 1 son

   ii) all daughters

   iii) alternating sexes

   iv) all sons

   v) atleast two daughters

2. In lab exercise 8, we discussed about phenylthiocarbamide tasters. PTC tasting, as you are aware is dominant to non-tasting. A taster man whose mother is a non-taster marries a taster woman who in a previous marriage had a non-taster daughter. What would be the probability of the couple having

   a) their first child a taster?

   b) their first child a non-taster boy?

   c) 7 children with 4 tasters and 3 non-tasters?

   d) 5 children, of whom 2 taster bodys 1 taster girl 1 non taster body and 1 non taster girl in that order.

3. Two parents have genotype Mm and suffer from migraine headache. What is the probablity that

   a) their first child will be a girl with migraine and their second a boy without the disorder.

   b) 4 children are born to them with 3 children born without migraine and one child with it.

EXPERIMENT 20 INVESTIGATION OF HUMAN KARYOTYPES

Structure

20.1 INTRODUCTION

In this laboratory exercise, you will learn to study and to identify the different groups of human chromosomes and prepare a karyotype of them from the figures provided. You will also learn about the abnormal chromosomal numbers that results in specific syndromes commonly met with in the human males and females.

Individual chromosomes are most easily studied during metaphase. At that time, each chromosome clearly shows the two chromatids connected by a centromere. It is possible to stop the process of mitosis in metaphase by chemical means and to photograph the chromosomes. These photographs of metaphase smears are arranged in a prescribed manner to obtain the karyotype of that individual.

Karyotyping

Geneticists have developed a system for identifying each of the 46 chromosomes. The 22 pairs of autosomes are numbered from 1 to 22 according to their length. The sex chromosomes constitute the pair 23. It is very difficult to arrange chromosomes exactly according to number. However, the 23 pairs have been arranged into 7 groups according to the size and location of centromere. The Table 20.1 gives this information. This table would be your key guide in the preparation and study of karyotypes in this experiment.

Table 20.1: The Seven Groups of Chromosomes.

Group	Chromosomes	Characteristic
A	1, 2, 3	Very long; centromere in the centre of chromosome
B	4 and 5	long; centromere away from centre of chromosomes
C	6, 7, 8, 9, 10, 11, 12, X	Medium length; centromeres in the centre or slightly away from center of chromosomes
D	13, 14, 15	Medium length; centromeres at or very near the end of chromosomes
E	16, 17, 18	Somewhat short; centromeres in the centre or away from the centre of chromosomes
F	19 and 20	Short; centromeres in the centre of chromosomes
G	21, 22, Y	Very short; centromeres at or very near the end of chromosomes

The chromosomes of the first 22 pairs (autosomal chromosomes) are similar in all human karyotypes. But the chromsomes of the twenty-third pair, the sex chromsomes are dissimilar in male, i.e., there is a larger X chromosome and a smaller Y chromosome. Females have two X chromosomes in their karyotype.

In this investigation you would study three abnormal karyotypes. i) Down's syndrome – an extra chromosome 21 is associated with this disorder. ii) A missing X chromosome in females causes Turner's syndrome; and iii) the occurrence of an extra X chromosome (XXY) in males leads to Klinefelter's syndrome.

Prior Reading for this Experiment

Units 8, 9 and 10 of Genetics course, (LSE-03)

Objectives

After doing this laboratory exercise you should be able to:

- prepare karyotypes from the xerox photographs of chromosomes provided;
- identify karyotype of normal male and female;
- diagnose the chromosomal disorders from the abnormal karyotypes provided.

20.2 MATERIALS REQUIRED

1. Human chromosome photographs
2. Human karyotype forms
3. Scissors
4. Pencil
5. Tape or Glue

20.3 PROCEDURE

Step 1 : Cut each individual chromosome from sheet I and II. Be careful not to cut any part of the chromosomes. Count how many you have. **Note: The chromosome cut-outs are very light and are easily lost. Should a chromosome be lost from the sheet set, the set would become incomplete and the whole exercise will be useless. So be very careful.**

Step 2 : Arrange the chromosomes in the karyotype forms a and b respectively according to the chromosome characteristics provided in Table 20.1, placing the short arm of each chromosome towards the top.

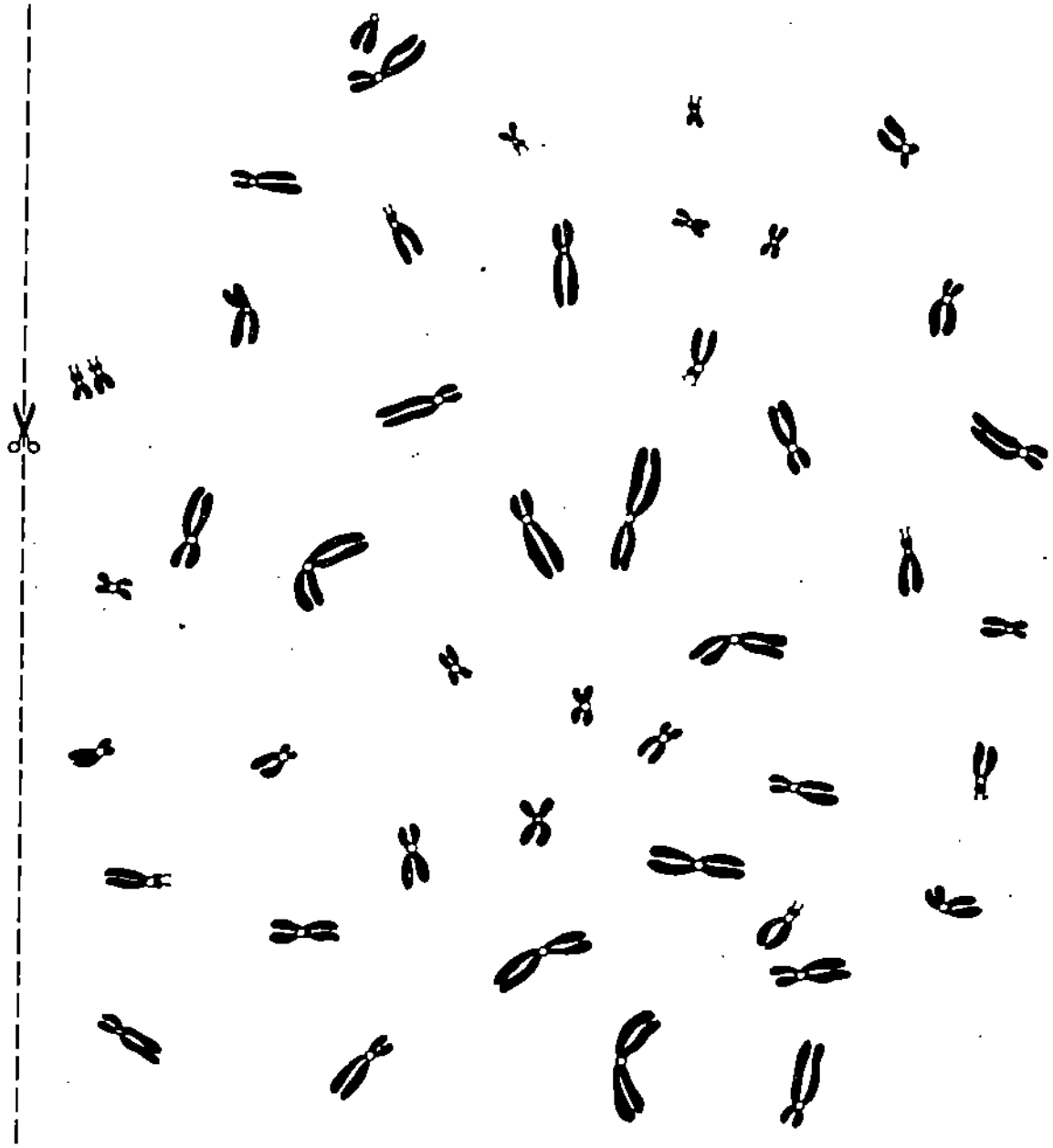
Step 3 : Pair the chromosomes referring to their banding pattern (if provided) and other features mentioned above.

Step 4 : After the chromosomes are arranged in order, glue or tape them in place.

Step 5 : Answer the questions based on sheet I and II about the karyotypes you have prepared.

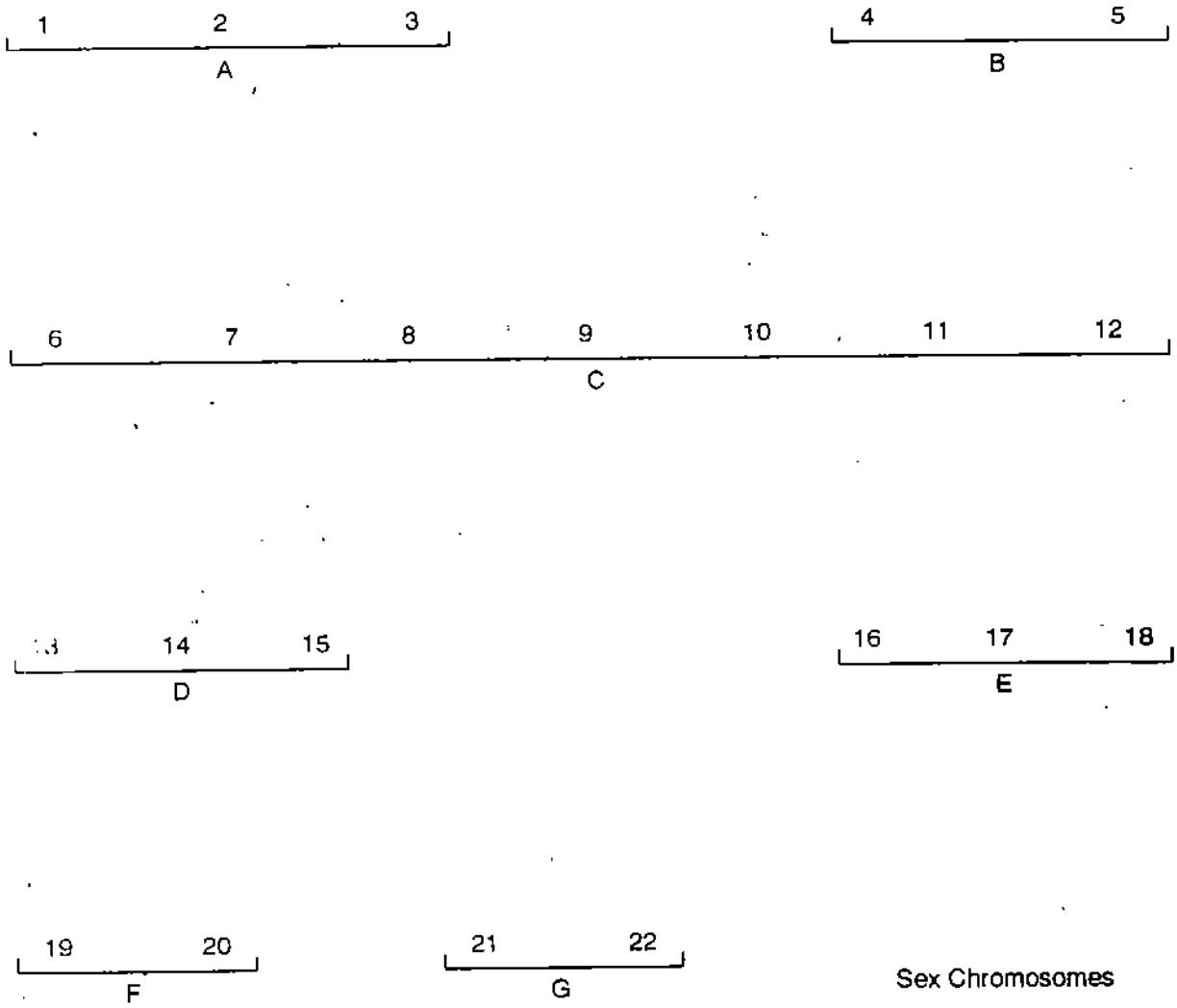
Step 6 : Study the karyotype sheets III, IV and V critically, and answer the questions based on them.

SHEET - I

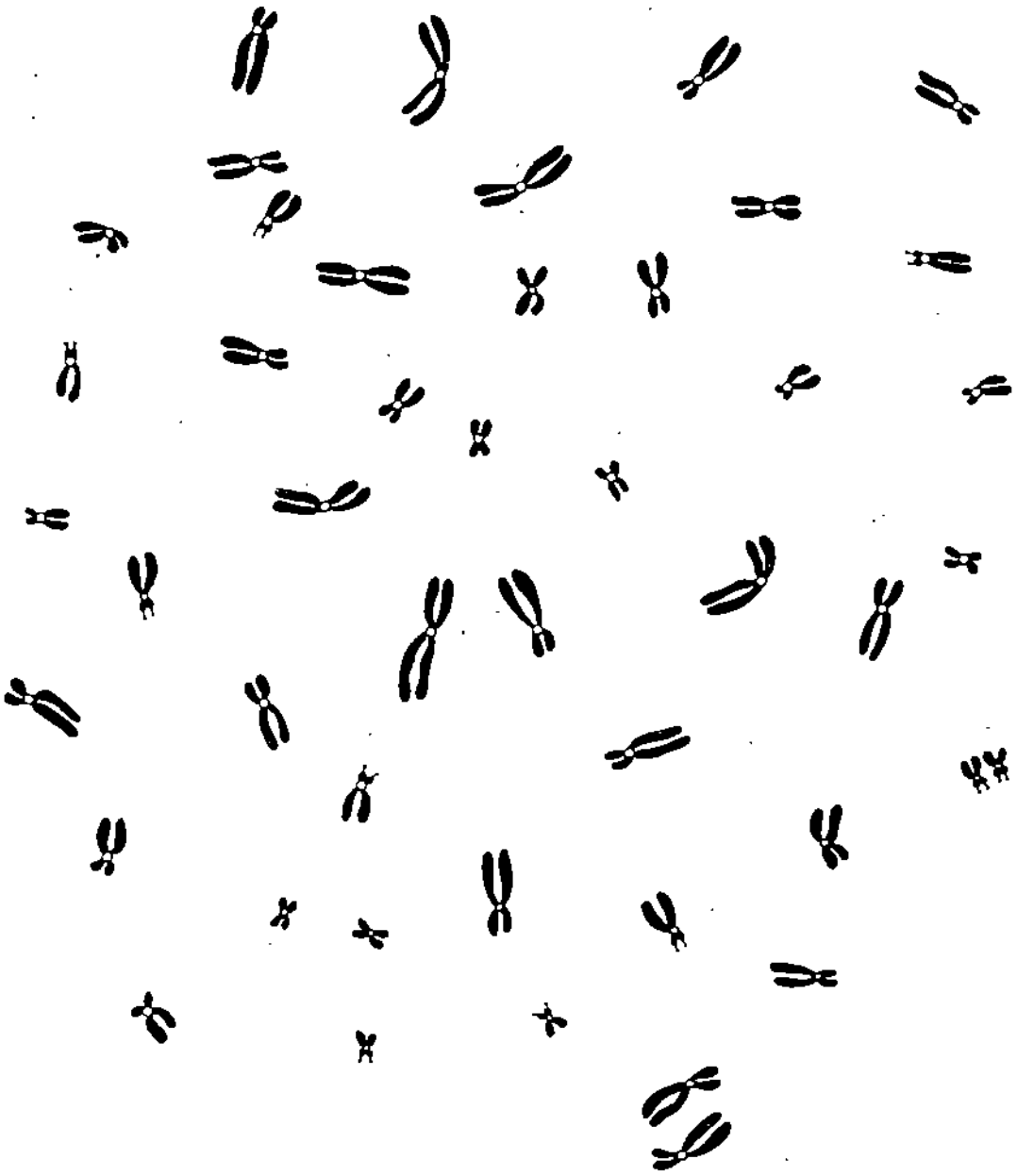


Hint : This is a chromosome spread from a normal human.

Karyotype Form a

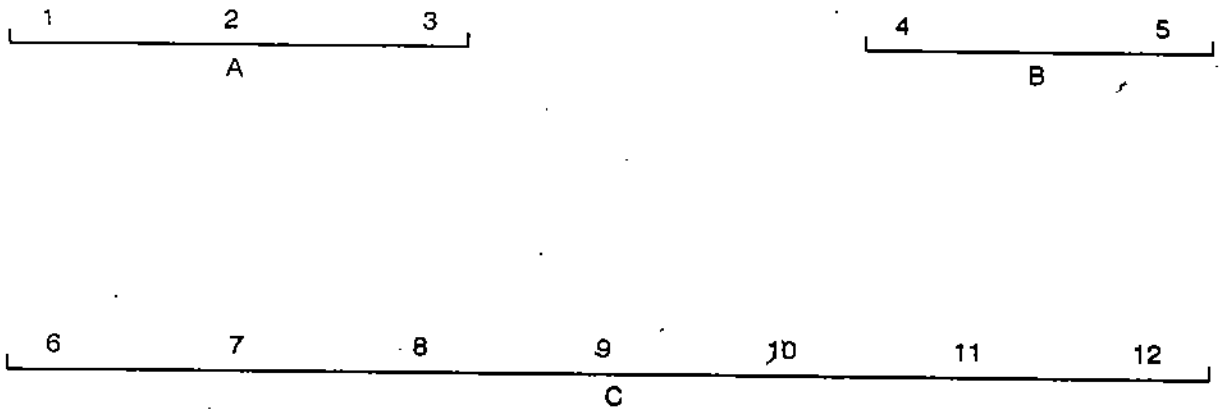


SHEET - II



Hint : This is a chromosome spread from a normal human.

Karyotype Form b



20.4 QUESTIONS BASED ON SHEETS I & II

1) Why must the photographs of individual chromosome be cut separately from sheet I and II, and pasted in order to karyotype it?

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2) Why metaphase smears are considered best for karyotype studies?

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3) Identify the sex of the individuals whose chromosomes appear in Sheet I and Sheet II respectively?

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4) Compare the two karyotypes you have made. What specific difference can you find?

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5) How important is this difference? Explain.

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6) For what kind of an individual would a genetic counsellor, most likely recommend karyotyping done? Why?

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7) a) What kind of difficulties did you have sorting the chromosomes into pairs? b) Into groups? c) Would someone preparing a karyotype in a genetics lab. have the same difficulties? Comment.

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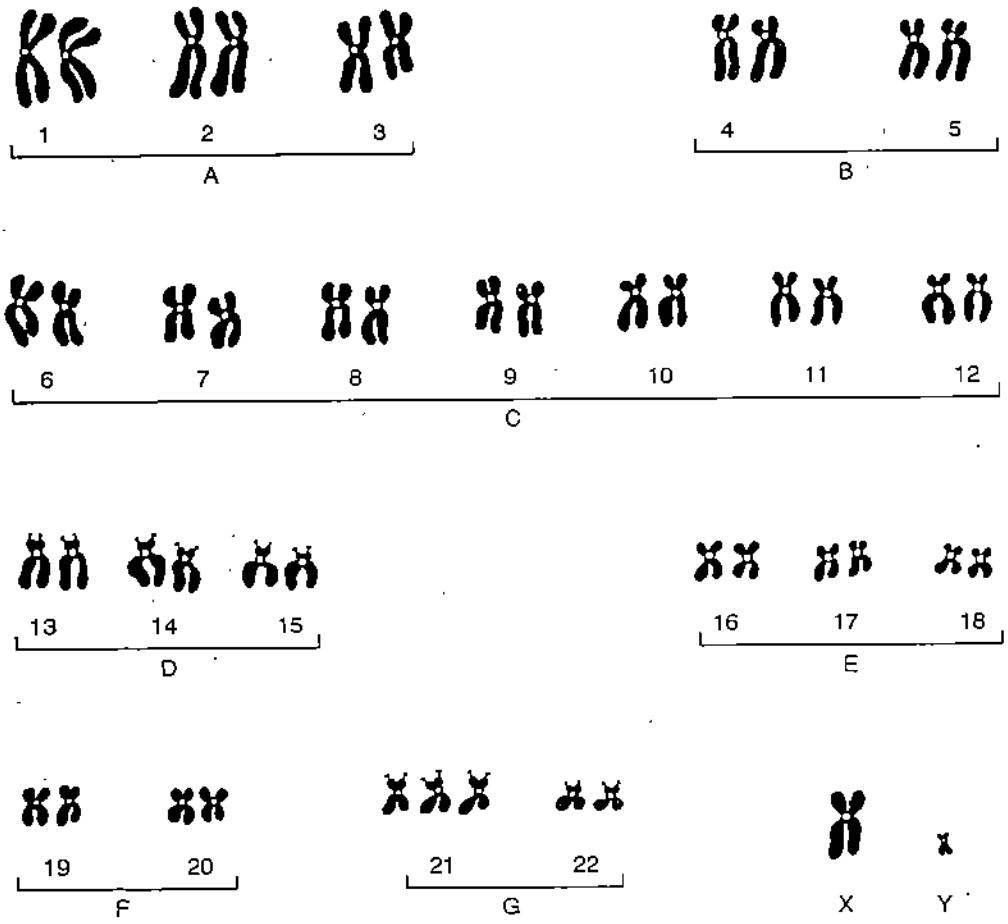
20.5 STUDY OF AN UNKNOWN KARYOTYPE FROM SHEET-III

This is a karyotype of an individual. Observe it and answer the questions given in section 20.6.

SHEET - III



20.6 QUESTIONS BASED ON SHEET-III

1) Is this a karyotype of a normal individual or does it show any abnormality?

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2) What is the feature that makes it different from the earlier two karyotypes?

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3) Based on (2), name the genetic condition that such persons have?

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4) What are the prominent physical features of such individuals?

