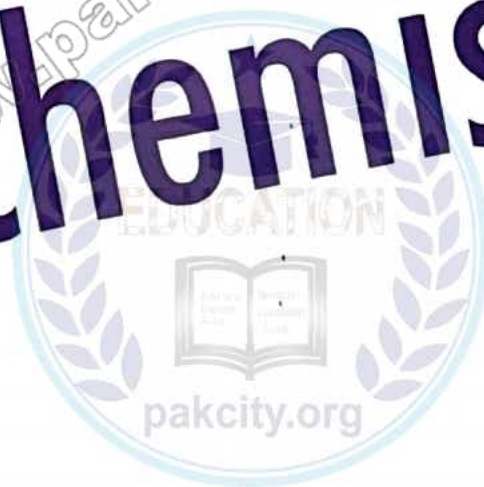


(1) Biochemistry



1

Experiment

IDENTIFICATION OF BIOCHEMICALS FROM BIOLOGICAL MATERIALS

Material:

Test tubes, Test tube stands, Test tube holder, Pipette, Burner, Beaker tripod stands wire gauze. Purpose of this practical is: to detection of proteins carbohydrates ant fats from given solutions.

Purpose of this practical is: To detection of proteins carbohydrates ant fats from given solutions.

Proteins:

The word "**protein**" is derived from the Greek word "*proteios*", which means "of primary importance". In fact, proteins plays an important role in all biochemical and physiological body processes; they act as enzymes, hormones, receptors, antibodies and are required for the structural integrity of cells.

Protein structure

Proteins are organic compounds made of "**amino acids**" joined together by "**peptide linkages**".

Essential and non-essential amino acids:

There are 20 standard amino acids which differ in their side chain (**R**). Some of them are considered "**essential**" since they cannot be synthesized in our body and must be therefore provided in the diet (e.g. tryptophan & phenylalanine), while others are "**non-essential**" and can be synthesized in the body (e.g. alanine & cysteine).

Amphoteric nature of amino acids:

As amino acids have both an "**amino**" gp and a "**carboxylic**" gp, they are considered as both "**base**" and "**acid**", i.e. they are amphoteric.

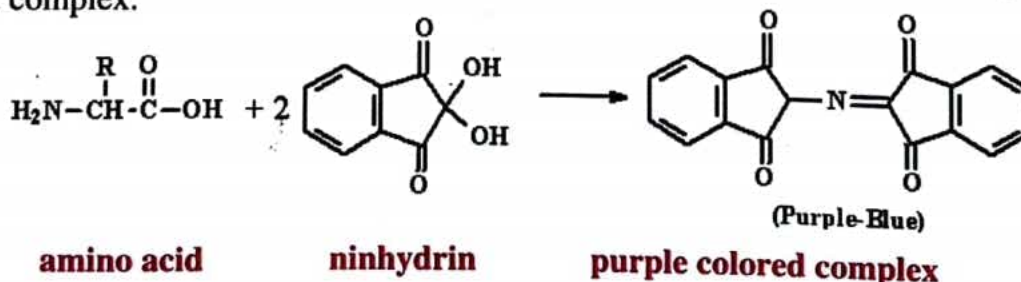
At a certain pH, the amino group can become protonated gaining a positive charge, and the acid group can become deprotonated gaining a negative charge. The resulting doubly charged ion is known as "**zwitterion**".

Practical

Using the provided solutions of **albumin** (egg white), **casein** (milk protein) and **gelatin** (animal collagenous material), perform the following:

A. General tests**B. Color reactions****C. Precipitation reactions****A. General tests for proteins****1. Ninhydrin reaction:****Principle:**

Ninhydrin reacts with amino acids in proteins at high temperature giving a purple colored complex.



Ninhydrin is most commonly used as a forensic chemical to detect "**fingerprints**", as amines left over from proteins sloughed off in fingerprints react with ninhydrin giving a characteristic purple color.

Procedure & observation:

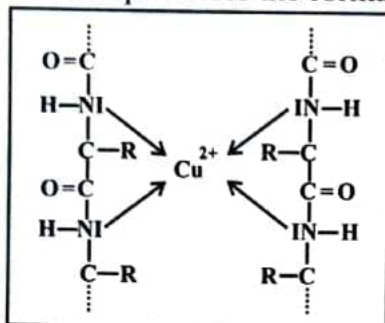
- To 1 ml amino acid solution in a test tube, add 1 drop of ninhydrin.

Put in a boiling water bath and observe the formation of a purple color.

2. Biuret test:

Principle:

The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a blue to violet complex known as the "**biuret complex**". **N.B.** Two peptide bonds at least are required for the formation of this complex.



Biuret complex

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 3 drops of 10% sodium hydroxide solution and 3-6 drops of 0.5% copper sulfate solution.
- Mix well; a blue to violet color is obtained with albumin, casein & gelatin.

B. Color reactions of proteins

1. Reduced sulfur test:

Principle:

Proteins containing sulfur (in **cysteine** and **cystine**) give a black deposit of lead sulfide (PbS) when heated with lead acetate in alkaline medium.

Procedure & observation:

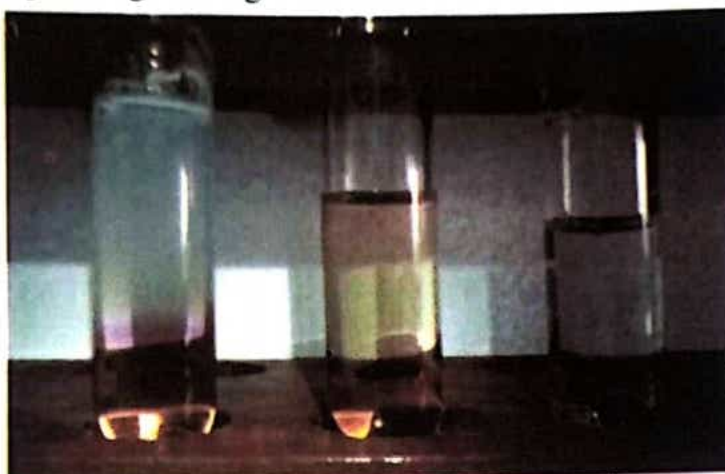
- To 1 ml of protein solution in a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate.

Mix well and put in a boiling water bath for few minutes; a black deposit is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.



Procedure & observation:

- To 1 ml of protein solution in a test tube, add 1 ml of Hopkins-Colé reagent and mix well.
- Incline the test tube and slowly add 1 ml of concentrated H_2SO_4 on the inner wall of the test tube to form 2 layers.
- Put the test tube in a boiling water bath for 2 minutes.
- A reddish violet ring is formed at the junction between the 2 layers with albumin and casein; gelatin gives negative results.

**C. Precipitation reactions of proteins**

Precipitation of proteins by heavy metals and alkaloidal reagents indicates the presence of both negative and positive charges and hence the **amphoteric** nature of proteins.

1. Precipitation by heavy metals:**Principle:**

Heavy metals (e.g. Hg^{2+} , Pb^{2+} , Cu^{2+}) are high molecular weight cations. The positive charge of these cations counteracts the negative charge of the carboxylate group in proteins giving a precipitate.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add 1 drop of lead acetate; a white ppt is obtained.
- To 1 ml of protein solution in a test tube, add 1 drop of 10% copper sulfate; a blue ppt is obtained.

**2. Precipitation by alkaloidal reagents:****Principle:**

Alkaloidal reagents (e.g. **tannate** & **trichloroacetate**) are high molecular weight anions. The negative charge of these anions counteracts the positive charge of the amino group in proteins giving a precipitate.

Procedure & observation:

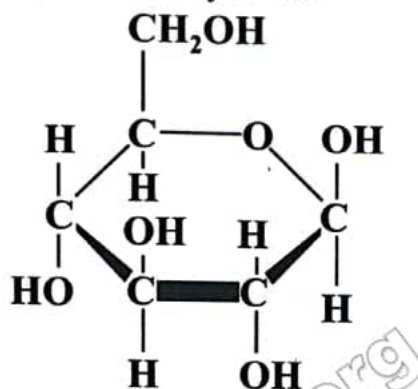
- To 1 ml of protein solution in a test tube, add tannic acid drop wise until a buff ppt is obtained.
- To 1 ml of protein solution in a test tube, add 1 ml of trichloroacetic acid (TCA);

Observation: While ppt is obtained.

Carbohydrates

To characterize carbohydrates present in an unknown solution on the basis of various chemical assays.

Carbohydrates are polyhydroxy aldehydes and ketones or substances that hydrolyze to yield polyhydroxy aldehydes and ketones. Aldehydes ($-CHO$) and ketones ($=CO$) constitute the major groups in carbohydrates.



Carbohydrates are mainly divided into monosaccharides, disaccharides and polysaccharides. The commonly occurring monosaccharides includes glucose, fructose, galactose, ribose, etc. The two monosaccharides combine together to form disaccharides which include sucrose, lactose and maltose. Starch and cellulose fall into the category of polysaccharides, which consist of many monosaccharide residues.

Materials Required:

- | | | |
|---------------|---------------|---------------------|
| 1) Glassware | 2) Test-tubes | 3) Test-tube-holder |
| 4) Water-bath | 5) Spatula | 6) Dropper |

Reagents Required:

- | | | |
|----------------------------------|-----------------------------------|-----------------------|
| 1) Molisch's-Reagent | 2) Iodine-solution | 3) Fehling's-reagentA |
| 4) Fehling's-reagentB | 5) Benedict's-qualitative-reagent | |
| 6) Barfoed's-reagent | 7) Seliwanoff's-reagent | 8) Bial's-reagent |
| 9) Phenylhydrazine-hydrochloride | | 10) Sodium-acetate |
| 11) Glacialacetic-acid | 12) Glucose,fructose | 13) Microscope |

1) Molisch's Test: (General test for carbohydrates)**Principle:**

This is a common test for all carbohydrates larger than tetroses. The test is on the basis that pentoses and hexoses are dehydrated by conc. Sulphuric acid to form furfural or hydroxymethylfurfural, respectively. These products condense with α -naphthol to form purple condensation product

Procedure:

In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of α -naphthol solution. Carefully incline the tube and pour dropwise conc. H_2SO_4 , using a dropper, along the sides of the tube.

Observation:

Violet colour at the junction of the two liquids:

Inference:

The sample contain carbohydrates

Molich's Test

- Carbohydrate is detected by Molisch's Test

2) Fehling's Test**Principle:**

This forms the reduction test of carbohydrates. Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or red cuprous oxide and is precipitated. Hence, formation of the yellow or brownish-red colored precipitate helps in the detection of reducing sugars in the test solution.

Procedure:

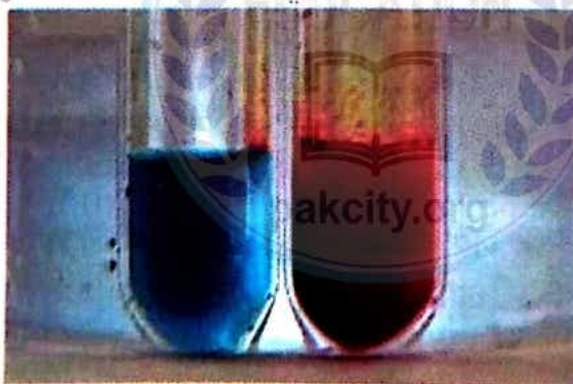
In a test tube, add 2 ml of the test carbohydrate solution and add equal volumes of Fehling A & Fehling B and place it in a boiling water bath for few minutes.. When the content of the test tube comes to boiling, mix them together and observe any change in color or precipitate formation.

Observation:

The production of yellow or brownish-red precipitate of cuprous oxide

Inference:

Indicates the presence of reducing sugars in the given sample.

**3) Benedict's Test: (Test for monosaccharides)****Principle:**

As in Fehling's test, free aldehyde or keto group in the reducing sugars reduce cupric hydroxide in alkaline medium to red colored cuprous oxide. Depending on the concentration of sugars, yellow to green color is developed. All monosaccharides are reducing sugars as they all have a free reactive carbonyl group. Some disaccharides, like maltose, have exposed carbonyl groups and are also reducing sugars, but less reactive than monosaccharides

Procedure:

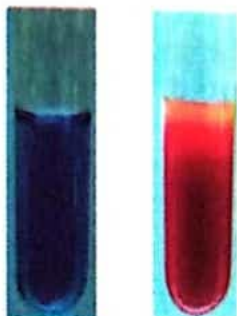
In the test tube with 2 ml of Benedict's reagent, add 5-6 drops of the test carbohydrate solution and mix well. Place the test tube in a boiling water bath for 5 minutes and observe any change in color or precipitate formation.

Observation:

Cool the solution. Observe the colour change from blue to green, yellow, orange or red depending upon the amount of reducing sugar present in the test sample.

Inference:**BENEDICT'S TEST****Procedure**

- Take 5 ml of Benedict's reagent.
- Add 8 drops of carbohydrate solution.
- Boil over a flame or in a boiling water bath for 2 minutes.
- Let the solution cool down.



(Negative Reaction) (Positive Reaction)

Benedict's Test Results

Blue solution Green / yellow ppt Orange red ppt Brick red ppt

4) Barfoed's Test: (Test for monosaccharides)**Principle:**

Barfoed's test is used to detect the presence of monosaccharide (reducing) sugars in solution. Barfoed's reagent, a mixture of ethanoic (acetic) acid and copper(II) acetate, is combined with the test solution and boiled. A red copper(II) oxide precipitate is formed which indicates the presence of reducing sugar. The reaction will be negative in the presence of disaccharide sugars because they are weaker reducing agents. This test is specific for monosaccharides. Due to the weakly acidic nature of Barfoed's reagent, it is reduced only by monosaccharides.

To 2 mL of the test solution add about 2-3 mL of Barfoed's reagent. Mix it well and boil it for one minute in the water bath and allow to stand for a few minutes.

Observation:

Formation of a red precipitate of cuprous oxide in the bottom and along the sides of the test tube immediately.

Inference:

Monosaccharides answer this test. Since Barfoed's reagent is slightly acidic, this test is specific for monosaccharides.

Barfoed's Test**Procedure:**

- To 2 ml of Barfoed's Reagent, add 2 ml of carbohydrate solution.
- Keep the test tubes in the boiling water bath for 3 minutes.
- Cool under running water.
- Over-heating should be avoided.



A scanty brick red precipitate is observed in a positive reaction.

5) Seliwanoff's Test: (Test for Ketoses)

Principle:

It is a color reaction specific for ketoses. When conc: HCl is added. Ketoses undergo dehydration to yield furfural derivatives more rapidly than aldoses. These derivatives form complexes with resorcinol to yield deep red color. The test reagent causes the dehydration of ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural reacts with resorcinol present in the test reagent to produce a red product within two minutes (reaction not shown). Aldohexoses reacts so more slowly to form the same product.

Procedure:

To 2 mL of Seliwanoff's reagent, add two drops of test solution. The mixture is heated to just boiling.

Observation:

A cherry red condensation product will be observed.

Inference:

Indicating the presence of ketoses in the test sample. There will be no significant change in colour produced for aldose sugar.

Seliwanoff's Test

Procedure:

- To 3 ml of Seliwanoff reagent add 1ml of fructose.
- Boil for 30 seconds only.
- Cool the solution.



A cherry red colour is observed in a positive reaction.

6) Bial's Test: (Test for pentoses and hexoses)

Principle:

Bial's test is used to distinguish between pentoses and hexoses. They react with Bial's reagent and are converted to furfural. Orcinol and furfural condense in the presence of ferric ion to form a colored product. Appearance of green colour or precipitate indicates the presence of pentoses and formation of muddy brown precipitate shows the presence of hexoses.

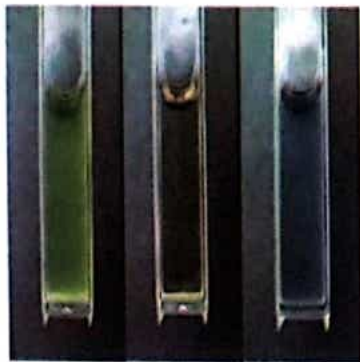
Procedure:

To 5 ml of Bial's reagent add 2–3 mL of test solution and warm gently in a hot water bath for 2 minutes .

Observation and Inference:

The formation of a bluish green product is indicative of pentoses. Hexoses generally react to form muddy brown products.

Bial's Test



- The formation of a bluish product.
- All other colors indicate a negative result for pentoses.
- Note that hexoses generally react to form green, red, or brown products.

7) Iodine Test: (Test for starch)

Principle:

This test is used for the detection of starch in the solution. The blue-black colour is due to the formation of starch-iodine complex. Starch contain polymer of α -amylose and amylopectin which forms a complex with iodine to give the blue black colour

Procedure:

Add 2 drops of iodine solution to about 2 mL of the solution.

Observation:

A blue-black colour is observed

Inference:

Which is indicative of presence of polysaccharides.



8) Osazone Test:(Test for glucose, fructose)

Principle:

The ketoses and aldoses react with phenylhydrazine to produce a phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone. Needle-shaped yellow osazone crystals are produced by glucose, fructose and mannose, whereas lactosazone produces mushroom shaped crystals. Crystals of different shapes will be shown by different osazones. Flower-shaped crystals are produced by maltose.

Procedure:

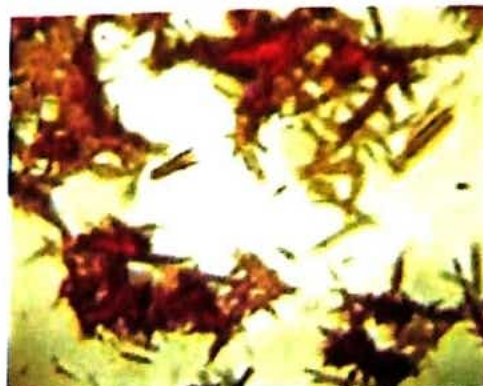
To 0.5 g of phenylhydrazine hydrochloride add 0.1 gram of sodium acetate and ten drops of glacial acetic acid. Add 5 mL of test solution to this mixture and heat under boiling water bath for about half an hour. Cool the solution slowly and examine the crystals under a microscope.

Observation and Inference:

Needle-shaped yellow osazone crystals will be observed for glucose and fructose, whereas lactosazone shows mushroom shaped and maltose produces flower-shaped crystals.

OSAZONE TEST

Needle shaped glucosazone crystals as viewed under the microscope



Galactosazone crystals as viewed under the microscope (Rhombic plates)



Sun flower shaped Maltosazone crystals as viewed under the microscope



Powder puff/hedge hog shaped crystals of lactose as viewed under the microscope

Lipids:

Chemically fats and oils are trimesters of glycerol and higher fatty acids. They are of animal or plant origin. Desi ghee is animal ghee while vanaspati ghee is vegetable ghee. Fats are solids while oils are liquids at ordinary temperature. Fats and oils may be saturated or unsaturated.

Saturated fat

Saturated fats contain only single bonds within the carbon chain. Saturated fats are of animal origin and are usually present in solid form. It increases the blood cholesterol level. Some examples are meat fat, butter etc. Coconut oil and palm oil also contain saturated fat.

Unsaturated fat

Unsaturated fats contain double bonds within the carbon chain. Unsaturated fat is found in fish like salmon and tuna, nuts, seeds etc.

General Test for Lipid:**1. Sudan III test Procedure:****Procedure:**

Take 0.5 ml ether or chloroform in a test tube and add 0.5 ml sample—drop by drop till the sample is fully dissolves. Add one drop of Sudan III reagent.

Observation:

Red colour appears.

**Inference:**

The sample contains fat.

2. Acrolein test:**Procedure:**

Take 0.5gm powdered sodium bi-sulphate (NaHSO_4) or potassium bi-sulphate (KHSO_4) in a clean dry test tube, add 3 to 4 drops of sample. Mix thoroughly and heat.

Observation:

An irritating smell of acrolein is felt.

Inference:

Sample contains fat.

3. Solubility Test for Lipid:**Procedure:**

Take five test tubes marking A, B, C, D, E. Put 5 ml—water, absolute alcohol, ether, chloroform and benzene one in each test tube. Add 3 to 4 drops of sample in each test tube, shake thoroughly, allow to stand.

Observation:

- (1) In test tube A drops of oils are seen floating on the surface of water,
- (2) In test tube B oil drops settle at the bottom of alcohol,
- (3) In test tubes C, D and E the sample is mixed.

Inference:

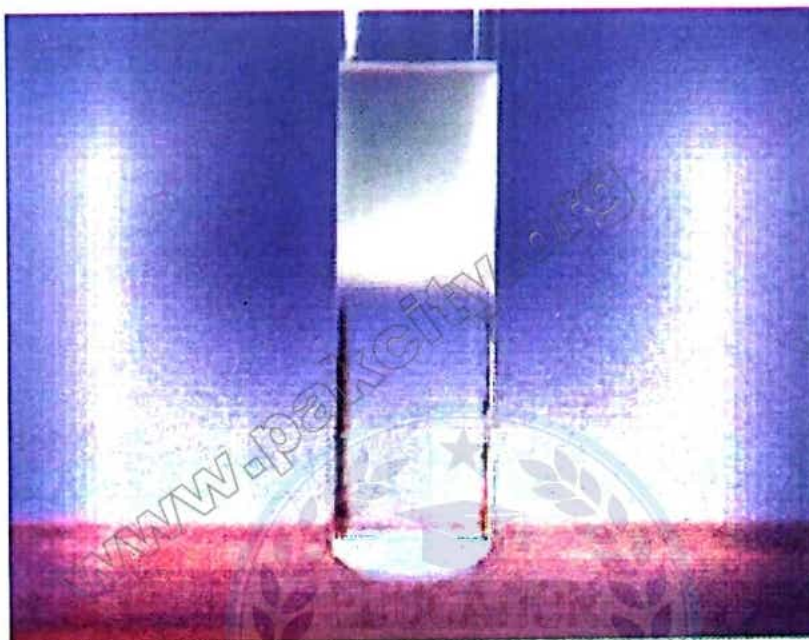
The sample contains fat, as it is not soluble in water (test tube A) but soluble only in organic solvents (test tube B, C, D, E) and sinks to the bottom in alcohol (B).

4. Emulsification Test for Lipid:**Procedure:**

Take 3 ml sample in a test tube, add 2 drops of oleic acid, shake well. Add 2 drops of the mixture to another test tube containing 3 ml 10% caustic soda.

Observation:

Emulsion is formed (acid neutralize alkali forming soap).

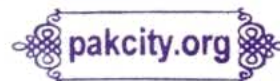
**Inference:**

The test sample contains fat.

2

Experiment

STUDY OF STARCH BREAKDOWN IN GERMINATING GRAM SEEDS

**Digestion:**

The breakdown of larger molecules into smaller molecules is called digestion.

Materials:

Test tubes, Test tube rack, Petri dish, Mortar with pestle, Filter paper, 1% iodine solution, 1% starch solution and Benedict solution.

Procedure:**Extraction of Enzymes from Gram Seeds:**

1. The germinating moist seeds are taken in mortar.
2. The seeds are ground with pestle. Now the mortar is filled with distil water.
3. The mixture is filtered with filter paper. The mixture obtained by filtration is called filtrate. This filtrate contains many enzymes like **diastase**. Half of this filtrate is boiled in a test tube.

Preparation of Test Tubes:

Two test tubes are taken. They are marked as A and B. Following substances are added in each tube:

Tube A:

2 ml starch solution + One drop of iodine solution + 2 ml of un-boiled filtrate.

Test B:

2 ml starch solution + One drop of iodine solution + 2 ml of boiled filtrate.



Negative Test
Tube A



Positive Test
Tube B

Observations:

After sometimes, blue colour of iodine disappears in test tube A. But it does not disappear in tube B. Now Benedict test is performed on both tubes.

Test tube	Result of iodine test	Benedict test	Result	Conclusion
Test tube A	Blue colour of iodine disappear	Add Benedict reagent	Red ppt.	Starch is digested
Test tube B	Blue colour of iodine does not disappear	Add Benedict reagent	No test	Starch is not digested

Result:

The disappearance of blue colour and red ppt. with Benedict solution indicates that digestion has taken place in tube A. Thus enzymes in test tube A are active. But the results of test tube B show that digestion does not take place in test tube B. Thus their enzymes are inactive.

VIVA VOCE



Q.1 Why are germinating seeds taken for extraction of enzyme?

Ans. The germinating seed breaks its stored starch into glucose. It obtains energy from it. So these seeds have a large number of digesting enzyme.

Q.2 Name a major enzyme present in germinating seed for digestion of starch.

Ans. Diastase enzyme.

Q.3 Why does the colour of iodine disappear in the tube A?

Ans. The tube A has active enzymes. They digest the starch into glucose. As iodine give blue colour only with starch. Therefore, its colour disappears.

Q.4 Why does the colour of iodine not disappear in the tube B?

Ans. The tube B has heat killed inactive enzymes. They cannot digest the starch into glucose. Therefore, the colour of iodine does not disappear.

Q.5 How enzymes become inactive by boiling or heating?

Ans. Heat increase vibrations in the enzyme. These vibrations denature the enzyme and it becomes inactive.

Q.6 What colour does Benedict's solution give with glucose and starch?

Ans. It gives green, yellow or red colour with glucose. It remains unchanged with starch.

Q.7

Ans. The seed digest its stored starch and change into glucose. This glucose is a source of energy for germinating seed.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Demonstrate the starch breakdown in germinating gram seeds.

Performance = 1, Apparatus = 1, Procedure = 1, observation and result = 1, Short question = 1/2 + 1/2 = 5.

(Multan Board 2004)

Q.1 Name the enzyme involved in starch breakdown in seed.

Ans. Diastase

Q.2 Which enzyme does involve in starch breakdown in oral cavity?

Ans. Amylase or amylopsin.

Experiment 2: Demonstrate the starch breakdown in germinating gram seeds.

(D.G. Khan Board 2003).

Q.1 Name the enzyme involved in starch breakdown in seed.

Ans. Diastase

Q.2 What is chemical nature of this enzyme?

Ans. Proteins



(2)

Enzyme



3

Experiment

STUDY OF EFFECTS OF TEMPERATURE, PH, ENZYME AND SUBSTRATE CONCENTRATION ON ENZYME ACTIVITY

PART A

EFFECTS OF TEMPERATURE ON ENZYME ACTIVITY

The rate of enzyme action increases with the increase of temperature. The reaction rate is doubled after every rise of 1°C in temperature. But very high temperature denature the enzyme. Therefore, rate of reaction is decreased at high temperature.

Materials:

5 test tubes, Test tube rack, Pepsin, Petri dish, Water bath, Blotting paper, Incubator, Thermometer, Toluene, Egg and Bunsen burner.

Procedure:

1. An egg is broken. Its egg white (albumen) is removed carefully. It is placed in Petri dish. The albumin is heated. Therefore, it coagulates and becomes solid. Remaining water from it is removed by blotting paper.
2. The coagulated albumin is cut into five squares. The size of each square should be 1 mm.
3. Now five test tubes are taken. One piece of the egg albumen is put in each of the tube.
4. 5 ml pepsin and 10 drops of 0.1 molar solution of HCl are added in these tubes. Two drops of toluene are added in each tube. It retards bacterial growth.
5. These tubes are put in separate five incubators. The temperature of the incubators is at 10°C ,

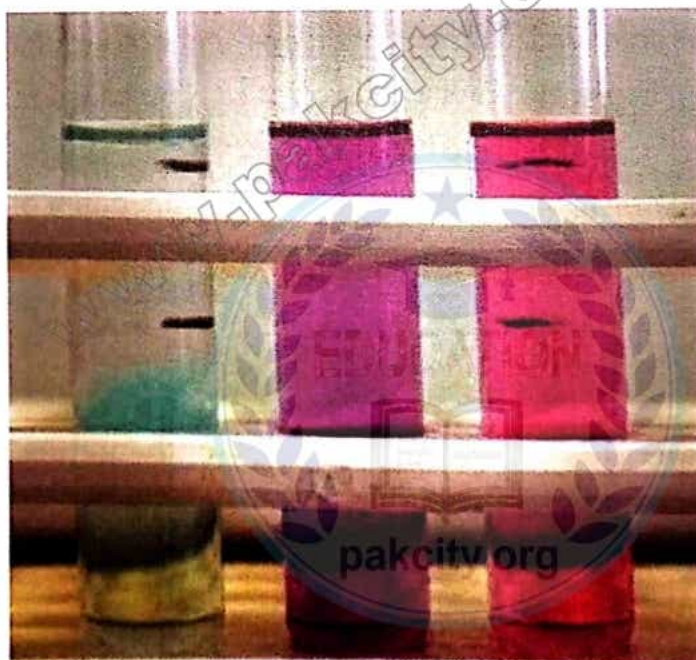


The test tubes are removed from the incubator after 4 or 5 hours. Biuret tests are performed on these test tubes to determine the presence or absence of proteins.

Number of test tube	Temperature at which it was kept in incubator	Biuret test	Result of the test	Conclusion
1.	10°C	Red colour	Protein is present	No digestion
2.	20°C	Pink colour	Protein is present	Slight digestion
3.	30°C	Violet or light pink colour	20% protein is present	80% digestion
4.	37°C	Colour is not changed	Protein absent	100% digestion
5.	50°C	Red colour	Protein is present	No digestion

Result:

The above data show that the rate of action of pepsin increases with the increase of temperature. It is most active at optimum temperature 37°C. But it is denatured and becomes inactive at 50°C.



Colour changes in the solution.

They range from no colour change (blue) to pink to deep violet.

<p>OBSERVATIONS</p> <ul style="list-style-type: none"> No change (solution remains blue) The solution turns from blue to violet (deep purple) The solution turns from blue to pink 	<p>INTERPRETATION</p> <ul style="list-style-type: none"> Proteins are not present Proteins are present Peptides are present (Peptides or peptones are short chains of amino acid residues)
--	--

PART B

EFFECTS OF pH ON ENZYME ACTIVITY

pH directly affects the enzyme activity. Every enzyme has **optimum pH**. It works efficiently at this pH. Any change in pH causes ionization of enzyme and hence break it. So reaction rate is retard.

Material:

Albumen of egg, Petri dish, 5 test tubes, Knife, Incubator, 0.5% pepsin solution, 0.1 M HCl and 0.1 M NaOH.

Procedure:

The white of egg is heated. It is coagulated. The coagulated albumen is cut in into 5 pieces. The five pieces are put in into five test tubes. 15 ml of pepsin is added in each tube. These tubes are labelled as A, B, C, D and E. Now the pH of these tubes is adjusted by following ways:

- 1. Tube A: Alkaline with pH 9:** 10 drops of 0.1 M NaOH are added.
- 2. Tube B: Slightly alkaline with pH 8:** 5 drops of 0.1 M NaOH are added.
- 3. Tube C: Neutral with pH 7:** 10 ml water is added.
- 4. Tube D: Slight acidic with pH 5:** 5 drops of 0.1 HCl is added.

These tubes are placed in incubator at 37°C.

- 5. Tube E: Acidic with pH 2:** 10 drops of 0.1 HCl is added.

Observation:

After 3 hours Biuret test is performed on each test tube. It gives following results:

Test tube	pH	Biuret test	Result of the test	Conclusion
Test tube A	9	Red ppt	Protein is present	No digestion
Test tube B	8	Red ppt	Protein is present	No digestion
Test tube C	7	Red ppt	Protein is present	No digestion
Test tube D	5	Pink ppt	Protein is present	Partial digestion
Test tube E	2	Unchanged	Protein is absent	Complete digestion

Result:

The observation shows that pepsin enzyme work in acidic condition. Its optimum pH is 2.

Note:

The antilog of hydrogen ion concentration is called pH. It means more hydrogen ions (H⁺) less pH and lesser hydrogen ions more pH. Acids have more hydrogen ions. So they have less pH. The bases have less hydrogen ion so they more pH.

Acidic = pH 1 to 6.9

Neural = pH 7 to 7.4

Alkaline = 7.5 to 14

PART C

EFFECTS OF ENZYME CONCENTRATION ON ENZYME ACTIVITY

The rate of reaction is increased with the increase of concentration of enzyme. There should be unlimited amount of substrate and temperature should be optimal.

Material:

Albumen of egg, Petri dish, 5 test tubes, Knife, Incubator, 0.5% pepsin solution and 0.1 M HCl.

Procedure:

The white of egg is heated. It is coagulated. The coagulated albumen is cut in into 5 pieces. The five pieces are put in five test tubes. Five drops of 0.1 M HCl is added in each tube. These tubes are labelled as A, B, C, D and E. Now 15 ml pepsin is added into each tube with following concentrations:

1. **Tube A:** 0.1% pepsin
2. **Tube B:** 0.5% pepsin
3. **Tube C:** 1% pepsin
4. **Tube D:** 1.5% pepsin
5. **Tube E:** 2% pepsin

These tubes are placed in incubator at 37°C.

Observation:

Biuret test is performed on each tube after 3 hours. It gives following results:

Test tubes	Pepsin concentration	Biuret test	Conclusion
Test tube A	0.1%	Red colour	Slight or no digestion
Test tube B	0.5%	Pink colour	Slight digestion
Test tube C	1%	Light pink colour	50% digestion
Test tube D	1.5%	Violet colour	80% digestion
Test tube E	2%	Unchanged	Complete digestion

Biuret test is performed on each tube after 3 hours. It gives following results:

Result:

The observations show that rate of activity of pepsin increases with the increased of enzyme concentration. This rate becomes maximum at 2% concentration of pepsin.

PART D**EFFECTS OF SUBSTRATE CONCENTRATION ON ENZYME ACTIVITY**

The enzyme activity increases with the increase of substrate concentration. But the amount of enzyme should be unlimited.

Material:

Albumen of egg, Petri dish, 5 test tubes, Knife, Spirit lamp, Incubator, 0.5 pepsin solution and 0.1 M HCl.

Procedure:

Five drops of 0.1 M HCl and 15 ml 0.5% pepsin are added in each tube. These tubes are labelled as A, B, C, D and E. The white of egg is heated. It is coagulated. The coagulated albumin is cut in into 10 pieces. The pieces are put in the test tube in following ways:

- | | |
|--------------------------------|-------------------------------|
| 1. Tube A: One piece | 2. Tube B: Two pieces |
| 3. Tube C: Three pieces | 4. Tube D: Four pieces |
| 5. Tube E: Five pieces | |

These tubes are placed in incubator at 37°C.

Observation:

After three to five hours, the tubes are removed from the incubator. Biuret test is performed in each test.

Test tubes	Number of egg pieces	Biuret test	Conclusion
Test tube A	1	Unchanged	Complete digestion
Test tube B	2	Unchanged	Complete digestion
Test tube C	3	V. light pink	80% digestion
Test tube D	4	Light pink colour	50% digestion
Test tube E	5	Red colour	No digestion

Result:

The Biuret test shows that complete digestion take place that same amount of enzyme can digest a large amount of proteins but on saturation of enzyme there is no further increase in rate of reaction..

VIVA VOCE



- Q.1** Why does the reaction rate increases with the increase of temperature up to optimum temperature?
- Ans.** Heat provides activation energy. Therefore, enzymes increase the catalytic activity.
- Q.2** What is optimum temperature of enzymes?
- Ans.** The temperature at which enzyme work efficiently is called optimum temperature.
- Q.3** What is optimum temperature of enzymes for man?
- Ans.** The normal body temperature of man 37C or 98.6F is the optimum temperature for the enzymes of man.
- Q.4** Why does reaction rate drop at high temperature?
- Ans.** High temperature destroys the globular structure of enzyme. It denatures enzyme. Thus rate of reaction is dropped.
- Q.5** Why do we always use HCl with pepsin?
- Ans.** Pepsin enzyme always works in acidic medium. Therefore, it is necessary to used HCl with pepsin.
- Q.6** Why do we use egg white in the experiment? Can we use egg yolk as well?
- Ans.** The egg white has protein called albumen. But egg yolk contains mostly lipids. As pepsin acts only on protein, so we use egg white in the experiments.
- Q.7** What is pH?
- Ans.** The antilog or negative log of hydrogen ion concentration is called pH.
- Q.8** What is range of pH for acidic solution?
- Ans.** The range of pH for acidic solution is 1 to 7.
- Q.9** What is neutral pH?
- Ans.** The neutral pH is 7 to 7.4.
- Q.10** What is range of alkaline pH?
- Ans.** The range of alkaline pH is 7.4 to 14.

Q.11 What is optimum pH of pepsin?

Ans. The optimum pH of pepsin is 2.

Q.12 Can pepsin work in intestine?

Ans. No, it cannot work in intestine because the pH of intestine is alkaline and pepsin can work only in acidic environment.

Q.13 What does happen with enzyme with the change of pH?

Ans. The change of pH causes ionization of enzyme. Now its active site cannot recognize its substrate. Further change in pH may destroy globular structure of enzyme.

Q.14 Name tests which are used for identification of proteins.

Ans. Million's test and Biuret test.

Q.15 Why does reaction rate increases with the increase of enzyme concentration?

Ans. The increase of concentration of enzymes increases the number of active sites of the enzymes. Therefore, rate of reaction is increased.

Q.16 What is the affect of substrate concentration on enzyme action?

Ans. The rate of reaction increases with the increase of substrate concentration when the amount of enzyme is unlimited.

Q.17 What is the use of toluene in these experiments?

Ans. Toluene retards the bacterial growth. Bacteria can destroy enzyme.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Demonstrate the effect of temperature on enzyme action.

(Performance = 1, Apparatus = 1, Procedure = 1, Observation and result = 1, Short question = 1/2 + 1/2 = 5)

(Bahawalpur Board 2004)

Q.1 What is an enzyme?

Ans. Enzymes are biological catalysts which speed up chemical reactions in the cells.

Q.2 What happens with enzyme at high temperature?

Ans. High temperature causes denaturation of enzyme.

Experiment 2: Demonstrate the effects of enzyme concentration on enzyme action.

(Multan Board 2003)

Q.1 Name some enzymes present in pancreases.

Ans. Trypsin, lipase and amylase.

Q.2 How does reaction rate increases with the increase of enzyme concentration?

Ans. At high concentration, more active sites are available for enzyme action.

(3) Microbiology



4

Experiment

LABORATORY SAFETY TECHNIQUES & USE OF MICROSCOPE
& MEASUREMENT OF MICROSCOPIC
OBJECTS BY MICROMETRY

The laboratory is a place where the students must remain very vigilant in order to avoid any mishap. Furthermore, the safety of all those working in the laboratory must be ensured. The following instructions must be strictly followed in this regard.

- Note and follow all the warning signs on the chemical containers.



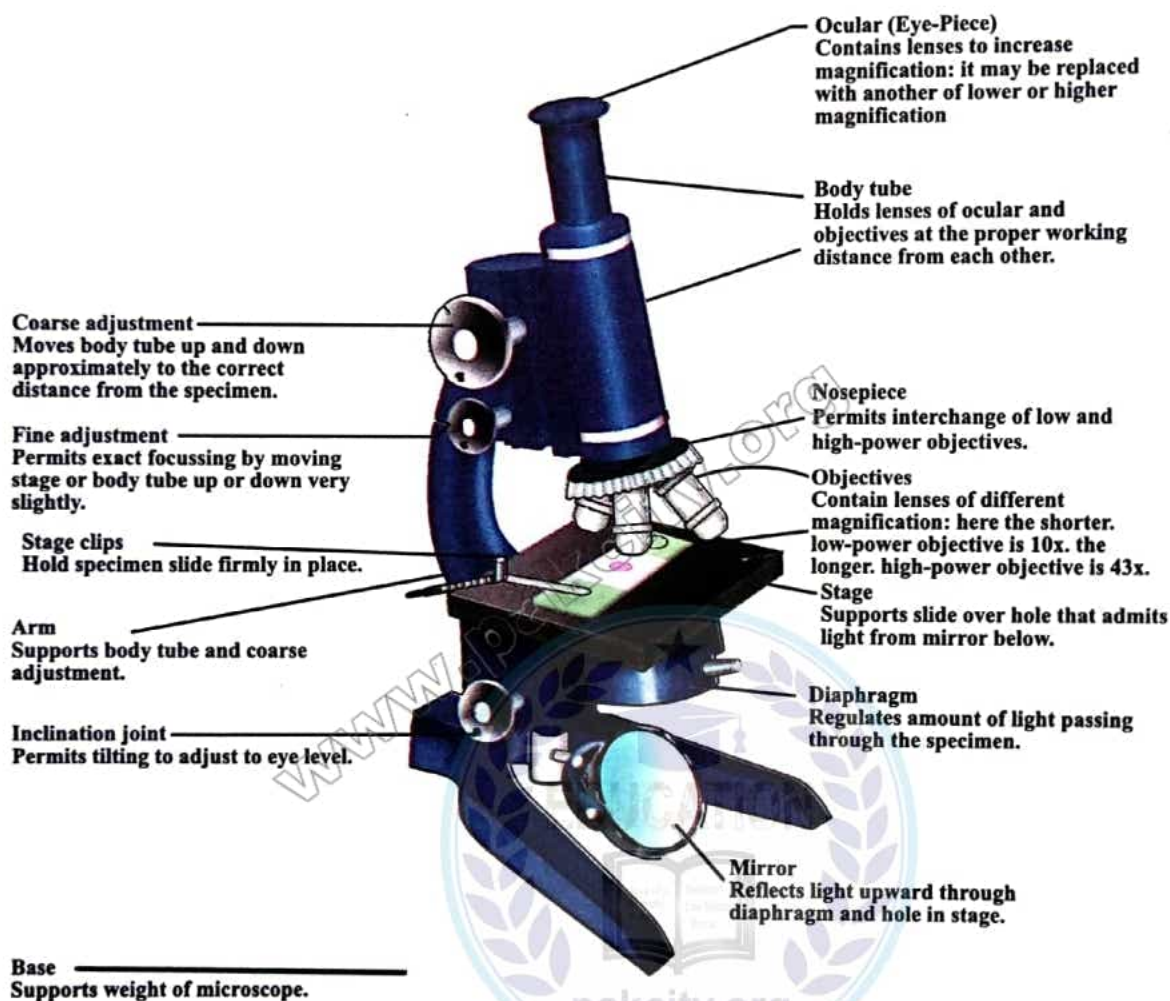
Fig. Signs for Hazardous Substances

- Wear a laboratory coat at all times.
- Avoid eating, drinking and smoking in the laboratory.
- Be careful with glassware.
- Do not use chipped or cracked glassware, it may break on heating or under slight strain.
- Treat all chemicals as hazardous.
- Must know what you are doing before you start the work.
- Work in a tidy manner and clean up afterwards.
- Heating anything with burner has its risks. Make sure to hold test tubes so that they point away from you and anyone else.
- Follow the instructions strictly e.g., heat gently, pour acid along the wall of the test tube, add drop by drop etc.
- Use pipette very carefully, particularly while sucking acids or use pipette filters.
- Report all accidents and dangers to the staff.
- Use water baths for heating flammable chemicals.
- Always wash your hands before leaving the laboratory.

PART-A

THE COMPOUND MICROSCOPE

The compound microscope is a very significant scientific tool for making biological studies. It is an instrument which is used to see and study such small objects which are otherwise invisible. Different types of microscopes with different magnifications are available but the most common one is the 'Monocular compound microscope' which magnifies upto 1,000 times. The most modern and the best available microscope is the "Electron microscope" which can magnify the objects upto 100,000 times. The power of a microscope to magnify the objects is known as its 'Magnification'.



PARTS OF A MONOCULAR COMPOUND MICROSCOPE

1. Ocular (Eye-Piece):

It is the part through which the observer looks at the specimen. It consists of a tube containing one lens which magnifies the image of the object being studied. The oculars may be of different powers such as 5X, 10X which means that different eyepieces can magnify the object to different extents. An eyepiece of 5X power (magnification) will magnify the image of an object 5 times and so on. The oculars of different magnifications can be used according to needs.

2. Body Tube:

It is a metallic tube which serves to keep eyepiece at its upper end and objects which are screwed to a revolving nose-piece, at its lower end. The body tube maintains a proper working distance between eyepiece and objective lenses.

3. Revolving Nose-Piece:

It is the circular projecting part present below the body tube. It can be rotated either way and permits interchange of objectives of different powers (magnifications).

4. Screws for Coarse Adjustment:

These are a pair of screws or knobs of large size. They move the body tube (or the stage in some microscopes) up and down for rough focussing of the object under low power. These screws should never be used while focussing under high power.

5. Screws for Fine Adjustment:

These are a pair of screws of smaller size situated below the larger ones. They also move the body tube (or in some microscopes, the stage) very slightly and permit exact and sharp focussing.

6. Objectives:

These are the metallic tubes containing lenses of different magnifications. These may be one, two or three in number. The magnifying power of each objective is engraved on it.

The magnifying power of the microscope can be calculated for different sets of eyepieces and objectives by just multiplying the powers of the eyepiece and the objective, being used at the time of study. For example, if the power of the eyepiece is 10X and that of the objective is 60X; the total magnification of the microscope shall be $10X \times 60X = 600X$ i.e., the microscope shall magnify the image 600 times the size of the object.

7. Stage:

It is the horizontal circular or squarish platform on which the glass slide carrying the object is placed for study. It has a hole in the centre which admits light coming from the mirror. The light must pass through the object for distinct vision.

8. Stage Clips:

Two clips are movably fixed on the stage for holding the slide firmly in place and prevent it from slipping away when the object is being studied through the eyepiece.

9. Diaphragm:

It is attached below the stage-hole and serves to regulate the amount of light passing through the object. Some microscopes are provided with a condenser (with an adjustable iris diaphragm) while others have a round plate-like diaphragm with holes of different sizes to achieve the purpose.

10. Mirror:

A movable plano-concave mirror is fixed some distance below the stage. It can be moved sideways, upwards or downward to focus and reflect light upward through the diaphragm, stage hole, objective and then to the eyepiece.

12. The Arm:

It is the curved metallic rod of the microscope which is fixed at the base at the inclination joint and supports the body tube and the screws for coarse and fine adjustment above. It is the part which is used for handling the microscope.

13. The Base (Foot):

It is the horseshoe-shaped basal heaviest part which supports the entire weight of the microscope.

Magnification of Microscope:

The numbers of times which a microscope increases an object as compared to its original size is called magnification of microscope. It is also called power of microscope. It is obtained by multiplying the power of ocular with power of objective. For example, if power of ocular is 10X and power of objective is 100X, and then magnification will be $10 \times 100 = 1000$. It means that this microscope can increase the object by 1000 times.

Procedure of Focusing Light:

1. Microscope is placed on a table.
2. The specimen (slide) is placed on the stage. It is held in its position by clips.
3. At start microscope is kept at low power.
4. Now light is focused on object by revolving the mirror. At one point there is maximum light available.
5. Coarse and fine adjustments are used to increase the visibility of specimen.
6. Now the specimen is observed at high and low power.

Care of the Microscope:

Following precautionary measures should be taken during use of microscope:

1. It should be lifted by placing one hand under the base. Other hand is used to hold the arm of the microscope.
2. It should not be placed near the edge of the table.
3. Mirror should be handled carefully. It may fall and break into pieces.
4. Clean the lens with lens paper before use.
5. Slide should be firmly held by the clips.
6. Microscope should be kept in its proper box.

PART B

MEASUREMENT OF MICROSCOPIC OBJECTS BY MICROMETRY

Material:

Compound microscope, ocular micrometer, stage micrometer, prepared slide.

Procedure:

1. Remove the eye piece from microscope and unscrew it. Insert ocular micrometer in eye piece diaphragm. Screw the eye-piece back and insert it in microscope.
2. Place stage micrometer on microscope stage and focus it first using the lower power and then high magnification power.
3. Adjust the ocular micrometer lines parallel with those of stage micrometer lines.
4. Make the zero lines of both micrometer coincide.
5. Now find that which line of ocular micrometer coincide with 10th (it could be 20th, 30th and so on) line of stage micrometer scale.
6. Then find the length of each division by equating them.

$$100 \text{ division of Stage micrometer} = 1 \text{ mm}$$

$$1 \text{ division of stage micrometer} = 0.01 \text{ mm}$$

Calculations:

Suppose, at low power objective

$$5 \text{ ocular micrometer division} = 10 \text{ stage micrometer divisions}$$

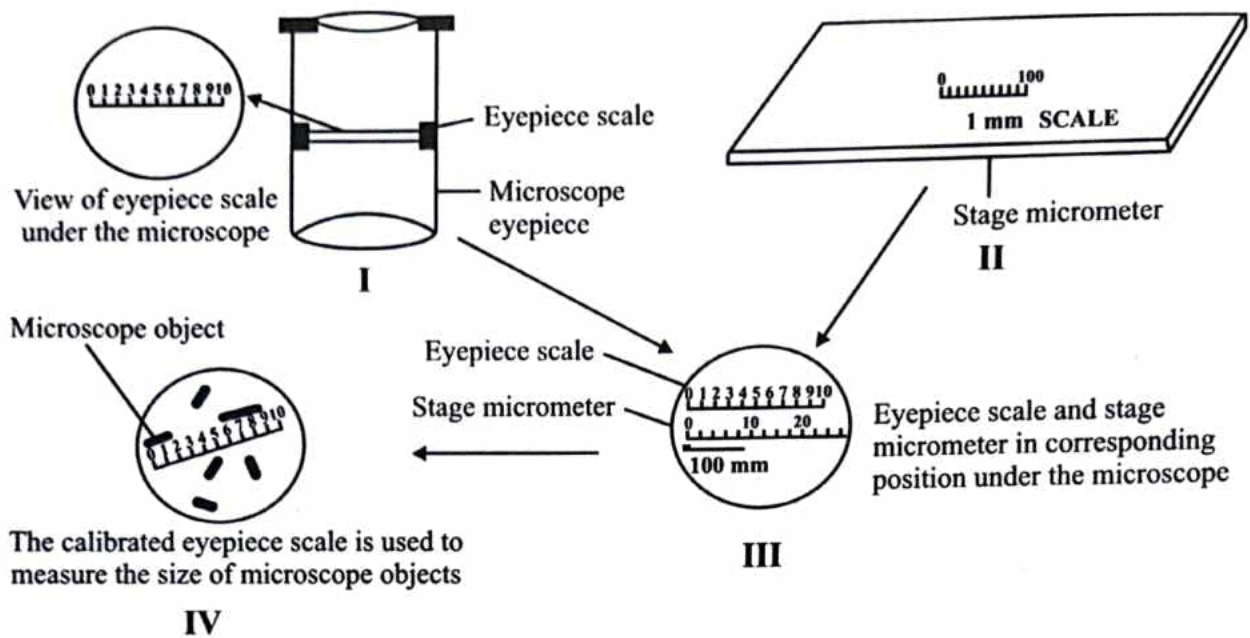
$$5 \text{ ocular micrometer division} = 0.1 \text{ mm}$$

$$1 \text{ ocular micrometer division} = 0.1/5 = 0.02 \text{ mm or } 20 \mu (1 \text{ mm} = 1000 \mu)$$

Measurement of Micro-organism by Using Ocular Micrometer:

After calculating the length of each division (calibrating factor) of ocular micrometer (at given magnification power e.g., 10X), replace the stage micrometer with prepared or provided slide of micro-organism.

Find the number of divisions of ocular micrometer that occupies by cell (micro-organism) under observation and multiply these divisions with calibrating factor (C.F). Determine 3 readings for length and width of micro-organism.



Observations:

Observation No.	Length (μ)	Width (μ)
1	$L_1 = \text{No. of divisions} \times \text{C.F}$	$W_1 = \text{No. of divisions} \times \text{C.F}$
2	$L_2 = \text{No. of divisions} \times \text{C.F}$	$W_2 = \text{No. of divisions} \times \text{C.F}$
3	$L_3 = \text{No. of divisions} \times \text{C.F}$	$W_3 = \text{No. of divisions} \times \text{C.F}$
Mean value		

Result: Size of microorganism = Length \times Width

VIVA VOCE

Q.1 Name two lenses of microscope.

Ans. Ocular and objective.

Q.2 What is difference between compound and electron microscopes?

Ans. The source of illumination for compound microscope is simple light. But the source of illumination for electron microscope is moving electron.

Q.3 Why is compound microscope called so?

Ans. Multiple lenses are used in compound microscope. Therefore, it is called compound microscope.

Q.4 What is meant by magnification of microscope?

Ans. The numbers of times which a microscope increases an object as compared to its original size is called magnification of microscope.

Q.5 Power of ocular of a microscope is 10X and power of its objective is 100X. Calculate its magnification.

Ans. Its magnification will $10 \times 100 = 1000$.

Q.6 What is meant by resolution of eye?

Ans. The minimum distance between two points in which our eye can differentiate is called resolution of eye.

Q.7 What is the function of diaphragm of microscope?

Ans. Diaphragm is used to adjust the intensity of light falling on slide.

Q.8 What is the difference between fine adjustment and coarse adjustments?

Ans. Fine adjustment is used when objective comes very near to slide. Coarse adjustment is used for random movement of body tube.

Q.9 What are Micron and Angstrom?

Ans. Both are unit of measurement of very small object. One micron = 10^6 and one Angstrom = 10^{10} .

Q.10 How many microns are present in one millimetre?

Ans. 0.001 microns are present in 1 mm.

Q.11 What is ocular micrometer?

Ans. The micrometer which is inserted into the eye piece of the microscope is called ocular micrometer.

Q.12 What is stage micrometer?

Ans. The micrometer which is adjusted on the stage of microscope is called stage micrometer.

Q.13 What is the length of scale of stage micrometer?

Ans. The scale of stage micrometer is equal to 1 mm.



Q.14 What is the width of one division of stage micrometer in mm and micron?

Ans. It is equal to 0.01 mm or 10 micron.

Q.15 Is ocular micrometer is not sufficient for measurement of an object? Why do we use stage micrometer?

Ans. The measurement of scale of ocular is unknown. Therefore, we use stage micrometer to measure the length of its one division.

Q.16 Do all the microscopes have same value of one division of ocular?

Ans. No, all the microscopes have different values of one division of ocular. That is why, we use stage micrometer separately for each microscope.

Q.17 What is microscope?

Ans. Microscope is an instrument use to observe the small creatures.

Q.18 Who did invent the first microscope?

Ans. First microscope was invented by Galileo.

Q.19 Name different types of microscopes.

Ans. Compound microscope, light microscope, dissecting microscope and electron microscope.

Q.20 What is power of microscope?

Ans. The magnification of microscope is called power of microscope.

Q.21 Name one cell which does not require microscope for its observation.

Ans. Egg of ostrich.

Q.22 Name longest cells of the body.

Ans. Cell of neuron.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Measure the length of and width of cell provided with the help of ocular and stage micrometers.

(Performance = 1, Apparatus = 1, Procedure and measurement = 1, Observation and result = 1, Short question = $1/2 + 1/2 = 5$)

(Multan Board 2004)

Q.1 What is the use of stage micrometer while we use ocular micrometer for direct measurement of cells?

Ans. The measurement of scale of ocular is unknown. Therefore, we use stage micrometer to measure the length of its one division.

Q.2 Can stage micrometer be used directly in measurement of cell?

Ans. Stage micrometer is placed on stage of microscope in place of slide. Thus this stage micrometer is replaced by slide. Therefore, it is impossible to measure cell directly with stage micrometer.

Experiment 2: Measure the length of and width of cell provided.

(Gujranwala, Multan, D.G. Khan Boards 2004)

Q.1 Why two micrometers i.e., stage and ocular are used?

Ans. The measurement of scale of ocular is unknown. Therefore, we use stage micrometer to measure the length of its one division. Similarly stage micrometer cannot be used for direct measurement because it has to be replaced by slide.

Q.2 Can any of the two micrometers be used directly to observe and measure the size of cells?

Ans. No, the size of one division of ocular is known and it is measured by stage micrometer. Similarly stage micrometer is to be replaced by slide for measurement.

Experiment 3: Measure the length of and width of cell provided with the help of ocular and stage micrometers.

(Bahawalpur, Lahore Boards 2004)

Q.1 What is ocular micrometer?

Ans. The micrometer which is inserted into the eye piece of the microscope is called ocular micrometer.

Q.2 What is the length of scale of stage micrometer?

Ans. The scale of stage micrometer is equal to 1 mm.

5

Experiment

MICROBIOLOGICAL TECHNIQUES

PART-I

Materials:

Inoculating needle and loop, Pipette, Bunsen burner or alcohol lamp, Petridishes, Test tubes, Autoclave or pressure cooker, Culture media, Cotton for plugs, Chemical disinfectants, Incubator, Inoculating chamber.

Procedure:**(i) Aseptic Technique:**

All the materials that are to be used in culturing the microorganisms should be clean and sterilized *i.e.*, free from microorganisms. A technique of using materials which have no living microorganisms on them is called an aseptic technique. Undesirable microorganisms which contaminate the preparation are called contaminants. In microbial work the contaminants can be removed or killed by heating under pressure, by flaming, or by chemical means such as the use of acids, alkalies, salts, alcohols, phenols and various other compounds. The chemical used in sterilization of glassware and equipments are called disinfectants and those used in sterilizing living things are called antiseptics.

(ii) Equipments:

(a) Autoclave: It is used to sterilize all equipment and media that will not be damaged by heat. If it is not available, pressure cooker may be used instead.

(b) Pipettes: These are of two types: (i) calibrated glass tubes into which liquid can be sucked, and (ii) medicine dropper pipettes provided with rubber bulbs. The pipette should be filled with a pipette filler. If it is not available, materials may be sucked in a pipette very carefully.

(iii) PetriDish:

It is a flat-bottomed glass dish with a small rim.

(iv) Inoculating Needles and Inoculating Loops:

These are used to transfer clusters of microorganisms from one medium to another. These should be sterilized in a flame and cooled before use.



Fig. Inoculating needles and loop

(a) Culture Media: Culture media may be corn meal Agar, Malt Agar, Potato Agar, Czapek dox agar, Plain Lemco Agar. the composition of these media is given:

PREPARATION OF CULTURE MEDIA FOR THE GROWTH OF NON-PATHOGENIC BACTERIA

Material:

Test tubes, Petri dishes, Flask, Meat, Powdered agar, Malt (چینی یا گنے کا شیرہ), Marmite, Potato, Dextrose and Autoclave (چیزوں کو گرم کرنے کے لیے اون کی طرح کا آلہ).

Procedure:

(a) Preparation of Culture Media of Meat Infusion and Agar (MIA):

1. 50 gram of meat is taken. It is grinded into small pieces.
2. This meat is placed in flask in one litre water.
3. The material is heated for 2 hours. It is cooled and. It is filtered with cotton wool.
4. 10 gram agar is added in the flask.
5. 1 litre distal water is added in the filtrate. The pH of the meat is adjusted at 7.3.
6. The medium is place is placed in autoclave for sterilization. Culture medium of meat and agar is ready for use.

(b) Preparation of Culture Medium of Malt, Mermite and Agar (MMA):

1. One litre of water is taken in flask.
2. 30 gram of Malt, 15 gram of Marmite and 10 gm of Agar are added in the water.
3. The mixture is heated for 10 minutes and filtered.
4. Media is transferred into petri dishes. Its pH is adjusted at 7.3.
5. The media is then transferred into autoclave for sterilization.
6. Media is now ready for use.

(c) Preparation of Culture Medium of Potato Dextrose and Agar:

1. A small piece of potato is taken in a flask. It is cut into small pieces. The pieces are put in 1 litre of water. The potato pieces are boiled in this water.
2. The pieces of potato are mashed in the flask with glass rod. This mashed potato is filtered through cotton wool.
3. Now dextrose and agar is added into the filtrate. The mixture is heated.
4. The medium is heat in autoclave for sterilization.
5. Media is now ready for use.

(b) Staining Techniques:**Gram Staining Technique of Bacteria:**

Primary stain: Crystal violet stain.

Secondary stain: Safranin stain (pink).

After staining with these two stains, the bacteria are treated with alcohol. The Gram positive bacteria retain the colour of primary stain. But Gram positive bacteria lose the colour of primary stain but retain the colour of secondary stain.

Material:

Glass slide, Cover slips, Curd, Hay infusion, Root nodules, Crystal violet stain, Safranin stain, Absolute alcohol, Blotting paper, Compound microscope, Spirit lamp, Inoculating needle.

Preparation of Stains:**1. Crystal Violet Stain:****Solution (a)**

Crystal violet = 2 gm

95% Alcohol = 20 ml

Solution (b)

Ammonium oxalate = 0.8 gm

Distilled water = 80 ml

Mix solutions 'a' and 'b'.

2. Gram's Iodine Solution:

Potassium iodide = 0.5 gm

Distilled water = 75 ml

Iodine = 0.25 gm

Dissolve potassium iodide in distilled water. Then add iodine and dissolve it. This is the stock solution. Just before use, 1 ml of this solution may be diluted with 14 ml of distilled water.

Procedure:**Extraction of Bacteria:**

Different bacteria can be extracted from different materials:

- 1. Curd :** Curd has bacterium call *Lactobacillus*. It is a gram positive bacterium.
- 2. Root nodule:** The roots of leguminous plants develop nodules for nitrogen fixation. The roots nodule contains bacterium called *Rhizobium*. *Rhizobium* is a gram negative bacteria.
- 3. Hay Infusion:** Hay infusion is produced by mixing hay in water. It is kept in air for some days. Therefore, bacteria produce in it. They start decomposition of hay. It contains both gram negative and gram positive bacteria.

Staining Procedure:

- 1. Smear formation:** A drop of distal water is taken on a slide. A drop of curd or hay infusion is placed on this distal water. This drop is spread on slide with the help of needle. Now it is called **smear**. In case of curd, the smear is washed with xylol. It removes fats.

2. Use of primary stain:

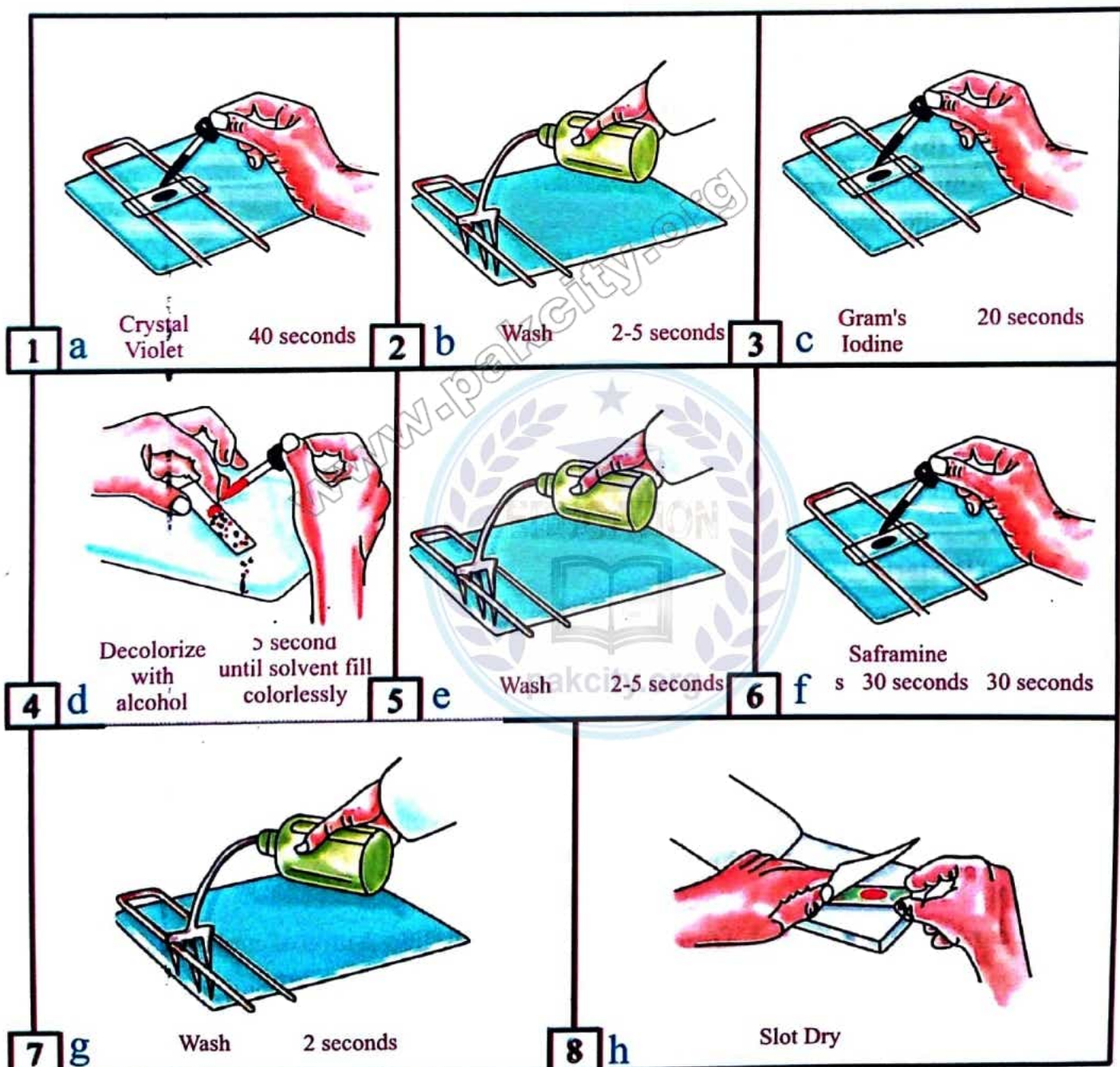
- (i) The smear is covered with 4 drops of **crystal violet stain** for one minute. The excess stain is removed by slightly turning slide in one direction.
- (ii) The slide is now covered with **iodine solution** for one minute. Excess iodine removed by slightly turning the slide.

3. **Decolourisation:** The slide is dipped in absolute alcohol for 2 to 3 minutes. It decolourizes gram negative bacteria. But gram positive bacteria retain the primary stain.

4. **Use of secondary stain:** Now the slide is dipped in Safranin stain (secondary stain) for 20-30 seconds. Excess stain is washed with water. Some bacteria become pink. They are stained by Safranin stain. They are confirmed to negative bacteria.

