Training Programme of KRPs of Western Region on Laboratory Skills in Biology at Senior Secondary School level

PROGRAMME CO-ORDINATOR

DR. DAKSHA M. PARMAR

विद्यया ऽमृतमश्नुते

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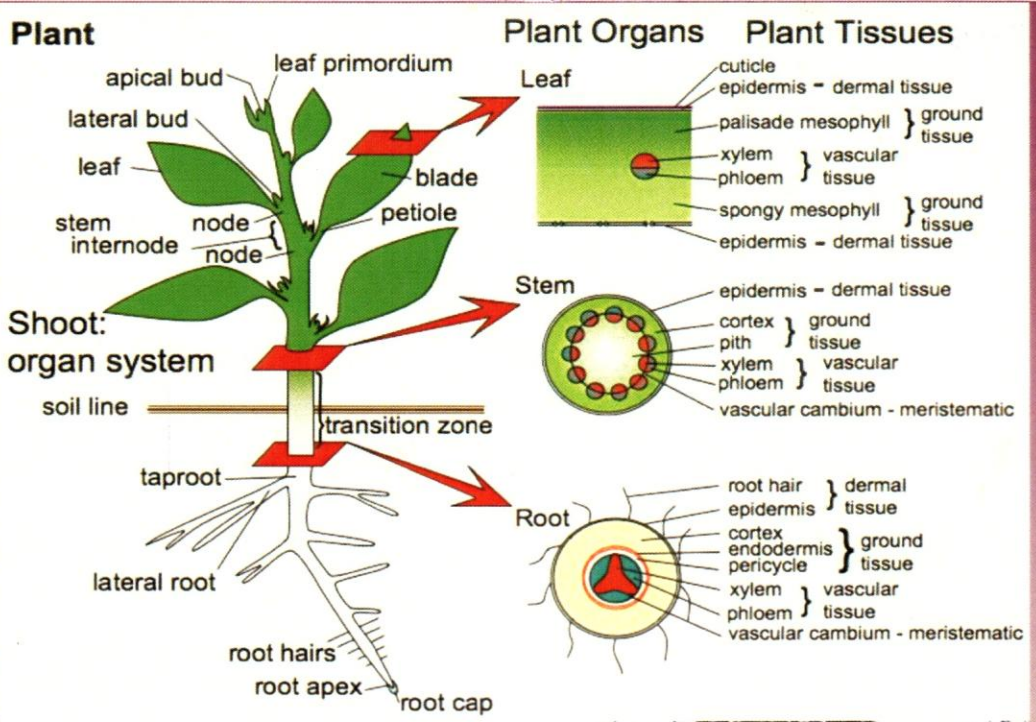

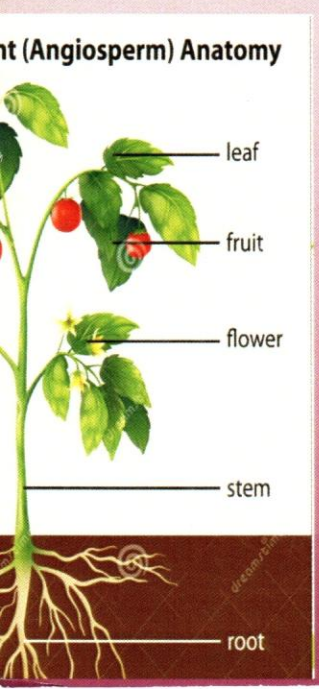

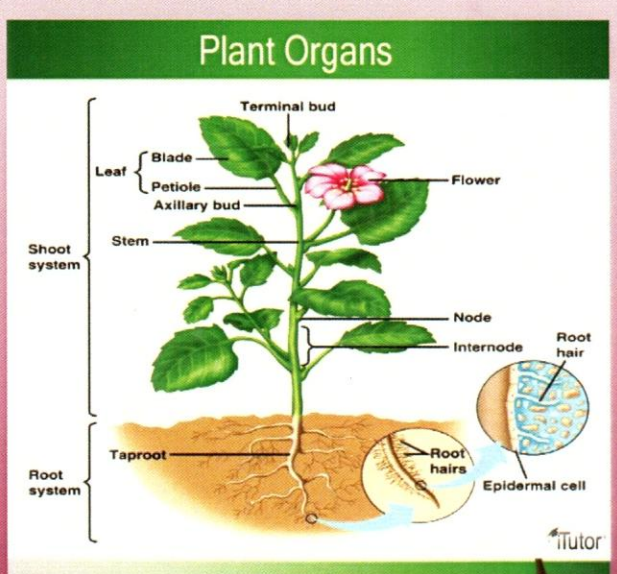

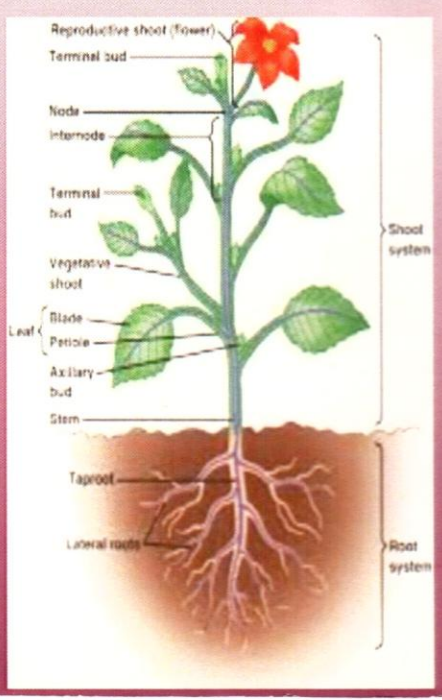

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Training Programme of KRPs of Western Region on Laboratory Skills in Biology at Senior Secondary School level

Chairperson:

Prof. N. Pradhan, Principal, Regional Institute of Education, Bhopal

Chief Advisor:

Prof. V.K. Kakaria, Head, DESM, Regional Institute of Education, Bhopal

Members of Content Development Committee:

Prof. J. Mandal

Prof. Reeta Sharma

Prof. Shaukat Saeed Khan

Dr. Ruchi Gautam

Dr. D.M. Parmar

Dr. Shruti Shrivastava

Dr. Sabiha Kamal Khan

Dr. Mulridhar

Coordinator:

Dr. Daksha M. Parmar

"Training of KRPs of Western Region on Laboratory Skills in Biology at Senior Secondary School Level"

APPROACH PAPER

Biology being one of the compulsory subjects at +2 levels besides Physics and Chemistry. Biology learning forms the basis of learning Botany, Zoology, Biochemistry, Biophysics, Life Sciences, and other allied subjects opted by the students at graduation level.

The Syllabus and Textbooks developed by NCERT on the guidelines of NCF-2005 signifies to implement this basic idea. Learner should get hands-on experience of the theory learnt during conduction of the practical and should be able to relate in understanding of the daily life principles involving Biology.

RIE- Bhopal caters to the entire western region including the states of Gujarat, Maharashtra, Madhya Pradesh, Goa, Chhattisgarh, Daman & Diu and Dadra & Nagar Haveli. In-service training programmes are an integral part of the activities carried out at Regional Institute of Education, Bhopal. It is our abiding duty to serve the states under our jurisdiction with the fruits of our experimentation in educational innovations and best practices.

The present training programme was conceptualized under the leadership of our Principal Prof. Nityanand Pradhan and under the expert guidance of Prof. V. K. Kakaria, Head, DESM and various faculties to deliberate on the hard spots pertaining to Biology Laboratory skills at the Senior Secondary level so as to provide the teachers with hands on experiential learning in Biology and to negate the learning gaps present, if any.

The teachers selected for the present programme represents the entire region for providing training at senior secondary level in Biology teaching i .e. class XI and Class XII.

Though it will not be possible for any body to cover the vast regime of entire syllabus of Biology of class XI and XII in just five days time period and

to do justice to the topics, however with a presumption that since the participants are practicing teachers hence very well conversant with the content therefore just a little brushing up of the topics by a panel of experts in the field along with the incorporation of latest information inputs and touching upon the philosophical background under the broad theme of Biology will do for the time being.

All this by relating the chosen topics to the present day needs of the learners and further to link the school subject knowledge with day to day living in the surrounding so as to sustain the interest of the pupils in the discipline and further reducing the non-comprehension burden of the learner was the justification behind the chosen topics.

The objectives framed are:

i) To familiarize the participants with the philosophy of NCF-2005 with special reference to Biology.

ii) To provide training to the participants to follow Constructivist approach in teaching-learning process at their end.

iii) To develop, understand, correlate and practice modern theoretical concepts of Biology teaching–learning by means of activities and experimentation.

iv) To correlate Biological concepts with other branches of sciences to understand and explain various phenomena for developing better understanding.

v) To emphasise the underlying principles in Biology as well as highlighting the interrelationships of Biology with other areas of knowledge.

vi) To develop strategies to use Biological skills in daily and real life situations.

vii) To get the feedback from the states agencies on the utilization of the prepared material.

(Dr. Daksha M. Parmar)
Programme Coordinator

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"Training of KRPs of Western Region on Laboratory Skills in Biology at Senior Secondary School Level"

Time Table

From 12th-15th December, 2017

Date & Day	Time	Topic
11.12.2017 (Monday)	9.30 - 10.00 a.m.	**Inaugural Session**
	10.00 - 11.30 a.m.	**Botany Practical** *Prof. Jaydeep Mandal*
	11.30 - 1.00 p.m.	**Botany Practical** *Prof. Shaukat Saeed Khan*
	Lunch Break	
	2.00 - 3.30 p.m.	**Zoology practical** *Dr. Sabiha Kamaal Khan*
	3.30 - 5.00 p.m.	**Zoology Practical** *Prof. V.K. Kakaria*
12.12.2017 (Tuesday)	9.30 - 11.00 a.m.	**Zoology Practical** *Dr. Murlidharan*
	11.30 a.m. - 1.00 p.m.	**Zoology Practical** *Dr. Sudhi Srivastava*
	Lunch Break	
	2.00 - 3.30 p.m.	**Botany Practical** *Prof. Shaukat Saeed Khan*
	3.30 - 5.00 p.m.	**Botany Practical** *Prof. Shaukat Saeed Khan*
13.12.2017 (Wednesday)	9.30 - 11.00 a.m.	**Zoology Practical** *Dr. Sabiha Kamal Khan*
	11.30 a.m. - 1.00 p.m.	**Botany Practical** *Dr. Ruchi Gautam & Prof. Reeta Sharma*
	Lunch Break	
	2.00 - 5.30 p.m.	**Photo sessions and Visit to places of Environmental concern** *Botany & Zoology Faculty*

14.12.2017 (Thursday)	9.30 - 11.00 a.m.	**Botany Practical** *Prof. Ajay Kumar Bhardwaj*
	11.30 a.m. - 1.00 p.m.	**Botany Practical** *Prof. Reeta Sharma & Dr. D.M. Parmar*
	Lunch Break	
	2.00 - 3.30 p.m.	**Zoology Practical** *Prof. Shariq Ali*
	3.30 - 5.00 p.m.	**Zoology Practical** *Dr. Murlidharan*
15.12.2017 (Friday)	9.30-11.00 a.m.	**Botany Practical** *Prof. Ajay Kumar Bhardwaj*
	11.30 a.m. - 1.00 p.m.	**Botany Practical** *Prof. Reeta Sharma & Dr. D.M. Parmar*
	Lunch Break	
	2.00-3.30 p.m.	**Zoology Practical** *Prof. Shariq Ali*
	3.30-4.30 p.m.	**Valedictory Session**

CURRICULAR EXPECTATIONS IN BIOLOGY AT SR. SEC. STAGE

Students at the senior secondary stage are expected to:

i. Develop interest to study biology as a separate discipline.
ii. To develop process skills including observation, drawing, critical thinking, problem solving, analytical approach & drawing conclusion.
iii. To develop sensitivity towards environmental concerns.
iv. To develop awareness about surroundings (flora & fauna) natural processes & phenomena.
v. To develop awareness about issues related to health, hygiene, sanitation & ethics.
vi. To prepare the learner to play a rational, responsible and informed role in the society by removing the prevalent myths & misconception.
vii. To appreciate the historical development & land mark discoveries in biology.
viii. To develop mathematical skills in students for better understanding of biological concepts
ix. To appreciate the contributions made by the scientist (women scientist in particular) that led to important discoveries.
x. To appreciate the diversity prevalent in the living world.
xi. To carry out investigatory projects for developing the future research capabilities.
xii. To develop an understanding about various biological phenomena and concepts related to day to day life.
xiii. To promote an interdisciplinary approach for better understanding of biology.
xiv. To provide orientation for professional / career opportunities available in medicine, agriculture, industry, teaching and research.
xv. To internalize acquire & appreciate values such as honesty, cooperation, collaboration & judicious use of resources.

LEARNING OUTCOMES IN BIOLOGY AT SR. SEC. STAGE

1. Appreciates the number and types of living organisms and understands the characteristics of living organisms such as metabolism, consciousness and cellular organization.
2. Classifies organisms into five kingdoms, understands the limitations of two kingdom classification and appreciates taxonomical categories and aids (herbarium, botanical gardens and museums) and relates classification with evolution.
3. Develops the ability to compare the characteristics of different kingdom Divisions/Phylum with emphasis on plant and animal kingdom providing suitable examples.
4. Differentiates morphological characteristics of various plant parts along with their modifications & describes important plant families using technical terms for various parts of a plant.
5. Classifies different tissues on the basis of their structural & functional organizations and recognizes their role in the formation of organs & organ system in plant & animals.
6. Explains the morphology & anatomy of some animals (earthworm, cockroach & frog).
7. Explains the process of secondary growth in dicot plants & appreciates the process of formation of wood.
8. Draws the structures of morphological & anatomical features of plants & animals.
9. Prepares slides to observe & identifies the anatomical structures of plants & animals.
10. Appreciates the biodiversity in the surroundings and causes of its depletion and efforts to conserve it
11. Discusses the environmental issues related to air, water and soil pollution and methods to overcome these.
12. Understands population growth models, population interaction, ecosystems and ecological succession
13. Appreciates the steps taken to solve the environmental problems by local people (Case Studies like Chipko movement, JFM, Narbada Bachao)
14. Conduct investigatory projects (cause effect relationship)
    - Soil quality

- Water pollution
- Air pollution

15. Appreciates the efforts and experiments carried out by scientists that led to scientific discoveries
16. Exhibits creativity in planning, designing and conducting experiment to establish scientific concepts, phenomena & processes related to digestion, circulation, excretion and control & coordination (reflex action), photosynthesis, respiration, transportation, role of plant growth regulators & mineral nutrition.
17. Explains various structures and related physiological processes, phenomena occurring in plants and animals to attain conceptual clarity as given below-

- Nutrition
- Digestion & absorption
- Locomotion & movement
- Respiration
- Transportation
- Excretion
- Control & coordination

18. Applies learning of scientific concepts in day to day life, application of growth hormones in agricultural fields, osmosis, apical dominance, parthenogenesis, vegetative propagation and fermentation.
19. Develops the ability to identify the causes of disorders related to various processes in plants and human body like digestion, circulation locomotion & movement and control & coordination
20. Draws labeled diagrams, flow charts & tables pertaining to structures & functioning of different organs and organ system in human body and plants parts involved in various physiological processes
21. Differentiates various modes of reproduction (sexual, asexual and vegetative) in plants
22. Explain the reproductive structures and the process of sexual reproduction in human beings
23. Develops awareness towards reproductive health, contraceptive, amniocentesis & STDs.
24. Draws labeled diagrams of reproductive structures in plants & animals.
25. Develops understanding of the Mendel's laws of inheritance and deviation with causes.

26. Differentiates between the terms like.
    - Dominant / recessive
    - Homozygous / Heterozygous
    - Phenotype / genotype.
27. Understand & draw structures of DNA and RNA .Explains & describe processes like transcription and translation in prokaryotic and eukaryotic cells, linkage and recombination, divergent and convergent evolution.
28. Discusses and appreciates scientific discoveries of genetic material, biogenesis and Human Genome Project.
29. Develops awareness about disorders and diseases caused by genetic variation.
30. Draws the diagrams flow charts graphs & tables to understand Mendelian ratios, protein synthesis and evolution.
31. Understand the concept of health and hygiene and develops awareness
32. About the common diseases in humans, concept of immunity.
33. Differentiates various drugs, alcohol and tobacco for their ill-effects and remediation.
34. Understands the necessity for enhancement of food production through improved practices in crop production dairy, poultry, agriculture, pisciculture.
35. Appreciates the role of microbes in household, industry and agriculture.
36. Prepares flow chart and diagrams for sewage treatment depicting the role of microbes and visit to a sewage treatment plant.
37. Appreciates uses of biotechnology in various fields – medicine, agriculture, industry and research.
38. Explains the tools, techniques and processes used in various biotechnological processes.
39. Develops the ability to understand the ethical issues related to biotechnological products.

Class XI; Unit III, Chapter 8

Aim: To study parts of a Dissecting and compound microscope

Learning Objectives:

1. **To acquaint the users with kinds of microscope**
2. **To inculcate laboratory skills of handling Microscopes**
3. **To facilitate the maximum use of microscopes at learner level**

Principle:

Magnified and real images of minute objects are obtained using combination of lenses. A compound microscope is a complex assemblage of such lenses enabling highly magnified images of the microscopic living organisms and intricate details of cells and tissues. A monocular mono-objective compound microscope is normally used in a biology laboratory.

Dissecting Microscope

It consists of following parts:

(i) **Base**—It is bifurcated and it supports the other parts of microscope.

(ii) **Stand**—It is short, hollow, cylindrical rod fixed at one end of base and is used to hold the microscope.

(iii)**Vertical limb**—It's a short, cylindrical rod that fits into the hollow of the stand and can be moved up and down with the help of adjustment screws.

(iv)**Folded arm**—It is the horizontal flat arm attached to vertical limb and at the other end, it has a lens fitted into it. It can be moved up and down along with movement of vertical limb, to focus an object and it can also be moved sideways. The lens can be 5x, 10x or 20x.

(v) **Stage**—A rectangular glass plate attached to stand and has two clips fitted on it, to hold the slide or the object in position.

(vi)**Mirror**—Concave, reflecting mirror is attached close to the lower end of the stand to reflect sunlight rays on to the stage.