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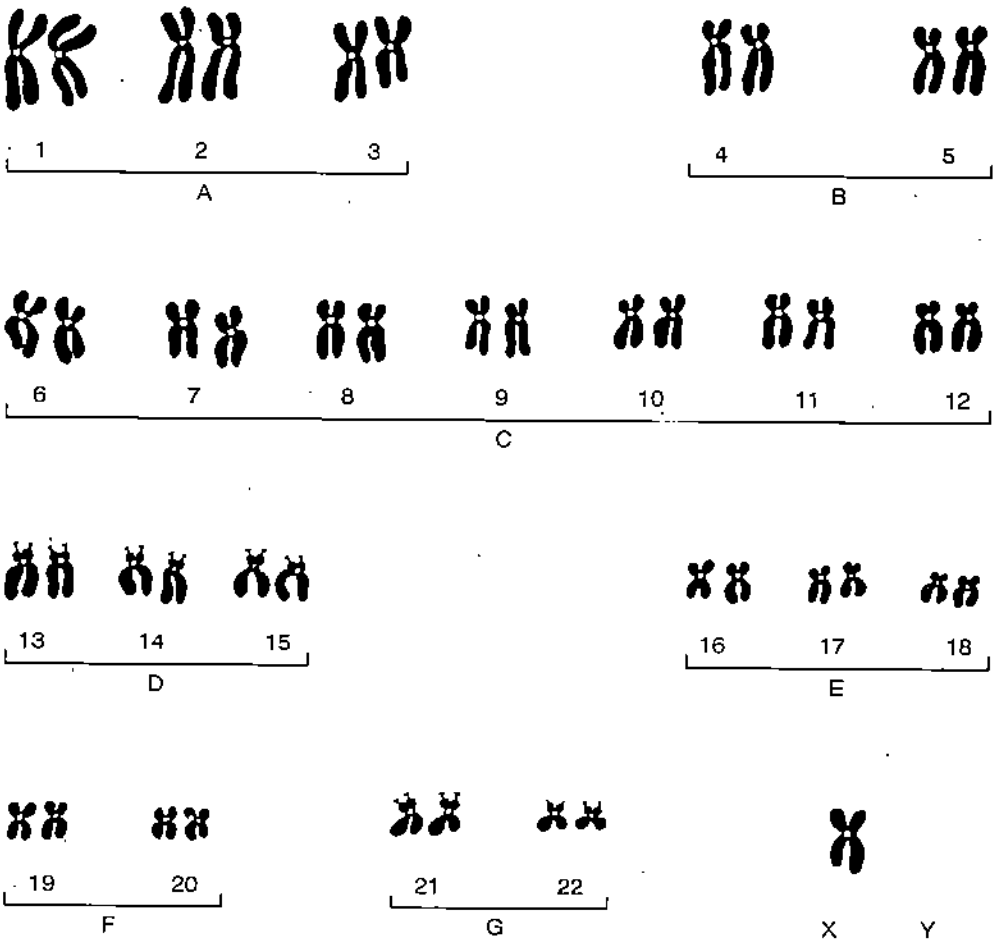
20.7 STUDY OF AN UNKNOWN KARYOTYPE SHEET-IV

This is a karyotype of an individual. Observe it and answer the questions given in section 20.8.

SHEET - IV



20.8 QUESTIONS BASED ON SHEET-IV

1) Does sheet IV show a normal karyotype or any abnormality?

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2) If found abnormal, then what is the difference?

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3) Such karyotype belongs to person with which kind of genetic abberration?

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4) What are the prominent physical features exhibited by such persons?

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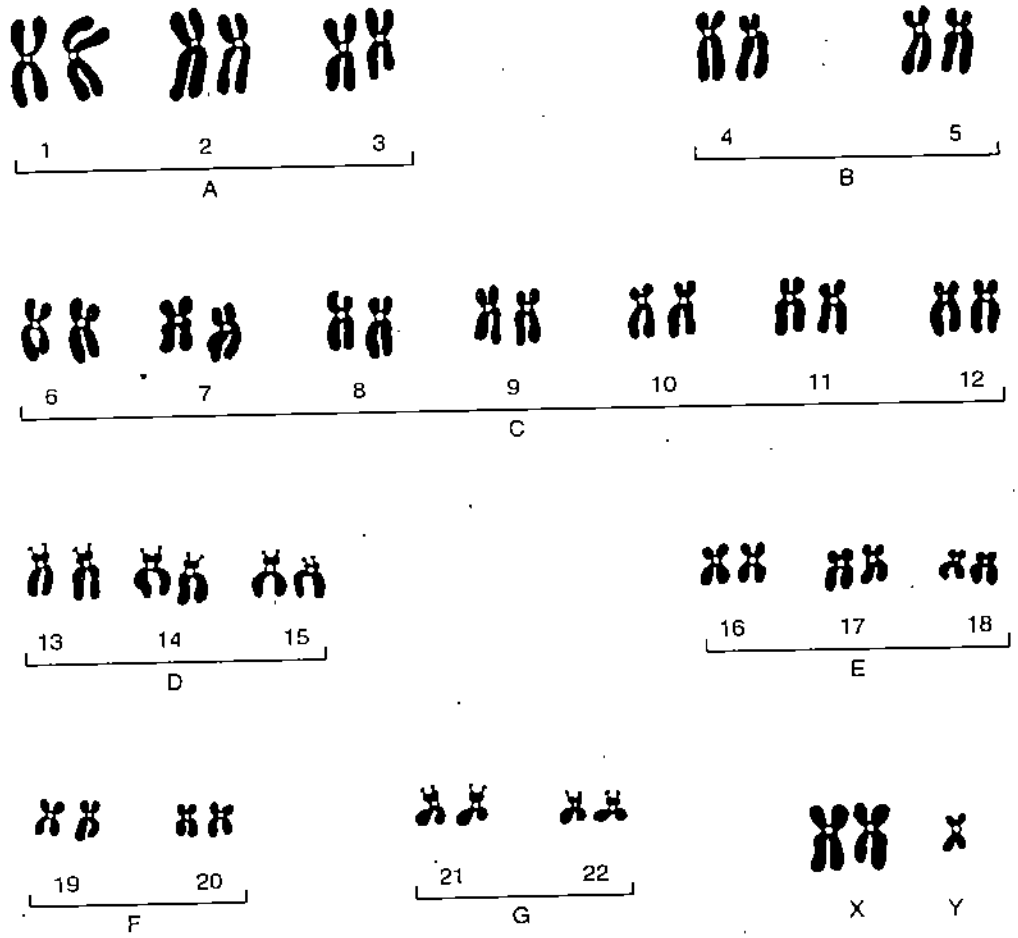
20.9 STUDY OF AN UNKNOWN KARYOTYPE FROM SHEET-V

This is a karyotype of an individual. Observe it and answer the questions given in section 20.10.

SHEET - V



20.10 QUESTIONS BASED ON SHEET-V

1) Does the sheet V show a normal karyotype or any abnormality?

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2) If you have found it abnormal, then what is the difference?

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3) This is a karyotype of an individual having which kind of genetic aberration?

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4) What are the prominent physical features that help in identifying such individuals?

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EXPERIMENT 21 HYDROGEN ION CONCENTRATION AND DETERMINATION OF pH

Structure

21.1 INTRODUCTION

Hydrogen ion concentration is the measure of acidity or alkalinity of a solution. Hydrogen ion concentration (H^+) is expressed in numbers known as pH, where pH means the logarithm of the reciprocal of H^+ concentration or the negative logarithm of H^+ concentration. It can be written as follows:

$$pH = \log \left(\frac{1^+}{H}\right) = - \log \left(H^+\right)$$

You have studied in Unit 4 of the Block 1 of LSE-01 Cell Biology, the concept of ionisation of water and the equillibrium constant (K_{eq}) of the dissociation of water. You have also studied there that the concentration of H^+ and OH^- are equal in pure water and is equal to 10^{-7} moles/litre. You were pointed out that the pH scale is based on the ion product of water (Kw). You may now go back to unit 4 to Block 1 of Cell Biology course and brush your memory as to how the acidity and alkalinity of solutions are calculated in terms of H^+ concentration. In this lab exercise you will learn certain procedures for determining the H^+ concentration of a few solutions of biological interest. You are aware that a solution with a neutral pH has a value of 7, solutions with pH below 7 are acidic and above 7 are alkaline.

A solution of pH 5 contains 10^{-5} moles of H^+ per litre and a solution of pH 8 contains 10^{-8} moles of H^+ per litre. Since the pH values are expressed in logarithmic scale, a difference in the pH value of 3 units would essentially mean that there is a thousand fold difference in the H^+ concentration between two solutions. The range of pH scale is from 0 to 14.

The hydrogen ion concentration is one of the most important chemical components of the habitat. It determines the nature of many chemicals reactions that occur in the environment and also affects the diversity and distribution of organisms. Various plants and animals often have differing requirements of pH in their habit. With this brief introduction to the concept of pH let us now learn the procedures for the determination of pH.

Objectives

At the end of this lab exercise you should be able to:

- define pH and know the basis for the pH calculations,
- determine the H^+ concentration of solutions using a pH meter and the relative uses of pH indicator papers and the pH meter.

21.2 MATERIALS REQUIRED

1. 250 ml beakers—3
2. pH indicator papers (narrow and wide range)
3. pH meter and the electrodes
4. Standard buffer solutions of pH 4 and pH 8.2
5. Filter paper
6. Distilled water
7. Lime water
8. Well water
9. Sodium bicarbonate solution (baking soda).

21.3 PROCEDURE

You will learn in this lab exercise to determine the pH of solutions by two different methods: 1) by using pH indicator paper. 2) by using pH meter. Let us now discuss each of these procedures.

21.3.1 pH Indicator Papers

pH indicator papers are provided as small narrow strips. This paper develops a colour when dipped into solutions, the pH of which are to be determined. The colour can be compared with the chart provided alongwith pH papers. Both wide and narrow range pH papers are available. Wide range pH papers give approximate pH values as 1, 2, 3, 4... etc. Narrow range pH papers give more precise pH values. They are available with the difference of 0.5 units such as 2.5, 3, 3.5, 4, 4.5., or 0.2 units such as 7, 7.2, 7.4, 7.6, 7.8, 8 ... etc. Let us now determine the pH of certain solutions using wide and narrow range of indicator papers.

1) Tear off 1 cm. length wide range pH indicator paper and dip it into the given solution, the pH of which is to be determined.

2) Compare the colour developed on the paper with the chart that is provided to you.

3) Record your results in the form of table provided below in your observation

note book. Repeat the procedure to determine the pH of other solutions as well and record your results.

4) Repeat the procedure with the narrow range pH paper and obtain a more precise pH of the same solutions. These results can also be recorded in the table.

S. No.	Test Solutions	Wide Range pH	Narrow Range pH
1.	Lime Water		
2.	Well Water		
3.	Sodium bicarbonate		
4.	Any other solution		

21.3.2 pH Meter

A pH meter as shown in Fig. 4.1 is a more sophisticated device for making accurate estimations of the hydrogen ion concentration of solutions. The instrument is provided with two electrodes, a reference electrode and a glass electrode and in some cases the two electrodes are supplied as a combined electrode. The potential difference between the two electrodes is amplified in an electrometer which is then read either on an analogue or a digital pH scale. Before making the measurements of the pH, the instrument needs to be standardised. For standardisation you should follow the procedures given below:

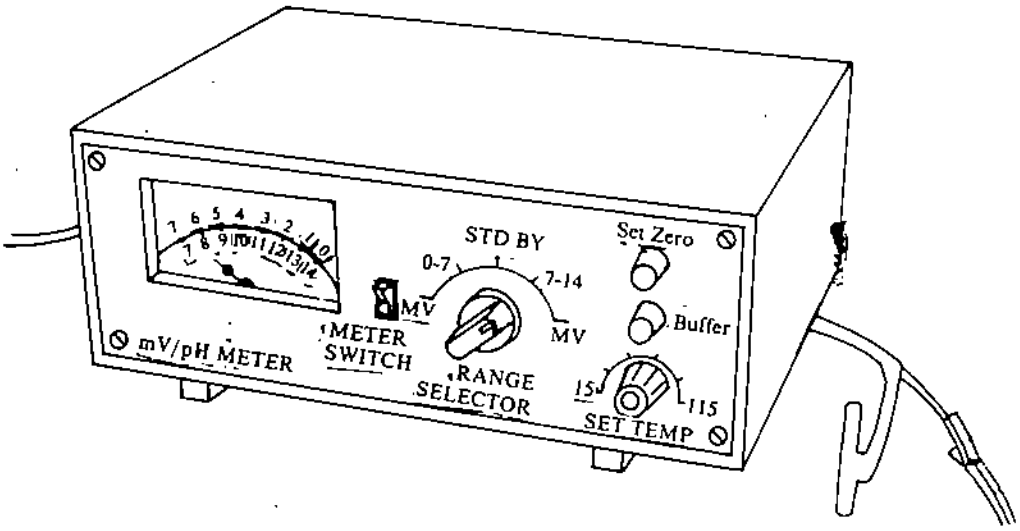

Fig. 21.1 : pH meter

1. Connect the instrument to the power supply.
2. Check whether the electrodes are properly connected to the instrument.
3. Wash the electrodes once with the distilled water and use the filter paper to dry it.
4. Dip the electrodes in a beaker containing distilled water.
5. The meter should read either zero on the analogue scale or 7 in a digital scale. If it does not read so, use the set zero knob of the instrument to read

the same. Remove the electrode from the distilled water, dry them with the filter paper and immerse them in a beaker containing standard buffer solution of pH 4. Turn the "selector knob" of the pH to 0-7 range or the acidic range.

6. Use the set buffer knob to read the pH 4 on the meter. Turn the selector knob to the middle position.

7. You may now remove the buffer solution, wash the electrodes with distilled water, dry them and immerse the electrodes in a standard buffer solution of pH 9.2.

8. Turn the selector knob to 7-14 range or the alkaline range and the meter should read pH 9.2. The selector knob be returned to middle position.

Now, the instrument is standardised. You may now use the instrument for determining the pH of the given solutions. For this: 1) Immerse the electrodes in the given solution whose pH is to be determined. 2) Turn the selector knob first to the 0 to 7 range. If the pH of the test solution is in the acidic range, you may read the same on the meter. It there is no change on the meter reading, it means the pH of the solution is on the alkaline range. You may turn the selector knob to the 7-14 range and read the pH of the solution on the meter. 3) If the pH of another solution is to be measured then you must wash the electodes with distilled water and dry them with the filter paper. 4) Make measurements of the solutions provided to you using the pH meter and record your results in the form of the table provided below in your observation note book.

S. No.	Solutions	pH

21.4 PRECAUTIONS

1. The electrodes are very delicate structures and therefore. need to be handled carefully.

2. The bulb at the tip of the glass electrode is also very sensitive and the glass membrane provided there should not be allowed to undergo abrasion.

3. The electrodes need to be kept clean and therefore, should be washed with distilled water after every use and dried with filter paper.

4. The tips of the electrodes should always be kept immersed in distilled water and should not be allowed to go dry.

5. The selector knob must always be brought to the middle position before the electrodes are removed from any solution.

6. Replace the buffer solutions in appropriate containers and do not contaminate them.

EXPERIMENT 22 ESTIMATION OF SALINITY OF WATER SAMPLES

Structure

22.1 INTRODUCTION

Salinity refers to the total amount of soluble salts dissolved in a kilogram of water collectively. The salts in water include such common ions as Ca^{2+}, Mg^{2+}, K^+, Na^+, Cl^-, SO^{2-}_4, HCO^-_3, and CO^{2}_3. These ions occur either naturally or added as pollutants to the environment. The ionic composition of water affects the distribution of animals and plants in water. And depending on whether organisms can tolerate wide fluctuations in salinity or not, they have been classified as euryhaline and stenohaline animals respectively. Many marine organisms are intolerant of dilution of sea water which happens due to the flow of rivers into them causing estuarine condition. These organisms fail to survive in estuaries. However, there are also certain marine organisms which can tolerate the diluting effect.

In this Lab exercise you will learn the method of estimation of the salinity of water samples by a titrimetric method. The titrimetric method can be regarded as accurate enough, although the method assumes that the percentage composition of chloride in sea water is constant in relation to all other dissolved minerals present. In many laboratories the titrimetry has been replaced by conductivity measurements since salinity relates to the total dissolved salts. However, we confine ourselves to the salinity measurement by chloride estimation.

Objectives

At the end of this lab exercise you should be able to:

- define the terms chlorinity and salinity of water samples,
- estimate the salinity of the water samples by volumetric method, and
- relate the salinity of the water to the life of the organisms.

22.2 MATERIALS REQUIRED

1. 100 ml. conical flasks—2

2. 10 ml. pipettes—2

3. 50 ml burette.

4. 0.05 N silver nitrate solution ($AgNO_3$).

5. 5% potassium chromate solution.

6. Water samples—(2 different water samples such as well water and river water)

22.3 PROCEDURE

1. Fill the burette with 0.01 N $AgNO_3$ solution.

2. Take 10 ml. of water sample A in a conical flask and add a few drops of 5% potassium chromate solution.

3. Titrate the water samples against $AgNO_3$ solution. The end point is the appearance of brickred colour.

4 Titrate the sample until the concordant values are obtained. You may have to titrate a minimum of two times.

5. Record your results in the form of following table in your record note book.

S. No.	Volume of water sample	Burette reading Initial	Final.	Volume of $AgNO_3$ consumed.

6. Repeat the experiment with sample B.

22.4 CALCULATIONS

Calculate the salinity of the water sample with the following formula in your record book.

Chlorosity of Water =

$$\frac{\text{Volume of } AgNO_3 \text{ consumed} \times \text{Normality of } AgNO_3}{\text{Volume of the sample.}}$$

$$\text{Chlorinity of Water} = \frac{\text{Chlorosity of water}}{\text{density of water}}$$

For practical purposes, the density of water can be takes as 1.
Salinity of Water = 0.03 + (1.805 x chlorinity of water)

= ..... parts per thousand

Calculate your results with the help of above formulae and report the salinity of the samples.

22.5 PRECAUTIONS

Check that your burette is properly filled without leaving any air column. For this you may have to open the stopcock of the burette and let some $AgNO_3$ run down. Make sure you fill the burette with $AgNO_3$ solution to read zero.

22.6 SAQ

1. You have estimated the salinity of water from two different sources. Do the salt content of the two waters differ? If your answer is yes, what are the factors responsible for the difference?

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EXPERIMENT 23 ESTIMATION OF DISSOLVED OXYGEN CONTENT OF WATER SAMPLES

Structure

23.1 INTRODUCTION

Oxygen is necessary for aerobic respiration. Aquatic organisms for respiration obtain the oxygen from water, where it remains in dissolved form. In addition the dissolved oxygen in water affects the oxidation-reduction state of many other chemical variables, such as nitrate and ammonia, sulphate and sulphite, and ferrous and ferric ions. The amount of oxygen present in aquatic environment is highly variable and generally low. Many factors such as temperature, salinity, respiration, photosynthesis and decomposition of decaying plants and animals affect the amount of dissolved oxygen. As such oxygen is not very soluble in water and the solubility decreases with increasing temperature. The photosynthetic acitivity of water plants increase the amount of dissolved oxygen during day time, whereas during night it becomes depleted due to respiration of plants and animals. During the process of decomposition microorganisms use the dissolved oxygen thus making it deficient. This adversely affects the other aquatic organisms. You can see in Table 23.1 the oxygen content in some respiratory media.

Table 23.1: Oxygen content of some samples of water and air

Samples	Dissolved Oxygen content millilitres/litre
Sea water at 5° C	6.4
Fresh water at 5° C	9.0
Fresh water at 25° C	5.8
Air	209.5

The amount of oxygen dissolved in water can be measured and is usually expressed as mg/l (equivalent to parts per million or ppm). There are two methods of estimating dissolved oxygen: by using oxygen electrodes and by Winkler's titration method.

Winkler's method is the most commonly used method for estimation of dissolved oxygen in water. In this lab exercise you will be estimating the dissolved oxygen by Winkler's method from at least from two different water sources such as a pond and a well, or tap water and well water, or a river and pond.

Objectives

At the end of this lab exercise you should be able to:

- describe the principle behind the estimation of the dissolved oxygen in water,
- perform the experimental procedure without any difficulty,
- become familiar with the calculations for the estimation of oxygen, and
- discuss that the oxygen content of the different aquatic habitats differ significantly.

23.2 PRINCIPLE

Winkler's method is a volumetric procedure in which manganous ions (Mn^{2+}) are oxidised into manganic ions (Mn^{3+}) which reacting with an alkali precipitates into $MnO(OH)_2$ and $Mn(OH)_2$. The extent of oxidation is directly related to the amount of dissolved oxygen. In the presence of iodide ions in dilute sulphuric acid, the manganese hydroxide is converted into manganous sulphate [$MnSO_4$] and simultaneously the iodide ions are oxidised to molecular iodine (I_2). It is the concentration of this iodine that is directly proportional to the concentration of oxygen in the original water sample. The amount of iodine liberated at the end of the reaction can be determined by titration with a thiosulphate solution using starch as an indicator to determine the end product.

23.3 MATERIALS REQUIRED

1. Burette and Burette stand
2. 300 ml. glass stoppered reagent bottles
3. 250 ml. conical flasks
4. 10 ml. pipettes
5. Measuring cylinder
6. $MnSO_4$ solution (36 gms of $MNSO_4$ dissolved in 100 ml. of distilled water.
7. Alkaline-iodide solution
   a) 100 gms of NaOH/100 ml. of distilled water
   b) 27 gms of NaI/100 ml. of distilled water
   c) Mix solutions a and b

8. Concentrated H_2SO_4

9. Starch solution 1 gm of starch per 100 ml. of distilled water. The water must be heated to bearable warmth and the starch dissolved in it.