Result: Pink bacteria \Rightarrow Gram negative bacteria

Purple bacteria \Rightarrow Gram positive bacteria



VIVA VOCE

- Q.1 Name one Gram negative bacteria.**
Ans. *Rhizobium*
- Q.2 Name one Gram positive bacteria.**
Ans. *Lactobacillus*.
- Q.3 Name different culture of bacteria.**
Ans. Meat infusion and agar media, Malt, marmite and agar medium, Potato, dextrose and agar medium.
- Q.4 What is autoclave?**
Ans. Autoclave is an oven like instrument. It is used to sterilize the compound at high temperature.
- Q.5 How is the pH of the culture media adjusted?**
Ans. The pH of culture media is adjusted by adding NaOH if it is acid or by adding HCl if it is alkaline.
- Q.6 What is the difference between pathogenic and non-pathogenic bacteria?**
Ans. The disease causing bacteria are called pathogenic bacteria and the bacteria which do not cause any disease are called non-pathogenic bacteria.
- Q.7 What type of bacteria are present in the curd, aerobic or anaerobic?**
Ans. Anaerobic are present in the curd.
- Q.8 What is the function of bacteria in root nodules?**
Ans. These are nitrogen fixing bacteria.
- Q.9 What is smear? How is it formed?**
Ans. Smear is a blot of a chemical. It is formed by pasting chemical on slide.
- Q.10 What are Gram positive bacteria?**
Ans. The bacteria which retain primary dye colour (violet) are called Gram positive bacteria.
- Q.11 What are Gram negative bacteria?**
Ans. The bacteria which retain secondary dye colour (pink) are called Gram negative bacteria.
- Q.12 Why do Gram negative bacteria decolorize by alcohol?**
Ans. The cell walls of Gram negative bacteria have more lipid contents than peptidoglycan. Lipid has less affinity for crystal violet dye. Therefore, crystal violet dye dissolves in alcohol and they become decolorized.
- Q.13 What is the difference between the cell wall of Gram positive and Gram negative bacteria?**
Ans. The cell wall of Gram positive bacteria has more peptidoglycan than Gram negative bacteria. Cell walls of Gram negative bacteria have more lipid contents than Gram positive bacteria.

Q.14 Crystal violet dye clearly differentiates the Gram positive and Gram negative bacteria. Then why do we use secondary stain?

Ans. Secondary stain (Safranin) stain is used to confirm the presence of Gram negative bacteria.

Q.15 What are bacteria?

Ans. Bacteria are unicellular prokaryotic organisms.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Devise a method to stain Gram positive and Gram negative bacteria.

Performance = 1, Apparatus = 1, Procedure = 1, Observation and result = 1, Short question = $1/2 + 1/2 = 5$

(Faisalabad Board 2004)

Q.1 Who did discover the Gram staining technique?

Ans. Sir Hans Gram.

Q.2 Name bacteria present in the curd. Is it Gram negative or Gram positive bacteria?

Ans. Lactobacillus. It is a Gram positive bacterium.



6

Experiment

STUDY OF ANIMAL AND PLANTS CELLS BY STAINING WITH
SAFRANINE, ACID FUCHSIN, METHYLENE
BLUE AND EOSINE

SECTION-I: ANIMAL CELLS

**Materials:**

Living frog, Chloroform, Compound microscope, Dissection box, Slides, Watch glasses, Camel hair brush, Cover slips, Dropper, Stain (methylene blue or safranin or eosin or acid fuchsin), Squamous epithelium of frog, Epithelial lining of frog, Epithelial lining of buccal cavity of frog.

Preparation of Stains:**1. Methylene Blue Solution:**

Methylene blue, saturated solution in absolute alcohol	=	30 ml
Add potassium hydroxide (KOH)	=	0.01 gm
Add Distilled water	=	100 ml

2. Safranin Stain:

Safranin	=	1 gm
95% alcohol	=	50 ml
Distilled water	=	50 ml
Dissolve safranin in alcohol. Then add distilled water.		

3. Acid Fuchsin Stain:

Acid fuchsin	=	0.5 gm
Distilled water	=	100 ml
Dissolve acid fuchsin in distilled water.		

4. Eosin (Aqueous):

Dissolve 1.0 gm of Eosin Y in 100 ml of distilled water.

5. Eosin (Alcoholic):

Dissolve 1.0 gm of Eosin Y in 100 ml of 70% alcohol.

PART-A

SQUAMOUS (PAVEMENT) EPITHELIUM OF FROG

Procedure:

1. Keep a frog in water for one or two days in a 1000 ml beaker. You will see that the epithelial lining (squamous epithelium) of skin is gradually cast off in the form of semi-transparent membranous flakes.
2. These flakes can also be preserved in formaline for future use. If these cannot be obtained readily, the skin of an anesthetized frog can be scrapped with the edge of a glass slide or a blunt knife. It will remove epithelial cells.
3. Place a small piece of epithelial tissue in a drop of water on a clean slide.
4. Stretch the tissue by using camel hairbrush and needle.
5. Put a drop of any of the stains mentioned above and let it remain for a minute or so.
6. Add a few drops of water and sponge out the stain with tissue paper.
7. Select the homogeneously stained portion of the tissue and trim it with the help of sharp scalpel to the size of about 3 mm square. Remove the surplus trimmings from the slide.
8. Put again a drop of water and place the cover slip on the tissue.

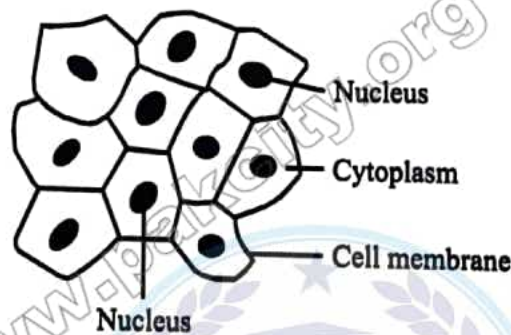


Fig. Surface view of squamous epithelial cells from Frogs skin

Observations:

1. Cells are flattened,
2. Roughly pentagonal or hexagonal
3. Cell are closely fitted without intercellular spaces, like a pavement.
4. The nuclei are distinct and darkly stained as compared to the cytoplasm.

VIVA VOCE

Q.1 What is the shape of the epithelial cells of the skin of frog?

Ans. These are roughly pentagonal or hexagonal in shape.

Q.2 How do the epithelial cells of the skin of frog relate to their function?

Ans. The epithelial cells being flattened from a continuous layer which is well suited for their protective function.

PART-B

HUMAN CHEEK CELL

Method:

1. Take a clean cotton swab and gently scrape the inside of your month.
2. Smear the cotton swab on the centre of the microscope slide for 2 to 3 seconds.
3. Add a drop of methylene blue solution and place a coverslip on top.
4. Remove any excess solution by allowing a paper towel to touch one side of the coverslip.

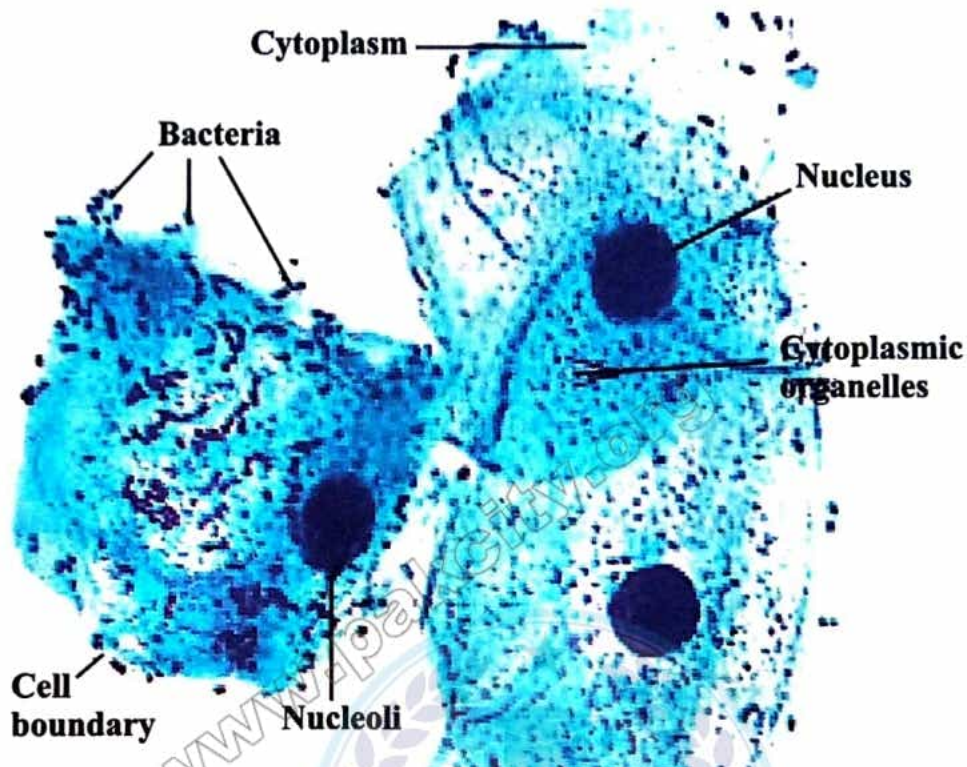


Fig. Human Cheek Cell

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SECTION-II: PLANT CELLS

Materials:

Compound microscope, Glass slides, Cover slips, Dropper, Dissection box, Camel hair brush, Watch glasses, Filter paper or paper towel, Onion bulb, Leaves of Bryophyllum or spinach, Methylene blue or safranin or acid fuchsin or eosin stain.

PART-A MESOPHYLL CELLS

Procedure:

1. Take a fresh leaf of Bryophyllum or spinach. Hold its lower surface towards you. Tear the leaf in the middle avoiding midrib and main veins.
2. Lower epidermis is peeled off exposing the underlying mesophyll cells.
3. Take a pinch or scratch the green portion with the help of the tip of a scalpel.
4. Dislodge these cells in a drop of water taken on the slide.
5. Spread the cells into layer by stirring with the needle.
6. Add a drop of stain and place a coverslip over it.
7. Examine the cells first under low and then high power of the microscope.

Spongy Mesophyll Layer



The cells in the spongy mesophyll layer are not as closely packed as the cells in the palisade mesophyll layer.

This creates air spaces inside the leaf to enable gases to move in and out.

Spongy mesophyll layer

There are not as many chloroplasts in the spongy mesophyll cells as there are in the palisade mesophyll cells - but photosynthesis still occurs in the spongy mesophyll

Observations:

1. Rectangular cells - The palisade cells.
2. Irregular shaped cells - The spongy cells.

Nuclei and cytoplasm would be seen clearly differentiated from the green chloroplasts.

VIVA VOCE**Q.1 What is Mesophyll?**

Ans. Mesophyll is the green tissue lying between the upper and lower epidermis.

Q.2 How many types of mesophyll are there?

Ans. There are two types of mesophyll: (i) palisade mesophyll and (ii) spongy mesophyll.

Q.3 What is the location and shape of palisade mesophyll?

Ans. It lies immediately below the upper epidermis and consists of one or two layers of thin-walled barrel-shaped cells arranged at right angles to the epidermis.

Q.4 What is the location and shape of spongy mesophyll?

Ans. It lies between palisade mesophyll and lower epidermis and consists of irregular cells.

PART-B**LEAF EPIDERMIS**

1. Cut off a small fragment of epidermis and immediately shift it in a drop of water taken on the slide.
2. Prepare a stained slide.
3. Observe the slide under low and high powers of the microscope.

Observations:

1. Kidney shaped guard cells bordering the stomata and surrounded by the epidermal cells.
2. The epidermal cells are irregular in shape but closely fitted by their edges with no interstitial spaces.
3. Notice the presence of chloroplasts only in the guard cells.

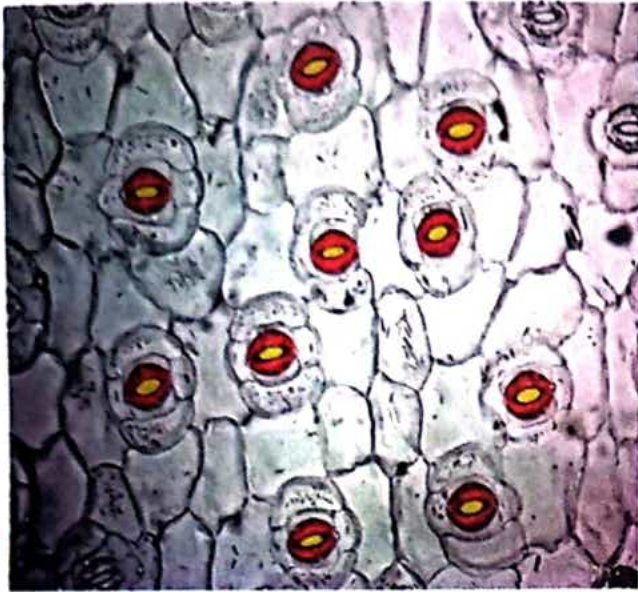


Fig. Lower epidermis of leaf

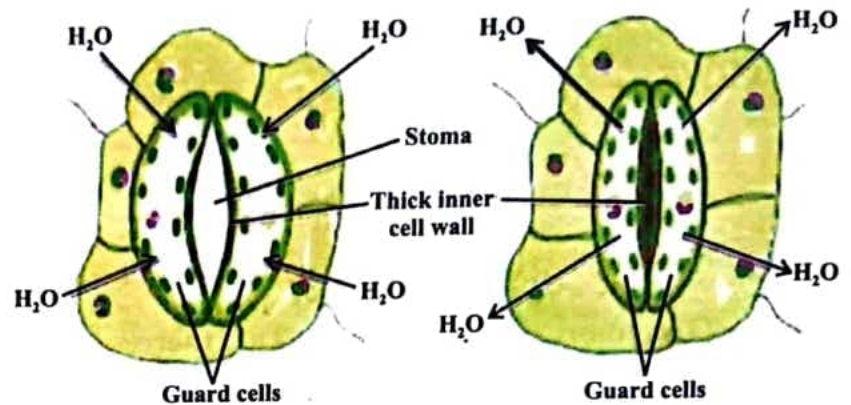


Fig. Lower epidermis of a leaf showing a stoma

VIVA VOCE

Q.1 What is the function of the stomata?

Ans. Stomata are meant for exchange of gases and water between leaf tissues and atmosphere.

Q.2 What are stomata?

Ans. Stomata are very small openings present in the lower epidermis of bifacial leaf.

Q.3 What are guard cells?

Ans. Guard cells are the kidney (bean) shaped cells which surround stomata.

Q.4 Which cells of the epidermis of leaf have chloroplast?

Ans. Only guard cells present in the epidermis of leaf have chloroplast.

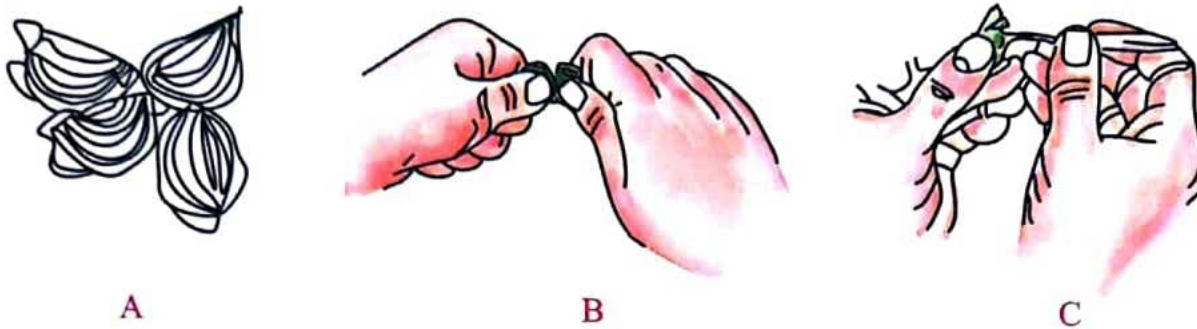
PART-C

ONION EPIDERMIS CELLS

Procedure:

1. Cut an onion bulb vertically into four pieces.
2. Each piece consists of concentric layers of fleshy scales.
3. Hold one of the scales with your fingers and thumbs of both hands, with its concave side facing you.

4. Now tear the scale from its outer convex side.
5. A very thin, transparent membrane, the epidermis pulls from the surface of the scale.
6. Peel it off. It shall come off in a sheet.
7. Transfer it to water in a watch glass and cut into small pieces of 1 or 2 mm square.



Preparing onion epidermis for examination

8. Take a drop of water in the centre of a clean slide. Put into it a small piece of epidermis with its outer surface (which was outermost on the scale) facing upwards.
9. Stretch completely to remove wrinkles, if any.
10. Cover with a cover glass. Remove excess of water around the coverslip with a filter paper.
11. Examine the cells of the epidermis under the low and high powers of the microscope.



Fig. How to run liquid under the cover glass

Observations:

1. Cells are rectangular in shape
2. Have thick cell walls.

Now take off the slide from microscope stage and stain the material.

The nuclei will be darkly stained. Study first under the low and then under the high power. You will find granular substance lying between the nucleus and the cell wall. This is the cytoplasm. It has been stained lightly.



Fig. Cells of onion epidermis

VIVA VOCE



Q.1 What is the shape of epidermal cells of onion?

Ans. They are rectangular in shape.

Q.2 Why do you consider these cells as plant cells?

Ans. These have thick cell walls which is a characteristic of plant cells.

Q.3 What is the shape and location of nucleus in an onion epidermal cell?

Ans. The nucleus is rounded or elliptical in shape and is situated either in the centre of the cell or on one side near the cell wall.

Q.4 What is the evidence that unstained cells of onion epidermis are living?

Ans. The streaming movement of the cytoplasm proves that the cells are living.

Q.5 How does the cytoplasm look like?

Ans. It looks like a fine granular substance.

Q.6 Where in the onion epidermal cells is most of the water found?

Ans. Most of the water is found in the vacuoles present in the cytoplasm.

Q.7 How do you account for any difference between the cytoplasm of the stained cells as well as that of the unstained ones?

Ans. The cytoplasm is not homogeneous substance, it bears a variety of structures, all of which are not equally stained. Due to this differential staining reaction, different components of the cytoplasm become well differentiated in the stained cells.

PREVIOUS BOARD EXPERIMENTS

Experiment 1: Prepare a temporary slide of the given material (Onion epidermis) and draw its labelled diagram. (1 + 1 = 2)

(Multan, Lahore, D.G. Khan Boards 2004)

Experiment 2: Prepare a temporary slide of the given material (bifacial leaf) and draw its labelled diagram (1 + 1 = 2).

(Multan, Lahore, D.G. Khan, Bahawalpur Boards 2004)

Experiment 3: Prepare a temporary slide of the given material (Squamous epithellium of frog) and draw its labelled diagram. (1 + 1 = 2)

(Multan, Lahore, D.G. Khan Boards 2004)

7

Experiment**TO FIND OUT THE EFFECTS OF DIFFERENT CONCENTRATIONS OF SALT OR SUGAR SOLUTIONS ON ANIMAL AND PLANT CELLS**

The difference of salt or sugar concentration causes osmosis. If there is more concentration of salt outside the cells than inside, then water moves from inner to outside. It causes shrinkage of cell. **The shrinkage of cell due to exosmosis is called plasmolysis.** If there is more concentration of salts inside than outside, then water moves from outside to inside. Therefore, the cell becomes swollen.

PART A**EFFECTS ON ANIMAL CELLS****Material:**

Blood cells, Cover slip, Dropper, Blotting paper, 0.9%, 0.5% and 3.0% NaCl solutions, Compound microscope.

Preparation of Solutions:

There are three types of solution:

1. Hypotonic Solution:

A dilute solution as compared to internal solution of cell is called hypotonic Environment. 0.5% NaCl solution is hypotonic solution for blood cells.

2. Isotonic Solution:

A solution with same concentration as compared to internal solution of cell is called isotonic Environment. 0.89% NaCl is an isotonic solution for blood cells.

3. Hypertonic:

A more concentrated solution as compared to external environment is called hypertonic environment. 3% or more is hypertonic solution for blood cells.

Procedure:

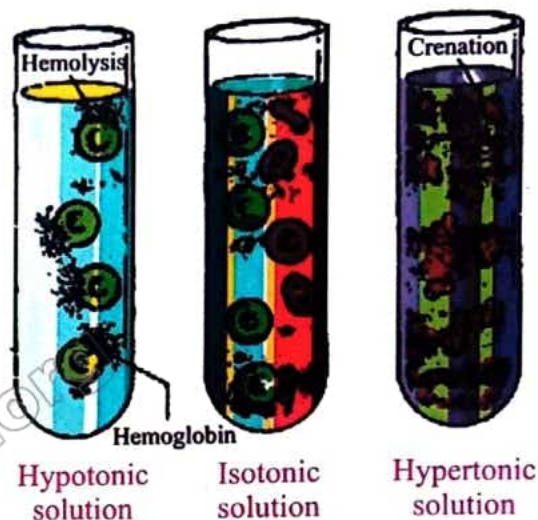
1. Three test tubes are taken. They are marked as A, B and C. 5 ml of 0.5% solution is taken in test tube A. 5 ml of 0.89% solution is taken in test tube B and 5 ml of 3% solution in test tube C.
2. A small amount of blood is taken. Its red blood cells are washed and 3 drops are transferred to each test tube.
3. After one hour, a small amount of blood is taken from each test tube on slides and observed under microscope.

Observation:

Test tubes	Solution	Classification	Observation	Result
Test tube A	0.5%	Hypotonic	RBC swelled and burst	Endosmosis of water
Test tube B	0.89%	Isotonic	No change	No osmosis
Test tube C	3%	Hypertonic	RBC shrink	Exosmosis of water and plasmolysis take place

Result:

Observation shows that RBCs gain water in hypotonic environment. They remain unaffected in isotonic environment. But they lose water in hypertonic environment and shrink. This condition is called plasmolysis.



PART B EFFECTS ON PLANT CELLS

Material:

Filament of spirogyra, Cover slip, Dropper, Blotting paper, 5%, 10% and 15% sugar solution, Compound microscope.

Procedure:

- One drop of water is taken on slide. A filament of spirogyra is placed in this water on slide. The slide is covered with cover slip. It is examined under microscope.
- Now cover slip is removed. A drop of 5% sugar solution is dropped on the filament with the help of a dropper. Now cover slip is again place over it. Extra water is removed by blotting paper. It is examined under microscope after few minutes.
- Same procedure is repeated with 10% and 15% solution.

Observation:

No.	Sugar solution	Observation	Conclusion
1.	Water	Cell remain unaffected	No plasmolysis
2.	5% sugar solution	Little shrinkage	Slight plasmolysis
3.	10% sugar solution	More shrinkage	10% plasmolysis
4.	15% sugar solution	More shrinkage	20% plasmolysis

Result:

The observation shows that plants loss water in concentrated solution. Therefore, it undergoes plasmolysis.



(a) Normal Spirogyra cells



(b) Plasmolysed Spirogyra cell

VIVA VOCE

Q.1 What is Plasmolysis?

Ans. The shrinkage of cell due to exosmosis is called plasmolysis.

Q.2 When does plasmolysis occur?

Ans. Plasmolysis occurs when cell is placed in more concentration (hypertonic environment). The cell loses water and shrink. It is called plasmolysis.

Q.3 What is the affect of plasmolysis on plant?

Ans. Wilting in plant cells takes place due to plasmolysis.

Q.4 Can a plant cell burst, if it is placed in hypertonic environment?

Ans. A plant cell has cell wall. Therefore, it cannot burst in hypertonic environment.

Q.5 Can 100% plasmolysis take place in plant cell?

Ans. Never, the cell wall of the plants cells allow it to shrink to certain degree.

Q.6 What is role of central vacuole in plant cells?

Ans. Large central vacuole of plant stores a large amount of fluid. It produces turgor in plant cells and plant does not wilt.

Q.7 Fluid in central vacuole in plants is hypo, hyper or isotonic than cytoplasm.

Ans. The fluid in plant cells is little bit hypertonic than outer cytoplasm.

Q.8 What is turgor pressure?

Ans. The hydrostatic pressure inside the cell is called turgor pressure.

Q.9 What is osmotic pressure?

Ans. The pressure which brings about osmosis is called osmotic pressure. Osmotic pressure is produced due to presence of solute in water.

Q.10 Which cell is more affected plant or animal by change in concentration?

Ans. Animal cells lack cell wall. So they are more affected by change in concentration than plant cells.

Q.11 Why do RBCs burst in hypotonic environment?

Ans. The red blood cells are without cell wall. So they burst in hypotonic environment.

Q.12 What does happen, if a freshwater animal is placed in marine water?

Ans. Freshwater animal is adapted for hypotonic environment. If it is placed in marine water which is hypertonic, it will lose water and shrink. It may cause its death.

Q.13 What does happen, if a marine animal is placed in fresh water?

Ans. Marine animal is adapted for hypertonic environment. Therefore, it gain water and swell or it can burst.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Set up and apparatus to study the effects of different concentrations of sugar solution on spirogyra cells.

Performance = 1, Apparatus = 1, Procedure and diagram = 1, Observation and result = 1, Short question = $1/2 + 1/2 = 5$

(Multan Board 2004)

Q.1 What is hypotonic solution?

Ans. A dilute solution as compared to external environment is called hypotonic solution.

Q.2 Define incipient plasmolysis.

Ans. The point at which plasmolysis is about to take place is called incipient plasmolysis.

Experiment 2: Demonstrate the process of plasmolysis and deplasmolysis in fresh filament of spirogyra.

(Multan Board 2004)

Q.1 What is the reason of exosmosis in this experiment?

Ans. The spirogyra filament was placed in hypertonic solution. So exosmosis takes place in it.

Q.2 If a fish of fresh water is put in sea water, what will you expect to happen?

Ans. The fish will lose water and shrink. So it will undergo plasmolysis.

Experiment 3: Demonstrate the affects of hypotonic, isotonic and hypertonic environments on red blood cells.

(Faisalabad Board 2004)

Q.1 Give examples of hypotonic and hypertonic environment.

Ans. The example of hypotonic environment is freshwater (pond) and the example of hypertonic environment is marine water (sea).

Q.2 What will happen if distal water is injected into the human blood in body?

Ans. The blood plasma will become hypotonic. There blood cells will absorb water and burst.

8

Experiment

INVESTIGATION OF BACTERIAL CONTENTS OF FRESH AND STALE MILK

The aims of the experiment are to determine the effect of leaving milk unrefrigerated for twentyfour hours and why milk becomes stale. Milk is almost a complete food for us and the experiment shows that it is also a good culture medium for certain bacteria.

The bacteria actively grow in the milk and consume its oxygen. As the milk gets staler and staler, its bacterial population goes on increasing and consequently, its oxygen content goes on reducing. This reduction in oxygen can be deducted by means of methylene blue solution which loses its colour as the oxygen content of the milk diminishes due to increased population of bacteria. The methylene blue solution will take less time to decolourize, if the number of bacteria in the milk is high and vice versa. The quality of the milk with respect to its staleness and the number of bacteria found in it can be rated by, the time it takes to decolourize the methylene blue solution. Each sample of milk can be rated with the help of the following table.

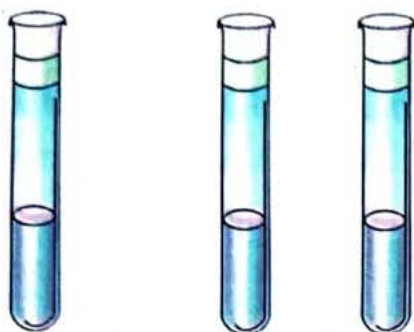
TABLE-I

Time to decolourize methylene blue in the milk	Rating of quality of milk	Degree of staleness of milk
Less than 20 minutes	Highly contaminated	Very stale
20 minutes to 2 hours	Poor	Less stale
2 – 5½ hours	Fair	Still less stale
5½ – 8 hours	Good	Fresh or very slightly stale
More than 8 hours	Excellent	Fresh

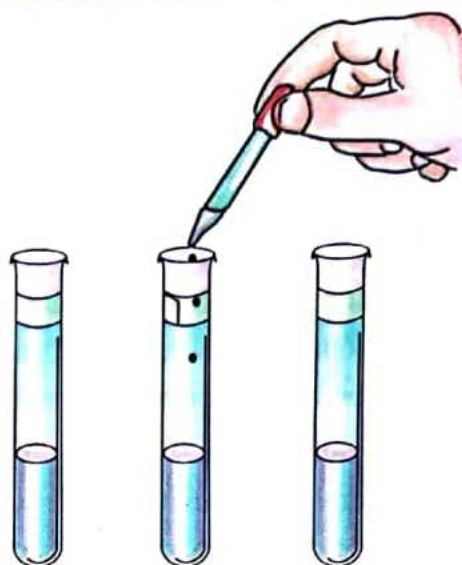
Materials: Test tubes; Test tube stand; Incubator or water bath; Thermometer for water bath; Pipettes; Cotton; Methylene blue solution; Samples of fresh milk and those of stale milk of varying age; Watch.

Preparation: Methylene blue solution can be prepared as described in the beginning of the notebook in the general instructions.

Procedure: With the help of pipette, fill one third of separate test tubes with different samples of the milk. Label the tubes as fresh milk, oneday stale, twodays stale etc. Add one ml (20 drops) of methylene blue solution to each tube. Shake the tubes for thorough mixing. Now plug the tubes with sterile cotton and place them in an incubator or water bath maintained at 37°C.



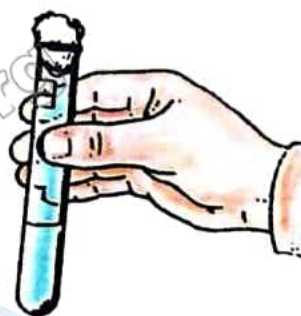
A. Fill the test tubes one-third full with samples of milk of various ages or from different sources. Label each tube for its contents.



B. Add 1 ml (20 drops) of methylene blue solution to each tube. Mix by shaking.



C. Plug the tubes with cotton and place them in a 37°C incubator or a hot water bath at 37°C.



D. Examine each tube periodically. Rate the quality of the milk according to its bacterial population as described in the text.

Examine the milk samples after intervals, notice the colour fading and record the time taken by each sample to decolorize completely, in the form of the following table and rate the quality of the milk according to Table-I.

OBSERVATIONS:

S.No.	Content of tube	Time duration	Rating of quality of milk
1.	Fresh milk	7 hours	Good
2.	Two day stale milk	17 minutes	Highly contaminated

Result: Fresh milk decolorized in 7 hours showing good quality while stale milk decolorized in 17 minutes showing very poor quality.

VIVA VOCE

Q.1 From where do the bacteria come in the milk?

Ans. The bacteria enter the milk during milking and handling, even under the most hygienic conditions.

Q.2 What causes the milk to become stale?

Ans. The lactic acid produced by lactobacillus causes the milk proteins to clot and thus milk becomes sour or stale.

Q.3 Which bacteria are commonly called lactic acid bacteria and why?

Ans. *Streptococcus lactis* and *lactobacillus* are commonly called lactic acid bacteria because they produce lactic acid during fermentation of milk sugar (lactose).

Q.4 What causes souring of milk?

Ans. The accumulation of lactic acid causes souring of milk.

Q.5 What is the colour of the colonies of *Streptococcus lactis* and *Lactobacillus*?

Ans. These colonies are chalky-white.

Q.6 What is the usual habitat of *Escherichia coli* and *alcaligenes* kinds of bacteria?

Ans. Both these kinds of bacteria are usually found in the human gut.

Q.7 How do the colonies of *Streptococci lactis* and *Lactobacilli* look like, besides their colour?

Ans. The colony of *Streptococcus lactis* is smooth-textured with entire edge whereas that of *Lactobacillus* has a rough surface texture with irregular edges.

Q.8 What is agar?

Ans. It is an extract of red algae.

Q.9 Why agar is said to be an ideal medium for bacteria culture?

Ans. First, it is attacked by only a few microbial species and does not soften until a temperature of 100°C is reached. Secondly, the liquid agar does not solidify until it is cooled to 42°C. These properties of agar help to refine the methods of obtaining pure culture.

Q.10 Why gelatin is not used as a jelling substance in the medium.

Ans. Gelatin melts at 28°C, which is below the best temperature for the growth of many bacteria. Moreover, many organisms make an enzyme, gelatinase, which digests gelatin and thus causes the medium to liquify. Whenever the medium liquifies, the separate pure colonies run together and mix.

Q.11 Define microbiology.

Ans. It is the study of microorganisms which include bacteria, viruses, fungi and protists such as protozoa and microscopic algae.

Q.12 What are bacteria?

Ans. The bacteria are the unicellular, prokaryotic organisms having a single chromosome.

Q.13 How do the fresh and stale milk differ with respect to the bacterial population?

Ans. The stale milk has more bacterial population than the fresh milk.

Q.14 Name some bacteria found in the milk.

Ans. These are Streptococcus lactis, Lactobacillus, Escherichia coli, Microbacterium, Alcaligenes and Brevibacterium.

Q.15 Of the following bacteria which ones are Gram +ve and which ones are Gram ve? Streptococcus, Escherichia coli, Lactobacillus, Alcaligenes.

Ans. Streptococcus and lactobacillus are Gram +ve whereas Escherichia coli and Alcaligenes are Gram ve.

Q.16 How does refrigeration save milk from bacteria?

Ans. The low temperature maintained in the refrigerator (4°C) allows only a very slow reproduction of bacteria and thus saves the milk from them.

Q.17 How does oxygen depletion in a medium affect the methylene blue and why?

Ans. The oxygen depletion causes decoloration of methylene blue by reducing it. Methylene blue becomes colourless in reduced state.

Q.18 Why are the milk samples placed at 37°C in the above experiment?

Ans. The enzymes function best and the bacterial growth is maximum, at this temperature.



(4)

Algae



9

Experiment

STUDY OF NOSTOC FROM FRESH MATERIAL
AND PREPARED SLIDES**Material:**

Microscope, Slide, Cover slip, Nostoc culture, Prepared slide of nostoc, Blotting paper.

Procedure:

1. A clean glass slide is taken. A small material from nostoc culture is placed on slide.
2. This material is spread on the glass slide with needle.
3. A cover slip is placed on it. The cover slip is slightly pressed by finger.
4. Excess water is removed by blotting paper.
5. Slide is examined under microscope.

Observation:

1. Nostoc is composed of filaments. Each filament is composed of chains of cells called **trichome**. Trichome is covered by mucilaginous sheath
2. Some colourless, barrel cells are present within the trichome. These cells are **heterocysts**.
3. Individual cell of trichome is composed of two parts: Peripheral part contains chlorophyll and central part contains chromosomes.

Result:

These observations confirm that the material provided is nostoc.

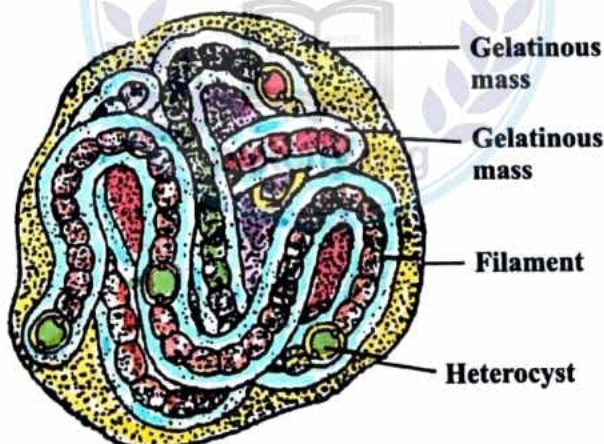
Reason of Identification: Presence of heterocysts

Fig. Nostoc filaments embedded in gelatinous mass

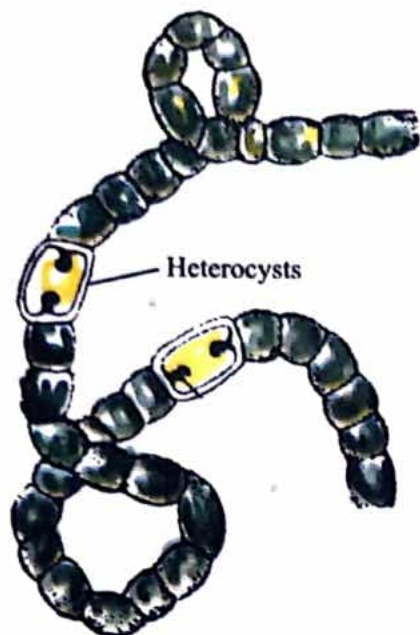


Fig. Nostoc. A vegetative filament

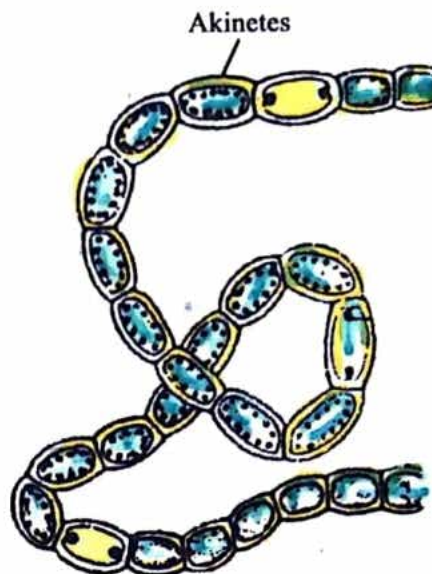


Fig. Nostoc. A filament with akinetes

VIVA VOCE

Q.1 What are cyanobacteria?

Ans. Cyanobacteria are photosynthetic oxygen releasing prokaryotes.

Q.2 What is the old name of cyanobacteria?

Ans. The old name of cyanobacteria is blue green algae.

Q.3 What is the difference between photosynthesis of cyanobacteria and photosynthesis of photosynthetic bacteria?

Ans. Cyanobacteria use water and release oxygen. Bacteria use H_2S and do not release oxygen.

Q.4 What pigments are present in cyanobacteria?

Ans. Chlorophyll and Phycocyanin.

Q.5 What is the function of heterocyst?

Ans. It is used for nitrogen fixation.

Q.6 How does reproduction take place in nostoc?

Ans. Reproduction in nostoc takes place by fragmentation and Akinete formation.

Q.7 What is Akinete?

Ans. A reproductive cell with thick wall and large food reserve is called Akinete.

Q.8 What is trichome?

Ans. Individual chain of cells in a filament is called trichome.

Q.9 What are hormogonia?

Ans. A broken piece of filament of cyanobacteria is called hormogonia.

Q.10 Why are the blue-green algae called so?

Ans. Because their cells contain, besides chlorophyll, a blue pigment known as phycocyanin.

Q.11 From where does the gelatinous mass come?

Ans. It is secreted by the filaments themselves?

Q.12 What is the function of heterocysts?

Ans. They serve for the storage of food.

Q.13 Do the cells of *Nostoc* lack nuclei?

Ans. The chromatin granules lying in the central colourless cytoplasmic region of each cell constitute a primitive type of nucleus.

Q.14 Are all the cells of a filament alike in their shape, size and function?

Ans. No. The heterocysts are different from other cells of the filament, in size and function.

Q.15 What is the filament of *Nostoc* known without mucilaginous sheath?

Ans. The filament without the mucilaginous sheath is known as trichome.

Q.16 What are akinetes in the life-history of *Nostoc*?

Ans. During the unfavourable conditions of drought all the cells of a mature filament of *Nostoc* except heterocysts enlarge, accumulate food, thicken their walls and are now known as akinetes which grow into new filaments on the return of favourable conditions.

Q.17 What are hormogonia in *Nostoc*?

Ans. The parts of the filaments of *Nostoc* lying between adjacent heterocysts are called hormogonia.

Q.18 What is the function of hormogonia of *Nostoc*?

Ans. The hormogonia may separate from the old filament at the heterocysts, grow and form new filaments. It means that they bring about asexual reproduction.

Q.19 What is the main difference between a prokaryotic and a eukaryotic cell?

Ans. A prokaryotic cell lacks a walled nucleus whereas the eukaryotic cell possesses a nucleus with a well-defined wall.

Q.20 What is the difference between a prokaryote and a eukaryote?

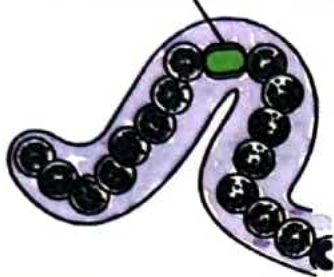
Ans. An organism having prokaryotic cells is the prokaryote while the one possessing eukaryotic cells is the eukaryote.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment: Prepare a temporary slide of the given material (Nostoc) and draw its labelled diagram. (1 + 1)

(Lahore, Multan, D.G. Khan, Faisalabad Board 2004)

A. Identify the specimen and give reason

No.	Slide	Identification	Reason
1.		Nostoc filament	Presence of heterocyst

B. Identify the specimen and give two important characters

No.	Slide	Identification	Reason
1.		Nostoc filament	<ol style="list-style-type: none"> 1. The colony is composed of filaments or trichomes 2. Heterocysts are present

10

Experiment

IDENTIFICATION OF CHLORELLA, PARAMECIUM, AMOEBA, ENTAMOEBA, PLASMODIUM, EUGLENA, VOLVOX, ULOTHRIX & ULVA FROM FRESH MATERIAL AND PREPARED SLIDE

Material:

Compound microscope, Prepared slides and fresh materials of chlorella, Paramecium, Amoeba, Entamoeba, Plasmodium, Eugleria, Ulothrix and Ulva.

Procedure:

One drop of fresh material is taken on slide. It is studied under microscope. The prepared slide of these organisms is studied under microscope at low and high power.

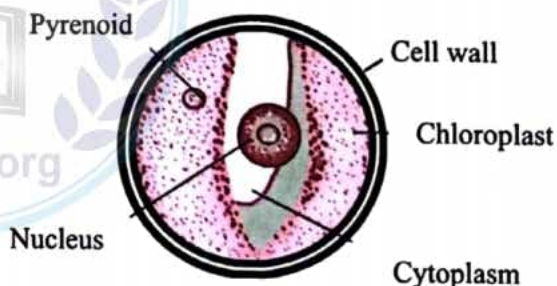
Observations:**CHLORELLA****Classification:**

Kingdom	Protista
Group	Plant like protists (algae)
Phylum	Chlorophyta

Characteristics:

1. These are unicellular algae.
2. Body is spherical.
3. A cup shaped chloroplast is present.
4. Single nucleus in cytoplasm.
5. A pyrenoid is present in chloroplast.
6. It is used for research on photosynthesis.

Reason for identification: Cup shaped chloroplasts a present



Q.1 Name the invertebrates in the cells of which can *Chlorella* grow.

Ans. These are the protozoans (*Stentor* and *Paramecium*); Sponges (*Ophrypodium spongilla*) and Coelenterates (*Hydra*) etc.

Q.2 Sometimes the cells of *Chlorella* occur in groups. Should the plant be called multicellular in such a case?

Ans. Although the cells of *Chlorella* sometimes occur in groups yet they do not constitute multicellular body because of having no "division of labour" among them. Every cell of the group is independent and it can perform all the vital functions.

Q.3 How does *Chlorella* reproduce?

Ans. The only method of reproduction in *Chlorella* is by means of **autospores** (aplanospores).

Q.4 What is the economic importance of *Chlorella*?

Ans. An antibiotic called *Chlorellin* which is useful for the control of bacterial diseases is prepared from *Chlorella*.

PARAMECIUM

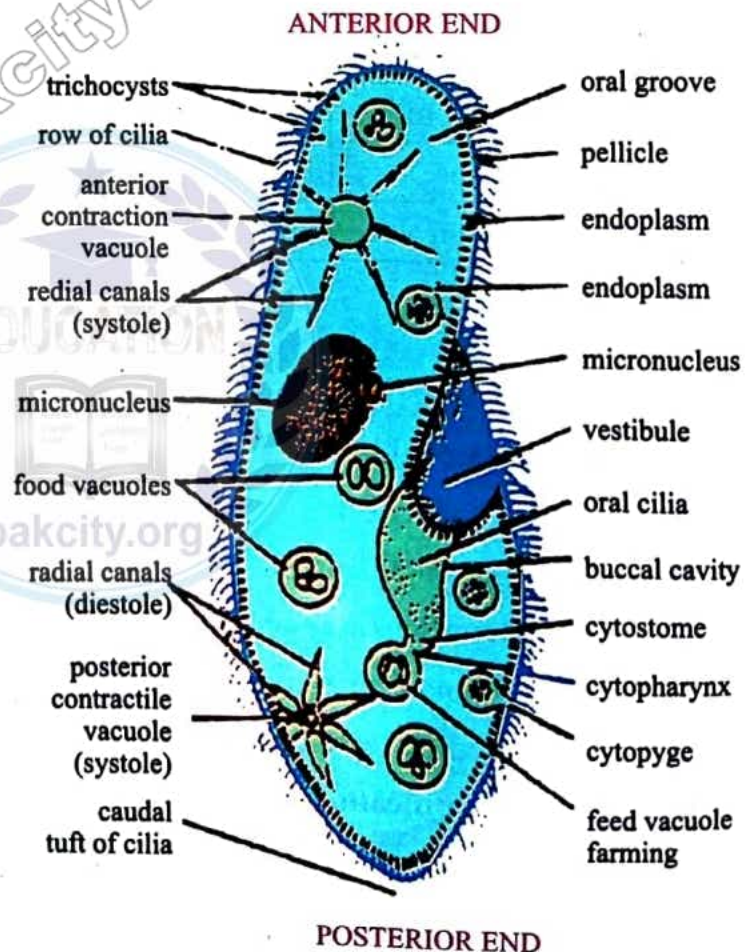
Classification:

Kingdom	Protista
Phylum	Protozoa
Group	Ciliates

Characteristics:

1. It is a unicellular organism.
2. Its body is covered by cilia.
3. Its outer covering is called pellicle.
4. Contractile vacuole is present.
5. It has two nuclei: micronuclei and macronuclei.
6. Sexual reproduction take place by conjugation.

Reason of Identification: Slipper like body covered with cilia.



VIVA VOCE

- Q.1** What is the general shape of *Paramecium*?
- Ans.** The *Paramecium* is elongated and cylindrical or slipperlike in shape.
- Q.2** How does a *Paramecium* move?
- Ans.** The *paramecium* moves by means of cilia.
- Q.3** What is the function of contractile vacuoles in *Paramecium*?
- Ans.** The contractile vacuoles serve to remove excess of water from the body.
- Q.4** How do the food vacuoles move through cytoplasm in *Paramecium*?
- Ans.** The food vacuoles move through the cytoplasm due to its (cytoplasm's) streaming movement.
- Q.5** What structures become distinctly visible after staining?
- Ans.** The nuclei and the food vacuoles become distinctly visible after staining.
- Q.6** What structures are no longer seen after staining which kills the *Paramecium*?
- Ans.** The contractile vacuoles become invisible after staining.
- Q.7** How does exchange of respiratory gases take place in *Paramecium*?
- Ans.** The exchange of respiratory gases between the body of the *Paramecium* and the surrounding water takes place through the pellicle.
- Q.8** Had the *Paramecium* been a plant cell what structures would not have been expected in such a cell?
- Ans.** If *Paramecium* had been a plant cell the cilia and food vacuoles would not have been expected there.

AMOEBA

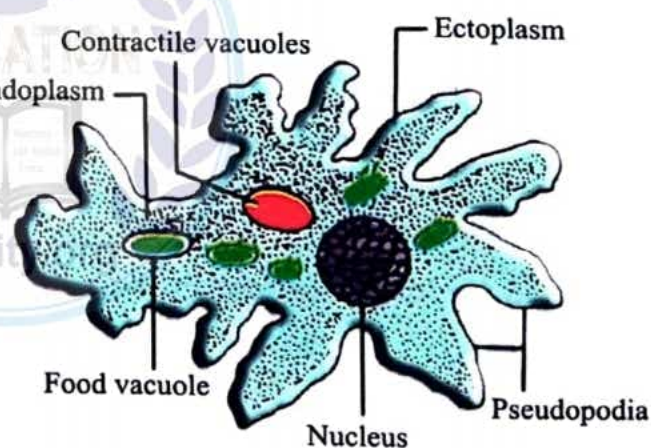
Classification:

Kingdom	Protista
Phylum	Protozoa
Group	Amoeba

Characteristics:

1. Amoeba is unicellular.
2. It has contractile vacuole.
3. It always changes its shape.
4. It moves by pseudopodia.
5. It reproduce asexually by binary fission and spore formation.

Reason of Identification: Irregular shape.



VIVA VOCE

Q.1 Name the phylum of *Amoeba*?

Ans. The phylum is Protozoa.

Q.2 What is the function of pseudopodia in *Amoeba*?

Ans. These are locomotary and feeding organs of *Amoeba*.

Q.3 What is the function of contractile vacuole in *Amoeba*?

Ans. It serves to give out surplus of water and to some extent wastes.

Q.4 How are food vacuoles formed in *Amoeba*?

Ans. The food particles along with some pond water are ingested into the endoplasm with the help of pseudopodia. The drops are the food vacuoles.

Q.5 What is the general shape of *Amoeba*?

Ans. *Amoeba* is irregular in shape due to formation of pseudopodia.

ENTAMOEBA

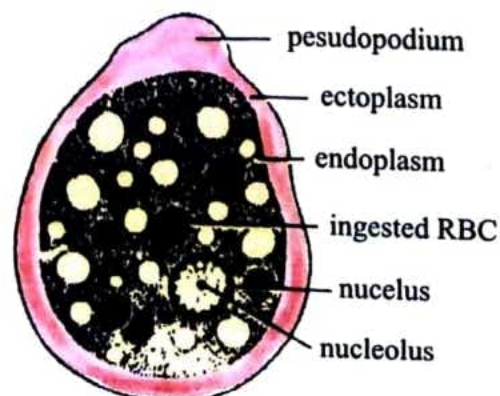
Classification:

Kingdom	Protista
Phylum	Protozoa
Group	<i>Amoeba</i>

Characteristics:

1. It is unicellular.
2. It is a human parasite.
3. It causes amoebic dysentery in man.
4. It moves by pseudopodia.
5. It reproduces asexually by binary fission and formation.

Reason for identification: Presence of blood cells in body



VIVA VOCE

Q.1 What is a parasite?

Ans. An organism which lives in (or on) the body of another organism (host) and derives food from it is called a parasite.

Q.2 What is a pathogenic parasite?

Ans. A parasite which causes disease to its host is known as a pathogenic parasite.

Q.3 How do the pseudopodia of *Amoeba* and *Entamoeba* differ?

Ans. The pseudopodia of *Amoeba* consist of both the ectoplasm as well as the endoplasm whereas those of *Entamoeba* consist of only the ectoplasm.

Q.4 Why is contractile vacuole absent in *Entamoeba*?

Ans. The function of the contractile vacuole is to give out surplus of water from the cell body. Being parasite, there is no surplus of water in *Entamoeba* and hence no need of the contractile vacuole.

Q.5 In which protozoans are found contractile vacuoles?

Ans. The contractile vacuoles are found in those protozoans which live in fresh water.

Q.6 Name the disease which is caused to man by *Entamoeba*.

Ans. It is Amoebic dysentery (Amoebiasis).

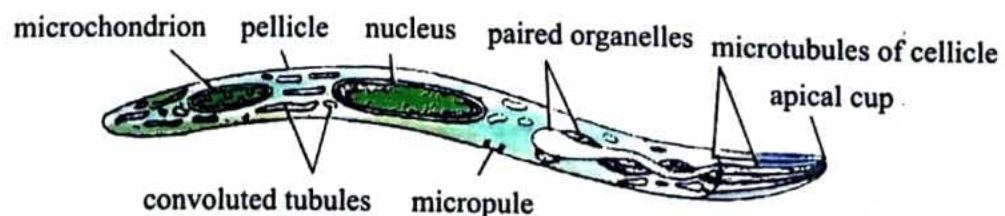
Q.7 Is there any dysentery, other than amoebic?

Ans. Yes. It is bacterial dysentery caused by bacteria. The symptoms of both kinds of dysentery are the same.

PLASMODIUM

Classification:

Kingdom	Protista
Phylum	Protozoa
Group	Apicomplexans



Characteristics:

1. It is unicellular.
2. It develops spore at some stage of their life.
3. It has no locomotory organs.
4. Its forms are sporozoites and merozoites.
5. It reproduces sexually by gamete formation.
6. It spends its life in two hosts: man and mosquito.
7. It causes malaria in man.

Reason for identification: Spindle shaped body

VIVA VOCE

Q.1 What is an "alternation of hosts"?

Ans. When a parasite needs two hosts for the completion of its life-history and the hosts alternate with each-other, it is called alternation of hosts as in the life cycle of malarial parasite.

Q.2 Does malarial parasite show "alternation of generations" and how?

Ans. Yes. The malarial parasite during its life history passes through two generations, an asexual and a sexual one and both the generations alternate with each-other.

Q.3 Is the sexual reproduction in malarial parasite isogamous or heterogamous?

Ans. It is heterogamous because the gametes are of two different types as regards their shape and size.

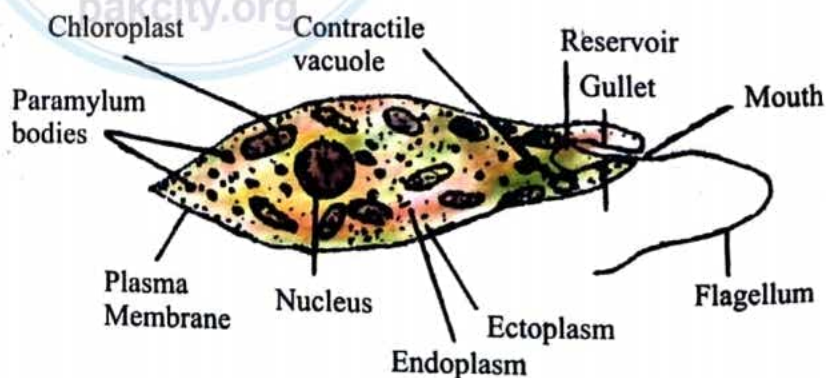
EUGLENA

Classification:

Kingdom	Protista
Group	Plant like protists (algae)
Phylum	Euglenoids

Characteristics:

1. It is unicellular.
2. It has two flagella. One long other short.
3. It has chlorophylls.
4. It lacks cell wall.
5. Contractile vacuoles are present.



Reason for identification: Presence characteristic chlorophylls and flagella

VIVA VOCE

Q.1 What is the normal shape of *Euglena*?

Ans. *Euglena* is normally spindle-shaped.

Q.2 What is pellicle in *Euglena*? What is its other name?

Ans. Pellicle is the outer thin flexible membrane around the body of *Euglena*. It is also called plasma membrane.

Q.3 What may be the total number of chloroplasts in the cell body of *Euglena*? What is their shape?

Ans. The total number of chloroplasts in the cell body of *Euglena* may be from 30 to 40. They are plate-like in shape.

Q.4 What is the shape of the gullet in *Euglena*?

Ans. It is tubular in shape.

Q.5 Name the locomotory organ of *Euglena*.

Ans. It is the flagellum.

Q.6 Describe mode of nutrition in *Euglena*.

Ans. It is autotrophic in the presence of light but heterotrophic in the absence of light.

Q.7 *Euglena* is a plant or animal. Explain.

Ans. *Euglena* possesses the characters of both the plants as well as the animals. Its plant-like characters are: Presence of chlorophyll and holophytic (autotrophic) mode of nutrition while the animal-like characters are: Absence of cell wall, holozoic (heterotrophic) nutrition and motility. Therefore, it cannot be definitely decided whether it is a plant or an animal.

Q.8 What is the nature of the stored food in *Euglena*?

Ans. The stored food in *Euglena* is a carbohydrate called Paramylum.

Q.9 Differentiate anterior end of *Euglena* from its posterior end.

Ans. The anterior end is blunt while the posterior one is pointed.

Q.10 Why is the shape of *Euglena* constantly changed during active locomotion (movement)?

Ans. It is so because of the absence of a rigid cell wall. The cell body is enclosed in a thin flexible plasma membrane which expands and contracts with the swelling and contraction of parts of the cell body.

VOLVOX

Classification:

Kingdom	Protista
Group	Plant likes protists (algae)
Phylum	Chlorophyta

Characteristics:

1. It is a colonial algae.
2. The colony is covered by mucilaginous sheath.
3. The colony moves by flagella.
4. Individual cells are oval or spherical in shape.
5. Some daughter colonies may be present parent colony.
6. Sexual reproduction takes place by oogamy.

Reason for identification: Rounded colony

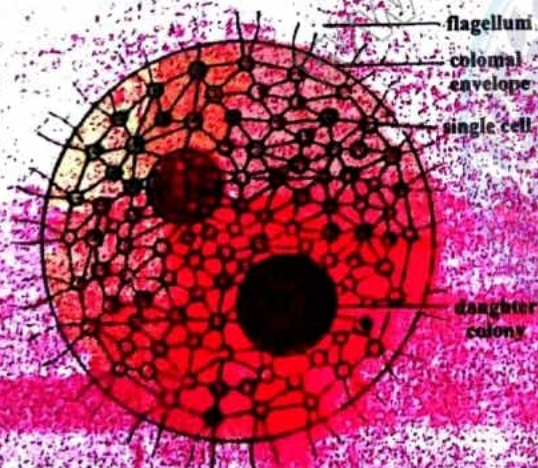


Fig. Volvox: Rounded colony with daughter colonies.

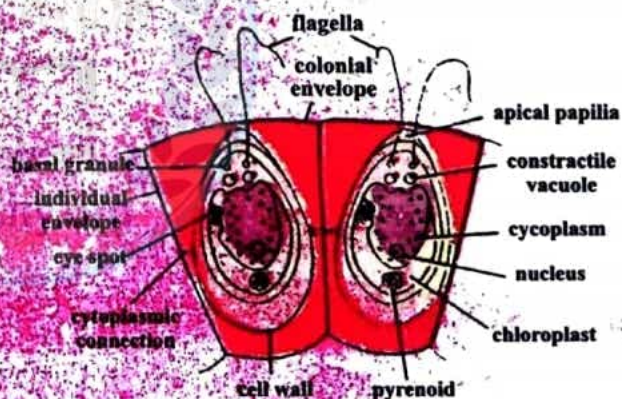


Fig. Volvox: A part of the colony to show cell arrangement and cell structure.

VIVA VOCE

Q.1 What is the origin of the name *Volvox*?

Ans. The name *Volvox* has been derived from a latin word *volvere* which means “to roll”.

Q.2 Does “division of labour” occur among the various cells of *Volvox*?

Ans. No. Every cell is independent and self-supporting.

Q.3 The *Volvox* grows in spring and then abruptly disappears and remains disappeared till the next spring. In which form does it pass winter?

Ans. The *Volvox* passes winter in resting condition as zygote or zygospore.



Q.4 What is the total number of the species of *Volvox*?

Ans. There are twenty species of *Volvox*.

Q.5 Name the most common species of *Volvox*.

Ans. It is *Volvox globator*.

Q.6 Is the *Volvox* colony really empty from within?

Ans. No. The centre of the colony may contain gelatinous material of watery consistency or water only.

Q.7 How does *Volvox* colony move in water and in which manner?

Ans. The *Volvox* colony rolls along in water due to the coordinated movements of its flagella.

Q.8 What is the total number of individual cells in a *Volvox* colony?

Ans. The total number of individual cells in a *Volvox* colony varies from 500 to 60,000 depending upon the species.

Q.9 What are plasmodesmae in *Volvox*?

Ans. The cytoplasmic strands which serve to connect the cells of the *Volvox* colony with one-another are known as plasmodesmae (single plasmodesma).

Q.10 Do both the flagella of *Volvox* differ in length?

Ans. No. Both the flagella are equal in length.

Q.11 What is the function of eye-spot?

Ans. It helps distinguish light and darkness.

Q.12 What is the function of pyrenoid?

Ans. It serves to convert sugar into starch, for storage.

ULTHRIX

Classification:

Kingdom	Protista
Group	Plant likes protists (algae)
Phylum	Chlorophyta

Characteristics:

1. It is **filamentous** algae.
2. The filament is attached by **holdfast**.
3. Chloroplast is **girdle** shaped.
4. There is thin layer of cytoplasm in it called **primordial utricle**.
5. One or more pyrenoid is present in chlorophylls.

Reason for identification: Filamentous body with girdle shaped chloroplast

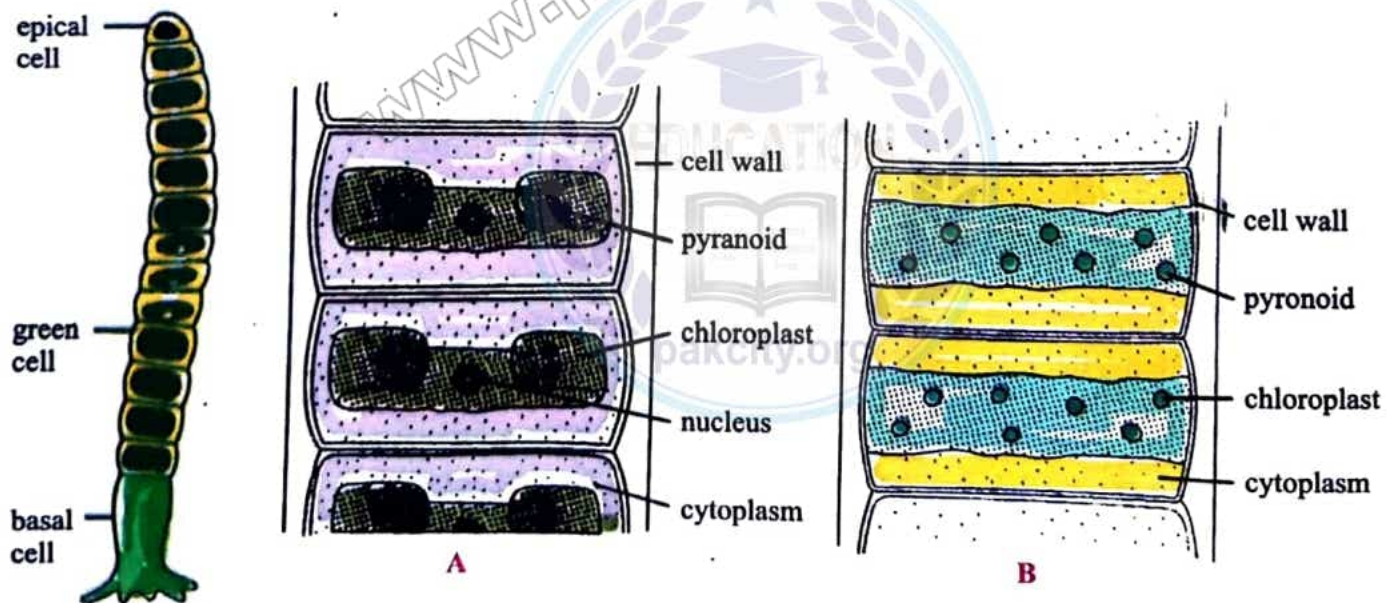


Fig. Ulothrix. Filament showing habit.

Fig. Ulothrix. A single cell viewed from either sides showing internal structures (Diagrammatic)

VIVA VOCE

Q.1 What is a thallus? Quote examples.

Ans. A plant body which is not differentiated into roots, stem and leaves is known as a thallus. The examples are algae, fungi and some bryophytes.

Q.2 Name a marine species of *Ulothrix*.

Ans. It is *Ulothrix flacca* which is found in the tide pools.

Q.3 What is the shape of the thallus of *Ulothrix*?

Ans. It is filamentous.

Q.4 What is the usual thickness of the filament of *Ulothrix*?

Ans. It is about 0.04 millimetres.

Q.5 What may be the total number of cells in a filament of *Ulothrix*?

Ans. The total number of cells in a filament of *Ulothrix* may be about one thousand or even more.

Q.6 What is the lowermost cell of *Ulothrix* called by which it attaches itself to some substratum?

Ans. It is called holdfast.

Q.7 Do the filaments of *Ulothrix* always remain unbranched?

Ans. No. The filaments may become branched during unfavourable conditions such as nutritional deficiency.



ULVA

Classification:

Kingdom	Protista
Group	Plant likes protists (algae)
Phylum	Chlorophyta

Characteristics:

1. Its body is **thallus**.
2. It is composed of sheet like multicellular body.
3. The plant body is attached with substratum by holdfast.
4. Asexual reproduction takes place by **zoospore** formation.
5. Sexual reproduction is **isogamy**.

Reason for identification: Sheet like body



Fig. *Ulva*. A thallus.

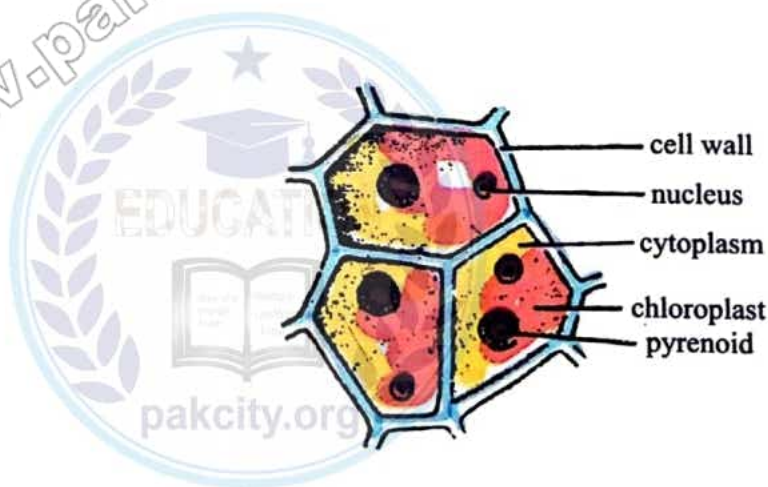


Fig. *Ulva*. Cells of thallus.

VIVA VOCE

Q.1 Explain low and high tides.

Ans. The waves of water constantly move up and down at the sea coast. The lowest permanent line of sea water is called low (Ebb) tide whereas the high temporary one is termed as the high (Flood) tide.

Q.2 What is tidal pool or tidal coast?

Ans. The part of the seacoast lying between the low and high tide lines is called as the tidal pool.

Q.3 What is division of labour? Does *Ulva* exhibit this phenomenon?

Ans. The assignment of different functions to different cells (organs) of the body of an organism is called division of labour. *Ulva* shows a very simple kind of division of labour in which the cells of the body are differentiated into the colourless ones of the rhizoids which serve to fix the plant to the substratum while the rest of the green cells carry on all the other functions.