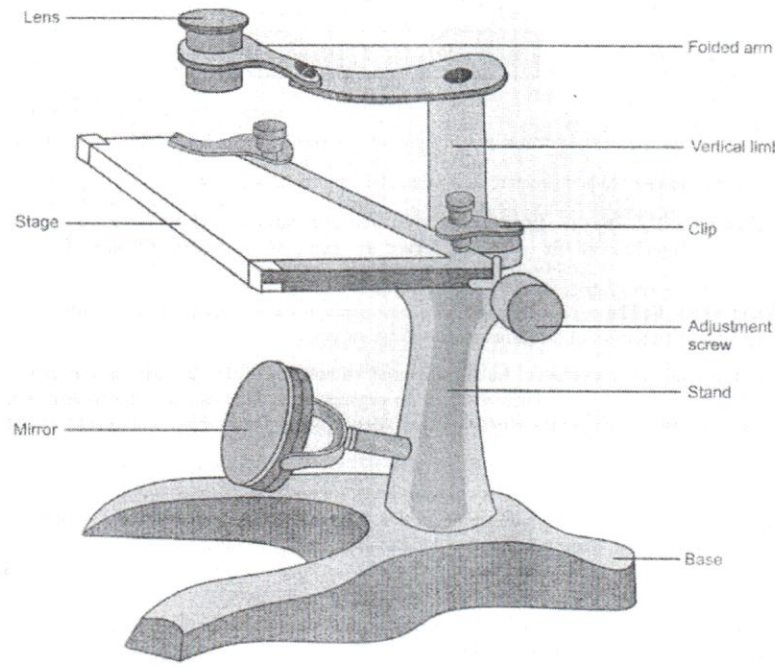


Fig. Dissecting microscope

Working of the Dissecting Microscope:

Object/Slide to be observed is placed on the stage. Lens is brought above the slide with the help of folded arm. Adjustment screws are used to move the vertical limb up and down to focus the slide.

Use of Dissecting Microscope

- It is used to dissect small objects and wherever low magnification is required.

Compound Microscope

Simple and dissecting microscopes are incapable of revealing details of cellular components because of limited power of magnification. It consists of more than one lens fitted one above the other at a proper distance in a cylindrical tube. An object magnified by one lens is further magnified by another lens.

Compound microscope has following parts :

Mechanical Parts:

(i) **Base** —It is a horse shoe-shaped structure and provides a stable support for the instrument.
(ii) **Arm**—It is a prominent curved part of instrument used for handling.
(iii)**Pillar**—It is a small vertical projection of base.

(iv)**Stage**—It is a flat square piece attached to lower end of arm and is used to keep an object to be magnified. It has an aperture in the centre for the light to pass.

(v) **Condenser**—It is a two lens system for focussing the beam of light at the object.

(vi)**Body tube**—It is a tubular part attached to upper part of arm and is movable.

(vii) **Nosepiece**—It is a circular metallic structure below the body tube to hold objective lenses.

(viii) **Coarse adjustment screw**—It is a bigger size screw to move body tube up and down for adjustment.

(ix)**Fine adjustment screw**—It is a smaller size screw for very slow movements for fine adjustments.

(x) **Mechanical stage**—This stage is equipped with scales to permit accurate mechanical positioning of the slide and any point on the object.

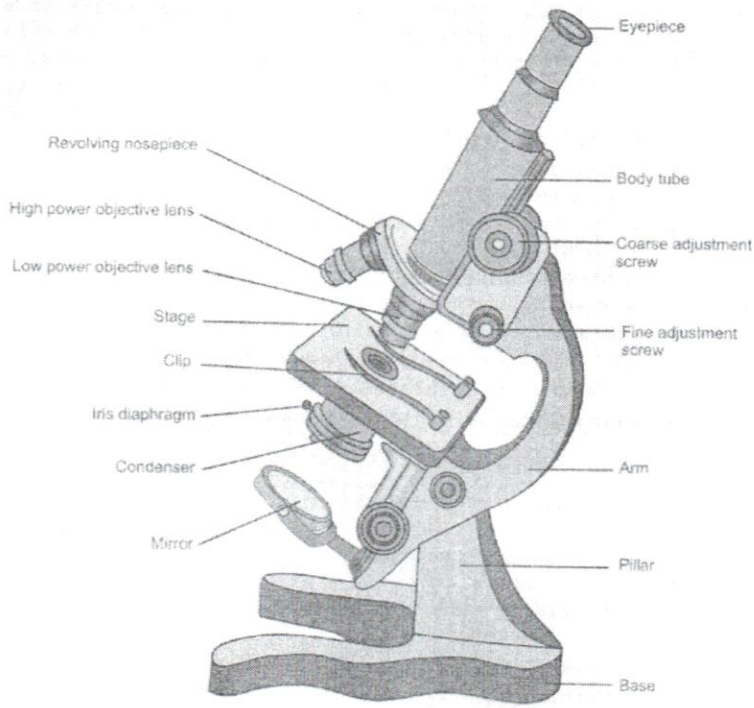


Fig. Compound microscope

Optical Parts:

(i) **Mirror**—It is attached on the pillar. It has two sides a plane and a concave and it is used to reflect rays of light into microscope.

(ii) **Objectives**—These are the lenses attached to the nosepiece. Usually these are 2-3 objectives of magnifications 10x (low), 45x (high) and 100x (Oil Immersion Lens) .

(iii)**Eyepiece**— It is a lens at the top of body tube through which an object is viewed. It can be of magnification 10x or 15x.

Objective Lenses:

It is a lens system made of many lenses in a definite pattern. The objective lenses are of three types:

i) **Low Power Objective:** Abbreviated as L.P. and marked with blue band with magnifying power 10x.

ii) **High Power Objective or High Dry Objective:** Abbreviated as H.P. and marked with a red band and magnifying power 40x. It is also called high dry objective because it is used without adding any fluid in the medium between specimen and objective.

iii) **Oil Immersion Objective:** Abbreviated as 0.I., marked with black band and magnifying power 100x and a drop of oil is added in the medium between the front lens and coverslip. Oil (fluid) has the same refractive index as the glass. Immersion oil is Cedar wood oil which has refractive index of 1.5. Oil is to touch the objective lens so that no air is there between coverslip and oil and thus put a drop of oil on coverslip and bring objective over it.

Cedar wood oil is used as immersion oil because:

- This oil is clear like water.
- It has same refractive index as glass, i.e., 1.5.
- It is non-volatile and hence does not evaporate.

Water cannot be used in place of oil because water will evaporate.

Refractive index of glass and oil should be same because the basic purpose is to get all the incident light rays from light hitting through the mirror, condensers and fall on specimen and details of specimen are possible if light rays do not diesizie from their path. In case of oil immersion, light passing through a point of specimen will not deviate and pass through reach our eyes and so even minute details of specimen are seen as throughout it is denser medium and air is absent. In dry objective, air is between specimen and objective and so there is change in density of medium, as glass is a denser and air is a rarer medium, so due to deviation of light rays from their path, all light will not reach eyepiece, thus details will not be clearly visible.

Eyepiece:

Eyepiece consists of two lenses but it should not be opened as once opened, all magnifications are disturbed. Eyepieces are of **Huygenian type**, i.e, if move the slide towards the left, the image will move towards the right and vice versa and its an important property of reverse orientation. Various modem microscopes are **parfocal,** i.e., while changing from low power objective to high power objective there is no need of focus change. In older types, focus had to be again adjusted after changing to high power.

Once the eyepiece is opened, parfocal property is lost. Eyepiece can be of following magnification — 5x, 10x, 15x, 20x. If eyepiece is 5x and objective lens is 10x then the object will be magnified 5 x 10 = 50 times.

If eyepiece is 10x and objective lens is 95x then the magnification of the object will be 95 x 10 = 950 times.

Image of the specimen is always formed within the eyepiece.

Resolving nosepiece and Numerical Aperture:

Ability to distinguish two close points as two separate points is known as resolving power.

Resolution = minimum distance at which two close points as two separate points is possible. Resolving power of human eye 100 microns. Microscope is an object which magnifies as well as resolves the object seen through it.

$$Resolving\ power = \frac{\lambda}{2NA} (\lambda = Wavelength\ of\ light\ source, NA = Numerical\ Aperture$$

As Numerical Aperture is negligible so it is ignored and thus

$$R = \frac{\lambda}{2}$$

So objects closer than half the wavelength of illuminating light, cannot be clearly distinguished in light microscope.

λ of visible light = 4000 Å) so if we take the average as 6000 Å so resolving power = $\frac{6000}{2}$ = 3000 Å.

Resolving power of light microscope 3000 Å or 300 nm or 0.30 μ.

Difference between theoretical and practical resolving power is due to : (i) Wind consists of dust particles in between eyepiece and specimen and that brings about interference. (ii) Numerical Aperture has been neglected at the time of calculations.

Numerical Aperture (NA) = ... sin θ]

.... = Refractive index of medium

sin = Pencil of rays, at point of specimen,

sum total of all the lenses of objective lens.

sin 0 = Perpendicular AB — Negligible

But that's negligible, hence Numerical Aperture =

Working of the Microscope

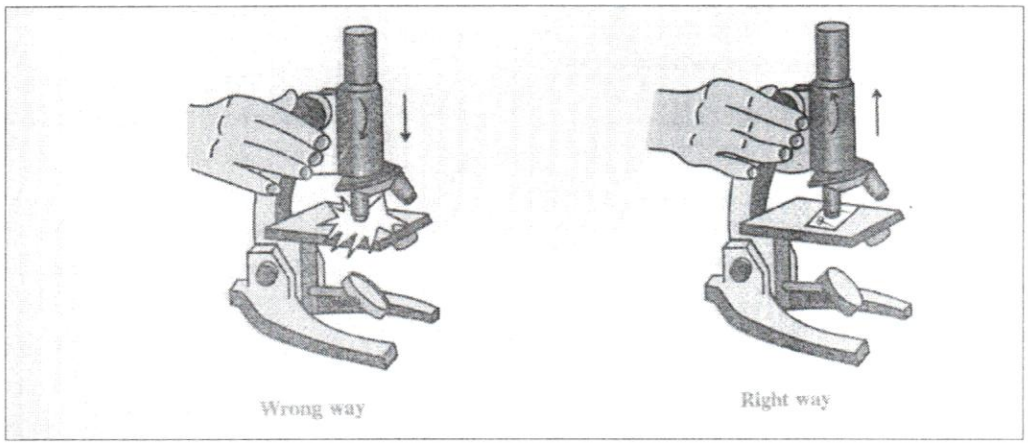
1. Adjust the light by turning the mirror towards the source of light and also by moving the condenser.
2. Place the slide on the stage such that object is just under the objective, i.e., it is in line with objective and eyepiece.
3. Locate the object and focus it with a low power objective using coarse adjustment.
4. To get higher magnification, turn the nosepiece to higher power, use only the fine adjustment at this stage to get a sharp image.

Use of the Microscope

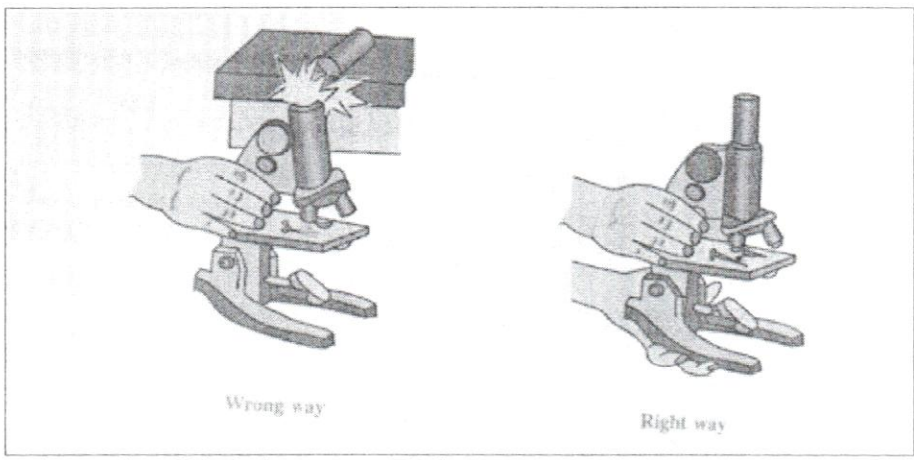
- It magnifies as well as resolves the object seen through it.
- It helps us to see minute details of tissues and cells.

Care of the Microscope

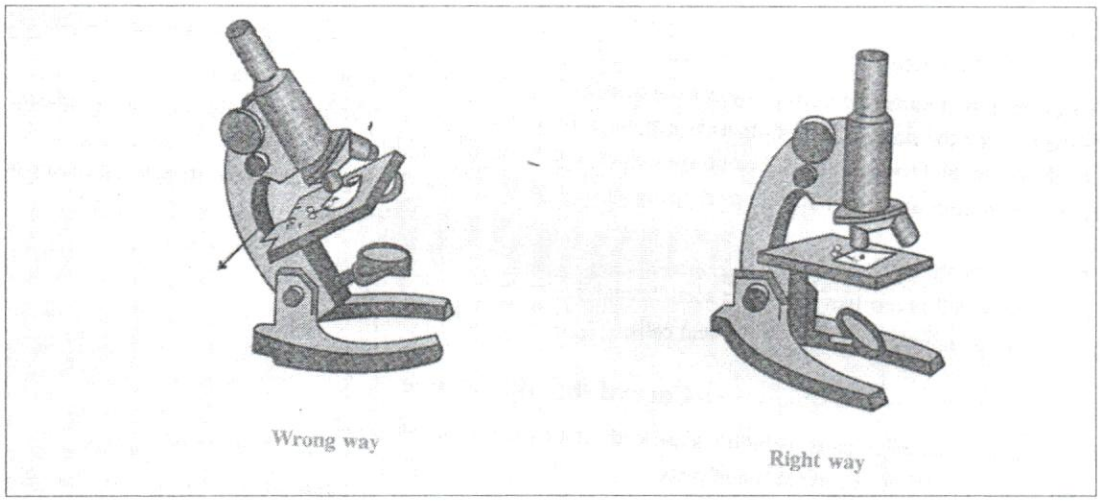
1. Clean the lenses and metal parts including stage with tissue paper or muslin cloth.
2. Do not remove objective lenses from nosepiece.
3. Be careful so that objective lens should not touch the slide in any case. While adjusting, do not move the lens downward, it should always be moved from bottom towards upward direction.



4. Keep the microscope covered when not in use.
5. Always hold the microscope with both the hands in an upright position while carrying it.



6. First focus the slide in low owes Right way p r and then only change to high power.
7. Do not use coarse adjustment screw while using high power objective lens.
8. Keep the microscope in upright position, do not move the stage in a slanting position.



Wrong way

Right way

Questions:

1. What is the magnification achieved when a 15X eye piece and a 40X objective are used together?
2. What should happen if direct sun rays are focused by the mirror into the body tube?
3. What is the difference between a dissecting and a compound microscope?
4. Which of the following parts provides support and supports the weight of a microscope?
   (a) Arm (b) Stage (c) Body tube (d) Foot
5. To which part of a microscope is the objectives fitted.
   (a) Nose piece (b) Diaphragm (c) Stage (d) Arm
6. Magnification power of a microscope is calculated by
   (a) magnifying power of eye piece × magnifying power of mirror.
   (b) magnifying power of eye piece × magnifying power of objective
   (c) magnifying power of objective × magnifying power of plane mirror.

This biology course offers a variety of laboratory exercises on current concepts in biology often using state of the art scientific equipment and technology. Various teaching techniques and materials will be employed enhance student understanding. Numerous laboratory methods will be utilized in demonstrations and student experiments. Safety instruction will be given and safe practices will be stressed in ãil laboratory work. Students will exercise critical thinking for solving problems and interpreting laboratory results.

For all students, faculty and staff using the Biology Labs

1- No food or drink is allowed in any biology lab. No gum chewing. Lunches, thermoses, and water bottles can be stored inside backpacks or purses. They are not allowed on lab benches, the floor, near computers, or in sinks.
2- Strict aseptic precautions should be observed when collecting blood or other specimens. It is better to wear the gloves.
3- Bench workers must wear laboratory coats and gloves.
4- Washing hands with soap and water should be a frequent practice particularly when leaving the work area.
5- Use of carpets and cushions should be discouraged. These are extremely difficult to clean or disinfect in case of spills. A smooth, polished floor is easiest to clean.
6- Mouth pipetting should be avoided as for as possible. Instead use rubber teats. However if it is to be done, it is better to plug upper end loosely with cotton wool. All care must be taken to ensure that fluid is not sucked into the mouth.
7- Never taste or smell any chemicals unless your teacher tells you it is safe to do so.
8- If re-useable glassware is used, it should be put in a strong disinfectant solution. At the end of the day transfer these to a strong detergent. Next day place these in boiling water for 10 to 20 minutes, cool and then wash with tap water.
9- All potentially infected material like stool, urine, bacterial cultures etc. must be incinerated after disinfections. Specimen containers should be such that spilling does not occur. Care should be taken in removing and replacing caps of containers.
10- In case of spilling or breakage in centrifuge or on bench or floor, first collect all glass pieces with forceps and then wash the area with strong disinfectant followed by washing with strong detergent. Then wash with water and wipe to dry.
11- Always alert your teacher to any spills on your skin or clothing.
12- All reagent containers must be of proper size and shape and properly stoppered. These shall never be left open. Containers must be clearly labeled. If these contain dangerous chemicals or infective material, a danger sign or poison label must be pasted on the container.
13- Be polite- no pushing, shoving, fighting or horseplay.
14- If you have allergies or asthma or think you might be pregnant please inform the instructor.
15- Always wear closed toed shoes and long pants or a long skirt during labs to protect your legs and feet from spills.
16- Tie or pin long hair, scarves and headwear out of the way to avoid contact with flames or chemicals.

17- Be sure you know how to use equipment before beginning an experiment. If you are unsure - ASK !
18- Organic fluids (e.g., ether, acetone, chloroform) or other volatile liquids should be used inside a fume hood.
19- Chemical wastes should never be placed in a sink drain without permission. Please consult your instructor in all cases.
20- Media with microorganisms (liquid or solid) should be placed in a Biohazard bag or labeled autoclavable beakers for sterilizing and disposal.
21- All glassware should be rinsed after use and test tubes placed upside down in the racks provided. Slides and coverslips should be discarded.
22- Electric and gas points should be checked from time to time for any leakage.
23- Students and instructors should know the locations and operation of all safety equipment. This includes fire extinguishers, eye washes, body showers, first aid kits, and the nearest exit from their room.

Common disinfectants:

a. FORMALINE 5 – 10 % solution for cleaning floors, surfaces and containers.
b. HYPOCHLORITE with 10 % available chlorine e.g. Clorox. This is used for overnight soaking of contaminated laboratory glassware, cleaning surfaces, floors and equipment.
c. GLUTARALDEHYDE 2 % is good for cleaning of infected metallic objects.
d. PHENOLS as Lysol, sudol etc. for soaking of infected material.

Detergents: A number of detergents are available in the market. Any one can be used.

Following Protocols

It is crucial that protocols are not only followed carefully, but that you understand the purpose of each step. Frequently complications will be encountered and you can effectively trouble shoot only if you understand how the protocol works.

Since we are attempting to give you a real research experience, the protocols are presented as they would be in a research lab. This means the protocols are written much more concisely than you may be used to. For example, a protocol will not include details like "label your tubes" as it is generally understood that unlabelled tubes will lead to disaster.

When following a protocol, you must think and anticipate.

Using a protocol effectively and efficiently

1. Read the entire protocol ahead of time, making a list of any equipment or components needed. Make sure you understand what you need to do in each step.
2. Check that you have all the components and equipment needed and that you know how to use them.
3. As you work through the protocol, read each step in its entirety, right before you do it.
4. Anticipate. For example, if you will need another set of labelled tubes in step 5, prepare these tubes during a 5 min in step 3 or well before.