10. 0.025N sodium thiosulphate ($Na_2S_2O_3$) solution. (6.205 gms of $Na_2S_2O_3 \cdot 5H_2O$ per 1000 ml. of distilled water).

23.4 PROCEDURE

From each sample obtain water carefully and without air bubbles in 300 ml glass stoppered reagent bottles. Label the bottles as A and B. For accurate determination of dissolved oxygen it is very necessary that special care in sampling and preparation of water samples should be taken. Any exposure of the sample to air will vitiate your results. Therefore, it is suggested that you collect water by keeping your bottle under the surface of water and allow the water to flow into the bottle very slowly without mixing with the air. It is also necessary that prior to the filling of the sample into the bottle, you determine the volume of the bottle. You may use a measuring cylinder for this purpose. Immediately after collecting the sample close the bottle with a glass stopper. This helps you to eliminate the air spaces. Now, you may add the various reagents to the sample as detailed below:

1. Remove the stoppers and add 2 ml. of $MnSO_4$ solution followed by 2 ml of alkaline-iodide solution in bottles A and B. Addition of these reagents should be done below the surface of water by dipping the pipette into the water thus preventing the contamination with air.

2. Stopper the bottles and gently tilt them several times for the solutions to mix. You will see the formation of yellowish brown precipitates of $Mn(OH)_2$ and $MnO(OH)_2$. Allow the precipitate to settle down and gently shake again.

3. Remove the stopper and add carefully 2 ml of conc. H_2SO_4 under the surface of prepared samples. Stopper the bottles again and mix well. The brown precipitate completely dissovles leaving a straw or brown coloured solution.

4. Transfer 50 ml of the contents of the sample bottle A to a 250 ml conical flask. Add 1 ml of starch indicator solution. The solution turns blue. Titrate this solution against 0.025N sodium thiosulphate solution.

   For titration you have to fill the burette with the thiosulphate solution. Open the stopcock of the burette and let the solution run down once. Refill the burette upto zero mark and perform the titration. The end point is the disappearance of the blue colour. Record the burette reading. You may repeat the titration till you get the concordant values. The concordant values may be obtained even at the end of the second titration if you do them carefully.

5. Repeat the above procedure with the sample B. Fill in the data in your observation note book in the form of the table provided below.

Sample	S. No.	Volume of the sample (me)	Burette reading initial	final	Volume of $Na_2S_2O_3$ consumed
A	1.	50	0	4.5	4.5
	2.	50	4.5	9.0	4.5

23.5 CALCULATIONS AND RESULTS

You can obtain the amount of dissolved oxygen per litre of water using the following calculations.

$$\text{Amount of oxygen/litre} = \frac{K \times 200 \times \text{vol. of } Na_2S_2O_3 \times 0.698}{\text{Volume of the sample}}$$

$$\text{where } K = \frac{\text{Volume of bottle}}{\text{volume of the bottle—volume of the reagent added}}$$

A sample calculation is shown below:

Volume of the bottle = 300 ml

Amount of reagent used = 4 ml (2 ml $MnSO_4$ + 2 ml Alkaline iodide)

$$K = \frac{300}{300-4} = \frac{300}{296} = 1.014$$

Volume of NaS_2O_3 consumed = 4.5 ml

$$\text{Amount of } O_2 = \frac{K \times 200 \times 4.5 \times 0.698}{50}$$

$$= \frac{1.014 \times 200 \times 4.5 \times 0.698}{50} = 12.74 \text{ mg/L}$$

23.7 SAQ

Do you find any difference in the oxygen content of the two water samples? If the answer is yes, how do you account for the difference?

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EXPERIMENT 24 STUDY OF COMMUNITY STRUCTURE BY QUADRAT, LINE AND BELT METHOD

Structure

24.1 INTRODUCTION

Plants growing together have mutual relationships among themselves and with the environment. Such a group of plants in one area forms a stand. Several similar stands represent a community which is a part of an ecological system (the ecosystem) in which transformation, accumulation, and flow of energy are involved. The functioning of this system is intimately related with the componer' of the community. The components vary in quality as well as in quantity a.. impart a structure to the community.

Community Structure

The structure of a community can be studied by taking into consideration a number of characters which are usually grouped under two heads viz. analytic and synthetic. Certain analytic characters viz. frequency, density, abundance and dominance can be expressed quantitatively while others viz. sociability, vitality, periodicity and straitification find only qualitative expression. Synthetic chracters include presence, constance and fidelity of components and may be computed from analytic characters of several stands of a community.

The analytic characters of a community are determined by means of three main sampling units—area, line and point, as employed in quadrat, transect and point method respectively.

Objectives

After doing this experiment you will be able to:

- describe about quadrat, line and belt methods and perform them,
- collect and identify the living organisms of the particular population,
- point out which is the most effective sampling method and how to use it properly in any given area,

- work out your sampling strategy beforehand and make best use of given time,
- know the limitation of apparatus and techniques you are going to use.

24.2 MATERIAL REQUIRED

Quadrats, pegs, thread, record book, magnifying glass, gloves.

24.3 METHODS

Quadrats: These have been used extensively in determining the distribution of plant communities but can also be used with slow moving invertebrates such as those which occur in leaf litter or in intertidal habitats.

Quadrats are sampling units of a known area. Now you must be thinking why sampling is necessary? It is seldom possible to count all the individual animals or plants within a given population. This would not only be extremely laborious and time consuming, but would almost certainly involve disturbance and damage to the habitat and population we wish to study. Thus by sampling, we aim to select for study a small representation of the total population. These sample units must be distinct, must not overlap and together they make up the total population. The number of individuals of a species in each sampling unit is then counted or estimated and from this information you can obtain frequency and distribution of that species in the population as a whole.

Now, let us study about the structure of quadrats. Usually the quadrats have a rectangular frame (Fig. 24.1) and come in a variety of dimensions. If frame is not available then you can make a quadrat of known dimension by using 4 pegs and string or strong thread. The pegs are inserted in the ground at four corners with equal distance from each other (Fig. 24.2). The distance dimension may be decided by oneself.

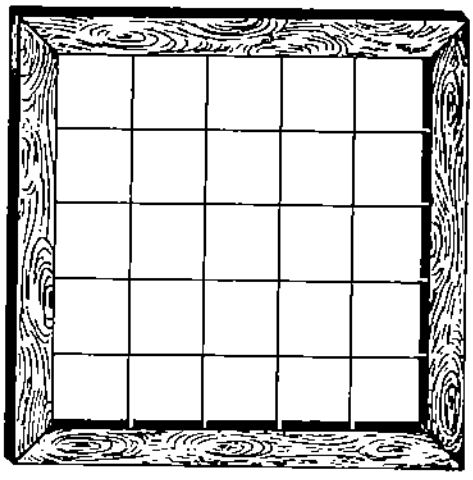

Fig. 24.1: Quadrat Frame. 0.5 cm wooden frame with wires fixed at 10 cm intervals.

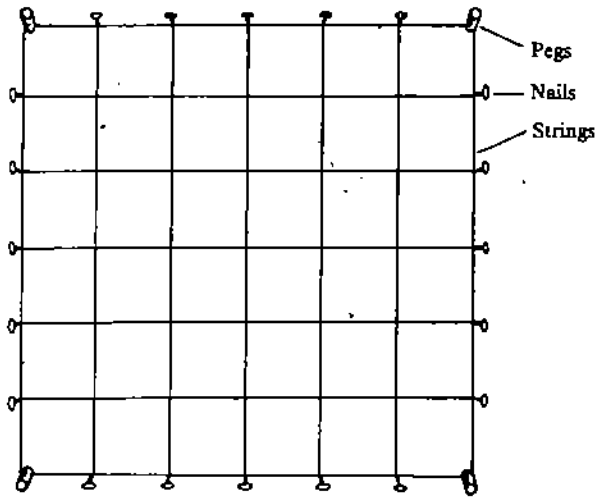

Fig. 24.2: Quadrat made from 4 pegs, strings and nails, fixed at equal distance and squares are made with help of a string.

When you use quadrat it is assumed that its contents will represent the whole sampling area. More commonly used are 1 m^2 and 0.25 m^2 frame quadrats. These are easily constructed out of wood and with cross wires or strings subdividing them at 10 cm intervals for the ease of counting.

Now, the question arises, how the size of the quadrat will be determined. If the dispersion of a population within the sampling area is truly random, then all the quadrat sizes would be equally efficient in the estimation of that population. However, the spatial dispersal of a population is seldom random or regular. An aggregated distribution (Fig. 24.3) is more likely with individuals found in paths. This is because several environmental factors will be unevenly distributed within a sampling area.

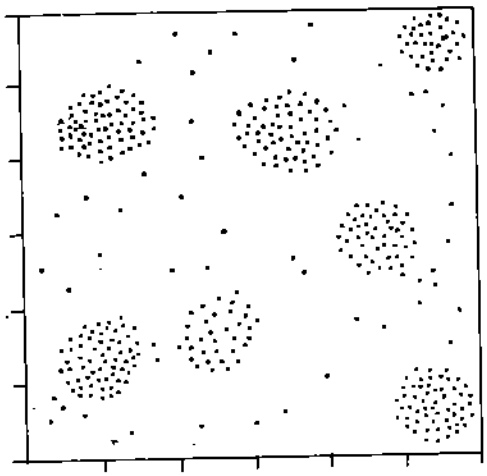

Fig. 24.3 : An aggregate distribution

Generally small size quadrat has been found to be more efficient than a large one when population is aggregated. Instantly the question comes to mind is why? The reasons are following:

- More small samples can be taken for the same amount of labour.
- More number of small quadrats cover a wider range of habitat than a few large ones and the sample will be more representative.
- Statistical error will be reduced as a sample of many small units will have more degree of freedom than a sample of a few large units.

Although a small quadrat may be theoretically best, there are practical considerations which set a lower limit on the size. Thus when sampling in a wood a small quadrat may undersample the dominant species of tree. In addition, the smaller the quadrat the greater the sampling error at its edges; are the plants on the edges of the quadrat to be included or not?

Thus, for determining the optimum quadrat size for a particular type of vegetation, a series of quadrats of increasing size are laid out. The cumulative number of plant species counted after each successive increase in quadrat size is then recorded e.g.

Quadrat size	Total number of species
0.025	10
1	14
4	19
8	22
16	25

Eventually a point is reached where a further large increase in quadrat size results in only a few extra species. Since the common species will have already been included, the extra time and effort required in recording very large quadrat is unproductive.

The optimum quadrat size is reached when a 1% increase in quadrat size produces no more than a 0.5% increase in the number of species present (Fig. 24.4).

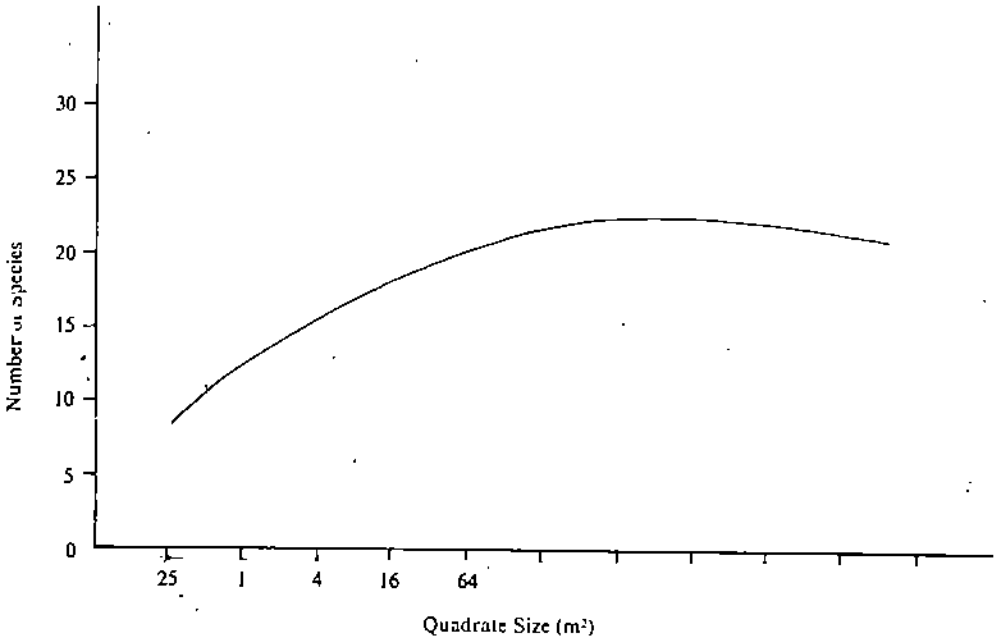

Fig. 24.4 : Graph to determine the optimum quadrat size

Then comes the number of quadrats as you have seen around yourself that a large variation is found when sampling in natural populations. In order to make our result statistically significant a large number of samples should be taken. However, sorting and counting all the species in a very large sample can be tedious and time consuming. A similar exercise enables us to estimate the optimum number of quadrats required when studying the species composition of a particular site. A series of quadrats of satisfactory minimum size is placed randomly across the sampling area. The cumulative number of species is recorded after each increase in quadrat number used; for example,

Number of Quadrats	Total number of species
1	14
4	34
8	37
16	40

Eventually a point is reached when all the common species have been identified and a further increase in quadrat number will not merit the time and effort required (Fig. 24.5). A satisfactory minimum number of quadrats is reached when a 1% increase in the number of quadrats has no more than a 0.5% increase in the number of species found.

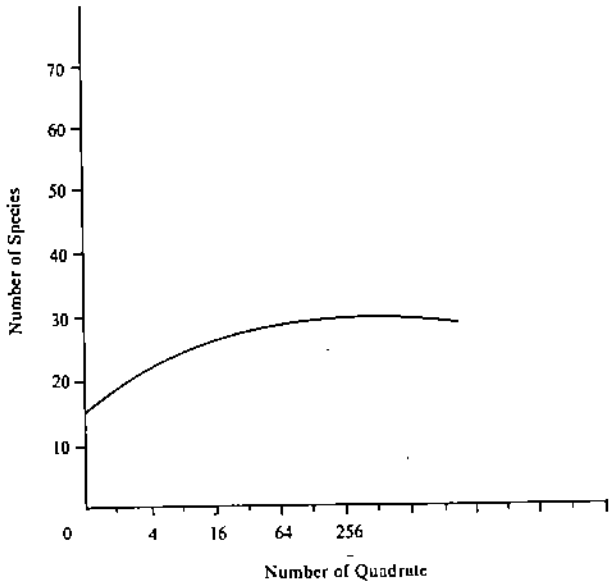

Fig. 24. 5 : Graph to determine number of quadrats

When the size and number of quadrats is known, we can study the structure of community by simply making the necessary observations. By the above described method you can make a quadrat of definite size by string and pegs or wooden quadrat of known size. The number of quadrats is already known. Now, you can make observations and record them in your notebook in the form of a table given below.

S.No.	Name of the Species	Quadrat laid down									
		1	2	3	4	5	6	7	8	9	10
1	A	—	—	—	—	—	—	6	—	4	—
2	B	4	2	1	—	—	—	3	—	2	—
3	C	1	8	—	10			11	7	8	2

24.4 TRANSECTS

The use of transects constitutes a form of systematic sampling but in this case the samples are studied in a linear fashion. Transects are useful for recording changes in the species composition of plant communities where some sort of transition exists, e.g. from water to land or from one soil type to another.

Commonly two types of transects are used:

1. Belt transect

This is a strip usually 0.5 m in width that is located across the study area in such a way as to highlight any transition. A tape or rope marked off at 0.5 m

intervals is laid across the sampling area and a 0.25 m^2 frame quadrat laid down at 0.5 m intervals by the side of the tape or rope to give a continuous belt transect. The animals and plants within each quadrat are identified and counted and an estimation made of their relative abundance. This procedure with a transect over 15 metres long is time consuming and it is more usual to carry out quadrat sampling at every metre interval to give a ladder transect.

As by doing this experiment you can see that this sort of sampling is quite intensive but it gives an accurate record of the organisms present. However, the length of the transect will be limited by the time available. The transect work will also require the recording of a profile indicating changes in the height of the ground.

Data Sheet for Belt Transect

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of species	**Quadrat number**														
.....															
.....															
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Line Transect Method

This method takes less time and is less quantitative and therefore, less representative. A tape or rope marked off at 0.5 m intervals is laid along the area of ground to be sampled. The plant species that are touched or covered by the line are then recorded either all the way along or else at regular intervals. There may be many species which do not touch the line and are therefore not recorded, thus the results may give a completely unrealistic sample of the community on which the line transect is made. However, if you want an impression of the main features of a transition in all, then this method has considerable use. The date sheet will be the same as belt transect method.

24.5 OBSERVATIONS AND RESULTS

Observations should be entered on the given data sheet, by recording + or – or

the number species are recorded. The species which is present in several quadrats is abundant and species present in few quadrats is rare one.

24.6 PRECAUTIONS

The most important precaution to keep in mind is conservation of the environment. The following points should be kep in mind:

1. The number of individual animals and plants collected should be kept to a minimum, depending upon the nature of the experiment. If there is need for quantitative study then number of sample taken should be strictly limited.

2. As far as possible all organisms removed during the exercise should be returned to their original habitat after the experiment is over.

3. The collection should be avoided from the same site as it would have adverse effect upon the density of plants and animal species within the community in question.

4. During collection, damage to the habitat should be avoided because it causes adverse effect on organisms found there.

Discussion

Now it should be clear that in order to determine the structure of community you have quardrat, belt transect and line transect methods. Now, you have to decide which of the methods would be followed to study the community structure.

The next and major problem is the problem of identification of plants and animals. The identification of plant and animals down to species level can be difficult and very time consuming. It will be good if you can identify the species by using prepared illustrations and diagnosic key. But this is difficult task as you have limited time. But the counsellor at your study centre will help you in identifying the plants and animal species.

EXPERIMENT 25 DETERMINATION OF DENSITY, FREQUENCY AND ABUNDANCE OF SPECIES BY QUADRAT METHOD

Structure

25.1 INTRODUCTION

In the previous experiment you have learnt how to study the community structure. But for a detailed knowledge you have to analyse the community structure quantitatively. In this experiment you can calculate abundance and frequency of the species studied.

Objectives

At the end of this experiment you should be able to:

- construct the quadrats
- determine the density, frequency and abundance of a given species in a specified habitat.

25.2 MATERIAL REQUIRED

4 Pegs,

1 meter scale,

string 50 m,

workbook,

bag for plant collection,

wooden quadrat of definite size (as determined in previous experiment),

graph sheet,

herbarium sheet,

cellotape.

25.3 PROCEDURE

You are aware of the required number of quadrats to be laid from experiment 24. Lay down the quadrats in the area to be studied. Note down the various species of plants present in each quadrat. It will be good if you can identify the species by yourself with the help of books or you may take the help of your counseller. Count total number of the individuals of each species.

25.4 OBSERVATIONS AND CALCULATIONS

Write down name of the species, total number of each species and then calculate the density of frequency in your observation note book in a tabular form as shown below:

Calculations

$$\text{Density} = \frac{\text{Total number of individuals of the species}}{\text{Total number of quadrats studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of the species}}{\text{Total number of quadrats in which the species has occured}}$$

$$\text{Frequency} = \frac{\text{Total number of quadrats in which species occured}}{\text{Total number of quadrats studied}} \times 100$$

The density values, thus obtained for each species, are to be expressed as individuals per unit area. For example, calculated density value for species A (Table 1) is:

1. This value is per 1600 cm^2 area (i.e. the area of the quadrat taken–40 x 40 cm^2). Actual density of this species would be as follows. In 1600 cm^2 area there is 1 individual.

In 100 x 100 cm^2, i.e. 10,000 cm^2 or (1 m x 1 m i.e. 1 m^2)

$$= \frac{10{,}000 \times 1}{1{,}600} = 6.25 \text{ individuals.}$$

Thus the density of species A is 6.25 per $meter^2$.

2. Similarly, you can calculate abundance and get an idea which species is found in abundance in particular area.

25.5 PRECAUTION

Same as described in earlier experiment.

Table 24.1: List of different species and other data as recorded by quadrat method. Area of the quadrat taken in the study 40 cm x 40 cm = 1600 cm²

S. No.	Name of the Species	Quardrats laid down 1 2 3 4 6 7 8 9 10	Total No. of individuals of the species	Total No. of quadrats in which species occured	Total No. of quadrats studied	Density	Abundance	Frequency
1	2	3	4	5	6	7	8	9
1.	Species A	4 – – 6 – – – – –	10	2	10	1*	5	12

* You should calculate the density as described in the method and convert this value nos./unit area

EXPERIMENT 26 STUDY OF XEROPHYTES, MESOPHYTES AND HYDROPHYTES

Structure

26.1 INTRODUCTION

This distribution of plants occur from extremes of north and south and east and west around the world. Thus certain groups of plants are adapted to unusual or extreme climatic conditions. These plants exhibit modifications in their anatomy and morphology that are related to specialised functions to a greater degree. The ecological groups of plants have much broader group. But they have to be classified on some basis. Water is one them. Warming (1909) classified plant communities on the basis of their dependence upon and relation to water. Water being most important occupies foremost position in distribution of vegetation and its structure. Warming primarily recognised three major groups of plants: i) Hydrophytes, ii) Mesophytes and iii) Xerophytes. Hydrophytes are those plants which live partly or fully submerged in water at least for some weeks. Mesophytes grow on land with a moderate supply of soil moisture. Xerophytes are those plants that live in dry or arid land.

Objectives

After doing this exercise you will be able to:

- describe and differenciate between hydrophytes, mesophytes and xerophytes,
- list various adaptations in hydrophytes, mesophytes and xerophytes with examples and illustrations.

26.2 CATEGORIES OF PLANTS BASED ON HABITAT

In this experiment you will be provided specimens and slides. From the given description you have to classify the plants into different catagories (described below).