Q.4 What is the structure of an individual green cell of the thallus of *Ulva*?

Ans. Each green cell of the thallus of *ulva* is isodiametric or elongated. It has a central nucleus surrounded by the cytoplasm, a parietally situated cup-shaped chloroplast with a single pyrenoid.

Q.5 What is the study of *algae* known as?

Ans. It is called phycology.


PREVIOUS BOARD EXPERIMENTS AND QUESTIONS


Experiment 1: Prepare a temporary slide of the given material (*Volvox*) and draw its labelled diagram (1 + 1 = 2)

(Sargodha Board 2004)

Experiment 2: Identify the given slide (*Euglena*) and draw its labelled diagram. (1 + 1 = 2)

(Faisalabad Board 2004)

No.	Slide	Identification	Reason of Identification
1.		Chlorella	Cup shaped chloroplast is present

No.	Slide	Identification	Two characters
1.		Chlorella	1. Cup shaped chloroplast is present. 2. Body is oval.

No.	Slide	Identification	Reason of Identification
2.		Paramecium	Slipper like shape

No.	Slide	Identification	Two characters
2.		Paramecium	<ol style="list-style-type: none"> 1. Cilia are present 2. Two nuclei micro and macronuclei.

No.	Slide	Identification	Reason of Identification
3.		Amoeba	Irregular shape

No.	Slide	Identification	Two characters
3.		Amoeba	<ol style="list-style-type: none"> 1. Irregular shape 2. Presence of pseudopodia


No.	Slide	Identification	Reason of Identification
4.		Euglena	Presence of characteristic chlorophylls and flagella

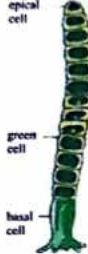
No.	Slide	Identification	Two characters
4.		Euglena	<ol style="list-style-type: none"> 1. Presence of characteristic chlorophylls 2. Flagella are present


No.	Slide	Identification	Reason of Identification
5.		Volvox	Rounded colony

No.	Slide	Identification	Two characters
5.		Volvox	<ol style="list-style-type: none"> 1. Rounded colony 2. Mucilaginous sheath is present on the body

No.	Slide	Identification	Reason of Identification
6.		Ulva	Sheet like body

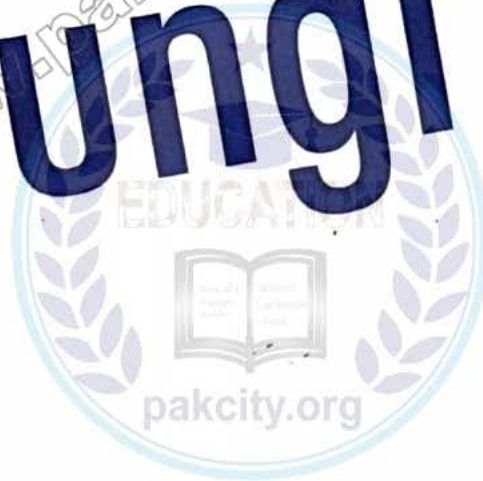
No.	Slide	Identification	Two characters
6.		Ulva	<ol style="list-style-type: none"> 1. Sheet like body 2. Chloroplast is present

No.	Slide	Identification	Reason of Identification
7.		Ulothrix	Filamentous body with girdle shaped chloroplast

No.	Slide	Identification	Two characters
7.		Ulothrix	<ol style="list-style-type: none"> 1. Filamentous body 2. Girdle shaped chloroplast

(5)

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Fungi



11

Experiment

STUDY OF YEAST, USTILAGO TRITICI AND PENICILLIUM FROM FRESH MATERIAL AND PREPARED SLIDES

Material:

Compound microscope, Prepared slides and fresh materials of yeast, Ustilago and Penicillium.

Preparations:**1. Pasteur's solution:**

Potassium sulphate	=	1 gm
Magnesium sulphate	=	0.1 gm
Ammonium tartrate	=	5 gm
Calcium sulphate	=	0.1 gm
Sucrose	=	75 gm
Distilled water	=	419 gm

Dissolve all the above ingredients in water in a flask.

2. Culture of Yeast:

Add a small bit of yeast cake to Pasteur's solution in the flask. Plug the mouth of the flask with a cotton swab and place it in a warm place at suitable temperature (about 30°C). After a few days the culture will be found to contain a large number of yeast cells.

Procedure:

1. Pour some yeast culture from the flask in the petridish or watch glass for experimental use.
2. Put with dropper, a drop of culture in the middle of a clean glass slide.
3. Cover with a cover slip.
4. Study under the low and high powers of the microscope.

Observation:**YEAST (SACCHAROMYCES)**

Kingdom	Fungi
Phylum	Ascomycota

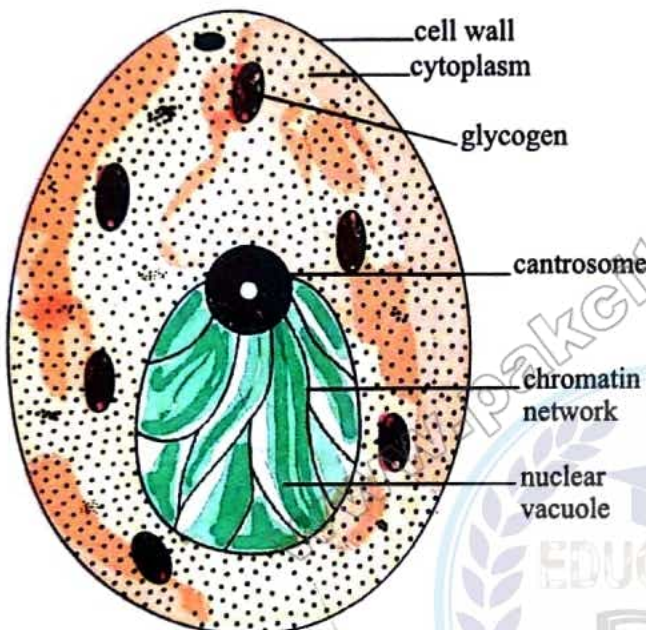
Habitat:

Yeast is saprophyte fungi. It grows on decay vegetables, ripe fruits. A few species are

Characteristics or Morphological Note:

1. Yeast is a unicellular and non-hyphal fungus.
2. Cells are spherical, oval or cylindrical.
3. Its cell wall is made up of chitin.
4. Its cytoplasm is granular.
5. Nucleus is covered by double membrane.
6. Vacuoles are present within the cells.
7. Asexual reproduction takes place by budding.
8. Ascospores and ascus are produced during sexual reproduction.

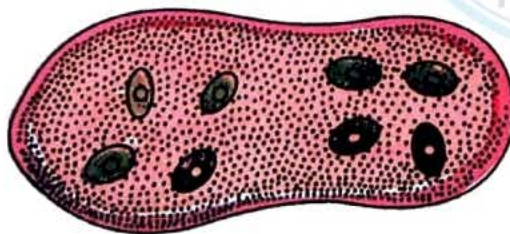
Reason for identification: Buds are present on cells



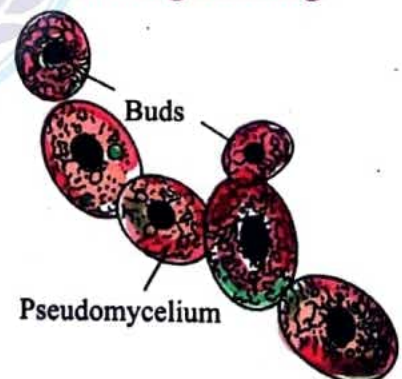
**Fig. *Saccharomyces* (Yeast).
A cell magnified**



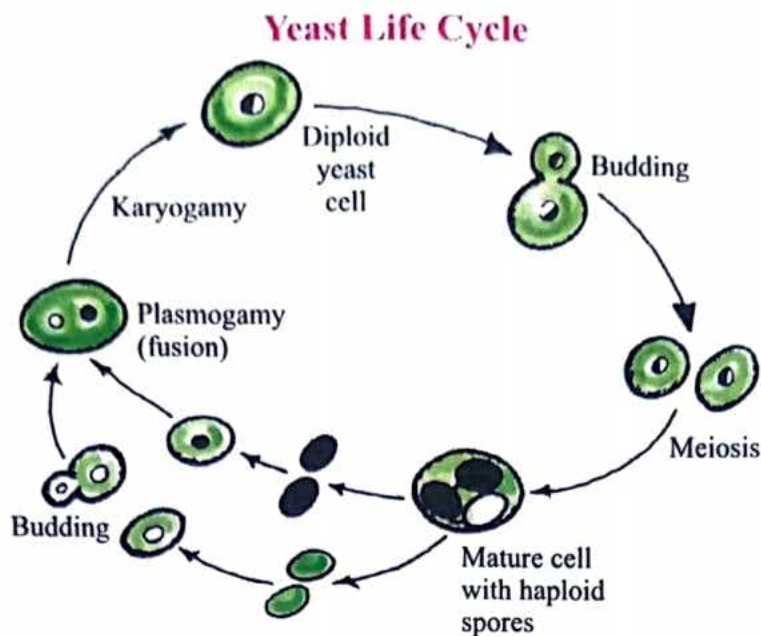
**Fig. *Saccharomyces*. Cells
showing budding**



**Fig. *Saccharomyces*. Ascus showing
ascospores (magnified)**



**Fig. *Saccharomyces*. Chain of yeast
cells forming pseudomycelium as a
result of budding**



VIVA VOCE

Q.1 Name the class of the Fungi to which belongs the yeast.

Ans. Its name is Ascomycetes. Its representatives are commonly called as sac-fungi.

Q.2 Where is yeast most abundantly found?

Ans. Yeast is most abundantly found in sugary substances containing small amounts of nitrogen and sulphur such as the nectar of flowers, surface of grapes, date-palm, sugar-cane, in the fruit juices and milk and in the soil of vine-yards etc.

Q.3 How are yeasts useful?

Ans. The yeasts are useful in many ways. They bring about fermentation of carbohydrates leading to the production of wine, beer, alcohol and bread in the breweries, distilleries and bakeries. They are valuable sources of food for us because of their high vitamin contents (Vitamins B, B₂, G), proteins, fats, carbohydrates and enzymes etc. They are also used in the treatment of certain diseases.

Q.4 How are yeasts harmful?

Ans. Some species of yeasts are pathogenic to certain plants causing leaf-curl diseases while some are pathogenic to man and animals.

Q.5 Which enzymes are commonly found in the yeast?

Ans. These are zymase and invertase.

Q.6 What are yeast cakes?

Ans. A large number of yeast cells are mixed with certain inert matter such as starch. The material is compressed and cut into cubes which are dried at 125°C. These dried cubes are called and sold as yeast cakes.

Q.7 Write down the equation for fermentation of glucose solution.

Ans. It is: $C_6H_{12}O_6 \xrightarrow{\text{Zymase}} = 2C_2H_5OH + 2CO_2 + \text{Energy (14 Kg. cal)}$
(Ethyl alcohol) Carbon dioxide

Q.8 What is mycelium?

Ans. The body of a fungus is called mycelium.

Q.9 Is the cell wall of yeast made up of cellulose, like common plants?

Ans. No. It is made up of chitin.

Q.10 Differentiate between ectoplasm and endoplasm.

Ans. If the cytoplasm of a cell is clearly distinguished into an outer and an inner (central) part, they are called ecto and endoplasm, respectively.

Q.11 Write down a peculiar feature of yeast.

Ans. Large vacuole contained in the nucleus of yeast cells.

Q.12 What is the nature of reserved food in yeast cells?

Ans. It is the granules of glycogen, oil-globules and proteins.

Q.13 What is pseudomycelium in the life of yeast?

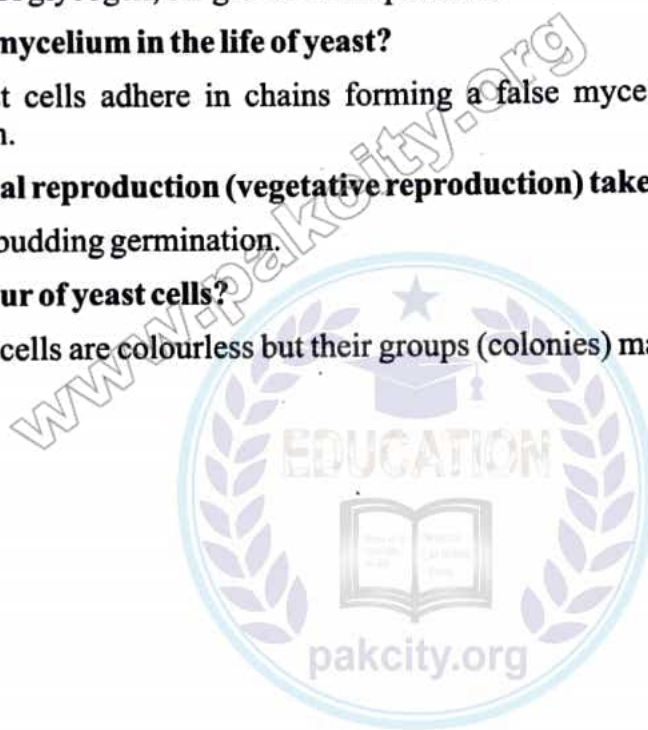
Ans. Sometimes yeast cells adhere in chains forming a false mycelium which is termed as pseudomycelium.

Q.14 How does asexual reproduction (vegetative reproduction) take place in yeast?

Ans. It takes place by budding germination.

Q.15 What is the colour of yeast cells?

Ans. Individual yeast cells are colourless but their groups (colonies) may appear white or creamy or brownish.



USTILAGO TRITICI

Classification:

Kingdom	Fungi
Phylum	Basidiomycota

Procedure:

1. Hold an infected ear of wheat in hand.
2. Look at the black powdery mass taking the place of grains.
3. Use hand lens, if desired. It consists of the smut spores or chlamydospores.
4. Now put the ear on a piece of white paper and tap it gently. The powdery mass consisting of chlamydospores will fall on the paper.
5. Lift the ear gently straight up from the paper by a forceps and place it on another paper.
6. With camel-hair brush pick up some of the material from the paper and place it on a slide.
7. Add a drop of glycerine-water solution.
8. Examine the chlamydospores under the low and high powers of the microscope.

Habitat:

It is a parasite on wheat, oat, rice and corn. It causes smut in these plants.

Characteristics or Morphological Note:

1. It is multicellular septate fungi.
2. Body is composed of uninucleate or dikaryotic hyphae.
3. It produces black dusty spores called **clamydospores**.
4. Clamydospore fall on ear of wheat. They grow and form hyphae. This hypae penetrates into the ovary of wheat. It becomes inactive in seed.
5. When in the next season, the seeds are grown. It germinates and forms a new mycelium.

Reason for identification: Black dusty power on the ear of wheat

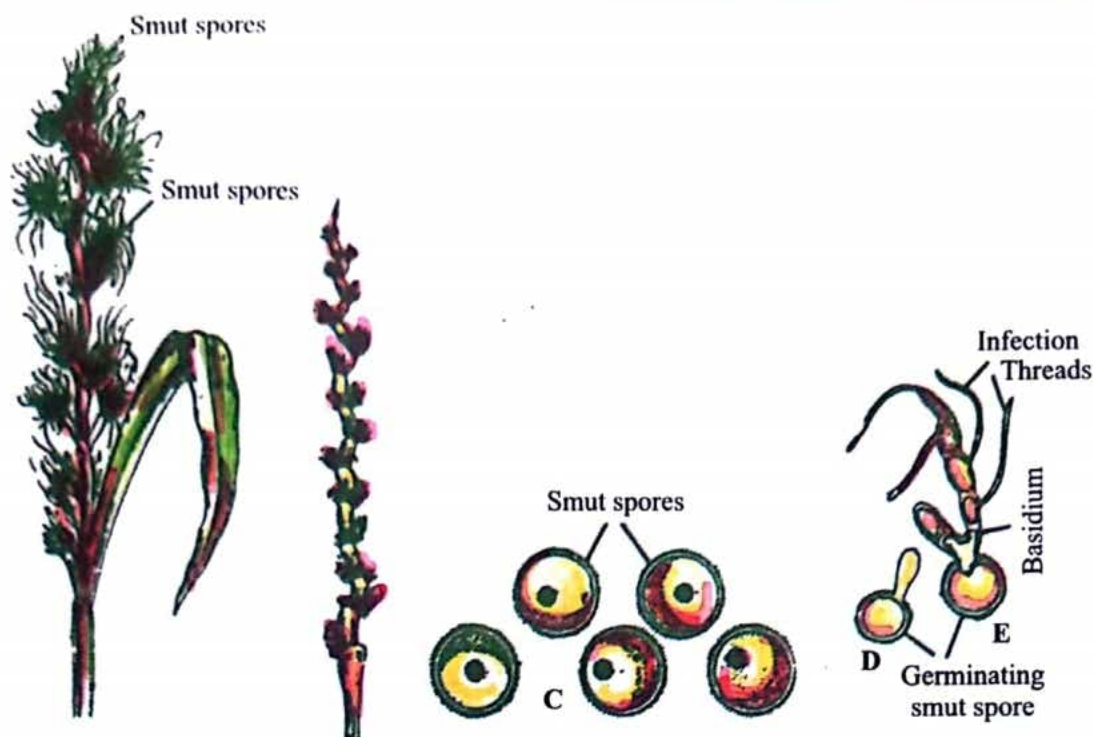


Fig. *Ustilago* (different stages)

VIVA VOCE

Q.1 Name the plants which are subjected to the disease “loose smut” (Kangiari) caused by different species of *Ustilago*.

Ans. The plants are wheat, barley and oat.

Q.2 What is the mode of life of *Ustilago*?

Ans. Various species of *Ustilago* live as parasites while many as saprophytes.

Q.3 Differentiate between monokaryotic and dikaryotic mycelium in *Ustilago*.

Ans. The mycelium of *Ustilago* is known as monokaryotic when it has only one nucleus in each cell and dikaryotic when it possesses two nuclei of different strains in each cell.

Q.4 How are the chlamydospores formed in *Ustilago*?

Ans. The chlamydospores are formed in the grains of the host by repeated division of the mycelium.

Q.5 What is the colour of the mature chlamydospores of *Ustilago*?

Ans. The mature chlamydospores have black soot-like colour.

Q.6 What is promycelium in the life-history of *Ustilago*?

Ans. The four-celled colourless hypha produced by the germination of chlamydospore of *Ustilago* is called promycelium.

Q.7 What are other names for the smut spores of *Ustilago*?

Ans. These are chlamydospores or teleutospores (teliospores).

Q.8 Is primary mycelium in *Ustilago* uninucleate or multinucleate?

Ans. It is uninucleate.

PENCILLIUM (BLUE GREEN MOLD)

Classification:

Kingdom	Fungi
Phylum	Basidiomycota

Materials:

Compound microscope, Slide, Cover glass (cover slip), Dissecting needle, Orange infected with *Penicillium*, Healthy orange, 70% Ethyl alcohol, Glycerine-water solution (1:1), Blotting or filter paper, Spirit lamp, A wide-mouthed jar with lid, Prepared and stained slides of *Penicillium*.

Preparation of Culture of *Penicillium* on an Orange:

Take a healthy orange, wash it with water, dry and then immerse in 70% ethyl alcohol for five minutes.

Take a dissecting needle, heat it on a flame, cool and rub it on the infected portion of the other (already infected) orange. Now puncture with this needle, the skin of the healthy orange at several places in small areas. Place this orange in the jar and put lid on it. After a few days *Penicillium* mould will grow on the healthy orange at the inoculated places which turn bluish-green. The culture on the orange can be readily used for study.

Procedure:

1. Pick up some mould from the infected orange with a needle and transfer it to the middle of a clean glass slide.
2. Add a drop or two of glycerine-water solution to the mould.
3. Spread the material.
4. Cover with a cover slip.
5. Examine under the microscope.

Observations:

Habitat:

It is saprophytic fungi. It is common found on decayed fruits and bread.

Characteristics or Morphological Note:

1. Its plant body is composed of septate hyphae.
2. Its cells are multinucleate.
3. Its mycelium produces long, erect **conidiophore**.
4. Conidiophore forms multiple branches called **sterigmata**. It gives it brush like appearance.
5. The sterigmata develop chains of blue green conidia.
6. Sexual reproduction rarely occurs in it. It produces ascocarp during sexual reproduction. It produces ascospores inside.

Reason for identification: Blue green powder and brush like appearance

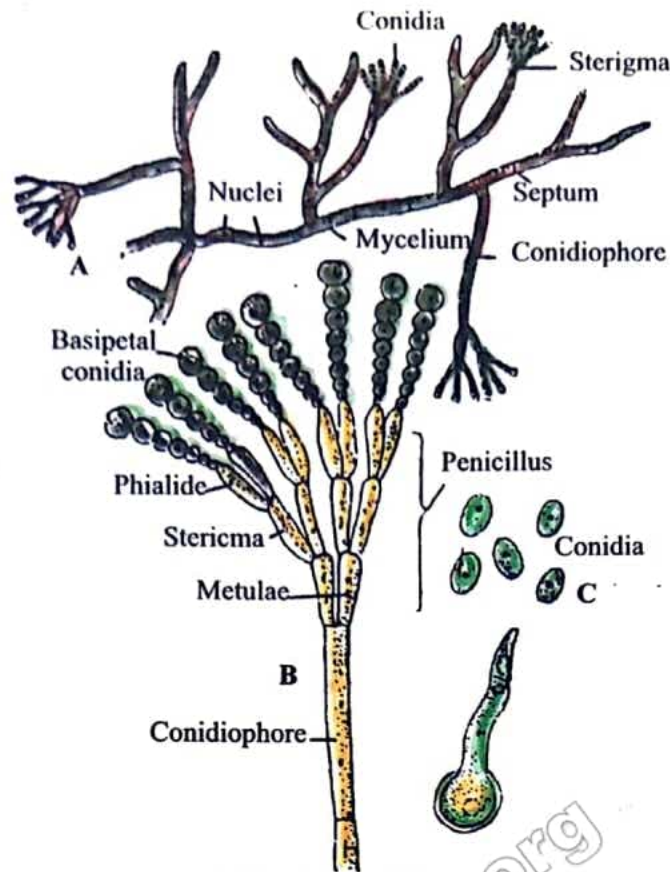


Fig. *Penicillium*

VIVA VOCE

Q.1 On what basis are *Penicillia* commonly called 'blue-moulds' or 'green moulds'?

Ans. They are called so on the basis of the colour of their conidia (spores).

Q.2 Name the species of *Penicillium* which spoil citrus fruits in storage.

Ans. The name of the species are *Penicillium italicum* and *P. digitatum*.

Q.3 Which species of *Penicillium* destroys apples, pears and grapes in storage?

Ans. It is the *Penicillium expansum*.

Q.4 Give the name of the mould from which antibiotic penicillin is prepared.

Ans. Penicillin is prepared from the mould *Penicillium notatum*.

Q.5 What are the benefits of some species of *Penicillium*?

Ans. A few species of *Penicillium* help produce organic acids such as citric, fumaric, oxalic, gluconic and gallic acids, cheeses and antibiotics.

Q.6 What is the purpose of washing the healthy orange with 70% alcohol during the preparation of culture of *Penicillium*?

Ans. It is washed to kill any previous growth *i.e.*, to disinfect it.

Q.7 When would growth be observed on the infected orange?

Ans. It depends on environmental conditions particularly suitable temperature.

Q.8 Name the species of *Penicillium* which is used to produce what anti-fungal antibiotic which is the only effective remedy for ringworm diseases including athlete's foot.

Ans. *Penicillium griseofulvum* is used to produce the anti-fungal antibiotic, *griseofulvin* which is the only effective remedy for ringworm diseases.

Q.9 What is the plural of *Penicillium*?

Ans. It is the penicillia.

Q.10 What is the fundamental difference between the algae and the fungi?

Ans. All the algae invariably possess chlorophyll and are, therefore, autotrophic in their mode of nutrition whereas the fungi lack chlorophyll and are heterotrophic in their mode of nutrition.

Q.11 What are the asexual reproductive cells of *Penicillium* known as?

Ans. The asexual reproductive cells of *Penicillium* are known as the conidia.

Q.12 What is the usual number of conidia on a sterigma?

Ans. Each sterigma bears a chain of 100 or more conidia.

Q.13 Differentiate between the mycelium and the hyphae.

Ans. The main body of a fungus is called mycelium which usually consists of simple or branched filaments known as the hyphae.

Q.14 What are conidiophores in the life-history of *Penicillium*?

Ans. The conidiophores are the conidia bearing hyphae which arise vertically from the main body (mycelium) of *Penicillium*.

Q.15 Name a parasitic and saprophytic fungus.

Ans. *Ustilago* is a parasitic fungus whereas *Penicillium* is a saprophytic one.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Write a morphological note on the specimen provided (*Penicillium*)

Identification = 1, Morphological note = 2, Diagram = 1, Short question = 1/2 + 1/2, Total = 5


(Gujranwala Board 2004)


Q.1 Which of the structure does look blue in the specimen?


Ans. Conidia


Q.2 Which structure does give brush like appearance?


Ans. Sterigmata


No.	Slide	Identification	Reason
1.		Yeasts	Buds are present on cells

No.	Slide	Identification	Two characters
1.		Yeasts	1. Buds are present on cells 2. Unicellular non-filamentous

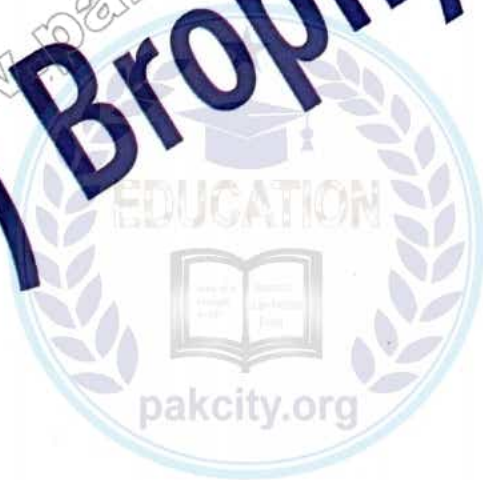
No.	Slide	Identification	Reason of Identification
2.		Ustilago (smut)	Black dusty powder on the ear of wheat

No.	Slide	Identification	Two characters
2.		Ustilago (smut)	<ol style="list-style-type: none"> 1. Black dusty powder on the ear of wheat. 2. Body composed of hyphae

No.	Slide	Identification	Reason of Identification
3.		Penicillium	Blue green powder and brush like appearance

No.	Slide	Identification	Two characters
3.		Penicillium	<ol style="list-style-type: none"> 1. Blue green conidia 2. Brush like appearance

(6)
Kingdom Plantae
(1) Bryophyta



12

Experiment

EXAMINATION OF MARCHANTIA AND FUNARIA (EXTERNAL MORPHOLOGY) FROM FRESH MATERIALS AND SEX ORGANS FROM PREPARED SLIDES

Marchantia and *Funaria* are *Bryophytes* which are the primitive land plants. They were the first to establish themselves on the land. On the basis of their appearance they are divided into two classes Liverworts-liver like and Mosses-Moss like *Bryophytes* complete their life cycle in two phases. A multicellular, Gametophyte-haploid phase alternates with multicellular sporophyte-Diploid phase. This phenomena is known as alternation of generation.

PART-I

STUDY OF *MARCHANTIA***Materials:**

Marchantia, male and female plants, Dissecting microscope, Razer blade, Watch glasses, Forceps, Prepared slides of T.S. of *Marchantia* thallus.

Procedure:

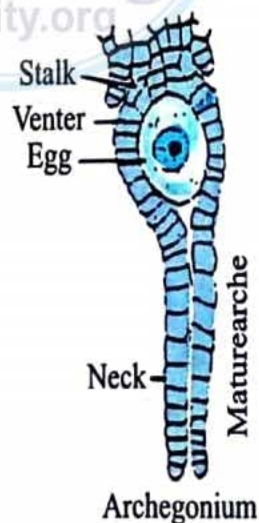
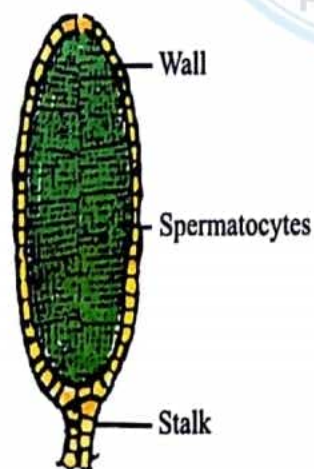
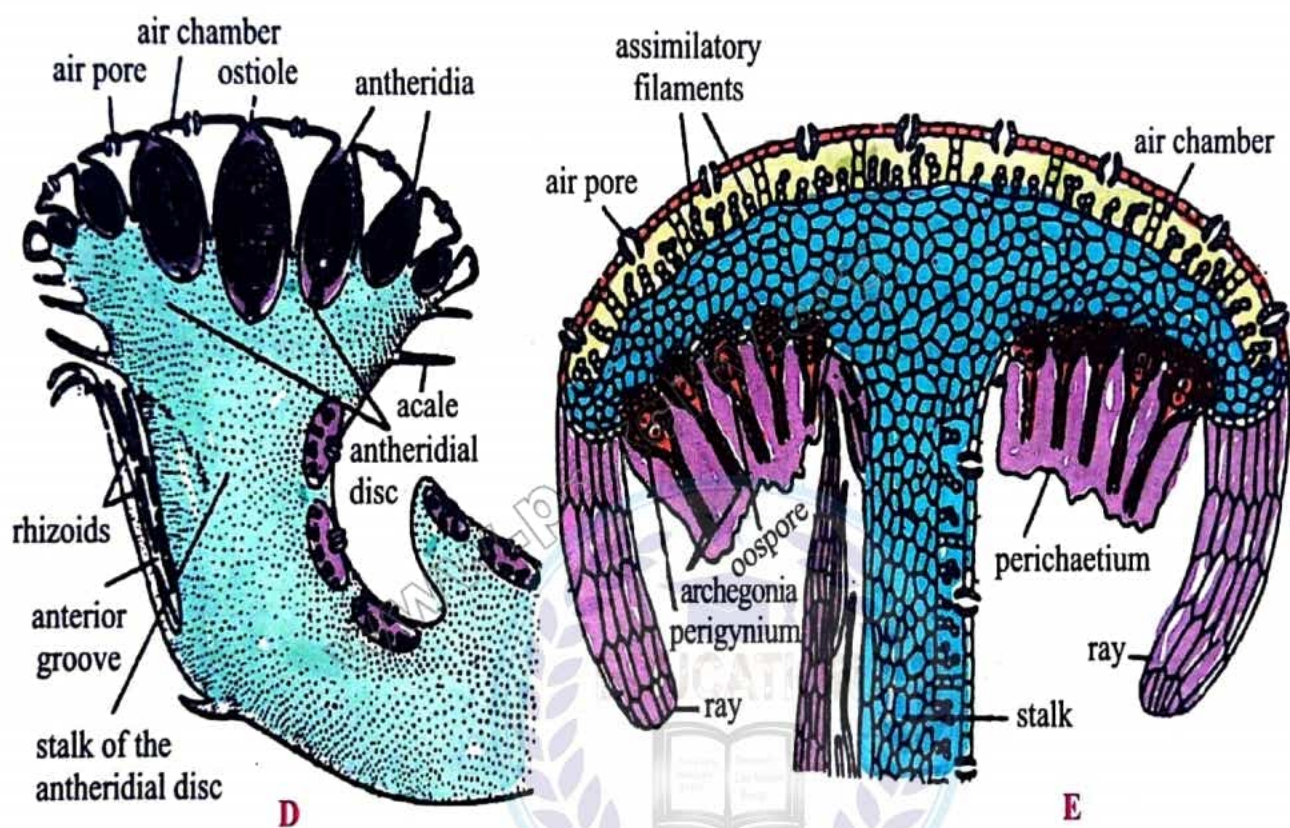
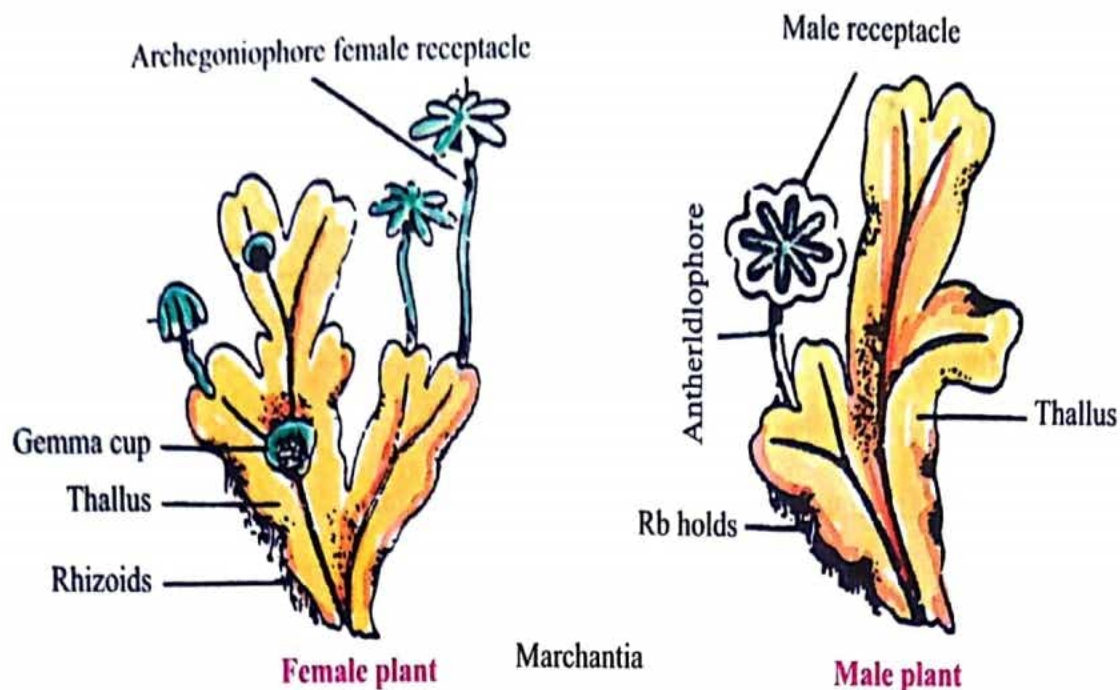
1. Take male and female *Marchantia* plants in watch glasses having water and study their different parts by hand lens.
2. Take a T.S. of *Marchantia* thallus and observe it under the microscope.

Classification:

Kingdom	Plantae
Phylum	Bryophyta
Sub-phylum	Hepaticopsida
Class	Hepaticeae

Study of External Morphology:**Marchantia:****Habitat:**

Marchantia is commonly found on damp ground by the sides of the streams, ditches and other shady and moist places.



***Marchantia* sp. A. Thalli, B. Female thallus. C. Male thallus, D. T.S. of antheridiophore with antheridia, E. T.S. of archegoniophore with archegonium**

on the lower surface of receptacle between rays. An egg or ovum develops in each archegonium.

Marchantia plant is a gametophyte. It is dioecious because male and female sex organs are born on separate plant.

Fertilization:

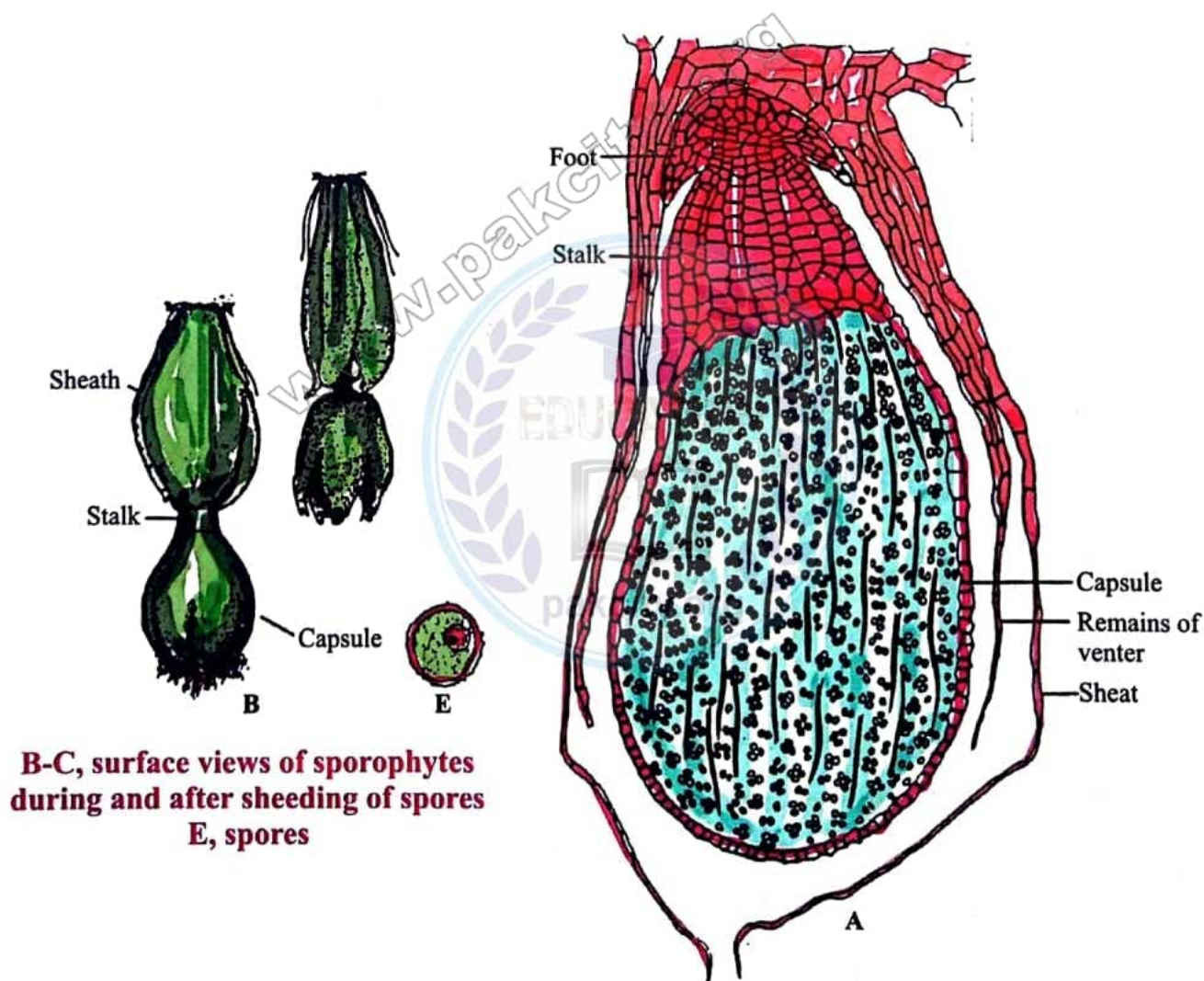
The spermatozoid swims in surrounding water to reach an egg and fertilizes it to form **oospore**. The oospore grows and forms the **sporogonium** or sporophyte.

Sporogonium:

The sporogonium consists of three parts:

- (i) A foot by which sporogonium is attached in the receptacle.
- (ii) A short **stalk** or **seta**.
- (iii) An oval **capsule** or **theca**.

Spores develop in each sporogonium which ruptures, on maturation and releases the spores in moist and shady places where each germinates into a thallus or a gametophyte. It means gametophyte is asexually reproducing generation.



**B-C, surface views of sporophytes during and after shedding of spores
E, spores**

Fig. *Marchantia polymorpha*

PART-II.

STUDY OF *FUNARIA*

Materials:

Funaria plant with sporophyte, Gametophytes with male and female sex organs or prepared slides of antheridium and archegonium, Slides, Cover slips, Dissecting needles, Dissecting microscope, Compound microscope, Glycerine-water solution.

Procedure:

1. Study a moss plant with sporophyte.
2. Break open the capsule of sporophyte with the help of a needle, in a drop of glycerine-water solution on a glass slide. Place a cover slip on it and examine under the microscope.
3. Take prepared slides of L.S. of antheridia and archegonium and observe them under the microscope.

Classification:

Kingdom	Plantae
Phylum	Bryophyta
Sub-phylum	Bryopsida
Class	Musci

Study of External Morphology:

Funaria (A Moss)

Funaria is a common moss which grows in large patches on moist ground, tree trunks and even on damp walls. It is about half an inch high. It shows alternation of generation.

Gametophyte:

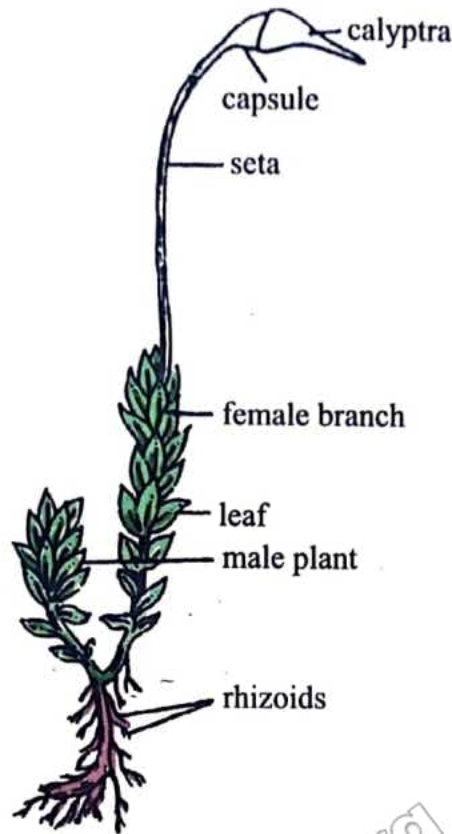
The gametophyte is the predominant generation of *Funaria* which reproduces sexually. It is differentiated into leaves and stem. There are no true roots.

Stem and Leaves:

Stem is small, feeble and erect. Rarely branches are present which arise beneath the leaves. The leaves are spirally arranged on the stem. Each leaf is simple, sessile and has a distinct midrib.

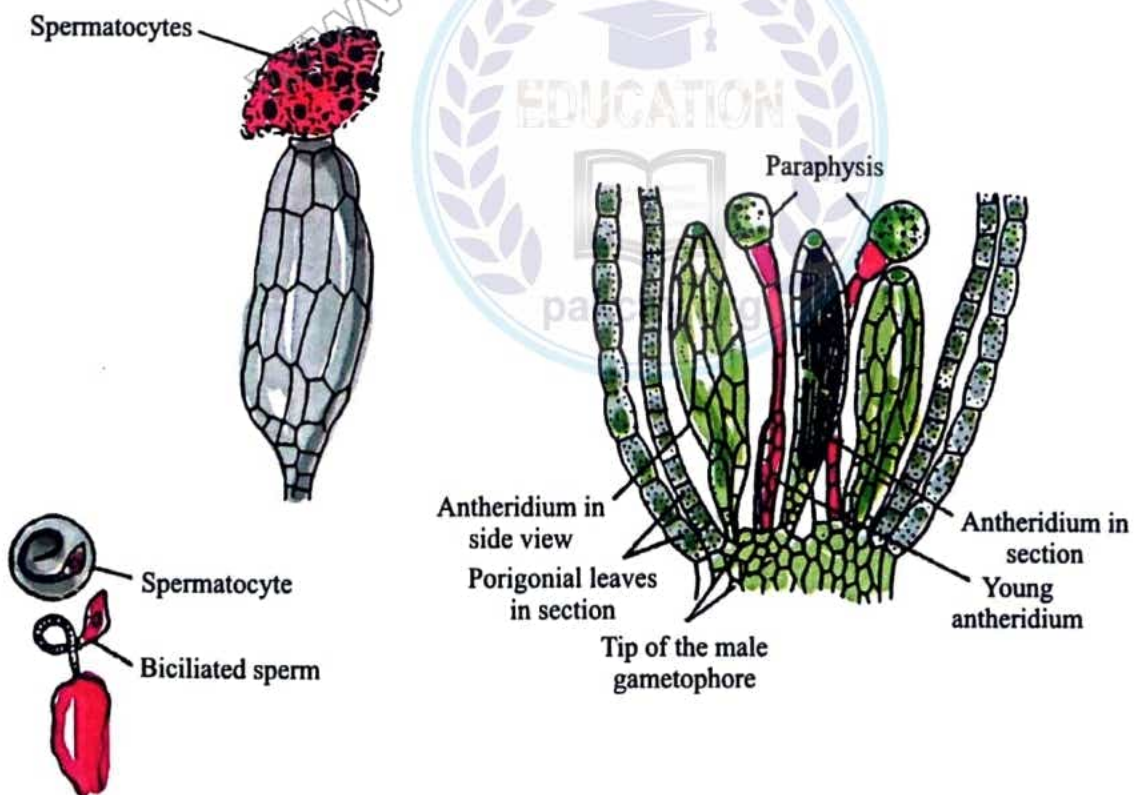
Rhizoids:

Numerous hair like branching structures called rhizoids develop from the base of the stem. They anchor the plant into soil.



Male Gametophyte:

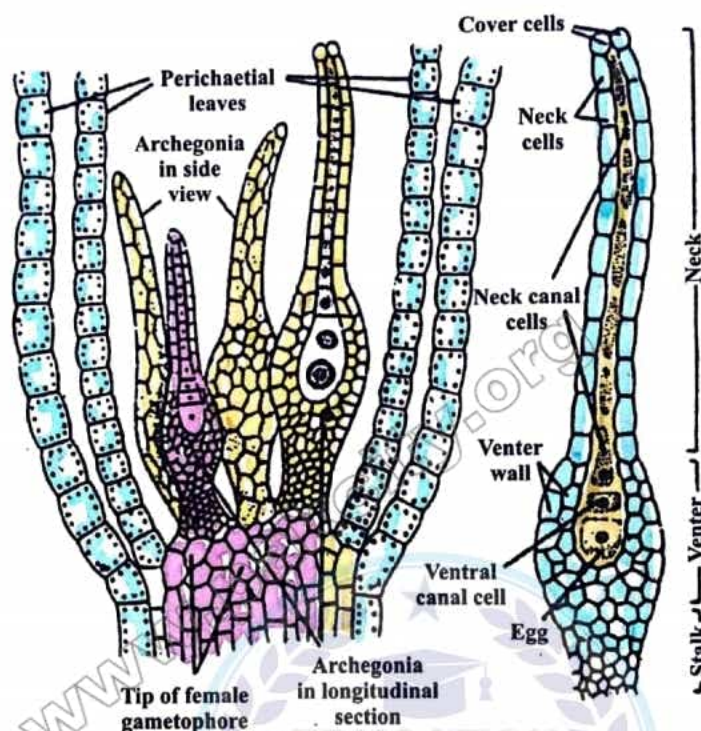
It is smaller in size and leaves are arranged in the form of a rosette at its apex. The male sex organs or **antheridia** are located at the tip of male gametophyte. Antheridia are protected by special leaves surrounding them. Antheridia are saclike structures which produces male gametes or sperms.



Female Gametophyte:

It is larger in size and bears a closed bud at the tip formed by the collection of leaves. Female sex organs called **archegonia** are present within the bud. Each archegonium consists of a long neck and a swollen base which contains the egg.

On maturation male gametes are released from the antheridia and swim in water to reach the archegonium. Here they fuse with eggs and form zygotes, which grow into sporophytes on the top of female gametophyte.

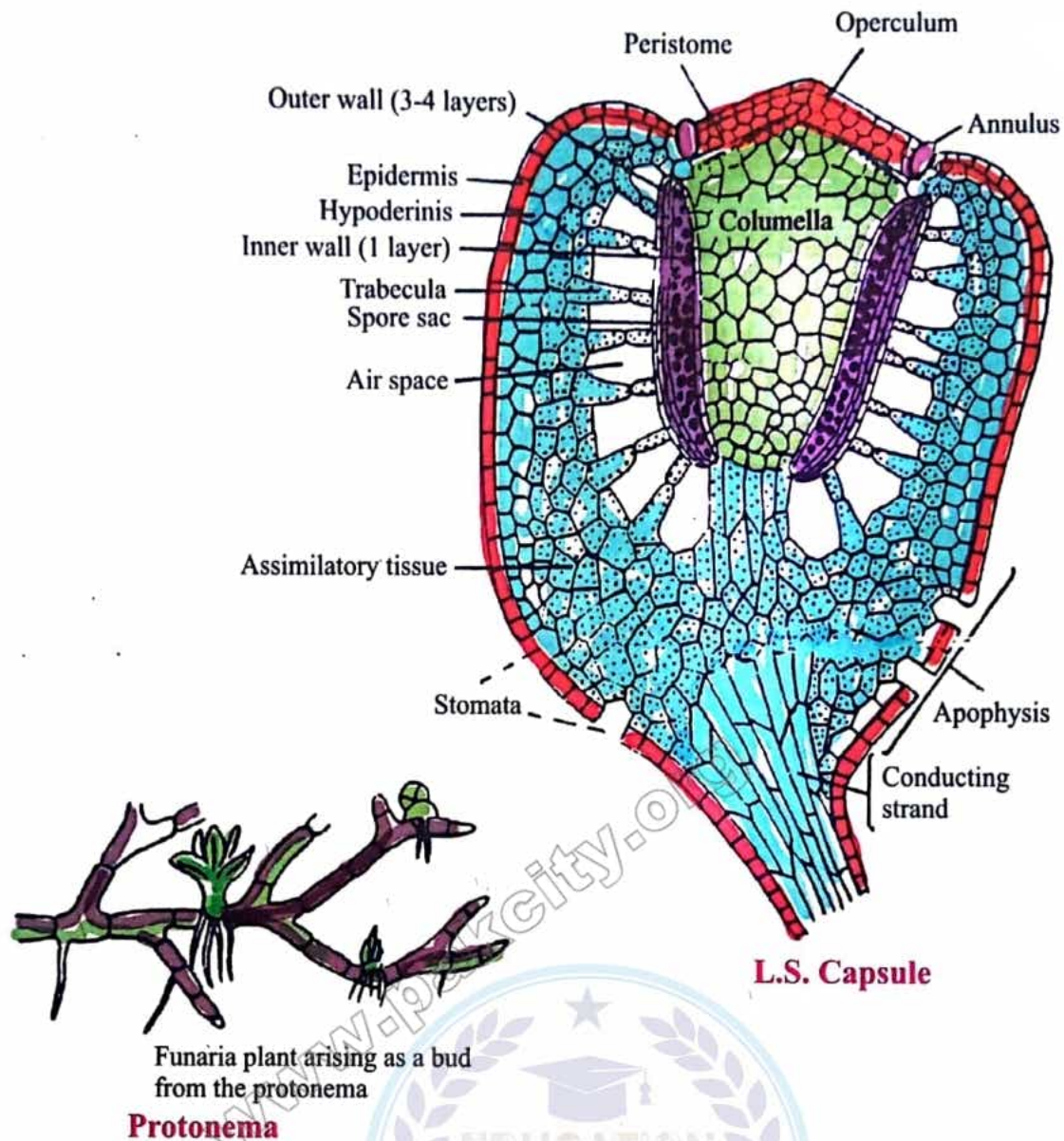


Sporophyte:

Sporophyte is a leafless non-photosynthetic plant that depends on the gametophyte for food, water and support. The sporophyte consists of three parts:

1. A **foot** which is embedded in the top of female shoot.
2. A **stalk** which is smooth and leafless.
3. A capsule or **sporangium** contains spores.

On maturation sporangium ruptures and a large number of thick walled spores are released. Each spore grows into a gametophyte.



VIVA VOCE

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Q.1 What is meant by dichotomous branches?

Ans. It means that every branch is divided into two branches.

Q.2 Why are bryophytes called amphibious plants?

Ans. Bryophytes need water for their reproduction. Therefore, they are called amphibious plants.

Q.3 What is meant by thallus?

Ans. A plant body that cannot be differentiated into root, stem and leaves is called thallus.

Q.4 What is the function of gemmae?

Ans. These gemmae are used for asexual reproduction.

Q.5 What is the adaptation in bryophyte for reducing loss of water?

Ans. A layer of cuticle is present in bryophytes. It prevents the loss of water from the surface of plant.

Q.6 What is the function of pores present on the upper surface of *Marchantia*?

Ans. These pores are used for exchange of gases. CO₂ moves inside by these pores.

Q.7 What is the function of rhizoids?

Ans. The function of rhizoids is to absorb water from soil.

Q.8 What are antheridiophore and archegoniophores?

Ans. Antheridiophore and archegoniophores are structure which arises from the thallus and give support of male and female receptacles.

Q.9 What type of alternation of generation does occur in bryophytes?

Ans. Heteromorphic alternation of generation.

Q.10 How can you differentiate between the male and female receptacles?

Ans. The male receptacle has wavy margin. But the female receptacle is starch shaped.

Q.11 Which is the dominant generation in bryophytes?

Ans. Gametophyte.

Q.12 Why does sporophyte generation depend on gametophyte?

Ans. Sporophyte is without chlorophylls and it needs support. Therefore, it depends on gametophyte for support and food.

Q.13 What are parts of antheridium of *Marchantia*?

Ans. Archegonia are composed of two parts. Lower swollen part is venter. Egg or oosphere is present in venter. The upper narrow part is neck.

Q.14 Differentiate between oosphere and oospore.

Ans. Oosphere is unfertilized egg. Oospore is fertilized egg or zygote.

Q.15 What is difference between *Marchantia* and *Funaria*?

Ans. *Marchantia* is liverwort and it has thallus body. *Funaria* is a moss. It has plant like body.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Write a morphological note on the specimen provided (*Marchantia*)

Identification = 1, Morphological note = 2, Diagram = 1, Short question = 1/2 + 1/2, Total = 5

(Multan Board 2003)

Q.1 Compare the sporophyte of bryophytes with the sporophyte of higher plants.

Ans. The sporophyte of bryophyte is dependant while the sporophyte of higher plants is independent and dominant.

Q.2 What are hair like structure present under the function of specimen? What is their function?

Ans. They are rhizoid. They absorb water.

Experiment 2: Identify the structures provided to you. Describe their structure and functions.
(Male and female receptacles of Marchantia)

(Bahawalpur Board 2003)

Q.1 What is the shape of the female receptacle?

Ans. It is star like.

Q.2 What structures are present on male receptacle? Give its function.

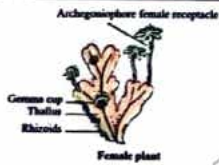
Ans. These structures are antheridium. They produce sperm or spermatozoids.


Experiment 3: Describe the external morphology of the specimen provided (Funaria)

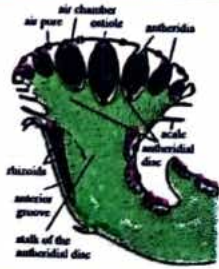
(Faisalabad Board 2004)

Q.1 Can sporophyte survive without gaemtophyte in this specimen?

Ans. Sporophyte is with out rhizoid and chlorophyll. Therefore, it cannot survive without gaemtophyte.

No.	Slide	Identification	Reason of Identification
1.		Female Marchantia	1. Star shaped female receptacle is present. 2. Rhizoid are present on lower surface.

No.	Slide	Identification	Two characters
2.		Male Marchantia	1. Disc shaped male receptacle is present. 2. Rhizoid are present on upper surface.

No.	Slide	Identification	Reason of Identification
3.		T.S. of male receptacle of Marchantia	Club shaped antheridia present

No.	Slide	Identification	Reason of Identification
4.		T.S. of female receptacle of Marchantia	Flask shaped archegonia is present

No.	Slide	Identification	Reason of Identification
5.		Funaria	Sporophyte is present on the gametophyte

No.	Slide	Identification	Reason of Identification
6.		Archegonia of Funaria	Flask shaped archegonia is present.

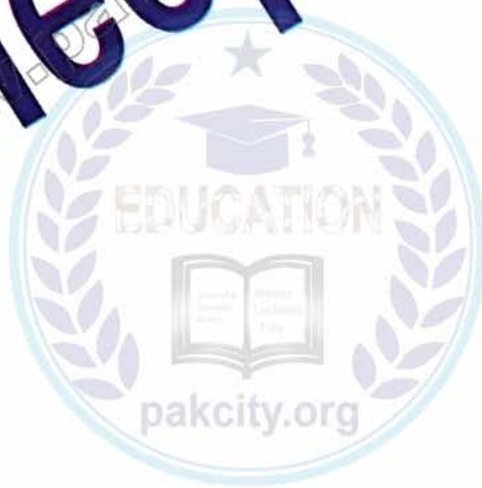
No.	Slide	Identification	Two characters
7.		Female Funaria	<ol style="list-style-type: none"> 1. Flask shaped archegonia is present. 2. Sporophyte is present on the gametophyte.

No.	Slide	Identification	Reason of Identification
9.		Antheridia of Funaria	Club shaped antheridia present.

No.	Slide	Identification	Two characters
10.		Male Funaria	<ol style="list-style-type: none"> 1. Club shaped antheridia present. 2. Rhizoids are present.

No.	Slide	Identification	Reason of Identification
11.		L.S of capsule of Funaria	Spores are present on the spore sacs.

(iii) Tracheophyta



13

Experiment

STUDY OF ADIANTUM (A) STUDY OF SPOROPHYTE PLANT BODY (B)
 PREPARATION OF SLIDE OF SPORANGIA (C) STUDY OF
 PROTHALLUS FROM FRESH OR PRESERVED
 MATERIAL/SLIDE

ADIANTUM

Classification:

Kingdom	Plantae
Division	Tracheophyta
Sub-division	Pteropsida
Class	Filicineae
Type	Adiantum (Fern)



Material:

Dissecting microscope, Compound microscope, Sporophyte and gametophyte of adiantum, Leaves, Sori, Prepared slide of T.S of the gametophyte, Slides and cover slip.

Procedure:

1. Sporophyte of Adiantum is taken. Its different parts are studied by hand lens and microscopes.
2. Sporangium of adiantum is removed from sorus. It is placed on slide in a drop of water and studied under microscope.
3. The prothellus or gametophyte is placed on slide. It is studied with low and high power of microscope.
4. A prepared slide of prothellus of Adiantum is taken. It is studied under microscope.

Observation:

Adiantum show alternation of generation. Main plant body is sporophyte. Gametophyte is in reduced form.

Study of Sporophyte:

The plant body is sporophyte. It is a small herb. It consists of stem, roots and leaves.

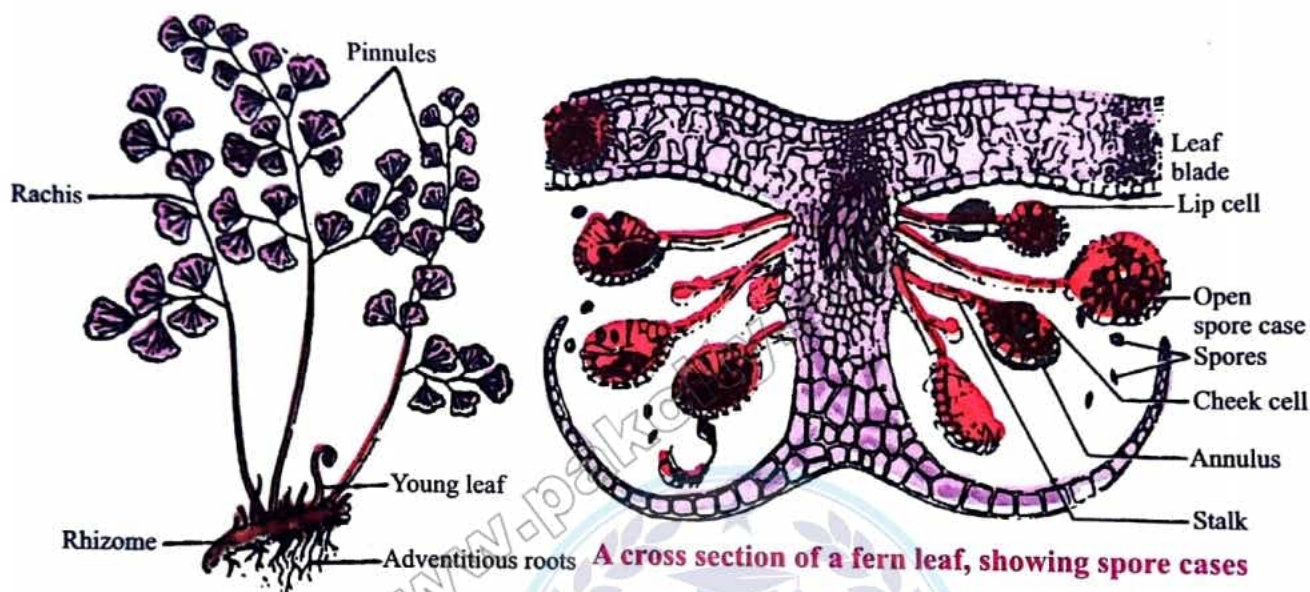
1. **Stem:** It has a short, thick and underground stem called **rhizome**. Rhizome is protected by brownish scales called **ramenta**.
2. **Roots:** It has fibrous **adventitious** roots.
3. **Leaves:** It has pinnately compound leaves or **fronds**. Fronds arise from the upper side of the rhizome. Young leaves show circinate vernation. The rachis has many **leaflets** called pinnae and pinnules. Pinnae and pinnules show dichotomous venation.
Pinnae are arised directly from the rachis. These are leaflet of first orders.
Pinnules: Each pinna has many pinnules. Pinnules are leaflets of second order

4. **Sporangium:** The sporangia are present in groups called **Sori**. Each sorus has a number of sporangia covered by false **indusium**.

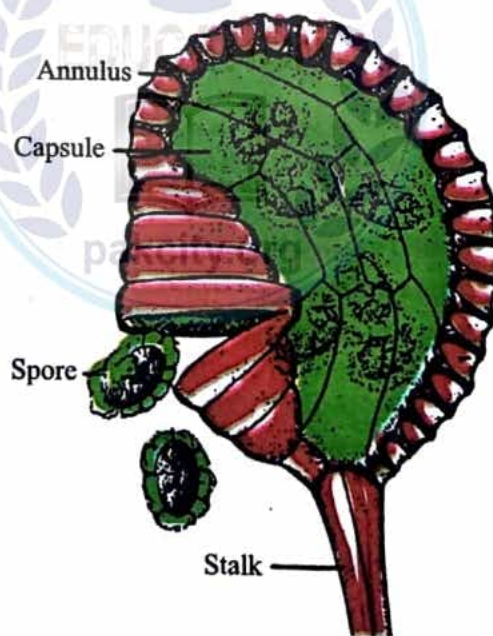
Each sporangium is slightly flattened. It has biconvex body called **capsule**. This capsule is borne on a multicellular stalk. The wall of capsule consists of a single layer of flat, thin walled cells. The edge of the capsule is made up of two parts:

- ◆ **Annulus:** The annulus form three fourth of the edge. The radial and inner walls of the annulus are thick.
- ◆ **Stomium:** The remaining fourth part is the stomium. The stomial cells are thin-walled.

Spore mother cells divide by meiosis within the sporangium and form haploid spores. The spores are dispersed by wind. Spore produces a haploid gametophyte or prothallus.



Adiantum (Sporophyte)



Fern sporangium

Study of Prothellus or Gametophyte:

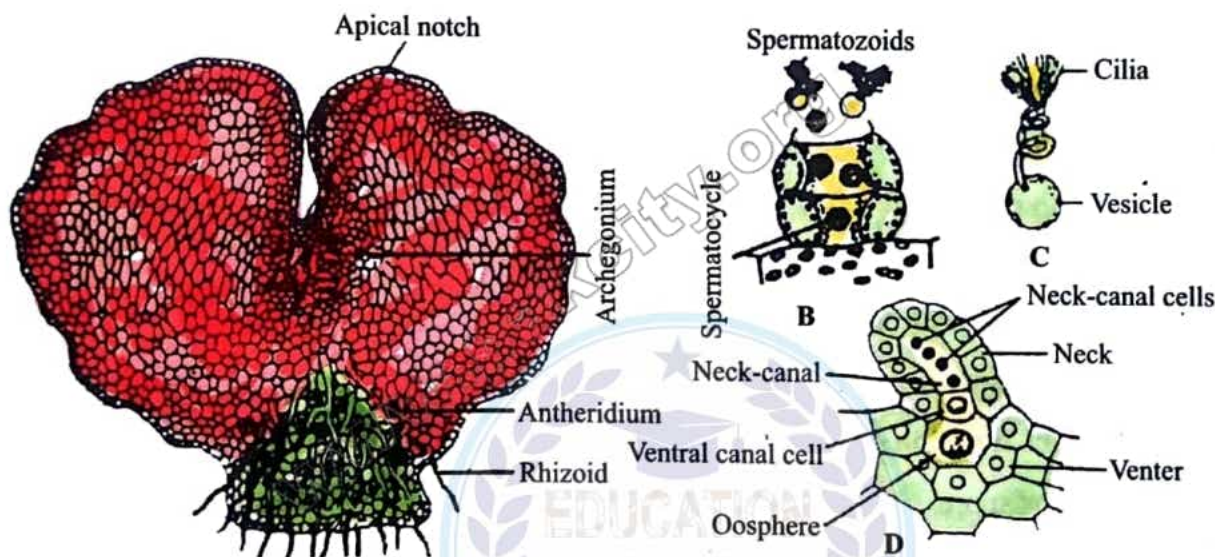
Prothallus is a small, flat and heart shaped structure. It is autotrophic. A notch is present at the anterior end of the prothallus. The growing point lies in this notch. It has unicellular **rhizoids** on its lower surface towards the posterior end. Prothallus is composed of rounded thin walled cells.

Reproduction Organs:

The prothallus is **monoecious** i.e., male and female sex organs are present on the same plant. These sex organs are present under the surface of prothallus.

- ◆ **Archegonia:** The archegonia are present near the notch. The archegonium consists of **venter** and a **neck**. The venter contains an egg or **oosphere**.
- ◆ **Antheridia:** The antheridia are scattered among the rhizoids. Each antheridium produces numerous spermatozoids. The spermatozoids are spirally coiled and multiciliated.

Spermatozoids or antherozoids in water and reach the archegonium. Fertilization occurs and **oospore** is formed. The oospore grows to form sporophyte.