Class XI; Unit III, Chapter 9

Aim: To detect the presence of carbohydrates like glucose, sucrose and starch.

Learning Objective:

(i) To design the experiment for the presence of carbohydrates like glucose, sucrose and starch, make observations, record data, data analysis and interpretation of data.

Principle:

Carbohydrates with the free aldehyde or ketone groups (i.e., simple sugars) reduce copper sulphate of Benedict and Fehling's regent to cuprous oxide forming a yellow or brownish-red coloured precipitate depending on the concentration of sugar.

Colour	Approximate amount of reducing sugar
No change of blue colour	Absence of reducing sugar
Blue changes to green precipitate	0.1-0.5% of reducing sugar
Blue changes to yellow precipitate	to 0.5 to 1% of reducing sugar
Blue changes to orange-red precipitate	1.0 to 2.0% of reducing sugar
Blue changes to brick-red precipitate	Over 2.0% of reducing sugar

Requirement:

Glasswares – test tubes, funnel, beaker; Chemicals – Benedict's solution, Fehling's solutions A and B; concentrated HCl, saturated solution of $NaOH_2$, dilute iodine solution; Equipments – water bath; Miscellaneous – test tube holder, test tube stand, spirit lamp.

Sample for test:

Fruit juice of apples/banana/leaves of onion/sugar cane extract, milk etc.

Preparation of regents:

i) Benedict's regent: Mix 173g of sodium citrate and 100g of anhydrous sodium carbonate in 600mL of water in a beaker and warm gently (solution A). Dissolve 17.3% of hydrated $CuSO_4$ in 100mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make upto the mark with water.

ii) Fehling's reagent A: Dissolve 6.93g of copper sulphate in 100mL of distilled water.

iii) Fehling's reagent b: 20g of KOH and 34.6g of sodium-potassium tartarate (Rochelle's salt) dissolve in 100 mL of distilled water.

A. Test for glucose:

Procedure:

Take small pieces of banana, apple and grapes. Crush them separately and strain their juices in different test tubes. Take milk in another test tube. Cut fresh leaves of onion bulb into small pieces and boil for 2 to 3 minutes in a test tube and filter it.

(a) Benedict's test:

- Take 2 mL of juice (fruit extract) / milk / onion leaf juice in a test tube.
- Add 2 mL of Benedict's reagent to it and boil. Direct heating of test tube should not be done. It should be carried out in a water bath at the boiling point of water.
- The colour changes from blue to green and finally to orange or brick red indicating the presence of simple sugar (glucose).

Benedict's test

Name of the food items	Colour of the Precipitate	Inference drawn
1. Apple juice	1. _____	1. _____
2. Banana extract	2. _____	2. _____
3. Grapes juice	3. _____	3. _____
4. Onion juice	4. _____	4. _____
5. Milk	5. _____	5. _____

(b) Fehling's test:

- Mix equal volume of Fehling's solution A and B (1-2mL) in a test tube.
- Add equal volume of the fruit juice or onion leaf juice or milk and place it in water-bath at the boiling of water.
- The initial blue colour turns green to yellow and finally a brick-red precipitate is formed

Fehling's test

Name of the food items	Colour of the Precipitate	Inference drawn
1. Apple juice	1. _____	1. _____
2. Banana extract	2. _____	2. _____
3. Grapes juice	3. _____	3. _____
4. Onion juice	4. _____	4. _____
5. Milk	5. _____	5. _____

B. Test for Sucrose:

Procedure:

Collect sugarcane juice or cut sugarbeet into small pieces. Crush them and strain their juice.

(a) Benedict's and Fehling's test:

- Perform Benedict's and Fehling's test with cane sugar or sugar beet juice as described above.
- No change of colour indicates the absence of simple sugars (i.e., monosaccharide).

(b) Hydrolysis test:

- Take 10mL of sample juice in a beaker, add few drops of concentrated HCl and boil.
- After cooling, add 4 mL of saturated solution of NaOH or Na_2CO_3 to neutralize the solution. Now divide the solution into two parts. Transfer one part in test tube 'A' and the other in test tube 'B'.
- Perform Benedict's test with sample in test tube 'A'. Do you observe any change in colour? If there is a change, it indicates the presence of simple sugar or monosaccharide's.

C. Test for starch (iodine test):

Principle:

Iodine specifically makes a blue-coloured complex with starch.

Procedure:

- Add 1 or 2 drops of dilute iodine solution to 2 to 3mL of extract.
- A blue-black colour shows the presence of starch.
- The blue colour disappears on heating and reappears on cooling.

Discussion:

The composition of blue coloured substance is not well defined. This may be an adsorption complex of starch with iodine rather than a definite compound.

Questions:

1. What is the use of HCl in the test for carbohydrate?
2. Why does the blue colour disappear on boiling and reappear on cooling in the test for starch.
3. Sucrose solution gives a negative test with Benedict's test. Why?
4. Will iodine test give a positive result with glucose, fructose or sucrose solution?
5. Why are monosaccharide's called reducing sugars?

Class XI; Unit III, Chapter 9

Aim: To detect the presence of fats (lipid) in different plants and animal materials.

Learning Objective:

1. To design the experiment for the presence of the presence of fats (lipid) in different plants and animal materials make observations, record data, data analysis and interpretation of data.

Principle:

Lipids (fats) are usually insoluble in water but soluble in organic solvent. Sudan III reagent gives a specific red colour with fats.

Requirement:

Glassware's; test tubes; Chemicals: Sudan III, ethyl alcohol, chloroform, ether, benzene; Miscellaneous – test tube holder, test tube stand, oil/ghee/butter.

Procedure:

(a) Solubility test

- Arrange five dry test tubes in a test tube stand.
- Add 2mL of water, ether, chloroform, benzene and ethyl alcohol to each test tube followed by one drop of mustard oil, ghee or butter.
- Shake thoroughly.
- Oil, ghee or butter is broken into small droplets in water which float at the surface indicating that fat is insoluble in water. But in acetone benzene and ethyl alcohol no such droplets are formed as the oil, ghee or butter dissolves in these organic solvents.

(b) Sudan III test

- Take 2mL of mustard oil in a test tube.
- Add equal volume of water to it and shake.
- Add a pinch of Sudan III and shake again.
- As the layers separate out, the lipid layer is seen to be stained red, whereas water layer remains uncoloured. This indicates the presence of lipids.

Questions:

1. What is the simplest form of fat?
2. Will fat dissolved in organic solvent give a positive result with Sudan III.

Class XI; Unit III, Chapter 9

Aim: To detect the presence of proteins.

Learning Objective:

(ii) To design the experiment for the presence of Protein, make observations, record data, data analysis and interpretation of data.

Principle: Proteins respond to some colour reactions due to the presence of one or more radicals or groups of the complex protein molecule. All proteins do not contain the same amino acids, and hence they do not respond to all colour reactions. Nitrogen atoms in the peptide chain form a complex (violet colour) with copper ions in the Biuret test. (Biuret test is for peptide bond in the molecule of a protein). Xanthoproteic test is specific for protein containing aromatic amino acids. The benzene ring in the amino acids is nitrated by heating with nitric acid and forms yellow intro-compounds which turns to orange colour with alkali.

Requirement: Glasswares: test tube, spirit lamp, Chemicals: 40% NaOH, 1% $CuSO_4$ solution, Concentrated HNO_3, 20% NaOH solution, Miscellaneous – test tube holder, test tube stand.

Procedure:

(a) Biuret test

- Take 2 mL of protein solution (milk, albumin of egg or gram seed extract) in a test tube.
- Add 1 mL of 40% NaOH solution and 1 or 2 drops of 1% $CuSO_4$ solution.
- A violet colour indicates the presence of proteins. Care must be taken that excess of copper sulphate is not added otherwise there will be blue colour instead of violet colour.

(b) Xanthoproteic test

- Add carefully 1mL of concentrated HNO_3 to 2mL of protein solution (albumin of egg, milk or gram seed extract).
- A white precipitate is formed.
- Boil the solution and the colour changes to yellow.
- Cool the test tube and add 2mL of 20% of NaOH (or ammonia solution) to make it alkaline.
- The colour changes to orange indicating the presence of proteins.

Discussion: A yellow stain is often observed on skin when it comes in contact with nitric acid. The reason of yellow stain is xanthoproteic reaction.

Questions:

1. Why does the skin turn yellow when it inadvertently comes in contact with HNO_3?
2. Why are only few drops of $CuSO_4$ solution added during the biurest test?

Class XI; Unit V, Chapter 19

Aim: To test the presence of sugar in the given sample of urine.

Learning Objective:

1. To design the experiment for the presence of sugar in the given sample of urine, make observations, record data, data analysis and interpretation of data.

Principle:

In normal urine, practically there is no glucose. Presence of glucose in urine is called glucosuria. To detect reducing sugars, such as glucose, fructose etc. in urine Benedict's or Fehling's tests are done.

$CuSO_4$ present in Benedict's solution or Fehling's solution is reduced on boiling by the reducing substances (glucose, fructose etc.) to form the coloured precipitate of cuprous oxide. The light green, green, yellow and brick red precipitates of cuprous oxides depend on the concentration of reducing substances present in urine.

$$\underset{\text{Glucose (reducing sugar)}}{CH_2OH(CHOH)_4CHO} + \underset{\text{Cupric sulphate (blue solution)}}{2Cu^{++}SO_4^{-}} + 2H_2O \xrightarrow{\text{heating}}$$

$$\underset{\text{Gluconic acid (oxidized sugar)}}{CH_2OH(CHOH)_4COOH} + \underset{\text{cupric sulphate (red precipitate)}}{Cu_2O} + 4H^{+} + SO_4$$

Requirement:

Glasswares: Test tubes, beakers, spirit lamp, pipette; Chemicals: Benedict's solution, Fehling's solution A and B. Seliwanoff's reagent, Miscellaneous – Test tube holder, test tube stand, urine sample.

Preparation of Reagents

(i) Benedict's reagent Mix: 173g of sodium citrate and 100g of anhydrous sodium carbonate in 600 mL of water in a beaker and warm gently (solution A). Dissolve 17.3g of hydrated $CuSO_4$ in 100 mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make upto the mark with water.

(ii) Fehling's reagent A: Dissolve 6.93g of copper sulphate in 100mL of distilled water.

(iii) Fehling's reagent B: 20g of KOH and 34.6g of sodium-potassium tartarate (Rochelle's salt) dissolved in 100mL of distilled water.

(iv) Seliwanoff's reagent: Dissolve 50 mg of resorchinol in 33 mL of concentrated hydrochloric acid and dilute it to 100 mL with distilled water.

(v) In absence of appropriate samples containing abnormal components of urine, these components (glucose, albumin) can be added in the normal urine sample.

Procedure:

(a) Benedict's test

- Take 5 mL of Benedict's reagent in a test tube. Add 0.5 mL (8 drops) of freshly passed urine to it.
- Boil for 2 minutes holding the test tube firmly with a test tube holder (during boiling, the contents of the test tube get a tendency to spurt out. Hence, it is wise to keep shaking the test tube after holding it in the inclined position near the flame to avoid cause overboiling.
- A light green, green, yellow and brick red precipitate indicates the presence of reducing substances in urine.
- The various coloured precipitates depend on the concentration of reducing sugars in urine which gives a rough estimate of the concentration given below:

Colour of precipitate	% of reducing sugar present
Light green	0.1 to 0.5
Green	0.5 to 1.0
Yellow	1.0 to 2.0
Brick red	above 2

(a) Fehling's test

- Take equal volume (2 mL) of Fehling's solution A and B in a test tube. Mix them well.
- Add the above solution drop wise to 1 mL of urine sample taken in a test tube. Heat the test tube after each drop is added.
- A yellow or orange or brick red precipitate is formed which indicates the presence of reducing sugar in urine.

Note: Benedict's and Fehling's test are not necessarily indicative of only glucose in urine but it may also indicates the presence of other reducing sugars, such as lactose (in case of pregnant woman and lactating mothers), fructose (in fructosuria), galactose (in galactosuria), homogentistic acid (in alkaptonuria), glucoronates and mucin.

(c) Seliwanoff's test: The qualitative Benedict's test is not very specific test for glucose, since other reducing sugars also give positive tests, such as fructose, galactose, lactose, maltose, pentose. Other urinary constituents, drugs and contaminants in the urine may give false positive results. For detecting whether glucose or fructose is present in the urine. Selfwanoff's test should be performed.

This test is to be performed when urine sample give positive test for Benedict's or Fehling's test.

- Take 3 mL of Seliwanoff's reagent and add 1 mL of urine sample.
- Boil for two minutes. Appearances of red to orange colour indicates the presence of fructose.
- If no colour appears in 2 minutes, continue boiling for 5 minutes.
- If faint orange or no colour appears, then it indicates the presence of glucose.

Discussion: Test is sensitive between 50-80 mg glucose/100 mL urine. Less than this amount in the urine will not be detected by this test.

Class XI; Unit V, Chapter 19

Aim: To detect the presence of albumin in the given sample of urine.

Learning Objective:

1. To design the experiment for the presence of albumin in the given sample of urine, make observations, record data, data analysis and interpretation of data.

Principle: Nitric acid causes the precipitations of albumin. When heated or treated with sulphosalicylic acid, albumin undergoes coagulation.

Requirement: Glasswares: Test tubes, graduated pipette (5 mL capacity), spirit lamp; Chemicals: Concentrated nitric acid, Robert's solution, Sulphosalicylic acid or a solution containing 13% salicylic acid and 20% sulphuric acid: Miscellaneous – test tube stand, test tube holder.

Procedure:

(a) Nitric acid ring test

- Take 5 mL of concentrated nitric acid in a test tube.
- Incline the tube and add the urine sample with the dropper, so that the latter flows down slowly along the side of the test tube to form a separate layer.
- A white ring develops at the junction of the two liquids which indicates the presence of albumin in the urine sample.

OR

- Take about 5 mL of Robert's solution in a test tube.
- Now incline the test tube and add 2 to 3 mL of the given sample of urine by means of a dropper long the inner side of the test tube so that it forms a layer over the Robert's solution.
- The presence of white ring at the junction of two layers indicates the presence of albumin in the sample.

(b) Heat coagulation test

- Take about 6 to 8 mL of urine in a test tube.
- Incline the test tube at an angle and heat the upper one-third of the test tube by a low flame.
- Turbidity develops in the heated portion of the urine.
- Add 1% of acetic acid drop by drop and boil or simply add a drop of 33% acetic acid.
- If the turbidity persists it confirms the presence of albumin in the urine sample (disappearance of turbidity, confirms the presence of phosphates).
- A yellow or orange or brick red precipitate is formed which indicates the presence of reducing sugar in urine.

Note: Benedict's and Fehling's test are not necessarily indicative of only glucose in urine but it may also indicates the presence of other reducing sugars, such as lactose (in case of pregnant woman and lactating mothers), fructose (in fructosuria), galactose (in galactosuria), homogentistic acid (in alkaptonuria), glucoronates and mucin.

(c) Seliwanoff's test: The qualitative Benedict's test is not very specific test for glucose, since other reducing sugars also give positive tests, such as fructose, galactose, lactose, maltose, pentose. Other urinary constituents, drugs and contaminants in the urine may give false positive results. For detecting whether glucose or fructose is present in the urine. Selfwanoff's test should be performed.

This test is to be performed when urine sample give positive test for Benedict's or Fehling's test.

- Take 3 mL of Seliwanoff's reagent and add 1 mL of urine sample.
- Boil for two minutes. Appearances of red to orange colour indicates the presence of fructose.
- If no colour appears in 2 minutes, continue boiling for 5 minutes.
- If faint orange or no colour appears, then it indicates the presence of glucose.

(c) Sulphosalicylic acid test

- Take 3 mL of urine in a test tube.
- Add a few drops of sulphosalicylic acid ad heat it gently.
- A whitish or cloudy turbid solution or precipitate (coagulation) in the solution indicates the presence of albumin in the urine sample.

Discussion: A trace of protein which is less than 250 mg (in 24 hours urine) is found in normal urine. Under pathological conditions like albuminuria, albumin is found in urine above normal level. This amount is so negligible that it escapes detection by any of the simple test. In kidney disturbance and in high blood pressure, albumin level is urine is significantly high.

Questions:

1. What is the colour of the urine and name the pigments responsible for this characteristics colour?
2. In which organ of our body, highly toxic ammonia is converted into urea?
3. Name the disorder that shows presence of excess urea in the urine.
4. Name the disorder in which glucose level is high in urine.
5. What do you call those animals that eliminate nitrogen mainly in the form of urea?
6. Which other organ of our body also excretes urea in small amount?
7. Which reagents will demonstrate presence of protein in urine?
8. What is the significance of appearance of different colours while performing Benedict's test?
9. What is the significance of performing Selfwanoff's test?

Class XI; Unit V, Chapter 19

Aim: To detect the presence of bile salts in the given sample of urine.

Learning Objective:

1. To design the experiment for the presence of bile salts in the given sample of urine, make observations, record data, data analysis and interpretation of data.

Principle: Old and damaged RBCs are removed from the circulation mostly in the spleen and to some extent in the liver by macrophages. Hemoglobin of the RBCs is broken down in the cytoplasm of macrophages. When iron is removed from heme component of hemoglobin, the iron-free portion of heme is converted to biliverdin, a green pigment, and then into bilirubin, a yellow orange pigment. Bilirubin enteres the blood and is transported in the liver from spleen. In the liver, bilirubin is secreted by liver cells into bile, which passes into the small intestine and then into the large intestine. Bilirubin is detected in urine in certain pathological conditions only.

Requirement: Test tubes, measuring cylinders (am mL), funnel, dropping pipette or drop bottle, Lugol's iodine solution, barium chloride solution (10%,) Fouchet reagent, solphur powder, concentrated nitric acid, test tube holder, test tube stand, filter paper, distilled water.

Preparation of reagents:

(i) Lugol's iodine solution: Dissolve 1g of iodine crystals and 2g of potassium iodide in 100 mL of distilled water.

(ii) Fouchet reagent: Dissolve 25 mL of trichloroachetic acid (TCA) in 75mL of distilled water. Now add 1g of ferric chloride to this solution and mix.

(iii) 10% Barium chloride solution: Dissolve 10g of $BaCl_2$ in 90 ml of distilled water and make up the solution to 100 mL with water.

Procedure:

(a) Lugol's Iodine Test: Pour 4 ml of urine sample into a test tube. Add 4 drops of lugol's iodine solution to this tube. Shake the tube well and observe. A faint yellow to brown colour indicates absence of bile pigments while light to dark green colour indicates the presence of bile pigments.

(b) Gmelins Test: Take 5 ml of concentrated nitric acid in a test tube. Add an equal volume of the given urine sample to it slowly along the sides of the test tube. Formation of a green, blue, yellow or red ring at the junction of the two solutions indicates the presence of bill pigments.

Procedure: Bile pigments present in the urine react with concentrated nitric acid and induces formation of a coloured ring at the junction between the urine and acid layer.