26.3 HYDROPHYTES

According to the way in which the hydrophytes develop in water they are subdivided into the following five catagories:

i) Free-Floating Hydrophytes

These types of plants remain in contact with water and air, but not soil. They float freely on the water surface. Leaves in some are very minute while in others quite large. Some of the free floating hydrophytes are *Trapa bispinosa, Azolla, Eichhornia crassipes, Salvinia, Wolffia, Pistia, Lemna* (Fig. 26.1).

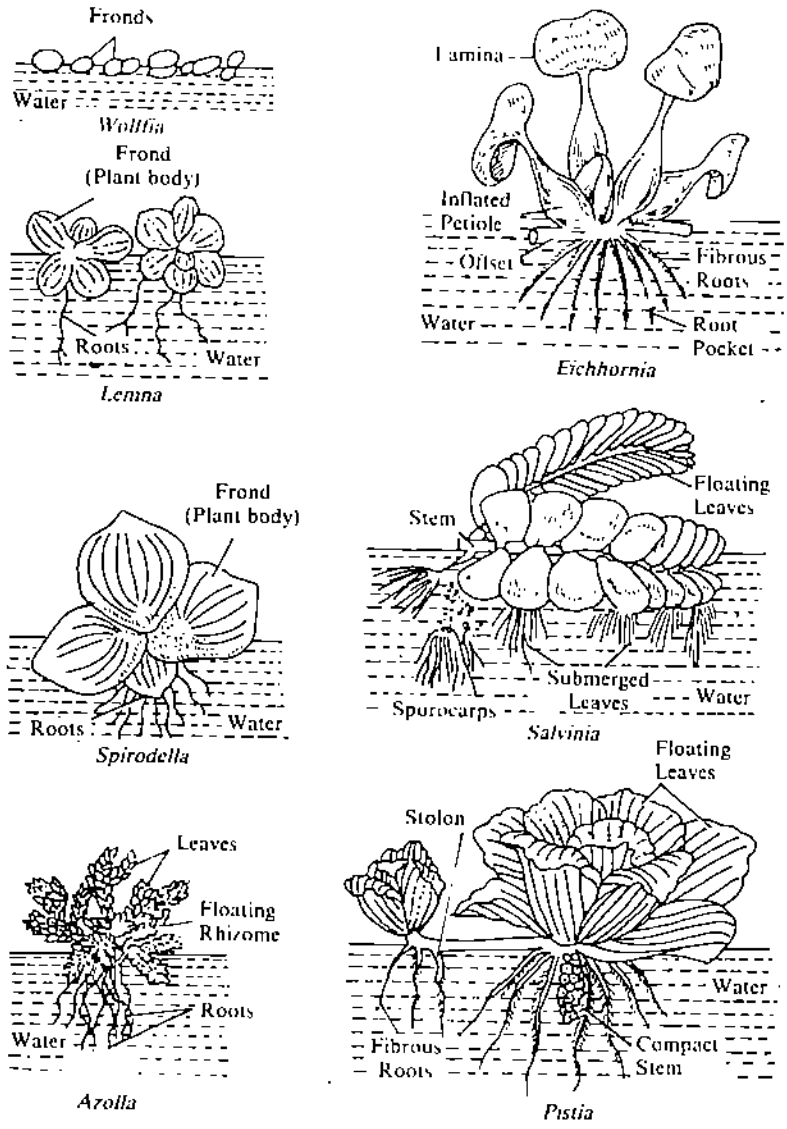

Fig. 26.1 : Free floating hydrophytes

ii) Rooted Hydrophytes with Floating Leaves

The roots of these type of hydrophytes are fixed in mud, but leaves have long petioles which keep them floating on the water surface. Except leaves, the rest of the plant body remains in water. Some examples are *Nelumbo nucifera, Nymphaea stellate* (water lily), *Trapa, Marsilea* (Fig. 26.2).

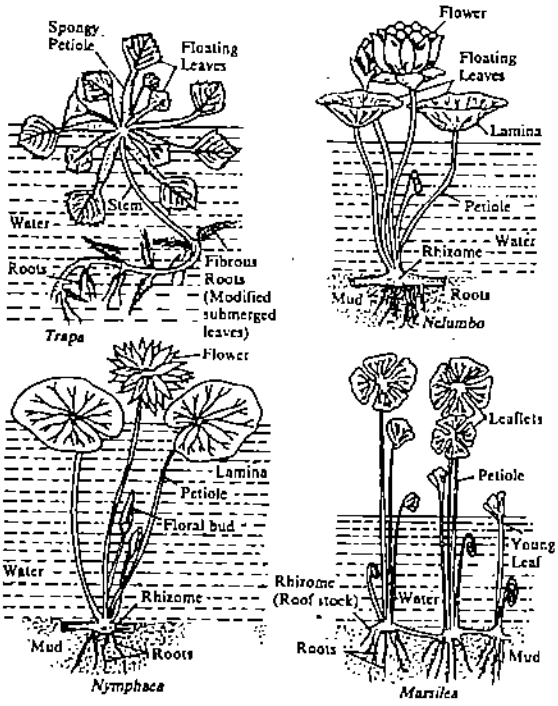

Fig. 26.2 : Rooted hydrophytes with floating leaves

iii) Submerged Floating Hydrophytes

These types of the plants are only in contact with water, being completely submerged and not rooted in the mud. Their stems are long and leaves generally small. Some examples are *Ceratophyllum, Utricularia.* (Fig. 26.3)

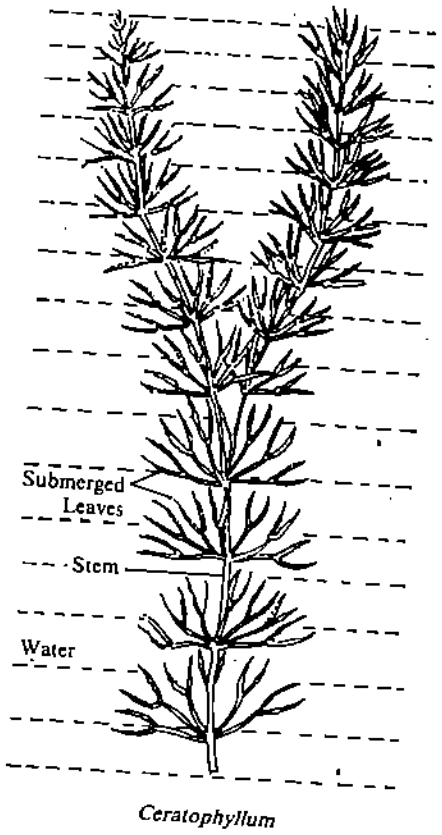

Fig. 26.3 : Submerged floating hydrophyte

iv) Rooted submerged hydrophytes

This type of hydrophytes remain completely submerged in water and rooted in soil. In some plants the stem is long, bearing small leaves at the nodes. In some plants stem is tuberous (corn-like) with long leaves, which are narrow, ribbon-shaped. Common example is *Vallisneria, Chara, Hydrilla, Potamogeton* (Fig. 26.4)

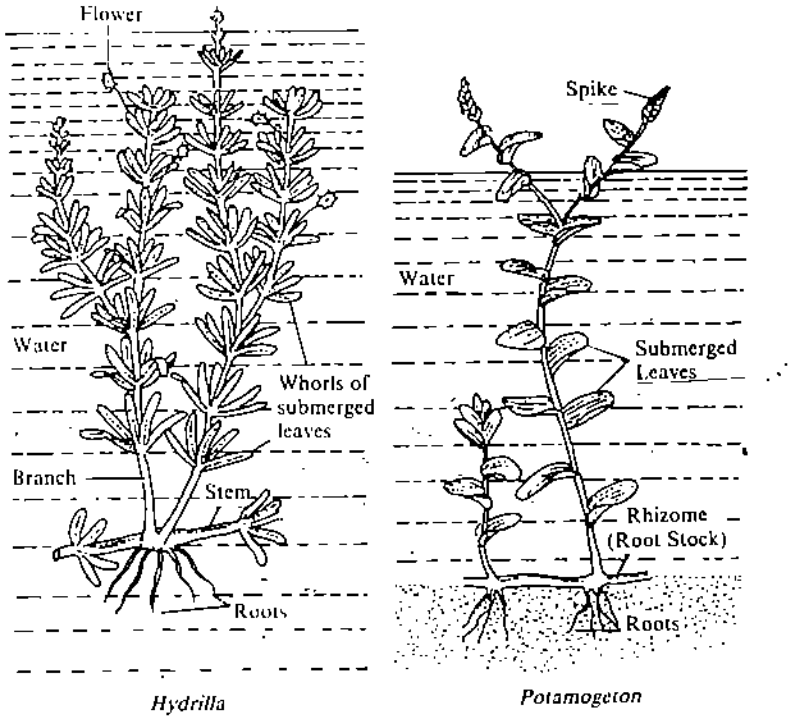

Fig. 26.4 : Rooted submerged hydrophytes

v) **Rooted emergent hydrophytes**

These types of plants grow in shallow water. These are such hydrophilous forms which require excess of water, but their shoots (assimilatory organs) are partly or completely exposed to air. The root system is completely under water, fixed in soil. In some plants, shoots are partly in water and partly emerging i.e. exposed to air. Whereas in some the shoots are completely exposed to air. Some common examples are *Sagittaria, Ranunculus, Cyperus* (Fig. 26.5).

Now let us see the ecological adaptations in hydrophytes. Though most of the features in hydrophytes are similar but they may differ from each other in some aspect. We will discuss all those features which enable them to become hydrophytes.

26.3.1 Morphological Features

1. Roots

There is plenty of water in the surroundings of hydrophytes; thus the root becomes of secondary importance hence less developed and insignificant in most of hydrophytes.

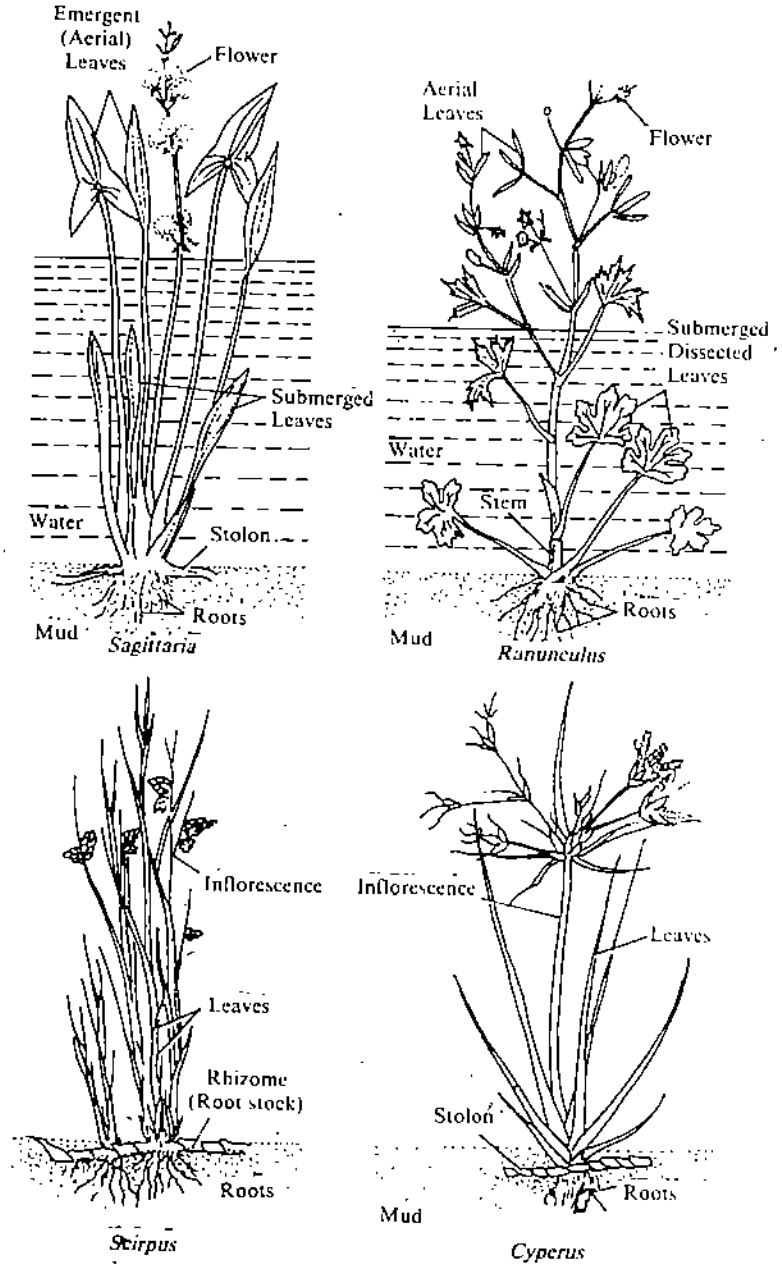

Fig. 26.5 : Rooted emergent hydrophytes

i) Roots may be completely absent or poorly developed as in *Wolffia, Salvinia*. However, in emergent forms, which grow in mud, roots are well developed with distinct root caps.

ii) Root hairs are absent or poorly developed.

iii) Root caps are usually absent, in some cases are replaced by root pockets as in *Eichhornia*.

iv) Roots when present are generally fibrous, adventitious, reduced in length, and unbranched or poorly branched.

2. Stems

i) In emerged forms the stem is long, slender, spongy and flexible. In

free-floating forms it may be slender, floating horizontally on water surface or thick, short, stoloniferous and spongy. In forms which are rooted with floating leaves it is rhizome.

ii) Vegetative propagation is by runners, stolons, stem and root tubers, dormant apices, offsets etc. are the common method of reproduction. Most of them are perennials.

3. Leaves

i) In submerged forms, leaves are thin, and are either long and ribbon-shaped as in *Vallisneria* or long and linear or finely dissected as in *Potamogeton*. Floating leaves are large, flat and entire with their upper surfaces coated with wax as in *Nymphaea*; their petioles long, flexible, and often covered with mucilage. In some cases petioles become swollen and spongy as in *Eichhornia*.

ii) Emergent forms show heterophylly with submerged, floating and aerial leaves as in *Ranunculus*.

iii) Submerged leaves are generally translucent.

4. Flowers and seeds

In submerged forms they are less common. Where flowers develop, seeds are rarely formed.

26.3.2 Anatomical features

Now, we will study about the anatomical features of roots of the hydrophytes.

1. Roots

i) Cuticle is either completely absent or if present it is thin and poorly developed.

ii) Epidermis is usually single layered and made up of thin walled parenchymatous cells.

iii) The cortex is well developed, thin walled and parenchymatous, major portion of which is occupied by well developed prominent air cavities—the 'arenchyma' which offers resistance to binding stress, increases buoyancy and allows a rapid gaseous exchange.

iv) In the given figure of *Potamogeton* you can see that vascular tissues are poorly developed and least differentiated in submerged form. The xylem vessels are less common and tracheids are present. In floating types vascular tissue are less developed while in emergent forms they are much distinct and well developed (Fig. 26.6).

v) The mechanical tissues are absent except in some emergent forms where pith is made up of sclerenchymatous cells.

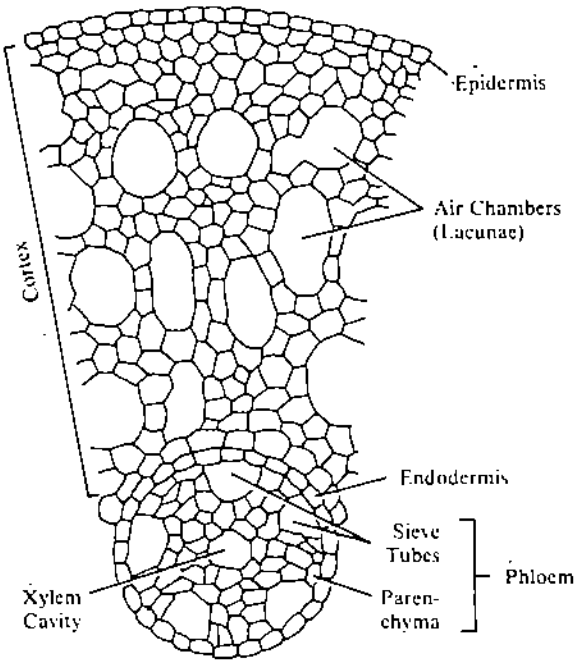

Fig. 26.6 : TS. root of *Potamogeton pectinatus* (submerged hydrophyte). Note the absence of root hairs and cuticle; undifferentiated broad cortex with air chambers; vascular tissues poorly developed, represented mainly by phloem; lack of mechanical tissues.

2. Stem

In the given figure of T.S. of stem of *Hydrilla* you can see that (Fig. 26.7)

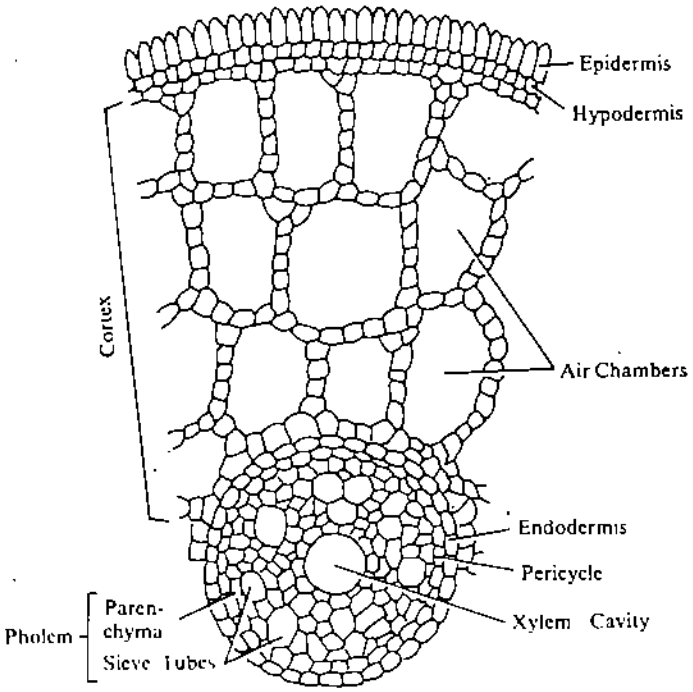

Fig. 26.7 : TS. stem of *Hydrilla* (submerged hydrophyte). Note, the absence of cuticle; thin-walled epidermis; undifferentiated cortex with air chambers; abundance of thin-walled elements; absence of mechanical tissues; reduced vascular elements, composed chiefly of phloem, xylem being represented only by a cavity in the centre.

i) Cuticle is developed or thin or entirely absent.

ii) Epidermis is single layered and made up of thin-walled parenchymatous cells while in emergent form cuticle as well as epidermis is generally well develop such as *Typha.*

iii) In floating and emergent form you can see hypodermis may be present as thin-walled parenchyma or collenchyma. It is completely absent in submerged form.

iv) Cortex is well developed. One of the important feature of the cortex is that it is thin walled and parenchymatous extensively transversed by air cavities. The cortical cells generally possess chloroplasts and are photosynthetic.

v) Endodermis is distinct, especially in rhizomes and similar organs.

vi) Generally, vascular bundle have no bundle sheaths. Vascular bundles are thin walled. But in emergent forms vascular elements are comparatively well differentiated and developed.

vii) Mechanical tissues are generally not present.

3. Leaves

You can see form the slides provided to you of different T.S. (of various plant leaves.)

You can see that internal structure of leaves show variation but some of the anatomical feature is common to the most of the leaves. From the given transverse section you can see that;

i) Cuticle is usually absent in submerged forms such as *Potamogeton* but in floating forms it is poorly developed confined to upper side and is very thin. In emergent form also cuticle is thin (Fig. 26.8).

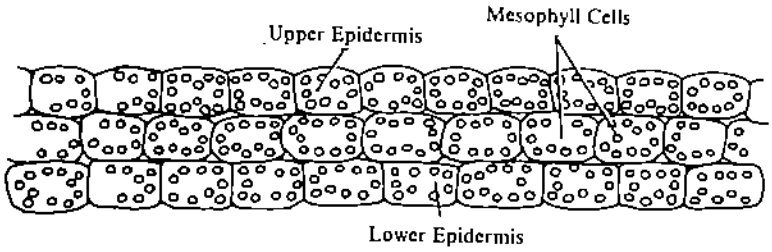

Fig. 26.8 : T.S. leaf (only lateral wing portion shown) of *Potamogeton pusillus* (submerged). Note, the absence of cuticle and stomata undifferentiated single-layered mesophyll between two epidermal layers.

ii) Epidermis is single-layered, made up of thin-walled cells with abundance of chloroplasts.

iii) Stomata are completely absent in submerged leaves, as in *Potamogeton.* In floating form stomata are confined only to the upper surface of leaf, whereas in emergent forms they are generally found on both of the surfaces of leaves.

iv) Mesophyll is undifferentiated in submerged leaves, and generally it is single layered. In floating leaves, mesophyll is differentiated (a) palisade and (b) spongy parenchyma with well-developed air cavities as in *Nymphaea.*

In emergent leaves also, mesophyll is well differentiated with air cavities (Fig. 26.9).