Prothellus or gametophyte of Adiantum

Reproductive organs of Adiantum

VIVA VOCE

Q.1 Why is Adiantum called as fern?

Ans. The vascular flowerless with feathery green leaves are called ferns.

Q.2 What is a rhizome?

Ans. Short thick underground stem is called rhizome.

Q.3 What are adventitious roots?

Ans. The roots which directly arised from the stem are called adventitious roots.

Q.4 What are fronds?

Ans. The feathery pinnately compound leaf is called frond. It is composed of rachis, pinnae and pinnule.

Q.5 Differentiate between pinnae and pinnule.

Ans. Pinnae are arised directly forms the rachis. These are leaflet of first orders. Each pinna has many pinnules. Pinnules are leaflets of second order.

Q.6 How can you differentiate between old and younger leaves of Adiantum?

Ans. The younger leaves are present near the growing tip of plant. They show circinate vernation (coiled). Old leaves are present at the lower surface and they do not show circinate vernation.

Q.7 What is sorus?

Ans. The sporangia are present in groups called Sori.

Q.8 What is annulus?

Ans. The annulus form three fourth of the edge of sporangium. The radial and inner walls of the annulus are thick.

Q.9 What is Stomium?

Ans. The fourth part of the wall of the sporangium is called stomium. The stomial cells are thin-walled.

Q.10 What cells are present at edge of capsules?

Ans. Annulus and stomium.

Q.11 What is the function of annulus and stomium?

Ans. Dispersal of spores.

Q.12 What structure does present under the reflex surface of leaflet of Adiantum?

Ans. Sorus.

Q.13 Which generation is dominant in Adiantum?

Ans. Sporophyte generation.

Q.14 What is shape of prothellus of Adiantum?

Ans. Prothallus is a small, flat and heart shaped structure.

Q.15 What is meant by monoecious plants?

Ans. Male and female sex organs are present on the same plant.

Q.16 Compare the gametophyte of adiantum with the gametophyte of bryophytes.

Ans. The gametophyte of bryophytes is dominant. But the gametophyte of Adiantum is reduced.

Q.17 Where are the reproductive organs of *Adiantum* located?

Ans. Reproductive organs are present under the surface of prothellus.

Q.18 Which generation is haploid and which generation is diploid in *Adiantum*?

Ans. Gametophyte is haploid and sporophyte is diploid.

Q.19 Which type of leaf is present in *Adiantum*?

Ans. Compound leaf.

Q.20 Why is *Adiantum* called as maiden hair fern?

Ans. The stipe of rachis is black smooth and shiny. Therefore, it is called maiden hair fern.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Study the specimen (*Adiantum*) and prepare a slide of specimen.

Identification and slide = 1, Morphological note = 2, Diagram = 1, Short question = 1/2 + 1/2,
Total = 5

(Multan Board 2004)

Q.1 What is the shape of young leaves?

Ans. They are coiled.

Q.2 What is the role of annulus?

Ans. Annulus forms protective coat of sporangium. It has role in splitting of sporangia.

Experiment 2: Write morphological note on the specimen provided (*Adiantum*).

Identification = 1, Morphological note = 2, Diagram = 1, Short question = 1/2 + 1/2, Total = 5

(Multan Board 2004)

Q.1 What are brown structures near the margin of leaflets?

Ans. They are sori.

Q.2 What types of cells help in dispersal of spores?

Ans. Annulus and stomium.

Experiment 3: Study the sporophyte of the given specimen. Prepared slide of its sporangium
(Specimen *Adiantum*)

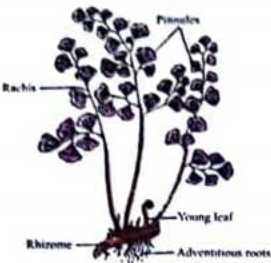
(D.G. Khan Board 2004)


Q.1 Name the part of leaves of the specimen.


Ans. The parts of leaf are rachis, pinnae and pinnule.


Q.2 What is sorus?

Ans. The sporangia are present in groups called Sori.

No.	Slide	Identification	Reason of Identification
1.		Fronds of Adiantum	Sori are present on the reflex leaf surface.

No.	Slide	Identification	Two characters
1.		Adiantum (Sporophyte)	<ol style="list-style-type: none"> Compound leaves or fronds are present. Under ground rhizome is present.

No.	Slide	Identification	Reason of Identification
2.		Sporangium of Adiantum	Thick walled annulus cells are present.

No.	Slide	Identification	Two characters
2.		Sporangium of Adiantum	<ol style="list-style-type: none"> Thick walled annulus cells are present. Stalk of the sporangium is present.

STUDY OF PINUS MALE AND FEMALE CONES FORM FRESH OR PRESERVED MATERIALS

14 Experiment

PART-A MALE CONE OF PINUS

Material:

Male cone, Dissecting and compound microscope, Blade, Glass slide, Cover slip.

Procedure:

1. External structure of male cone is observed with the help of hand lens or dissecting microscope.
2. A scale of male cone is placed on slide. It is studied under compound microscope.
3. Microscope is taken on slide. It is crushed. Microspores or pollen grains are observed under microscope.



Fig. B-Male cone, C-scale, D-pollen grain

Observation:

Male cone has small size. Following structures are observed in male cone.

1. **Micro Sporophylls:** Male cone has central axis. Many spirally arranged leaves are attached on this central axis. These leaves are called microsporophyll.
2. **Microsporangia or Pollen Sac:** Two microsporangia are attached on the surface of each sporophyll.
3. **Microspores or Pollen Grain:** A large number of pollen grains are produced in each microsporangium. The pollen grain has two large **air sacs**. These air sacs help in the dispersal of microspore. Pollen grains reach the female gametophyte by wind.
4. **Pollen Tube or Male Gametophyte:** Pollen tube germinates to produce male gametophyte in the form of pollen tube. Pollen tube has sperms.

Reason for identification: Male cone has very small size and it has small microsporophyll.

PART-B**FEMALE CONE OF PINUS****Material:**

Female cone, Hand lens, Blade.

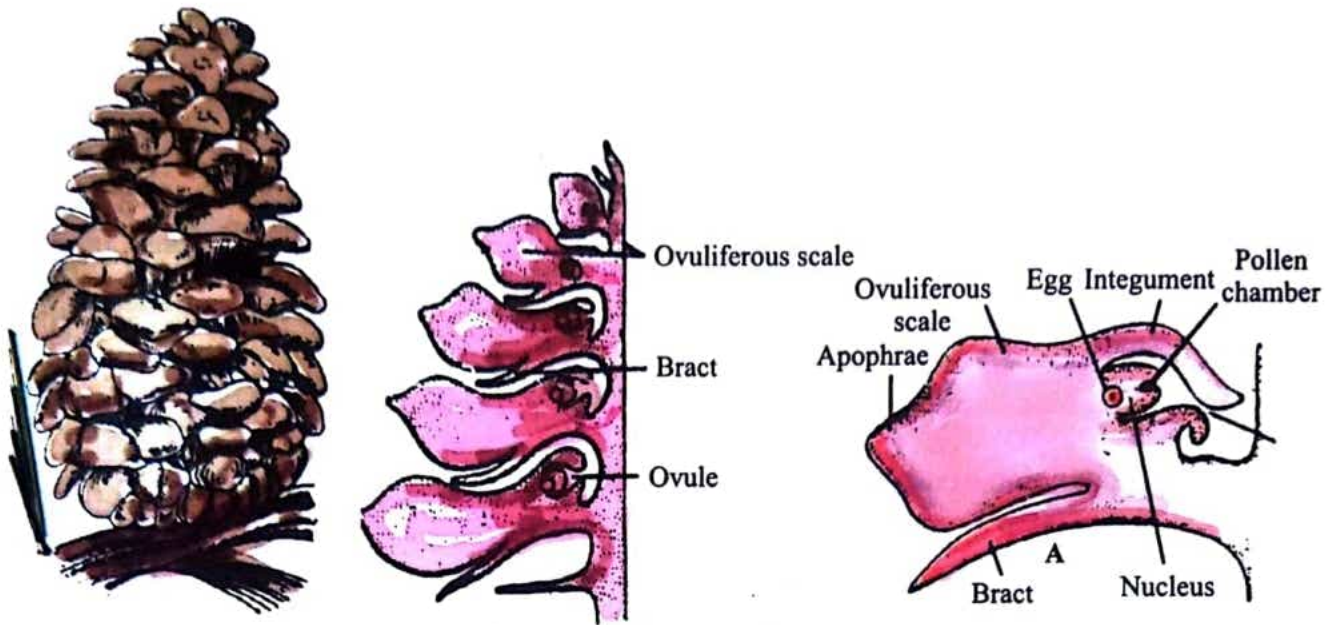
Procedure:

External structure of male cone is observed with the help of hand lens or dissecting microscope.

Observation:

Male cone is much large than female cone. It consists of following parts:

1. **Megasporophylls:** Female cone has central axis. Many megasporophylls are attached on this central axis. These sporophylls are hard and woody structure. They are spirally arranged. Each sporophyll is composed of two scales.
 - ◆ Upper ovuliferous scales
 - ◆ Lower bract scale
2. **Ovule:** There are two depressions on the upper surface of each ovuliferous scales. Two sporangia are attached in these depressions.



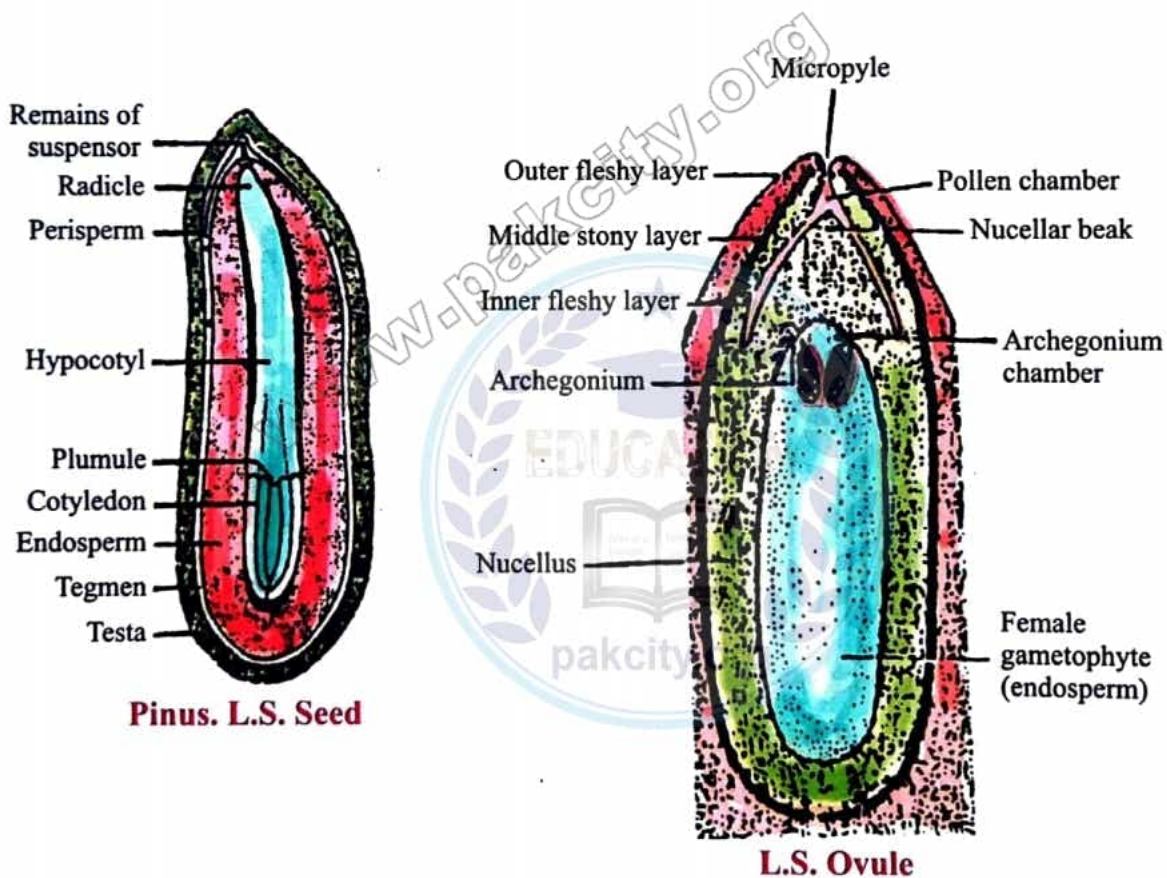
- Megaspores:** Four megaspores are produced in ovule by meiosis. Three megaspores degenerate. Only one functional megaspore is left.
- Female Gametophyte:** The functional megaspore germinates to produce female gametophyte. The female gametophyte has archegonia. These archegonia have eggs.

Reason for identification: Female cone has large size. It has large scales.

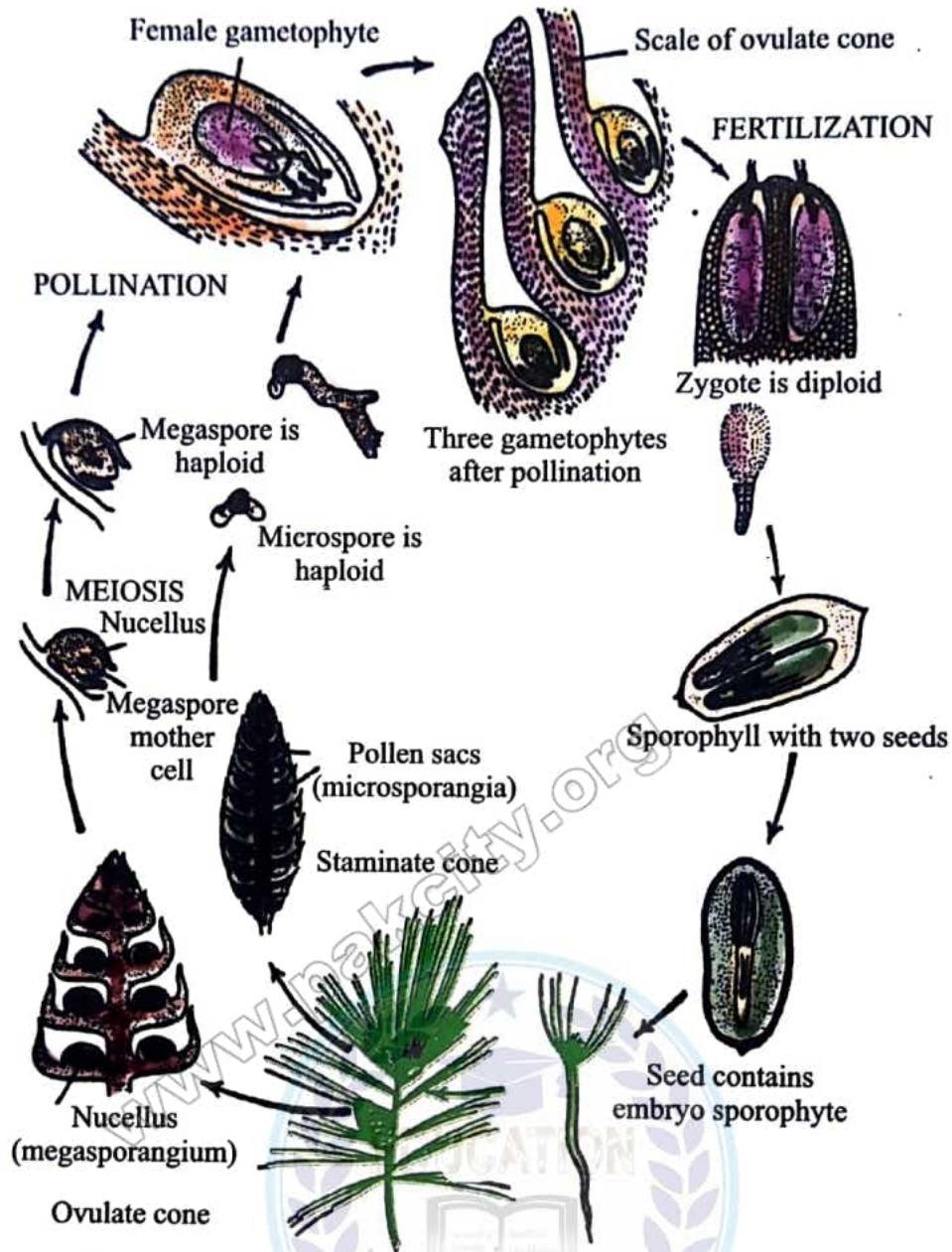
Seed:

Take a seed of *Pinus longifolia* and *P. giradiana*. The seed of *P. longifolia* has a papery wing for better dispersal.

Cut a longitudinal section of seed, it is covered with tough hard covering testa, lined internally by thin membranous covering called tegmen. An embryo is centrally located having radicle to the upper pointed end followed by hypocotyle and then cotyledons. Embryo is enclosed in the endosperm the food for next generation.



LIFE CYCLE



The diploid sporophyte produces two kinds of cones
The Life History of a Pine Tree.

VIVA VOCE

Q.1 What is a sporophyll?

Ans. The reproductive leaves on which sporangia are attached are called sporophylls.

Q.2 How can you differentiate between male and female cone?

Ans. Male cone has much smaller size than female cone.

Q.3 Where are the male cones present on the tree?

Ans. Male cones are present on the tip of braches.

Q.4 Why do pollen grains have air sacs?

Ans. These are sacs are used for dispersal of pollens through wind.

Q.5 What is shape of leaves of pinus plant?

Ans. Needle like.

Q.6 Why is the shape of leaves of pinus plant needle like?

Ans. This shape helps to reduce to the loss of water by transpiration.

Q.7 Where is the male gametophyte located?

Ans. Pollen with sperms is the male gametophyte.

Q.8 Name woody structure of female pinus cone which are arranged spirally.

Ans. They are megasporophylls.

Q.9 How many types of scales are present in megasporophylls?

Ans. Two types: Bract scale and woody ovuliferous scale.

Q.10 Why are they scales of the female cone woody in nature?

Ans. The woody scales protect the megasporangia.

Q.11 Ovules are located on which of the scale of female cone.

Ans. Ovuliferous.

Q.12 Why is the female cone sticky in nature?

Ans. The female cone secretes gum like material. It makes the cone sticky for attachment of pollen grain.

Q.13 What is difference between ovule and megasporangium?

Ans. The integumented megasporangium with female gametophyte is called ovule.

Q.14 Where is the female gametophyte located?

Ans. Female gametophyte is located within the ovule.

Q.15 Where is the egg of the pinus located?

Ans. Egg is located within the archeogonium in ovule.

Q.16 Write common name of seed of pinus?

Ans. Chilgoza.

Q.17 How much time does female cone take to form seed inside?

Ans. Three years.

Q.18 What are gymnosperms?

Ans. The naked seed plants are called gymnosperms.

Q.19 Why is pinus called as conifer?

Ans. Pinus develop cones. Therefore, it is called conifer.

Q.20 Define ovule.

Ans. Integumented indehiscent megasporangium is called ovule.

Q.21 Define seed.

Ans. A fertilized ovule is called seed.

Q.22 What is the function of pollen tube?

Ans. Pollen tube is use to transfer the sperm into ovule.

Q.23 Why is the pinus called as evergreen tree?

Ans. Pinus never shed its leaves. Therefore, it is called evergreen plant.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Write morphological note on male cone of pinus.

Slide preparation = 1, Morphological note = 2, Diagram = 1, Short question = 1/2 + 1/2, Total = 5


(Rawalpindi Board 2003)


Q.1 Name the woody structure present in the given specimen.

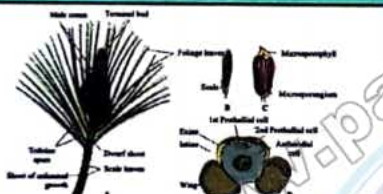
Ans. Sporophylls in the form of scales.


Q.2 On which scale are the ovules present?

Ans. Ovuliferous scales.

No.	Slide	Identification	Reason of Identification
1.		Female	Female cone has large size. It has large scales.

No.	Slide	Identification	Two characters
1.		Female cone	<ol style="list-style-type: none"> 1. It has large scales, bract and ovuliferous scale. 2. Ovules are present on the scales.

No.	Slide	Identification	Reason of Identification
2.		Male cone of pinus	Male cone has very small size and it has small spirally arranged microsporophyll.

No.	Slide	Identification	Two characters
2.		Male cone of pinus	<ol style="list-style-type: none"> 1. Spirally arranged microsporophyll. 2. Microsporangia are present on sporophylls.

15A Experiment

STUDY OF FLOWER

Flower is a modification of branch and is the reproductive part of the plant.

Material:

Flowers Solanum nigrum, Sweet pea, Avena, Brassica Dissecting box slides compound microscope.

Procedure:

Take the above mentioned flowers and note its following parts.

Axis of Flower or Stalk:

It is the small stalk or branch like structure which get enlarged at its apex to form **Thalamus** or **Receptacle**. It may be flat rounded conical or cup like. Flower parts or floral leaves are arranged on it in the form of whorls. The floral leaves are as under:

Calyx or Sepals:

These form the outer most circle of small triangular in shape usually green leaves. They enclose and protect inner parts of flower, specially in the bud condition. The sepals may be free or united called polysepalous when free and Gamosepalous when united.

Corolla Petals:

They are brightly coloured leaves usually other than green, forming the second whorl. They are meant for the attraction of insects. They may be polypetalous or free and gamopetalous or united.

Androecium:

These are modified stamens floral leaves forming third whorl. These are also called male parts. Each stamen consist of a stalk or rod like **filament** and lobed structures at the tip called **anthers**. These are made of pollen sacs. Take anther on a slide in water or glycerine water, tease and break them open. Study under the microscope. A large number of rough surfaced particles called **pollen grains** are visible.

Pollination:

These pollen grains are transferred to the stigma of female part. This transfer is called pollination.

Gynocium Pistil:

It is the inner most whorl which consist of flask shape structure called carpel, or pistil and collectively Gynaecium. It is the female part of the flower. Carpels may be free or united. Each carpel consist of flattened rounded tip stigma, neck shape style and lower broad part ovary. Cut a longitudinal and transverse section of carpel. A number ovules may be visible with naked eye, and its part of attachment is called placenta.

Perianth:

Calyx and corolla are collectively also called perianth and are non-essential parts of flower. Stamen and carpel are essential parts of flower.

Terminology to Explain Flower:

1. **Bracteate:** The flower which arises in the axil of bract.
2. **Ebracteate:** Flower with out bract.
3. **Pedicillate:** Flower present on the pedicil or stalk.
4. **Sessile:** Flowers without pedicil.

5. **Complete:** Flowering having both sepal and petal.
6. **Incomplete Flower:** A flower without one or more of the normal parts, as carpels, sepals, petals, pistils, or stamens.
7. **Bisexual or Hermaphrodite:** Flower having both stamens and carpels are called Bisexual.
8. **Unisexual:** Flower having either stamen or carpels.
 - (i) Flower having only stamens are called male or staminate.
 - (ii) Flower having only pistil are pistillate or female.
 - (iii) Flower without any pistil or stamen is Neuter.
9. **Actinomorphic:** Flower having its parts regularly arranged or flower can be divided into 2 equal halves at more than two planes. (= **Radially symmetrical**)
10. **Zygomorphic Irregular:** (= Bilateral Symmetry) Flower which can be divided into 2 equal halves only at one plane.

11. **Hypogynous:**

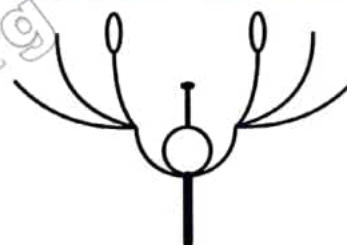
- Gynoecium present above all other floral parts.
- Ovary is superior E.g. Brinjal, China rose



Hypogynous Superior ovary

12. **Perigynous:**

- Gynoecium present at the centre
- Other part almost at same level
- Ovary half inferior E.g. Plum, Ros



Perigynous

13. **Epigynous:**

- Ovary enclosed by thalamus and gets fused with it
- Other parts above ovary
- Ovary inferior E.g. Guava, Cucumber



Epigynous Inferior ovary

14. **Perianth:** Collective term for sepals and petals
many be free or united

15. **Sepaloid:** green in colour

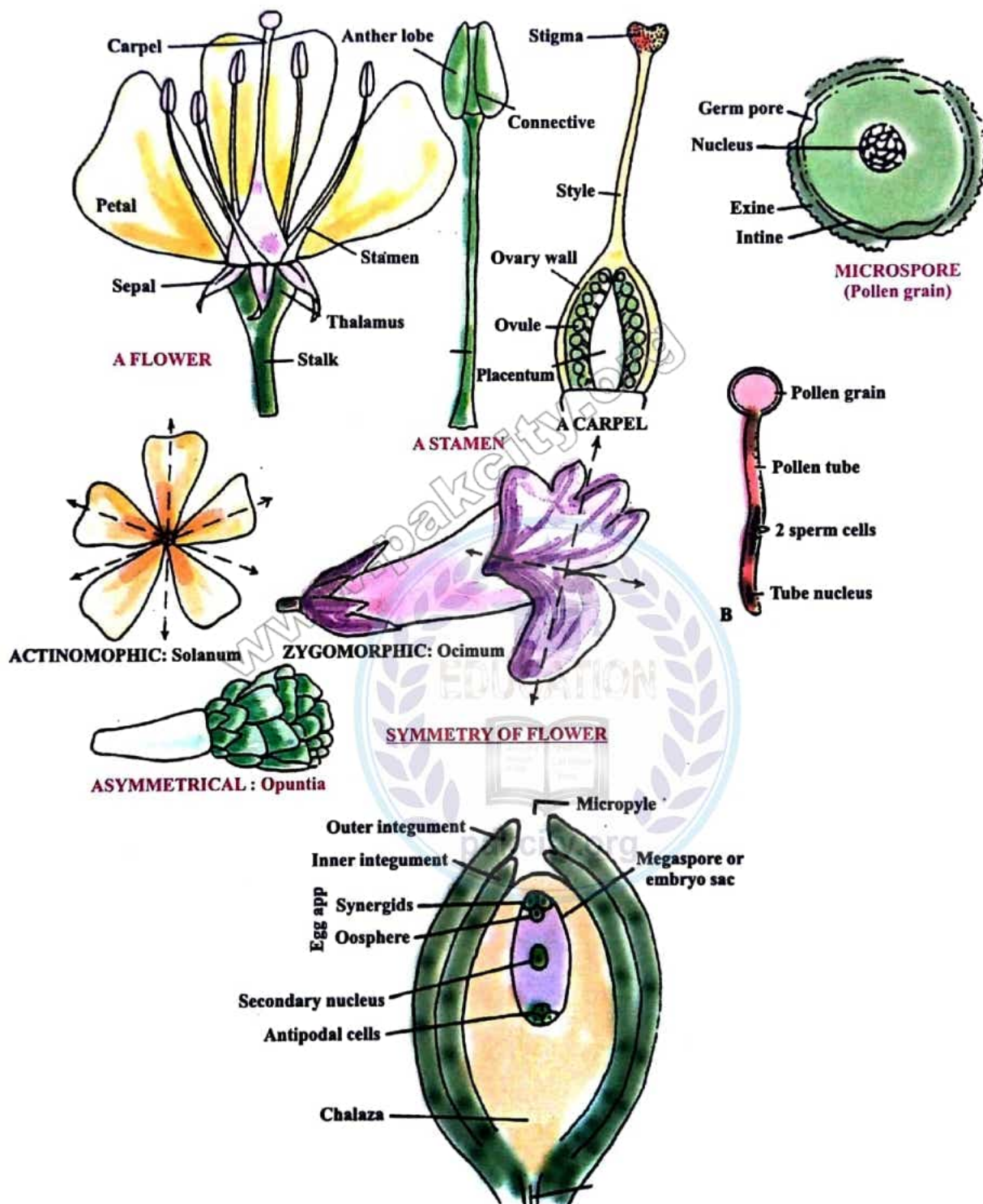
16. **Petaloid:** coloured other than green

Calyx:

Petaloid:	Sepals having the colour of petal
Polysepalous:	Sepal free
Gamosepalous:	Sepals united
Bilabiate:	Sepals fused in two
Bilipped:	Groups like two lips
Companulate:	Sepals fused to form bell like shape

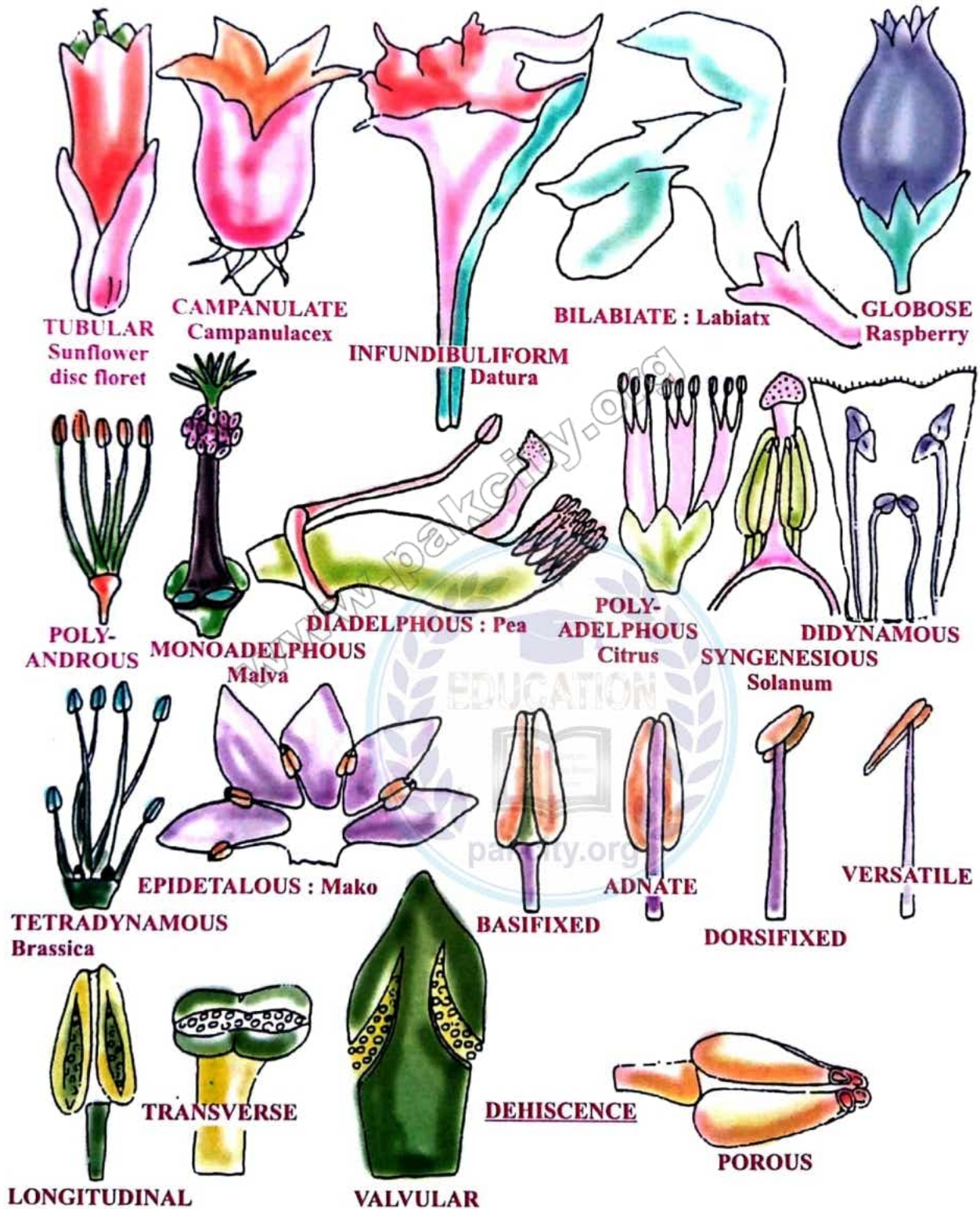
Corolla:

- (a) **Sepaloid:** Petal green in colour
- (b) **Polypetalous:** Petals free
- (c) **Gamopetalous:** Petal united
- (d) **Bilabiate:** Petals forming
- (e) **Bilipped:** Two lips



Androecium:

- (a) **Polyandrous:** Stamens free
- (b) **Adelphous:** Filaments united anther free
- (c) **Staminal tube:** Stamens united to form a tube around the style
- (d) **Epipetalous:** Stamen attached to the petals
- (e) **Basifixed:** Filament attached to the base of anthers
- (f) **Versatile:** Filament attached to side of anther, anthers can swing



Gynaecium:

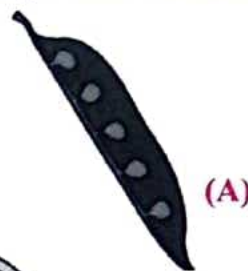
- (a) **Monocarpellary:** Carpel one
 (b) **Bicarpellary:** Carpels two
 (c) **Tricarpellary:** Carpels three
 (d) **Polycarpellary:** Carpels more than three
 (e) **Apocarpous:** Carpels free
 (f) **Syncarpous:** Carpels united

Placentation

Placentation refers to the arrangement of ovules inside the ovary. It is of five basic types.

(A) Marginal placentation

The ovary in which the placenta forms a ridge along the ventral suture of the ovary and the ovules develop on two separate rows is known to have marginal placentation. This type of placentation is found in peas.

**(B) Parietal placentation**

When the ovules develop on the inner walls of the ovary, the ovary is said to have parietal placentation.

**(C) Axile placentation**

In axile placentation, the placenta is axial and ovules are attached to it. Example include China rose, lemon, and



tomato.

(D) Basal placentation

The ovary in which the placenta develops from its base and a single ovule is found attached to the base is said to have basal placentation. It is found in marigold and sunflower.

**(E) Free central placentation**

In free central placentation, the ovules develop on the central axis while the septa are absent. This type of placentation is found in Dianthus and primrose.

**Symmetry:**

Actinomorphic

⊕

Zygomorphic

†

Sex:

Bisexual

♂
+

Male

♂

Female

♀

Sepals:

Free

K number

United

K (number)

Corolla:

Free

C

C_{number}

United (number)

C_(number)**Androecium:**

Free

A

A number

United

A (number)

Epipetalous

C **Carpel:**

Free Apocarpous

G number

United Syncarpous

G (number)

Superior

G number

Inferior

G number**For example floral formula of *Petunia* may be written as:**

15B**Experiment**

DIFFERENT TYPES OF INFLORESCENCE



Inflorescence:

Development of flowers in group on the same shoot is called Inflorescence.

Flower when present in singles are called **solitary**.

Material:

Inflorescence of various types:

Pea, Brassica salvia Avena saliva and others, hand lens or Dissecting microscope.

Following are some types of inflorescence:

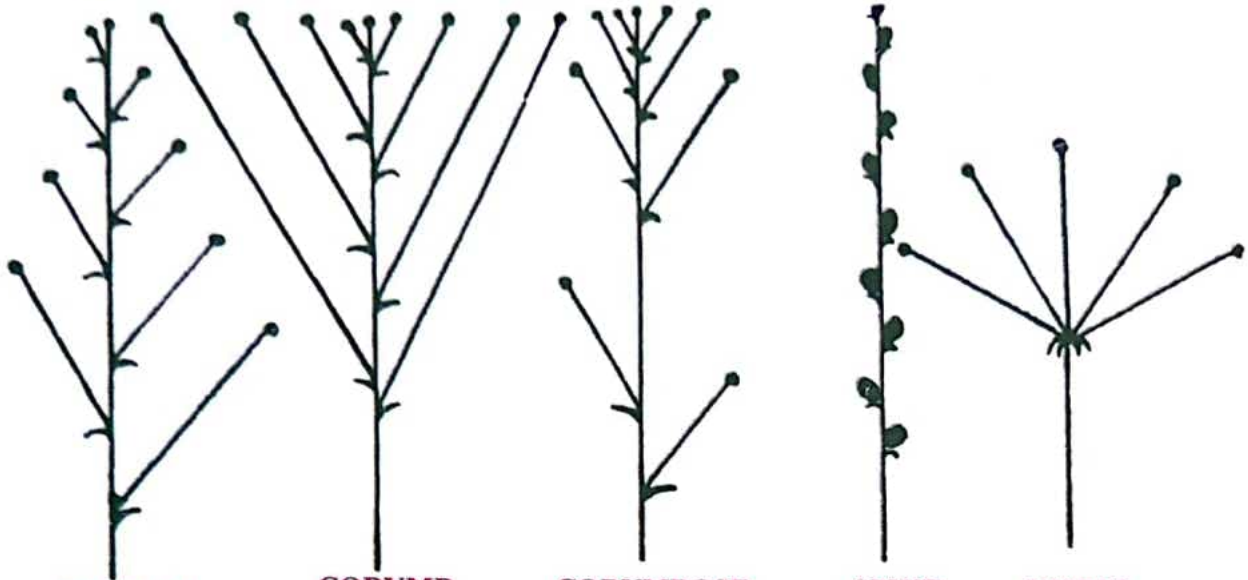
1. **Racemose:** In this type of inflorescence main axis keeps on growing bearing flowers on the side and axis ends in a flower. Oldest flower is towards the base and youngest toward the apex and youngest are towards the base and lateral side.
2. **Cymose:** The stem ends in a flower and lateral branches or branch originate below the flower each branch in turn bears flower.

Following are the types of racemose:

1. **Typical Raceme:** The main axis of stem is long and bear branches terminating in flowers. The young flowers are towards the apex and old towards the lower side as in radish Brassica and Cassia fistule.
2. **Spike:** The main axis is long but bears sessile flowers i.e., without pedicel e.g., wheat, bottle brush.
3. **Catkin:** It is a type of spike having unisexual flowers either pistillate or staminate e.g., Mulberry and Willow.
4. **Corymb:** The pedicel of lower flowers is large and that of upper is small forming umbrella like structure e.g., candy tuft.
5. **Umbel:** The main axis is short and all the flower appear to originate from same level and reach at the same level. Onion and cherry are examples. In a number of cases a number of Umbels originate from one point called Umbel of Umbel or Compound Umbel. e.g. Carrot.
6. **Capitulum:** The main axis is reduced to flat concave or convex disc called receptacle. Flowers are small and sessile called florets, and are crowded together and appear like a single flower. e.g. Sunflower and other composite family members.
7. **Panicle:** A branched raceme is called panicle e.g. grapes and oats.

Subject:-

INFLORESCENCE



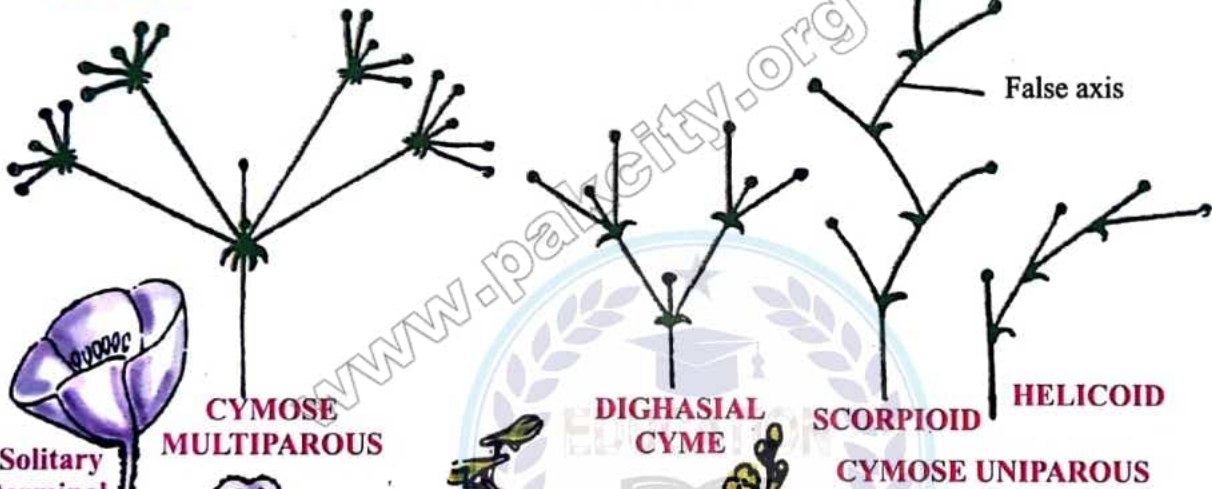
TYPICAL RACEME

CORYMB

CORYMBOSE RACEME

SPIKE

UMBEL



CYMOSE MULTIPAROUS

DIGHASIAL CYME

SCORPIOID

HELICOID

CYMOSE UNIPAROUS

False axis



Solitary terminal

POPPY



Solitary axillary

RACEME : Larkapur

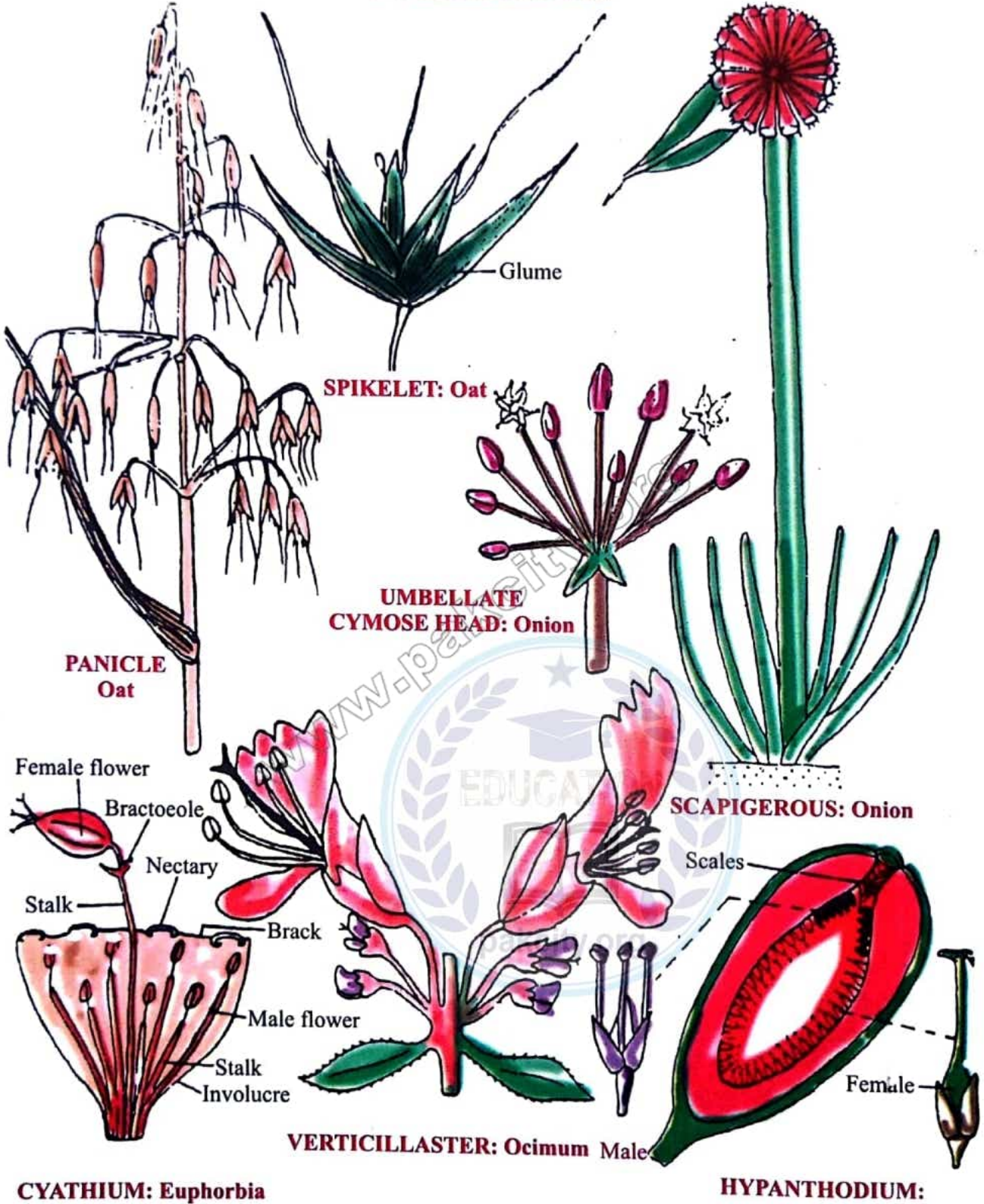


SPIKE : Verbena



CATKIN : Mulberry

INFLORESCENCE



8. **Cymose:** Apex ends in a flower, below which daughter axis is produces one, daughter axis which again ends in flower and so on e.g. Silene.

Cymose are of following types:

- (a) **Uniparous (Monochasial):** The main axis of stem ends in a flower. Below the flower, flower bearing branch develops. New branches may be formed on the left and right sides alternately and is called **Scorpioid** is Freasia, henbane. In **Helicoid** the flowers are formed on the same side as in Begonia day lily etc.
- (b) **Biparous or Dichasial:** The main axis ends in flower, two branches are from lower side of the flower reach flower in turn bear two branches. e.g. Straw berry, Ipomoea.
- (c) **Multiparous (Poly Chasium):** In this case three or more branches originate from the lower side of flower e.g. Euphorbia.



16

Experiment

Description of Flower in Technical Terms

DICOTYLEDONS

ROSA INDICA (ROSE vern. GULAB)(Family: *ROSACEAE*)

- Leaf:** Alternate; Petiolate; Stepulate, Stipules adnate; Pinnate compound.
- Inflorescence:** Large solitary or paired terminal flowers on short lateral branches.
- Flower:** Ebracteate; Pedicellate; Complete; Bisexual (hermaphrodite); Actinomorphic; Perigynous; Differently coloured, usually pink; Scented.
- Calyx:** Sepals 5, Gamosepalous; Imbricate; Persistent; Hairy; Green; Inferior.
- Corolla:** Petals 5 or numerous; Polypetalous; Imbricate; Rosaceous; Large; Variously coloured; Scented; Inferior.
- Androecium:** Stamens indefinite; Polyandrous; Arranged in many whorls of 5 each; Inferior; Filaments slender and unequal; Anthers small, ditheous (2-celled) and dorsifixed.
- Gynoecium:** Polycarpellary; Apocarpous; Style apical and short; Stigma capitate; Ovary simple and superior; Unilocular; One ovule in each loculus; Placentation basal.
- Floral Formula:** $\otimes \frac{\ominus}{\oplus} K_{(5)} C_{5-\infty} A_{\infty} G_{\underline{\infty}}$



A Flowering Branch



U.S. Flower



Floral Diagram

Fig. *Rosa indica* (Rose Gulab)

Solanum nigrum* (Nightshade vern. Mako)*(Family: Solanaceae)****Leaf:**

Ramal & Cauline, Alternate, but opposite in floral region, petiolate, simple, exstipulate, ovate, dentate.

Inflorescence:

(Uniparous scorpioid cyme).

**Scorpioid
(Uniparous cyme)****Flower:**

Pedicellate; Ebracteate; Complete; Hermaphrodite; Actinomorphic; Pentamerous; Hypogynous; Wheel-shaped; White.

Calyx:

Sepals 5; Gamosepalous; Campanulate; Hairy; Inferior; Green.

Corolla:

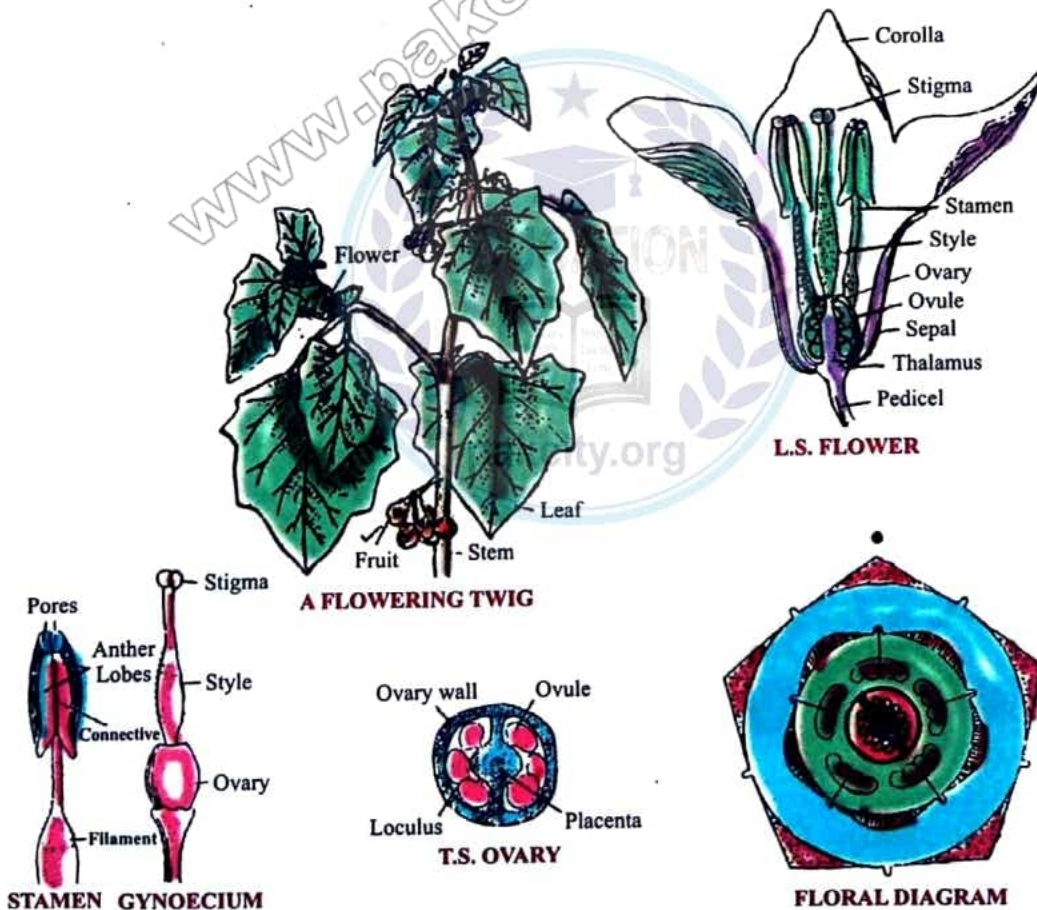
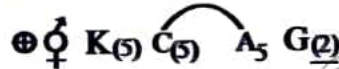
Petals 5; Gamopetalous; Inferior; Rotate; White; Non-scented.

Androecium:

Stamens 5; Polyandrous; Epipetalous (alternating with the petals); Inferior; Filament short; Anthers basifixed and apparently united into a cone.

Gynoecium:

Bicarpellary; Syncarpous; Style long and terminal; Stigma capitate and bilobed; Ovary superior; bilocular; Placentation axile; Numerous ovules in each loculus.

Floral Formula:

Cassia fistula (Amaltas)

(Family: *Leguminosae*, Sub-family: *Caesalpinaceae*)

- Leaf:** Alternate; stipulate, stipules very small, pinnate compound leaf-let. Opposite; sub-sessile; ovate; Globus, Margin entire; Apex acute vention unicostate and reticulate.
- Inflorescence:** Axillary or Extra axillary, long pendulous, compound raceme.
- Flower:** Pedicellate; Bracteate; Complete; Hermaphrodite; Zygomorphic; Pentamerous; Slightly perigynous; Non-scented; Yellow.
- Calyx:** Sepals 5; Polysepalous; Imbricate; Odd sepal anterior; Inferior; Slightly petaloid.
- Corolla:** Petals 5; Polypetalous; Ascending imbricate *i.e.*, the posterior petal is overlapped by the two postero-lateral ones which in their turn are overlapped by the two antero-lateral ones; Inferior; Yellow.
- Androecium:** Stamens 10 in Polyandrous; Inferior; Filaments of variable lengths; Anthers dorsifixed, 3 posterior stamens reduced to staminodes.
- Gynoecium:** Monocarpellary; Ovary superior; Shortly stalked and curved; Unilocular; Ovules many; Style short; Stigma capitate and hairy; Placentation marginal.
- Floral Formula:** $\uparrow \text{♀ } K_5 C_5 A_{3+3+4} G_1$

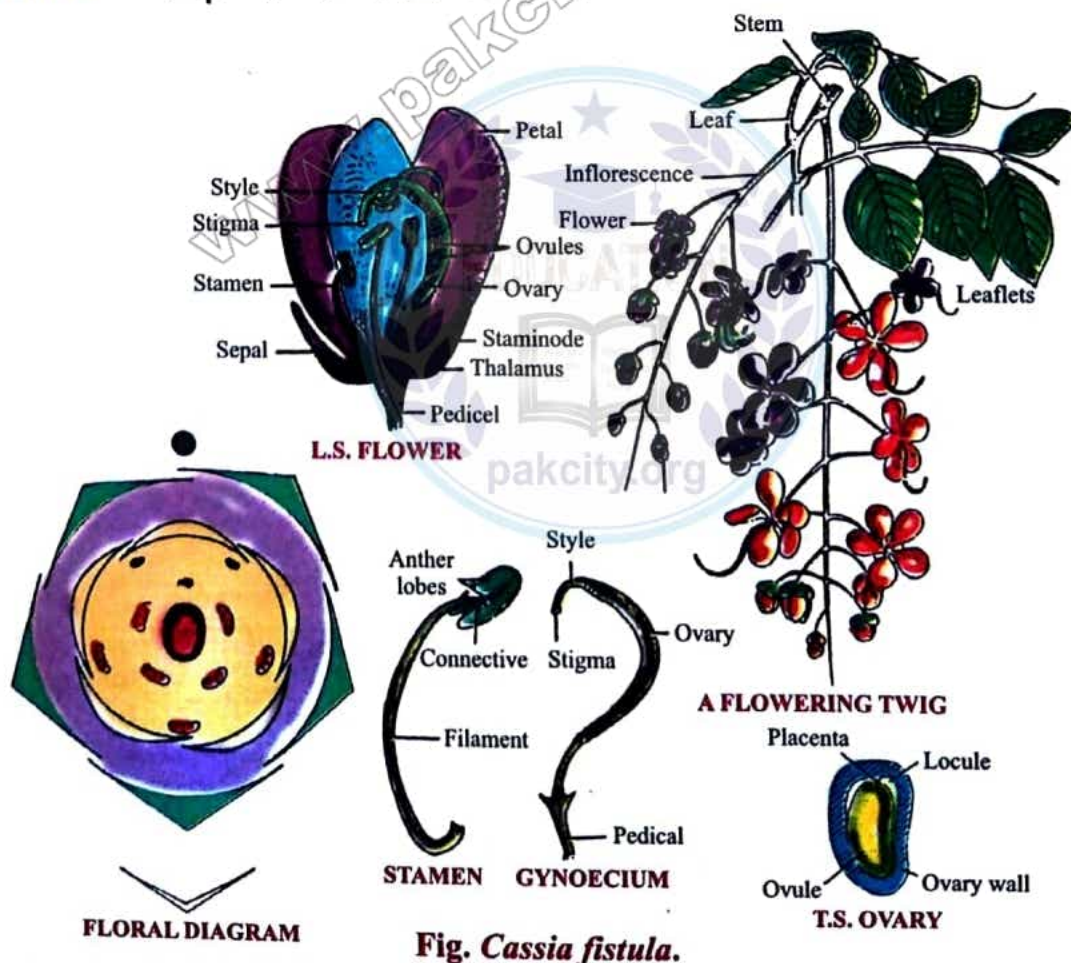


Fig. *Cassia fistula*.

Lathyrus odoratus, Sweet pea (Phool Matar)

Flowers from January to March

(Family: Leguminosae, Sub-family: Papilionaceae)

- Leaf:** Cauline and ramal, compound, upper leaf-let modified into tendrils, alternate, petiolate, stipulate (stipule leafy).
- Inflorescence:** Solitary axillary or Pedunculate raceme.
- Flower:** Pedicellate; Bracteate; Complete; Hermaphrodite; Zygomorphic; Pentamerous; Slightly perigynous; Papilionaceous; Usually white, sometimes pink or otherwise coloured.
- Calyx:** Sepals 5; Gamosepalous; Campanulate; Inferior; Green; Hairy.
- Corolla:** Petals 5, 3 free and 2 anterior ones united; Papilionaceous *i.e.*, the posterior large petal is called *standard*, the lateral two are wings (*alae*) and the two anterior ones are united to form the keel (*Carina*); Inferior; White or pink etc.; Non-scented.
- Androecium:** Stamens 10; Diadelphous (9 united to form a tube around the ovary and one free); Inferior; Anthers basifixed.
- Gynoecium:** Monocarpellary; Style long, slightly hairy and bent towards the posterior side; Ovary superior; Unilocular; Ovules many; Placentation marginal; Stigma slightly hairy.

Floral Formula:

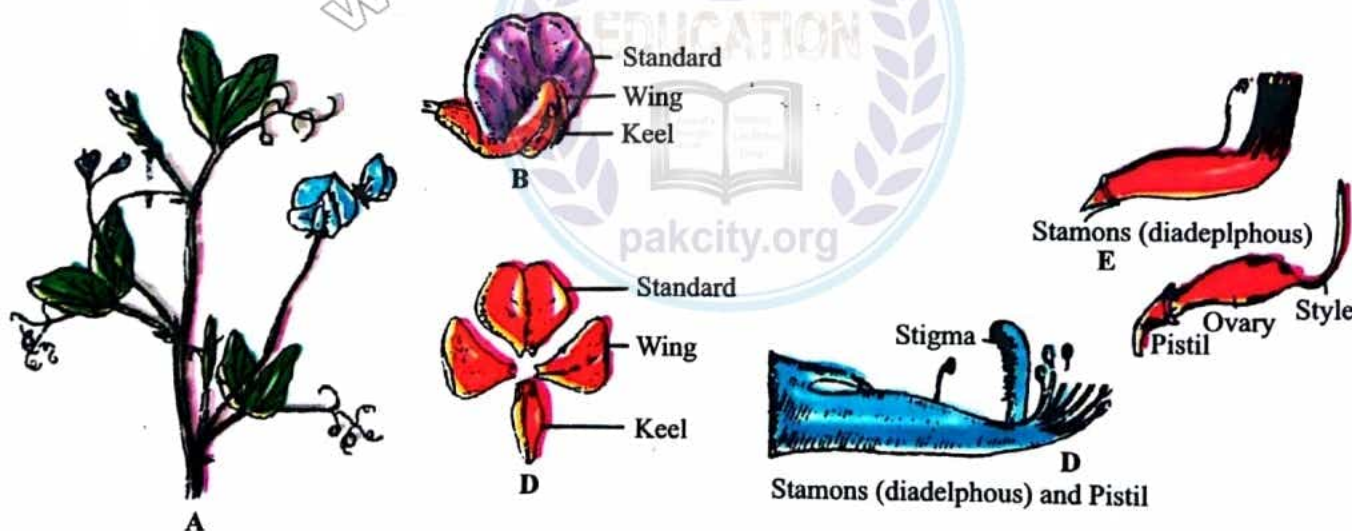
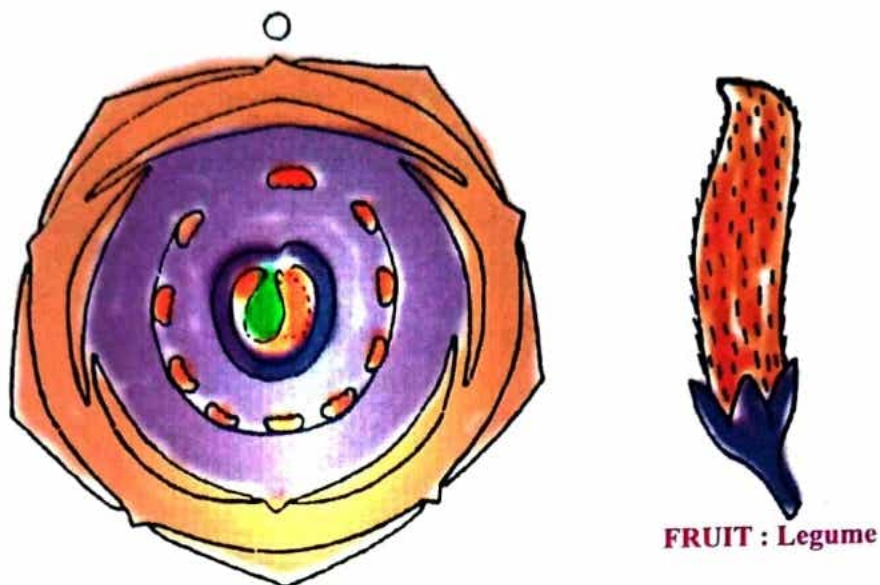


Fig. *Lathyrus odoratus*. A, Portion of the plant. B, Flower. C, Vertical Section of Flower. D, Ground Plan of Corolla. E, Floral Diagram.

**OPENED FRUIT**

FAMILY GRAMINEAE (Poaceae)

Avena Sativa

Leaf: Cauline, alternate, sheathing sessile, linear, acute.

Venation: Multicostate, parallel venation.

Inflorescence: Panicle of spikelets.

***Avena sativa* (jai):** It has an inflorescence called Panicle of Spikelets. The main axis is long and bears lateral branches in a raceme manner e.g., the lower, older branches are longer than upper younger ones. Each lateral branch bears a group of three sessile flowers (Spikelets).

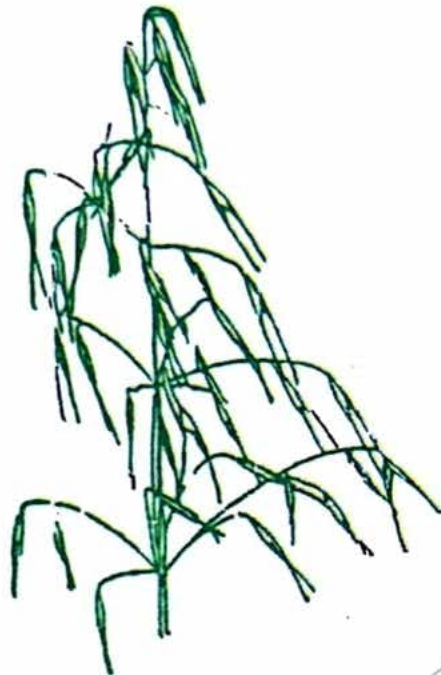
Flower: Bracteate, arising in the axil of inferior palea, opposite to which lies the superior palea. Incomplete, bisexual, zygomorphic, on account of position of lodicules, hypogynous.

Parienth: Represented by two minute scaly lodicules which are present on short axis within the two palea.

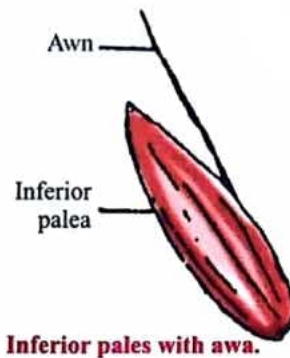
Androecium: Stamen three, polyandrous, filament long, anthers versatile.

Gynaecium: Monocarpellary, superior, unilocular with single basal ovule, style short, single, stigmas two feathery.

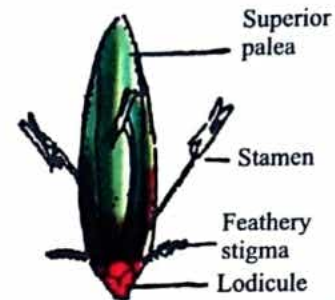
Floral Formula: $\dagger, \text{♀}, P_2 (\text{lodicules}), A_3, \underline{G}_1$.



Panicle of Spikelets

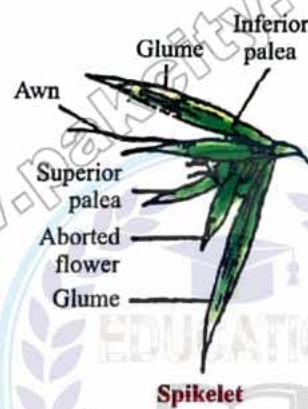


Inferior pales with awa.

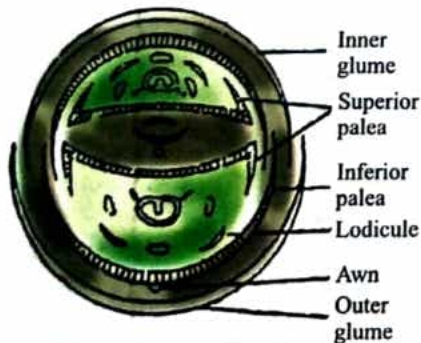
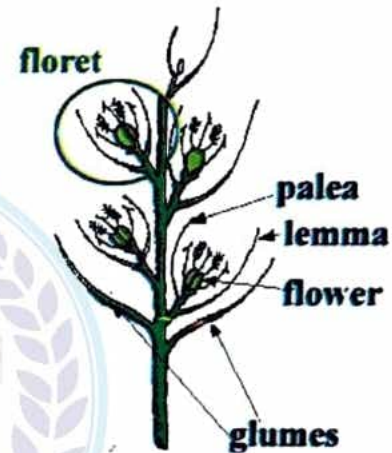


Flower with inferior palea somoved.

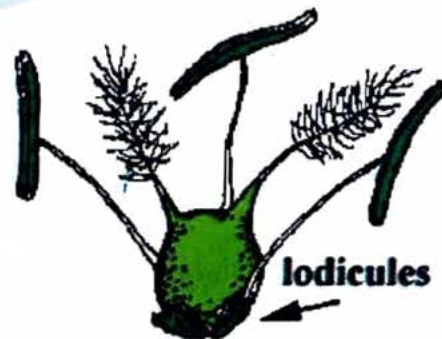
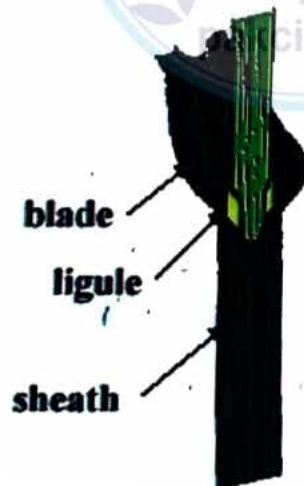
Floral diagram



Spikelet



Floral diagram of a spikelet



lodicules

VIVA VOCE

Q.1 What are essential parts of flower?

Ans. Stamen and carpels are essential parts of flower.