(c) Fouchets test: Take 5 mL of the given urine sample. Add 2-5 mL of $BaCl_2$ to this test tube and mix the two solution. A precipitate will appear. Now filter the mixture. The precipitate containing the bile pigments remains on the filter paper. Add 2 drops of Fouchet

reagent to the precipitate on the filter paper. If the precipitate turns green, it shows the presence of bile pigments.

Discussion: The colourless bilrubin is oxidized by the ferric ion of ferric chloride (present in the Fouchet Regent) to green biliverdin.

Bilirubin + Fe^{+++}	Biliverdin + Fe^{++}
(colourless)	(green)

Questions:

1. Give the names of the pigments found in bile.
2. Which organ of the body produces bil pigments?
3. Which pigment provides colouration to the bile?
4. What are the functions of bile pigments?
5. How are the bile pigments produced?
6. Mention the name of the diseases during which excretion of bile pigments
7. What are the different tests to detect the presence of bile pigments in urine?

Class XI; Unit V, Chapter 19

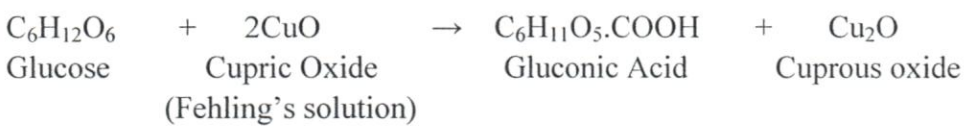
Aim: To estimate the amount of glucose in the given unknown solution.

Learning Objective:

1. To design the experiment to estimate the amount of glucose in the given unknown solution, make observations, record data, data analysis and interpretation of data.

Principle: A freshly prepared Fehling's solution is first standardized by titration against a standard solution of pure glucose A.R. The standardized Fehling's solution is then used to determine the amount of glucose in an unknown sample or solution by direct titration.

The Fehling's solution being a solution of cupric ions is blue in colour and at the end point changes to red colour precipitate of cuprous oxide. As the supernatant liquid is blue and the precipitate is red in colour, there may be some difficulty in determination of end point accurately. Hence sometimes a methylene-blue indicator is employed for accurate determination of the end point.



Theory

Chemicals needed

Fehling's stock solution A made by dissolving ~70 g CuSO4·5H2O in DDI water and diluting to 1.000 L

Fehling's stock solution B made by dissolving ~350 g KNaC4H4O6·4H2O (potassium sodium tartrate tetrahydrate) and 100.0 g NaOH in DDI water and diluting to 1.000 L. After sitting overnight, any sediment is filtered out.

Methylene Blue indicator, 1 % aqueous solution Dextrose, Unknown dextrose sample

Dextrose standard solution

1. Weigh accurately ~2.5 g of dried dextrose (i.e. glucose).
2. Dissolve in DDI water and dilute to 500.0 mL in a volumetric flask.
3. Mix well.

Fehling's Standardization Procedure

1. Accurately transfer 10.00 mL Fehling's solution A and 10.00 mL Fehling's solution B into a 250.0 mL Erlenmeyer flask. Use a separate volumetric pipet for each solution. Add approximately 30 mL of DDI water. Mix well.
2. Fill buret with dextrose standard. Make sure to drain to remove any bubbles.
3. Place flask on a hot plate turned to a medium heat. Heat to at least 70 °C but below boiling (use a thermometer). Throughout the titration, continue to monitor the temperature to make sure to keep the temperature of the solution consistently above 70°C. Remember that the addition of the dextrose titrant will cause the solution to cool.
4. Add dextrose standard until blue color nearly disappears. The mixture will remain a murky blue-gray with a reddish precipitate but won't completely clear up.

5. After the color mostly disappears (or after ~10 mL of added dextrose, whichever comes first), add 1 or 2 drops (no more) of methylene blue indicator.
6. Continue adding dextrose standard until the blue color disappears entirely and only a colorless solution with a red precipitate is present. It will help to hold a piece of white paper behind the flask. Just before the last few drops. At the end point
7. Using the concentration of dextrose in your titrant and the volume of the dextrose solution used for the titration, determine the mass of dextrose that reacts per mL of Fehling's reagent. ("Fehling's reagent" refers to the mix of 10 mL each of stock solutions A and B.)
8. Repeat the titration at least three times. Clean-up. Extra dextrose solution may be disposed of in the sink. The red precipitate (Cu2O) should be collected by gravity filtration on filter paper and placed in the appropriate container in the hood. The remaining liquid may be disposed of in the sink. Any excess Fehling's solution A should be placed in the copper waste container in the hood. Excess Fehling's solution B may be disposed of in the sink.

Unknown Determination

1. Quantitatively transfer the solution to a 500.0 mL volumetric flask, dissolve in DDI water and dilute to 500.0 mL. Mix well.
2. Fill buret with this dextrose unknown. Repeat titration as performed to standardize the Fehling's solution.
3. Repeat the titration using your unknown dextrose solution at least three times.
4. Use the mass of glucose that reacts per mL of Fehling's reagent (determined in standardization procedure step 7) to find the average concentration of dextrose in your prepared unknown (the 500.0 mL diluted solution).

From the final reading the normality of titrant can be calculated by the equation:

$$N_1V_1 = N_2V_2$$

After finding the normality, the amount of substance in the whole of the given solution can be calculated by the equation:

$$\frac{\text{Atomic weight} \times \text{Normality} \times \text{Volume of the given solution upto which it is made up}}{1000}$$

- **Note:** 10 times dilute the stock solution and that is used as titrant.
- Atomic weight of Glucose=180.1559 g/mol.

Observations and Calculations:

No.	Volume of Tirate (mL)	Burette Reading (mL)		Volume of Tirant used (mL)
		Initial	Final	
1				
2				
3				

Normality of Titrate used, N1=.....N.
Volume of Titrate used, V1 =.....mL.
Volume of Titrant Used, V2 =.....mL.
Therefore, the Normality of Titrant N2= $\frac{N1 \times V1}{V2}$ =.....N.

The amount of substance in the whole of the given solution =

$$\frac{\text{Atomic weight x Normality x Volume of the given solution upto which it is made up}}{1000}$$

=.....g.

Result:

- The amount of substance in the whole of the given solution =.....g.

Class XI; Unit V, Chapter 19

Aim: To Estimate the Saponification value of oils.

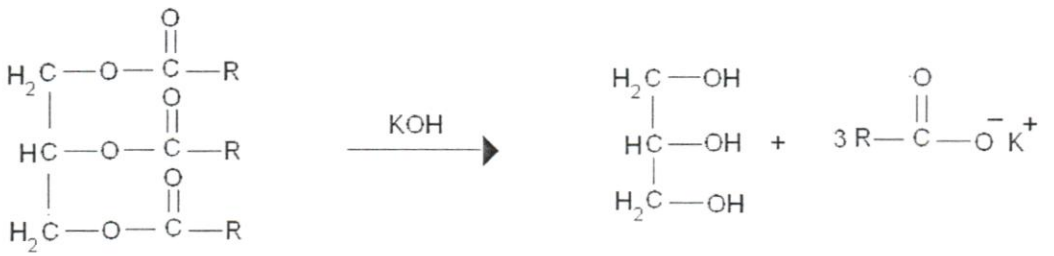
Learning Objective:

1. To design the experiment to estimate the Saponification value of oils, make observations, record data, data analysis and interpretation of data.

Theory: Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid.

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain, the less acid is liberated per gram of fat hydrolysed. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight .

Principle: Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NaOH) yield glycerol and potassium or sodium salts of fatty acids (soap) .



Procedure:

Materials Required:

1) Fats and Oils [coconut oil, sunflower oil]
2) Conical Flask
3) 100ml beaker
4) Weigh Balance
5) Dropper
6) Reflux condenser
7) Boiling Water bath
8) Glass pipette (25ml)
9) Burette

Reagents Required:

1) Ethanolic KOH(95% ethanol, v/v)
2) Potassium hydroxide [0.5N]
3) Fat solvent
4) Hydrochloric acid[0.5N]
5) Phenolphthalein indicator

Procedure:

1. Weigh 1g of fat in a tared beaker and dissolve in about 3ml of the fat solvent [ethanol /ether mixture].
2. Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent.
3. Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser.
4. Set up another reflux condenser as the blank with all other reagents present except the fat.
5. Place both the flasks in a boiling water bath for 30 minutes.
6. Cool the flasks to room temperature.
7. Now add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl .
8. Note down the endpoint of blank and test.
9. The difference between the blank and test reading gives the number of milliliters of 0.5N KOH required to saponify 1g of fat.
10. Calculate the saponification value using the formula :

Saponification value or number of fat = mg of KOH consumed by 1g of fat.
Weight of KOH = Normality of KOH * Equivalent weight* volume of KOH in litres
Volume of KOH consumed by 1g fat = [Blank – test]ml

Class XI; Unit V, Chapter 19

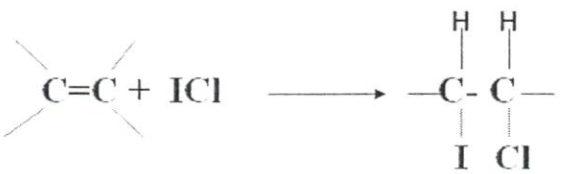
Aim: To determine the iodine value of fats and oils and thus estimate the unsaturation of the fats and oils.

Learning Objective:

1. To design the experiment to determine the iodine value of fats and oils and thus estimate the unsaturation of the fats and oils, record data, data analysis and interpretation of data.

Theory: Saturated fatty acids contain only single bond between the carbon atoms and are tend to be solids at room temperature. Unsaturated fatty acids contain double bonds between the carbon atom in addition to the single bonds present in the fatty acid chain. They are likely to exists as liquids at room temperature.
Unsaturated fatty acids can be converted into saturated by the process of hydrogenation. Depending upon the degree of unsaturation, the fatty acids can combine with oxygen or halogens to form saturated fatty acids

Principle: Fatty acids react with a halogen [iodine] resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monochloride reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.



Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple coloured product and thus the endpoint can be observed.

Important note:

1. Iodine monochloride is caustic. So handle the reagent with gloves.
2. For better results, perform the experiments without any time gap during addition of reagents as the liberated iodine is susceptible to oxidation by light.

Materials Required:

- Iodine Monochloride Reagent
- Potassium Iodide
- Standardized 0.1 N Sodium thiosulphate
- 1% Starch indicator solution

- Reagent bottle
- Chloroform
- Fat sample in chloroform
- Iodination flask
- Burette and burette stand with magnetic stirrer
- Glass pipette
- Measuring cylinder
- Distilled water

Method:

1. Arrange all the reagent solutions prepared and the requirements on the table.
2. Pipette out 10ml of fat sample dissolved in chloroform to an iodination flask labeled as "TEST".
3. Add 20ml of Iodine Monochloride reagent in to the flask. Mix the contents in the flask thoroughly.
4. Then the flask is allowed to stand for a half an hour incubation in dark.
5. Set up a BLANK in another iodination flask by adding 10ml Chloroform to the flask.
6. Add to the BLANK, 20ml of Iodine Monochloride reagent and mix the contents in the flask thoroughly.
7. Incubate the BLANK in dark for 30 minutes.
8. Mean while, Take out the TEST from incubation after 30 minutes and add 10 ml of potassium iodide solution into the flask.
9. Rinse the stopper and the sides of the flask using 50 ml distilled water.
10. Titrate the "TEST" against standardized sodium thiosulphate solution until a pale straw colour is observed.
11. Add about 1ml starch indicator into the contents in the flask, a purple colour is observed.
12. Continue the titration until the color of the solution in the flask turns colourless.
13. The disappearance of the blue colour is recorded as the end point of the titration.
14. Similarly, the procedure is repeated for the flask labeled 'Blank'.
15. Record the endpoint values of the BLANK .
16. Calculate the iodine number using the equation below:

Volume of Sodium thiosulphate used = [Blank- Test] ml

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Equivalent Weight of Iodine = 127
Normality of sodium thiosulphate ($Na_2S_2O_3$) = 0.1

Class XI; Unit V, Chapter 19

Aim: To perform the isoelectric precipitation of casein present in milk.

Learning Objective:

1. To design the experiment to perform the isoelectric precipitation of casein present in milk, record data, data analysis and interpretation of data.

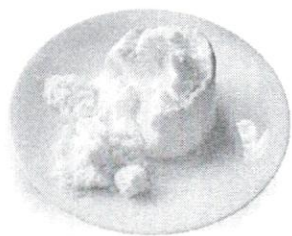
Principle: Casein, like proteins, are made up of many hundreds of individual amino acids. Each may have a positive or a negative charge, depending on the pH of the [milk] system. At some pH value, all the positive charges and all the negative charges on the [casein] protein will be in balance, so that the net charge on the protein will be zero.
That pH value is known as the isoelectric point (IEP) of the protein and is generally the pH at which the protein is least soluble. For casein, the IEP is approximately 4.6 and it is the pH value at which acid casein is precipitated.

Materials required:

1) Raw milk - 100ml
2) 0.2N HCl - 50ml
3) Diethyl ether - 50ml
4) 50% Ethanol - 50ml
5) Whatman No 1 filter paper strip (Size 25×50mm) - 2 no.

Procedure:

1. Measure 100ml of milk in a measuring cylinder and transfer 25ml of milk to four Oakridge centrifuge tubes each.
2. Centrifuge the milk in a centrifuge at 4000rpm at room temperature (25- 30o C) for 20 minutes. This is done to remove the fats and lipids from the mixture.
3. After centrifugation, carefully remove the fats and lipids from the surface of the milk with a spatula.
4. Then transfer the milk from all the tubes into a beaker and add equal volume of distilled water and stir well. Now check the pH.
5. Start adding 0.2N HCl drop by drop into the milk mixture and stir well.
6. Note the PH at which precipitation (white curdy substances) appears. The pH should be 4.6.
7. Take the curdy precipitate and allow it to sediment.
8. Now decant the supernatant using a filter paper and funnel and wash the precipitate with distilled water to remove the salts, then wash with diethyl ether and ethanol.
9. Dry the precipitate and take the weight of the casein and record it.



Class XI; Unit V, Chapter 19

Aim: Estimation of Vitamin C content in Different Types of Fruits

Learning Objective:

1. To design the experiment to perform the isoelectric precipitation of casein present in milk, record data, data analysis and interpretation of data.

Introduction: Vitamins are organic compounds whose traces are vitally essential for proper use of macronutrients (carbohydrates, lipids and proteins) in cell metabolism and hence, for proper growth and function. The term `vitamin' (L. vita = life + min = amine or vitally essential amine) was first used by **Funk**. Vitamins are relatively simpler organic compounds synthesized mostly by plants and certain intestinal bacteria. Hence, animals obtain most of these in traces **(=micronutrients)** from their food. Except for some, the vitamins are not generally stored in the body. Their useless excess is readily excreted out with urine. That is why, their regular, rather daily supply to the body from food is essential.

About 20 different types of vitamins have been properly discovered. These fall into two main categories those soluble in water and those soluble in fats. The water soluble vitamins include vitamin B-complex and vitamin C. The fat soluble vitamins include vitamin A, D, E and K.

Vitamin C also called **ascorbic acid** is a water soluble vitamin present naturally in many fruits and vegetables, especially in sour fruits like, lemon, orange, unripe mango, ambla, tomato etc. It is essential for the formation and growth of connective tissues, cartilages, bones, teeth etc. Playing an important role in iron metabolism, it is also required for the formation of RBCs. Its deficiency causes **scurvy** which is characterised by spongy and bleeding gums, loose and falling teeth, fragility of blood vessels, extensive haemorrhages in skin, fragility of bones, swollen joints, exhaustion, high fever and nervous breakdown.

Material Required: Different types of sour fruits (such as lemon, orange, unripe mango, ambla, tomato, guava etc.) and some non sour fruits like (apple, grapes, etc.), acidic 2, 6 dichlorophenol-indophenol solution, acetic acid, chloroform, ascorbic acid, distilled water, juicer, conical flasks, pipettes, burette, beaker etc.

Experimental Procedure:

i. Extract out juice from the fruits separately using juicer and keep these in separate beakers.
ii. Take out 10 ml juice of each type and dilute it to 100 ml with distilled water in separate test tubes.
iii. Take 10 ml of diluted fruit juice in a conical flask and add 2 ml of acetic acid and 2 ml of chloroform into its.
iv. Titrate the mixture with the acidic 2, 6-dichlorophenol-indophenol solution taken in a burette, till the colour of chloroform layer changes from colourless to pink.
v. Titrate 10 ml of standard ascorbic acid solution (i.e., ascorbic acid solution of known concentration) with acidic 2, 6-dichlorophenol-indophenol solution as above.

vi. Also titrate 10 ml of distilled water containing 2 ml of acetic acid and 2 ml of chloroform (i.e., blank solution) in the same way.

vii. Repeat each of the titrations to get at least three concordant readings.

Observation: Record your data and calculate the concentration of vitamin C for each type of fruit using the following formula

Concentration of vitamin $C = \frac{X-Y}{X-Z} \times$ volume of fruit juice

$\times$ Dilution of the juice = mg/100 ml of juice

where X = volume of 2, 6-dichlorophenol indophenol used in titrating the fruit juice

Y = volume of 2, 6-dichlorophenol-indophenol used in titrating the standard ascorbic acid solution

Z = volume of 2, 6-dichlorophenol-indophenol used in titrating the blank solution.

Present the data (i.e., concentration of vitamin C) of different fruits in a table given below :

S. No.	*Name of fruit*	*Concentration of vitamin C*
1.	Lemon	-
2.	Orange	-
3.	Unripe mango	-
4.	Ambla	-
5.	Tomato	-
6.	Guava	-
7.	Apple	-
8.	Grape	-

Conclusion: Different fruits contain different concentration of vitamin C. The concentration of vita-min C is more in sour fruits as compare to the non-sour fruits.

Reference: Give list of books and other reading materials you have consulted for the present project.

Viva Voce:

Q.1. What are vitamins?

Q.2. Who introduced the term 'vitamins' for the first time?

Q.3. How many type of vitamins have been discovered so far?

Q.4. What is hypervitaminosis?

Class XII; Unit VII, Chapter 8

Aim: To find out your own blood group.

Learning Objective:

1. To design the experiment to determine types of blood group, record data, data analysis and interpretation of data.

Determination of Blood group of human blood

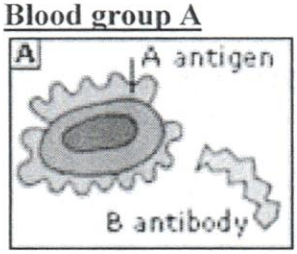
Requirement: Three glass slides, Anti sera A, Antisera B, Antisera D with separate droppers and Lancet.

Principle: The principle of the experiment is to find out the blood group by mixing the blood with the Antiserum and observe the agglutination by antigen antibody reaction.