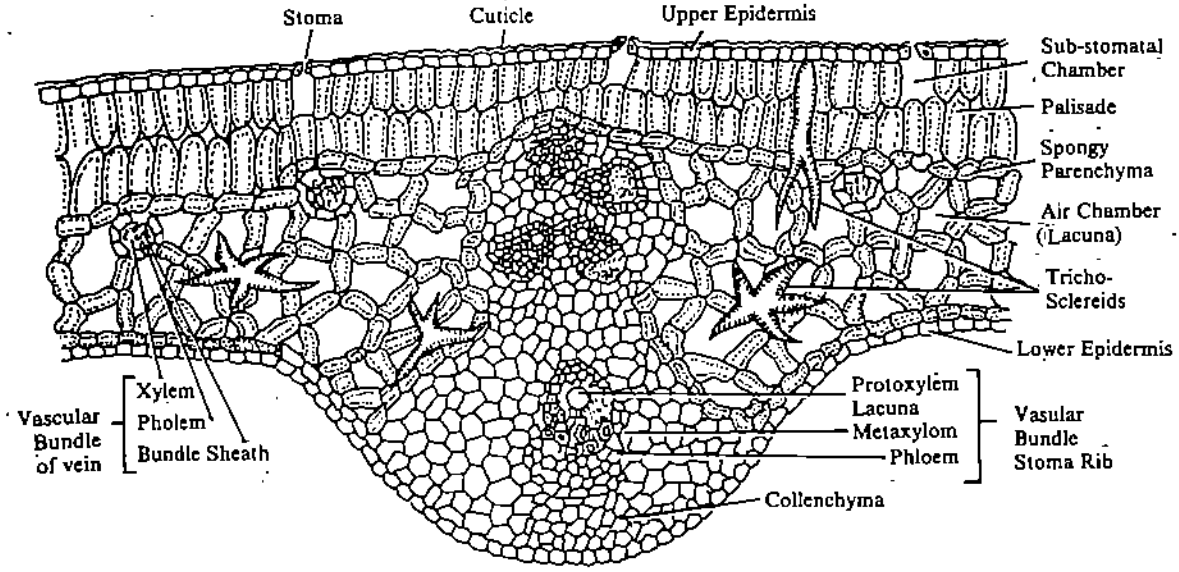

Fig. 26.9 : T.S. leaf of *Nymphaea* (floating-leaves). Note, the thin cuticle; stomata being confined only to the upper surface; thin-walled epidermal cells; abundance of air chambers in spongy parenchyma; absence of mechanical tissues (only sclereids present): reduced vascular elements represented mainly by phloem, xylem being much reduced

v) Vascular tissues are very much reduced and sometimes difficult to be differentiated into xylem and phloem. Whenever differentiated into xylem, elements are thin walled and phloem being well developed as in *Nymphaea*. However, in aerial leaves, vascular elements are comparatively well differentiated with vessels in xylem.

vi) Mechanical tissues are absent.

vii) The petioles, wherever found is well developed and also possess internally the various tissues characteristic of a typical hydrophyte i.e. abundance of aerenchyma, thin walled cells, lack of differentiation in vascular tissues and absence of any lignified mechanical tissues as in *Nymphaea* (Fig. 26.10).

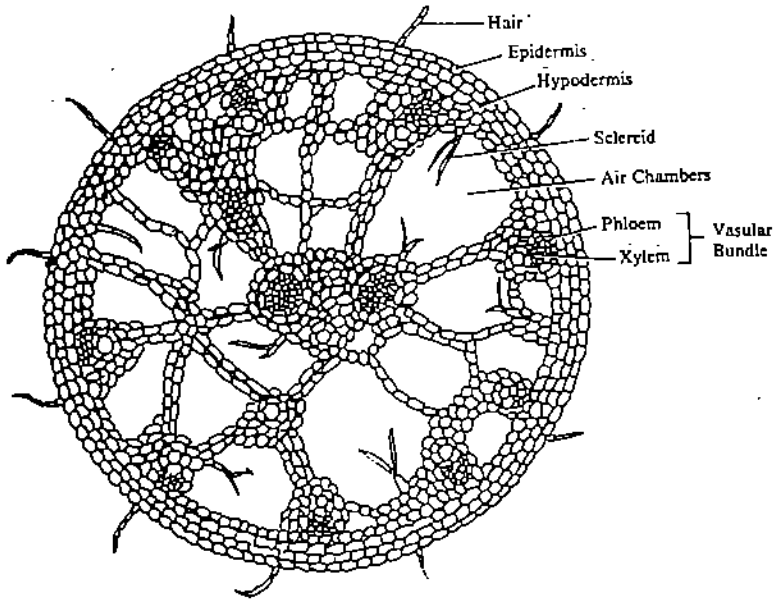

Fig. 26.10 : T.S. petiole of *Nymphaea* (floating-leaved). Note, the absence of cuticle; thin-walled epidermal cells; reduced mechanical tissues represented only by a few layers of collenchymatous hypodermis; abundance of aerenchyma; vascular elements with abundance of phloem, xylem being represented by lacunae.

26.4 MESOPHYTES

Mesophytes are plants that normally grow in habitat where water is neither scarce nor abundant. In such habitats the pore space in soil is occupied almost equally by water and soil atmosphere. This condition of water and air is very suitable for plant growth and hence in mesophytic condition the growth of forests and crop plants is best. In mesophytes, no adaptation is necessary unless the habitat is specialised in some other way. Mesophytes are very extensive on the surface of land and most crops like wheat, maize barley, peas, gram or sugarcane or species in grassland, meadows tropical and temperate forests are all mesophytes.

Morpho-anatomical features

i) Root system is well developed. Roots are generally branched with root caps and root hairs.

ii) Stems are generally aerial, solid and freely branched.

iii) Leaves are generally large, broad, thin and varied in shapes mostly oriented horizontally, green, without hair of waxy coatings.

iv) Cuticle in all aerial parts moderately developed.

v) Epidermis well developed, without any hair or waxy coatings and cells without chlorplasts.

vi) You can observe that stomata are generally present on both surfaces of leaves. Guard cell show frequent movements.

vii) Mesophyll in leaves is well differentiated into palisade and spongy paranchyma with many intercellular spaces.

viii) Vascular and mechanical tissues both are well developed and differentiated.

ix) During noon hours there may be temporary wilting.

26.5 XEROPHYTES

There have been many interpretations of the term xerophytes, Sometimes they are loosely defined as plants of dry habitats. A truly ecological definition approaching as near as possible a quantitative basis is that xerophytes are plants which grow on substrata which usually become greatly depleted of gravitational ground water to a depth of least 20-25 cm during the course of a normal season. Some examples of xerophytes are *Aloe, Euphorbia, Opuntia, Agave Bryophyllum, Yucca, Tradescantia* (Fig 26.11).

Thus, in arid zones, all plants not confined to the margins of streams or lakes have been considered as xerophytes, whereas in regions of heavy rainfall the class would be represented only by shallow-rooted plants of sandy soils, by plants of dry ridgetops, and by algae, mosses and lichens which grow on trees barks or rock surfaces etc.

The true nature of xerophytes is not clearly understood. For example, it is

difficult to decide whether a xerophyte is really xerophilous and occurs only in dry habitats and deserts or it is merely drought-resistant.

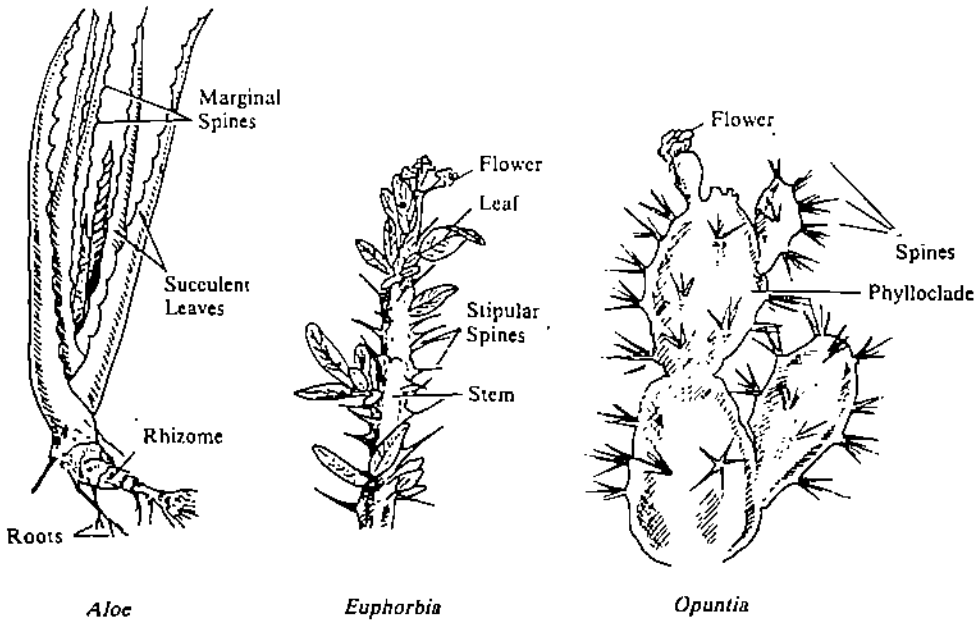

Fig. 26.11 : Succulent xerophytes

26.5.1 Morphological Features

1 Roots

In contrast with hydrophytes which develop in conditions with plenty of water, Xerophytes develop under water deficient conditions. The main purpose of roots is to secure water, which is present in less amount and in deep layers of soil. The root system is the most important organ for the survival of plant and thus is very well developed. The roots have following characteristics.

i) It is very well developed and in some cases is several times longer than shoot. Roots are long, tap roots with extensive branching spread over wide areas.

ii) Root hair and root caps are very well developed.

2. Stems

i) Mostly the growth of stem is stunted, woody, dry, hard, ridged and covered with thick bark.

ii) In some as *Saccharum* stem becomes underground, whereas in opuntia (Fig. 26.11) if becomes fleshy, green, leaf-life (phylloclade) covered with spines. In *Euphorbia* also (Fig. 26.11) it becomes fleshy and green.

iii) On stems and leaves, there are generally hairs and/or waxy coatings.

3. Leaves

i) Leaves are very much reduced, small, scale-like appearing only for a brief period, sometimes modified into spines. Lamina may be long, narrow or needle—like as *Pinus* or divided into many leaflets as in *Acaia* (Fig. 26.12).

ii) Foliage leaves when present, may become thick, fleshy and succulent or tough and leathery in texture.

iii) Leaf surface is mostly shiny and glazed to reflect light and heat.

iv) In some species leaves become folded and rolled in such a manner that the sunken stomata become hidden and thus rate of transpiration is minimised.

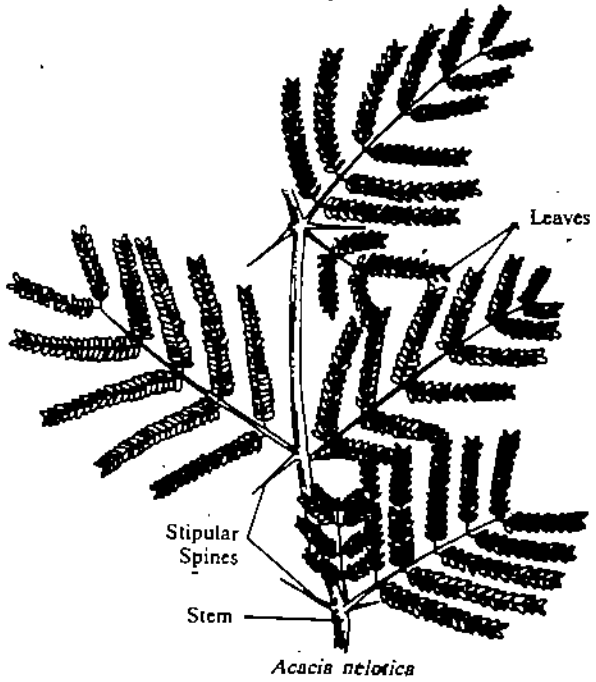

Fig. 26.12 : Non-succulent perennials

26.5.2 Anatomical features

1. Roots

i) Root hairs and root caps are well developed. In *Opuntia* root hairs develop even at the root tips.

ii) In *Asparagus* roots may become fleshy to store water.

2. Stems

i) In succulent fleshy xerophytes, such as *Casuarina*, following cheif characteristics are present (Fig. 26.13).

a) Cuticle is very thick,

b) Epidermis is well developed, with heavily thickened cell walls,

c) Hypodermis is several-layered and sclerenchymatous.

d) Stomata are of sunken type

e) Vascular tissues are very well develop, differentiated, heavily lignified. vascular bundles have well developed several layered bundle sheath.

f) Mechanical tissues are very well developed

i) Bark is very well developed

ii) Oil and resin are often present

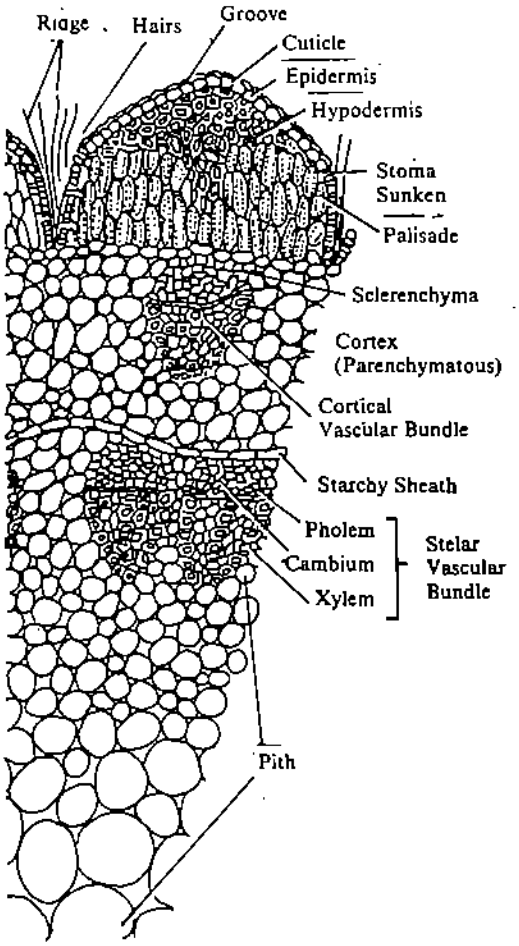

Fig. 26.13 : T.S. stem (a part) of *Casuarina*. Note, the thick cuticle; sunken stomata confined only to grooves; presence of hairs in grooves; sclerenchymatous hypodermis; green palisade region of subhypodermal cortex; well-developed vascular tissues and mechanical tissues

3. Leaves

i) In succulent leaves of malacophyllous xerophytes, such as *Peperomia*, epidermal cells of leaves serve as water-storage organs (Fig. 26.14).

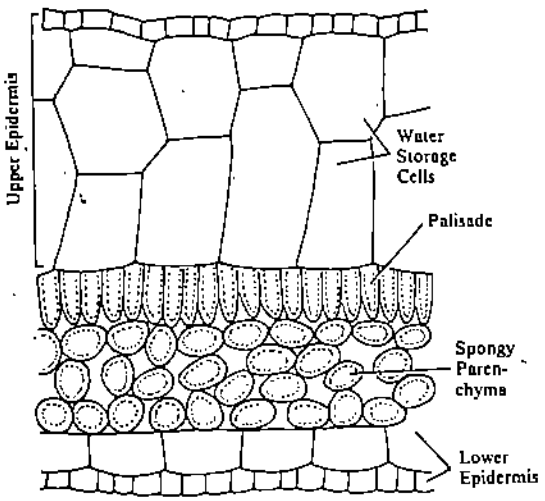

Fig. 26.14 : T.S. leaf (lateral wing portion only) of *Peperomia* showing epidermal water storage tissue. Note epidermins is many layered, inner layers of which have large thin-walled cells acting as water storage.

Similarly, succulent leaves of *Aloe* have prominent water—storage regions in their mesophyll. The cuticle is thick and outer walls of epidermal cells are heavily deposited with cutin and cellulose.

ii) In non succulent xerophytes, such as *Nerium* (Fig. 26.15) and *Pinus* have following characteristic features:

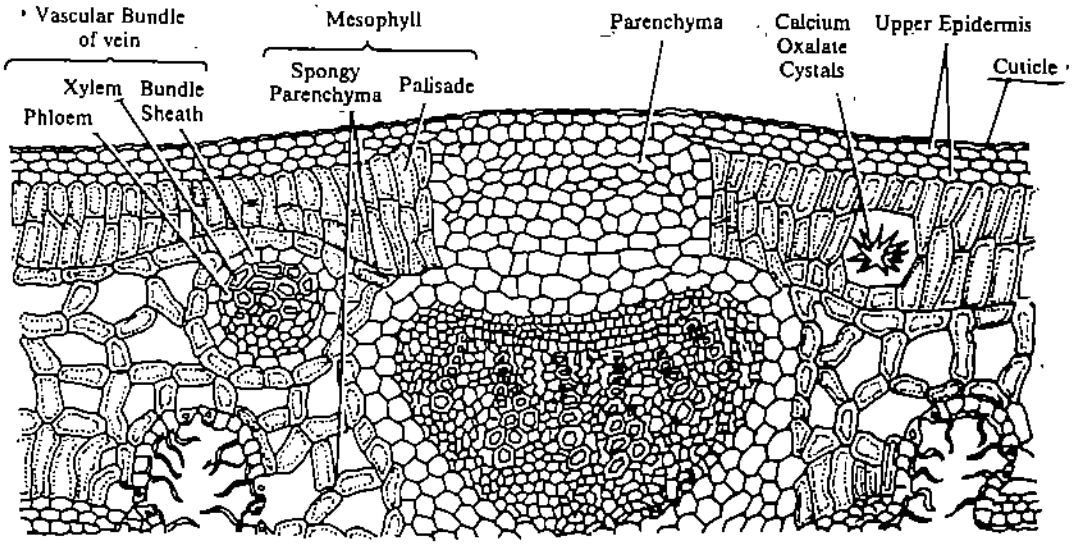

Fig. 26.15 : T.S. leaf of Nerium (non-succulent perennial). Note, thick cuticle on both sides; multiseriate epidermis; stomata of sunken type, moreover situated inside the stomatal pits, confined only to lower epidermis; well differentiated mesophyll with palisade on both the sides though abundant on the adaxial one; vascular tissues well-differentiated.

a) The cuticle is heavy and well developed.

b) Epidermis is several layered in *Nerium* and in *Pinus* hypodermis is several layer.

c) Mesophyll is very well differentiated into palisade and spongy parenchyma.

d) Stomata are of sunken type confined to lower epidermis, in some xerophyte, for example in *Nerium* (Fig. 26.15) stomata are situated in pits lined with hairs.

e) Vascular tissues are very well developed, differentiated into xylem with lignified elements and phloem.

f) Mechanical tissue are very well developed, including several kinds of sclereids.

In this exercise some example of hydrophytes, mesophyte and xerophyte have been described. Various morphological and anatomical features are described in detail. Now after studying the given description you will be asked to classify unknown specimens.

26.6 SELF-ASSESSMENT QUESTIONS

1) A hydrophytic plant is given to you. Examine the leaves of the plant. How would this condition of finely dissected leaves be advantageous to the plant?

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2) You are given the prepared slide, examine the slide of cross section of the leaf. Draw a diagram and locate the large air spaces. List several ways in which these air spaces may be of benefit in a hydric habitat.

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3) Remove the hydrophytic plants from the water, see how flaccid it becomes. Why is it so and why do submerged hydrophytes not need large amount of supporting tissues?

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4) Examine the prepared slides of *Hydrilla* stem and *Potamogeton* leaves. Locate the vascular tissues and note the absence of xylem. What is the process by which water enters aquatic plants?

5) Examine the epidermis and cuticle in cross section and transverse section of stem and leaves of hydrophytic plants. Would you expect to find guard cells in submerged hydrophytes? If not, why?

6) On floating hydrophytes where will you see stomata and why?

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7) List out various morpholgical and anatomical modifications in the hydrophytic plants you have studied.

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MESOPHYTES

1) Some plants are given to you. Study the morphology of whole plant and take T.S. of leaves and stem. Study various structure. Is some adaptation are seen, if not why?

You have to study:

a) Root system

b) Leaves, morphology and transverse sections

   i) Structure of epidermis

   ii) Presence of stomata

   iii) Differentiation of mesophyll

c) T.S. of Stem

   i) vascular tissues

   ii) mechanical tissues

Xerophytic Adaptations

Xerophytes have evolved many adaptations to prevent desiccation of the plant. You are provided with some slides of xerophytic plant. You should study carefully and try to answer the following questions:

1) Examine the slides of cross section of *Pine* leaf and locate the stomata. Draw a full labelled diagram. How does this position of stomata reduce the rate of transpiration.

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2) The prepared slide of xerophytic plant show waxy material called cutin. In addition epidermal cells are lignified. Explain how it cut down loss of water from plant.

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3) In some xerophytic plants leaves may be fleshy, in others, the stem is fleshy. In the given plant, note the absence or greatly reduced number, of leaves on the succulent stem. What is the primary photosynthetic organ in these plants.

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4) You are provided with a succulent leaf, cut a thin cross section and mount it in a drop of water on a slide. Examine the C.S. under the microscope. What are the xerophytic characteristics you observe microscopically. Make a labelled diagram and write down the xerophytic adaption carefully.

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5) You are provided with the six plant species. Write down the characteristic in the provided space. After writing the characteristic properties decide the habitat of the plant.

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Species	CHARACTERISTICS						
	Air Chambers	Supporting tissues	Protective tissues	Leaf modification (Stomata, motor cells, other)	Vascular tissues	Water storage tissues	Habitat and reason for choice xeric/hydric

EXPERIMENT 27 STUDY OF ANIMAL AND PLANT RELATIONSHIPS

Structure

27.1 INTRODUCTION

Organisms living in any habitat have evolved adaptations which assure their survival in a specific environment but may require entirely a different set of adaptations in an ecologically different situation. For example, while nematodes are found in almost every ecological niche (aspect of the environment), the nematodes living in or near the roots of lake plants probably could not adapt themselves to living in roots of cactus in a desert nor in an deodar forest nor in a glassland, although other nematodes are found in each of these niche.

Furthermore organisms in a particular habitat have to adapt and associate with other animals and plants occurring in that environment.

In some organisms such associations become almost compulsory for their survival and the organisms thus associated with one another always occur together. Increase or decrease in the population of one of the partners in such an association would lead to an increase or decrease in the other partner as well.