Q.2 What are non-essential parts?

Ans. Sepals and petals are non-essential parts.

Q.3 What is the function of Petals?

Ans. They attract insect for pollination.

Q.4 What is pollination?

Ans. Transfer of pollen grains from the anther to the stigma is called pollination.

Q.5 Which part give rise to seed?

Ans. Ovule give rise to seed.

Q.6 Which parts forms fruit?

Ans. Fruit is formed from ovary.

Q.7 What is the function of sepals?

Ans. Sepals protect the other parts.

Q.8 What is pedicel?

Ans. It is the stalk on which floral parts are present.

Q.9 What is meant by superior and inferior ovary?

Ans. When ovary is above other parts of flower it is called superior ovary when other parts are above the ovary it is called inferior ovary.

Q.10 Define the following terms:

Polysepalous

Polypetalous

Gamopetalous

Perianth

Ans. Polysepalous	=	Flower with free sepals
Polypetalous	=	Flower with free petals
Gamopetalous	=	Flower with united petals
Perianth	=	Sepals and petals collectively are called perianth.

Q.11 Define inflorescence.

Ans. Group or bunch of flowers present on the same branch.

Q.12 What is difference between raceme and spike?

Ans. Flower of raceme are pedicilate while spike has sessile flowers.

Q.13 What is the difference between Spike and Catkin?

Ans. Spike has bisexual flowers and catkin has unisexual flowers.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Describe following parts of provided flowering shoot (*Avena sativa*) in technical terms. (1 + 1 + 3)

(Multan, D.G. Khan Boards 2004)

1. Corolla
2. Androecium
3. Floral formula

Experiment 2: Describe following parts of provided flower (*Cassia fistula*) in technical terms.
(1 + 1 + 1 = 3)

(Multan, D.G. Khan Boards 2004)

1. Inflorescence
2. Corolla
3. Androecium

Experiment 3: Describe following parts of provided flowering shoot (*Lathyrus*) in technical terms. (1 = 1 + 1 = 3)

(Multan, Bahawalpur, Lahore, D.G. Khan Boards 2004)

1. Androecium
2. Gynaecium
3. Floral formula

Experiment 4: Describe following parts of provided flowering shoot (*Solanum nigrum*) in technical terms. (1 + 1 + 1 = 3)

(Gujranwala, Lahore, Rawalpindi Boards 2004)

1. Flower
2. Corolla
3. Androecium

Experiment 5: Describe following parts of provided flowering shoot (*Avena sativa*) in technical terms. (1 + 1 + 1 = 3)

(Multan 2004)

1. Perianth
2. Androecium
3. Floral formula

Experiment 6: Describe following parts of provided flowering shoot (*Rose*) in technical terms. (1 + 1 + 1 = 3)

(Multan, Bahawalpur Boards 2004)

1. Corolla
2. Androecium
3. Floral formula

17

Experiment

STUDY OF INTERNAL STRUCTURE OF MONOCOT
AND DICOT ROOT, STEM AND LEAF FROM
PREPARED SLIDES

PART-I

(A) INTERNAL STRUCTURE OF ROOTS

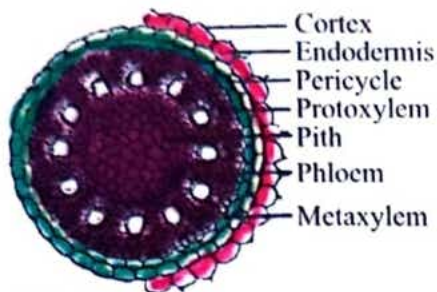
Materials:

Compound microscope, Prepared slides of T.S. of Dicot and Monocot roots.

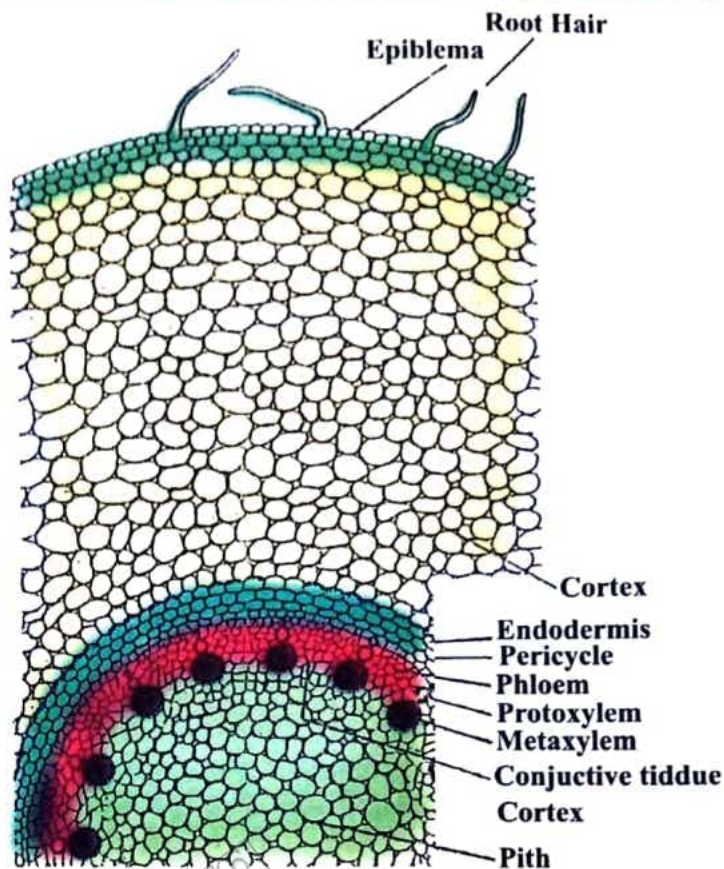
Procedure:

1. Take a prepared slide of T.S. of dicot root and observe it carefully under the microscope.
2. Take a prepared slide of T.S. of monocot root and observe it carefully under the microscope.

T.S. OF DICOT ROOT	T.S. OF MONOCOT ROOT
<p>It shows following characters:</p> <ol style="list-style-type: none"> 1. Epidermis: It is a single outermost layer. Its cells have thin wall. They form root hairs. 2. Cortex: It is composed of many cells. These cells are thin walled. Intracellular spaces are present between them. 3. Endodermis: It is present inner to cortex. Its cells have thick walls. 4. Pericycle: It is present inner to endodermis. It is composed of thin wall cells. 5. Vascular bundle: The xylem cells are in the centre. They give star shaped appearance. 6. Pith: Pith is absent in dicot root. <i>Reason of identification: The xylem cells are in the centre and give star shaped appearance.</i> 	<ol style="list-style-type: none"> 1. Epidermis: It is a single outermost layer. Its cells, have thin wall. They form root hairs. 2. Cortex: It is composed of many cells. These cells are thin wall. Intracellular spaces are present between them. 3. Endodermis: It is present inner to cortex. Its cells have thick walls. 4. Pericycle: It is present inner to endodermis. It is composed of thin wall cells. 5. Vascular bundle: Xylem and phloem are arranged in rings. They alternate with each other. 6. Pith: Pith is present in monocot root. <i>Reason of identification: Xylem and phloem are arranged in rings and pith present in the centre.</i>



T.S. of Monocot root
(epidermis and cortex is removed)



Zea mays, transverse section of a monocot root.



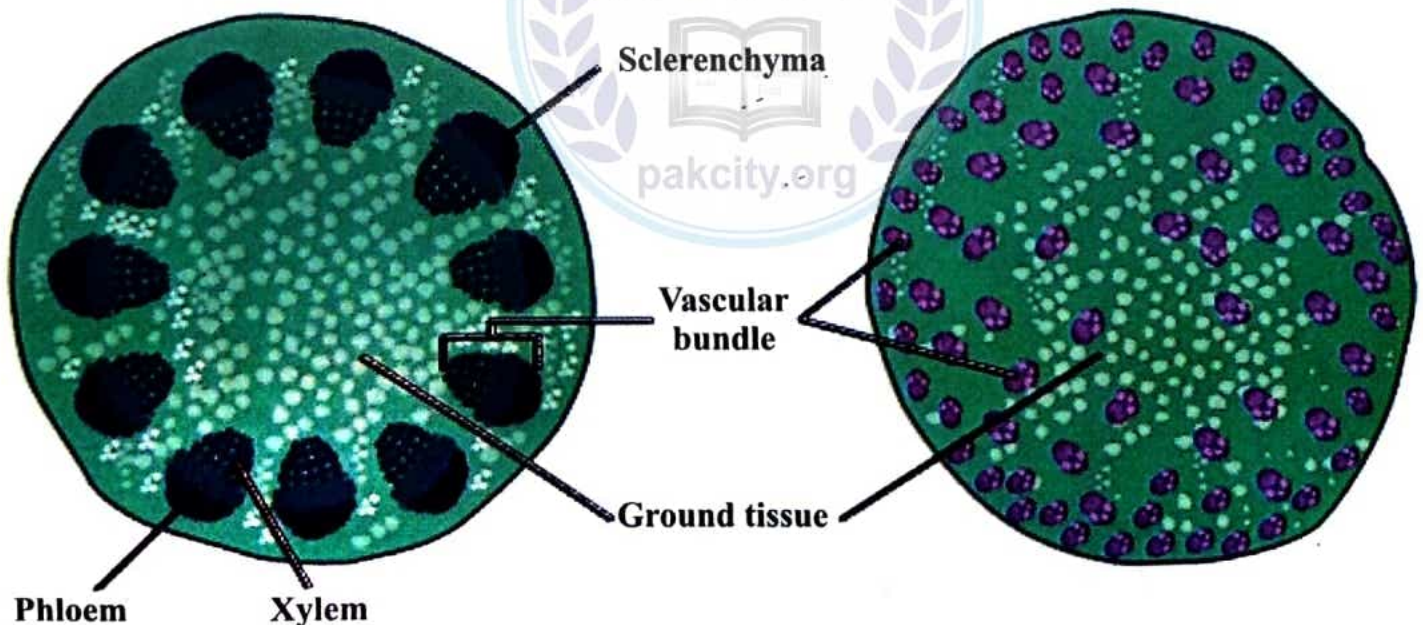
T.S. of Dicot Root

(B) INTERNAL STRUCTURE OF STEM

DICOT STEM	MONOCOT STEM
<ol style="list-style-type: none"> 1. It is differentiated into cortex, pericycle and pith. 2. The vascular bundles are arranged in a ring and have a uniform size. 3. Cambium is present in each bundle. 4. The xylem vessels are arranged in radial rows. 5. Lysigenous cavity is absent. 6. Vascular bundles are never enclosed in sheaths of dead cells. 7. Medullary rays are present between the vascular bundles. 8. Pith is present in the centre due to ring-like arrangement of vascular bundles. <p>Reason of identification: Vascular bundles are arranged in rings</p>	<ol style="list-style-type: none"> 1. It is not differentiated into cortex, pericycle and pith. There is, on the other hand, a ground tissue extending from the hypodermis to the centre. 2. The vascular bundles are irregularly scattered in the ground tissue. They are smaller towards the periphery and larger towards the centre. 3. Cambium is absent. 4. The xylem is Y-shaped having four distinct vessels, the two larger ones lying at the arms and two smaller ones at the apex of 'Y'. 5. Lysigenous cavity is present in the protoxylem. 6. Each vascular bundle is enclosed in a sheath of dead cells. 7. Medullary rays are not present. 8. Pith is absent because of the scattered arrangement of vascular bundles. <p>Reason of identification: Vascular bundles are scattered in the ground tissues.</p>

Dicot Stem

Monocot stem



(C) INTERNAL STRUCTURE OF LEAF

1. Epidermis:

There are two layers of epidermis: upper and lower epidermis. Epidermis is covered by cuticle. The lower epidermis has stomata with guard cells.

2. Mesophyll:

The green tissues between the upper and lower epidermis are called mesophyll. There are two types of mesophyll:

- ◆ **Palisade mesophyll:** The cells are present under upper epidermis. They are present at right angle to the epidermis.
- ◆ **Spongy mesophyll:** These cells are arranged very loosely and they have large intracellular spaces.

3. Vascular Tissues:

Vascular tissues form midrib and veins of the leaf. These are present between palisade and spongy layers.

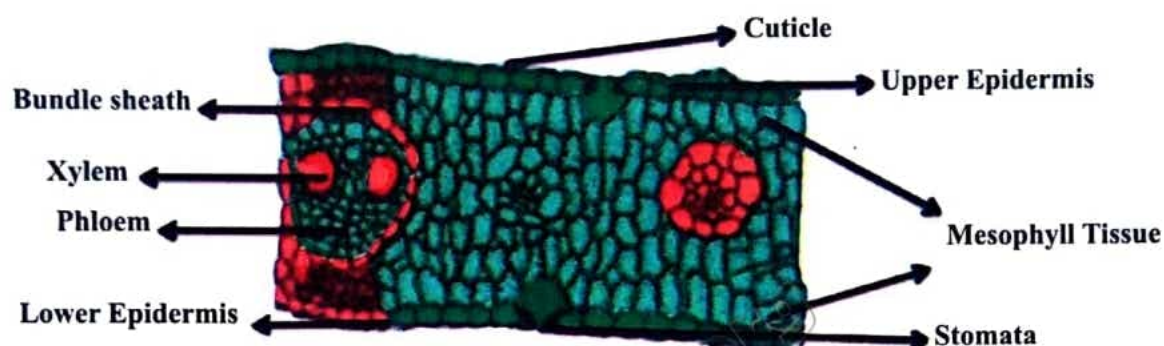
Reason of identification: Presence of guard cells and mesophyll cells and mesophyll cells.



Fig. Difference between mono and dicot leaf



Internal Structure of dicot leaf



Internal Structure of Monocot leaf

Characters	Dicot leaf	Monocot leaf
1. Nature of orientation	Typically dorsiventral	Typically iso-bilateral
2. Stomata	Hypostomatic	Amphistomatic
3. Motor cells	Absent	Present in the upper epidermis
4. Mesophyll	Differentiated into palisade and spongy parenchyma	Undifferentiated
5. Veins	Irregularly scattered	Undifferentiated
6. Xylem vessels	Many protoxylem and metaxylem vessels in each bundle	Two protoxylem and two metaxylem vessels in each bundle
7. Bundle sheath extensions	made up of collenchyma	Made up of sclerenchyma

KEY: Identification between Root and Stem:

ROOT	STEM
(i) The vascular bundles are radial and exarch.	(i) The vascular bundles are conjoint, collateral and endarch.
(ii) The endodermis is quite conspicuous and ring-like.	(ii) The endodermis is the inconspicuous and may or may not present.
(iii) The epidermal hairs are unicellular and tubular.	(iii) The epidermal hairs are multicellular.

Identification between Monocot Stem & Dicot Stem:

MONOCOT STEM	DICOT STEM
(i) The vascular bundles are conjoint, collateral and closed (i.e., cambium absent).	(i) The vascular bundles are conjoint, collateral and open (i.e., cambium present).
(ii) Vascular bundles are scattered and many; the larger bundles are towards the centre and smaller towards periphery; usually each bundle is surrounded by a sclerenchymatous bundle sheath.	(ii) The vascular bundles are arranged in a ring; generally they are uniform in size; no bundle sheath.
(iii) Phloem is represented by sieve tubes and companion cells only.	(iii) Phloem is represented by sieve tubes, companion cells and phloem parenchyma.
(iv) The ground tissue is present; it extends from periphery to centre; usually hypodermis is sclerenchymatous.	(iv) Cortex and pericycle are well marked and differentiated; usually hypodermis is collenchymatous.

Identification between Monocot Root & Dicot Root:

MONOCOT STEM	DICOT STEM
(i) Xylem bundles are numerous (12-20), polyarch, rarely limited in number.	(i) Xylem bundles vary from 2 to 6 (di-tohexarch), rarely more.
(ii) Pith is large and well developed.	(ii) Pith is small or absent.
(iii) The cambium is altogether absent.	(iii) The cambium appears later.

VIVA VOCE

Q.1 What is bleeding?

Ans. The flow of sap from the cut, pruned, tapped or wounded plant or wounded plant with a considerable force is called bleeding.

Q.2 What is difference between vascular tissues of monocot and dicot stems?

Ans. The vascular tissues of monocot are scattered and the vascular tissues of dicot are present in rings.

Q.3 What are mesophyll cells?

Ans. The green tissues between the upper and lower epidermis are called mesophyll.

Q.4 What is difference between the epidermis of stem and leaves?

Ans. The epidermis of stem is without stomata but they are present in the epidermis of leaves.

Q.5 What are isobilateral leaves?

Ans. In these leaves, the stomata are present in both upper and lower epidermis, e.g., lily and maize (corn) leaves.

Q.7 What is root cap?

Ans. The protective covering of the root is called root cap.

Q.8 What is rhizome?

Ans. Underground stem which store food is called rhizome.

Q.9 What are root hairs?

Ans. The microscopic outgrowth of the epidermal cells is called root hairs. They increase the surface area for absorption of water.

Q.10 What is difference between dicot and monocot roots?

Ans. Pith is present in monocot stem but it is absent in dicot stem.

Q.11 What is the function of root?

Ans. It absorbs and anchors the plants. In some plant it also store food.

Q.12 What is the function of endodermis of root?

Ans. Endodermis have angular thickening. Therefore, it does not allow water to move outward.

Q.13 What is root pressure?

Ans. The osmotic pressure created in the root which forces water and dissolved ions up into the xylem is called root pressure.

Q.14 What is difference between epidermis of root and stem?

Ans. The epidermis of root has root hairs but absent in stem epidermis. The epidermis of stem has cuticle but absent in root.

Q.15 What is the function of cuticle of stem?

Ans. It reduces the loss of water by evaporation.

Q.16 Name some underground stems.

Ans. Rhizome, tuber, bulb.

Q.17 Name some roots which store food.

Ans. Turnip, carrot, radish, sugar beet.

Q.18 What is ascent of sap?

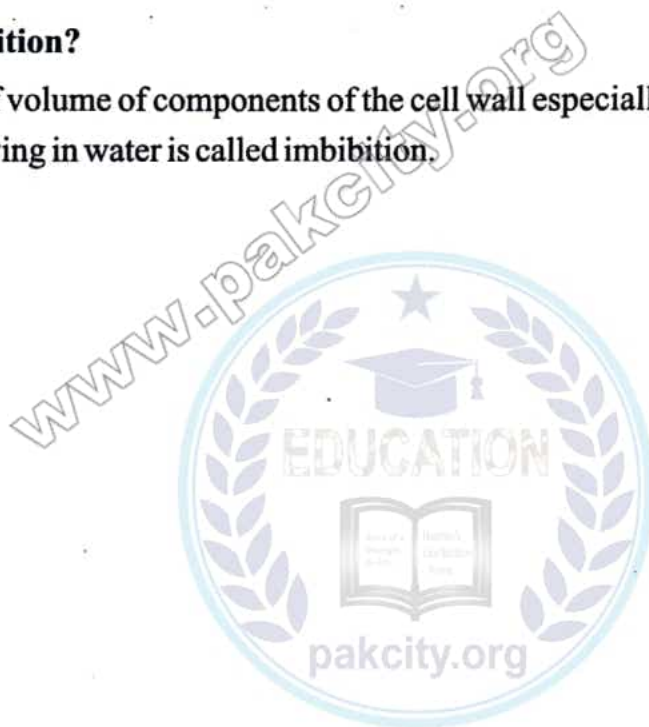
Ans. The upward movement water through the xylem from roots to leaves is called ascent of sap.

Q.19 What is guttation?

Ans. The loss of liquid water through the water secreting glands or hydathodes in leaves is called guttation.

Q.20 What is imbibition?

Ans. The increase of volume of components of the cell wall especially cellulose, pectin and lignin without dissolving in water is called imbibition.



(7)
Kingdom Animalia



18**Experiment****GENERAL SURVEY OF ANIMAL KINGDOM
(MAJOR PHYLA)**

The animals are differentiated into two basic kinds: (i) **Invertebrates** which lack a vertebral column or backbone (ii) **Vertebrates** which possess a vertebral column. Anyhow, the animals are divided up into different phyla which are described in this experiment.

Materials:

Compound microscope, Dissecting microscope, Prepared slides of microscopic animals, Museum specimens, Stuffed animals.

The students are required to study the various animals belonging to different phyla under the microscope, in the museum and the field.

(i) Phylum Protozoa:

1. The protozoans are unicellular animals. The single cell of the body carries on all the life processes. Some form colonies.
2. Majority are uninucleate but some are multinucleate.
3. Most are fresh-watered or marine but some are found in moist earth while others are parasites of many animals and plants.
4. The nutrition may be holophytic, or parasitic.
5. Reproduction takes place asexually by **binary fission, multiple fission or budding** and sexually by conjugation.
6. The locomotary organs in motile forms may be **pseudopodia, flagella or cilia**.
7. In some protozoans the body is covered by calcareous or chitinous shell.

Examples: Free-living - Amoeba, Paramecium, Euglena.

Parasitic: Plasmodium causes malaria, **Entamoeba** causes dysentery, **Trypanosoma** causes African sleeping sickness, **Opalina**.

Shelled - Foraminifera.

Colonial - Vorticella, Volvox.

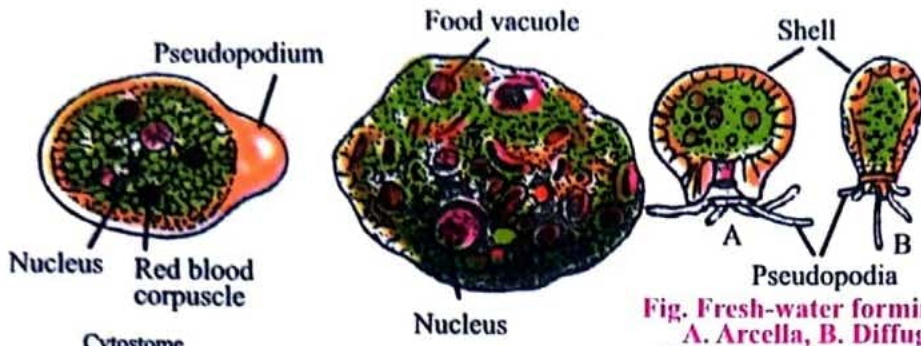


Fig. Fresh-water foraminifera
A. Arcella, B. Diffugia



Fig. Shells of four species of foraminifera

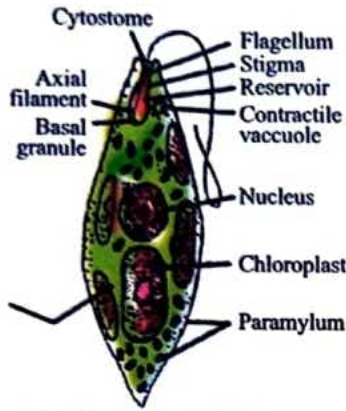


Fig. Englena viridis

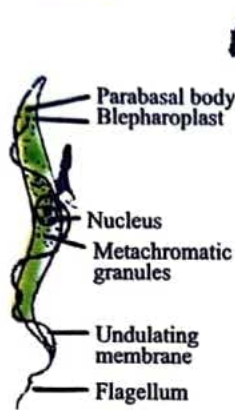
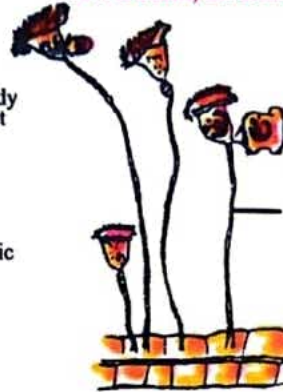
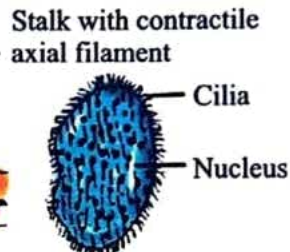


Fig. Trypanosoma gambiense

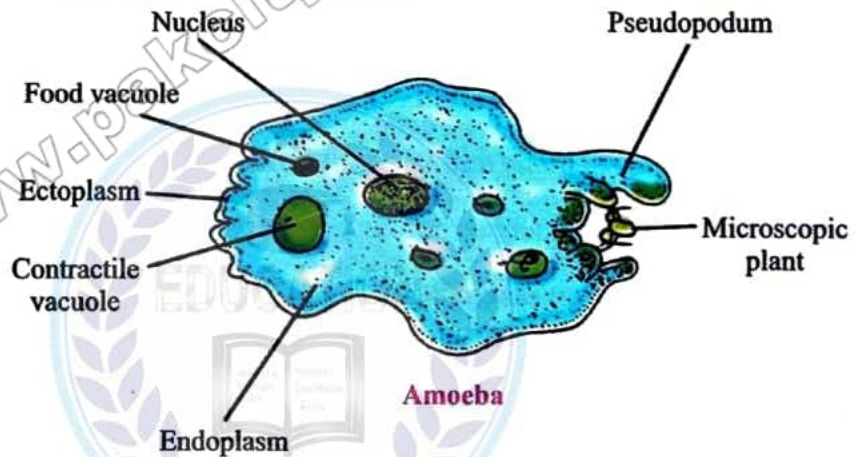


Vorticella

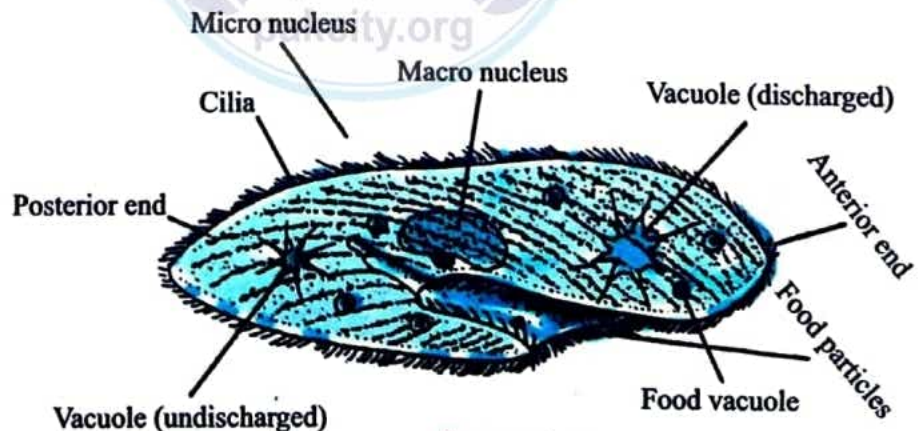


Oplanina ranarum

PHYLUM PROTOZOA



Amoeba



Paramecium

PHYLUM PORIFERA

Porifers are the most primitive animals.

Definition:

The Porifers are pore-bearing animals, (Latin porus = pore, ferre = to bear), commonly called the sponges.

Habitat:

An animals are aquatic. Out of total 10,000 species, 150 species live in fresh water while others are marine.

Organization:

These animals are multicellular however, there is no tissue organization and have no true organs.

Symmetry:

Sponges lack symmetry.

Body Wall:

Body wall is formed of an outer layer, ectoderm or **pinacoderm**, made up of **pinacocytes** and an inner layer **choanoderm** made of collared flagellated cells called **choanocytes**. Between these two layers is present gelatinous mesenchyme, which may contain amoeboid cells and **spicules** or sponging fibers.

Size:

The poriferans range in size from few millimeters wide to more than one metre tall. They are macroscopic.

Canal System:

There is a single cavity inside the body, the spongocoel. In most sponges this spongocoel may be divided into flagellated chambers or canals. The spongocoel if present intact, flagellated chambers, and canals are all lined by flagellated choanocytes.

Pores:

Numerous pores are present in the body wall. The pores through which water enters the body are called **Ostia** and pore through which the water leaves the body is known as **Osculum**.

Respiratory & Circulatory System:

There are no respiratory or circulatory organs.

Nutrition:

Sponges depend upon the food coming to them along with water currents brought about by movement of flagella of choanocytes. Food includes small animals, zooplankton, plants and phytoplankton, which constitute about 20% of their food. The food enters the spongocoel cavity through ostia. The flagellated cells, Choanocytes, ingest the food.

Excretion:

The waste products either diffuse out of the sponge directly through the body wall or flow out through osculum.

Locomotion:

The adult sponges are stationary, spending their lives attached to the rocks at the bottom or other solid objects. However, their larvae are able to move.

Nervous System:

There is no definite nervous system, however neuro-sensory and neuron cells are present which seem to coordinate the flow of water.

Pores:

The skeleton is in the form of variously shaped needle-like structures called **spicules** of calcium carbonate (CaCO_3) or silica (SiO_2). The bath sponge has a skeleton of spongin fibers. The skeleton is present among and provides support. Spicules are also present around osculum and ostia.

Reproduction:

Both asexual and sexual methods of reproduction take place in sponges.



CHARACTERISTIC FEATURES OF PHYLUM COELENTERATA / CNIDARIA

Definition:

These are simplest metazoa. The name cnidaria has been given to this group of animals due to the presence of special cells called cnidocytes or **cnidoblasts**. These cells give rise to nematocysts-the stinging cells, characteristic of this group.

Size:

The coelenterates range in size from microscopic *Hydra* to macroscopic *Branchioceranthus*, a hydrozoan polyp that may reach two metres in length.

Habitat:

The coelenterates are aquatic found both in marine and freshwater habitats.

Body Wall:

Cnidarians are **diploblastic** i.e., have body wall of two layers; the outer **ectoderm** and inner **endoderm**. Between the two layers is a jelly-like **mesogloea**.

Development:

The ectoderm forms outer covering and some cells of this layer in most animals give rise to nematocysts while the endoderm cells become specialized for digestion of food.

Body Cavity:

In these animals there is only one cavity, which serves as digestive as well as body cavity, which is called **gastrovascular cavity** or **enteron**. The enteron opens to the outside by only one opening the **mouth**.

Symmetry:

In coelenterates, the body is in the form of a cylinder radially symmetrical and any plane (direction) will divide the body into two similar halves.

Tentacles:

The mouth is surrounded by a series of tentacles. These bear stinging cells called nematocysts. These are organs of defense and offense. They paralyze the prey that comes to the animal, which is then drawn towards mouth and is ingested.

Nutrition:

They are heterotrophic, carnivores and feed upon small organism, which come into contact with them. These organisms are immobilized by nematocysts and taken into the digestive cavity as food.

Colony:

Cnidarians are found in two basic forms of zooids, the **polyps** and the **medusae**. They form the colony.

Polymorphism:

The occurrence of structurally and functionally more than two different types of individuals, called the zooids within the same organism is called polymorphism. It is the characteristic feature of coelenterates.

Polyps: Polyps or hydroids are cylindrical animals and are nutritive in function, hence named as gastrozooids.

Medusa: The medusa or gonozooids are umbrella-like, free swimming and involved in sexual reproduction as they have gonads.

Blastostyle: According to some there is a third kind of zooid called blastostyle. It is protective in nature.

Nervous System:

The nervous system is in the form a network of neuron cells forming an irregular net or plexus in the body wall. There is no central nervous system.

Skeleton:

Many colonial coelenterates such as corals produce a hard exoskeleton formed of **calcium carbonate** (CaCO_3). It is secreted by epidermal cells that take lime from seawater. The skeleton of coral is responsible for formation of small **coral islands** or large **coral reefs**.

Locomotion:

Sessile: Most species are sessile, for example *Hydra*, *Obelia*, *Corals*, etc.

Colonial: Others are colonial. The individual members of colony are called zooids, for example the *sea fans*. Some of colonial members have up to five different types of zooids, performing different functions for colony e.g. *Physalia* (Portuguese man of war).

Motile: Some are free-living and motile, for example *jellyfish*.

Reproduction:

In Coelenterates reproduction takes place by asexual as well as sexual means.

Asexual Reproduction: Budding, Blastostyle.

Sexual Reproduction: The Medusa when released in water develops reproductive organs which gives rise to gametes that unite to form zygote from which colony is again formed.

Corals:

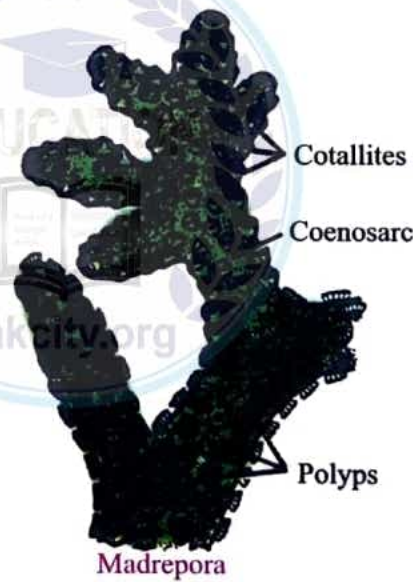
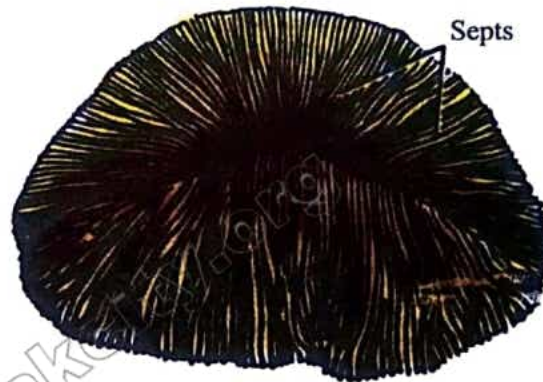
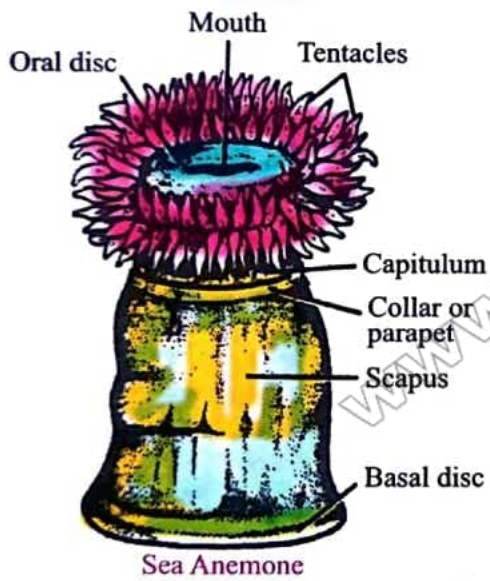
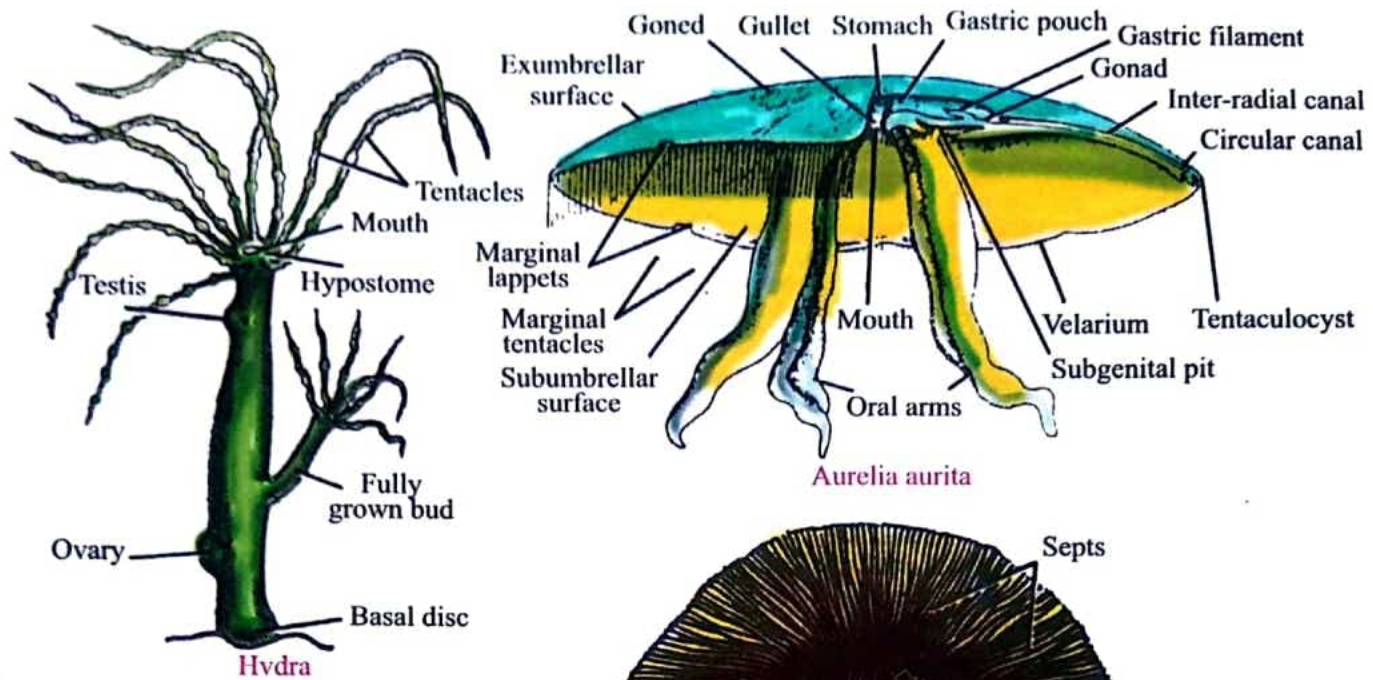
Corals are formed the secretions produced by specialized polyps, which become covered by stony cups due to hardening of their secretions. A polyp can pass out its tentacle from the mouth of the stony cup for the purpose of feeding and withdraw itself where not feeding. The stony network or masses of such colonial coelenterates are called **corals**.

Coral Reefs:

Living polyps are found on the surface layer of corals where as underneath the mass are dead stony structures only. These stony masses of calcium carbonates (lime-stone) are called **coral reefs**.

Coral reefs are found in the coastal waters of Florida, West Indies, East Coast of Africa, Australia and Island of Coral Sea.

PHYLUM COELENTERATA



PHYLUM PLATYHELMINTHES

General Characteristics:

The name Platyhelminthes means “flatworms” as their bodies are dorsoventrally compressed and soft.

Size:

They are **macroscopic**. Their size ranges from few millimeters (10 mm in case of *Planaria* to several meters in worms).

Habitat:

Most of the Platyhelminthes are **endoparasites**, i.e., live inside their hosts e.g., tapeworm (*Taenia solium*), liver flukes (*Fasciola hepatica*), and blood flukes. Some of them cause diseases in humans.

A few species are **free living** and found in freshwater, for example *Planaria*.

Advancement:

In the Platyhelminthes we find for the first time the aggregation of tissues into organ such as the eyespots and reproductive organs, which are usually made up of more than one kind of tissues.

Body Wall:

The Platyhelminthes are triploblastic. There is development of a third layer, the **mesoderm**, which separates the **ectoderm** and **endoderm**.

Body Cavity:

The Platyhelminthes are **acoelomates** as the body cavity is absent.

Symmetry:

The Platyhelminthes exhibit **bilateral symmetry**.

Segmentation:

Body is **unsegmented** except in tapeworm where proglottids are present.

Nutrition:

The parasitic species absorb nutrients from the hosts. The free-living species (*Planaria*) feed on small animals and bodies of dead and decaying animals.

Digestive System:

Digestive system is branched and has a single opening, the **mouth**. The digestive system is poorly developed in some species or may be absent as in the tapeworms.

Excretory System:

The excretory system consists of branching tubes ending in bulb-like cells, the **flame cells**.

Nervous System:

A well-developed nervous system is present in the form of either a simple **network of nerves** or **ganglia**. The sense organs are present at the anterior end.

Locomotion:

The free-living forms are motile. They move by cilia present on their undersides e.g. *Planaria*.



In parasitic forms the movement is restricted.

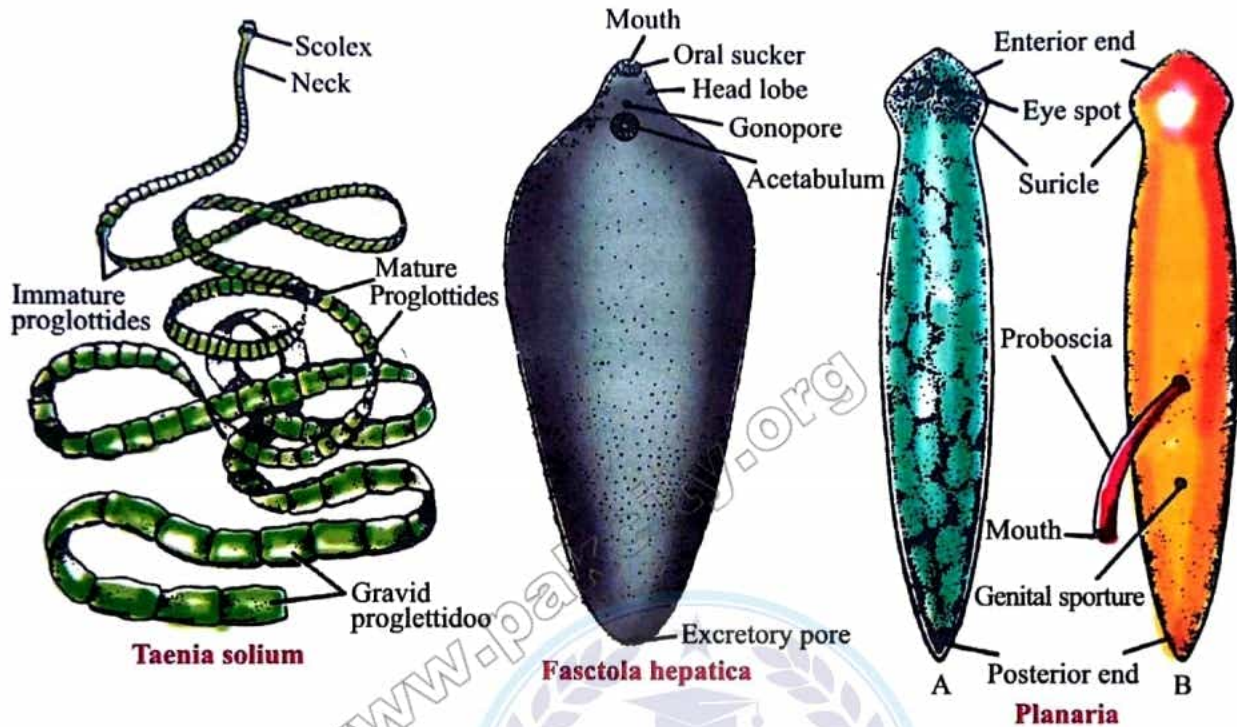
Reproduction:

The Platyhelminthes reproduce both by sexual and asexual means.

A sexual reproduction is by **fragmentation**.

The sexually reproducing species are **hermaphrodite**, i.e., both male and female reproductive organs are present in the same individual. Larval form is usually present.

Respiratory and circulatory systems are absent.



ASCHELMINTHES (PHYLUM NEMATODA) THE ROUND WORMS GENERAL CHARACTERISTICS

Definition:

The name Nematoda means "pointed ends". The animals included in this group have elongated worm-like body with pointed ends.

Size:

The nematodes range from small microscopic forms to some reaching a length of up to metre.

Segmentation:

The bodies of these animals are unsegmented, which acts a hydrostatic skeleton.

Body Wall:

The nematodes are triplobalstic, possess three layered body wall.

Structure:

One end of the body is anterior, however the head is not clearly marked and there are no special sense organs at this end.

Symmetry:

The nematodes exhibit bilateral symmetry.

Body Cavity:

The body cavity is pseudocoelom. It is derived from the hollow space situated in the blastula at an early stage in embryological development, and not from the mesoderm. It consists of a number of vacuolated cells filled with a protein-rich fluid. It is considered to be forerunner of the coelom.

Cuticle:

There is a definite cuticle secreted by ectoderm.

Digestive System:

The digestive system is in the form of alimentary canal with two openings. The opening at the anterior end is mouth and at the posterior end is the anus. In parasitic nematodes the system is simple. A space is present between the body and alimentary canal. It provides "tube within tube" type structure in nematodes.

Excretory System:

The excretory system consists of two longitudinally running canals, which unite at the anterior end to form a single canal that opens to the exterior through an excretory pore on the ventral surface.

Nervous System:

There is a nerve ring around the pharynx, which give rise to dorsal, ventral and lateral nerve cords running throughout the length of the worms.

Sense Organs:

The sense organs are in the form of sensory papillae present on the lips at the anterior end.

Respiratory System:

The respiratory systems is absent. The gaseous exchange takes place through surface layer.

Reproduction:

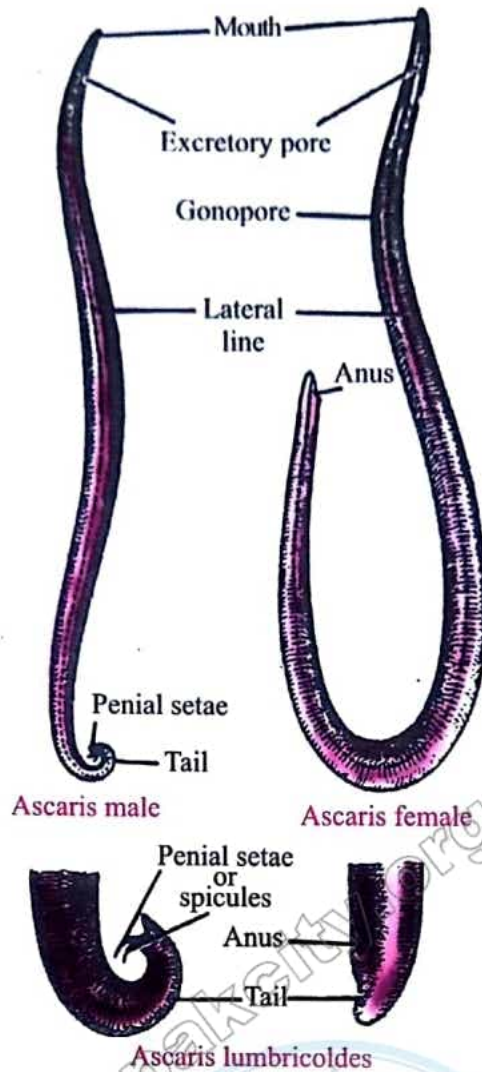
The sexes are separate. The female gonads are ovaries and these produce eggs. These male gonads are testes, which produce sperms. A larval stage is present in the life cycle.

Circulatory System:

The circulatory systems is absent.

Locomotion:

Locomotion is by undulating waves of contraction and relaxation of muscles. These muscles layer is divided into four longitudinal parts, two dorso-laterals and two ventro-laterals. The muscles are absent, therefore the bending is in dorso-ventral plane only.



PHYLUM ANNELIDA

General Characteristics:

Definition:

They are segmented and commonly called annelids (from the Latin word for little ring”).

Habitat & Mode of Living:

The annelids include worms, many living in marine e.g., (*Nereis*) along the coasts, freshwater or damp soil habitats. e.g., **Earthworms**. Some are parasites, for example, *Hirudo* (**Leech**).

Steameric Segmentation:

The body is metamericly segmented. The body becomes divided transversely into a number of similar parts or segments. These segments originate in mesoderm. The subdivisions may be indicated externally by constrictions of the body surface.

Internally the segments are separated from each other by **septa** extending across the coelom. However the various systems of body such as gut, blood vessels, and nerve cord are continuous throughout the body, penetrating each individual segment.

Body Wall:

The animals are triploblastic.

Body Cavity:

The animals are coelomate, i.e., they have a true body cavity i.e., the mesoderm splits into parietal layer, which lines the body wall and the visceral layer which covers the alimentary canal. The space between the two layers of mesoderm is filled in by **coelomic fluid**. The coelomic fluid separates the, body wall from alimentary canal. This incompressible fluid in the coelom in many annelids is confined by the partitions separating the segments and it.

Symmetry:

The annelids exhibit bilateral symmetry.

Body Structure:

The annelids show specialization of body structures. The organ systems are well developed.

Digestive System:

It is in the form of alimentary canal, which is divided into distinct parts, each performing a specific function. It has two openings, the **mouth** at the anterior end, and the **anus** at the posterior end. The mouth is overhung by a lobed structure, the **prostomium**. In parasitic species, the digestive system is poorly developed.

Excretory System:

Excretion takes place by specialized structures called nephridia. These are ciliated organs presented in each segment in the body cavity.

Nervous System:

A well-developed central nervous system is present in annelids. It comprises of a simple **brain** and a solid ventral **nerve cord**. Nerves arise in each segment from the nerve cord.

Circulatory System:

Annelids are the first group of invertebrates, which have developed a closed circulatory system a system in which a circulatory fluid called **blood**, flows in a network of vessels known as **blood vessels**. It transports gases and nutrients.

Respiratory System:

The respiratory system is absent. The exchange of gases is by diffusion across through skin in blood capillaries. The skin is kept moist by mucus, and coelomic fluid passing out through closed pass and the gases are transported by blood.

Muscular System:

The body contains muscles, which help in locomotion. The muscles are of two types:

- (a) **Circular Muscles:** These are arranged along the radius of the body.
- (b) **Longitudinal Muscles:** These are arranged along the length of the body.

Skeleton:

The coelomic fluids of annelids provide a form of hydrostatic skeleton against which its muscles could act during locomotion and burrowing.

Locomotion:

The locomotion is brought by the interaction of muscles and hydrostatic skeleton. Contraction of circular muscles produces a pressure in the coelomic fluid that forces the body to elongate. Similarly contraction of longitudinal muscles produces a pressure in the coelomic fluid that would cause the body to widen. Most annelids possess chitinous cheatae or setae embedded in sacs (earthworm) or parapodia present in the body wall (Nereis). These help in locomotion. The setae are absent in leech.

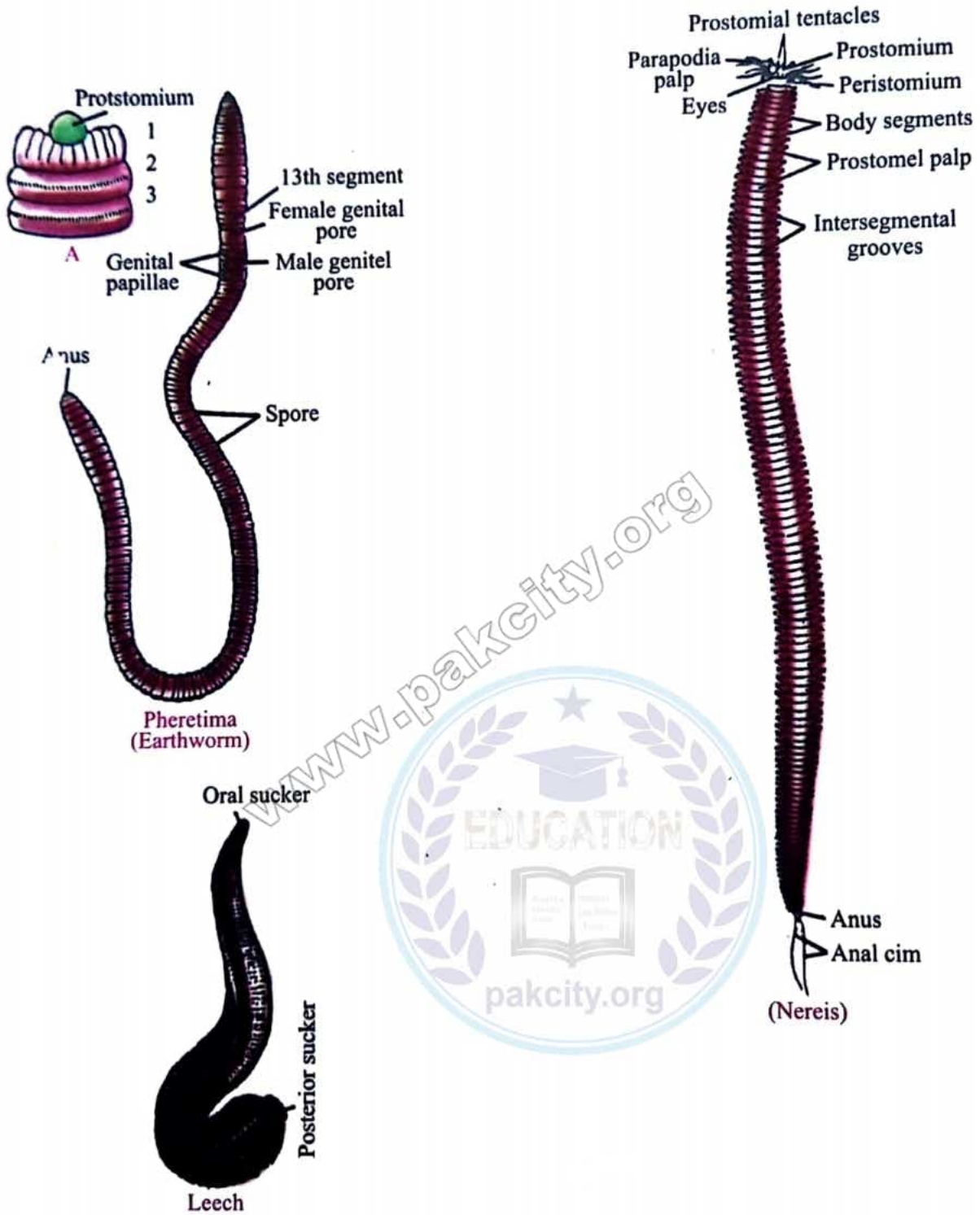
Reproduction:

The common mode of reproduction is sexual.

Classification of Annelida

Character	Polychaeta	Oligochaeta	Hirudinea
Structure	They have a distinct head region with eyes and structures called palps and tentacles.	These animals have external and internal segments. Head region not prominent or distinct.	They have body with fixed number of segments. Each segment has additional circular rings or markings called annuli. No distinct head.
Locomotion	Organs of locomotion are parapodia.	Organs of locomotion are setae.	No organs of locomotion.
Trachophore larvae	Present.	Not present.	Present.
Habitat	Mostly aquatic (marine).	May be terrestrial or aquatic.	Aquatic.
Examples	Neries chaetopterus	Lumbricus terrestris. Pheretima posthuma.	Hirudo medicinalis.

PHYLUM ANNELIDA



PHYLUM ARTHROPODA

General Characteristics:

Definition:

It is the largest phylum. They are commonly called arthropods (arthros = joined + pods = feet).

The body is divided into distinct regions:

1. The head
2. Thorax
3. Abdomen

The body is covered with waterproof chitinous cuticle secreted by the epidermis.

Habitat:

Arthropods have exploited every type of habitat on land and in water. The aquatic species include both freshwater and marine. Most of these can fly therefore visit air periodically.

Structure:

Arthropods have different shapes. Some are worm-like while the others are flying insects.

Segmentation:

The arthropod body is segmented. Each segment is attached to its neighbour by means of a modified portion of cuticle, which is thin and flexible.

Appendages:

Appendages are joined, which have been modified to perform various functions such as walking, flying etc.

Body Wall:

They are triploblastic.

Symmetry:

They possess bilateral symmetry.

Body Cavity:

The coelom is not present as the main body cavity. Instead a **haemocoel** has developed. It is reduced coelom and communicates with blood vascular system.

Digestive System:

The digestive system is in the form of alimentary canal with two openings, the mouth and anus. The food comprises of small plants and animals.

Excretory System:

A well-developed excretory system comprising of **malpighian tubules** is present in arthropods. The nitrogenous wastes are excreted in the form of solid uric acid.

Nervous System:

A highly developed nervous system is present. It consists of paired ganglia (**simple brain**) connected to a ventral **nerve cord**. A ganglion is present in each segment. Nerves arise from these ganglia.

Sense Organs:

The sensory organs are a pair of compound **eyes** and **antennae**.

Respiratory System:

Most arthropods possess **tracheal system** for the exchange of gases. Each **trachea** opens to the exterior through a pore.

Some arthropods respire through **gills**.

Circulatory System:

The blood vascular system is of **open type**. The blood flows above the body tissue bathing it for most part of the body. However, there is a primitive heart and a main blood vessel. Blood is colourless as it is without haemoglobin.

Skeleton:

They possess **external skeleton** or **exoskeleton**. It is in the form of an outer covering, the cuticle, light in weight; and formed chiefly of chitin. It provides a surface for the attachment of muscles which help in locomotion.

Locomotion:

The arthropods exhibit active and swift movements. They can swim, crawl or fly. The organs of locomotion are paired legs and paired wings.

Reproduction:

In termites, there is an ability of parthenogenesis otherwise they reproduce sexually. The sexes are separate. The gonads are testes in males and ovaries in females.

Metamorphosis:

Life histories of insects are characterized by **metamorphosis** (meta = change morphed = form). This is an abrupt change of form or structure during the life cycle.

Examples:

Insects (cockroaches, butterflies, mosquitoes, ants), prawns, squids, spiders, scorpions, centipedes and millipedes are some common examples of arthropods.

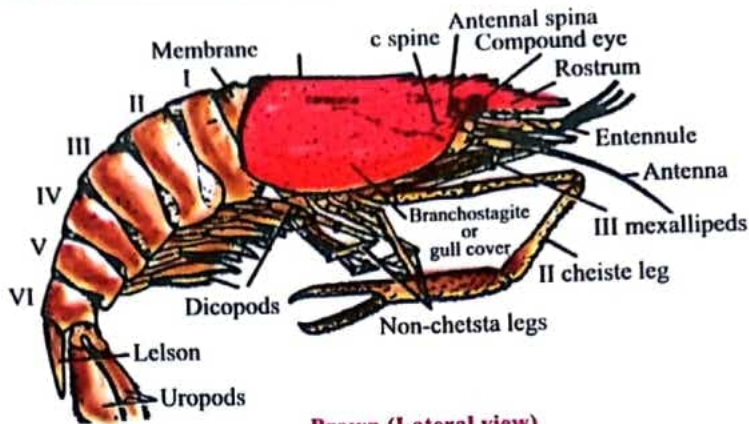
Classification of Arthropods

Character	Crustacea	Insecta	Arachnida	Myriapoda
Habitat	Aquatic	Found everywhere	Few aquatic terrestrial	Terrestrial
Sexes	Mostly separate	Separate & oviparous	Separate & oviparous	Separate and oviparous
Head parts	2 pair of antennal appendages. 1 pair of mandibles & 2 pairs of maxillae.	A pair of antennae & compound eyes on head	No appendages of antennae & no jaws. A pair of chelicerae with claws & two pairs as pedipalps.	A pair of antennae and a pair of eyes.
Body segments	On the dorsal side of the cephalothorax, the exoskeleton is in the form of carapace. In exoskeleton, deposition of salts in addition to chitin makes it more firm.	Three distinct regions; Head: Usually vertical to the body and jaws are ventral. Thorax: Three segments. Abdomen:- Varying number of segments.	Anterior segments fused to form a cephalothorax. Abdomen may be segmented or unsegmented with or without appendages.	A large number of segments each having a pair of legs.
Respiration	Through gills	Tracheae	By gills, lungs or book lungs.	Tracheae
Example	Daphnia, cyclops, crabs, lobsters, prawn, woodlouse etc.	Dragon fly, butterfly, moths etc.	Spider, ticks, mites, scorpions etc.	Millipedes, centipedes etc.

PHYLUM ARTHROPODA



Cyclops



Prawn (Lateral view)



Crab



Centipede



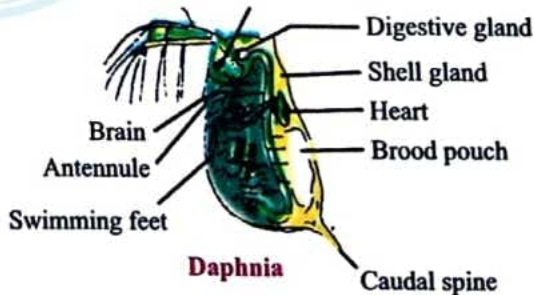
Dragonfly



Millipede



Daphnia



Daphnia



Grasshopper



Scorpion



Fig. Silver fish



Fig. Mole-cricket



Fig. Locust



Fig. Praying mantis, *Mantis religiosa*.



Fig. Dragon-fly



Fig. Stick-insect.



Fig. Leaf-insect.



Fig. Red cotton-bug.



Fig. The common green katydid.



Fig. House-fly



Fig. A water-beetle



Fig. Potato beetle



A



B

Fig. A, body louse; B, head louse



Fig. -Gypsy moth

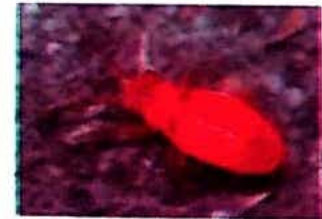


Fig. Red-bug.



Fig. -Privet hawk moth



Fig. -Stag beetle

PHYLUM MOLLUSCA

GENERAL CHARACTERISTICS

- 50,000 or more species of molluscs.
- Characteristics:
 - **Bilateral symmetry.**
 - **Body enclosed by a blanket-like mantle that secretes a shell** made of calcium or some other stiff structure.
 - A mantle cavity between the mantle and the internal organs; the anus, reproductive, and excretory ducts open into the mantle cavity.
 - A ventral, muscular foot that is highly modified among the various groups of molluscs.

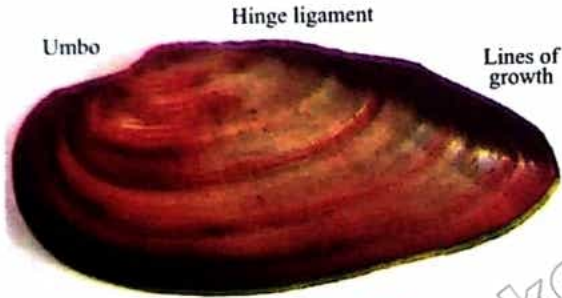
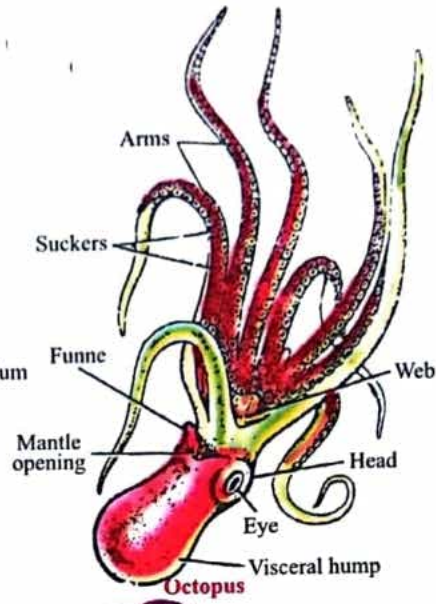
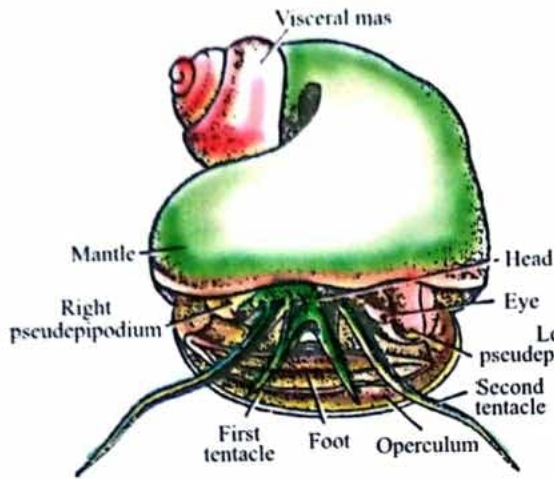
CHARACTERISTICS:

- Body of parts: head foot and visceral mass.
- Mantle that secretes a calcareous shell and covers the visceral mass.
- **Shell:** Monovalvular or bivalvular or absent or internal Bilateral symmetry.
- Mantle cavity function in excretion, gas exchange elimination wastes products.
- **Open circulatory system** in all except in one class cephalopod.
- Protostome characteristics.
- **Sense organs:** Eyes, Statocyst, sense of touch, smell and gustatory.

Table: Major Classes of Phylum Mollusca

Class and Examples	Main Characteristics
Polyplacophora (chitons) (see figure)	Marine; shell with eight plates; foot used for locomotion; head reduced
Gastropoda (snails, slugs) (see figures)	Marine, freshwater, or terrestrial; asymmetric body, usually with a coiled shell; shell reduced or absent in some; foot for locomotion; radula present
Bivalvia (clams, mussels, scallops, oysters) (see figures)	Marine and freshwater, flattened shell with two valves; head reduced; paired gills; most are filter-feeders; mantle forms siphons
Cephalopoda (squids, octapuses, chambered nautilus) (see figures)	Marine; head surrounded by grasping tentacles, usually with suckers; shell external, internal, or absent; mouth with or without radula; locomotion by jet propulsion using siphon made from mantle

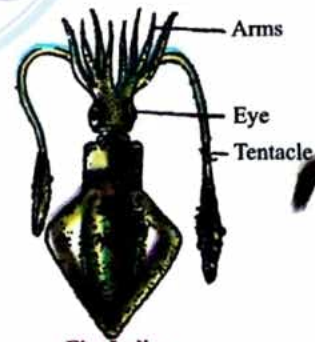
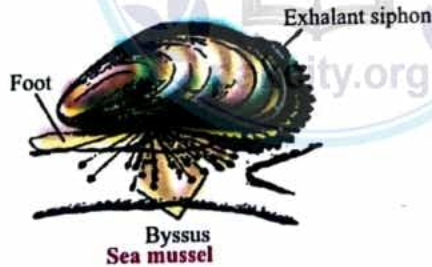
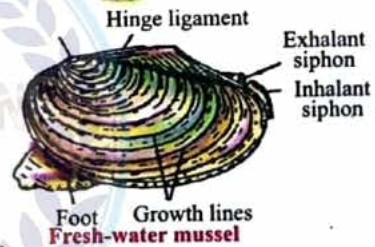
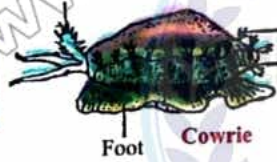
PHYLUM MOLLUSCA



Unio. Entire animal with shell



Shell of Triton



PHYLUM ECHINODERMATA

General Characteristics:

There are over 5,000 known species of echinoderms.

Habitat:

The Echinodermata are exclusively marine and most of them are found at the bottom along shorelines in shallow seas. Most species are free-living, however some are attached to the substratum.

Body Wall:

Echinoderms are triploblastic.

Body Cavity:

They are coelomates.

Symmetry:

All the larval forms of these animals exhibit **bilateral symmetry** but the adults show **radial symmetry**, which is an adaptation for their special mode of life.