Theoretical Explanation: At least 100 antigen can be recognized and more than 15 systems of well define antigens have been known, out of many antigen type A and type B are important which constitute OAB system. People are Rh +ve or Rh –ve In OAB system either both agglutinogens are present on the surface of RBC ,one of them present or both of them are absent on the surface of RBC .On this basis the population may be divided in to four blood group i.e .O,A,B,AB These are developed genetically according to genotypes. Blood group with their genotypes and Agglutinogen+agglutinins: Genotype Agglutinogens Agglutinins Blood group % OO - Anti A& Anti B O 47% OA or AA A Anti B A 41% OB or BB B Anti A B 9% AB AB - AB 3% The agglutinins are globulin which develops spontaneously due to A and B antigens which enter the body in food, bacteria and in other ways. Most naturally occurring antibodies to ABO antigen are Ig M class Rh antibodies are Ig G. Blood group antigens are present in surface of RBCs.

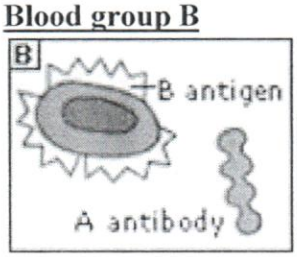
ABO blood grouping system: According to the AB0 blood group system there are four different kinds of blood groups: **A, B, AB and O** (null).

Blood group A



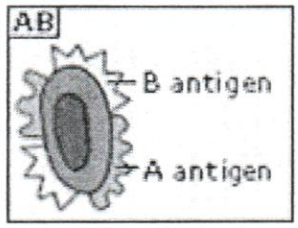
Blood group A: If you belong to the blood group A, you have A antigen on the surface of your red blood cells and B antibodies in your blood plasma

Blood group B

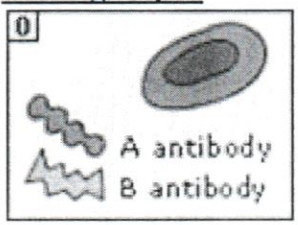


Blood group B: If you belong to the blood group B, you have B antigen on the surface of your red blood cells and A antibodies in your blood plasma

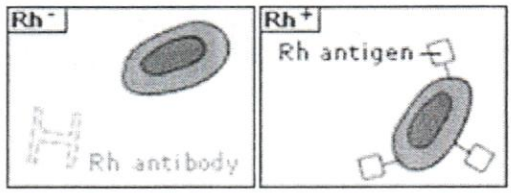
Blood group AB



Blood group O



Rh factor



Rh (Rhesus) factor is found on the RBC's surface in most people. Like A and B, this is also an antigen and those who have it are called Rh+. Those who lack the antigen on the surface of RBCs are called Rh-. A person with Rh- blood does not have Rh antibodies naturally in the blood plasma. But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies (as the immune system is triggered by the presence of an unknown antigen in the system). A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.

Principle behind blood tests: *Blood clumping or Agglutination observation.*

The serum of a person may cause agglutination of the red blood corpuscles (R.B.C.) of another person. Depending on the presence or absence of two agglutinogens A and B in erythrocytes and two specific agglutinins anti-A (α) and anti-B (β) in the serum, human blood groups are designated as A, B, AB and O. An R.B.C. may have either or both factors (A, B, AB) or none at all. A serum may have either or both factors (α, β, αβ) or none at all.
If the corpuscles contain A, the serum contains β, the blood group is A. If the corpuscles contain B, the serum contains a the blood group is B. If the corpuscles contain both A and B, the serum is free from α and β, the blood group is AB (universal receiver). If the corpuscles contain neither A nor B, the serum contains both α and β, the blood group is O (universal donor).

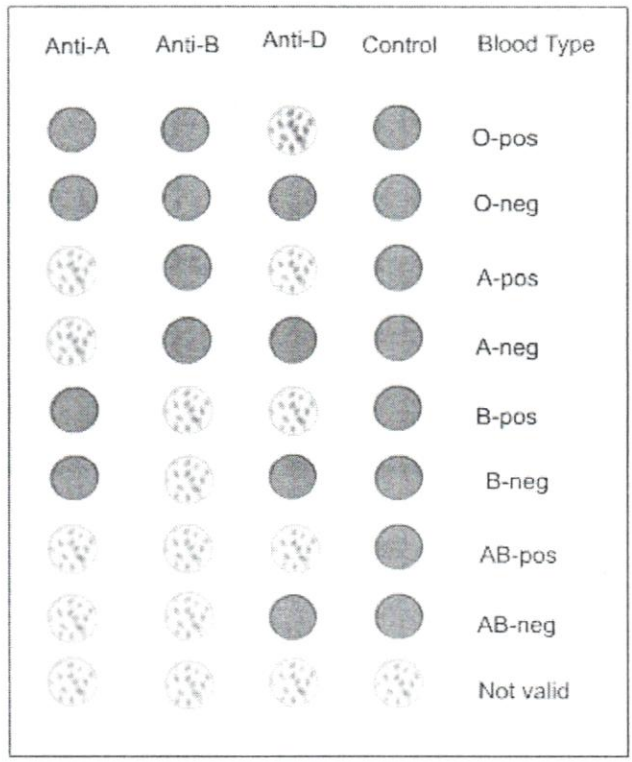


Image Courtesy :-
http://nobelprize.org/nobel_prizes/medicine/laureates/1930/landsteiner.jpg

Blood Group	Antigens	Antibodies	Can give blood to	Can receive blood from
AB Rh^+	A, B and Rh	None	AB Rh^+	AB Rh^+ AB Rh^- A Rh^+ A Rh^- B Rh^+ B Rh^- 0 Rh^+ 0 Rh^-
AB Rh^-	A and B	None (Can develop Rh antibodies)	AB Rh^- AB Rh^+	AB Rh^- A Rh^- B Rh^- 0 Rh^-
A Rh^+	A and Rh	B	A Rh^+ AB Rh^+	A Rh^+ A Rh^- 0 Rh^+ 0 Rh^-
A Rh^-	A	B (Can develop Rh antibodies)	A Rh^- A Rh^+ AB Rh^-	A Rh^- 0 Rh^-

			AB Rh^+	
B Rh^+	B and Rh	A	B Rh^+ AB Rh^+	B Rh^+ B Rh^- 0 Rh^+ 0 Rh-
B Rh^-	B	A (Can develop Rh antibodies)	B Rh^- B Rh^+ AB Rh^- AB Rh^+	B Rh^- 0 Rh^-
0 Rh^+	Rh	A and B	0 Rh^+ A Rh^+ B Rh^+ AB Rh^+	0 Rh^+ 0 Rh^-
0 Rh^-	None	A and B (Can develop Rh antibodies)	AB Rh^+ AB Rh^- A Rh^+ A Rh^- B Rh^+ B Rh^- 0 Rh^+ 0 Rh^-	0 Rh^-

https://www.nobelprize.org/educational/medicine/landsteiner/readmore.html

Materials Required:

- Monoclonal Antibodies (Anti-A, B and D)
- Blood Lancet
- Alcohol swabs
- Tooth picks
- Sterile cotton balls
- Clean glass slide
- Ice tray
- Biohazard disposal container

Procedure:

1. Set the table with all the materials required. Remember to place the Monoclonal Antibody (Mab) kit in an Ice tray.
2. Open an Alcohol swab, and rub it at the area from where the blood will be sampled (finger tip). (Discard the swab)
3. Open the Lancet cover, put pressure at the tip of the finger from where blood will be sampled (maintain it). Prick the finger tip with the opened Lancet.(Discard the Lancet)
4. As blood starts oozing out, make 1 drop fall on the three depressions of the glass slide. (in clinical setup, there will be a fourth well used as a control).
5. Place a cotton ball at the site where it was pricked. Using the thumb, put pressure on the area to stop blood flow.

6. Take the Anti-A (blue) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 1st spot. Place the bottle back in ice.
7. Take the Anti-B (yellow) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 2nd spot. Place the bottle back in ice.
8. Take the Anti-D (colorless) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 3rd spot. Place the bottle back in ice.
9. Take a tooth pick and mix the content in each well. Discard the tooth pick after using in one well (take a new one for the next well).
10. After mixing, wait for a while to observe the result.

Questions

1. What are the consequences of Rh incompatibility and what does it lead to?
2. Hemolytic disease of new born due to maternal fetal ABO in compatibility occurs more frequently as a result of which specific mismatch?
3. Two parents with blood types A and O have a child who has type O blood. What is the probability that their next child will be type A?
4. A woman is married for a 2nd time. Her first husband was blood type A and her son by that marriage was type O. Her new husband is type B and their child is type AB. What is the woman's blood group?

Class XII; Unit VII, Chapter 8

Aim: Estimation of Haemoglobin (Hb count) with the help of Sahli's Haemoglobinometer (acid haematin method)

Learning Objectives:

1. To design the experiment to perform the Estimation of Haemoglobin (Hb count) with the help of Sahli's Haemoglobinometer, record data, data analysis and interpretation of data.

Principle: Anticoagulated blood is added to the 0.1 N HCl and kept for 5-7 minutes to form acid haematin. The color of this acid haematin should be matched with the solution, present in the calibration tube. Distilled water is added to the acid haematin until the color matches and the final reading is directly noted from the graduation in the calibration tube. [Please note that 100 percent on the scale corresponds to 14.5gm % to 15gm %].

Requirements: Sahli's haemoglobinometer, Hydrochloric acid, distilled water.

Procedure:

a. After ensuring the hemoglobin pipette and tube are dry,add N/10 HCl into the tube upto mark 2g%

b. Mix the EDTA sample gently and fill the pipette with 0.02ml blood. Make sure that no air bubbles enter into the pipette. If it enters, discard and pipette again. Wipe the external surface of the pipette to remove any excess blood.

c. Add the blood into the tube containing HCl. Wash out the contents of the hemoglobin pipette by drawing in and blowing out the acid few times so that the blood is mixed with the acid thoroughly.

d. Allow to stand undisturbed for 10min.(This is because, maximum conversion of hemoglobin to acid hematin, occurs in the first ten minutes)

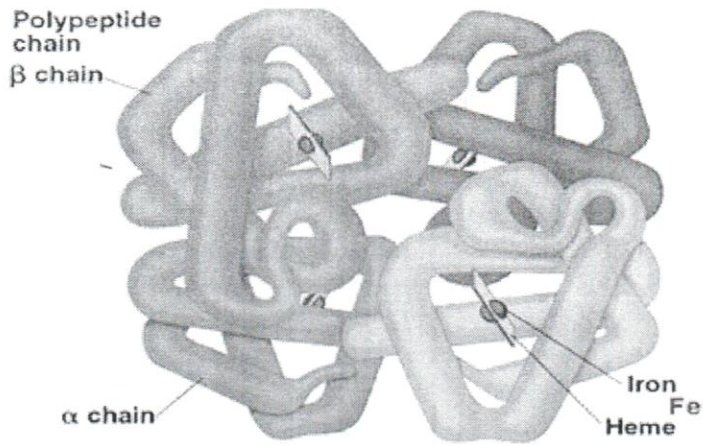
e. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop. Stir with the glass rod till it's color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.(Note that the stirrer should be above the level of the solution and not out of the tube)

f. The reading of the lower meniscus of the solution should be noted as the result. Express the hemoglobin content as g%.

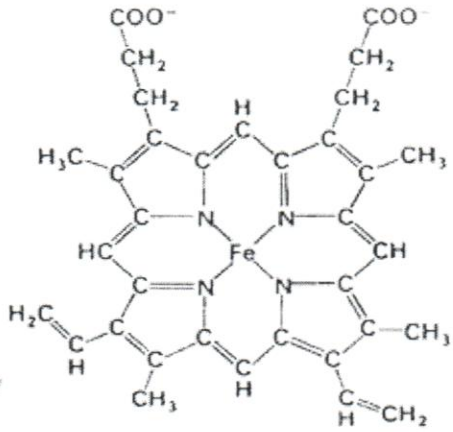
Result: The Hb estimation of the given sample is ..... g/100 ml of blood/.....g/dl of blood/.....G%.

Precautions

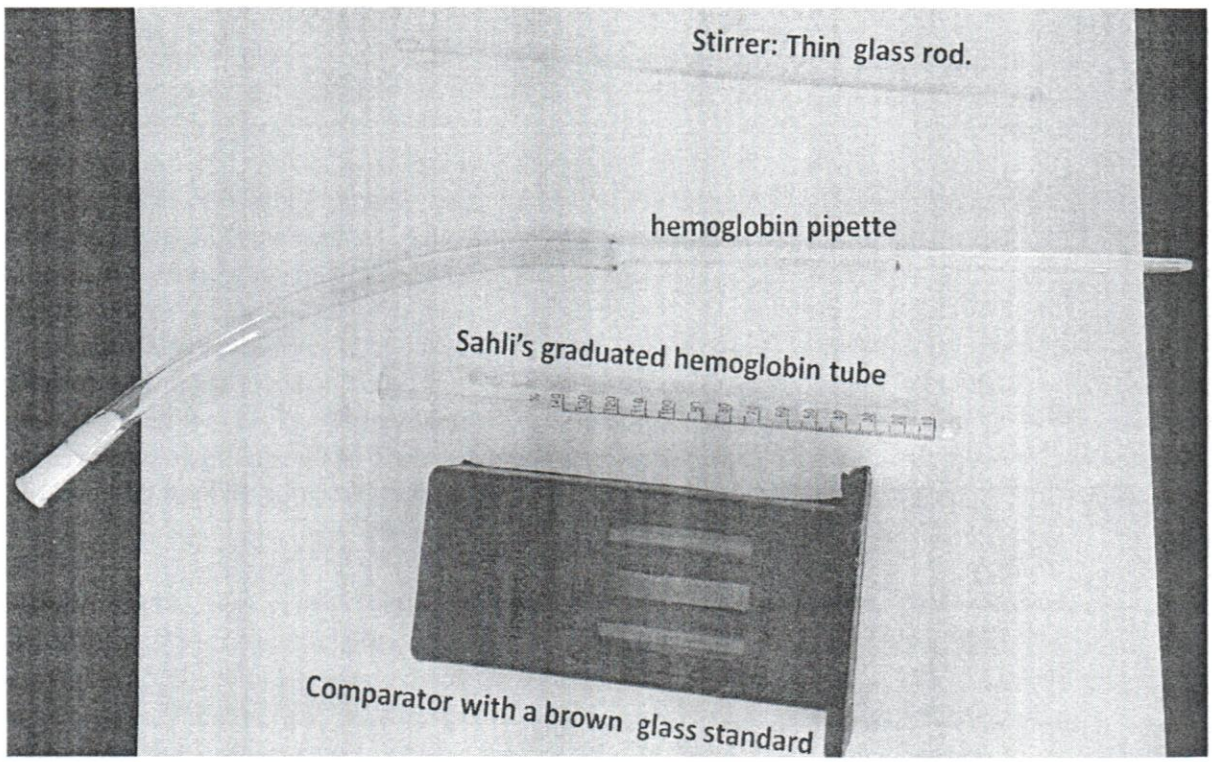
i. Pipetting of blood should be done cautiously
ii. Mix the blood properly with HCl by using stirrer
iii. Match the color



Hemoglobin



Heme
(Fe-protoporphyrin IX)



Sahli's Hemoglobinometer Parts-

a. **Sahli's graduated hemoglobin tube** (marked in grams percent g% (2-24) and percentage % (10 -140)

b. **Comparator** with a brown glass standard. opaque white glass is present at the back to provide uniform illumination.

c. **Sahli's pipette or hemoglobin pipette** (marked at 20µl or 0.02 ml). No bulb

d. **Stirrer:** Thin glass rod.

Questions

1. What are the indications of hemoglobin estimation?
2. What are the different methods of hemoglobin estimation
3. What is principle of acid hematin method of hemoglobin estimation?
4. Explain the procedure of Sahli's method of estimation of hemoglobin.
5. What are the advantages and disadvantages of this method?
6. What are the other colorimetric method of hemoglobin estimation

Class XII; Unit VII, Chapter 8

Aim: To prepare Haemin/ Teachmans crystals from human blood.

Learning Objectives:

1. To design the experiment to prepare Haemin/ Teachmans crystals from human blood, record data, data analysis and interpretation of data.

Learning Objective: The student should be able to:

1) Prepare the crystals from dried blood on some cloth, paper, file, floor etc
2) Recognize the human blood.
3) Know the medico legal value of this experiment.
4) Differentiate the human blood from animal blood from human blood or some other stain.

Requirement: Two glass slides, 2cover slips, Bunsen burner, solution of glacial acetic acid mixed with sodium chloride solution and the material for pricking the finger.

Principle of Experiment: The principle is to prepre the hydrochloride of haemoglobin (haemin's crystals by heating it with glacial acetic acid and sodium chloride solution and observe the shape of crystal.

Theory: Heamin's crystals are derived from Hb and are hydrochloride of haematin which is a ferric (Fe++) compound and obtained by gently heating hemoglobin with glacial acetic acid and sodium chloride solution. Under microscope they appear as rhomboid shape and reddish brown in color.

$$Oxy.Hb \longrightarrow Hb + O2$$

$$CH3COOHNa + HCl + HCl \text{ of } Hb \longrightarrow Oxy.Hb + CH3COOH + NaCl$$

This practical has a great medico legal importance and is performed to distinguish between human blood stain and some other spots. Human blood may be detected from suspected blood stain as the crystals of this shape are the only formed form of human blood.

Procedure: There are two methods to obtain Heamin's crystals.

Fresh blood method of direct method:

1. Sterilize the finger with spirit swab and prick it with prestirilized blood lancet.
2. Take two slides and put one drop of blood in center of each slide which has been cleaned properly.
3. Keep one slide on the table; leave the blood to dry NaCl proceeds with the other slide.
4. Put a drop of solution of with glacial acetic acid and sodium chloride solution on the fresh blood.
5. Put cover slip and heat the slide very gently on the flame in a wave like manner .Avoid continuous heating so as to prevent blood from burning.

6. Heat the slide till the fumes (bubbles) appear around the edges of the cover slip.
7. Stop heating and wait till the slides cool down. 8. Observe the slide under microscope first in low power 10x then in high power 45x

Dry Blood Method of Indirect Method:

1. Take the slide which was previously left on the table and the blood was allowed to clot.
2. Scrape the dry blood, mash it in powder form and collect it in the center of slide.
3. Put a drop of glacial acetic acid and sodium chloride solution and put cover slip on it.
4. Heat the slide till the fumes (bubbles) appear around the edges of the cover slip.
5. Wait till the slides cool down. Observe the slide under microscope .The crystal formed by this method relatively small in size than obtained by fresh blood method.

Medicolegal Significance: This experiment has no clinical value but has great medico legal importance because Heamin's crystals are formed only by human blood not from animal blood or any other stain .In animal blood the shape of crystal are entirely different .

Precautions:

1. Avoid continuous heating of slide on the flame as over heating will burn the blood.
2. Heat the slide till the fumes (bubbles) appear around the edges of the cover slip to void under heating.
3. If the crystals are not formed raises the cover slip put one drop of glacial acetic acid and sodium chloride solution and repeat the procedure.