Associations that occur among organims may be between individuals of the same species (intra specific association) or between individuals of different species (inter specific association. Intra specific associations which form the basis of social organisations as in bees and termites are not going to be a part of this present laboratory exercise. We will be only studying the interspecific associations with the help of both plant and animal examples.

Interspecific associations are referred to as 'symbiosis'. This word 'symbiosis' is derived from a Greek word and literally means 'living together'. Symbiotic relationships fall into four categories. 1) Mutualism, 2) Commensalism, 3) Parasitism, 4) Predator relationship. We will study each of these with specific examples.

Before starting the exercise we would first like to refresh your memory about the main types of interspecific association, defining them briefly.

1) Mutualism—is the association between two organisms in which each partner benefits from the other.

2) Commensalims—refers to a relationship in which one of the two organisms, the commensal gains from the association, while the other, the host, neither gains nor loses.

3) Parasitism—This is an association in which one organism, the parasite benefits at the expense or harm of the other (the host).

4) Predation—This is an association in which a free living organism, the predator kills and devours another organism, which is called the prey. Predation differs from parasitism in the sense that parasites that live on or in the host derive nourishment without killing it, while predators kill and destroy their prey.

Objectives

This laboratory exercise should enable you to:

- define interspecific and intraspecific associations
- describe with examples the different types of associations—mutualism, commensalism, parasitism and predation,
- Observe and examine slides, specimens and models of organisms
- accurately draw and observe both external and internal structure of organisms
- prepare temporary slides.

27.2 MATERIALS REQUIRED

Preserved	:	*Taenia solium* (Tape worm)
		Dodder plant, (*Cuscuta*) parasitic on plant stem
Plant mounts or preserved	:	lichen types
Slides	:	T.S. of lichen thallus Wood eating termites *Trichonympha* or *Pyrsonympha* *Entameoba gingivalis* T.S. of the association of dodder plant and its host *Taenia solium*. scolex and mature gravid proglottids *Pediculus humanus* (human head louse)
Specimens/models	:	Suckerfish (*Echeneis*) *Drosera* (Sun dew plant)
Other materials	:	0.9% Nacl Slides and cover slips Pasteur pipettes Tooth picks

27.3 MUTUALISM

Mutualism is an association in which both the partners benefit. Under mutualism we shall consider two examples:

1) Lichens—the association between a fungus and an algae.

2) Wood eating termites and the wood digesting flagellate protozoans, housed in their gut.

1) Lichens

Lichens are formed due to an intimate association of a fungus and an algae. Lichens have different shapes: crustose (appressed to a substratum), foliose (leaflike lobes) or fruticose (erect or pendant branching structures).

Step I—Note the colour and morphological appearance of the lichens and draw the outline diagrams of them in your observation notebook.

Step II—Compare your diagrams and observations with Fig. 27.1.

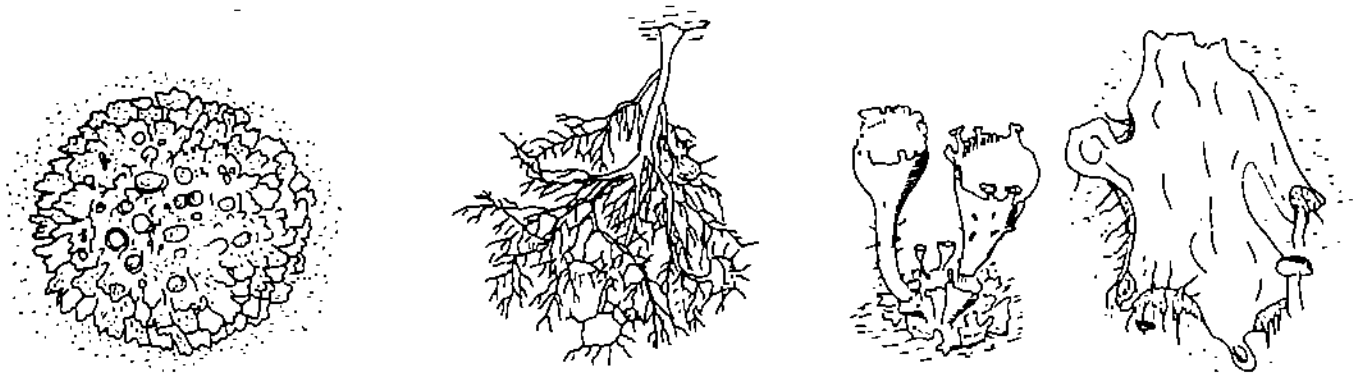

Fig. 27.1 : Three types of lichens—lichens are formed by mutualistic union between an algae and fungus

StepIII Looking at the lichen can you make out the two members of the association?

.....

StepIV Which member do you think is more evident?

.....

StepV Obtain a transverse section of a prepared slide of lichen from your counsellor and examine it under the microscope. Note the close association of the hyphae and algae cells within the thallus of lichen, and compare it with Fig. 27.2.

StepVI Looking at the slide, can you infer which member provides oxygen and organic food and which provides anchorage, inorganic food and affords protection from drying?

.....

.....

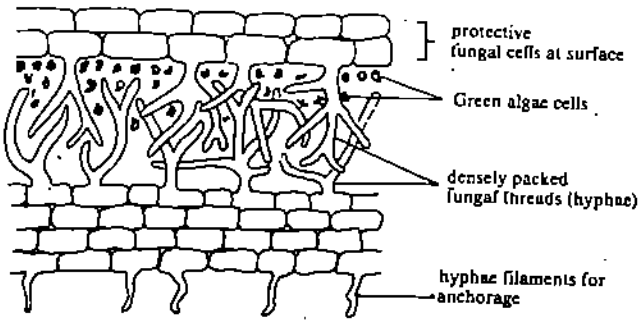

Fig. 27.2 : Cross section of a lichen showing the algae cells, among the fungal hyphae. It is the fungus that gives lichen its form

2. Wood Eating termites and Flagellate Protozoans

Mutualism is further examplified by the relationship between wood eating termites and certain flagellate protozoans. Termites which feed on wood do not have the enzyme cellulase needed to digest the cellulose of the wood. Flagellate protozoans such as *Trichonympha or Pyrsonympha* harboured in the gut of the termites help them in digesting the cellulose by releasing cellulase in the gut lumen. In turn, the protozoans get a safe place to live in (Fig. 27.3).

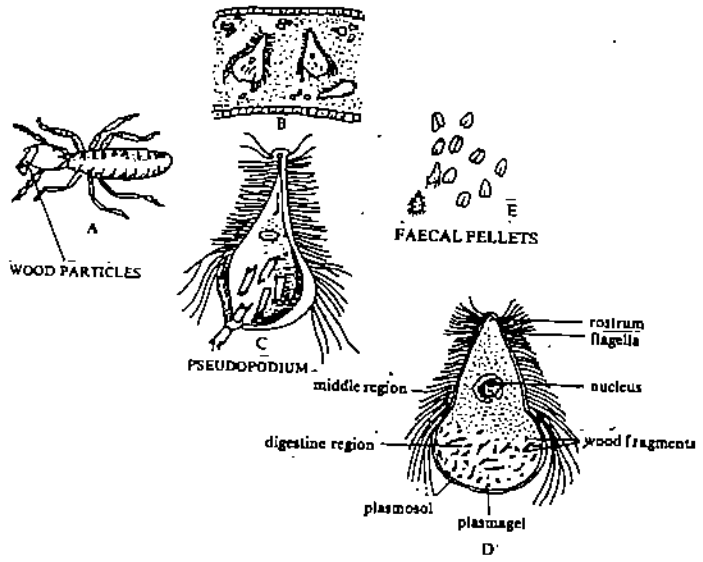

Fig. 27.3 : Mutualism between termite and *Trichonympha*. (A) Worker termite eating wood particles; (B) T.S. intestine of termite showing *Trichonympha*; (C) Enlarged view of *Trichonympha*. (D) Optical section of a trichonymphid (E) faecal pellets.

Step I—Examine the termite specimen provided to you, under the dissecting microscope, and compare it with Fig. 27.3.

Step II—Examine a slide of flagllate protozoans *Trichonympha* or *Pyrosonympha* provided by your councellor, under the microscope and draw a diagram.

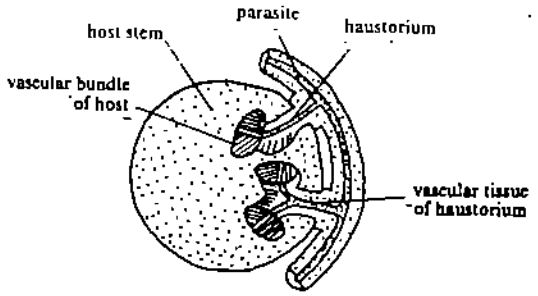

Fig. 27.8 : A transverse section of the dodder plant and its host in very close contact. Note the vascular tissue of the haustoria of the parasite in contact with the vascular bundle of the host.

ep I—Examine with the help of Fig. 27.7 the specimen of the dodder plant ovided to you. Can you observe any leaves on the dodder plant specimen.

ep II—If yes, what is the colour of the leaves?

.....

ep III—Does the colour of the leaves, suggest the mode of nutrition of the dder plant, that is whether it is autotrophic or heterotrophic?

.....

ep IV—Can you explain why the dodder plant is considered a parasite and t a fungus?

.....

.....

ep V—Examine under the microscope the transverse section (T.S) of the dder plant in close association with its host plant in the slide provided. Make neat diagram of your observations and compare it with Fig. 27.8.

Animal parasites

Pediculus humanus found in the human head is an ecto parasite.

ep I—Examine a slide of head louse under the low power of the microscope d compare it with Fig. 27.9. What morphological features do you observe?

dy divided into .....

ings .....

mber of pairs of legs .....

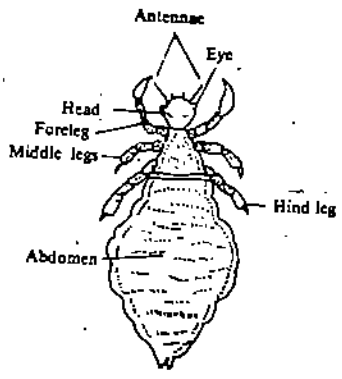

Fig. 27.9 : *Pediculus humanus* (head-louse)

Step II—What are the adaptations of the ectoparasite in order to live and feed on the human host?

.....

.....

b) *Taenia solium* (pork tape worm) is a helminth parasite which is found in the small intestine of humans.

Step I—The adult form of *T. solium* is found in the humans which are the primary host, while the larvae forms occur in the pigs which are the secondary host. Thus the life cycle of *T. solium* is a complicated one, involving two hosts (Fig. 27.10). Figure 27.10 shows the mode of transmission, as well as the number and location of the larval stages.

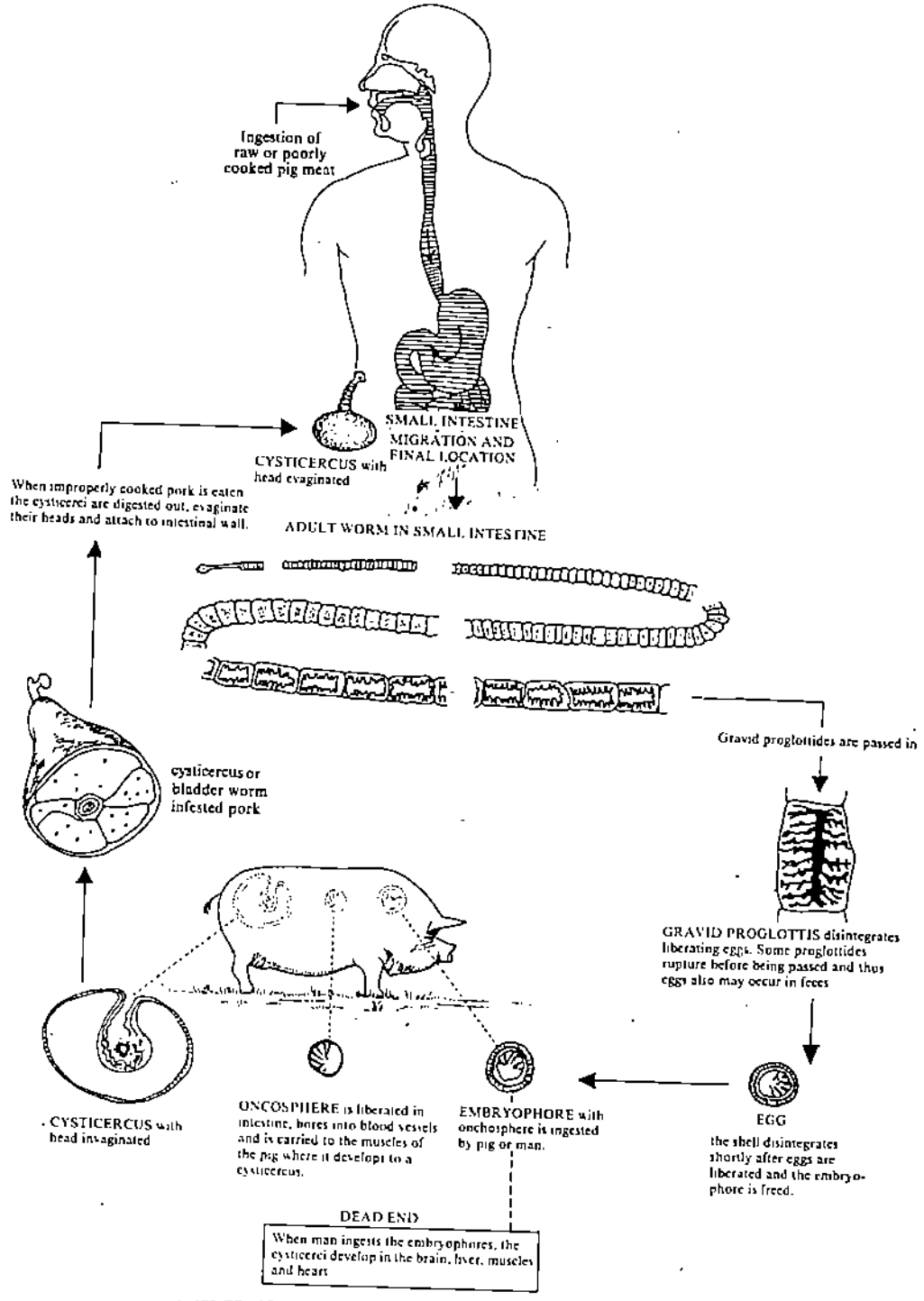

Fig. 27.10 : Life cycle of *Taenie solium*

Step II—How many larvae stages occur in the life cycle of *T. solium*?

.....

Step III—Examine a preserved specimen of the parasite and identify the three regions scolex, neck and the segmented buds. Each segment is known as a proglottid and a tapeworm has several proglottids. Also, observe under the microscope permanent slides of scolex and gravid proglottids and compare with 27.11.

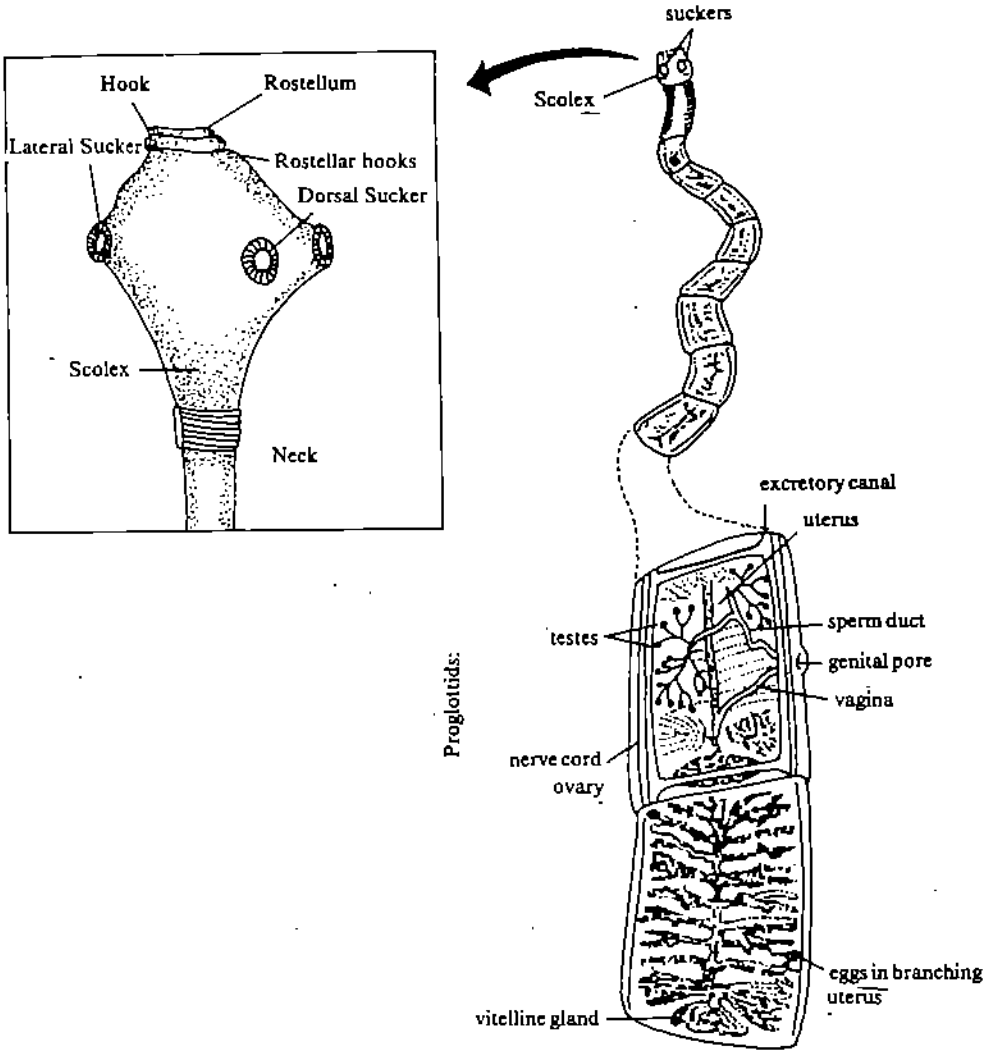

Fig. 27.11 : a) Scolex of *Taenia solium*
b) Details of mature proglottids of *T. solium*

Step IV—Note the various modifications given below in Table 27.11 and if possible write down the reasons for such modifications. For studying the slide you can consult Fig. 27.10 and 27.11.

Table 27.1

	Parasitic adaptations	Reasons
1)	Scolex (head) has suckers and hooks	
2)	Mouth is absent in the scolex (which is, thus only meant for attachment to the host)	
3)	Body is long and flat	
4)	Body is covered by a cuticle	
5)	Circulatory system absent	
6)	Digestive system is absent	
7)	Nervous system and muscular system is poorly developed	
8)	The cuticle of tapeworm is thick and it produces antienzymes	
9)	Tapeworm body is segmented and each segment is called proglottid which has both male and female reproductive organs.	
10)	The lower segments of the tapeworm called the mature proglottids, lose their reproductive organs, enlarge and become filled with a large number of eggs	
11)	From time to time 2-3 of the mature proglottids drop off from the tapeworm and pass out of the host body with the host's faecal matter	
12)	The mode of respiration is anaerobic	

Step V—Check your answers with the reasons we have listed out in table 27.2.

Table 27.2

	Parasitic adaptations	Reasons
1)	Scolex (head) has suckers and hooks	The suckers and hooks of the tapeworm are used for clinging to the wall of the small intestine of human host.
2)	Mouth is absent in the scolex (which is, thus only meant for attachment to the host)	The absence of mouth indicates that the parasite does not need to eat food

(*Contd.*)

3)	Body is long and flat	Body is long and flat, so that it provides a large surface area for absorption of the food, already digested by the host. The food in liquid form is absorbed along the entire length of body of the worm.
4)	Body is covered by a cuticle	Cuticle makes the parasite resistant to the action of digestive enzymes
5)	Circulatory system absent	Both these systems are absent as the tapeworm being parasitic does not
6)	Digestive system absent	need either the circulatory or digestive system.
7)	Nervous system and muscular system is poorly decreased	Both these systems are Poorly developed because the parasite remains at one place, and does not require locomotion for searching for food or for reproducing.
8)	The cuticle of tapeworm is thick and it produces antienzyme	These two features help to protect the worm from the action of the digestive enzymes of the host.
9)	Tapeworm body is segmented and each segment called proglottid, which has both male and female reproduction organs.	The presence of both male and female reproductive organs, ensures fertilization as the worm may not be able to find its specific partner, within its host body. Thus fertilization is not left to chance.
10)	The lower segments of the tapeworm called the mature proglottids, lose their reproductive organs, enlarge and become filled with a large number of eggs	The larger number of eggs which are greatly in excess are essential for ensuring continuity as further development of the parasite is outside the host body and so is dependent on the chance of the availability of the secondary host, the pig.
11)	From time to time 2-3 of the mature proglottids drop off from the tapeworm and pass out of the host body with the hosts faecal matter	Each of the mature proglottids protects the eggs by assuming the function of an egg sac with lots of eggs. The eggs are released when the proglottid disintegrates.

(*Contd.*)

12) The mode of respiration is anserobic	The tapeworm has adopted an anaerobic mode of respiration as it lives in a dark and oxygen deficient environment within the host body.

27.6 PREDATION

You are aware that some animals are predatory in nature. A lizard eating an insect, a cat eating a mouse are all very common examples of predation and do not need any description. You will be provided here with just one example of predation. The example chosen by us is unusual as we have chosen a predator, basically an insectivore which belongs to the plant world, *Drosera* plant (sun dew plant).

Sun dew plant

Step I— Examine the model/specimen of *Drosera* provided by your counsellor and draw a diagram of it.

Step II—Compare the diagram with Fig. 27.12.