Structure:

The mouth is on lower surface and anus is on upper surface.

Shape:

The body may be flattened like biscuit (sea dollar); with short arms (starfish); globular (sea urchin); star-shaped (brittle star with long arms) or elongated (sea cucumber). There is a central disc from which arms radiate.

Water Vascular System:

A characteristic **water vascular system** is present in their coelom. It is a complex system of tubes and spaces surrounding the mouth and passing into the arms and tube feet. The water circulates through these channels. Water enters these canals through a sieve-like plate called **madreporite**.

Level of Organization:

The echinoderms exhibit low degree of organization. There are specialized organs for digestion and reproduction but respiratory, circulatory, excretory and nervous systems are poorly developed. A **nerve ring** is present around the pharyngeal region.

Reproduction:

The sexes are separate and the fertilization is external. The larvae are complex, exhibit bilateral symmetry and resemble those of chordates.

Locomotion:

The free-living species move with the help of **tube feet**. Each foot is a soft sac-like structure present along the edges of grooves present below the arms.

Skeleton:

The body is covered by delicate epidermis. The mesodermal cells develop a firm calcareous **exoskeleton**. It may be called **endoskeleton** because it is below the epidermis and is formed by the mesoderm.

Regeneration:

Regeneration is common among echinoderms. It is the ability to reform organs. Starfish, sea cucumber, sea lily, brittle star and sea urchin exhibit characteristics. In fact any piece containing a part of disc can regenerate.

Examples:

The common examples are:

Asterias (sea star), sea urchin, sea cucumber, sea-dollar, brittle star.



Fig. -Sea lily

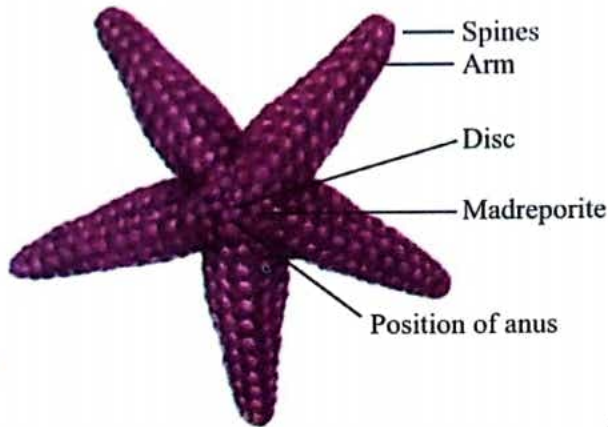


Fig. - Starfish seen from the aboral surface.

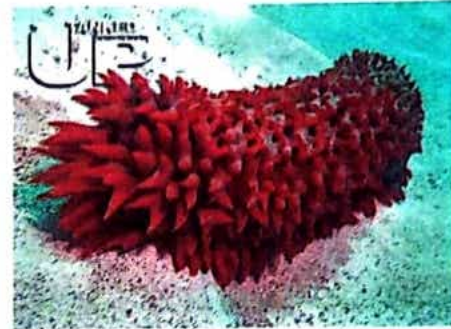


Fig. -A Sea-cucumber



Fig. -A Sea-urchin seen from the oral side.



Fig. -Cake-urchin

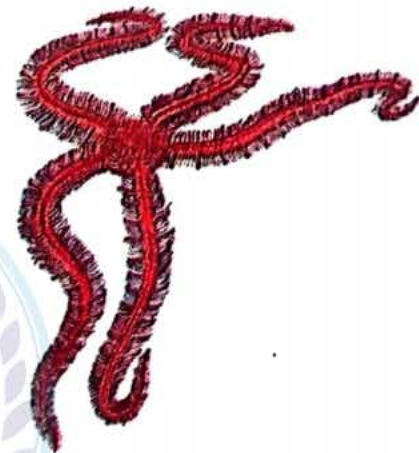


Fig. -Brittle-star (aboral view)



Fig. -A Feather star

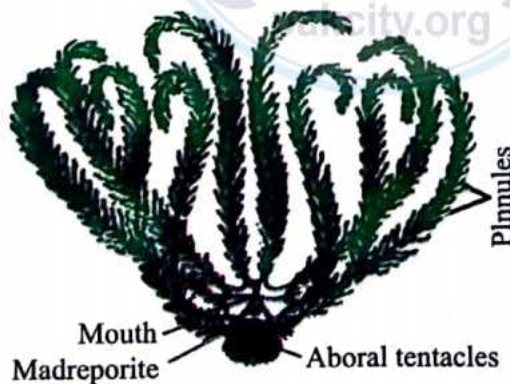


Fig. -A Feather star



PHYLUM CHORDATA

1. The organ systems in chordates are well-developed particularly the nervous system.
2. They are mostly bilaterally symmetrical.
3. Most of their skeleton is internal.
4. Their most basic embryonic features are the notochord, dorsal nerve tube and paired slits in the pharynx.

The Phylum is divided into four sub-phyla, of which **Craniata** or **Vertebrata** is the most important.

The vertebrates possess a vertebral column in the adult stage and two pairs of limbs which except in fishes are of the pentadactyl type:

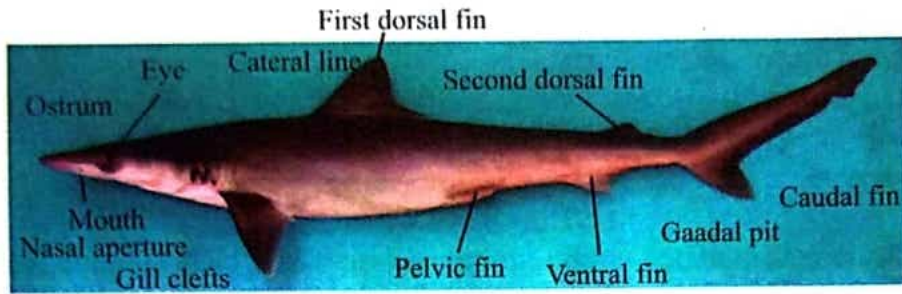
The sub-phylum **Vertebrata** is divided into the following five classes:

1. Class Pisces
2. Class Amphibia
3. Class Reptilia
4. Class Aves
5. Class Mammalia

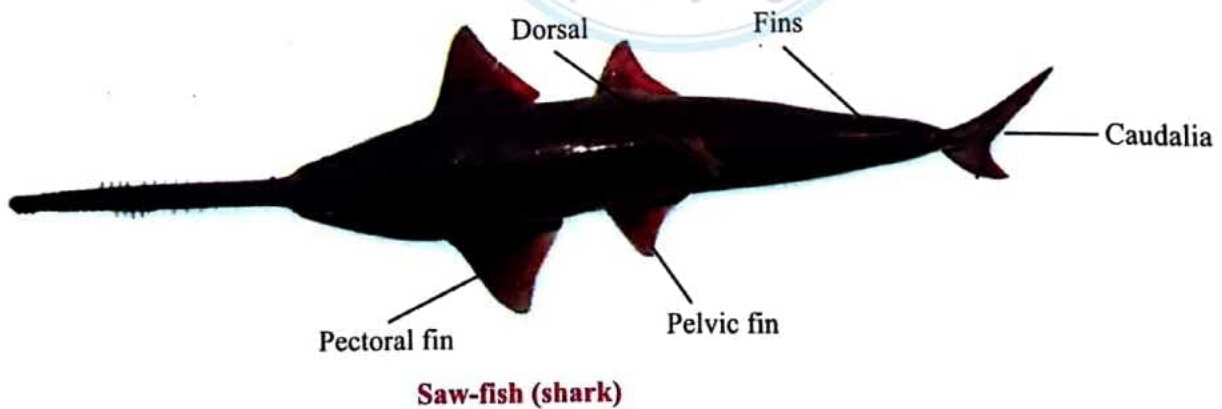
SUPER CLASS PISCES

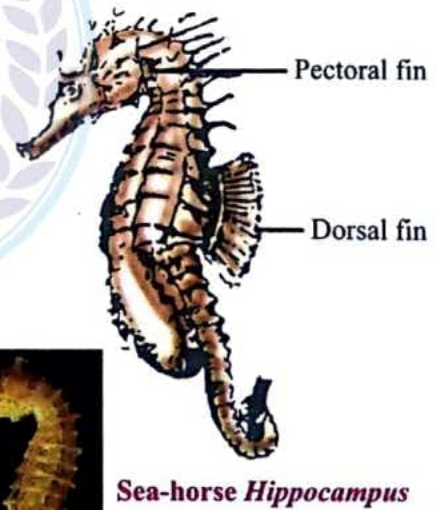
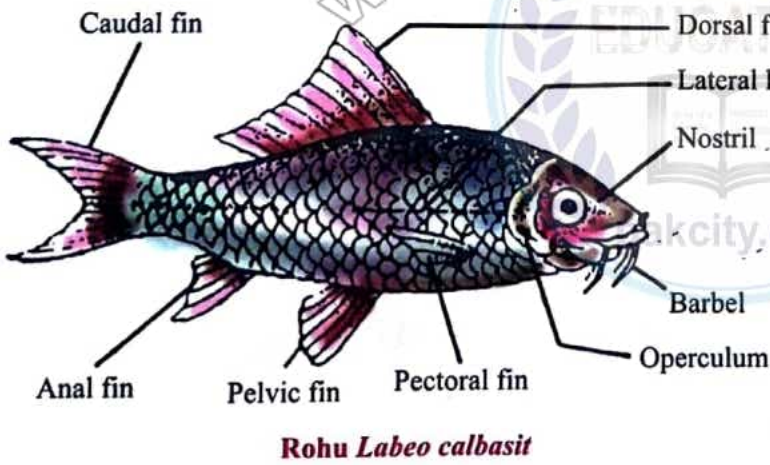
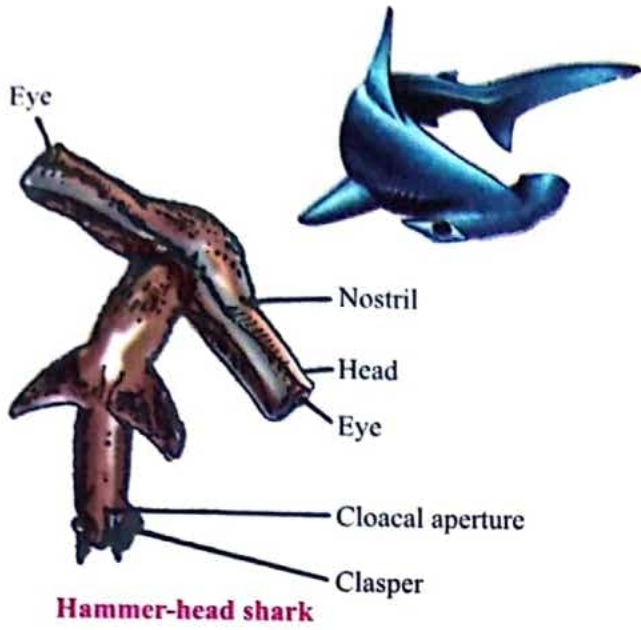
Super class pisces includes three important classes i.e., cyclostomata, chonrichthyes and osteichthyes.

Feature	Cyclostomata	Chondrichthyes	Osteichthyes
Common name	Most primitive, jawless	Cartilaginous	Bony
Body	Long, eel-like	Fusiform	Small fishes
Scales (exoskeleton)	No	Placoid	Dermal scales, ganoid, cycloid or etenoid
Appendage (fins)	No paired appendages	Both paired and unpaired fins present, anterior pair of fins (pectoral fins) are enlarged	Both types, single median and paired
Mouth	Ventral suctorial	Ventral, olfactory sacs not connected	Terminal, jaws with or without teeth
Endoskeleton	Cartilaginous	Cartilaginous	Bony
Digestive system	No stomach	J-shaped stomach	Normal stomach
Circulatory system	Heart with one auricle	Many pairs of aortic arches	2-chambered heart, one atrium, one ventricle, nucleated RBCs
Respiratory system	6-14 pairs of gills	5-7 pairs of gills, no operculum	Gills supported by gill arches and operculum
Swim bladder	No	No	Present
Sexes	Separate in lamprey, hermaphrodite in hagfishes	Separate	Separate, paired gonads
Fertilization and development	External, long larval period in lamprey	Internal, oviparous or viviparous	External
Examples	Lampreys, Hagfishes	Shark (dog fish), Skates and Rays	Perch, Trout, Rohu, Palaice



Scoliodon





Class Amphibia:

- (i) The amphibians lead a double life, living both in water (in embryonic condition) and on land and hence the name amphibia (amphi, two; bios, life).
- (ii) They are cold-blooded animals.
- (iii) They breathe by gills in the larval stage and by lungs in the adult.
- (iv) The skin is usually soft and glandular containing mucous-secreting glands. It sometimes supplements the lungs in breathing.
- (v) The limbs are five-fingered or pentadactyle type.
- (vi) There is a common hole for the exist of undigested food, urine and gametes known as the cloacal aperture.
- (vii) The heart is five-chambered.
- (viii) The development is usually accompanied by metamorphosis.

Examples: Salamanders; Newts; Necturus; Frogs; Toads; Caecilians.

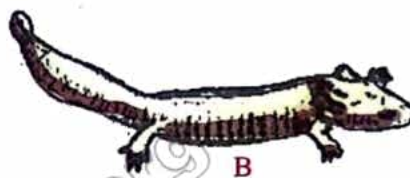


Fig. Tailed amphibians. A-Ambystoma (or Amblystoma) tigrinum; B-Necturus maculosus

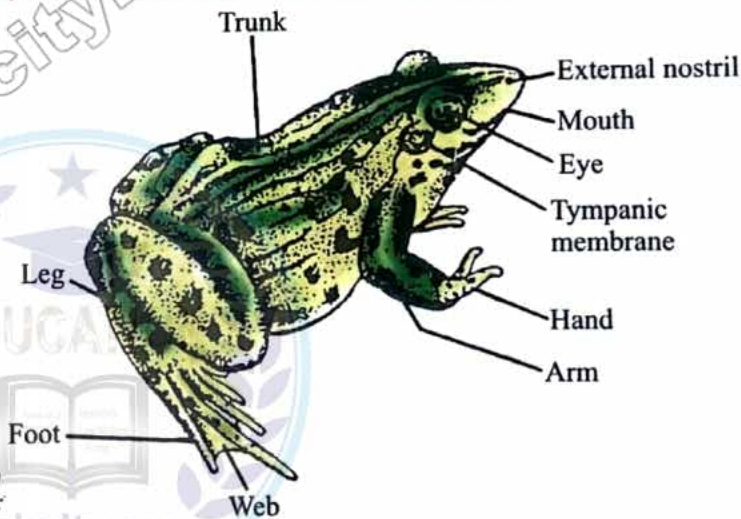
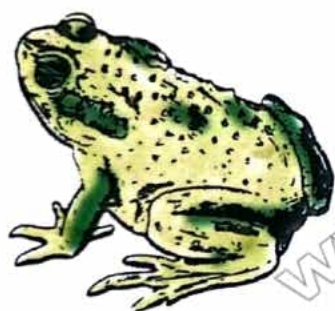


Fig. Marbled Salamander

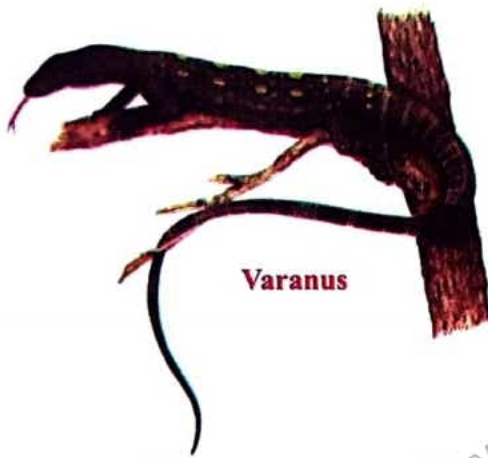
Fig. -A, Toad; B, Javan flying frog; C, Tree frog.

Class Reptilia:

- (i) The reptiles are cold-blooded animals.
- (ii) The body is covered by horny epidermal scales and sometimes also by dermal bony plates.
- (iii) They are predominantly terrestrial but some have secondarily adopted aquatic life.
- (iv) They are essentially tetrapods with pentadactyle limbs which are sometimes greatly modified or even absent as in snakes.
- (v) The heart is imperfectly four-chambered.
- (vi) Teeth are present in jaws except in turtles and tortoises.
- (vii) The eggs are large with sufficient yolk.
- (viii) Three embryonic membranes, the **amnion chorion** and **allantois chorion** are formed during development.

Examples: Sphenodon; Turtles; Tortoises; Lizards; Snakes; Alligator; Crocodile; Chamaeleon (Girgit); *Uromastix* (Sahnda).

CLASS REPTILIA



Varanus



Snake



Wall Gecko Lizard

Giant Tortoise



Flying Lizard



Uromastix hardwickii (Lateral view)



Chamaeleon

Class Aves:

- (i) The aves or birds are essentially adapted to an **aerial** life but some are, however, flightless, of which few live in water. In flying birds the forelimbs are modified into **wings**. In flightless birds the wings are **vestigial**. In aquatic birds they are modified into paddles or flippers.
- (ii) The greater part of the body is covered with **feathers**.
- (iii) The bones are **pneumatic** *i.e.*, they contain air spaces which are connected with a system of air-sacs in the body.
- (iv) Teeth are absent and the jaws are modified into beak which is covered with a horny sheath.
- (v) The sternum is broad with a prominent ventral **keel**.
- (vi) The heart is four-chambered.
- (vii) All the birds are warm-blooded, **oviparous** vertebrates.
- (viii) The eggs have much yolk and are enclosed in calcareous shells.
- (ix) The alimentary canal ends in a cloaca.
- (x) Amnion and allantois chorion are formed during development.

Examples: Pigeon, Parrot, Hen, Sparrow, Wood-pecker, Stork, Eagle, Kite, Heron, Cuckoo, Kiwi, Ostrich, Cassowary, Penguin etc.



Fig. Flying birds. A, stork, B, parrot

CLASS AVES



Parrot



The Blue rock-pigeon



Ostrich



Kiwi



Owl



Penguin

Class Mammalia:

- (i) The mammals are warm-blooded vertebrates which have hair on the skin.
- (ii) The skin is provided with **sweat** and **sebaceous** glands and in the female with mammary glands.
- (iii) They are mostly terrestrial, through some live in fresh or marine water. The limbs are accordingly modified for walking, running, climbing or swimming.
- (iv) A muscular partition or diaphragm divides the body cavity into an anterior thoracic and a posterior abdominal cavity.
- (v) The teeth are present which are varied in structure and function.
- (vi) The heart is four-chambered and the brain is highly developed.
- (vii) The urino-genital aperture is separate from anus but Prototherians (Duckbill) have a common cloacal aperture.
- (viii) All the mammals are **viviparous** except Prototherians which are oviparous.
- (ix) They form amnion and allantois chorion during development.

The class mammalia is divided into three sub-classes:

(a) Subclass Prototheria (Monotremata) or Egg-laying Mammals:

The prototherians lay eggs but suckle the youngones with milk. They have a cloaca and cloacal aperture instead of separate anus and urinogenital aperture. **Examples:** *Tachyglossus* (Echidna); Duck-bill Platypus (*Ornithorhyncus*).



Fig. -Duckbill



Fig. Spiny anteater (Echidna)



Fig. -Asiatic Pangolin
(Scaly Anteater)



Kangaroo



Fig. -Ard-vark

(b) Subclass Metatheria (Marsupialia) or Pouched Mammals:

The marsupials are mostly found in Australia while a few live in America. The female has a pouch or marsupium on its belly in which are kept newly born, helpless young till they are able to fend for themselves. **Examples:** Kangaroo; Koala; Wombat; Bandicoot; Opossum; Flying Phalanger.

(c) Subclass Eutheria (Placentalia) or True Mammals:

All the mammals except the prototherians and marsupials are included in this subclass. The eutherians develop **placenta** by which the embryo remains attached with the uterus of the mother till the development is completed.

The subclass is divided into several orders, of which the following are important:

- Order Edentata:** **Examples:** Sloths; *Armadillo*; Scaly ant-eater (*Pangolin*).
- Order Rodentia:** The rodents gnaw their food with their chisel-shaped front teeth. **Examples:** Rats; Squirrels; Mice; Porcupine; Rabbit; Hare.
- Order Insectivora:** **Examples:** Hedgehog; Mole; Shrew.
- Order Chiroptera (Flying-mammals):** **Examples:** Bat; Flying Fox.
- Order Carnivora:** **Examples:** Cat; Dog; Lion; Tiger; Wolf; Fox; Bear; Leopard; Jackal.
- Order Cetacea (Aquatic, fish-like mammals):** **Examples:** Whale; Dolphin, Porpoise.
- Order Perissodactyla:** Hoofed mammals with odd number of toes in each foot.
Examples: Horse; Ass; Zebra; Rhinoceros; Tapir.
- Order Artiodactyla:** Hoofed mammals with even number of toes in each foot.
Examples: Camel; Ox; Sheep; Goat; Giraffe; Deer; Pig; Sea horse (Hippopotamus).
- Order Proboscidea:** **Example:** Elephant.
- Order Primates:** **Examples:** Monkey; Baboon; Gibbon; Gorilla;

CLASS MAMMALIA



Common porcupine



Bat



Armadillo



Dolphin

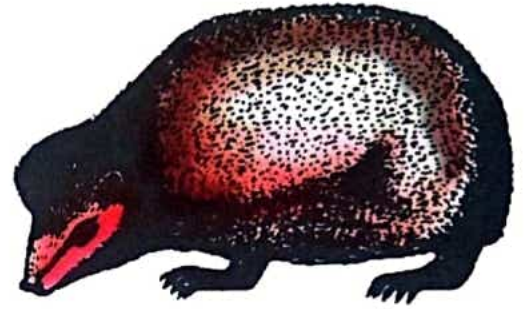


Fig. -Hedgehog



Fig. The zebra



Fig. Giraffe



Fig. The Roe Deer

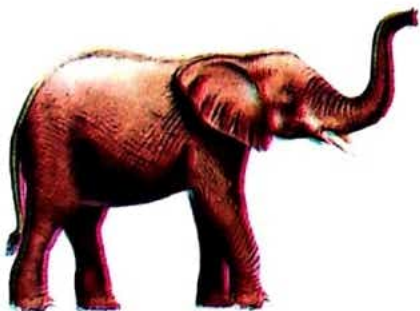


Fig. Elephant



Fig. Monkey

VIVA VOCE

Q.1 What is the basis of biological classification?

Ans. The biological classification is based on the fundamental similarities of structure and functions and the evolutionary relationship existing among different organisms.

Q.2 Define systematics or taxonomy.

Ans. The naming and classification (arrangement) of living beings into groups and sub-groups on the basis of their evolutionary relationship is called taxonomy or systematics.

Q.3 Differentiate between parazoa and metazoa giving examples.

Ans. The simple animals that represent an early stage of multicellular animals are called parazoa which includes only one phylum, the Porifera. All the multicellular animals except Porifera are known as the Metazoa.

Q.4 Why is the phylum Coelenterata called so?

Ans. The animals of this phylum possess a single cavity in the body. Because it serves both as coelom as well as the enteron (digestive cavity) it is known as the coelenteron and hence the name of the phylum as coelenterata.

Q.5 What are warm-blooded animals (homiotherms or endotherms)?

Ans. The animals which maintain a constant and high body temperature are called warm-blooded animals.

Q.6 What is the difference between anus and cloaca?

Ans. The cloaca is the common aperture for the exit of undigested food particles, gametes and urine. While anus is the aperture (opening) for the discharge of undigested food only.

Q.7 Differentiate between oviparous and viviparous animals?

Ans. The animals that lay or spawn their eggs are called oviparous animals while the animals which give birth to young ones are called viviparous.

Q.8 What is the characteristic feature of mammals?

Ans. Feathers.

Q.9 What are two most remarkable features of mammals?

Ans. 1-hair 2-mammary glands.

Q.10 Name the sub-classes of Class Mammalia?

Ans.

1.	Prototheria	Egg-laying mammals
2.	Methatheria	Pournched mammals
3.	Eutheria	Placental mammals

Q.11 Give the classification of man?

Ans. Phylum	Chordata	Subphylum	Vertabrata
Class	Mammalia	Subclass	Eutheria
Order	Primates	Family	Hominidae
Genus	Homo	Species	Homo sapiens

Q.12 Name the phylum of the following animals? Flat fish, Jelly fish, Star fish, Cuttle fish, Silver fish.

Ans. Flat fish	Chordata
Jelly fish	Cnidaria
Star fish	Echinodermata
Cuttle fish	Mollusca
Silver fish	Arthropoda

Q.13 Name the class and phylum of the following: Fly, Flying fish, Horse, Sea-horse, Duck, Duck-billed platypus, Whale and wolf.

Ans. Animals	Phylum	Class
Fly	Arthropoda	Insecta
Flying fish	Chordata	Osteichthyes
Horse	Chordata	Mammalia
Sea-horse	Chordata	Osteichthyes
Whale	Chordata	Mammalia
Wolf	Chordata	Mammalia
Duck	Chordata	Aves
Duck-billed platypus	Chordata	Mammalia

Q.14 Give examples of some flightless or running birds?

Ans. Ostrich, Kiwi.

Q.15 Name an aquatic birds.

Ans. Penguin.



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Experiment

EXTRACTION AND CHROMATOGRAPHY OF LEAF CHLOROPLAST PIGMENTS

The colour of plants and their parts is due to certain pigments, the most common of which is green, found in the chloroplasts of the photosynthetic cells of the leaves. In this experiment you will determine whether the green colour is due to one or more pigments.

Materials:

Fresh or frozen spinach (bean or grass or geranium) leaves; 80% Acetone; 90% Acetone-Petroleum ether mixture (1:9); Pestle and mortar; Filter paper or Chromatography papers Whatman No. 1; Funnel; Dropper; Glass wool; Beakers. Spirit lamp or Bunsen burner; Tripod stand; Forceps or tongs; Ethyl alcohol; Water bath; Pins; Pencil; Test tube with cork lid (fitted with pin); Sand.

Procedure:**1. Extraction of Chlorophyll and other Pigments:**

Take some green leaves of spinach (or others) in a mortar. Grind the leaves along with some sand (sand will facilitate grinding). Add to this a small quantity of acetone and mix thoroughly with the help of mortar. Filter this mixture, through the glass wool, in a beaker. The filtrate is a raw solution of leaf pigments including chlorophyll.



pakcity.org

A. Using a mortar and pestle, grind two or three spinach leaves in 5 ml of acetone

Separation of Different Pigments:

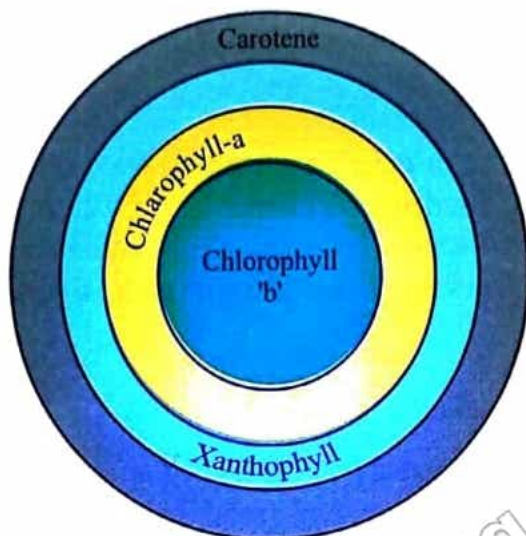
Chromatography is a process by which different components of a solution can be separated by means of a suitable solvent. Different substances show different degrees of solubility or different rates of flow of their molecules, in the same solvent. Due to this differential solubility, it is possible to separate different constituents of a solution. There are different techniques for this purpose, of which two are given below:

Method-I:

1. Take a filter paper. Put some filtrate or chlorophyll extract, drop by drop, in the centre of the filter paper (chromatographic paper).
2. Before the filtrate dries, pour some acetone-petroleum ether mixture over it.
3. The mixture will flow around and will carry with it various leaf pigments with different speeds.
4. This will result in the formation of a paper chromatogram, consisting of different pigments arranged in different concentric circles or zones.

Observations:

1. The outermost zone will be yellow in colour representing the **carotene**.
2. Next to this will be the yellowish-brown zone of **xanthophyll**.
3. Inner to this will be the blue-green zone of **chlorophyll-a**.
4. The innermost or central zone will be olive-green zone of **chlorophyll - b**.

**Fig. Paper Chromatogram****Method-II:**

1. Cut a strip of filter paper (chromatographic paper) of sufficient length to reach near the bottom of a test tube and of such width that its edges do not touch the sides.
2. Draw a pencil line across the strip 3 cm from the end.
3. Fold the other end over through 90° and by means of a pin attach it to the cork to be fitted in the mouth of the test tube.
4. Using the head of a pin as a dropper, place a drop of chlorophyll extract at the centre of the pencil line.
5. Let the drop dry, then put a second drop over the first also allow it to dry.
6. Repeat similar dropping for about ten minutes, building up a small but concentrated area (spot).

B. Paint a narrow band or chlorophyll extract across the paper strip. Allow the extract to dry. Repeat this several more times, allowing the extract to dry between each application.



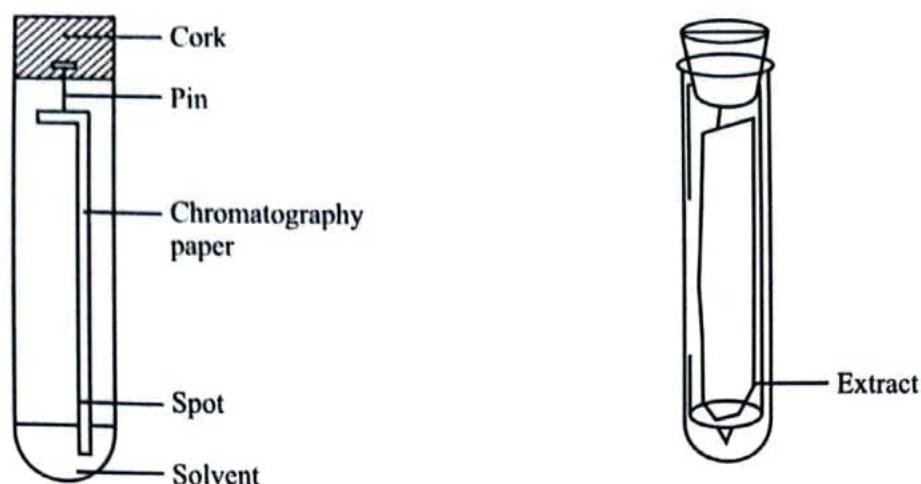


Fig. Separation of chlorophyll pigments using paper chromatography

7. Now pour some acetone-petroleum ether solution into the test tube to a depth of about 1.5 cm.
8. Insert the strip of filter paper into the test tube and close its mouth by the cork.
9. The bottom edge of the strip should dip in the solution but the coloured spot should not touch it.

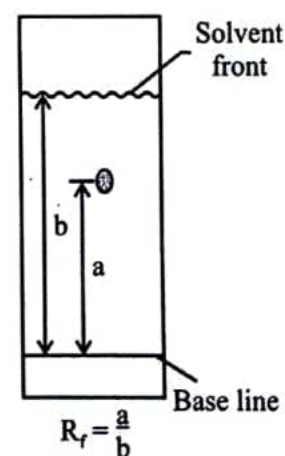
Observations:

1. The acetone-ether solution gradually rises up the strip carrying with it various leaf pigments, with different speeds.
2. After about ten minutes, when the ether solution is about 2 cm from the top edge, remove the strip and allow it to dry. A chromatogram consisting of different pigments arranged in four transverse bands would be formed. The pigments would separate in the following order from the dipped end of the strip to its bent end:
 1. Chlorophyll - b - Olive-green
 2. Chlorophyll - a - Blue-green
 3. Xanthophyll - Yellow-brown
 4. Carotene - Yellow

Calculations:

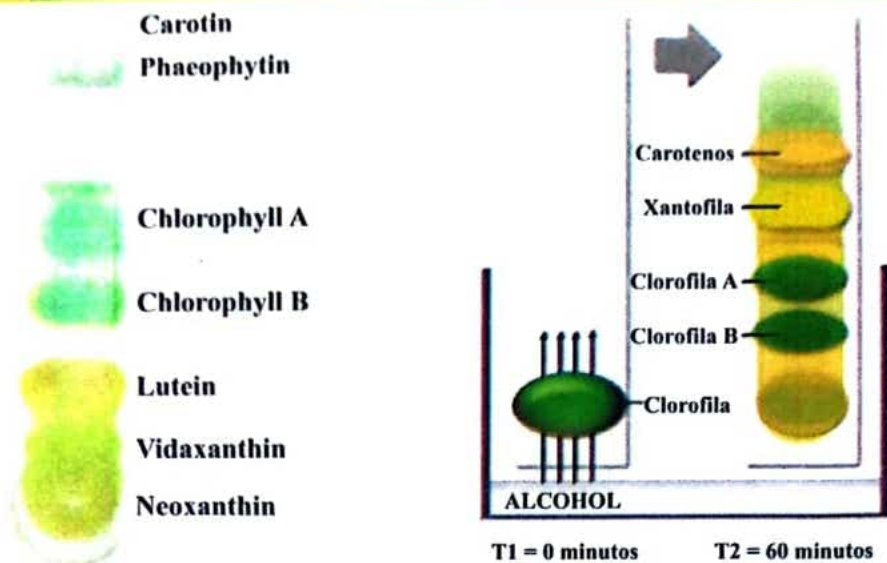
The movement of the solute relative to the solvent (100 parts of light petroleum : 12 parts of 90% propanone) front on a chromatographic system is constant for that solute. This can be expressed in the term R_f (Reference flow).

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent front}}$$



Result:

The following colour bands should be shown and find their R_f values.



Colour of spot	Pigments present	R_f value
Yellow	Carotene	
Yellow-grey	Phaeophytin	
Yellow-brown	Xanthophylls (often differentiates into two spots)	
Blue-green	Chlorophyll a	
Green	Chlorophyll b	

Precautions:

1. Always use a fresh filtrate (extract) to prepare a paper chromatogram.
2. Apply the solvent in single stroke.
3. Drop the extract exactly in the centre of filter paper.
4. Drop should not exceeds 0.5 cm in diameter.

VIVA VOCE

Q.1 What are photosynthetic pigments?

Ans. These are the various pigments found in thylakoids of chloroplast like chlorophyll, carotene, xanthophylls etc. which carry out photosynthesis.

Q.2 What is chlorophyll?

Ans. It is the green pigment located within the chloroplast. It is the key light capturing molecule which absorbs violet, blue and red light but reflects green and therefore appears green.

Q.3 What is meant by chromatography?

Ans. It is a term for a wide variety of techniques in which a mixture of dissolved components is fractionated as it moves through some time of porous matrix.

Q.4 What material is used for better crushing?

Ans. Sand is used.

Q.5 What chemical is added before filtration?

Ans. Acetone.

Q.6 Which paper is used in paper chromatography?

Ans. Watmann paper.

Q.7 How can you prepare a solvent?

Ans. A solvent can be prepared by mixing 100 parts of petroleum ether in 12 part of acetone.

Q.8 Which pigment is found in centre of paper chromatogram?

Ans. Chlorophyll 'b'.

Q.9 What is the colour of chlorophyll 'a'?

Ans. Blue green.

Q.10 What is the colour of carotene?

Ans. Yellow.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Extract the pigment of leaf and prepare a paper chromatograph.

Performance = 1, Apparatus = 1, Procedure = 1, Observation and result = 1, Short question = 1/2 + 1/2 = 5.

(Multan Board 2004)

Q.1 Name different pigment found in leaf.

Ans. Chlorophyll a, Chlorophyll b, Xanthophyll and Carotene.

Q.2 Why do they stay in different layers?

Ans. Different colours have different rate of movements and they absorb different wavelengths of light. Therefore, they stay in different layers on filter paper.

Experiment 2: Extract the pigment of leaf by chromatography.

(Faisalabad Board 2004)

Q.1 What is chromatography?

Ans. The technique in which different colours are separated from each other on the basis of their rate of movement is called chromatography.

Q.2 What is the use of alcohol in this experiment?

Ans. Different pigments are dissolved in alcohol. Therefore, alcohol is used to extract pigment from cells.

(9)

Digestion



20

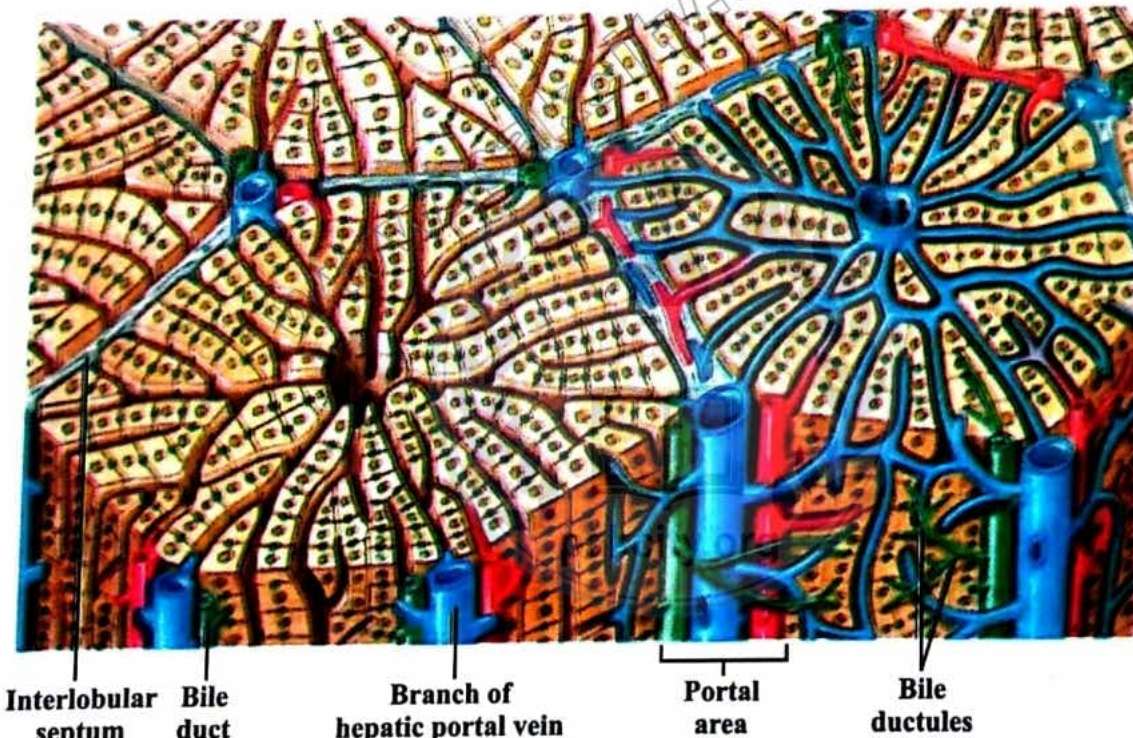
Experiment

STUDY OF T.S. OF LIVER, STOMACH, SMALL AND LARGE INTESTINE OF MAN FROM PREPARED SLIDES.

T.S. (Transverse Section) of Liver:

1. The T.S of liver has central **venule**.
2. Around the central venule are arranged most of the **hepatic cells**.
3. Between hepatic cells are present hexagonal structures called **hepatic lobules**.
4. Hepatic lobules contain:
 - A hepatic artery
 - A branch of hepatic vein
 - A branch of bile duct

Reason of identification: It has central venule and hepatic cells.



Diagrammatic view of lobular organization

Fig. Section of liver

T.S. of Stomach:

T.S of stomach is composed of following four layers.

1. Mucosa:

T.S of stomach shows inner most layer called mucosa. The inner layer of mucosa has epithelial tissues. Mucosa has many folds. These folds are gastric glands.

2. Sub Mucosa:

It is composed of irregular connective tissues.

3. Muscular Layer:

These muscles are present in three layers.

4. Serosa:

It forms outermost layer of stomach.

Reason of identification: It has folds of gastric glands.

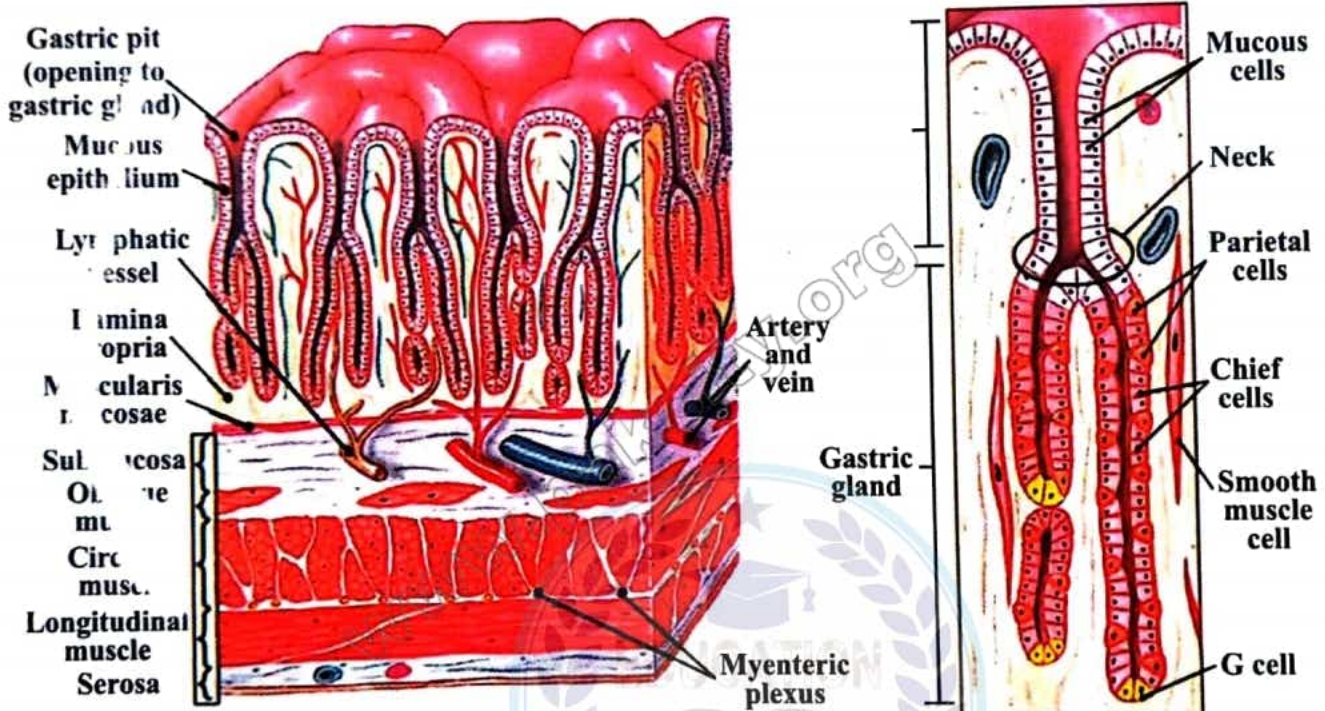
LINING AND GASTRIC GLANDS OF STOMACH

Fig. Martini, Anatomy & Physiology, Prentice Hall, 2001

T.S of Small Intestine:

T.S of intestine is composed of following four layers.

1. Mucosa:

It forms villi and intestinal gland. The villi form finger like projection in the cavity of intestine.

2. Sub Mucosa:

It is composed of connective tissues. It has circular folds.

3. Muscular Layers:

It is composed of two muscular layers.

4. Serosa:

It is the outmost layer of intestine.

Reason of identification: It has finger like projections called villi.

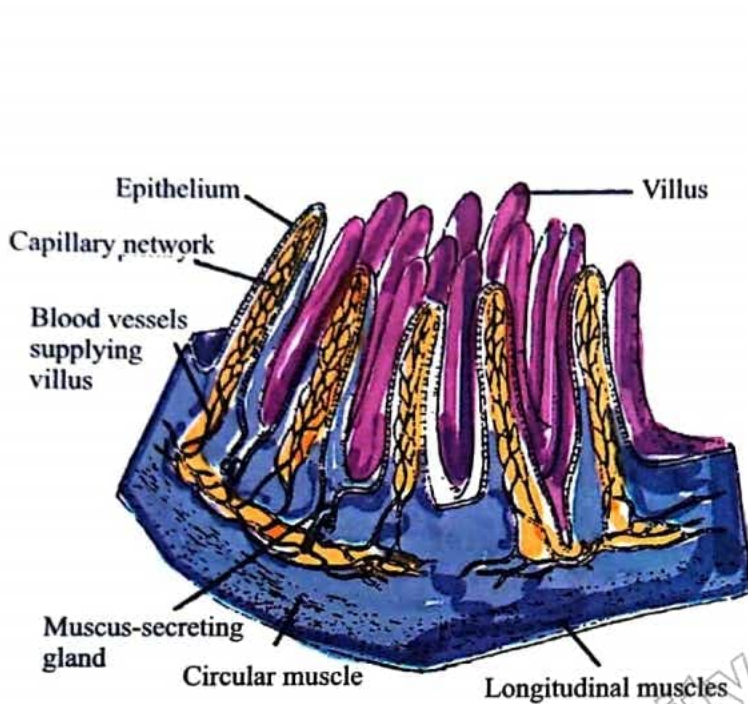


Fig. A part of T.S. of ileum

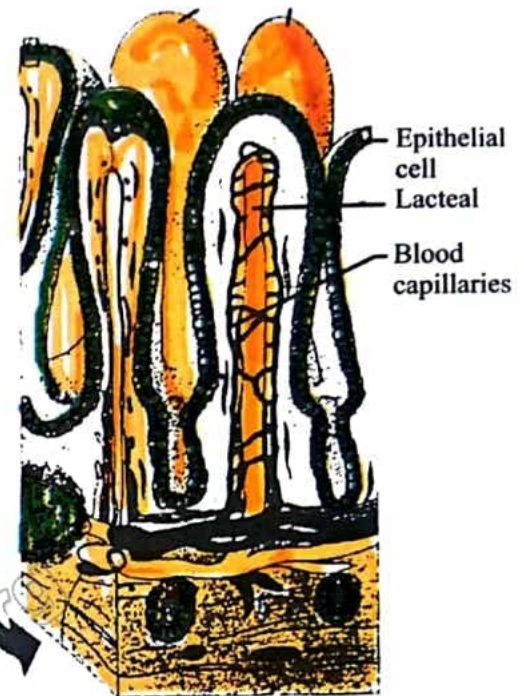


Fig. Villus structure

T.S of Large Intestine:

T.S of large intestine is composed of following four layers.

1. Mucosa:

It is smooth layer. It has no gland.

2. Sub Mucosa:

It is composed of connective tissues.

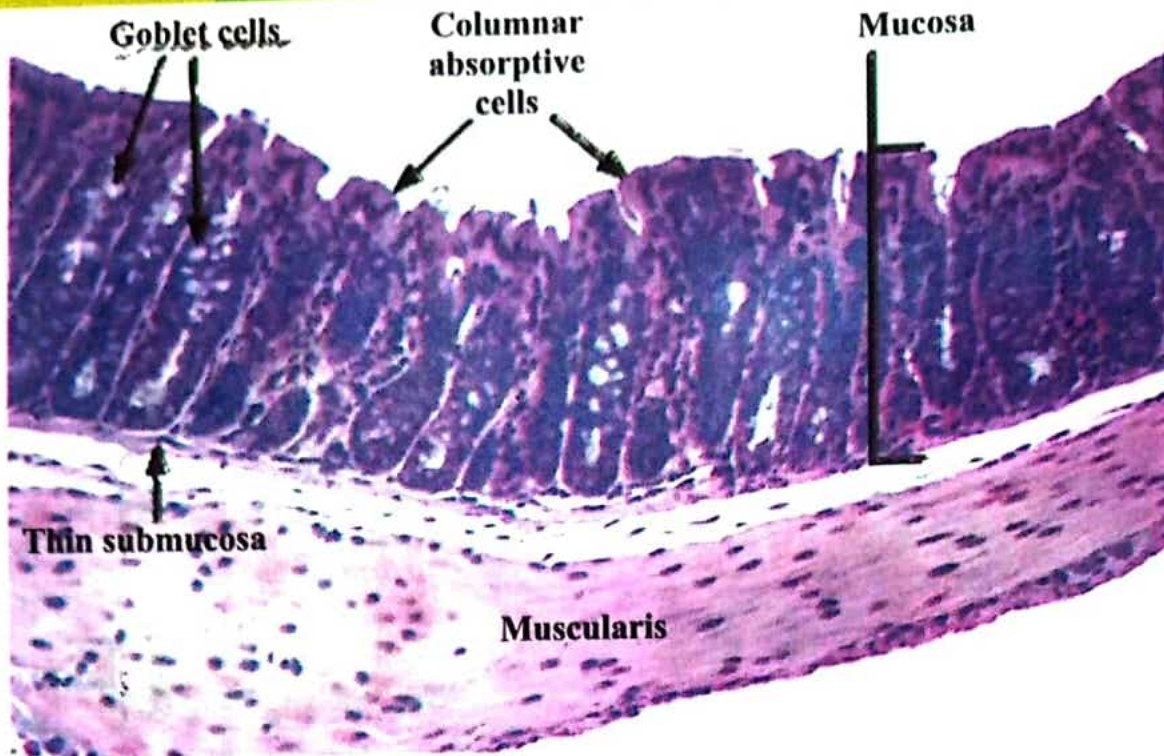
3. Muscular Layers:

It is composed of two muscular layers. Inner layer of circular muscles is well developed but outer longitudinal muscles are not well developed.

4. Serosa:

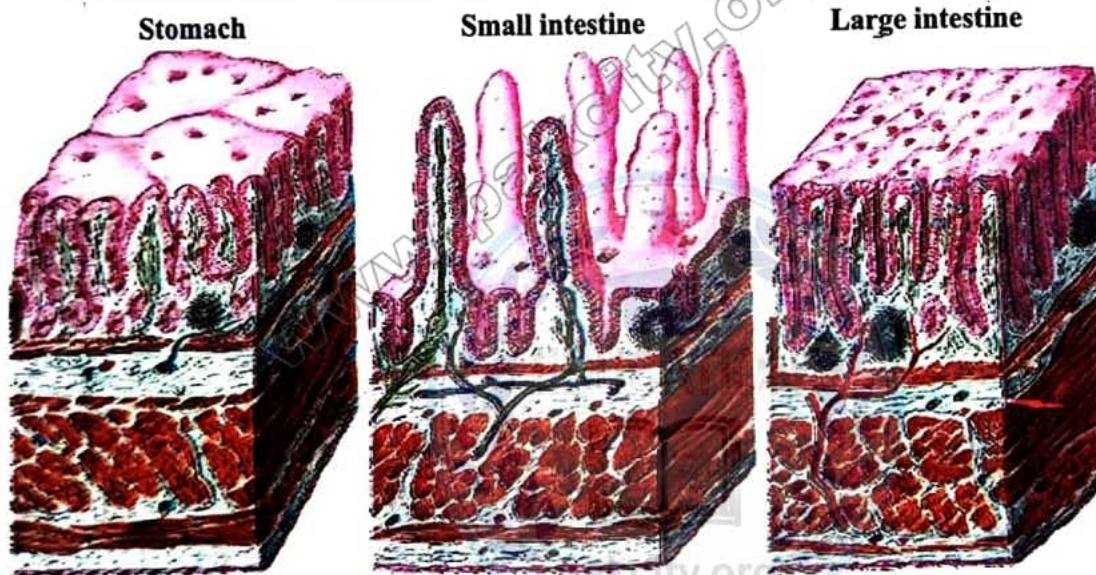
It is the outmost layer of intestine.

Reason of identification: The inner layer mucosa is flat without any gland.



TS of Large Intestine

COMPARISON OF HISTOLOGY OF STOMACH, SMALL-AND LARGE INTESTINES



VIVA VOCE

Q.1 Name the inclusions of submucosa.

Ans. These are connective tissue, nerve fibres, lymphatics and blood vessels.

Q.2 What is the function of the goblet cells of the mucous membrane?

Ans. The goblet cells secrete mucous for the lubrication of food.

Q.3 Differentiate between the mucosae of the human stomach and small intestine.

Ans. The mucosa of human stomach forms gastric glands whereas that of small intestine gives rise to the villi.

- Q.4** Name the cells which line gastric glands of man.
- Ans.** These are zymogen or peptic cells, parietal or oxyntic cells and the goblet or mucous cells.
- Q.5** Write down the functions of zymogen and oxyntic cell of the gastric glands of man.
- Ans.** The zymogen cells secrete pepsin while the oxyntic cells secrete hydrochloric acid.
- Q.6** What is Glisson's capsule in the human liver?
- Ans.** It is the connective tissue sheath enclosing the lobules of the liver.
- Q.7** What does the words 'intra' and 'inter' mean?
- Ans.** Intra means 'within' and inter means 'in between'.
- Q.8** Explain sinusoids in the liver of man.
- Ans.** The sinusoids are the fine tubules or passages connecting the intra and interlobular veins.
- Q.9** Name the cells of the human liver which secrete bile.
- Ans.** These cells are called *hepatocytes*.
- Q.10** What are lobules in the human liver?
- Ans.** The two lobes of the human liver are divided up into many functional units which are called lobules.
- Q.11** What are Kupffer cells in the human liver? What is their function?
- Ans.** The kupffer cells are phagocytic cells which break down old and worn out red blood cells and ingest pathogens.
- Q.12** Name the cellular coats which constitute the wall of the human gut.
- Ans.** These are from out inwards: Serous coat, muscular coat, submucosa and mucosa.
- Q.13** What is muscularis mucosae in the wall of gastrointestinal tract of man?
- Ans.** The muscularis mucosae is a narrow muscular band running in the submucosa just close to mucosa.
- Q.14** What does gastrointestinal tract represent?
- Ans.** It represents the stomach and intestine of the alimentary canal.
- Q.15** Name the cells of the mucosa (mucous membrane) of the human gut.
- Ans.** The human mucosa is composed of the columnar and goblet cells.

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Experiment

EXPOSURE OF DIGESTIVE SYSTEM OF FROG AND COCKROACH

THE FROG

The frog lives in damp places near water. It crawls on land by both pairs of limbs, but usually jumps about with the strong hind-limb. When in water, it swims with the hind-limbs which have webbed toes. It is a carnivorous animal feeding upon insects, worms and other small animals which it captures by its sticky tongue. The frogs go in hibernation during the severe winter season in order to protect themselves from the frost.

Classification:

Phylum	Chordata
Class	Amphibia
Order	Anura
Species	<i>Rana tigrina</i>



Materials:

Pithed frog (or chloroformed frog); Dissection box; Dissecting dish; Nails; Hammer; Bone cutter; Hand lens.

Pithing:

A frog can be pithed by severing (breaking) the connection between the brain and the spinal cord (by means of a fine needle) at the junction of vertebral column and the skull. This is a simple, painless procedure when properly done. The frog is immediately killed at the moment of pithing but certain body processes continue to operate for some time.

Procedure of Dissection:

1. A chloroformed or pithed frog is placed on board. Its ventral side is kept upward.
2. The limbs of the frog are fixed by nails.
3. The skin of the frog is lifted with the help of forceps. A cut is made in the skin in abdominal region. Then the skin is cut from abdomen to lower jaw.
4. **Anterior abdominal vein** passes through the midline of abdomen. It should not be cut. Therefore, body wall is raised and two lateral cuts are made in it. The body wall is cut from these two lateral sides.
5. The lower side of the mid section (with abdominal vein) is tied with a thread. After that, it is cut. Now bleeding does not take place from abdominal vein.
6. The sternum is cut at anterior side.
7. Now system is present in the abdominal cavity. The membrane between different organs is broken and different parts of digestive system are exposed.

Observation:

1. **Buccal Cavity:** Buccal cavity opens out side by mouth. Mouth is bounded by upper and lower jaw. Teeth are present on upper jaw. Tongue is attached at the anterior end but free at posterior end.
2. **Pharynx:** It is present at the posterior end of buccal cavity. It has a slit like opening called **glottis**. Glottis opens into lung. **Vocal sacs** are present at the floor of pharynx.
3. **Oesophagus:** Pharynx opens into oesophagus. It is short tube.
4. **Stomach:** Oesophagus opens into cardiac end of stomach. Its pyloric end opens into duodenum.
5. **Duodenum:** It is first part of intestine.
6. **Ileum:** It is second part of small intestine.
7. **Rectum (large intestine):** It is a wider tube. It opens into rectum.
8. **Cloaca:** It is a small chamber. It has urinary bladder on one side. It opens outside by cloacal aperture.

Glands:

There are three glands:

1. **Liver:**

It is present at the anterior part of the body. It has two lobes. **Gall bladder** is present in the right lobe. Bile duct arises from gall bladder and opens in duodenum.

2. **Pancreas:**

It is flat and pinkish gland present between stomach and duodenum. It opens into duodenum by **pancreatic duct**.

3. **Spleen:**

It is pinkish pear shaped structure. It is present in front of rectum.

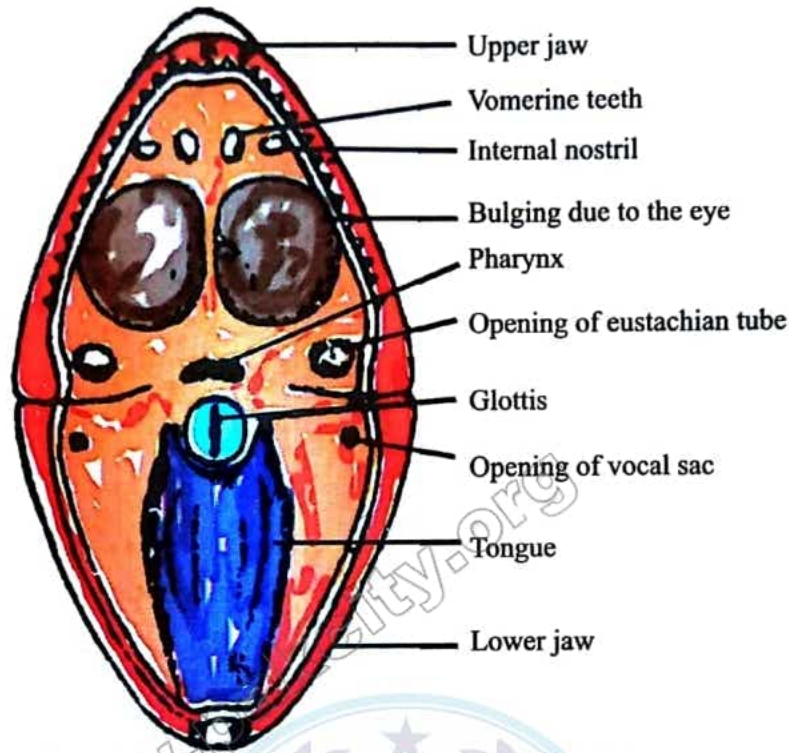


Fig. The buccal cavity of the male frog.

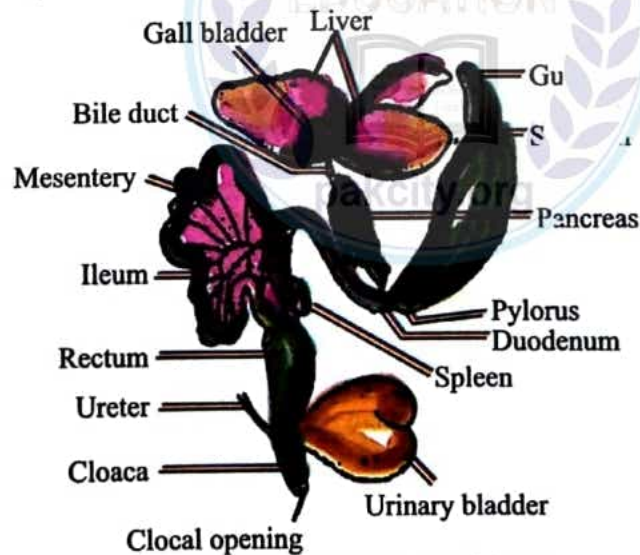


Fig. Digestive tube of frog

EXPOSURE OF DIGESTIVE SYSTEM OF COCKROACH

The cockroach belong to phylum Arthropoda, class insecta. It is widely distributed in the world. It is found commonly in keticheris, backeries, sewage and restaurants. It is active during night nocturnal) but remain hidden during day in holes, crevices and dark places. *Periplanata americana*, is most common in Pakistan, both the males and females of *P. americana* are winged, while females of *Blata orientalis* are wingless.

Classification:

Periplaneta Americana (Cockroach)

Phylum	Arthropoda
Class	Hexapoda
Order	Blattaria
Species	Periplaneta americana

Study of External Characters:

The body is 2-4 cm in length and 1 cm in width. The body is covered by an exoskeleton of chitin. The exoskeleton is in the form of plates. It is reddish brown in colour and divisible into three regions: **(1) Heads, (2) Thorax and (3) Abdomen**. A short **neck** connects the head with the torax.

1. Head:

Head is roughly oval, flattened structure attached anteriorly at right angle to the body axis. It contains a pair of long hair-like **antenna**, a pair of compound eyes and mouth parts.

2. Thorax:

It is composed of three segments **prothorax, mesothorax and metathorax**. Each segment on ventral side bear paired, jointed walking **legs**. Each leg is composed of **coax, femur, tibia** and **tarsus**. Each leg ends in a claw.

3. Abdomen:

The abdomen has ten segments. Each thoracic and abdominal segment is covered dorsally by a chitinous plate, the **tergum**, and ventrally by the **sternum**. 8th and 9th terga are small and are overlapped by the 7th. The 10th segment lacks sternum. A pair of jointed spindle shaped bodies called **and style** arise from 9th sternum in male cockroach. Between 10th tergum and 9th is the **anus** surrounded by two hard plates called **podical plates**.

Material:

Dissection box, Chloroformed cockroach, Dissecting board, Hand lens, Water in beaker, Cotton, Hammer, Nails.

Procedure of Dissection:

1. Cockroach is dissected from dorsal side. The wings of the killed cockroach are cut.
2. The abdominal plate **terga** and **sterna** meet at the lateral side. This meeting place of these plates is cut.
3. The animal is fixed on board with pins.
4. The terga are removed one by one.
5. Now abdominal cavity contains mass of white matter. It is removed with cotton.
6. Different parts of the digestive system of cockroach are exposed and studied.



Observation:

The alimentary canal of cockroach is composed of three parts:

1. Foregut:

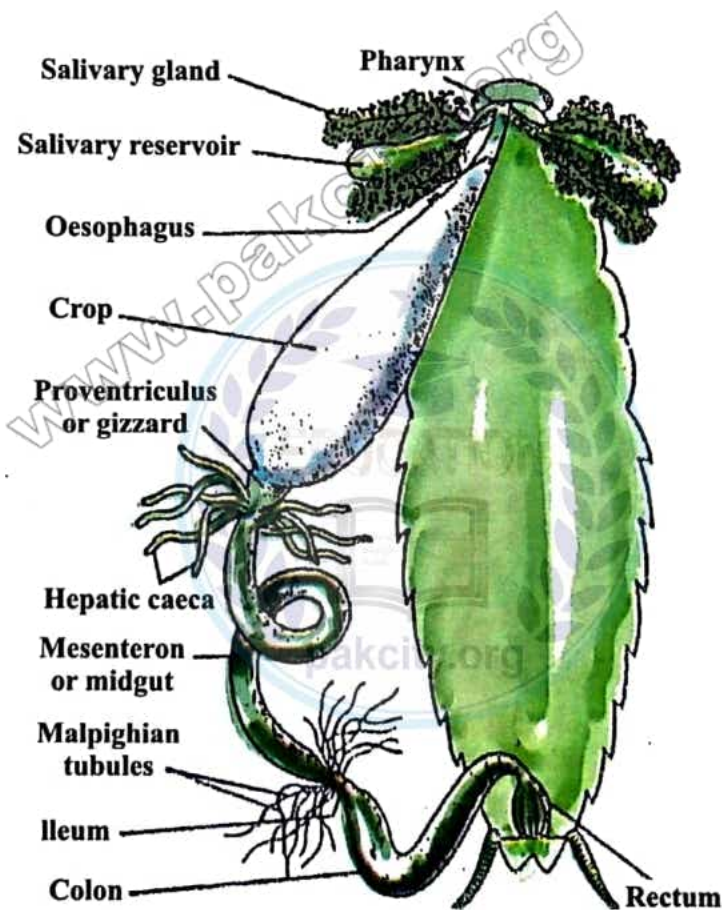
The foregut is composed of mouth cavity, **pharynx**, **crop** and **gizzard**. Crop stores food and gizzard is used to grind the food. Two pairs of salivary glands are present in the thorax region of the body. These salivary glands secrete saliva and pour this saliva into the mouth cavity.

2. Mid gut:

The mid gut is short narrow tube called **stomach**. Short finger like hollow tubes open into the anterior end of the mid gut. These tubes are called **hepatic caecae**. These caecae secrete digestive juices.

3. Hind gut:

The hind gut is a long coiled tube. The terminal (last) part of which forms a thick walled chamber called **rectum**. The rectum opens to the exterior through anus.



Digestive system

VIVA VOCE

Q.1 What is the function of crop?

Ans. It stores the food temporarily and then passes it to the next part of the alimentary canal.

Q.2 What is function of gizzard?

Ans. It is concerned with the grinding of food.

Q.3 What is the name of last portion of colon?

Ans. The rectum.

Q.4 Where do you find the anal cerci?

Ans. They are present under the lateral edges of 10th tergum.

Q.5 How are different parts of digestive system of frog attached with each other?

Ans. They are attached by mesentery.

Q.6 Name the abdominal plate of cockroach?

Ans. Abdominal plates of cockroach are **terga** and **sterna**.

Q.7 From which side does we dissect cockroach? Give reason.

Ans. Cockroach is dissected from dorsal side between nerve cord and other vital organs are present at ventral side.

Q.8 What is the general difference between dissection of invertebrates and chordates?

Ans. All the invertebrates are dissected from dorsal side and all the chordates are dissected from ventral side.

Q.9 Name the part of foregut of cockroach.

Ans. The parts of foregut of cockroach are **pharynx**, **crop** and **gizzard**.