Figure Haemin Crystals

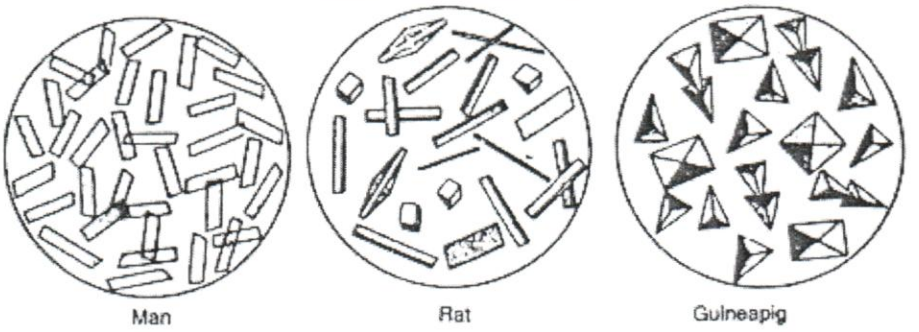


Fig. 33.4 : Haemin crystals

Man: Rhomboidal plates and prisms, often arranged in star-shaped clusters with round edges.
Rat: Narrow plates with varying width to needles, blunt at both the ends.
Guinea pig: Triangular plates often arranged in the form of squares.

Class XII; Unit VII, Chapter 5

Aim: To study pedigree charts of genetic traits like rolling of tongue, blood groups, widow's peak, colour blindness.

Learning Objectives:

1. To design the experiment to study pedigree charts of genetic traits like rolling of tongue, blood groups, widow's peak, colour blindness.

Requirements:

Pedigree charts for various traits.

Theory:

Pedigree:

A record of inheritance of certain genetic traits for two or more generations presented in the form of a diagram or family tree is called **pedigree**. It is employed in human beings and domesticated animals mainly pets. Because (a) cones crosses/mating cannot be carried out in human beings (b) generation time is long (c) number of offsprings per cour small. So the inheritance of traits in humans can be studied by pedigree analysis.

Advantages of Pedigree Analysis:

(iii) Pedigree analysis indicates Mendel's principles are also applicable to human genetics with some modification.

(iv) It helps to find whether the trait is dominant or recessive.

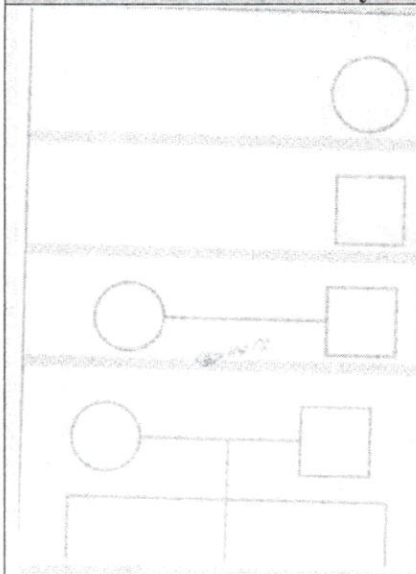
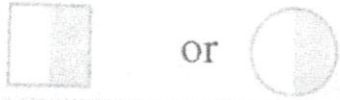
(v) It helps the genetic counsellors to advice the intending couples about the possibility of having children with defects like kaemophilia, colour blindness, sickle cell aliaemi, etc.

(vi) Carriers cannot be identified unless an affected child is borne to the carrier individual or without studying the pedigree

(vii) It helps in predictions regarding a character in children if related individuals or those with a family history for such are married.

(viii) Recessive traits are expressed in cases where both the parents are closely related. Symbols Used in Pedigree Analysis

Symbols used in Pedigree Analysis

Symbol		Meaning of Symbol
	Circle	Female individual
	Square	Male Individual
	Horizontal line connecting circle and square	Marriage
	Line perpendicular to marriage bar	Offsprings
	Shaded square or circle	Affected individual
	Half shaded square or circle	Heterozygous for a character
	Dot in middle of square or circle	Carrier of sex linked recessive character
	Rhombus with a number	Offsprings where sex is unspecified and no. indicates no. of offspring
	Two horizontal lines connecting square and circle	Marriage between close relatives.

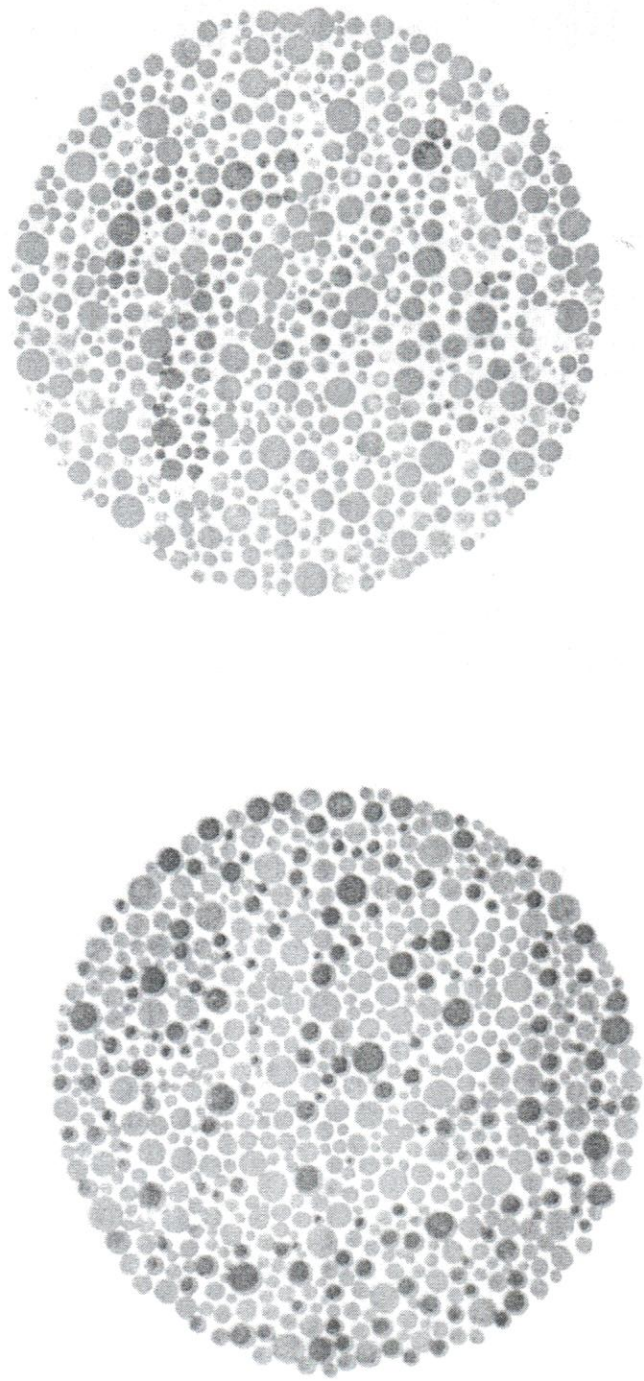


Chart to test the colour blindness disease in an individual

Common Genetic Traits:

1. **PTC Taster:** PTC (i.e., Phenyl thiocarbamide) paper has a sour taste. It is a genetic dominant trait. Genotype TT and Tt are tasters of PTC whereas tt are non-tasters, i.e, they do not taste PTC paper as bitter.
2. **Rolling of Tongue :** It is the ability of an individual to roll the tongue into U-shaped tube. It is also a dominant trait and inability to roll the tongue is recessive.

Tongue roller Non-roller

Fig. Tongue rolling trait

3. **Blood Groups :** It is a case of multiple alleles as there are 3 alleles-V, r, and VP and -113 are co-dominant and get straight hair lines
4. **Widow's Peak :** It is a hair line that forms distinctpeak as it crosses the forehead is said to have widow's peak. The homozygous dominant or heterozygous individuals have V-shaped front hair line whereas homozygous recessive individuals have straight hair

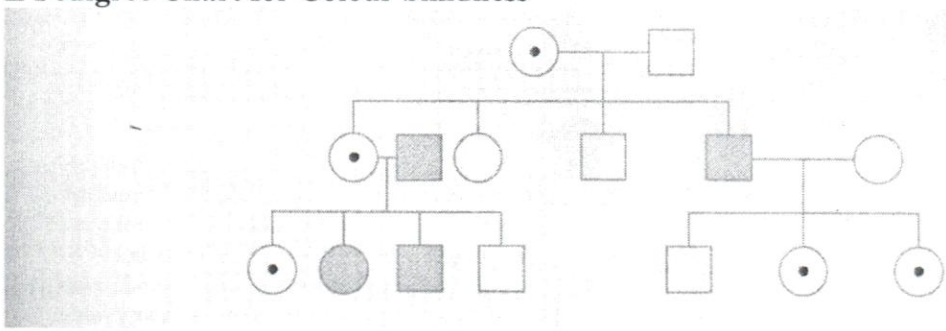
Widow's peak Straight hairline

5. **Sex Linked Inheritance:** The inheritance of such traits depend on the sex of the individual as the alleles for these traits are present on sex chromosome 'X'. 'Y' chromosome does not carry any allele for these traits. Such traits are more visible in males as compared to females because males have only one allele for that trait. e.g., Colour blindness, haemophilia.
6. **Fused Ear Lobe:** It is a trait due to recessive gene.

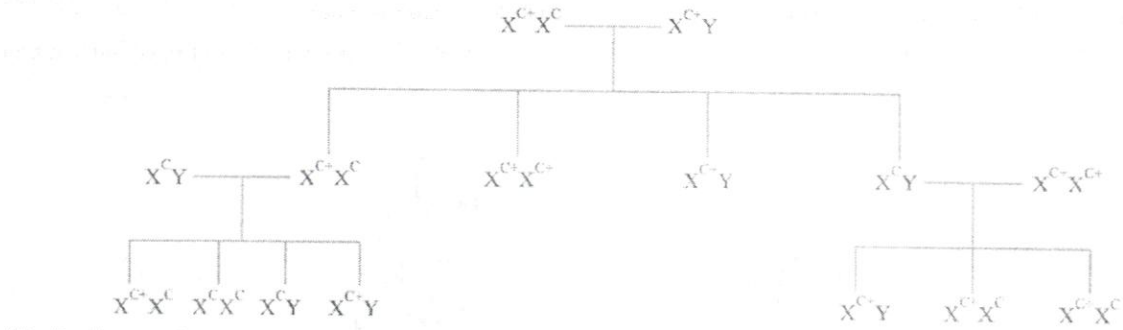
Procedure:

1. Observe the pedigree chart of various traits.
2. Try to find out the genotypes of the individuals.

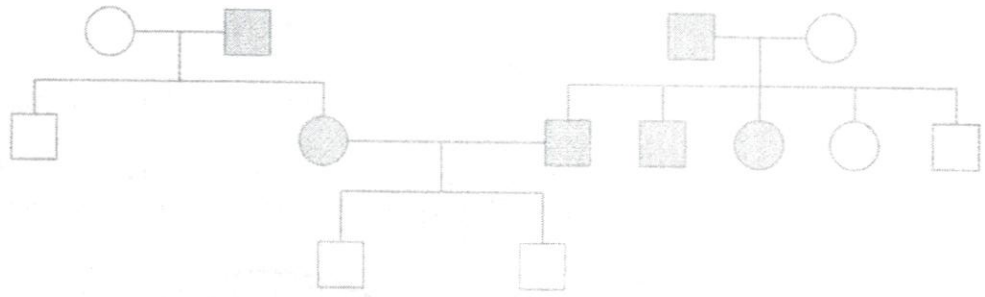
L Pedigree Chart for Colour blindness



Solution: As colour blindness is a sex linked character so the alleles have to be shown on sex chromosom (X^{C+} - Normal allele, X^{c} Colour blindness allele)

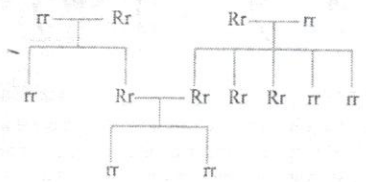


II. Pedigree Chart for Rolling Tongue

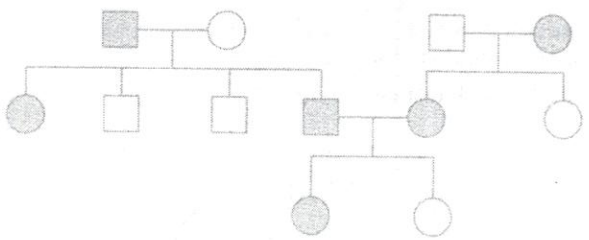


Solution : (*i*) As Rolling of tongue is a dominant trait and non-rollers are homozygous recessive.

(*ii*) Empty ◯ or □ represents normal trait and solid ● or ■ represent trait so possible solution is :

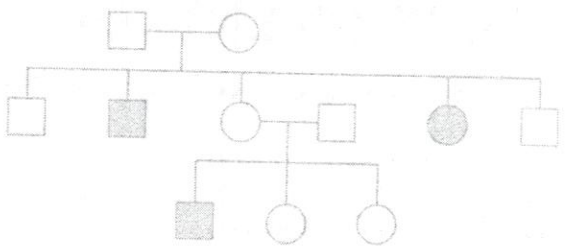


III. Pedigree Chart for Widow's Peak



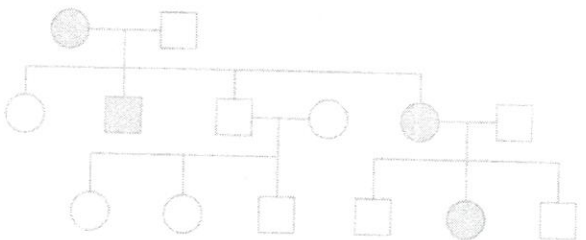
Hint for the solution to the problem is :

IV. Pedigree Chart for Sickle Cell Anaemia



[**Hint :** Sickle cell anaemia is an autosomal recessive disease.]

V. Pedigree Chart for Myotonic Dystrophy



[**Hint :** It is an autosomal dominant character.]

Questions:

Q. 1. What is pedigree analysis ?

Q. 2. What does • represent?

Q. 3. How many blood groups are there in human beings?

Q. 4. Name the universal recipient.

Q. 5. Name the universal donor and why are they called so?

Class XII; Unit VII, Chapter 6

Aim: To verify Mendel's law of Segregation.

Learning Objectives:

1. To design the experiment to verify Mendel's law of Segregation

Principle: When two pure lines with contrasting forms of a particular character (phenotypes) are crossed to produce the next generation (F_1 generation), all the members of the progeny are of only one phenotype i.e. of one of the two parents. The phenotype that appears is called dominant, and the one that does not appear is called recessive. When the F_1 plants are selfed, the progeny i.e. the F_2 generation is in the ration of 3 dominant: 1 recessive (3/4: ¼ or 75%: 25%). This reappearance of the recessive phenotype in F_2 generation verifies law of segregation.

Requirement: 64 yellow and 64 green plastic heads, all the exactly same shape and size, (when beads are not available, pea seeds may be coloured using paint, these beads represent the gametes of a specific trait), plastic beakers/petri dishes and a napkin/hand towel.

Procedure: Students have to work in pairs to perform the experiment. The following steps are to be strictly followed in the sequence mentioned below.

(i) Put 64 yellow beads in one beaker/petridish and 64 green beads in the other to represent respectively male and female gametes. Let the yellow bead be indicated by "Y" and green beads by "y".

(ii) Take a bead from each container and place them together (it represents fertilization) on the napkin spread before you on the table. (One student to take out beads and to put in the hand of the other student who will put them on the table.

(iii) Just like the previous step, continue to pick beads and arrange them in pairs. Thus 64 pairs of beads are obtained representing the 64 heterozygous F_1 progeny.

(iv) Put 32 F_1 progeny in one petridish and the remaining 32 in another petridish (representing the F_1 males and females)

(v) Stir the beads of each petridish with a pencil/pen for about 10 times taking care that no beads fall off.

(vi) To obtain the F_2 generation, one student would withdraw one bead from one beaker labeled male and one from the other beaker labeled female keeping his/her eyes closed (to ensure randomness), and put them together on the napkin spread over the table. Continue this process till all the beads are paired. Thus 64 offsprings of F2 are obtained.

(vii) Note the genotype (YY or Yy or yy) of each pair, and their possible phenotype.

(viii) Have six repeats of the experiment (steps i to vii) with partners changing their roles. Pool all the data from the six repeats together.

(ix) Calculate the genotypic and phenotypic ratios of your pooled data. Note the larger the sample size, more accurate is the result.

Observation: Record the result in the following table

Generation	Repeat No.	Total no. of individuals	Genotype (s) YY Yy yy	Phenotype (s)
F_1	1.			
	2.			
	3.			
	4.			
	5.			
	6.			
		Total		
F_2	1.			
	2.			
	3.			
	4.			
	5.			
	6.			
		Total		

Phenotypic ratio in F1.....

in F2.....

Genotypic ratio in F1.....

in F2.....

Discussion: The results are so because each diploid individual contains two copies of every gene -one copy on each of the two homologous chromosomes. These two copies of the gene may be of similar type (YY or yy) or are dissimilar Yy. The former (YY or yy) are called homozygous for that particular character, and the Yy are called heterozygous ones. The pure lines in the above cross are homozygous ones, which contributed only one copy of their gene (as a result of meiosis) to their F_1 progeny to restore its diploid nature with genotype Yy (heterozygous) where only one form **(allele)** is expressed (dominant) and the other form **(allele)** is not expressed (recessive). This is the phenomenon of **Dominance.**

When the F1 individuals are crossed together to raise the F_2 generation, each F1 individual produces two types of gametes: 50% having dominant allele, and the remaining 50% having recessive allele. These gametes undergo random fusion during fertilization to produce the F2 generation. According to simple probability of mixing of opposite sex gametes (sperms and ova), offsprings of three genotypes are likely to appear as follows: [(half of gametes of Y type + half of remaining gamete y type) X (half gametes of Yr type + half of remaining gamete ofy type)] = One-fourth of F2 individuals of YY phenotype + half of F2 individual Yy type + one-fourth of F2 individual of yy type. Among these proportion of dominant phenotype would be ❑ YY+ = ❑ yellow and recessive phenotype D yy i.e. green phenotypes in 3:1 or 75%:25% ratio.

This ratio of 3:1 in the F2 suggests that in the F_1 heterozygotes, the recessive allele does not get destroyed and remains only in the recessive (dormant) state to get an opportunity to express itself when it has separated from the influence of the dominant allele (Y). This is called Law of Segregation of the alleles.

Questions:

1. Do you expect the same results in terms of 3:1 ratio in F_2 if you had started with smaller number of beads (say 10 beads)?

Class XII; Unit VII, Chapter 6

Aim: To verify the Mendel's Law of Independent Assortment

Learning Objectives:

1. To design the experiment to verify the Mendel's Law of Independent Assortment

Principle: In a dihybrid cross, the segregation of one gene pair is independent of the segregation of the other pair. It means that genes of taco different traits assort independently to give a probability ratio equal to segregration probability ratio of one allele pair X segregation probability ratio of other allele pair, which comes to, (3:1) X (3:1) = 9:3:3:1 Requirement: Plastic beakers; 64 plastic beads each of yellow, green, red and white to represent, yellow and green colour of seed coat and red and white flowers respectively and napkin/hand towel

Procedure: Students are to work in pair.