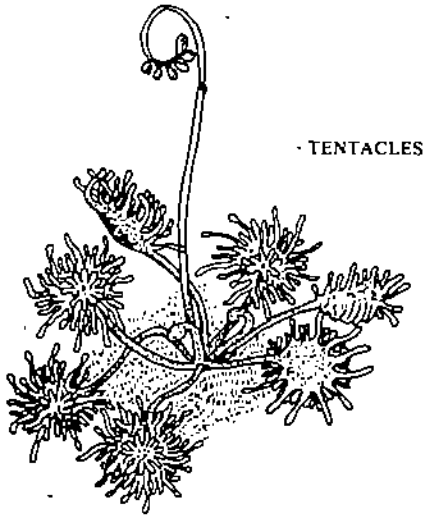

Fig. 27.12 : Predatory insectivorous plant—*Drosera* (Sun Dew) Plant

Step III—Where do you think are the insects trapped?

.....

Step IV—Examine the leaf of *Drosera*. Observe how it is stalked and its lamina is rolled or folded to form a globular or flat, surface, bearing numerous tentacles which move when touched by insects. These tentacles also secrete a viscid, sticky fluid which appears as shining droplets and is attractive to the insects. When the insect alights on a leaf, the tentacles trap it by bending over its body. The sticky fluid further prevents the escape of the insects.

EXPERIMENT 28 STUDY OF FAUNAL COMPOSITION OF CHOSEN HABITATS

Structure

28.1 INTRODUCTION

You already know that a particular environment where an organism lives is called its habitat. The fauna and flora within the habitat form a community. The ecosystem is formed by the habitat and the community and the dynamic interactions between them. Ecological studies show that ecosystems are dynamic and changing. The change may be seasonal or over a long period of years. Further more, most species show a remarkable degree of adaptations in order to live successfully in a particular environment.

In the present laboratory exercise, we have chosen specific examples of animals which show considerable adaptations to their habitat.

Habitat	Examples
Aquatic:	
Fresh water	Carp (*Labeo rohita*)
Marine	
Deep sea (benthic)	Angler fish (*Lophius*)
Intertidal zone	
i) Rocky shore —	Acorn barnacle (*Balanus*)
ii) Sandy shore —	Mole sand crab (*Hippa*)
Terrestrial:	
Desert —	Horned toad (*Phyrnosoma*)

b) Sublittoral zone.		Forms the basin of the ocean whose depth is upto 200 meters.
c) Deep sea zone.		It forms the basin of the ocean which is below the depth of sunlight penetration (i.e. below 200 meters).
4. **Water below the pelagic zone and without sunlight.**		Forms the water zone below 200 meters of depth where light is unable to penetrate.
a) Mesopelagic zone		Uppermost zone of water of the aphotic area extending from 200 meters to about 700 or 1000 meters below.
b) Bathypelagic zone.		Extending from below the mesoplagic zone upto a depth of 2000 to 4000 meters.
c) Abyssalpelagic zone		Below the bathypelagic zone extending upto 6000 meters.
d) Hadalpelgic zone		The water in the deep sea trenches between 6000 to 10,000 meters.
II Fresh Water	**Average Salinity**	
	< 5 ppt	

Lentic (eg. ponds, lakes) (Fig. 28.3)

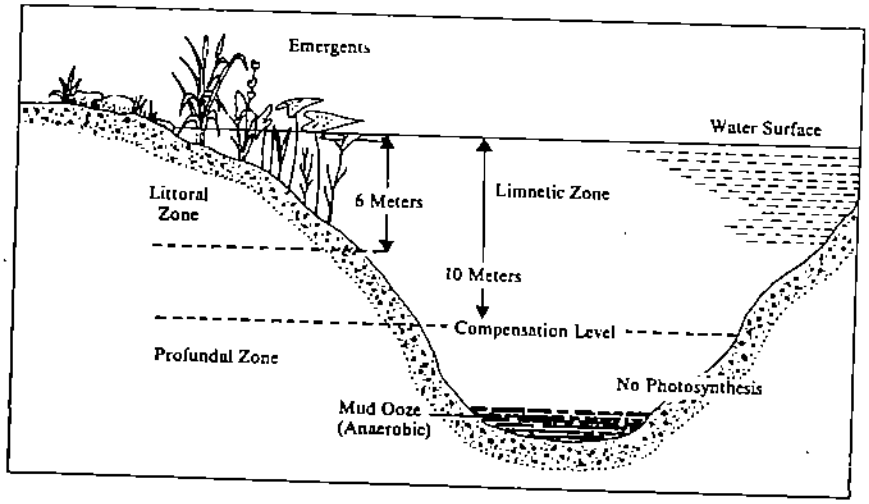

Fig. 28.3 : The various habitats in deep pond or lake

1 Littoral zone		Extending from the edge of water upto a depth of 6 meters.
2. Limnetic zone		The lighted zone extending beyond the littoral zone.
3. Profundal zone		Deep water zone where light is absent.

27.4 COMMENSALISM

Commensalism is a relationship in which one of the partners in the association benefits; mainly in terms of transporation or shelter and the other is neither benefited nor harmed. Under commensalism we shall look into two examples.

1) The protozoan *Entameoba gingivalis* found in the mouth of humans.

2) Sucker fish (*Echeneis*) which often attaches to bigger fishes such as sharks and get transported.

1) ***Entameoba gingivalis***

It is a permanent endo commensal occurring in the gum pockets of humans, where it gains shelter and food. This organism is found usually in those people who have gum disease although the disease as such is not caused by them but by baeteria.

Step I—Obtain a slide of *E. gingivalis* and examine it under the low power objective of the microscope, with rather weak ilumination. Can you observe the nucleus and food vacules. (Fig. 27.4).

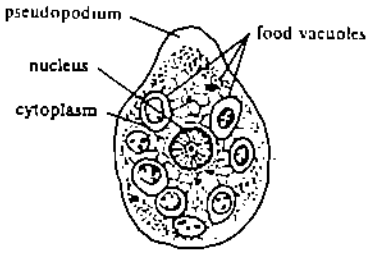

Fig. 27.4 : *E. gingivalis*

Step II—Prepare a temporary slide of *E. gingivalis*. In order to prepare a slide, place a drop of 0.9% Nacl solution on a glass slide. Gently insert a tooth pick into your gum pocket, at the base of your teeth and remove some material. Transfer this material onto the saline drop. Cover with a coverslip and examine under the microscope. Look for rather large cells which are mobile and have pseudopodia. How different do the *E. gingivalis* protozoans look in the permanent and temporary slides?

2 **Sucker Fish (*Echeneis*)**

Sucker fish, an ectocommensal attaches itself to larger fishes, mostly sharks, by means of a sucker present on its dorsal side. This attachment provides free transport to the sucker fish to different places. (Fig. 27.5).

Step I—Examine the specimen of sucker fish with the help of Fig. 27.6. Note the location of the sucker, which is a modified dorsal fish.

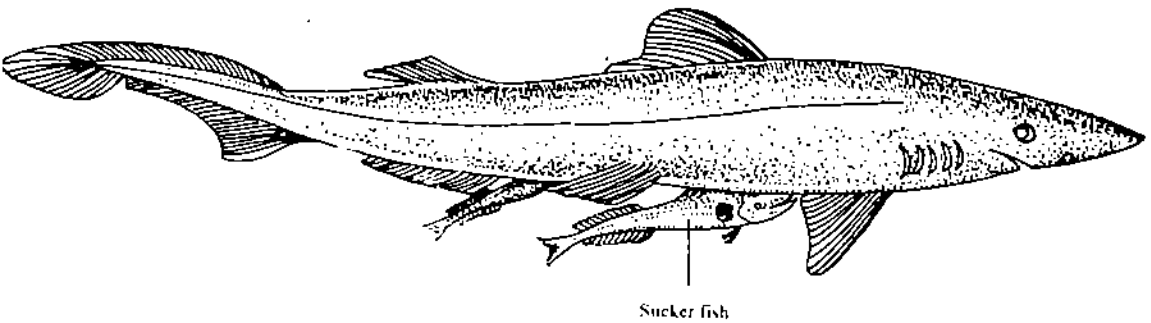

Fig. 27.5 : Shark fish with sucker fish and pilot fish

Step II—Observe the sucking disk of the sucker. Describe the sucker and make a neat diagram and compare it with Fig. 27.6.

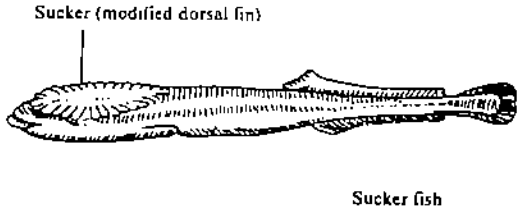

Fig. 27.6 : Diagram of the sucking disk of sucker fish

27.5 PARASITISM

Parasitism as defined earlier, is an association between two organisms in which one benefits at the expense of the other. In this association the parasite may live either on the surface (ectoparasite) or within the body of the host (endo parasite). The parasite obtains its food as well as the shelter from the host.

There have been many instances of host parasitic relationships. In every such relationship the parasite has evolved adequate adaptations depending on the hos The site of parasitisation, and the type of food it feeds. We shall look into examples of parasitism.

1) Plant Parasite

Dodder plant (*Cuscuta*) parasitic on plants

2) Animal parasites

i) Human head louse (*Pediculus humanus*) parasitic on human head.

ii) Tape worm (*Taenia solium*) found in the small intestine of humans.

1) Plant parasite

Dodder plant is parasitic on another plant, as you can see in Fig. 27.7 and 27.8

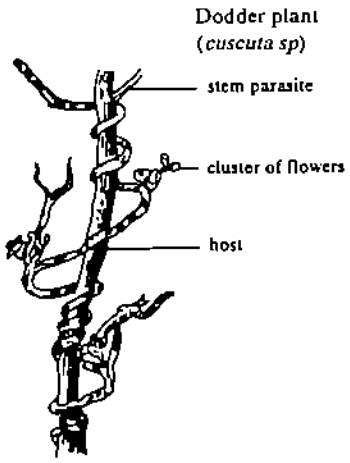

Fig. 27.7 : Dodder plant

Lotic (example rivers—and spring)		
1. Flowing zone.		Water depth variable shallow near the shore and may be extremely deep in the middle.
2. Rapid Riffle zone.		Shallow water zone, water flows rapidly.
3. Pools.		The quiet unflowing parts of the river.

Table 28.2: Terrestrial Habitats (Fig. 28.4)

Biome	Soil	Vegetation	Average Yearly Temperature Range	Average Yearly Precipitation
Polar	Sparse, very low in nutrients, frozen much of year	Mosses, lichens, small flowering plants along coast	–40°C–4°C	< 10 cm
Tund	Thin, moist topsoil over permafrost; nutrient-poor; slightly acidic	Mosses, lichens, dwarf woody plants	–26°C–4°C	< 25 cm
Conifer s forest	Low in nutrients highly acidic	Needle-leafed evergreen trees	–10°C–14°C	35-75 cm
Deci.ou forest	Moist, moderate, nutrient levels	Broad-leafed deciduous trees and shrubs	6°C-28°C	75-125 cm
Grassland	Very rich in nutrients, deep layer of topsoil	Dense, tall grasses in moist areas; short clumped grasses in drier areas	0°C-25°C	25-75 cm
Desert	ry, sandy, utrient-poor	Succulent plants; scattered grasses and sagebrush	24°C-34°C	< 25 cm
Tropical rain fore	hin, moist, ow in nutrients	Broad leaf evergreen trees and shrubs	25°C- 27°C	200-400 cm
Temper drain forest	Moist, nutrient-rich, highly ac ic	Giant needle-leafed evergreen trees	10°C-20°C	200-400

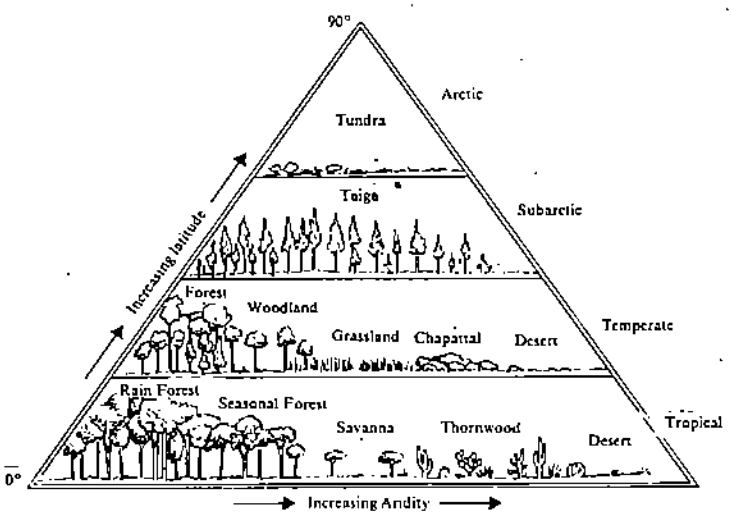

Fig. 28.4 : Terrestrial Habitats

Among various ecosystems that have been listed, we shall confine ourselves as we stated before to the study of a few specific habitats and a few chosen animals adapted to them. Let us start with specific animal 'fish' adapted to the aquatic medium.

28.4 AQUATIC HABITAT

All types of water ecosystems whether marine or fresh water have a unifying characteristic namely the water medium. Aquatic animals thus have developed cartain adaptations to live successfully in this medium.

Let us consider fish as an animal ideally adapted to an aquatic medium. Fishes whether marine or fresh water have to adapt to living in water and so have a numbers of common adaptations. We have chosen fish as it is a primary aquatic animal found **both in fresh and salt waters,** whose ancestors are believed to be always aquatic. For this purpose we have chosen a typical fish namely i) Carp (*Labeo rohita*) occurring in fresh water and ii) a highly modified fish *Lophius* angler fish occurring in the deep waters of the ocean whose adaptations enable it to live successfully.

You should keep in mind that the adaptations of *Labeo rohita* considered here are fairly representative for both fresh and marine water fishes.

28.5 FRESH WATER HABITATS—CARP

Examine the *Labeo* rohita and compare its features with Fig. 28.5

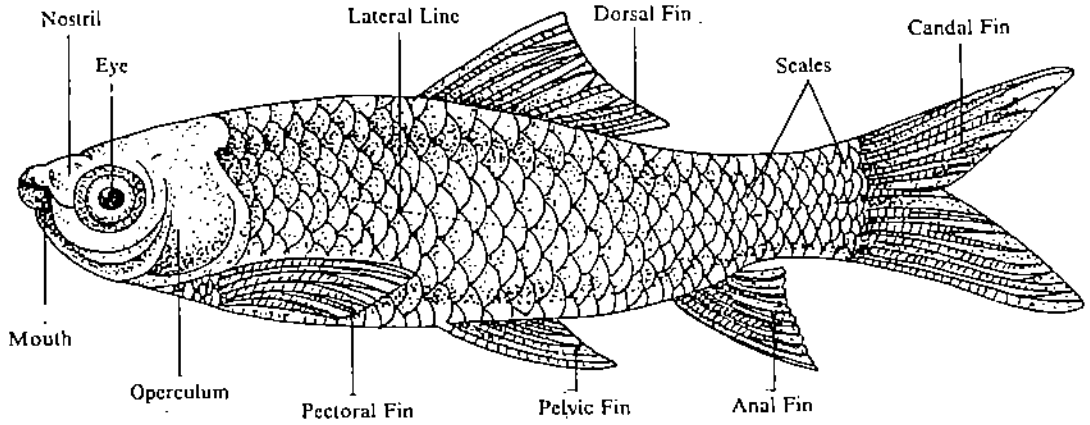

Fig. 28.5 : Carp (*Labeo rohita*)

1. *Labeo rohito* is a bony fish and is commonly known as carp in English and Rohu in Hindi. It is about 1 meter in length and weighs 4 kg.

2. Note the shape of the body. It appears spindle shaped, with compressed head and subconical snout. What possible reason can you attribute to the shape?

   .....

   The shape is ideal for offering minimum resistence to water while swimming.

3. Observe the body of *Labeo*. It is covered with large overlapping cycloid scales. In some fishes including this you may observe a thin layer of mucous covering the body. Both these features, further help to reduce friction in water, so that swimming is easier for the fish.

4. In the fish you will see that limbs are absent, instead it has fins. Note the shape, number and position of the fins in the *Labeo*. You will observe—two paired pectoral fins, two paired pelvic fins; and one or two median—dorsal, ventral and caudal (tail)—fins. The pectoral, pelvic and anal dorso-ventral fins act primarily as balancers, which push the fish. The pectoral and pelvic fins control stabilty and help in steering. These fins are thus concerned mainly with causing the fish to move up and down. The median fins (dorso-ventral) control rolling in fishes. See Fig. 28.6.

5. The tail is the major locomotory organ, for propelling the fish forward. Its lashing movement is possible due to the flexible nature of the vertebral column. Individual vertebrae of the column are joined with one another by suifficiently elastic ligaments which allow some movement to the spine as a whole. In some fish the skeleton is of cartilage and so is much softer and more flexible, thus allowing more movement. The movement involved can be seen in Fig. 28.6. The two sideways thrusts being equal cancel one another so that the final result is a forward not sideways movement of the animal under water.

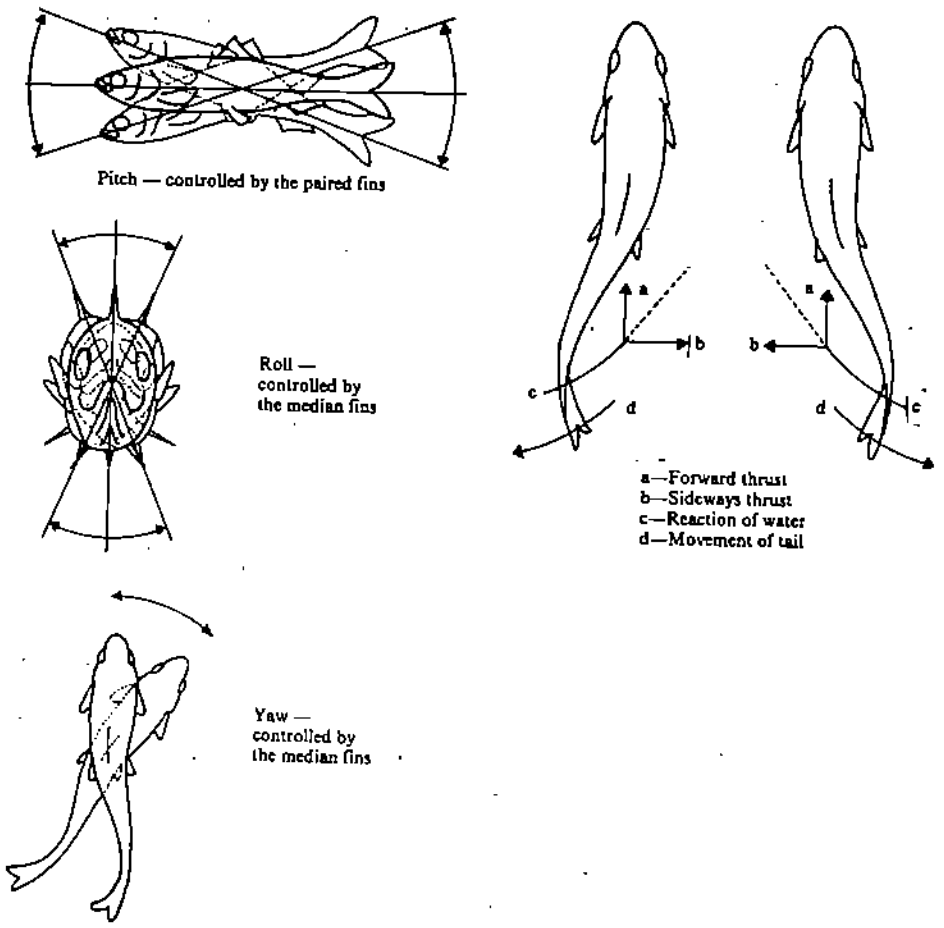

Fig. 28.6 : The forces which cause swimming movements of the fish

6. The movement of the fish is also helped by the alternate contraction and relaxation of the longitudinally arranged muscle blocks, present in the opposite side of the back bone. Fig. 28.7.

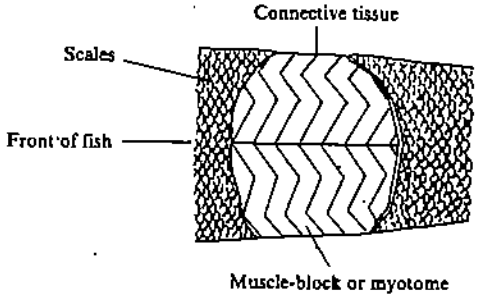

Fig. 28.7 : Arrangement of blocks of muscle down the back of a fish. Part of the skin has been removed to expose the muscles.

7. You are also aware that for respiring on land, you need lungs. The fishes however live in water and can not use lungs for respiring. Instead they have developed gills which allow them to exchange respiratory gases in water. (Fig. 28.8)

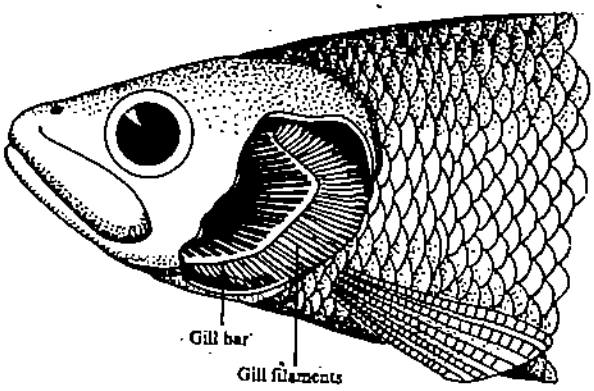

Fig. 28.8 : Gills of a bony fish. The operculum has been cut away to show the gills which lie below it. Gill filaments are divided and subdivided to provide a large surface area for absorption or oxygen in respiration

8. Note the operculum in the *Labeo*. If possible lift the operculum up. You can see that the operculum is large and hangs on either side enclosing the gill and brachia chamber. The gills you will see are richly supplied with blood vessels for facilitating the gas exchange.