Q.10 What is the function of crop of cockroach?

Ans. Crop is used for storage of food.

Q.11 What is the function of gizzard of cockroach?

Ans. It is used for grinding of food.

Q.12 What are hepatic caecae? Give their function.

Ans. Short finger like hollow tubes open into the anterior end of the mid gut. These tubes are called **hepatic caecae**. These caecae secrete digestive juices.

Q.13 What are amphibians?

Ans. The animals which live on land and water at the same time are called amphibians.

Q.14 What is peristalsis?

Ans. The rhythmic wave like contraction of the wall of alimentary canal is called peristalsis.

Q.15 What is digestion?

Ans. The breakdown of large non-diffusible molecules into small diffusible molecules is called digestion.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Dissect the animal provided (Cockroach). Exposed its digestive system and show it to examiner and draw its labelled diagram. Dissection = 1, Demonstration = 1, Labelled diagram = 1 + 1, Short questions = 1/2 + 1/2, Total = 5.

(Multan Board 2004)

Q.1 What is the function of rectum?

Ans. Rectum is used to store wastes.

Q.2 Which surface of animal is more suitable for dissection, dorsal or ventral? Why?

Ans. Dorsal surface of animal is more suitable for dissection because dorsal surface is without nerve cord and other vital organs.

Experiment 2: Dissect the animal provided (frog). Exposed its digestive system and draw its labelled diagram.

(Multan Board 2004)

Q.1 What is external feature of male animal?

Ans. It has a nuptial pad in its forelimb.

Q.2 Why they are not visible in winter?

Ans. The frogs are in hibernation in winter. Therefore, they are not visible in winter.

Experiment 3: Dissect the animal provided (frog). Exposed its digestive system and draw its labelled diagram.

(Multan, Faisalabad Boards 2004)

Q.1 What major care should be taken while dissecting animal from ventral side?

Ans. The anterior abdominal vein is present in the ventral abdominal wall. It is cut, it causes major bleeding. Therefore, care should be taken not to cut abdominal vein from ventral side.

Q.2 Why is animal dissected from ventral side?

Ans. The dorsal side has nerve cord and other vital organs are attached to dorsal body wall. Therefore, animal is cut from ventral side.

Experiment 4: Dissect the animal provided (Cockroach). Draw a labelled sketch of its digestive system and show its different part to the examiner.

(Multan, D.G. Khan Boards 2004)

Q.1 How many salivary glands are present in it?

Ans. Two pairs of salivary glands are present in it.

Q.2 What is the function of mouth parts?

Ans. They are used for grinding cutting and capturing of food.

22

Experiment

DIGESTION OF CARBOHYDRATE
BY ENZYME IN SALIVA**Materials:**

Test tubes, Test tube holders and stand.

Graduated pipette, Wooden stand, Water bath to maintain 37°C temperature. Thermometer, 0.4 starch solution, Benedict solution, NaCl, HCl, Iodine solution, Rubber or Chewgum.

Procedure:

There are 3 pairs of saliva secreting glands in the oral cavity. (Sublingual, Sub mandibular paratoid).

Saliva contain Ptyaline Mucin, Ptyaline digest the starch.

Take a rubber and chew it. Saliva secretion will start. Collect 4 ml saliva in test tube, Add 4 ml of water in it. Filter the enzyme preparation.

Take four test tubes. Take 10 ml of 0.4% starch solution in each test tube. Iodine test will show presence of starch, as the starch will change blue.

Benedict solution test will show that no glucose is present in the starch solution.

Starch sol. + Benedict sol. + Boil + No change + Absence of glucose.

Experimental Tube:

1. Take 10 ml of starch solution add 2 ml of ptyaline solution.

Control Tubes:

2. 10 ml of starch sol + 2 ml of water.
3. 10 ml of starch sol + Boiled ptyaline sol.

Place these tubes in water bath at 37°C temperature.

Test all the above test tubes for benedict test after 5 minutes.

Observations:

1. Tube No. 1 will show positive benedict test.

The blue colour of starch will become brown as there is no more starch.

2. Tube No. 2 and 3 Negative for Benedict test presence of glucose can also be tested by Cole reagent of Fehling solution.



10 ml starch
solu. + 2 ml
ptyaline solu.



10 ml starch
solu. + 2 ml
boiled H₂O



10 ml starch
solu. + 2 ml
boiled ptyaline

Result: Saliva enzymes converted the starch into glucose.

(10) Respiration



23

Experiment

INVESTIGATION OF STOMATAL DISTRIBUTION USING CLEAR NAIL VARNISH OR EPIDERMIS PEEL

In bifacial leaves (dicotyledonous leaves) the stomata are more numerous on the lower surface than on the upper. The isobilateral leaves (monocotyledonous leaves), on the other hand, have similar upper and lower surfaces with similar stomatal distributions. However, the number of stomata is fewer in plants adapted to dry conditions.

Materials:

Microscope, Glass slides, Cover slips, Fine forceps, Clear nail varnish, Fresh fully expanded leaves, preferably of *Bryophyllum* or *Rhoeo discolor*, Watch glasses, Camel-hair brush, razor blade, Forceps, Scissors.

Procedure:

1. A layer of nail varnish is spread on the surface of leaf. A thin layer of epidermis under the layer of varnish is removed with the help of forceps. It is placed on slide. It is covered with cover slip. It is observed under microscope.
2. Numbers of stomata are counted in small area of epidermis.
3. The diameter of the focus area of slide is measured with a ruler. Its area is measured by formula: πr^2 . Then number of stomata per square centimetre is calculated. It is called **stomatal density**. Suppose

$$\begin{aligned} \text{Diameter} &= 1.2 \text{ mm}, & \pi &= 3.142 \\ r &= \frac{1}{2} d = \frac{1}{2} \times 1.2 = 0.6 \text{ mm} = 0.06 \text{ cm} \end{aligned}$$

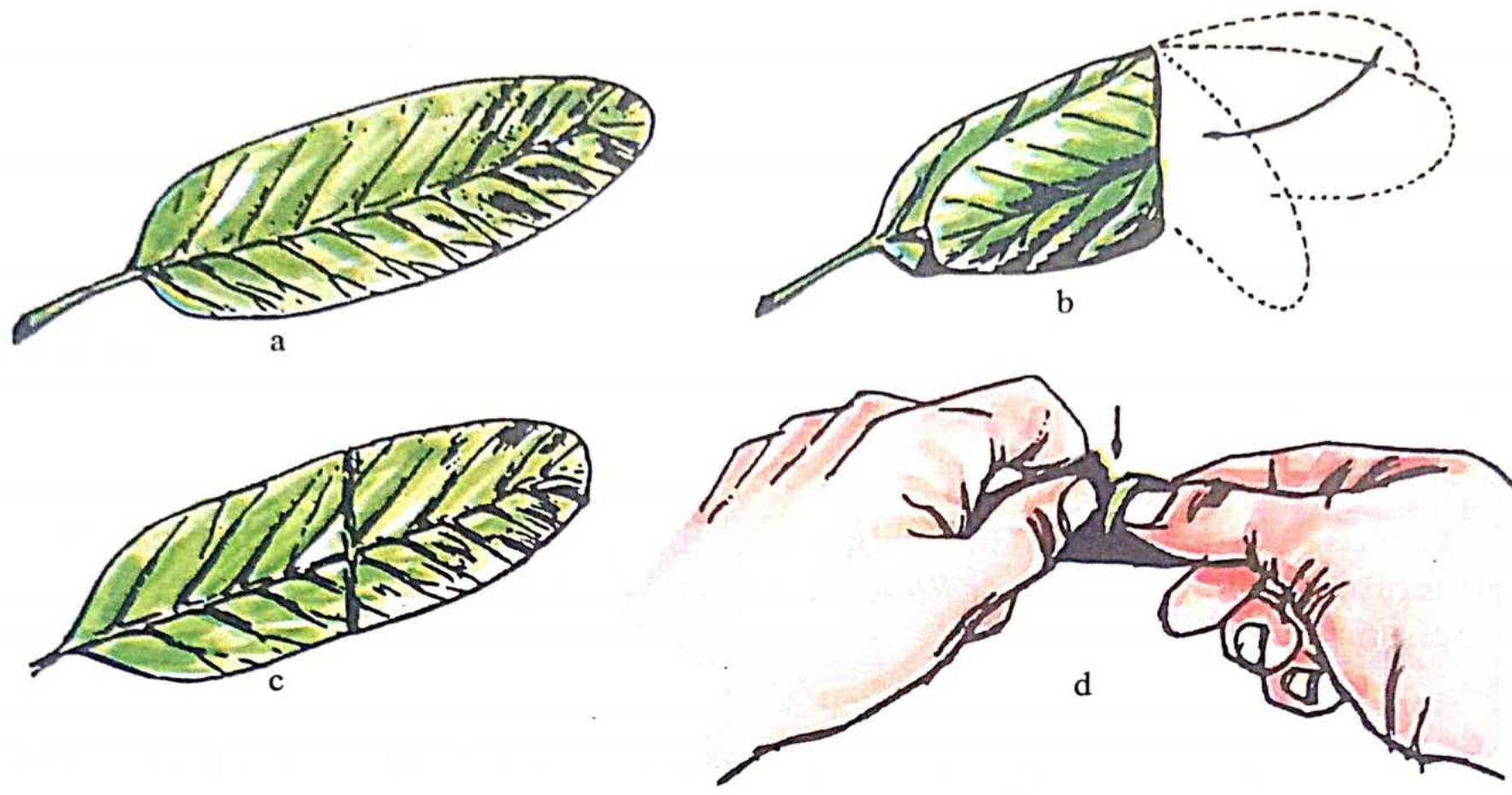
$$\text{Area of the field of view} = \pi r^2 = 3.142 (0.06)^2 = 0.0113$$

4. The number of stomata on the upper and lower epidermis is compared. This process is repeated with three plants.

Observation:

Compare the densities of the stomata in the upper and lower epidermises of the same leaf. You will notice that the number of stomata is far greater in the lower epidermis than in the upper epidermis of a bifacial leaf. e.g.

No.	Name of plant (leaf)	Upper epidermis per cm ³	Lower epidermis per cm ³
1.	Tomato (Dicot)	1200	13,000
2.	Oak (Dicot)	0	45,000
3.	Oat (Monocot)	2,500	2,300
4.	Maize (Monocot)	52000	6800



Tearing a leaf at an angle

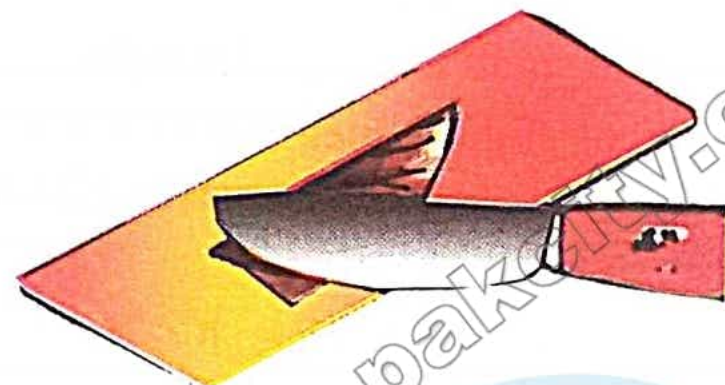


Fig. Trim away thick portion

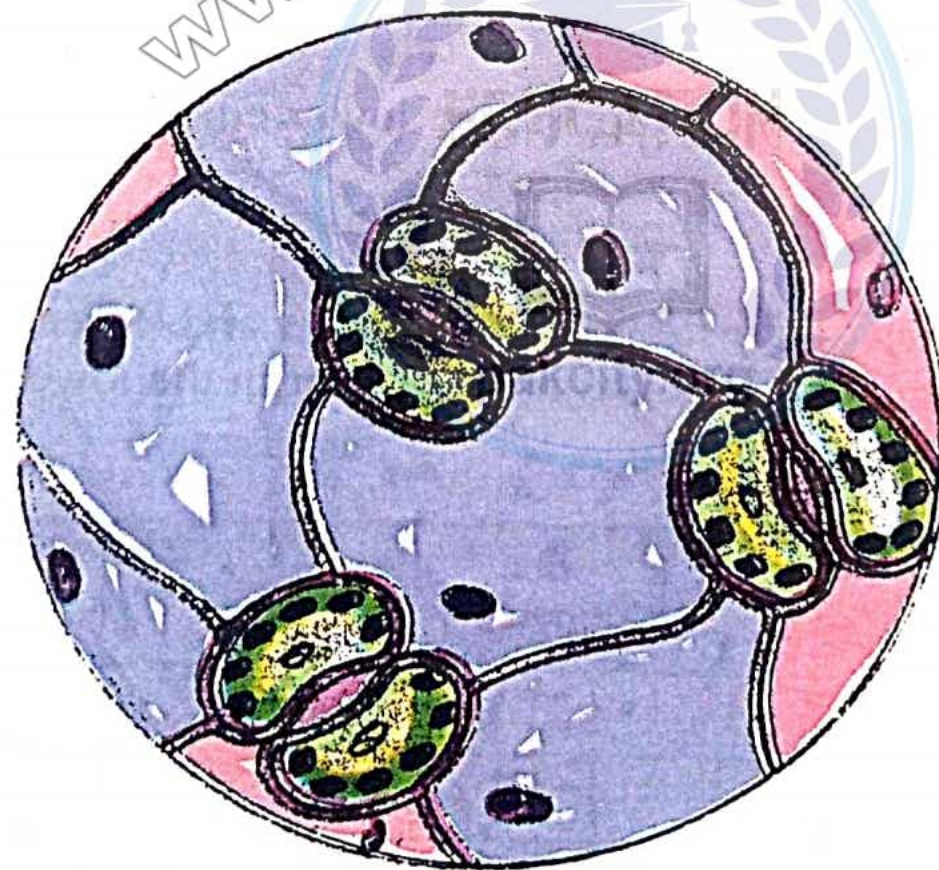


Fig. Preparation of slide of leaf epidermis

VIVA VOCE

Q.1 Can you calculate or estimate the number of stomata in upper or lower surface of leaf?

Ans. Yes, the number of stomata in upper or lower surface of leaf can be calculated or estimated.

Q.2 Were the stomata open or closed? Explained why?

Ans. Stomata were open. Because epidermis was placed in pure water. Water entered the cells. The guard cells became turgid resulting in opening of stomata.

Q.3 What would be the effect on a plant, if stomata are completely closed, all the time in 24 hours period?

Ans.

1. Rate of transpiration would be decreased. But stomatal transpiration would be totally ceased.
2. Photosynthesis would be stopped due to non-availability of CO₂ from atmosphere.
3. Respiration would be stopped due to non-availability of O₂ from respiration.

Q.4 If we put a plant in the dark, the stomata close. Why?

Ans. In dark no photosynthesis occurs in guard cells. Sugar level is decreased in guard cells. Water comes out of guard cells into the surrounding epidermal cell. Guard cells become flaccid and stomata are closed.

Q.5 Are stomata present only in leaves? If not, where else they are found?

Ans. No, stomata are not only present in leaves. They are also found on epidermis of herbaceous stems.

Q.6 What is the distribution of stomata in a monocot and dicot leaf?

Ans. In monocot leaf, stomata are present in both surfaces of leaves. In dicot leaf, stomata are present only in lower surface (ventral surface) of leaf.

Q.7 What are hypostomatous plants?

Ans. The plants in which stomata are found only in lower epidermis of leaf are called hypostomatous e.g., *Tradescantia*, *Zabrina*, *Colocasia* etc.

Q.8 What are epistomatous plants?

Ans. The plants in which stomata are found only in upper epidermis of leaf, are called epistomatous plants. e.g., *Typha* and *Nymphaea* etc.

Q.9 What are Amphistomatous plants?

Ans. The plants in which stomata are present on both dorsal and ventral surfaces of leaf are called amphistomatous plants e.g., *Ocimum* (Niazbo).

Q.10 Define leaf area index.

Ans. It is the ratio between the area of leaf surface exposed to light and the area of ground surface.

$$\text{LAI} = \frac{\text{Leaf area exposed to light (cm}^2\text{)}}{\text{Area of ground surface (cm}^2\text{)}}$$

It is helpful in determining the productivity of the plant. For maximum net production, L.A.I. should be more than 4.

24

Experiment**INVESTIGATION OF EFFECTS OF DIFFERENT CONCENTRATION OF GLUCOSE ON OPENING & CLOSING OF STOMATA****Material:**

Fresh leaves, Compound microscope, Glass slide, Cover slip, Dropper, Different concentrations of glucose solution (1%, 20%, 40%, 60%, 80%).

Procedure:

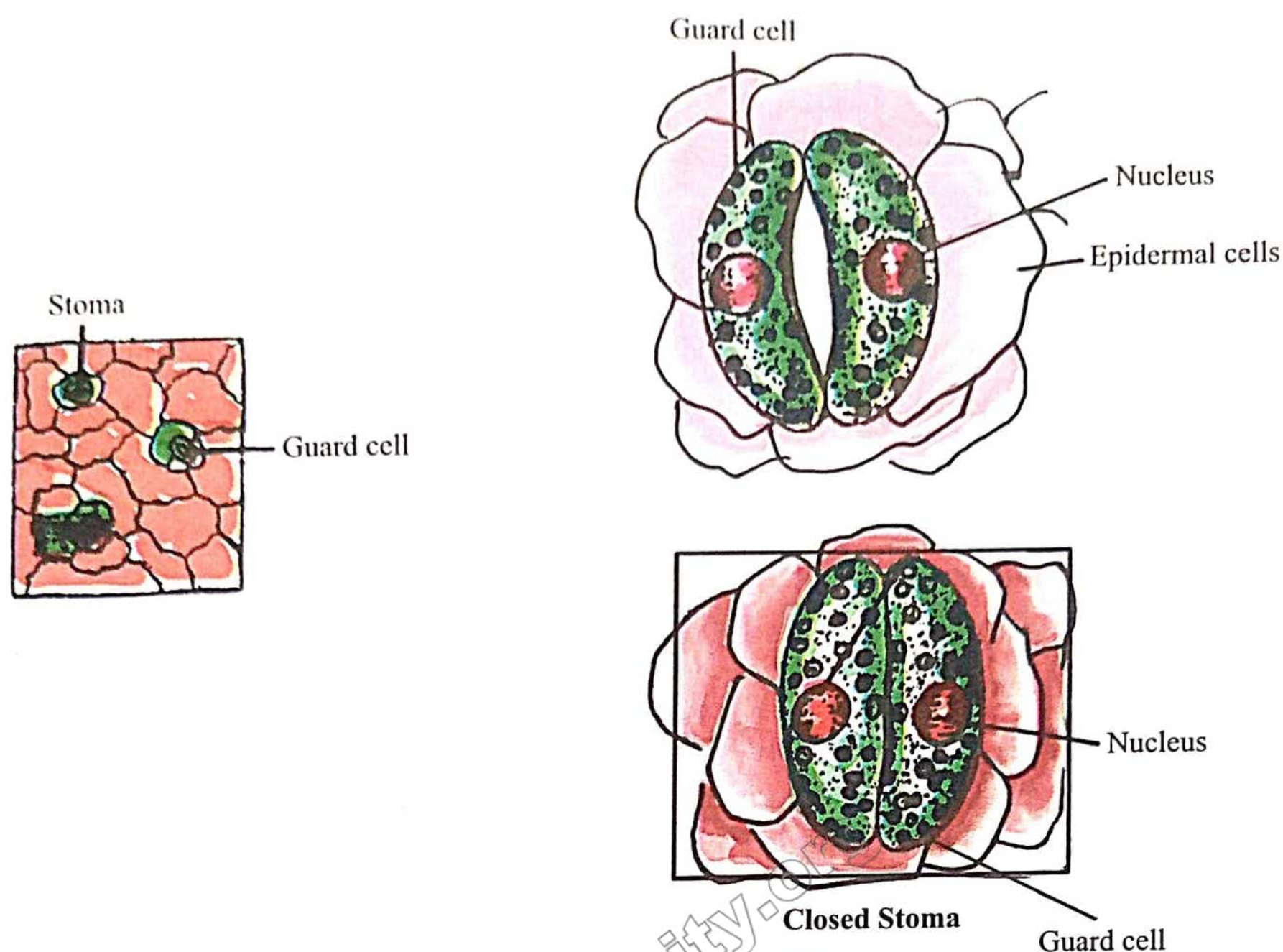
1. A leaf is taken. Its lower surface is brought in front of eyes.
2. Now the leaf is folded and bent. It tears up the lower epidermis.
3. The torn epidermis is peeled off from the surface of leaf. It separates a piece of lower epidermis from the surface of leaf.
4. This piece is cut from the remaining part.
5. A slide is taken. A drop of water is placed on it. The small piece of epidermis is spread over this slide. A cover slip is placed on slide and examined under microscope.
6. Now water is removed from under the surface of cover slip with the help of blotting paper. A drop of 1% sugar solution is dropped there with the help of dropper. It is observed under microscope. The size of stomata and shape of guard cells is noted.
7. This procedure is repeated with 40% and 80% sugar solutions.

Observation:

No.	Conc. of solution	Shape of guard cells	Degree of opening of stomata
1.	Pure water	Bean shaped	Completely open
2.	40% sugar solution	Less bean shaped	Open partially
3.	80% sugar solution	Flat inner surface	Completely closed

Results:

These observations proved that the guard cells lose water in sugar solution. Therefore, they lose turgidity and become less bean shaped. In highly concentrated solution they lose maximum water and stomata are completely closed.



VIVA VOCE

Q.1 Other than their shape, do you notice any difference between epidermal cells and guard cells?

Ans. The guard cells have chloroplasts while the epidermal cells have no chloroplasts.

Q.2 What differences in thickness do you see in the walls of guard cells?

Ans. The inner walls of the guard cells (those towards the opening) are thicker than their outer walls.

Q.3 What are the stomata?

Ans. The stomata are minutes pores, each surrounded by a pair of guard cells and situated in the lower epidermis of a bifacial leaf.

Q.4 What gases are exchanged through the stomata during respiration and photosynthesis?

Ans. Carbon dioxide and oxygen are exchanged through the stomata during respiration and photosynthesis.

Q.5 What gas will enter the stomata in greater amounts than it leaves, during photosynthesis?

Ans. The carbon dioxide enters the stomata in greater amounts than it leaves during photosynthesis.

- Q.6** What gas leaves the stomata in greater amounts than it enters, during photosynthesis?
- Ans.** It is the oxygen.
- Q.7** What changes did you observe when you added a drop of 5% glucose solution to the slide of leaf epidermis?
- Ans.** The stomata were closed.
- Q.8** Why are stomata closed at night?
- Ans.** At night there is no synthesis of soluble sugar and hence guard cells lose water by osmosis and the stomata are closed.
- Q.9**
- Ans.** At day time photosynthesis take place in guard cells and glucose is produced. It increases its osmotic concentration. Therefore, it absorbs water and become turgid and opened.
- Q.10** Why do stomata get closed at night?
- Ans.** At night there is no photosynthesis. The glucose is changed into starch. Therefore the osmotic concentration of glucose is reduced and it loses water. Thus it is closed.
- Q.11** Why guard cells become kidney shaped, when they absorb water?
- Ans.** The cell wall of guard cells is thicker inside (towards stomata) than out side. Thus it absorbs water and becomes kidney shaped.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Study the effect of different concentration of glucose solution on the opening and closing of stomata.

Performance = 1, Apparatus = 1, Procedure and diagram = 1, Observation and result = 1, Short question = $1/2 + 1/2 = 5$

(Multan Board 2004)

- Q.1** Why are stomata remained open at day time?
- Ans.** At day time photosynthesis take place in guard cells and glucose is produced. It increases its osmotic concentration. Therefore, it absorbs water and become turgid and opened.
- Q.2** Why do stomata get closed at night?
- Ans.** At night there is no photosynthesis. The glucose is changed into starch. Therefore the osmotic concentration of glucose is reduced and it loses water. Thus it is closed.

25

Experiment

EXPOSURE OF RESPIRATORY SYSTEM OF FROG

Material:

Dissection box, Chloroformed or pithed frog, Dissecting board, Hand lens, Water in beaker, Cotton, Hammer, Nails.



Procedure of Dissection:

1. A chloroformed or pithed frog is placed on board. Its ventral side is kept upward.
2. The limbs of the frog are fixed by nails.
3. The skin of the frog is lifted with the help of forceps. A cut is made in the skin in abdominal region. Then the skin is cut from abdomen to lower jaw.
4. **Anterior abdominal vein** passes through the midline of abdomen. It should not be cut. Therefore, body wall is raised and two lateral cuts are made in it. The body wall is cut from these two lateral sides.
5. The lower side of the mid section (with abdominal vein) is tied with a thread. After that, it is cut. Now bleeding does not take place from abdominal vein.
6. The sternum is cut at anterior side. Jaw is exposed from upper side.
7. Now respiratory organs are studied.

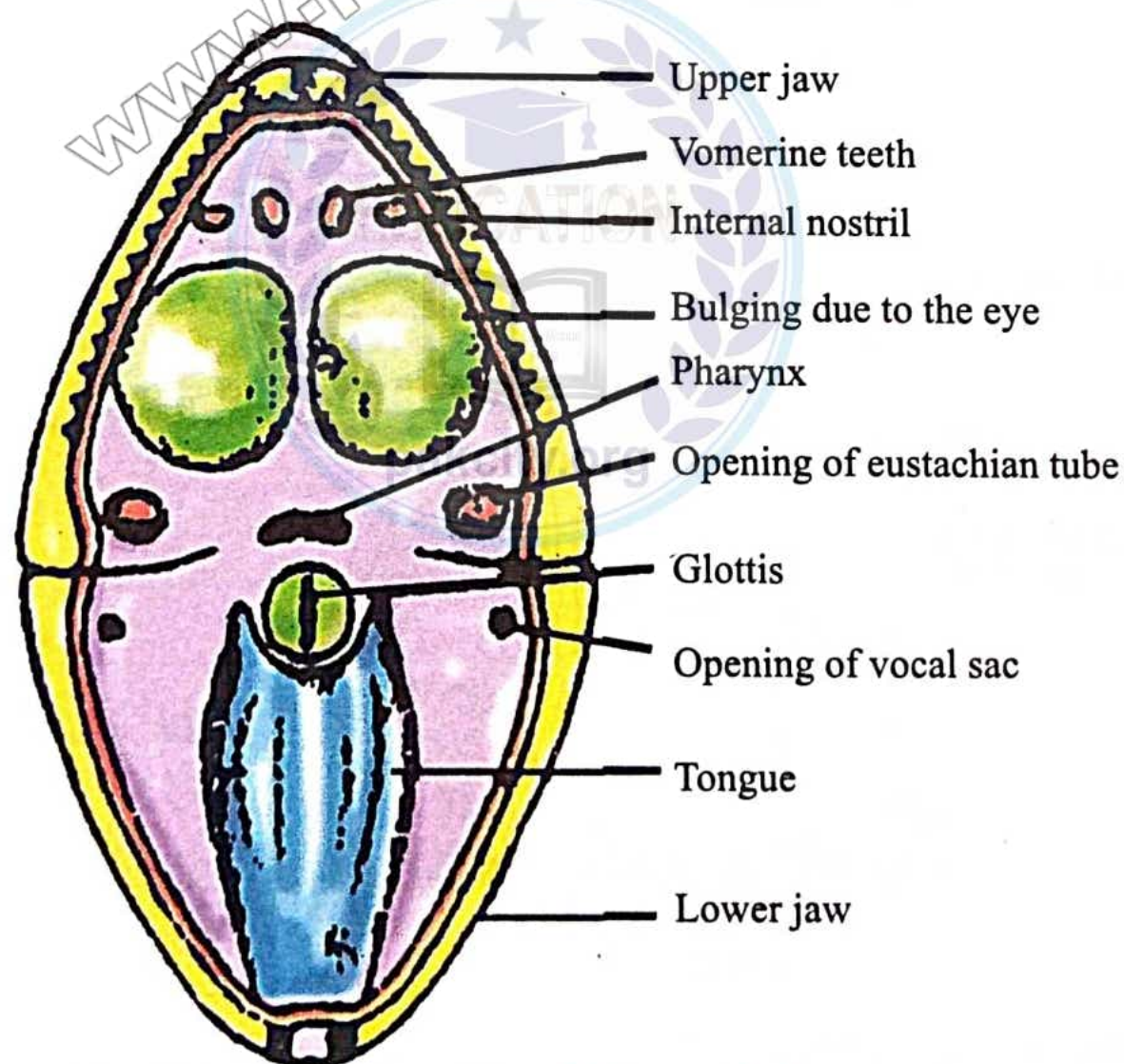


Fig. The buccal cavity of the male frog.

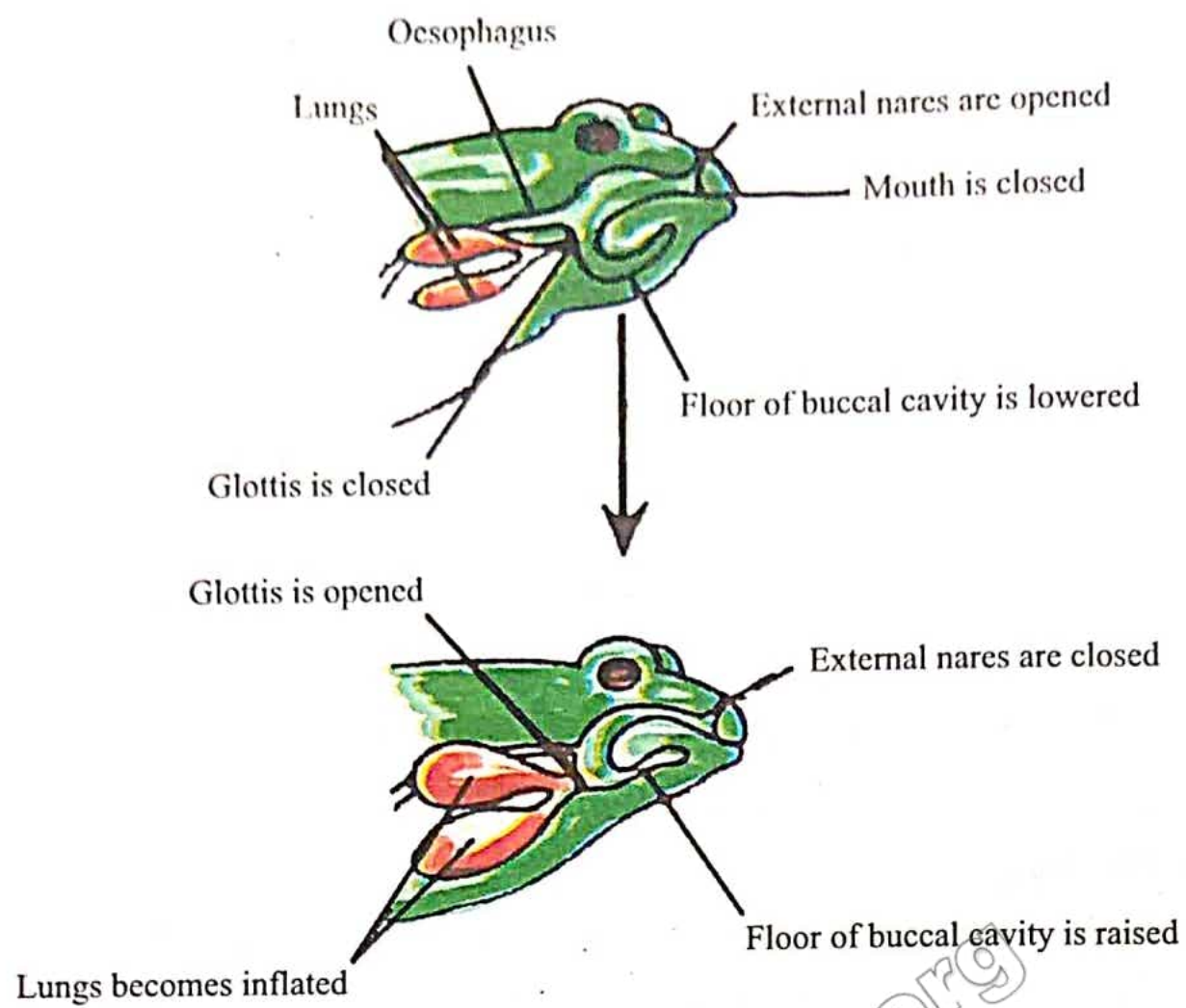


Fig. The two stages of Frog's breathing

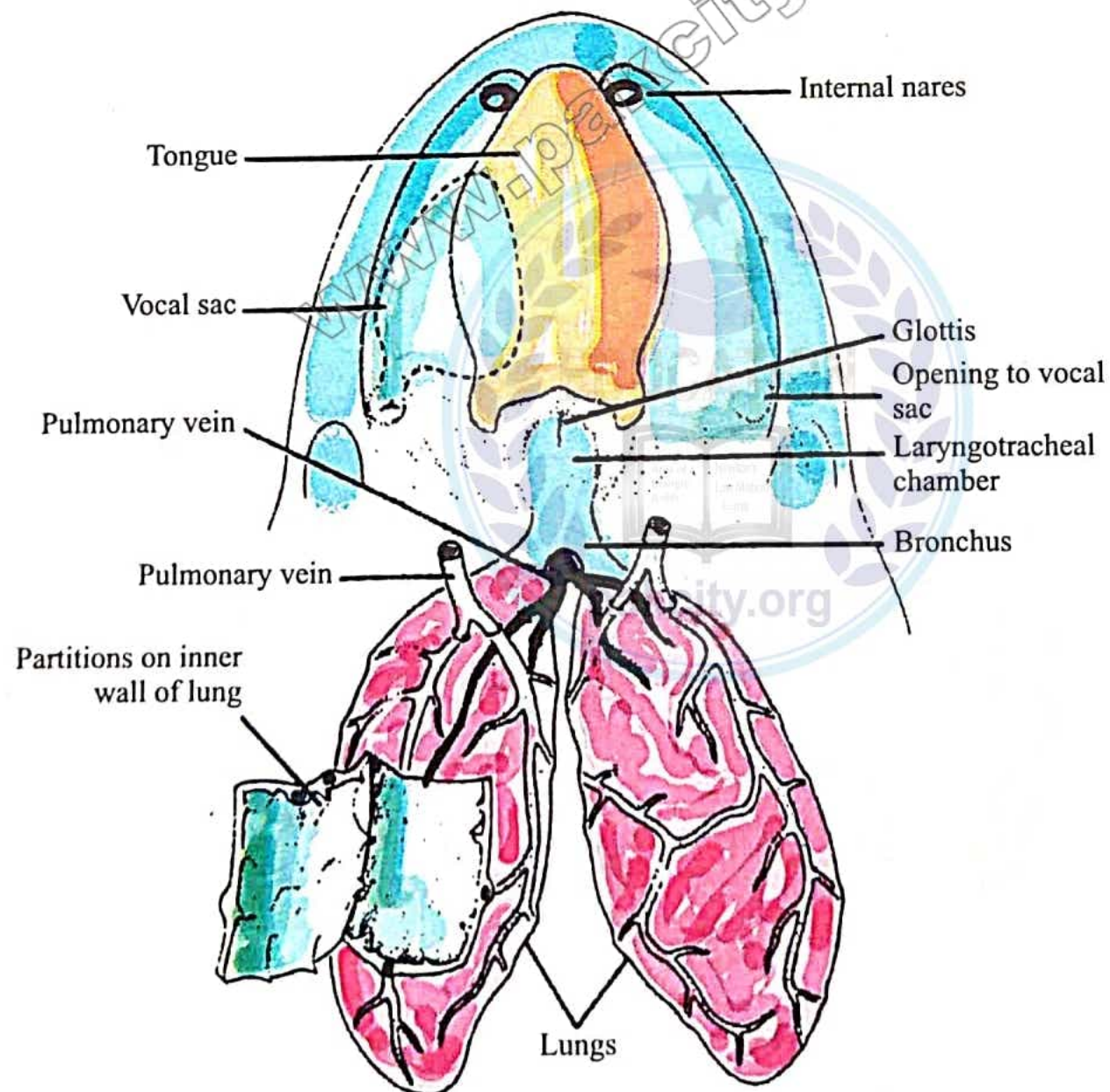
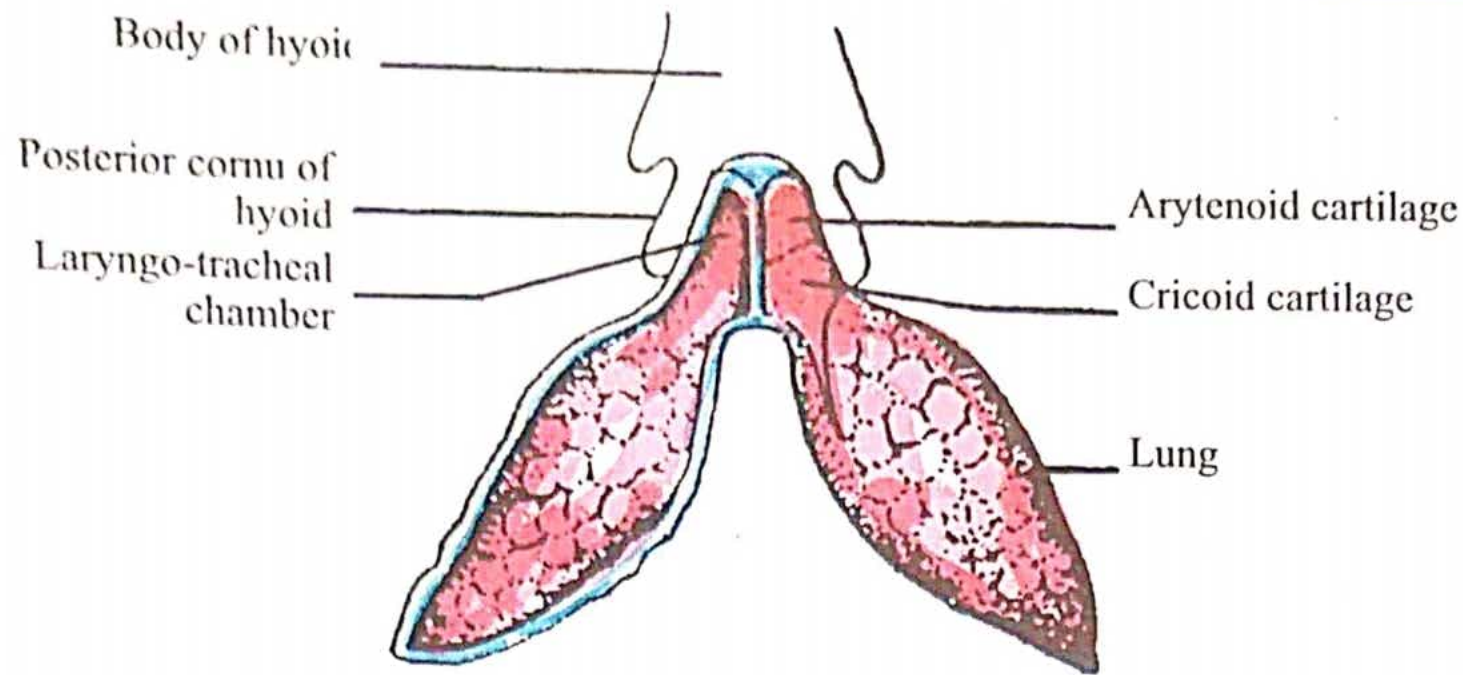


Fig. Dorsal view of floor of mouth and respiratory system of frog



Observation:

Respiration in frog takes place through lungs, skin and buccal chambers. These organs are richly supplied with blood vessels.

1. **Cutaneous Respiration:**

The gaseous exchange through skin is called cutaneous respiration.

2. **Buccal Respiration:**

The gaseous exchange in the buccal cavity is called buccal respiration.

3. **Pulmonary Respiration:**

The gaseous exchange through the lung is known as pulmonary respiration. The lungs of the frog are simple. When they are fully expanded, they look sacs like balloons. There are thin walled chambers in the inner surface of the lungs. These chambers increase the surface area. The wall of these air chambers are richly supplied with capillaries. These blood containing areas of the lungs are the main sites of the gaseous exchange. The process of respiration is divided into two parts: inspiration and expiration.

VIVA VOCE

Q.1 What are the different ways of respiration in frog?

Ans. The different ways of respiration in frog are: (1) Cutaneous respiration through the moist skin. (2) Buccal respiration through the lining of the buccal cavity. (3) Pulmonary respiration through the lungs.

Q.2 What is the snout?

Ans. The prolonged part of the face including the mouth and the nose is called snout.

Q.3 To which organs of the higher animals does the laryngo-tracheal chamber of the frog correspond to?

Ans. The laryngo-tracheal chamber of frog corresponds to the larynx (sound box) as well as the trachea (wind pipe) of the higher animals.

Q.4 Frog lacks a long trachea unlike other animals. Why?

Ans. The frog lacks a long trachea due to the absence of neck.

- Q.5** Name the cartilages which support the walls of the laryngo-tracheal chamber of frog.
- Ans.** These are a pair of flat, crescentic **arytenoid** cartilages and an irregular ring-like cartilage, the **cricoid**.
- Q.6** What are **alveoli** in the lungs of frog?
- Ans.** The inner surface of each lung of frog is highly folded to form a large number of small air sacs called as *alveoli*.
- Q.7** What is the space between the external and internal nares (nostrils) of frog known as? What is its function?
- Ans.** The space is known as the olfactory chamber. It is the sense organ of smell.
- Q.8** Why are the respiratory organs of vertebrates richly supplied with blood capillaries?
- Ans.** It is such so that the oxygen may enter the blood which by its circulation might carry it to the tissues.
- Q.9** What are the elastic, membranous folds, which produce voice in frog, known as?
- Ans.** These are known as the *vocal cords*.
- Q.10** What is the **glottis** in frog?
- Ans.** Glottis is a slit-like opening in the floor of the buccal cavity near the pharynx of frog by which the laryngo-tracheal chamber communicates with the buccal cavity.
- Q.11** Name the two phases of respiration in animals.
- Ans.** These are inspiration (breathing in) and expiration (breathing out).
- Q.12** What characteristics of the skin of frog make it suitable for respiration?
- Ans.** The skin of frog is thin, moist and richly supplied with blood capillaries for an easy exchange of respiratory gases.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Dissect the animal provided (frog). Exposed its respiratory system and show it to examiner and draw its labelled diagram. Dissection = 1, Demonstration = 1, labelled diagram = 1 + 1, Short questions = 1/2 + 1/2, Total = 5.

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(Bahawalpur Board 2004)

- Q.1** What is the difference between lung of frog and lung of man?
- Ans.** The lung of frog is balloon like. It is without alveoli. The lung man is compact and it has millions of alveoli.
- Q.2** What is buccal respiration?
- Ans.** The gaseous exchange in the buccal cavity is called buccal respiration.

(11) Transportation (1) In Plants



26

Experiment**DEMONSTRATION OF OSMOSIS IN LIVING PLANT CELLS**

The shrinkage of protoplasm is caused by exosmosis of water because water passes out of vacuole by osmosis. The solution outside the cell is more concentrated than the cell sap. This phenomenon is called 'Plasmolysis' and the cell in this condition is said to be plasmolysed. The reversal of the above phenomenon is called 'Deplasmolysis'.

Deplasmolysis:

1. If the dissolved salts and sugars in the cell sap are replaced by water, giving it a low osmotic potential (To the above slide add water).
2. Water enters by osmosis passing the permeable cell wall.
3. Cell sap volume increases and pushed outwards on the cell wall making the cell turgid. (Returned to its normal state.)

Material:

Microscope, Slide, Cover slip, Scalpel, Forceps, Onion bulb, Distilled water, 1M sucrose solution.

Procedure:**Plasmolysis:**

1. An onion bulb is cut into two pieces.
2. A scale from the onion is removed.
3. Now the scale is folded inward and bent, it tears up the epidermis.
4. The torn epidermis is peeled off from the surface of scale. It separates a piece of epidermis from the surface of scale.
5. A slide is taken. A drop of water is placed on it. The small piece of epidermis is spread over this slide. It is observed under microscope. Diagram of some epidermal cells is drawn.
6. Another strip of onion epidermis is prepared in a similar way. It is placed on 1M sucrose solution on slide. It is observed under microscope and its diagram is drawn. Some cells shown plasmolysis.

Deplasmolysis:

Now the cover slip of plasmolysed epidermis is lifted. Distilled water is poured in it. Cover slip is again fixed and it is observed under microscope.

Observation:

No.	Medium on slide	Observation	Conclusion
1.	Distal water	Normal shape	No osmosis
2.	0.1M sucrose solution	Shrinkage	Plasmolysis take place
3.	Distal water on slide 2	Normal shape	Deplasmolysis takes place

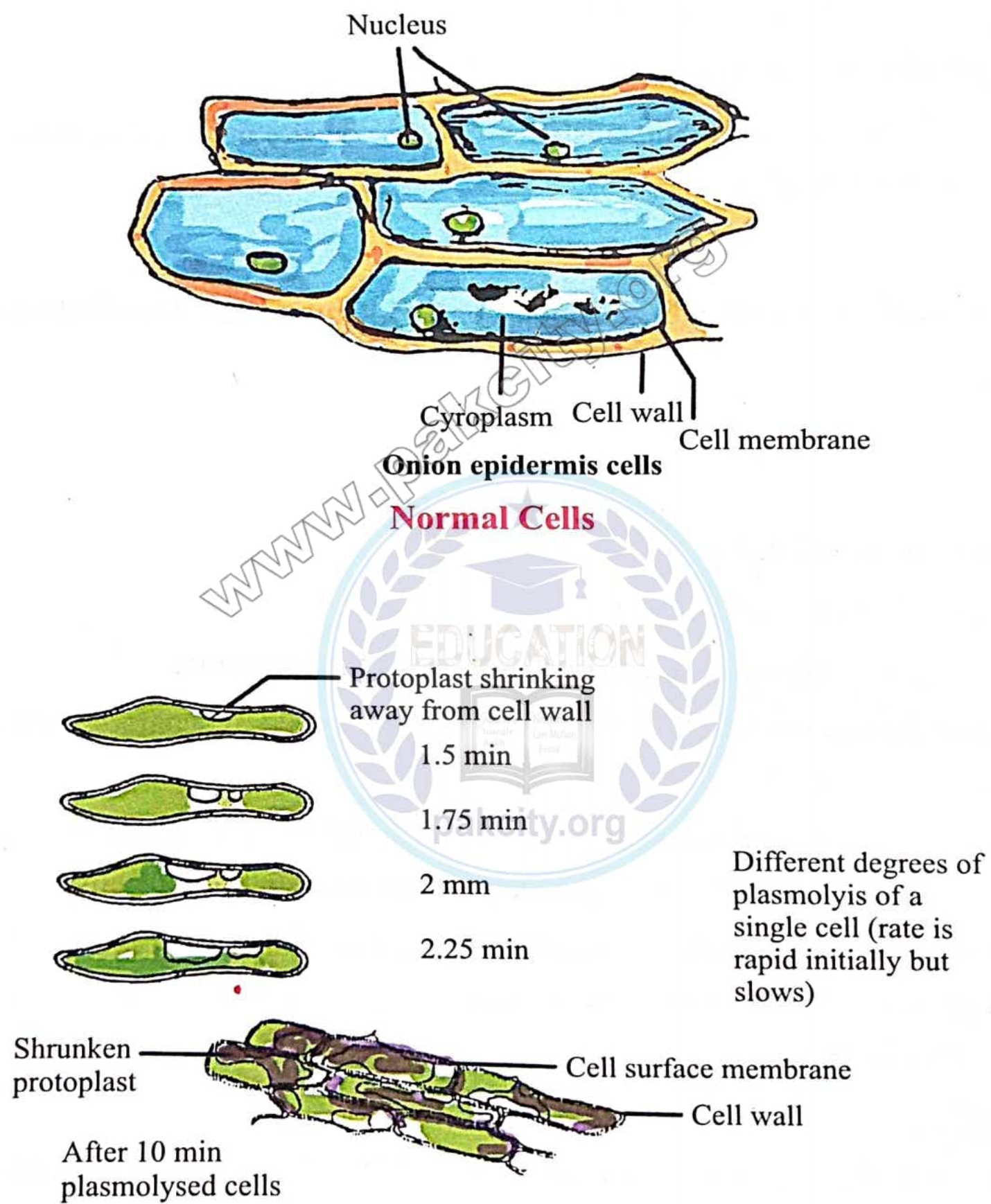
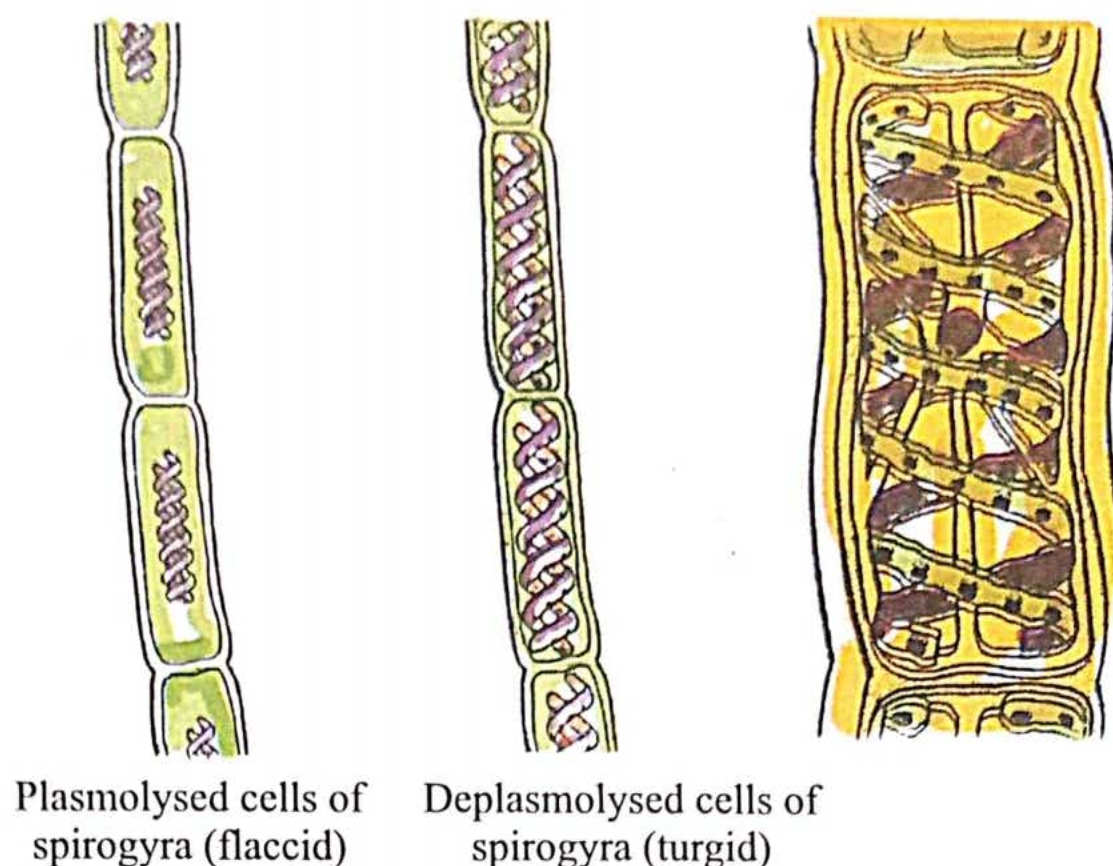


Fig. Appearance of onion epidermal cells during plasmolysis

(b) Plasmolysis and Deplasmolysis in Spirogyra:**Procedure:**

1. Take some fresh filaments of spirogyra and keep them in a clean glass slide in a drop of water.
2. Examine it under microscope.
3. You will observe that cells are fully distended or turgid.
4. To this material, add a drop of 5% solution of sugar or common salt.
5. Again, observe it under microscope.

You will notice that:



Demonstration of osmosis cells of spirogyra

Observations:

The vacuole shrink, pulling the cytoplasm away from the cell wall, leaving the cell flaccid.

VIVA VOCE

Q.1 Define osmosis.

Ans. Osmosis is defined as the movement of water molecules from a region of higher water potential to a region of lower water potential through a partially permeable membrane.

Q.2 What is plasmolysis?

Ans. The shrinkage of protoplasm is called plasmolysis.

Q.3 What is deplasmolysis?

Ans. The return of plasmolysed cell to its original position is called deplasmolysis.

Q.4 What is tonoplast?

Ans. The membrane of vacuole is called tonoplast.

Q.5 What is the significance of osmosis in plants?

Ans. It is responsible for transportation of materials through vascular tissues (xylem and phloem) and cell turgor.

Q.6 What is partially or differentially or semi-permeable membrane? Also give some example.

Ans. A membrane that allows some kinds of molecules to pass through it and not others is called partially or differentially or semi-permeable membrane. Cell membrane of living cells, egg shell membrane etc.

Q.7 Differentiate between endosmosis and exosmosis.

Ans. If water moves by osmosis into a cell the process is called endosmosis while if water moves out of the cell by osmosis, the process is called exosmosis.

27

Experiment**DETERMINATION OF MEAN SOLUTE POTENTIAL OF THE CELL SAP USING METHOD OF INCIPIENT PLASMOLYSIS****Materials:**

Microscope, 3 glass slides, distilled water, onion bulb, forceps, solutions of sucrose of different concentration (0.3 M, 0.4 M and 0.5 M).

Procedure:

Performed the following steps:

- (i) A fleshy scale from an onion bulb was removed and three pieces of the inner epidermis were cut.
- (ii) Each piece of epidermis was taken with forceps and put on different petridishes.
- (iii) Poured about 20 ml of 0.3 M, 0.4 M and 0.5 M sucrose solutions in different petridishes.
- (iv) Leave these petridishes for 20 minutes.
- (v) After 20 minutes each piece of epidermis was removed from the solution and put on separate slide, with a drop of same solution on it.
- (vi) After 20 minutes each piece of epidermis was removed from the solution and put on separate slide, with even a slight pulling of cytoplasm from cell wall was counted as plasmolysed.
- (vii) Counted the number of plasmolysed cells on each slide.

Observation:

It was observed that 50% of the cells of the tissue kept in 0.4 M solution were plasmolysed (a condition known as incipient plasmolysis).

Material:

6 Petri dishes, 6 test tubes, onion bulb, pipettes, beaker, brush, distilled water, slide, cover slips, microscope, razor, graph paper, distilled water, 1.0 M sucrose solution.

Procedure:

1. 0.30M, 0.35M, 0.40M, 0.45M, 0.50M, 0.60M solution are prepared by following ratios of distilled water and volume of sucrose solutions.

Concentration of sucrose solution	Volume of distilled water/cm ³	Volume of 1M sucrose solution
0.30M	14	6
0.35M	13	7
0.40M	12	8
0.45M	11	9
0.50M	10	10
0.60M	8	12

- Six Petri dishes are taken. The six solutions are put in each dish. The concentration of dish is marked on it by maker.
- A scale of bulb is taken. Its epidermis is removed and cut into six pieces. These six pieces are transferred in to above six Petri dishes for 20-30 minutes.
- The piece of epidermal tissue is removed from first (0.30M) Petri dish. It is placed on slide. Cover slip is put on it. It is examined under microscope. 100 cells are counted in a small area. Numbers of plasmolysed cell are noted out of these 100 cells. This process is repeated for all the remaining five Petri dishes.

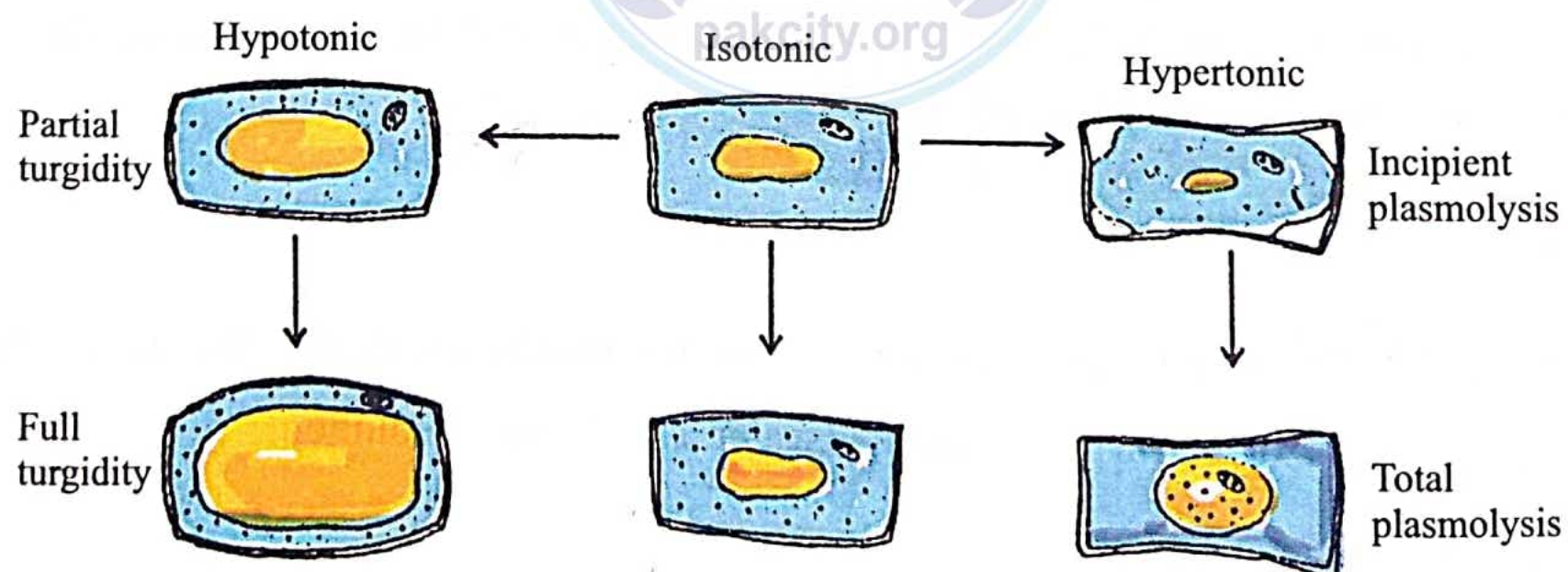
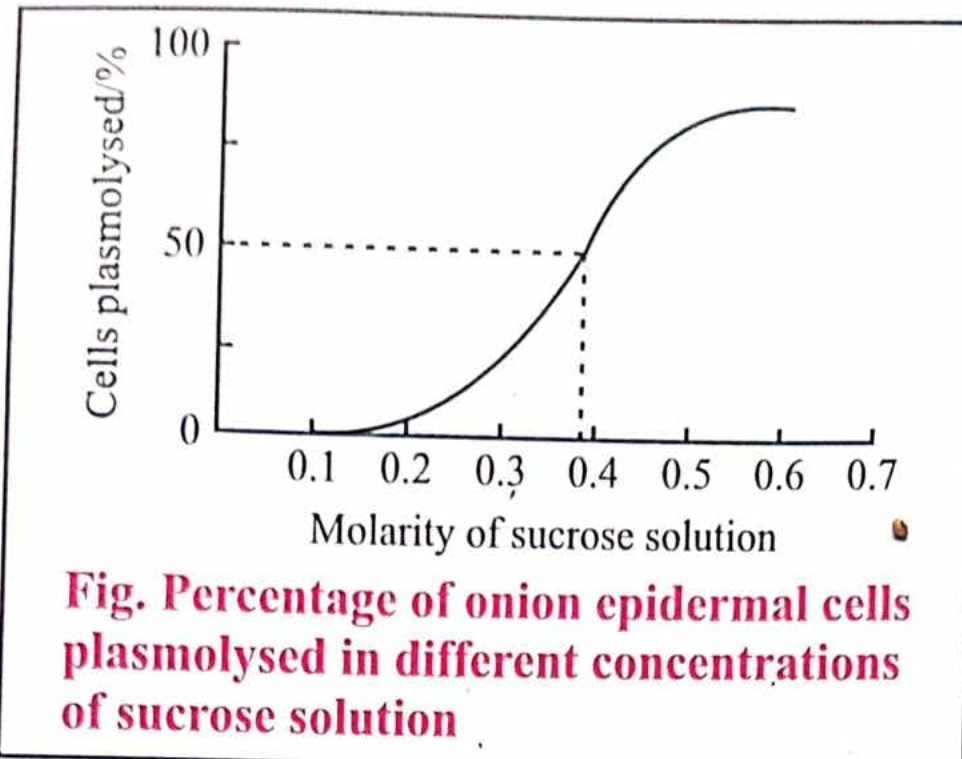


Fig. Cells showing turgidity, incipient plasmolysis and total plasmolysis



SOLUTION POTENTIALS OF GIVEN SUCROSE SOLUTIONS AT 20 °C

Concentration of sucrose solution (molarity)	Solute potential/kPa	Solute potential/atm
0.05	-130	-1.3
0.10	-260	-2.6
0.15	-410	-4.0
0.20	-540	-5.3
0.25	-680	-6.7
0.30	-820	-8.6
0.35	-970	-9.6
0.40	-1120	-11.1
0.45	-1280	-12.6
0.50	-1450	-14.3
0.55	-1620	-16.0
0.60	-1800	-17.8
0.65	-1980	-19.5
0.70	-2180	-21.5
0.75	-2370	-23.3
0.80	-2580	-25.5
0.85	-2790	-27.5
0.90	-3010	-29.7
0.95	-3250	-32.1
1.00	-3510	-34.6
1.50	-6670	-65.8
2.00	-11810	-116.6

VIVA VOCE

Q.1 What is plasmolysis?

Ans. The shrinkage of protoplasm is called plasmolysis.

Q.2 What is incipient plasmolysis?

Ans. When water comes out from the cell sap into the outer solution (exosmosis), the protoplasm begins to contract from the cell wall. This is called incipient plasmolysis.

Q.3 When is deplasmolysis?

Ans. When the plasmolysed tissue is placed in water, the water enters into the cell sap (endosmosis), the cells become turgid and the protoplasm again assumes its normal shape and position. This phenomenon is called deplasmolysis.

Q.4 Why do we use Rhoecol leaves?

Ans. Because the cell contains coloured protoplast.

Q.5 Do all cells of a tissue have similar solute potential?

Ans. No, the solute potential varies between cells in the same tissue.



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Experiment

TO INVESTIGATE AND MEASURE FACTORS AFFECTING THE RATE OF TRANSPIRATION USING POTOMETER

The loss of water from the surface of plant especially stomata is called transpiration. It is measured by potometer.

Material:

Ganong's potometer, A leafy branch, Glass rod, Vaseline, Beaker, Rubber tube, Stand.

Procedure:

1. Ganong's potometer is fixed horizontally on stand. It is filled with water. Its bend tube is dipped in a beaker of water.
2. A branch of a plant is cut under water.
3. The leafy shoot is fixed in the side arm of potometer with a cork. Vaseline is applied on loose part of potometer. It makes the potometer airtight.
4. The tap of the reservoir is open for a moment. It produces air bubble in the horizontal tube of the potometer. This air bubble is brought near the bend of tube. Then tap of potometer is closed. It is also made airtight. The reading of air bubbles is noted.
5. The apparatus is placed in air to sunlight some time. After sometime, the air bubble starts moving. Its new reading is noted. The time taken by bubble to cover this distance is also noted. Different readings are taken after different time intervals.

Effect of Different Factors on Rate of Transpiration:

The effect of different factors on transpiration can be measured by following way:

1. **Wind:** Electric fan is used.
2. **Humidity:** The shoot is enclosed in a plastic bag.
3. **Darkness:** Shoot is enclosed in a black polythene bag.
4. **Vaseline:** Vaseline is applied on the lower surface of leaf.
5. **Temperature:** Apparatus is placed near room heater.
6. Removing half of leaves.

Observation:

No. of reading	Factor applied	Time in hours	Distance covered in cm	Rate of transpiration
1.	Wind	1 hour	22 cm	22 cm/h
2.	High temperature	1 hour	20 cm	20 cm/h
3.	Humidity	1 hour	8 cm	8 cm/h
4.	Darkness	1 hour	4 cm	4 cm/h

Objectives:

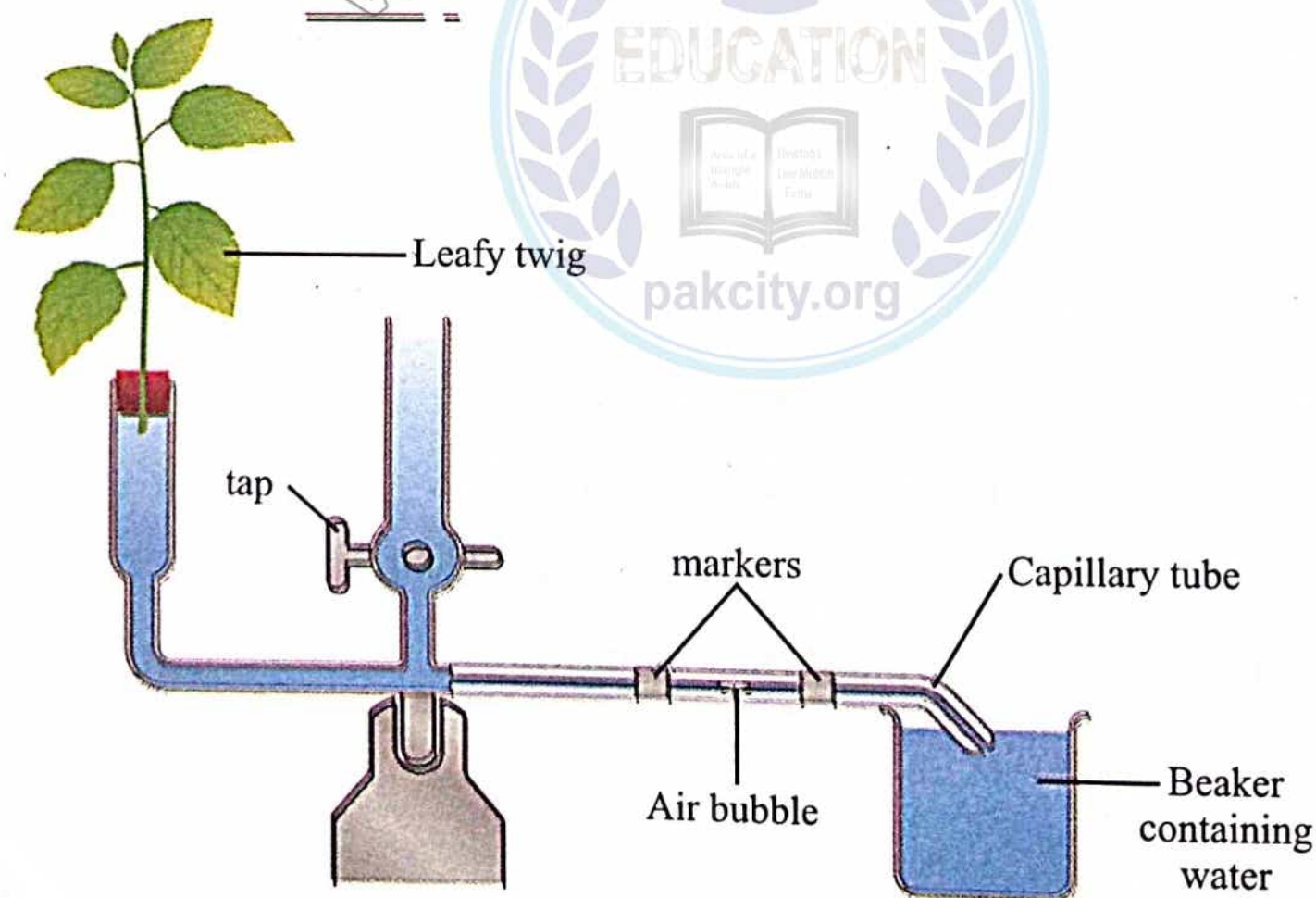
1. Demonstration of transpiration in plants.
2. Measurement of rate of transpiration in plants.
3. Study of factors affecting the rate of transpiration.