The following steps are to be followed sequentially:

(i) Place 64 beads of each colour in four separate beakers.

(ii) Put the beakers containing the yellow and red beads on your left side, and those containing the green and white beads on your right side. The beakers on your left side represent plants bearing yellow seed and red flower (dominant character YY, RR). Beakers on the right side represent plants bearing green seeds and white flowers (recessive character yy, rr). These are the two parental types having contrasting forms of two different characters.

(iii) Stir the beads in each beaker with a pencil/pen. Each bead now represents alleles in the male and female gametes.

(iv) Pick up one yellow, one green, one red and one white bead, and put them together on the napkin spread on the table.

(v) Continue picking up and putting together of the beads of all colours as mentioned in the previous step, till all the beads are utilised.

(vi) Note that in all, 64 such 4-bead clusters are obtained representing the F_1 individuals. Ascertain their genotype and phenotype.

(vii) Next step is to cross these F1 individuals to raise the F2 generation. Let us suppose half of the 4-bead clusters (32 clusters) represent the male parents and the remaining half (32 clusters) the female parents. Now put the 32 red and 32 white beads together in one beaker (numbered-I), and similarly put 32 yellow and 32 green beads together in other beaker (numbered-ti). These two beakers represent F. female. Similarly put remaining 32 red 4- 32 white beads in beaker numbered-ill, and 32 yellow and 32 green one in beaker numbered-IV to represent the F, male. The arrangement can be presented as below

Female F_1	Male F_1
32 red + 32 white (Beaker I)	32 red+32 white (Beaker III)
32 yellow + 32 green (Beaker II)	32 yellow + 32 green (Beaker IV)

(viii) Stir the beads in each beaker with a pencil. In order to raise the F2 generation, pick up (with eyes closed) one bead from the beaker-I of female and one bead from the beaker-Ill of the male, and put into the palm of the partner student. Similarly, pick up one bead each from the beaker-11 of female and beaker IV of male to put in the palm of the partner. This partner would now keep all the four beads together (to represent the F2 individual). Continue this process till all beads are utilised. At the end, 64 F2 individuals (each represented by a 4-bead cluster) are obtained.

(ix) Determine the genotype and phenotype of each of the 64 F2 individuals and write down the number of individuals of different genotypes and phenotypes in the tabular form (given below), remembering that (yellow seed colour) is dominant over y (green seed) and R (red flower) is dominant over r (white flower).

(x) Repeat the whole procedure (steps i to ix) six times, and tabulate your results.

Observation: Tabulate the results as follow:

Symbol (-) indicates the presence of corresponding dominant or recessive allele e.g. Y or y and R or r.

Summarise your results (adding together the data of all the six repeats)

Fl Generation

(a) Total number of individuals: _____

(b) Phenotype (s) _____

(c) Genotype (s) _____

Generation & Repeat	**Total number of individuals**	**Genotype**				**Phenotype**			
		Y-R-	**y-rr**	**yyR-**	**Yyrr**	**Yellow Red**	**Yellow White**	**Green Red**	**Gr wh**
F_1									
1.									
2.									
3.									
4.									
5.									
6.									
Total									
F_2									
1.									
2.									
3.									

4. 5. 6. Total									

F2 Generation

(a) Total number of individuals _____

(b) Phenotypes _____

(c) Number of individuals in each phenotypic class.

Number	*Phenotype*
_____	_____
_____	_____

(d) Phenotypic ratio _____

(e) Genotypic ratio _____

(f) Number of individuals of each genotypic class:

Number	*Phenotype*
_____	_____
_____	_____

(g) Genotypic Ratio _____

Discussion: The four phenotypic classes in the F2 generation are in ratio of 9:3:3:1 as expected from the **Law of Independent Assortment.** The genotypic ratio would be (1 :2:4:2): (2:1):(2:1):1.

Note:

1. In case six repeats of the experimental procedure are not feasible due to time limitations, either the number of repeats be slashed down to three or the data from single repeat of six different pair of students may be pooled together to make the final calculations.
2. This Law of Independent Assortment was later found to be true only for traits present on two different homologous pair of chromosomes, that is, the two are not linked together. The linked traits do not assort independently, rather they are inherited together (linked) except when crossingover separates them.
3. It is quiet likely that you may not find your data exactly in the expected ratio, instead almost -approximate to it. The statistical significance of this deviation from the exact expected ratio due to probality can be checked using chi-square (x^2) test, about which you will study in higher classes.

Questions:

1. Linked traits fail to assort independently. Explain.
2. How is independent assortment of alleles important from the point of view of variation?

Learning Objectives:

To inculcate laboratory skills of handling apparatus, designing the experiment, making observations, recording data, analysis and interpretation of data, drawing diagrams.

Aim: To determine water potential of the cell cap by plasmolytic method.

Requirements: *Rhoeo* leaves, forceps, needle brush, slides, cover slides, watch glasses, sucrose, microscope.

Principle: The free energy per mole of a substance is called its chemical potential. Water molecules also posse's kinetic energy and in liquid and gaseous form they are in random motion that is both raped and castanet. The chemical potential of water is called water potential. As pure water will have greater kinetic energy or water potential as coppered to a solution. By conversion the water potential of pure water of staudandard temperature is taken to be zero (Ψ =0).

When a cell becomes turgid, the pressure against the cell wall increases. This is called pressure potential and is denoted by Ψ 1. Water potential of a cell is affected by both solute and pressure potential. The relationship between them is as follows:

$$\Psi w = \Psi s + \Psi p$$

If Ψp is zero then Ψw will be equal to Ψs

Plasinolysis occurs when water movement of the cell and cell membrane shrinks away from the cell wall. This occurs when the cell is placed in a hypotonic solution. When the cell is placed in an isotonic solution there is no net flow of water towards inside or outside and the external concentration balances the solute concentration of the cytoplasum and the cell is in the flaccid condition where pressure potential will be zero and the water potential will be equal to solute potential of the cytoplasum.

By plaswolytic method we determine the molar concentration (M) of the isotonic solution at incipient plaswalysis. The water potential can then be calculated as:

Ψw = **Mirt**

Where M	=	molar concentration of isotonic solution
i	=	iorization constant
r	=	gas constant = (.0087 bars)
t	=	Room temp + 273

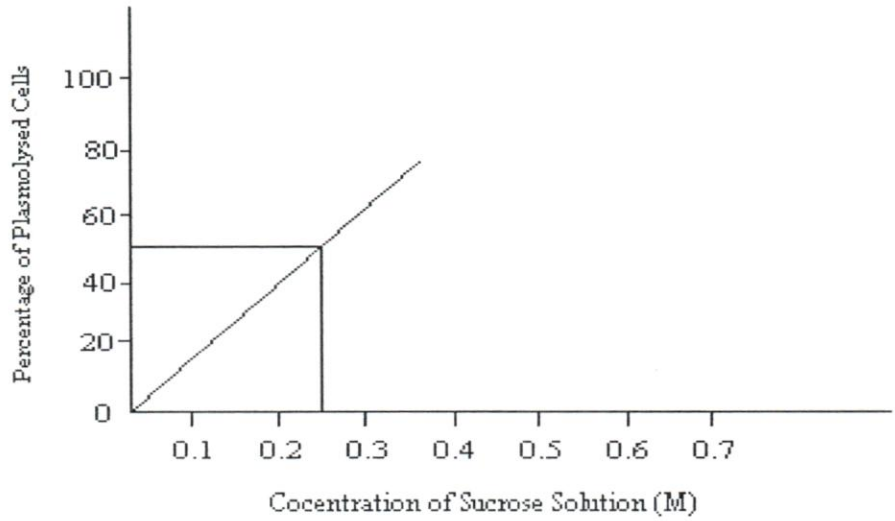
Procedure:

1. Prepare thin one cell thick peels from the lower surface of *Rhoeo* leaf and put these peels in a watch glass containing water.
2. From 1 M solution of sucrose prepare seven dilutors – 0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 0.6 M and 0.7 M and put them in seven different watch glasses.
3. Put two pieces of leaf seeds each of these dilutroies.
4. After five minutes take out a peel and mount on a slide putting a cover ship.
5. Observe these peels under a microscope counting the number of plasmolysed and unplasmolysed cells for each concentration of sucrose.

Observations and Calculations: On the basis of observations made prepare a table as follows:

S. No.	Concentration of sucrose solution	No. of plasmolysed cells	Total No. of cells	% of plasmolysed cells
1.	0.1 M			
2.	0.2 M			
3.	0.3 M			
4.	0.4 M			
5.	0.5 M			
6.	0.6 M			
7.	0.7 M			

Prepare a graph on the basis of above tabulated observations.



Aim: To educate the participants about prevailing preventive diseases

Theory: Diseases are of 2 types

(i) Infectious: Infectious diseases are disorders caused by microorganisms like bacteria, viral, fungi & parasites and can be spread from one person to another directly or indirectly.

(ii) Non-infectious: Non-infectious diseases do not spread and are due to changes in physiology of human body or accidents.

Broadly, infectious diseases are classified as:

1. Airborne infectious diseases
2. Food & water borne infectious diseases
3. Vector borne diseases.

Airborne infectious diseases:

- Influenza / Flu - Viral cause
- Pharyngitis - Viral or Bacterial
- Common Cold - Viral
- Chicken Pox
- Mumps
- Measles
- Pulmonary tuberculosis - Bacterial always
- Meningococcal meningitis
- Pneumonias

Symptoms

- Inflammation of nose, throat, sinuses or lungs
- Coughing
- Sneezing / rummy or stuffy nose
- Congestion
- Headache
- Swollen glands
- Body aches
- Fever
- Loss of appetite
- Fatigue / weakness
- Rashes

Treatment

- Adequate rest
- Keep hydrated with esteem inhalation
- Gargling & clean habits

- Hot beverages
- Simple & digestible diet
- Antihistaminic + Antipyretics
- Antibiotics at times.

Food & water borne diseases:

- Diarrhea / Dysentery
- Amoebiasis
- Cholera
- Typhoid
- Hepatitis 'A' or Viral hepatitis
- Jaundice
- Poliomyelitis
- Rat fever
- Swimmer's ear / otitis externa

Symptoms

- Loose motions
- Vomiting and Nausea
- Pain in abdomen
- Fatigue, weakness, lethargy
- Fever
- Dehydration
- Anorexia (reduced appetite)

Treatment

- Rest
- Proper hydration
- Antidiarrhoreals / Antiamoebics
- Antiemetic + Antipyretics
- Stool binders
- Antacids + Probiotics
- Easily digestible simple food
- Clean habits

Vector borne diseases:

- Diseases: disease transmitted by infected insects, ticks, bug, sandflies, rats.
- 17% of all infectious diseases 7,00,000 deaths annually.
- Malaria - Plague
- Dengue - Relapsing fever

- Chickunguniya - Japanese encephalitis
- Leishmaniasis
- Schistosomiasis - Zika
- Rickettslosis

Symptoms

- Loose motions
- Vomiting and Nausea
- Pain in abdomen
- Fever
- Fatigue, weakness, lethargy
- Dehydration

Aides Mesquites: Bite during daytime

- Chikungunya
- Dengue Fever
- Lymphatic Filariasis
- Yellow fever

Anopheles: Bite from dusk to dawn

- Malaria
- Lymphatic Filariasis

Culex: Bite during daytime

- Japanese Encephatitis
- Filariasis
- West mile fever

Sandflies

- Leismariasis

Ticks

- Rickettsial fever
- Relapsing fever
- Tick borne encephalitis

Fleas

- Plague
- Rickettsiosis

Malaria: Caused by *Plasmodium vivax, Plasmodium culax, Plasmodium Aedes, Plasmodium falciparum.*

- Transmitted by Mosquitoes

Symptoms

- High grade fever with chills
- Pain in abdomen
- Headache, bodyaches
- Nausea & Vomiting
- *Plasmodium vivax* cause, simple Malaria treatable with Chloroquine
- *Plasmodium falciparum* cause cerebral Malaria

Treatment

- Anti-malarial
- Supportive treatment
- Cerebral Malaria is dangerous and patient needs to be hospitalized

Chikungunya: Viral disease transmitted by *Aedes* Mosquito

- Moderate to high fever with severe joints & body pains are characteristics
- Patient may have joint pairs for quite a long time after cure.

Dengue Syndrome: Caused by a virus, spread by *Aedes* mosquito

Symptoms

- Febrile illness of 2-7 days duration
- Headache
- Pain around eyes
- Bodyaches, joint pains
- Rashes – patechiae
- Pain epigastrium
- Hemorrhage
- Anorexia
- Vomiting
- Epitasis / Gum bleeding

Dengue infection may be:

- Asymptomatic
- Classic Dengue fever
- Dengue Hemorrhagic fever with shock
- Dengue Hemorrhagic fever without shock
- 3 & 4 types are dangerous

Treatment

- Symptomatic & Supportive Rehydration

- If platelet count < 10,000/mm^3 plasma with platelet concentrate is given iv.

Prevention

- Avoid crowded places
- Clean habits
- Wash hands with soap & water frequently & before & after food.
- Hand sanitizers
- Avoid touching face, mouth, nose, eyes
- Stay away from people affected with cough and cold / flu
- Avoid close contact with affected people
- Wear face mask
- Cover mouth while sneezing or coughing with tissue or hanky
- Avoid hand shake
- Proper sleep
- Proper intake of food & water
- No road side eating habits
- Lots of fruits & vegetables.
- Decrease Stress
- VACCINATION on time
- Use mosquito nets, mosquito repellants
- Cover body fully in mornings & evenings
- Do not let water collect
- Use of spray on stagnant & dirty water bodies.
- Avoid use of fish in ponds which eat mosquito larvae

Experiments based on Human Physiology

Exercise:1

Aim: To prepare a permanent stained mount of placoid scale.

Requirements: Fish scales, Slides, cover slip, test tubes, test tube holder, KoH, Alcohol Series, Eosine, DPX and Compound Microscope.

Procedure:

- Cut a small piece of skin from the dorsal surface of fish (obtained from fish market).
- Boil it in 5% KOH in a test tube until a brown residue settles at the bottom of the test tube.
- This brown residue is placoid scale which have been detached from skin during boiling.
- Let the residue to settle down on the bottom of the test tube.
- Remove the KOH solution.
- Wash scales in water
- Dehydrate up to 70% alcohol.
- Stain in borax carmine.
- Mount in DPX/ Canada balsam.

Results & observations:

1. Placoid scales are minute, transparent, dermal denticles embedded in the skin in oblique rows.
2. It has diamond shaped basal plate having trident spine projecting out from skin.

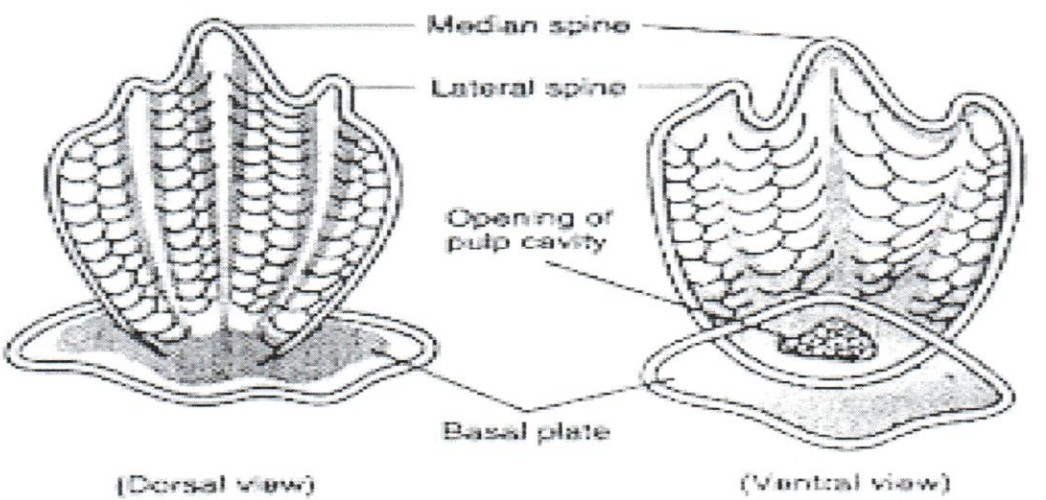


Fig. 3.3(a) : Placoid

Exercise: 2

Aim: To prepare a permanent stained mount of Cycloid scales. Labeo (Rohu)

Requirements: Fish scales, Slides, cover slip, test tubes, test tube holder, KoH, Alcohol Series, Eosine, DPX and Compound Microscope.

Procedure:

- Take few scales from the body of fish and stain in picro indigo carmine / borax carmine.
- Dehydrate upto 70% alcohol.
- Mount in DPX / Canada balsam.

Results & observations

1. Cycloid scales bear concentric lines of growth around a central nucleus. This lines shows its age.

Questions:

1. What are different types of scales in fishes?
2. How would you differentiate between different types of scales

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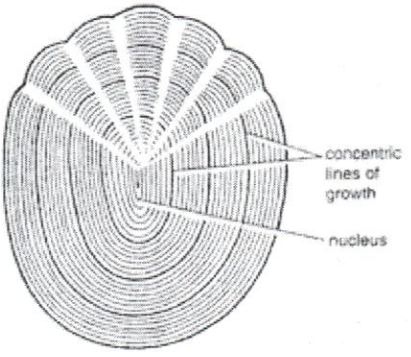


Cycloid scale

Exercise: 3

Aim: To prepare a permanent stained mount of stripped muscles in the given Animal tissue (Goat / Chicken can be obtained from meat shop).

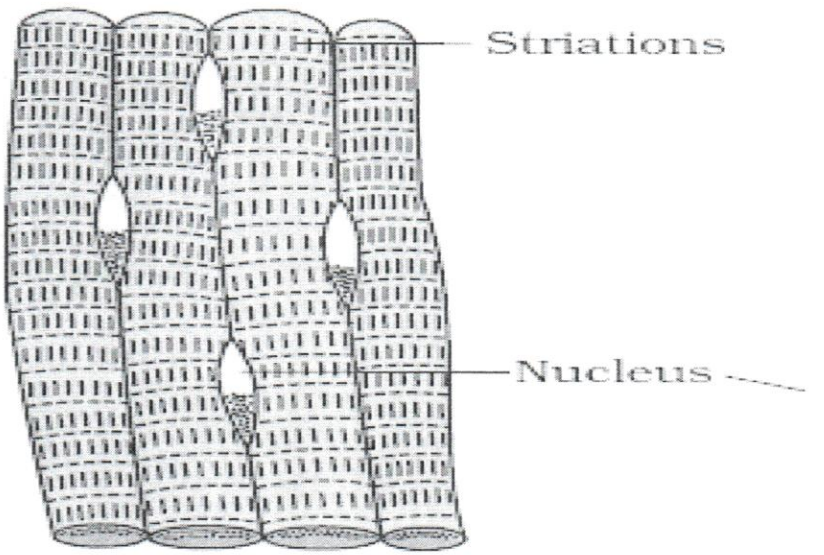
Requirements: Thigh muscles of Goat/ Chick, Slides, cover slip, needle, Brush, pointed forceps, Alcohol Series, Eosine, DPX and Compound Microscope,

Procedure:

- Take few muscles from thigh region of goat / chicken.
- Tease the muscles with the help of needles.
- Take few fibers and fix them in absolute alcohol followed by washing in water.
- Dehydrate upto 70% alcohol.
- Stain in eosin.
- Finally mount in DPX/ Canada balsam.
- Observe the slide under low power and high power.