9. The *Labeo* including other bony fishes also have a long air filled sac—the swim or air bladder. This makes the animal bouyant, so that it does not sink when it stops swimming. Cartiligionous fishes on the other hand lack this blader and so sink if they stop swimming. At different depths, the air pressure in the swim bladder is regulated by the fish.

10. Observe a lateral line on both side of the body in the lateral region. These are sensory in nature and have a number of neuromast organs. These act as rheoreceptors and help in echolocating objects in water.

11. Other sense organs are well developed. The sense of smell is particularly acute. The eyes are well developed too, though not as sharp as the sense of smell. The external ears are absent. The internal ear is present and is functional.

12. Salt and fresh water bony fish have opposite problems with maintaining the salt balance in their bodies. Salt water has a higher concentration of salts than found in blood. Thus salt water bony fish tend to lose water from their cells by osmosis. Scales which are impermeable to water help prevent the loss of water from gills of salt water fish and actively transport salt out of the body. Their kidneys excrete only small quantities of highly concentrated urine. This helps them conserve water in bodies. Fresh water fish have an opposite problem. They tend to absorb water from their surroundings by osmosis. The scales of fresh water fish, therefore, keep water out of the body. Further the gills of these fishes actively transport what little salt is available in the surrounding water into the body. Kidney also helps by excreting large quantities of water. Only a few bony fish like salmon can go from salt to fresh water. The gills and kidneys of these fishes are so adapted that they can reverse their water and salt transport functions.

SAQ 1

Wha. do you think would be the main problem faced by a fresh water fish if it is moved to the sea.

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28.6 DEEP SEA HABITATS—ANGLER FISH

You know the basic adaptations of fishes. However, fishes of various ecosystems and zones do show other specific adaptations. Let us consider the adaptations of deep sea fishes with the help of angler fish as an example.

1. It is a marine benthic fish of the deep sea.

2. Consider the body of the fish. It appears depressed and dorsove ventrally flattened, due to the extreme pressure of water from above. Fig. 28.9.

3. You will observe the head and anterior part of the body are very large and without scales. The mouth is large with strong recurved teeth so that no prey big or small can escape it, in the poor nutrient zone, in which it lives. Often it swallows a prey much larger than itself. The large mouth or its wide opening is possible due to the manner in which the mouth and skull are hinged.

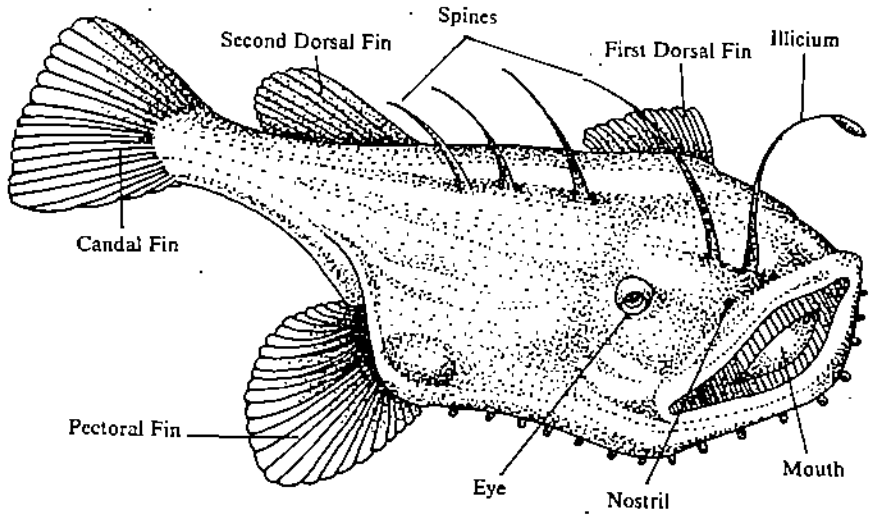

Fig. 28.9 : Angler fish (*Lophius*)

4. Observe the eyes which are large and lateral so that the fish can get help from very small amount of light available in their dark habitat.

5. Gill opening you will see is protected by being located in the lower arch of the pectoral fin.

6. In Angler fishes, a bait for living prey is present. This bait is formed by the modification of the first dorsal fin. It appears like a long rod like structure bearing a fleshy mass or bait at its tip called illicium. This bait lures preys like worms and small fishes. When the prey comes near the bait, the angler fish attacks it fercoiondy and eats it. In some angler fishes the bait is made more attractive by being luminescent.

7. The pecteral and caudal fins are absent as the waters are calm and mobility is limited due to extreme pressure and darkness.

8. Finding mates in the dark environment is difficult. To over come the difficu y of finding mates for ensuring reproduction the Angler fishes have camparatively smaller males actually attached ectoparasitically, permanently to the large female. Fig. 28.10.

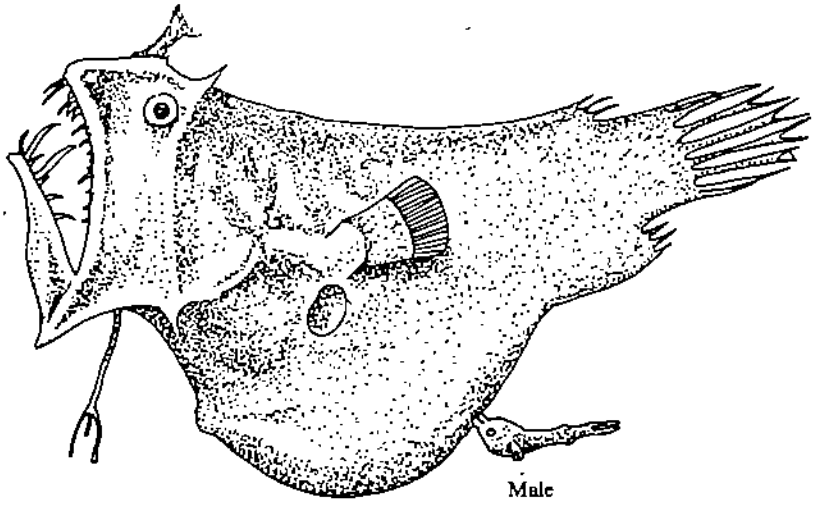

Fig. 28.10 : Large female angler fish with attached small parastic male

SAQ 2

Draw diagrams of both carp and angler fish and write down the differences and similarities between them in your record file.

28.7 INTERTIDAL ZONE—ACORN BARNACLE AND SAND MOLE CRAB

The sea shore both rocky and sandy lies between high and low tide marks and as you can expect the main dominating habitat factor here is the alternating exposure to air and flooding by sea water to which organism are subjected. The other changing physical conditions here are variations in temperature, periodic drying and wetting, frequent flooding with salt or fresh water resulting in fluctuations in pH, buffeting action waves etc. Such conditions would lead us to believe that organisms here would be few. However, it is not so and animals are particularly well represented by several species. Some of them show remarkable adaptations to life in the intertidal zone.

Most animals of this intertidal zone are basically marine in origin so they have to acquire adaptations which help them to minimize or avoid stress caused by daily exposure to air and sunlight and the force of the tidal waves.

You can study the adaptations in two animals belonging to the class crustacea of the phylum arthropod in sandy and rocky shores namely:

i) Acorn barnacle (*Balanus*) rocky shores.

ii) Sandy mole crab (*Hippa*) of sandy shores.

Both these animals are very different and very well adapted to their particular environment.

28.7.1 Rocky Shore Habitat—Acorn Barnacle

Acorn barnacle is a sessile animal found attached in great numbers to the rocky shore or to the mollusc shells in between tide marks in the shallow waters. (Fig. 28.11).

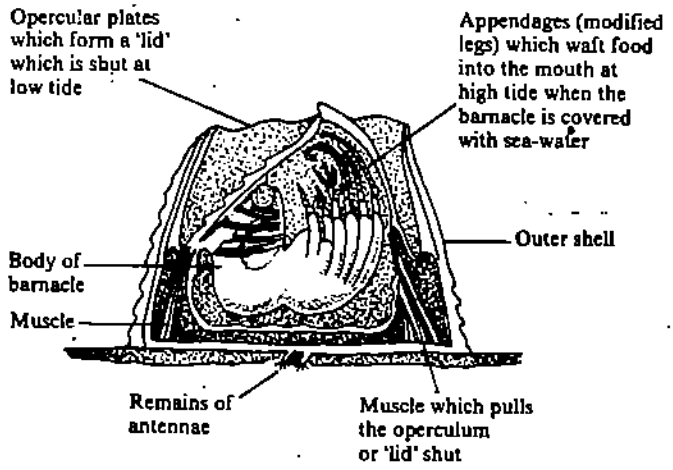

Fig. 28.11 : Vertical section of Acorn barnacle. The free swimming larva attaches itself to a rough surface by its head. The hard shell is secreted round the larva and forms the familiar object, often seen in a mass on the seaside posts and rocks withstanding the roughest seas

The acorn barnacle's main adaptive features as you can observe are:

1. It is sessile and is strongly attached to the rocky substratum. This way it avoids being swept off by the tidal waves.

2. The acorn barnacle as shown in Fig. 28.12 consists of a mantle which surrounds the body of the animal. This mantle is covered by a hard shell in the adult. This shell has six calcareous plates, an unpaired carina, an unpaired rostrum and two pairs of carino-lateral plates. Edges of the plates overlap and fit together forming a cylinder. Outer surface of each plate show three divisions-a central portion and two wings.

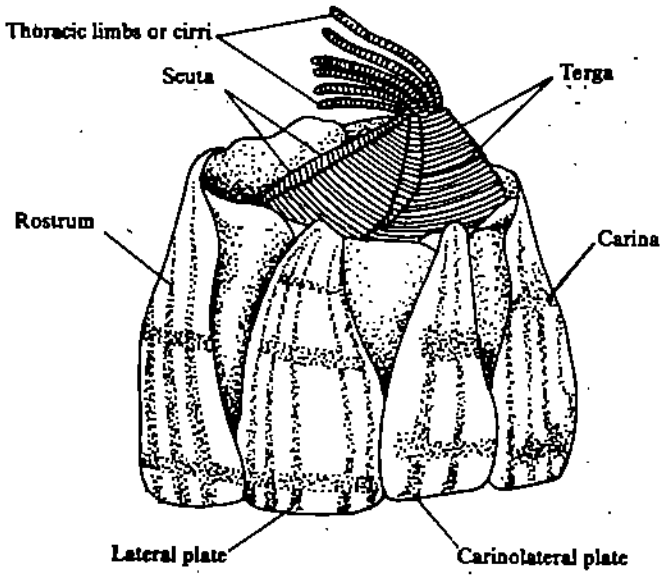

Fig. 28.12 : External lateral view of acorn barnacle

What do you think is the function of the shell? It helps to protect the soft body of the animal from the force of the tidal waves, from predators and from dessication.

3. Examine the opening of the shell and mantle you will see a four fold lid consisting of two scuta and two terga. When the tide is out, the mantle plates close for protection as well as to prevent dessication by evaporation of its body water.

4. You will see that the animal has six pairs of delicate fringed legs which are contained within the shell when the tide is out. However, in the water, these legs are protruded through the opening to collect and propel food to the mouth.

5. The Acorn barnacle has a method also of maintaining its body heat. It loses water when it becomes warm to cool itself, as it has developed a strategy which prevents excessive dessication due to water loss. It does so by keeping an extra supply of water within its mantle cavity which compensates for the water loss.

6. The animal being sessile, is unable to actively seek its mate. Thus in order to ensure reproduction, the barnacles have adapted two strategies i) Either the male and female live in permanent association or ii) the animals may be hermaphrodites.

7. The life cycle of this animal has also a free swimming nauplius larvae. The mobility allows the larvae to get themselves distribuited along the habitat and thus avoid crowding.

28.7.2 Sandy Shore Habitat—Sand Mole Crab

The animals of the sandy shore can not find a firm foothold as their substratum keeps shifting with the action of the tidal waves. Due to this most of the animals here are of the burrowing type. The adaptations of sand mole crab (*Hippa*) are as follows:

1. The *Hippa* or sand mole crab as the name suggests burrows into the sand. It has the ability to burrow very swiftly into the sand, Keeping its mouth parts above, to filter food from the water. Fig. 28.13.

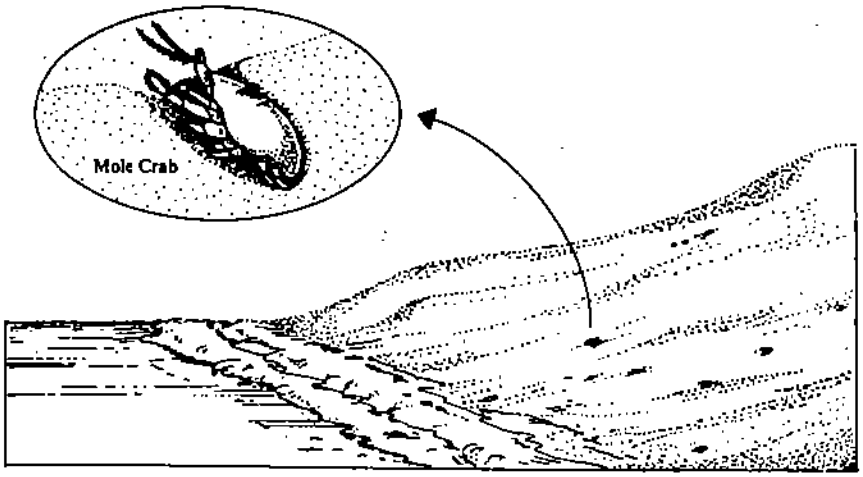

Fig. 28.13 : Sand mole crab avoids predaters, tidal wave action and dessication by burrowing into the sand

2. It has also developed a strategy which prevents the clogging of its respiratory surfaces due to suspended sand. Its antennae are held together in such a manner as to form a tube to the surface, through which only water enters into the branchial chamber. The sand particles are prevented from entering as well, since the antennae are densely clothed with closely spaced hairs.

3. Observe the body of the sand mole crab. It is reduced and elyptical (oval) and is provided with wings which cover the legs also on the under surface. Fig. 28.14. For most of the other adaptations as well you can take the help of Fig. 28.4.

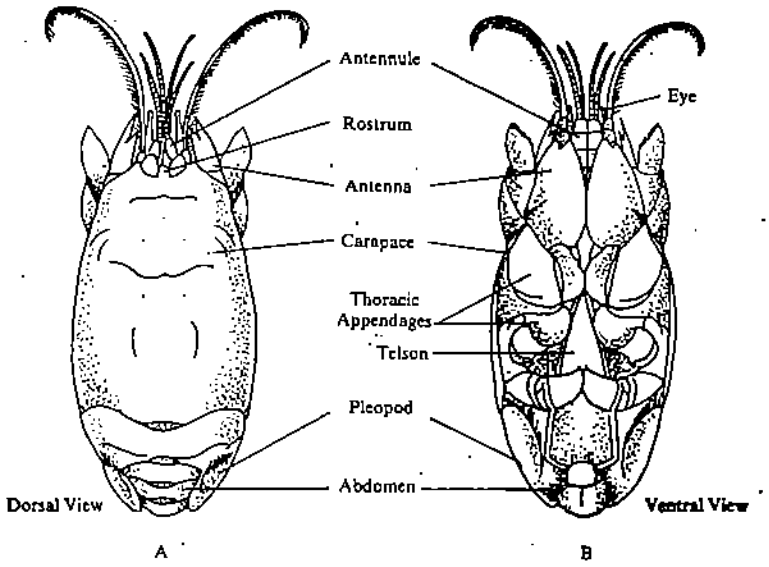

Fig. 28.14 : The dorsal and ventral view of sand mole crab (*Hippa*)

4. The head you can see has a pair of stalked compound eyes, a pair of short antennules, a pair of long hairy antennane and reduced rostrum. The third maxillipides are broad.

5. The thorax you will observe has 7 pairs of appendages of which the first two are partially chelate.

6. The rest of the appendages are used for digging.

7. You wil observe a gill attached to each thoracic leg (appendage).

8. The last one or two pairs of the appendages are usually smaller and are often concealed by carapace.

9. Abdomen you will observe is more or less reduced, often soft, and bent upon itself. The abdominal pleura is small.

10. The first three abdominal appendages called pleopods bear swimming appendags and produce water currents to bathe the gills. The last three called uropods are posteriarly directed and are used for swift directed, darting movement.

11. The animal's ability to burrow quickly in the sand helps it evade the force of the tidal waves, predators and dessication.

SAQ 3

You have examined both the Acorn barnacle and sand mole crab. Both belong to subphylum crustacea. Note similarities in these animals in the table given below and check your answers with your counsellor.

Crustacea	Body segments 2	Appendages, 2 pairs antennae, variable number of walking legs.	Respiration By gills
Acorn barnacle			
Sand mole crab.			

28.8 TERRESTRIAL HABITAT—DESERT—HORNED TOAD

A look at Table 28.2—Terrestrial Habitats would indicate to you that the unfavourable conditions existing in the deserts which are arid zones would make it impossible for life to exist. This is not so however. Surprisingly a large number of animals thrive here. Most of these animals are drought evaders or resisters and are particularly adapted to these drastic conditions. Most of the adaptations of the animals are mainly associated with the conservation of water, protection against extreme heat and cold and obtaining food. The adaptation strategies vary in different animals.

We have choosen the horned toad (*Phyrnosoma*), for studying its adaptations to the harsh desert environment. *Phyrnosoma* (Fig. 28.15). Fig. 28.15 will help you in studying most of the adaptative features of the *Phyrnosoma*. So consult it as much as possible.

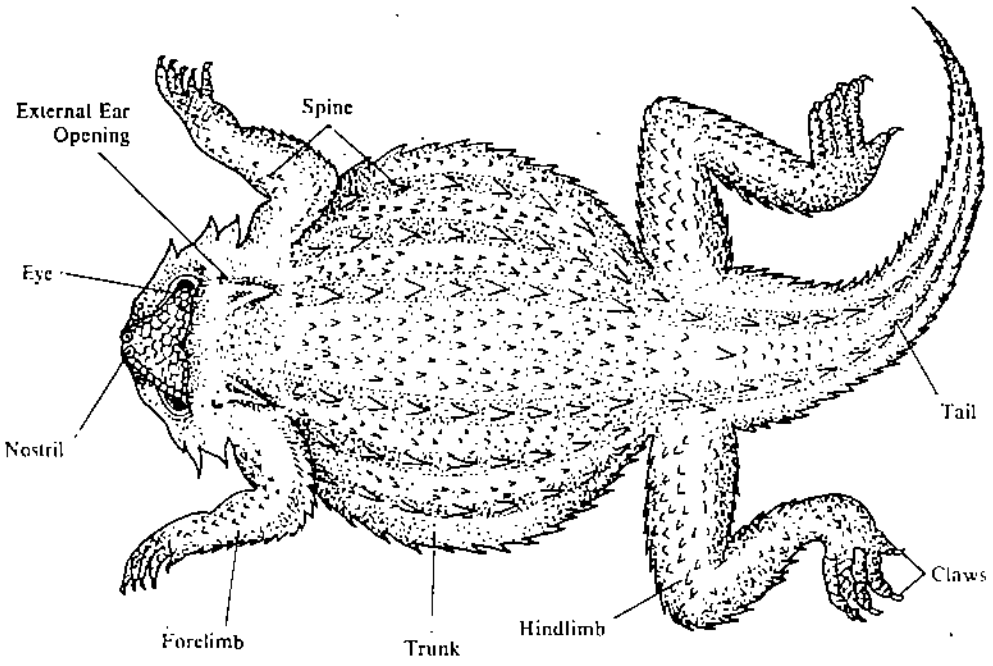

Fig. 28.15 : The horned toad *Phyrnosoma*. Its body has a thick spine covered hide to protect it from its enemies and to avoid loss of water

1. It is commonly called Horned toad, though in reality it is a reptile.

2. Examine the body of this reptile. It has a wedge shaped head bearing enlarged horn like scales. Its under surface has keeled scales.

3. Its hide you will notice, is with protective armour. This helps to protect it from the hot sun and its enemies to some extent. Further the skin being almost impermeable prevents water loss from general body surface.

4. The dorsal surface, you will observe is yellowish grey and blends easily with its surrounding, camouflaging it from its enemies and preys.

5. Count the spikes on the head. You will notice five spikes on each side—one post-orbital, three temporal and one occipital.

6. You will see that the sides of the lower jaw project in the shape of prominent ledges and are protected by a series of small spines.

7. Tongue is fleshy, non protrusible. Eyelids are complete. Teeth are usually homodont or pleurodent.

8. Nostrils are turned upward and have valves which prevent sand from entering the nostrils during sand storms and burrowing.

9. Tail is short and spiny as you will observe.

10. Being a reptile it is poikilothermal. However, despite this it is well adjusted for desert life by being acclimatized to high temperatures. Due to this it basks in the sun as long as it can. Later when it becomes too hot it moves to the shade.

11. It avoids adverse conditions of the surface by burrowing quickly with its wedge shaped, horned head which is particularly useful for burrowing in the sand. It usually burrow as night approaches.

12. The *Phyronosoma* can live without water for long periods. Its thick, spiny almost impermeable hide also helps in preventing excess loss of water from its general body surface, thus conserving its internal moisture.

13. It feeds on small insects and ants.

14. It protects itself from its enemies by squirting tiny streams of blood from the eye, which can go upto a distance of two feet.

15. Ears are absent.

SAQ 4

Draw a labelled diagram of horned toad.

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