Results (Observations):

1. The air bubble moves forwards gradually and slowly in the graduated capillary tube towards the leafy shoot.
2. The air bubble does not move when the stomata are blocked by applying vaseline to the lower epidermis.

Conclusions:

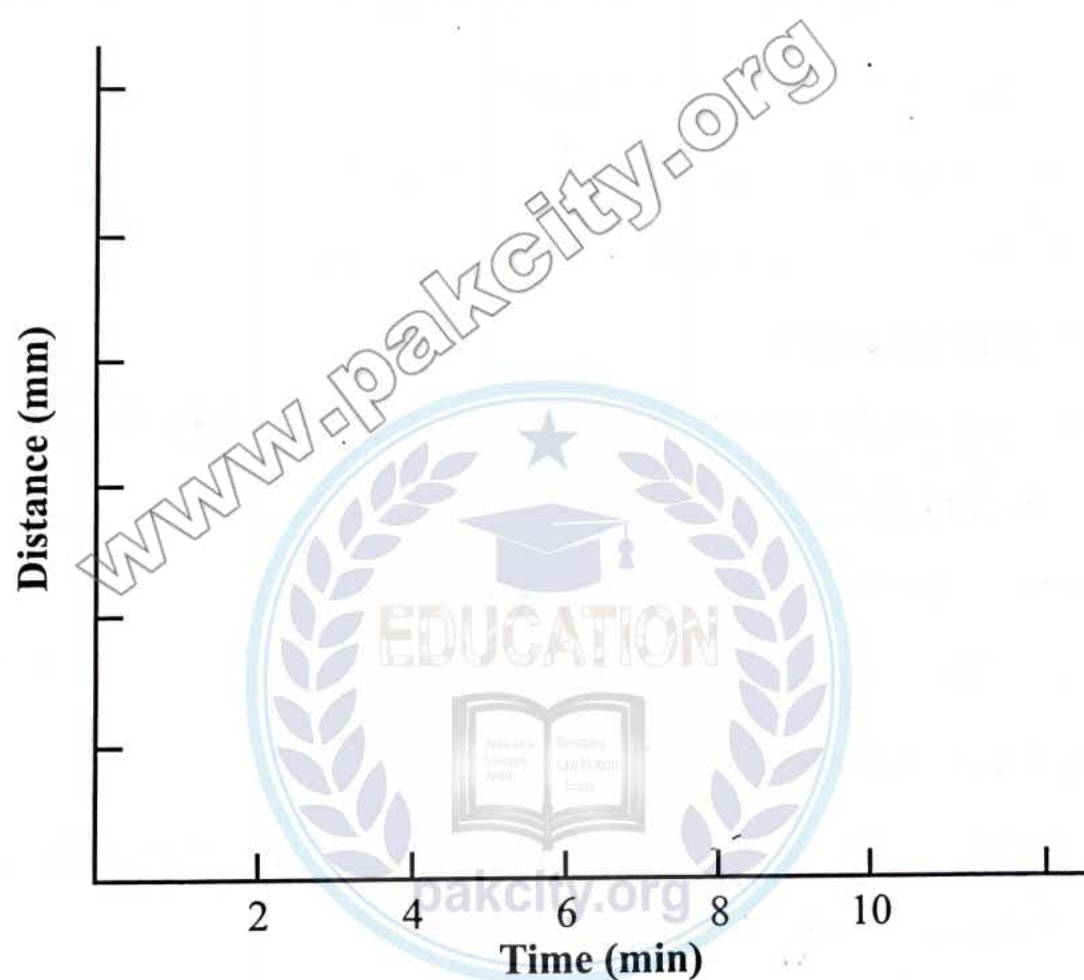
1. The plant loses water by transpiration.
2. The rate of transpiration is variable under different conditions.

**Fig. Ganong's potometer**

Note your own readings and complete this table:

Transpiration Data

Experimental conditions	Time (minutes)									
	1	2	3	4	5	6	7	8	9	10
	Distance (mm)									
Control										
Wind										
Humidity										
Darkness										
Removing half of leaves										
Applying vaseline on leaves										



Comparison of transpiration rates under various environmental conditions

Observation:

Rate of transpiration is determined by the following formula.

Distance traveled by air bubble in horizontal tube = X

Time taken to travel = Y

Rate of transpiration = $\frac{X}{Y}$ per minute

Conclusion:

You have seen that under different conditions, the rate of transpiration is variable, under wind condition, the plant loses more water by evaporation, so the rate of transpiration increase. And if we remove leaves, the total number of stomata of the shoot are reduced and this decreases the rate of transpiration. Similarly, if the lower epidermis of the leaf is covered with vaseline, then stomata on the lower surface are closed by vaseline and there is no transpiration.

VIVA VOCE

Q.1 What is potometer?

Ans. The apparatus used to measure the rate of water uptake by a shoot is called potometer.

Q.2 What is transpiration?

Ans. The loss of water from the surface of plant especially stomata is called transpiration. It is measured by potometer.

Q.3 Why is vaseline applied on loose part of potometer?

Ans. Vaseline is used make the potometer airtight.

Q.4 Why is it necessary to make the potometer airtight?

Ans. Potometer cannot retain water in it if is not made airtight.

Q.5 Why is shoot cut underwater?

Ans. If the shoot is not cut under water, air can enter into xylem tissues. Thus it will become difficult to obtain exact reading.

Q.6 What does happen with transpiration in wind?

Ans. Transpiration increases in wind.

Q.7 Why does transpiration reduce in darkness?

Ans. In darkness, the stomatal are closed. Thus rate of transpiration is reduced.

Q.8 What is the equipment called potometer used for?

Ans. The potometer is used for measuring the rate of transpiration of a living plant. It is also used for comparing the rates of transpiration of different plants and for studying the effect of different environmental conditions on the rate of transpiration.

Q.9 Differentiate between evaporation and transpiration.

Ans. Evaporation is the loss of water vapours from the surface of a non-living thing whereas transpiration is the loss of water vapours from the surface of a living plant.

Q.10 What does surface of a plant mean?

Ans. The surface of a plant means the leaves.

Q.11 What are the different types of transpiration? How much of the total transpiration occurs through them?

Ans. The transpiration is of three types viz. stomatal transpiration, cuticular transpiration and lenticular transpiration. Of the total transpiration 90% occurs by the first, 9% by the second and 1% by the third.

Q.12 When is the transpiration more active, in sunlight or in darkness and why?

Ans. The stomata open in the sunlight making the transpiration more active than the darkness during which stomata are closed.

Q.13 Why does rate of transpiration decrease in humidity?

Ans. In humid condition, air is already saturated with water. Therefore, it cannot absorb more water. Hence rate of transpiration is reduced.

Q.14 What is affect of temperature on transpiration?

Ans. Transpiration increases with the increase of temperature.

Q.15 What is cell sap?

Ans. Water plus dissolved salt and sugar present in large vacuole of the plants cells is called sap.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Measure the rate of transpiration under different factor with the help of potometer.

Material = 1, Procedure and diagram = 2, Observation and result = 1, Short question = 1/2 + 1/2, Total = 5.

(Gujranwala Board 2004)

Q.1 What is the role of stomata in transpiration?

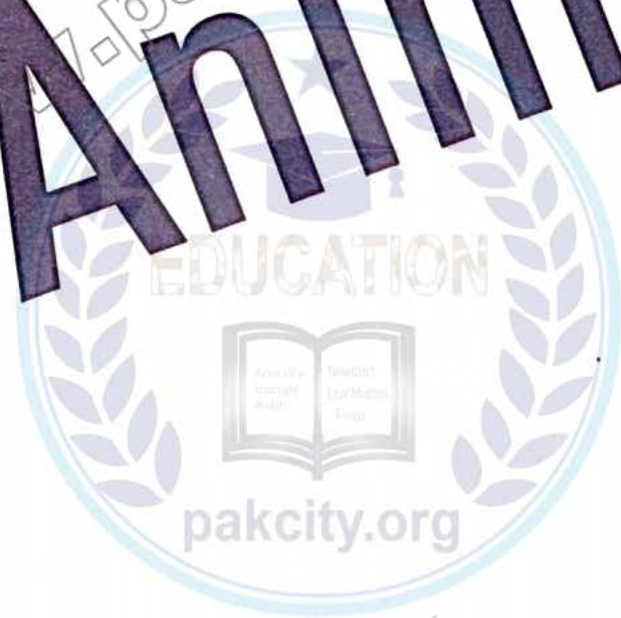
Ans. 90 percent transpiration takes place through stomata.

Q.2 What are the effects of temperature and humidity on transpiration?

Ans. Transpiration increases with the increase in temperature but decreases with the decrease in humidity.

(iii)

In Animals



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Experiment

STUDY OF PREPARED STAINED SLIDE OF HUMAN BLOOD INCLUDING IDENTIFICATION OF PHAGOCYTES AND PREPARATION OF SLIDES OF BLOOD SMEAR OF FROG

Material:

Prepared slides of human blood, Fresh blood of frog, Microscope, Glass slide cover slip, Ringer's solution, Methylene blue.





Procedure:






1. A drop of blood of frog is put on slide.
2. A drop of Ringer's solution and a drop of methylene blue are mixed with this blood.
3. It is mixed with the help of tooth pick and smear is formed.
4. A cover slip is put on it. It is observed under microscope.
5. Prepared slide of human is also observed under microscope.

Observation:

Following blood cells are observed in the smear of blood and prepared slide of blood:

Different cell types comparing their characteristics and functions

Cell type	Description	Average number present	Major function
Red blood cell (erythrocyte)	 Biconcave disc without nucleus, Approximately 8 μm in diameter	5,00,000 per mm^3	Transports oxygen and a small amount of carbon dioxide.
White-blood cell (leucocytes)		7500 per mm^3	
(a) Granulocytes			
1. Neutrophil	 About twice the size of red cells, nucleus two to five lobed	62% of white cells	Destroys small particles by phagocytosis
2. Eosinophil	 About twice the size of red cells, nucleus bilobed	2% of white cells	Inactivates inflammation-producing substances; attacks parasites

3. Basophil		About twice the size of red cells nucleus bilobed	Less than 1% of white cells	Releases heparin to prevent blood clots and histamine, which causes inflammation
(b) Agranulocytes		Two to three times larger than red cells, nuclear shape from round to lobed	3% of white cells	Gives rise to macrophage, which destroys larger particle by phagocytosis
4. Monocyte		Slightly larger than red cell, nucleus nearly fills cell	32% of white cells	Functions in the immune response by producing anti bodies
5. Lymphocyte		Membrane bounded cytoplasmic fragment of cells in bone marrow called megakaryocytes	250,000 per mm ³	Involved in blood clotting
Platelets				

PART-B

PREPARATION OF SLIDE OF BLOOD SMEAR OF FROG

Materials:

A living frog; A 5 ml syringe; An anticoagulant i.e. Disodium salt of ethylene diamino tetra acetic acid (EDTA) or 10% Sodium citrate or 1% Potassium oxalate; Microscope; Glass slides; Spirit lamp; Absolute alcohol; Chloroform; Distilled water; Alcoholic methylene blue solution; Filter paper.

Procedure:

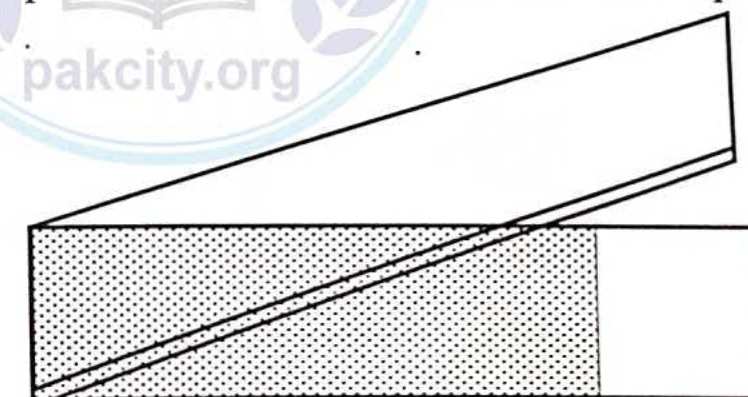
- Put about 0.5 gm of any of the above anticoagulant in a 5 ml blood taken directly from the vein of an anaesthetized frog.
- Mix thoroughly.
- Place a drop or two of the blood from the syringe at one end of a clean slide.
- Hold another slide in contact with the blood at an angle of about 45°. Draw the second slide over the first to produce a film (smear) of even thickness.
- Allow the smear to dry in the air or by gentle heat well above the flame of a spirit lamp.



(a) place a drop of blood on a slide



(b) the second slide is kept just in front of the drop so that it comes in contact with the drop



(c) the 2nd slide is pushed forwards smoothly

Fig. Preparation of smear

6. Fix the smear by dipping the slide in absolute alcohol.
7. Pour few drops of alcoholic methylene blue solution over the smear and leave for three or four minutes.
8. Wash off the slide under a gentle stream of water.
9. Blot dry the slide with filter paper.
10. Watch the slide under the low and high powers of the microscope.

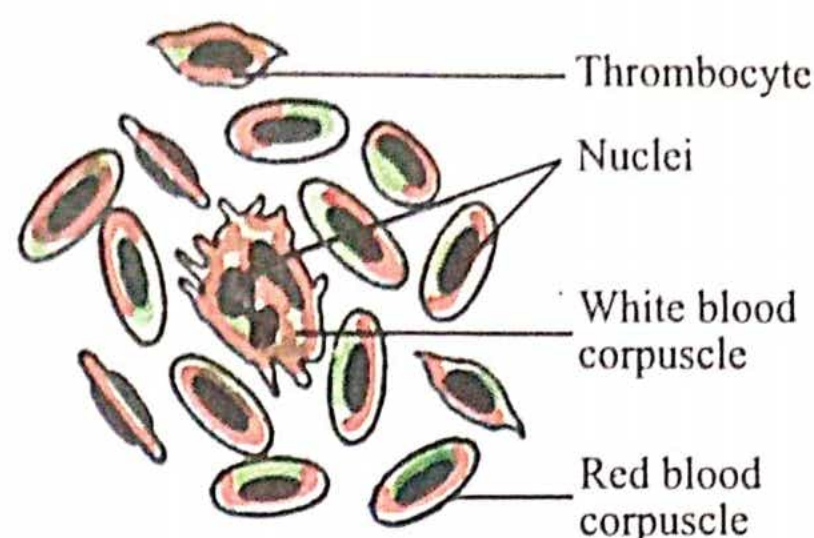


Fig. Blood of frog

Observation:

1. The erythrocytes are thin, biconcave oval disc-like cells, each having a large oval nucleus in the centre.
2. The leucocytes are smaller and much fewer in number than the erythrocytes. They are granular, nucleated and amoeboid i.e. irregular in shape.
3. Non-nucleated disc-like cells called platelets (Thrombocytes) are also present in the blood but they disintegrate as soon as the blood is exposed.

VIVA VOCE

Q.1 What is blood?

Ans. Blood is a medium composed of plasma and blood cells which dissolves nutrients, gases, hormone and wastes and transports these materials through the body.

Q.2 What is plasma?

Ans. Plasma is primarily water in which proteins, salts, nutrients and wastes are dissolved.

Q.3 Name different types of blood cells.

Ans. Red blood cells, white blood cells and platelets.

Q.4 What is the shape and function of RBC?

Ans. Biconcave disc without nucleus. Their function is transport of oxygen and a small amount of carbon dioxide.

Q.5 Name different types of white blood cells.

Ans. Neutrophils, Eosinophil, Basophil, Monocytes, Lamphocyte.

Q.6 What is the function of neutrophils?

Ans. Destroy small particle by phgocytosis.

Q.7 How can you differentiate between neutrophils and basophils?

Ans. The nucleus of Neutrophils is two to five lobed and the nucleus of basophils is bilobed.

Q.8 What is the function of platelets?

Ans. They are involved in blood clothing.

Q.9 What is an anticoagulant?

Ans. The anticoagulant is a chemical substance which prevents the clotting (coagulation) of blood.

Q.10 What does a smear mean?

Ans. It means a thin film (layer).

Q.11 Write down the composition of the blood of frog.

Ans. The blood of frog consists of a liquid part, the plasma in which are found floating three kinds of blood cells viz. the red blood cells (erythrocytes), the colourless or white blood cells (leucocytes) and the blood platelets (thrombocytes).

Q.12 Why are red blood corpuscles called so?

Ans. They are called so because they contain a red-coloured protein known as the haemoglobin.

Q.13 What is the function of the haemoglobin?

Ans. The haemoglobin serves to carry oxygen from the lungs to the tissues.

Q.14 What is the shape of red blood cells of frog?

Ans. The red blood cells of frog are biconvex, oval and disc-like.

Q.15 What is the shape of the white blood cells or leucocytes of frog?

Ans. They are irregular (amoeboid) in shape.

Q.16 How do the three kinds of blood cells of frog differ in size and number?

Ans. The erythrocytes are the largest and most numerous; the leucocytes are smaller and lesser in number; whereas the platelets are the smallest and the fewest.

Q.17 What is the function of the blood platelets?

Ans. The blood platelets help in the clotting of blood.

Q.18 What functions do the white blood cells perform?

Ans. The white blood cells protect the body from the attack of harmful bacteria either by engulfing them or by neutralizing their poisonous secretions.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Prepare the slide from given material (blood smear of frog) and prepared its labelled diagram. 1 + 1 = 2

(Multan, Bahawalpur, Lahore Boards 2004)

Experiment 2: Study the blood cells from prepared slide and prepared slide of blood of frog.

Material = 1, **Procedure and diagram** = 2, **Observation and result** = 1, **Short question** = 1/2 + 1/2, **Total** = 5.

(Rawalpindi Board 2004)

Q.1 Name the cells which are biconcave.

Ans. Red blood cells.

Q.2 Which of the cells is involved in phagocytosis?

Ans. Neutrophils.

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Experiment

STUDY OF STRUCTURE OF ARTERY, VEIN AND CAPILLARY FROM T.S OF THEIR PREPARED SLIDES

Materials:

Microscope prepared slides of artery, Vein and capillary and microscope.

Procedure:

Prepared slide of T.S of artery vein and capillary is studied under microscope.



Observation:

	Artes	Capillaries	Veins
Function	Carry blood away from the heart at high pressure	- Supply all cells with their requirements - Take away waste products	Return blood to the heart at low pressure
Structure of wall	- Narrow - Varies with heartbeat (increase as a pulse of blood passes through)	- Very narrow - Just wide enough for a red blood cell to pass through	Wide
Valves	(-)	(-)	(+) Prevent backflow
How structure fits function	- Strength and elasticity needed to withstand the pulsing of the blood, prevent bursting and maintain pressure wave - Helps to maintain high blood pressure, preventing blood flowing backwards	- No need for strong walls, as most of the blood pressure has been lost - Thin walls and narrow lumen bring blood into close contact with body tissue, allowing diffusion of materials between capillary and surrounding tissues. - White blood cells can squeeze between cells of the wall	- No need for strong walls, as most of the blood pressure has been lost - Wide lumen offers less resistance to blood flow

BLOOD VESSELS

- 3 major types
Arteries
Capillaries
Veins
- 2 sub-types
Arteries
Venues
- 60,000 miles or
100,000km of
blood vessels

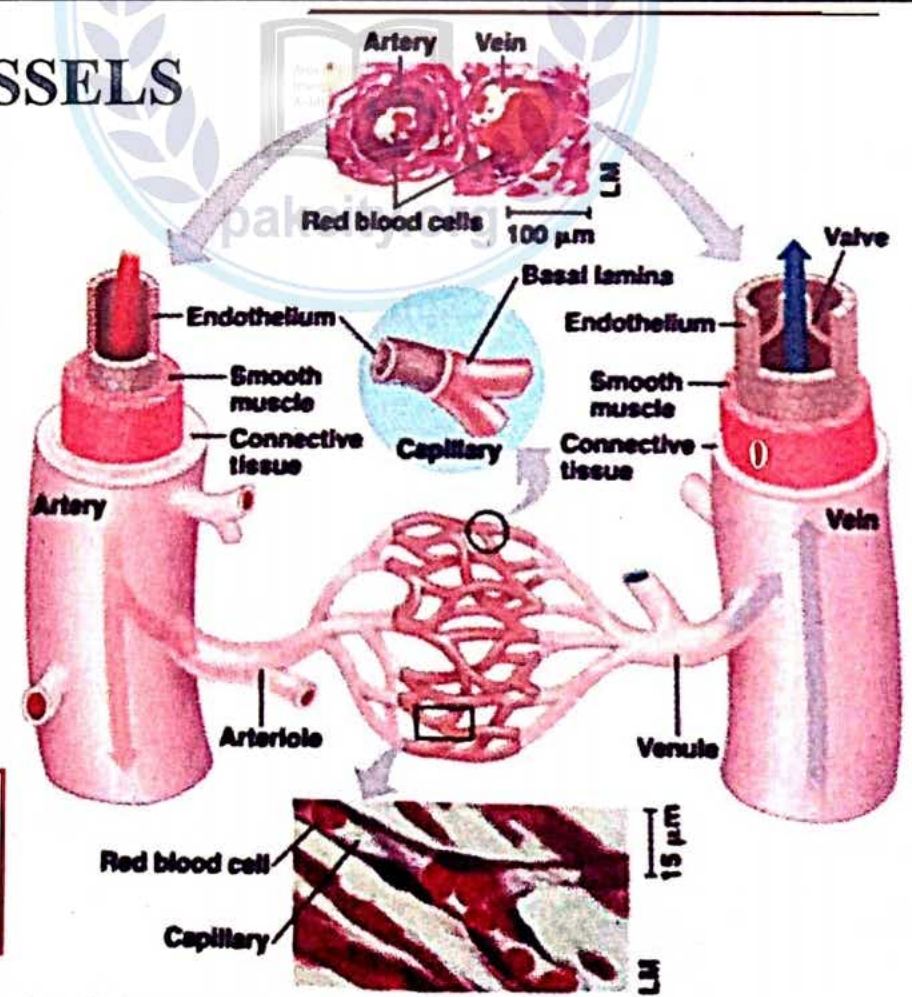


Fig. Human Blood Vessels

VIVA VOCE

- Q.1** Where is blood pressure highest or lowest, in arteries or veins?
Ans. The highest blood pressure is in arteries and the lowest in veins.
- Q.2** Which blood, oxygenated or deoxygenated is present in arteries.
Ans. Arteries contain oxygenated blood except pulmonary artery. Veins contain deoxygenated blood except pulmonary veins.
- Q.3** In which blood vessels semilunar valves are present.
Ans. Semilunar valves are present in veins.
- Q.4** In which vessels pulse is felt?
Ans. Pulse is felt only in arteries, particularly where the arteries are very close to the skin.
- Q.5** What is arteriosclerosis?
Ans. Hardening of the arteries is called arteriosclerosis.
- Q.6** Arteries are said to be more elastic. What is its significance?
Ans. This is to withstand the blood pressure and for the steady flow of blood.
- Q.7** What is the function of capillaries?
Ans. The capillaries bring about an exchange of materials (food, oxygen, CO₂ and urea) between the blood and the tissue, by the process of diffusion.
- Q.8** Name the three coats or tunics of the wall of the artery and the vein.
Ans. These are tunica interna, tunica media and tunica externa.
- Q.9** Which of the three tunics is thinner in veins than in arteries.
Ans. Tunica media is thinner in veins than in arteries.
- Q.10** Which is more elastic, artery or vein?
Ans. Artery is more elastic than the vein.
- Q.11** What is the wall of the capillary made up of?
Ans. The wall of the capillary is made up of a single layer of endothelium.
- Q.12** Name the different kinds of the blood vessels which carry the blood from the heart to the tissues.
Ans. These are the arteries, arterioles and capillaries.
- Q.13** Name, in order, different kinds of blood vessels which bring the blood from the tissues to the heart.
Ans. These are capillaries, venules and veins.
- Q.14** How many beds of capillaries are found in a tissue?
Ans. Two, one of the artery and the other of the vein.
- Q.15** What are collagen fibres?
Ans. These are the fibres which are made of protein called collagen, the main part of the fibrous connective tissue.

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Experiment

STUDY OF EFFECTS OF ACETYLCHOLINE AND ADRENALINE ON THE HEARTBEAT OF FROG

The cardiac muscles of heart are controlled by two systems **Autonomic nervous system**. Autonomic nervous system has two parts:

Sympathetic nervous system: Its nerve endings secrete adrenaline. Adrenaline increases the heart beat.

Parasympathetic nervous system: Its nerve endings release acetylcholine. Acetylcholine retards heart beat.

Material:

Pithed frog, Petri dishes, Scissor, Forceps, Adrenaline solution, Acetylcholine, Ringer's solution, Stop watch.

Procedure:

1. The pithed frog is dissected.
2. The ribs are cut and heart is exposed.
3. The pericardium is removed.
4. All the blood vessels of the heart are cut and heart is freed from the chest cavity. It is transferred into Petri dish containing Ringer.

Recording of Heart Beat:

1. **Ringer's solution:** The heart is placed in Ringer's solution. It makes the heart wet and heart beats normally. Now heart beat is noted.
2. **Adrenaline:** One part adrenaline and three parts Ringer's solution are mixed in Petri dish. The heart is transferred into this solution. Again heart beat is noted. Now heart is washed with Ringer's solution.
3. **Acetylcholine:**
One part acetylcholine and three parts Ringer's solution are mixed in Petri dish. Now heart is transferred into this solution. Heart beat is noted and heart is again washed with Ringer's solution.

Observation:

No.	Chemicals	Heart beat rate/min	Affects on heart
1.	Simple Ringer's solution	40	Normal
2.	Ringer's solution + Adrenaline	60	Fast
3.	Ringer's solution + Acetylcholine	32	Slow

Result:

The heart beat of frog increase in adrenaline but it decreases in acetylcholine.

VIVA VOCE

Q.1 What is the effect of adrenaline on heart beat?

Ans. It increases the rate of heart beat.

Q.2 Name the substance involved in the action of the sympathetic nerve endings?

Ans. It is the adrenaline.

Q.3 How does the heart respond to acetylcholine?

Ans. The heart responds to acetylcholine by decreasing its rate of beating.

Q.4 What chemical transmitter substance is secreted by the stimulation of parasympathetic nervous system?

Ans. It is acetylcholine.

Q.5 What is Ringer's solution for frog and what is its significance?

Ans. Ringer's solution (for frog) is a special preparation in which various organs of the frog can be kept alive for considerable period.

Q.6 What is autonomic nervous system?

Ans. The part of the nervous system which controls involuntary movements of the body is called autonomic nervous system.

Q.7 Which drug should be given to a case of heart depression?

Ans. Adrenaline should be given which has a stimulating effect on the heart.

Q.8 Do adrenaline and acetylcholine affect the striped (voluntary) or skeletal muscles?

Ans. The acetylcholinẽ may stimulate these muscles to contract but adrenaline does not have any effect on these muscles.

Q.9 What are “Inhibitors” and “Accelerators”?

Ans. The chemicals which slow down activities of the parts of the body of an animal are called “Inhibitors” and those which speed up activities are known as “Accelerators”.

Q.10 Suggest alternate words for inhibitors and accelerators.

Ans. These are “tranquilizers” and “stimulants” respectively.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Dissect the animal provided to expose its heart for the study of effect of drugs on heart beat rate.

Material = 1, Procedure = 2, Observation and result = 1, Short question = 1/2 + 1/2, Total = 5

(Multan Board 2004)

Q.1 What is the effect of cold water on heart beat?

Ans. Heart beat slows down.

Q.2 What is the effect of hot water on heart beat?

Ans. Heart beat increases.

Experiment 2: Study the effect of acetylcholine and adrenaline on the heart of frog.

(Multan Board 2004)

Q.1 Why heart is placed in Ringer's solution?

Ans. It keeps the heart tissue alive.

Q.2 What is function of sympathetic nerve?

Ans. It increases the heart beat.



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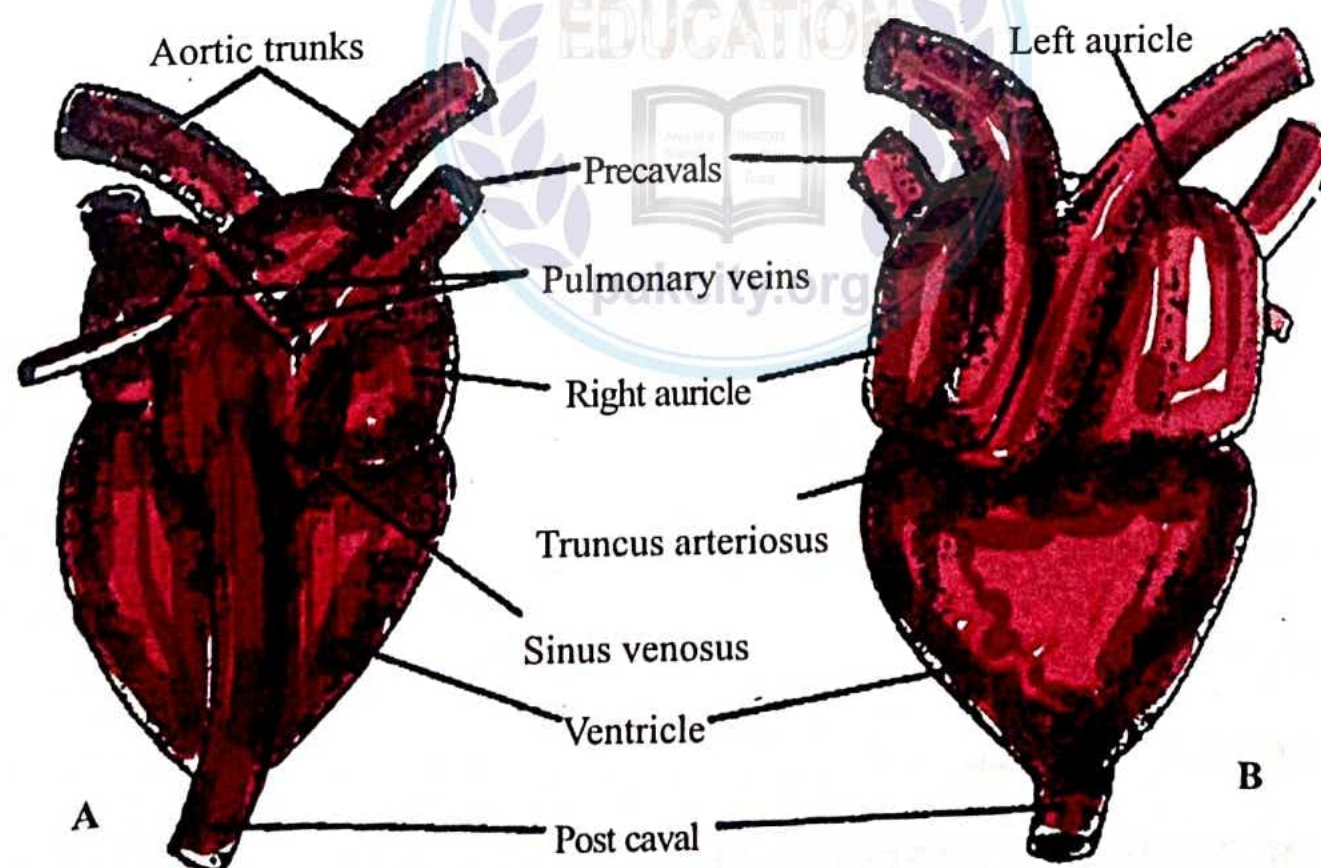
Experiment

EXPOSURE OF BLOOD CIRCULATORY SYSTEM OF FROG
(HEART AND MAIN BLOOD VESSELS)**Material:**

Pithed or chloroformed frog, Dissecting board, Dissecting box, Beaker, Hand lens, Hammer, Nail, Cotton.

Procedure:

1. A chloroformed or pithed frog is placed on board. Its ventral side is kept upward.
2. The limbs of the frog are fixed by nails.
3. The skin of the frog is lifted with the help of forceps. A cut is made in the skin in abdominal region. Then the skin is cut from abdomen to lower jaw.
4. **Anterior abdominal vein** passes through the midline of abdomen. It should not be cut. Therefore, body wall is raised and two lateral cuts are made in it. The body wall is cut from these two lateral sides.
5. The lower side of the mid section (with abdominal vein) is tied with a thread. After that, it is cut. Now bleeding does not take place from abdominal vein.
6. The sternum is cut at anterior side.
7. The outer membranes present on the heart are removed.
8. Now different arteries and veins are exposed and studied.



Heart A. Dorsal view. B. Ventral view

HEART:

The hearts of the amphibians have three chambers. They have two auricles and one ventricle. Additionally, sinus venosus and truncus arteriosus are also present.

1. **Sinus venosus:** It receives deoxygenated blood from two **superior vena cava** (precavals) and one **inferior vena cave** (postcaval).
2. **Right auricle:** The deoxygenated blood is passed from sinus venosus into right auricle.
3. **Left auricle:** The oxygenated blood from lung is transferred through pulmonary veins into left auricle.
4. **Ventricle:** There is complete mixing of oxygenated and deoxygenated bloods in the ventricle.
5. **Truncus arteriosus:** The truncus arteriosus pushes the blood into two **carotid**, two **systemic** and two **pulmocutaneous** arches. Thus arteriosus gives two branches. Each branch gives the branches. These are:

ARTERIAL SYSTEM:

1. **Carotid artery:** It supplies blood to head and brain. It further divides into internal and external carotid arteries.
2. **Pulmocutaneous artery:** It supplies blood to lung and skin.
3. **Systemic arch:** The systemic artery of each side form loop and move downward. Both these fuse to form **dorsal aorta**. Each systemic arch also give following arteries.
 - ◆ **Occipito vertebral artery:** Jaw and vertebral column.
 - ◆ **Subclavian artery:** Forelimb.
 - ◆ **Oesophageal artery:** Oesophagus.

Dorsal Aorta:

Dorsal aorta gives following arteries:

1. **Coeliaco mesenteric artery:** Digestive system.
2. **Renal arteries:** Kidney.
3. **Genital arteries:** Gonads.
4. **Posterior mesenteric artery:** Rectum.
5. **Iliac arties:** Hind limbs.

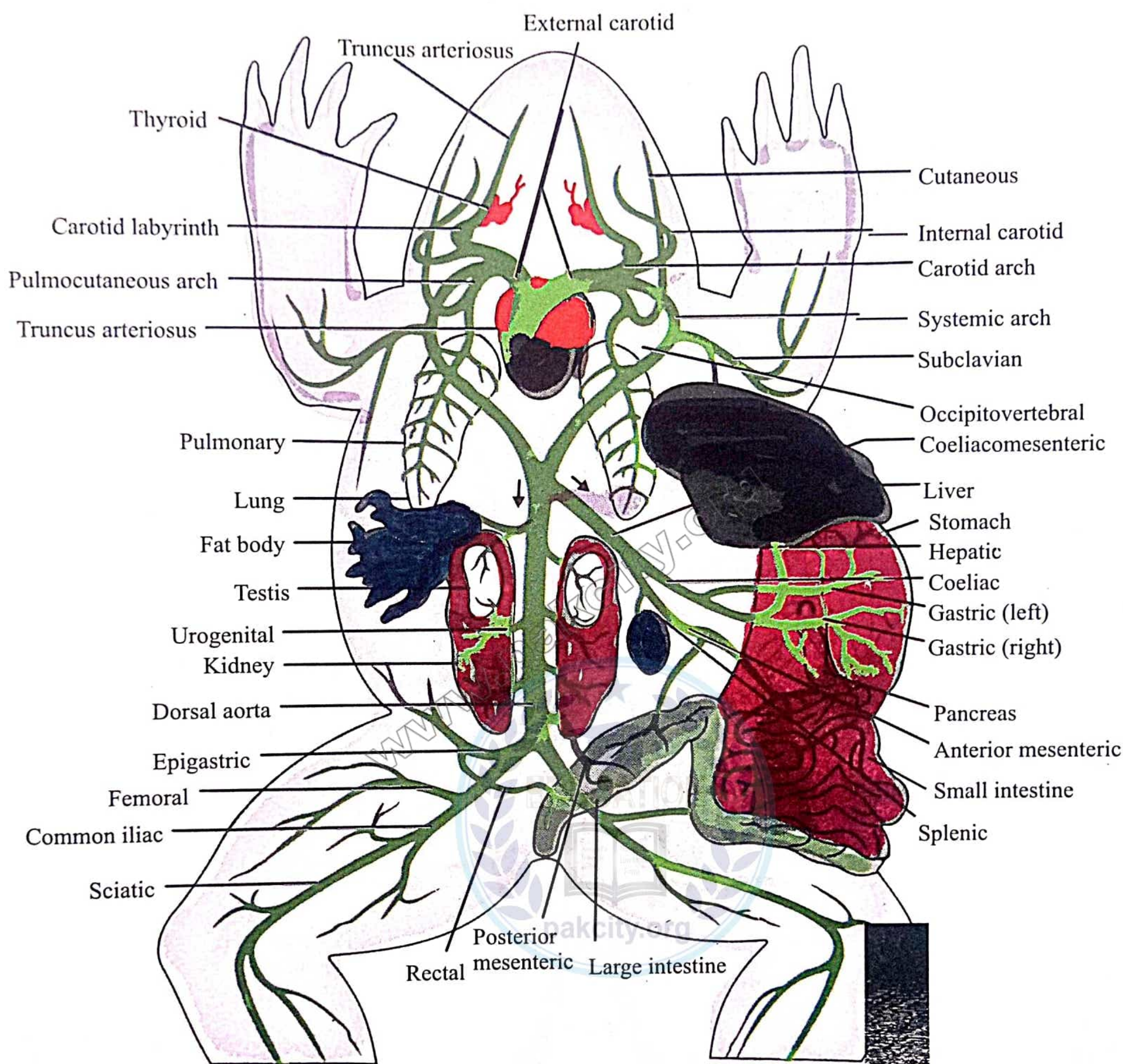


Fig. Arterial system of the frog (within body wall). Names of various organs are in boldface.

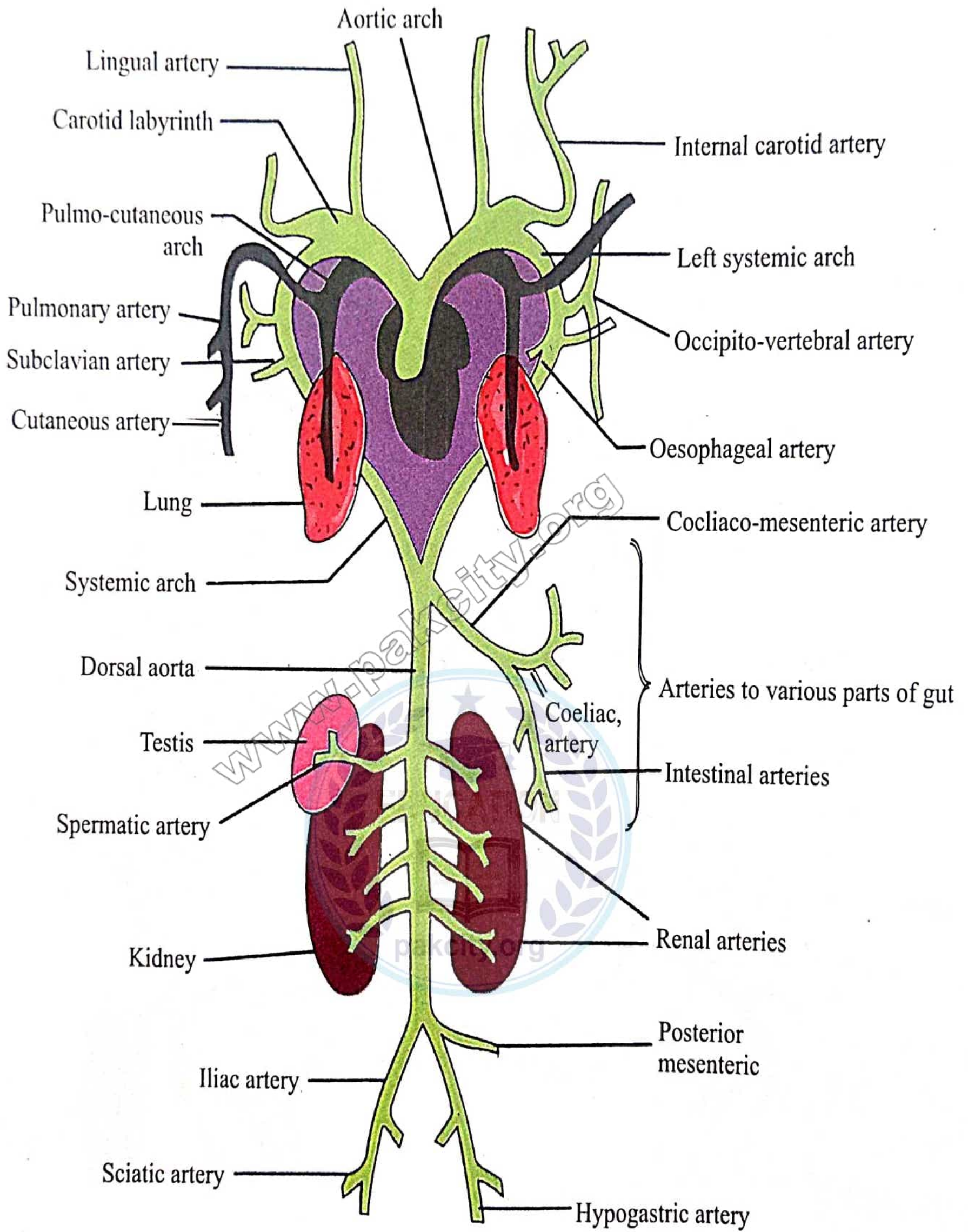


Fig. Arterial system of frog (without body wall)

VENOUS SYSTEM:

There are three veins which bring blood from different parts of the body and opens into sinus venosus: These are precavals, postcaval and pulmonary veins.

Precavals:

There are two precavals: right precaval and left precaval. Each precaval receives three branches.

1. **External jugular vein:** Tongue and lower jaw.
2. **Innominate vein:** Brain and shoulder.
3. **Subclavian vein:** Fore limb and skin.

Postcaval:

It receives following branches:

1. **Renal veins:** Kidney.
2. **Genital veins:** Gonads.
3. **Hepatic veins:** Liver.

Renal Portal Veins:

It collects blood from legs and opens into kidneys. Renal portal vein receives following veins:

◆ **Sciatic vein:** Inner side of leg.

◆ **Lumber vein:** Abdomen.

Renal veins arise from kidney and transfer blood to postcaval. It forms renal portal system.

Anterior Abdominal Veins:

Two pelvic veins fuse to form anterior abdominal veins. It opens into liver.

Hepatic Portal Vein:

It collects blood from digestive system and open into liver. Hepatic portal veins receive followings veins:

◆ **Gastric vein:** Stomach

◆ **Intestinal vein:** Intestine

◆ **Splenic vein:** Spleen

◆ **Pancreatic vein:** Pancreas

Hepatic veins arise from liver and open into postcaval. It forms hepatic portal system. It forms hepatic portal system.

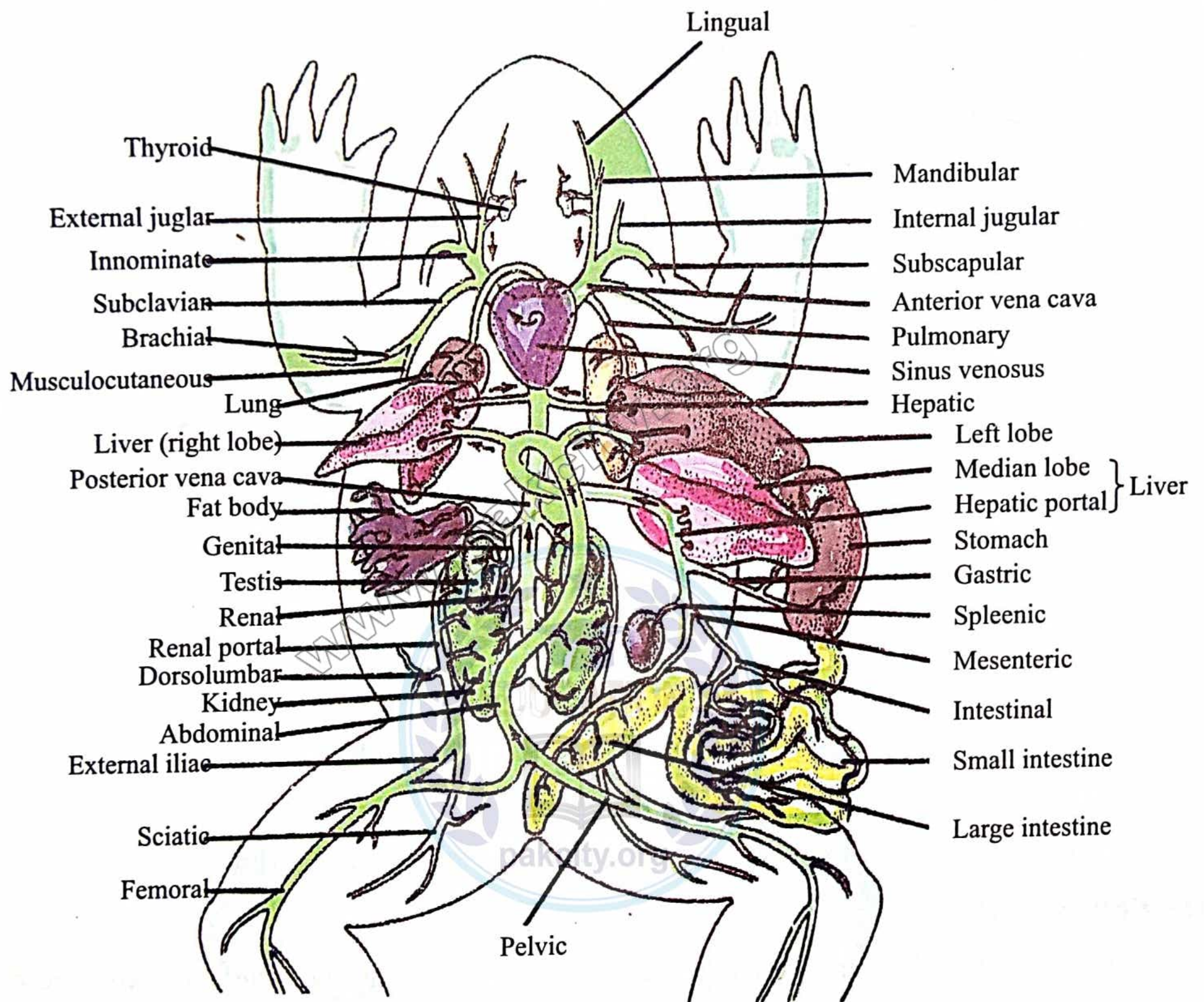


Fig. Venous system of frog

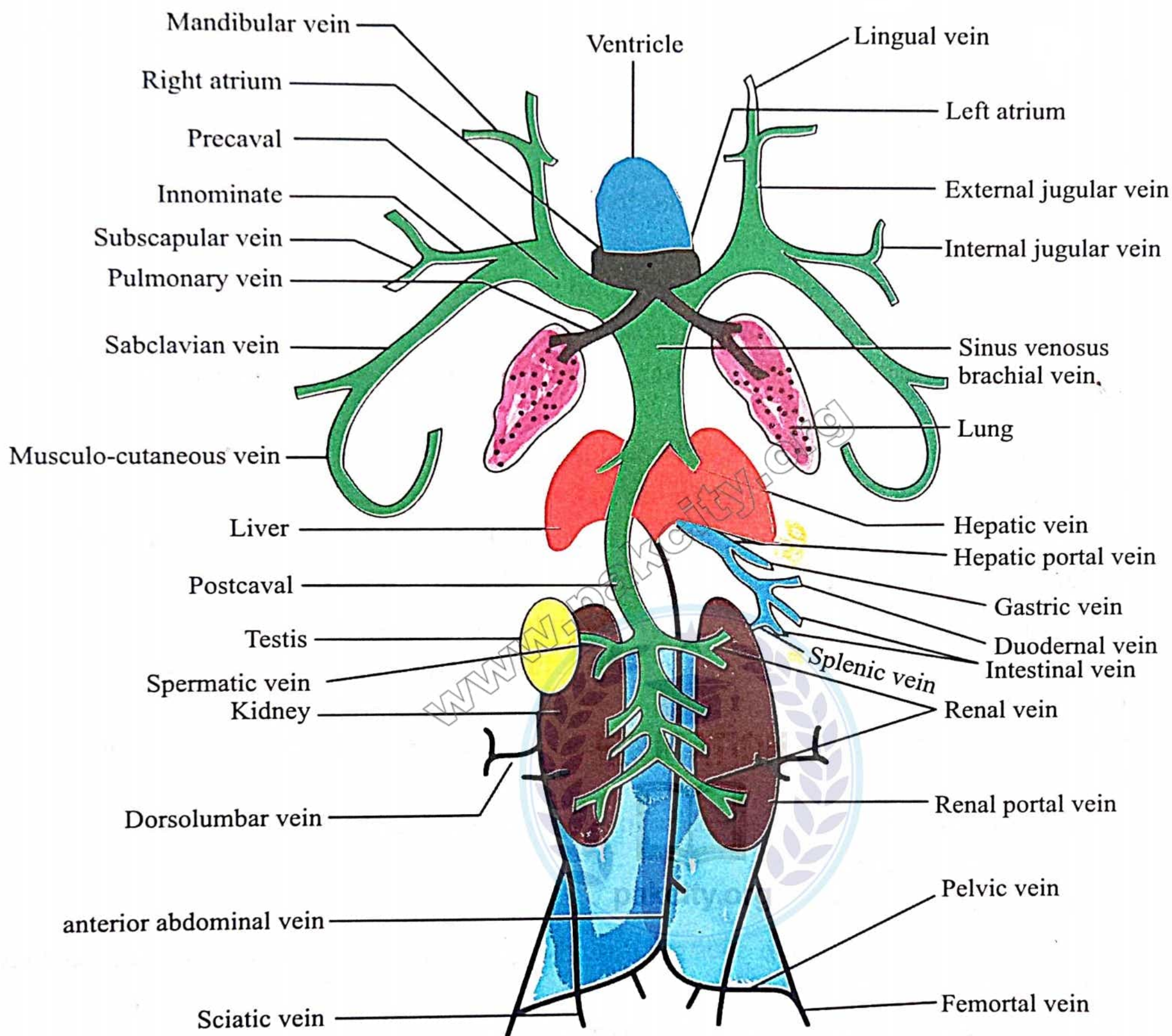


Fig. Venous system of frog (without body wall)

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Dissect the animal provided (frog) to expose its arterial system and draw its labelled diagram.

Dissection = 1, Demonstration = 1, Labelled diagram = 1 + 1, Short questions = 1/2 + 1/2, Total 5
(Multan Board 2004)

Q.1 Which artery supply blood to kidney?

Ans. Renal artery.

Q.2 Name the artery which supplies blood to skin and lungs. Which type of blood does it carry?

Ans. Pulmocutaneous artery.

Experiment 2: Dissect the animal provided (frog) to expose its venous system and draw its labelled diagram.

(D.G Khan, Multan Boards 2004)

Q.1 What is portal system? Give its one example.

Ans. A blood system in which vein does not directly open into postcaval through and organ like kidney or liver. Its example is renal portal system.

Q.2 What is the origin of sciatic vein?

Ans. Internal leg.

Experiment 3: Dissect the animal provided (frog) to expose its heart and draw its labelled diagram.

(Lahore, Sargodha Boards 2004)

Q.1 Is heart of frog is efficient heart? Why?

Ans. No, there is mixing of oxygenated and deoxygenated bloods. Therefore it is not an efficient heart.

Q.2 How many chambers are present in the heart of frog?

Ans. Three chamber.

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Experiment

MEASUREMENT OF BLOOD PRESSURE DURING REST
& AFTER EXERCISE WITH B.P APPARATUS

The hydrostatic force which pushes up the blood against the walls of the blood vessels is called blood pressure. The heart generates two pressures:

- ◆ **Systolic pressure:** The contraction phase of cardiac cycle is called systole. The systolic pressure is 120 mm Hg. It is the highest pressure.
- ◆ **Diastolic pressure:** The relaxation phase of the heart is called diastole. The diastolic pressure of the normal individual is between 75-85 mm Hg.

Material:

Stethoscope, Sphygmomanometer (B.P apparatus).

**Procedure:**

1. The cuff of B.P apparatus is wrapped around the upper arm.
2. Stethoscope is placed above the joint of elbow.
3. The cuff is inflated with the help of balloon of B.P apparatus. The level of mercury of needle of B.P apparatus is raised up 150 mm Hg. It closes the artery. Therefore, no sound is heard from it.
4. Now the valve of the balloon is opened very slowly. It deflated the pressure from cuff slowly. The sounds from stethoscope are heard attentively during this deflation.
 - ◆ **Systolic pressure:** After sometime, first sound of pulse is listened from stethoscope. The reading on scale is noted. It is a systolic pressure.
 - ◆ **Diastolic pressure:** The cuff is deflated further. A point comes after sometime, when the sound of pulse disappears. The reading is noted. It is diastolic pressure.

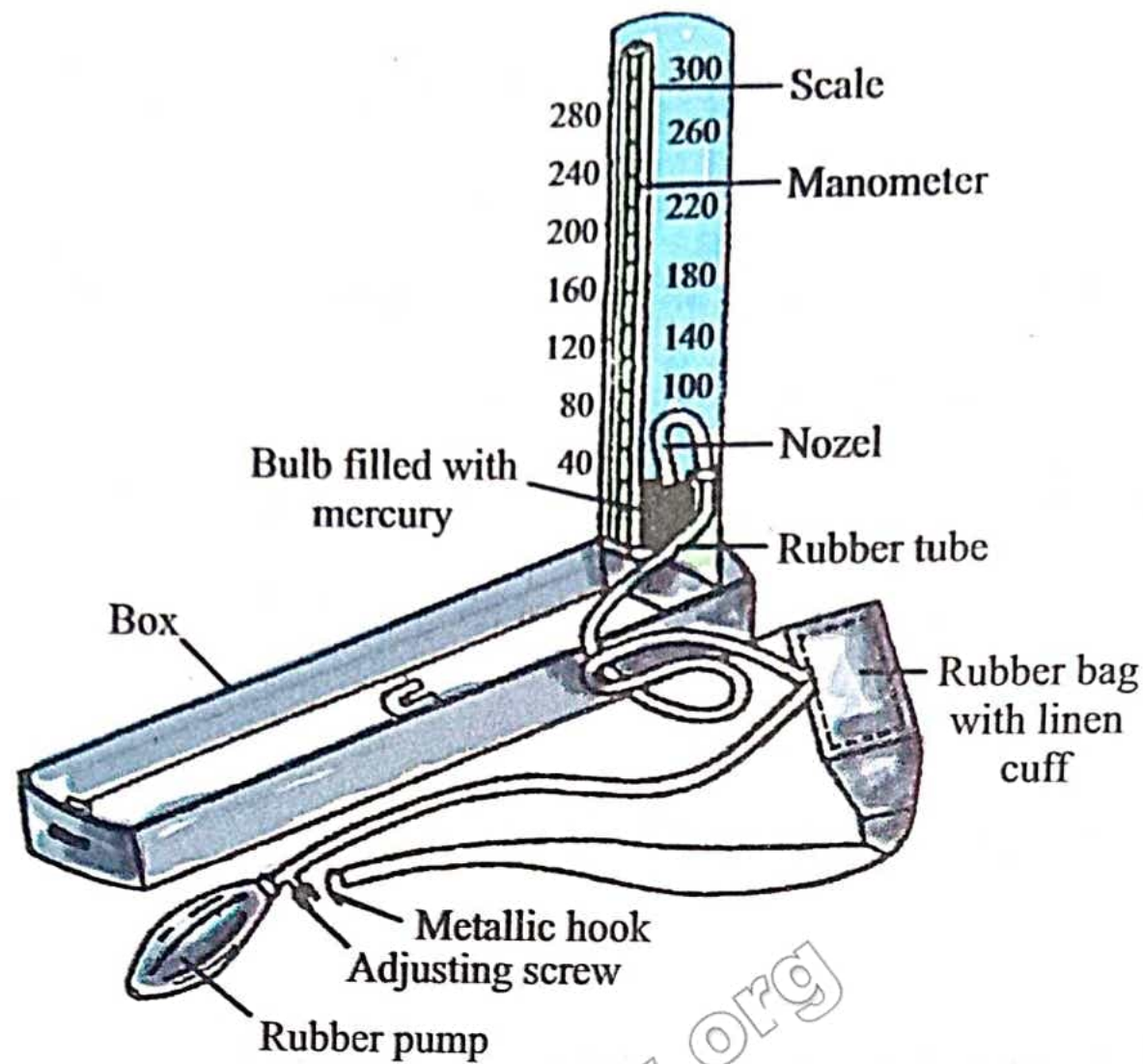


Fig. Sphygmomanometer (B.P. apparatus)

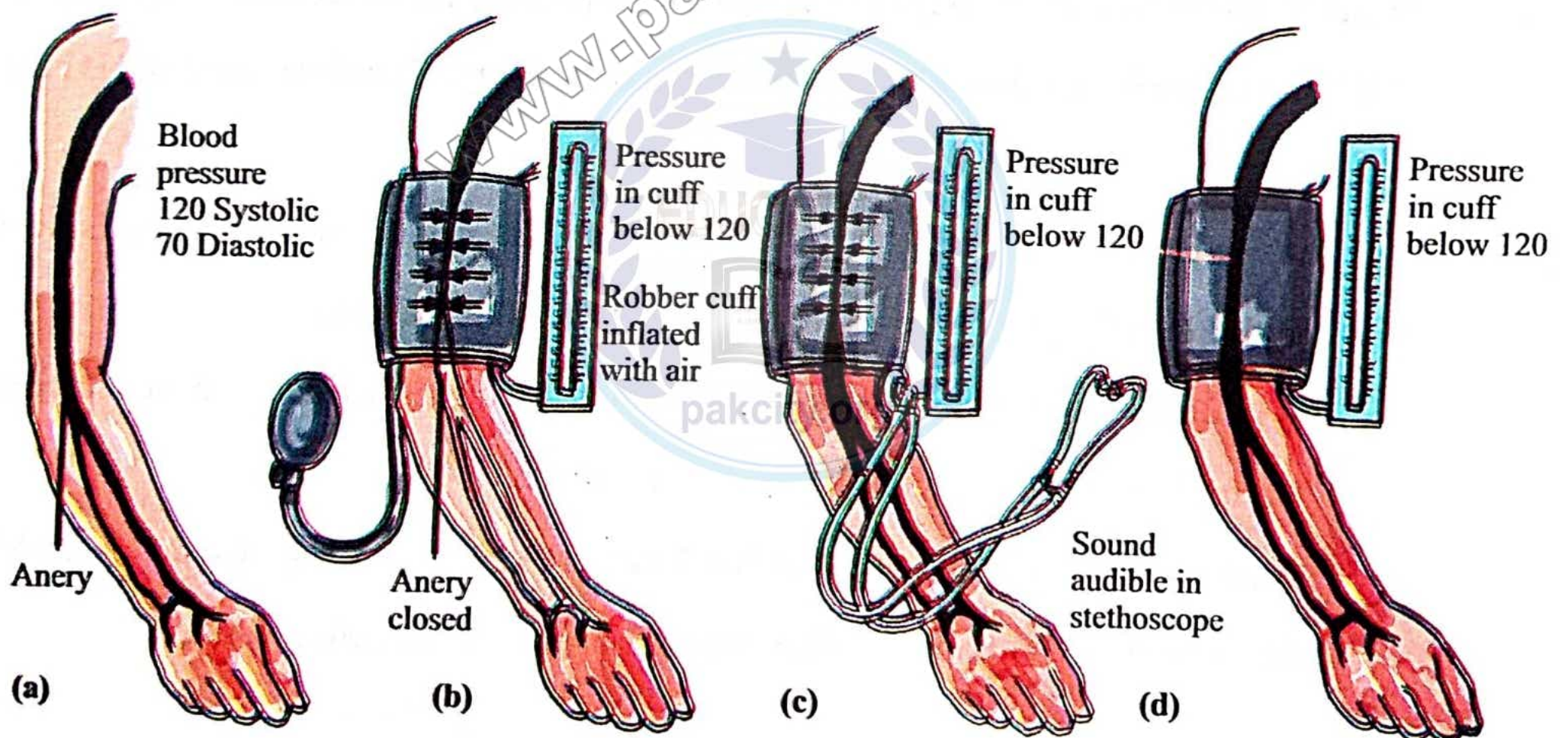


Fig. (a) Normal circulation of blood, (b) Inflating of blood vessel, (c) Systolic pressure, (d) Diastolic pressure

Observation:

No.	Persons	At rest (mm Hg)		After exercise (mm Hg)	
		Systolic	Diastolic	Systolic	Diastolic
1.	A	120	75	135	90
2.	B	120	80	130	85
3.	C	115	75	125	85

Result:

These observations show that average normal blood pressure at rest is 120/80. During exercise this pressure is increased up to 135/90 or more.

VIVA VOCE

Q.1 Define Blood Pressure.

Ans. The force developed by the blood pushing against the walls of the blood vessels is called blood pressure.

Q.2 What does pulse measure?

Ans. It measures heart rate and to some extent blood pressure and cardiac output.

Q.3 What is silent killer?

Ans. Blood pressure is said to be the silent killer.

Q.4 Upon what factors does blood pressure depend.

Ans. There are several factors, but the main are: heart rate; stroke volume; resistance to blood flow by blood vessels; strength of heart beat.

Q.5 What causes the blood pressure to increase?

Ans. **Physical activity:** kidney malfunctioning, narrowing and hardening of the arteries are the main causes of increase in blood pressure.

Q.6 What is the name of the apparatus which is used to measure the blood pressure?

Ans. Its name is *sphygmomanometer*.

Q.7 What is the name of the apparatus which is used to hear the pulse during blood pressure measurement?

Ans. It is called *stethoscope*.

Q.8 Explain systolic blood pressure.

Ans. it is the force with which blood pushes against the arterial walls during the contraction phase of ventricle.

Q.9 Define diastolic blood pressure.

Ans. The blood pressure experienced at the time of relaxation phase of ventricle is called diastolic blood pressure.

Q.10 What is the typical blood pressure of a healthy young man?

Ans. It is about 120/80 mm Hg.

Q.11 What is the typical blood pressure of a healthy young woman?

Ans. It is 8 to 10 mm Hg less than the young man.

Q.12 Which factor is primarily responsible for diastolic blood pressure?

Ans. It is the volume of the blood.

Q.13 What is pulse pressure and what is its normal value?

Ans. The difference between the systolic and the diastolic pressures is called as the **pulse pressure** whose normal value is from 30 to 50 mm Hg.

Q.14 What are the lower and upper limits of the systolic pressure in the normal adults?

Ans. The lower limit of systolic pressure in the normal adult is approximately 105 mm Hg and the upper limit as 150 mm Hg.

Q.15 How can the cuff of the sphygmomanometer be deflated?

Ans. The cuff can be deflated by opening the air valve of the air bulb by turning it anticlockwise.

Q.16 Name the artery of the arm which is compressed when the cuff is inflated.

Ans. The name of the artery is the brachial artery.

Q.17 What is the name of the artery over which fingers are placed at the wrist, for feeling the pulse?

Ans. Its name is radial artery.

Q.18 How much blood is normally found in the human body?

Ans. It is about five liter.

Q.19 Differentiate between hypertension and hypotension.

Ans. An increase in the normal systolic blood pressure is called hypertension whereas a fall in it is known as the hypotension.

Q.20 Why is blood pressure maximum in arteries?

Ans. It is so, because the blood is directly pumped into the arteries, when the ventricles contract.

PREVIOUS BOARD EXPERIMENTS AND QUESTIONS

Experiment 1: Measure the blood pressure at rest and during exercise.

Material = 1, Procedure = 2, Observation and result = 1, Short question = 1/2 + 1/2, Total = 5

(Lahore, Rawalpindi Boards 2004)

Q.1 Name the instrument used for measurement of blood pressure.

Ans. Sphygmomanometer.

Q.2 Differentiate between systolic and diastolic pressures.

Ans. The contraction phase of cardiac cycle is called systole. The relaxation phase of the heart is called diastole.