Results & observations:

1. Striated muscle fibers are covered by delicate membrane called sarcolemma
2. Striated muscle fibers are nucleated
3. These cells shows alternate dark and light bands, due to which they are called striated muscles.
4. Mayofibrils are present in sarcoplasm



Questions:

1. Where would you find stripped muscles
2. What are characteristic features of stripped muscles

Exercise: 4

Aim: To prepare a permanent stained mount of unstripped muscles in the given Animal tissue (Goat / Chicken can be obtained from meat shop).

Requirements: Animal tissue, Slides, cover slip, test tubes, Test tube holder, KOH, Alcohol Series, Eosin, DPX and Compound Microscope.

Procedure:

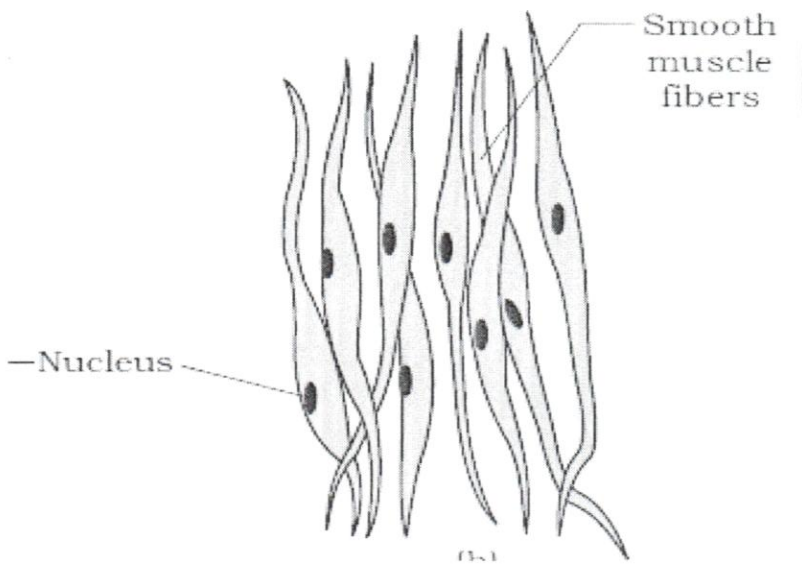
- Take small piece of intestine from Goat / Chicken.
- Dehydrate up to 70% alcohol.
- Stain in eosin.
- Finally mount in DPX/ Canada balsam.
- Observe the slide under low power and high power.

Results & observations:

1. Each muscle fiber or cell is spindle shaped and involuntary.
2. Sarcolemma is absent.
3. Light and dark bands are absent.
4. Myofibrils are present in sarcoplasm.

Questions:

1. What are characteristic features of smooth muscles?
2. How would you differentiate Striated muscles from smooth muscles?



Exercise: 5

Aim: To prepare permanent slide of haeme crystal from given blood sample.

Requirements: Blood, Glacial Acetic acid, dropper, slide, cover slip, sprit lamp and Microscope.

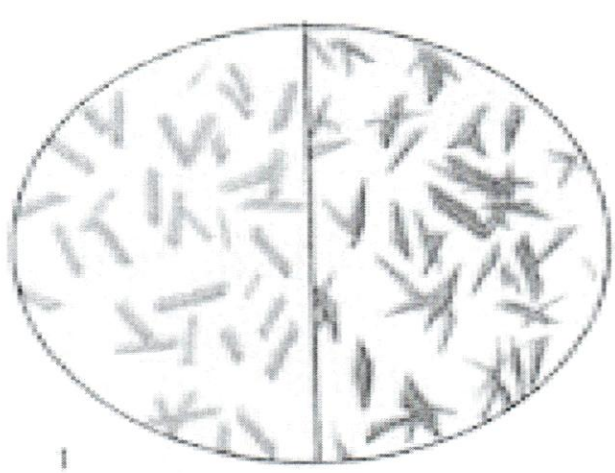
Principal:

- When hemoglobin or dry blood is heated with a few drops of glacial acetic acid, and, if necessary, a small crystal of NaCl, there are formed yellowish, microscopic crystals called hemin, or Teichmann's crystals. These crystals of hemin, which is the hydrochloride of heme, are very characteristic of blood and, therefore, furnish us with a *reliable and delicate test for blood.* However, this test does not enable us to differentiate between the blood of man and that of the lower animals.

Methods:

1. With the help of dropper take a drop of blood on the slide and prepare a thin film.
2. Allow it to dry for few minutes.
3. Pore few drops of Glacial acetic acid on blood film.
4. The mixture is covered with a cover slip and the slide heated over the flame of a spirit lamp. The reaction is complete with the beginning of boiling of the mixture and the slide is quickly removed from the flame.
5. The preparation is allowed to cool and examine under a microscope, initially under low magnification and then under high magnification.

Observations & Results:



Questions:

1. What is the aim of this experiment?
2. Why providing proper heat is a crucial step in haeme crystal slide preparation?

Exercise: 6

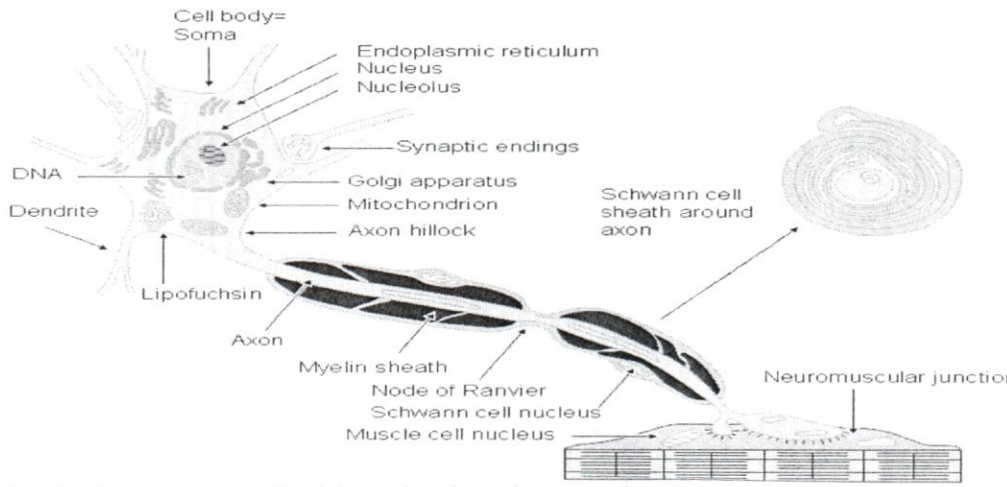
Aim: To study different types of neurons from spinal cord of Goat / Chicken can be obtained from meat shop).

Requirements: Animal tissue, Slides, cover slip, Methylene blue stain, DPX and Compound Microscope

Principle: The human central nervous system (CNS) contains about 100 billion neurons. Neurons are basic building blocks of the nervous system.Neurons in human brain are present in many different shapes and size.

Procedure:

- Spinal cord of Goat/ chicken obtained from the meat shop.
- Cut the tissue into pieces and keep in 0.9% Nacl, in refrigerator.
- Take a clean slide
- Keep a small piece of grey matter on slide.
- Slightly tease the material with needle.
- Add 2 drops of methylene blue stain. Leave it for 5 minutes
- Place cover slip on the tissue.
- Gently tap on cover slip
- Observe under microscope.

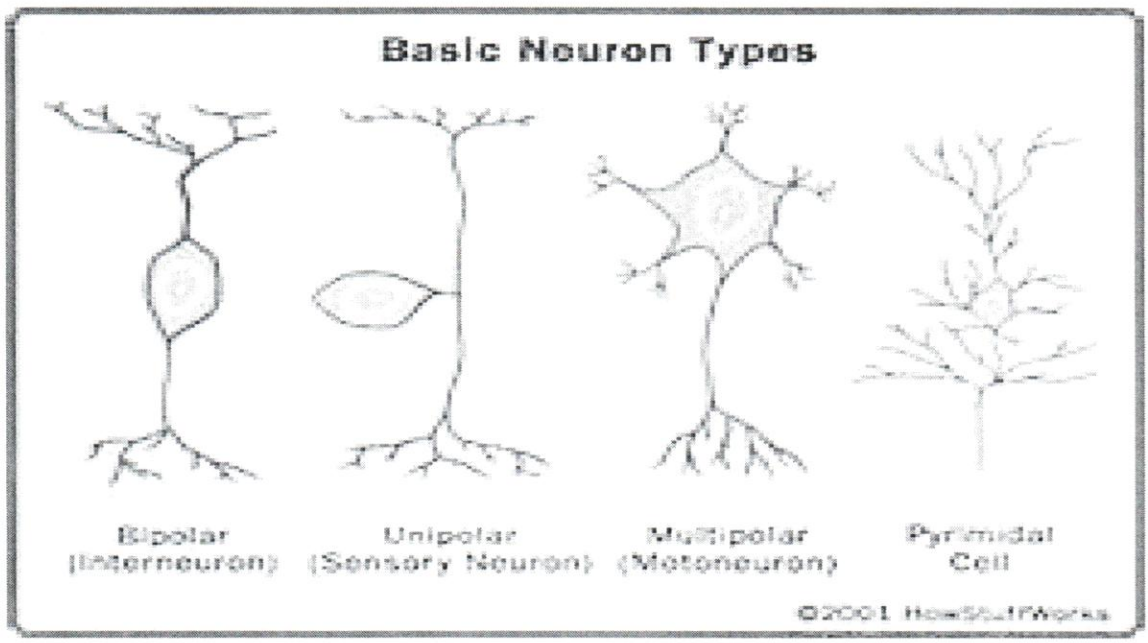


Results & observations: Darkly stained nucleus can be seen

1. A typical neuron consists of five to seven dendrites which extend out from the cell body or soma.
2. It has a long fibrous axon that originates from the cell body.
3. Axons of some neurons possess sheath of myelin. Such neurons are called myelinated neurons.
4. The myelin sheath leaves some uncovered areas on axon these uncovered areas are called nodes of Ranviers.

Types of neurons: Based on the morphological features like number of dendrites, soma shape etc neurons are classified into different types viz;

1. **Multipolar neuron**
   - Found in cerebral cortex
   - These neurons possess three or more dendrites and one axon.
   - They vary in size from large to very small
   - Soma shape is round or ellipsoidal.
2. **Bipolar neuron**
   - Found in retina of eye
   - Cone axon and one dendrite
3. **Unipolar neuron**
   - Found only in embryonic stage.
   - They possess cell body with one axon only.
4. **Pyramidal neuron**
   - **Found in cerebral cortex.**
   - These neurons possess single apical dendrite and a basal skirt of basal dendrites.
   - Soma shape is pyramidal or triangular.



Questions:

1. What are different types of neurons
2. How can you differentiate myelinated and non myelinated fibers
3. What are nodes of Ranvier.

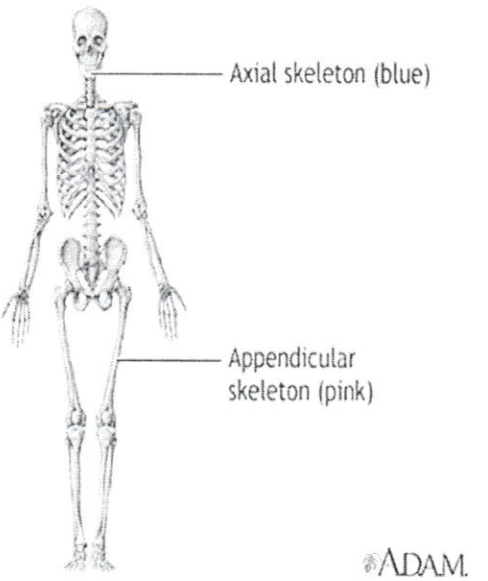
Exercise: 7

Aim: To study human skeleton system (Bones and Joints)

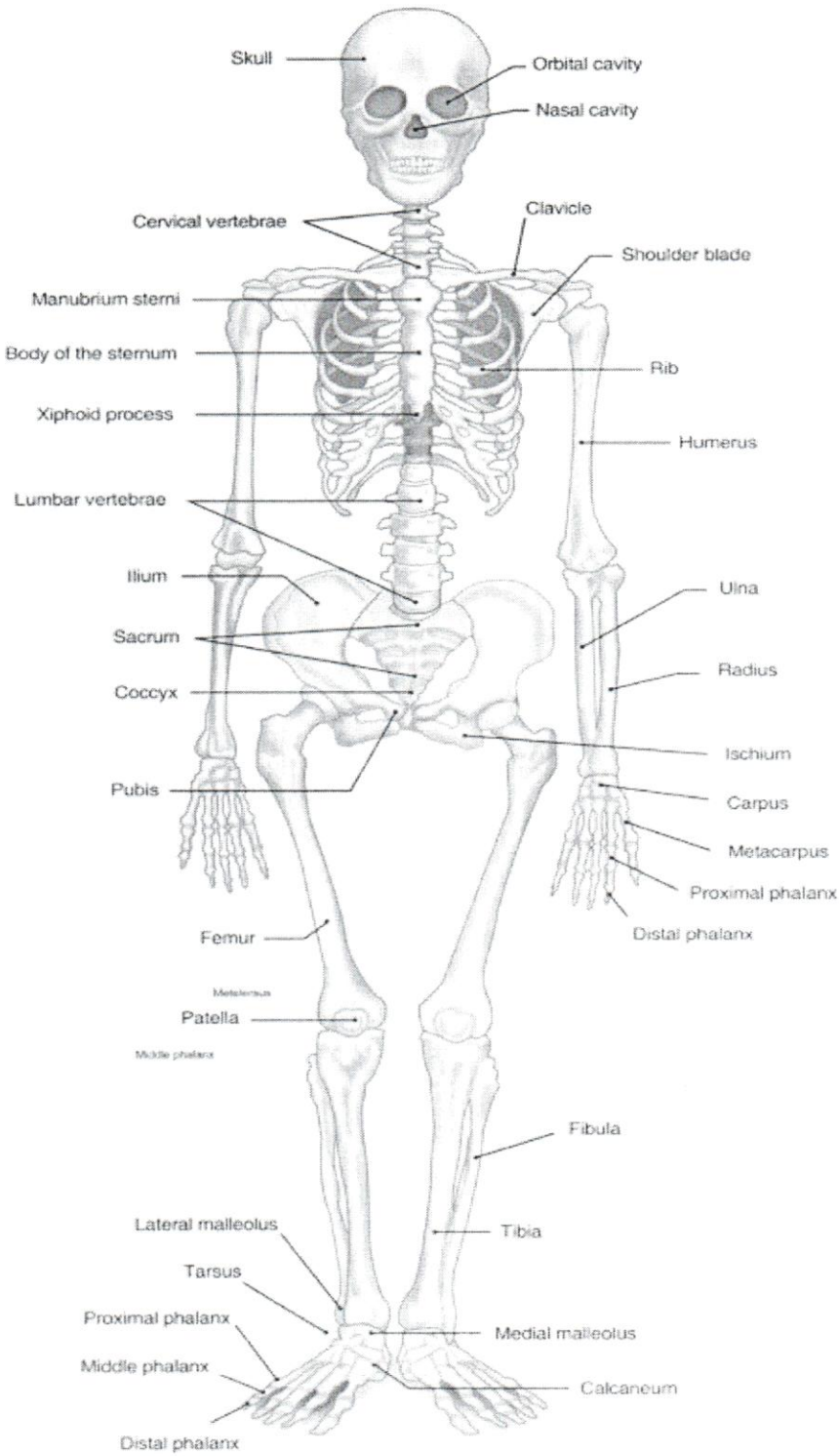
(Skull, Vertebral formula, No of vertebrae types of vertebrae and their morphology.

The Skeletal System

- **Parts of the skeletal system**
  - Bones (skeleton)
  - Joints
  - Cartilages
  - Ligaments
- **Divided into two divisions**
  - Axial skeleton
  - Appendicular skeleton



The Human Skeleton



1. Human skull:

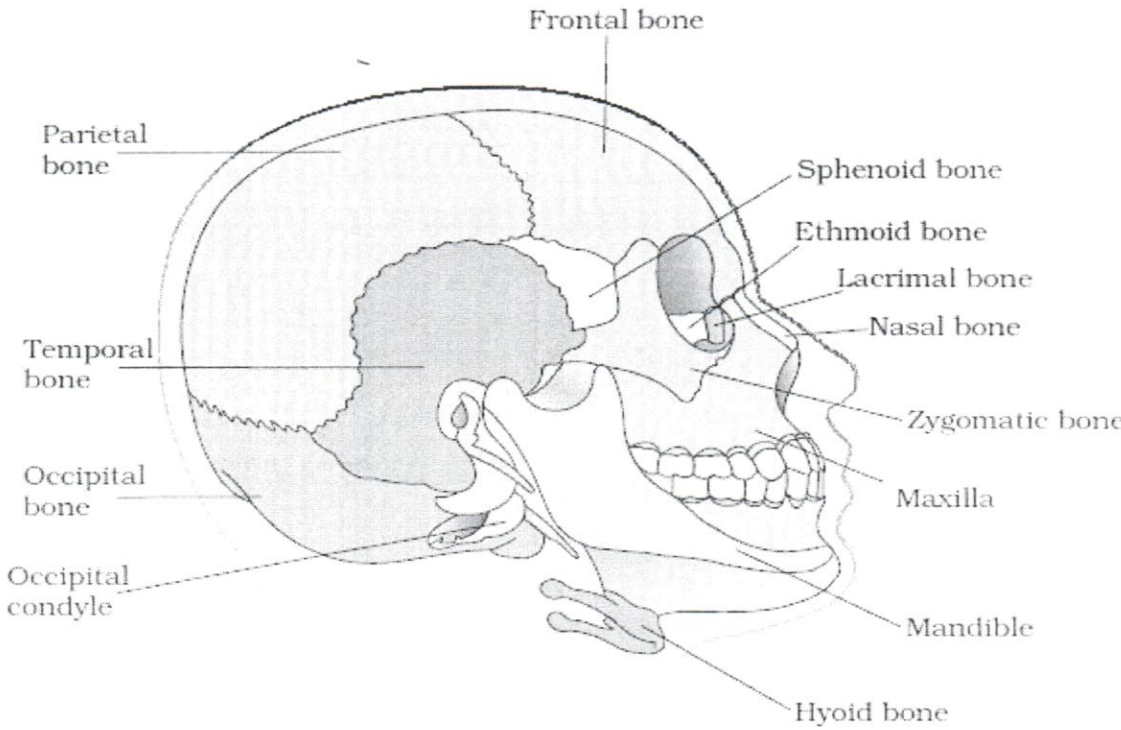


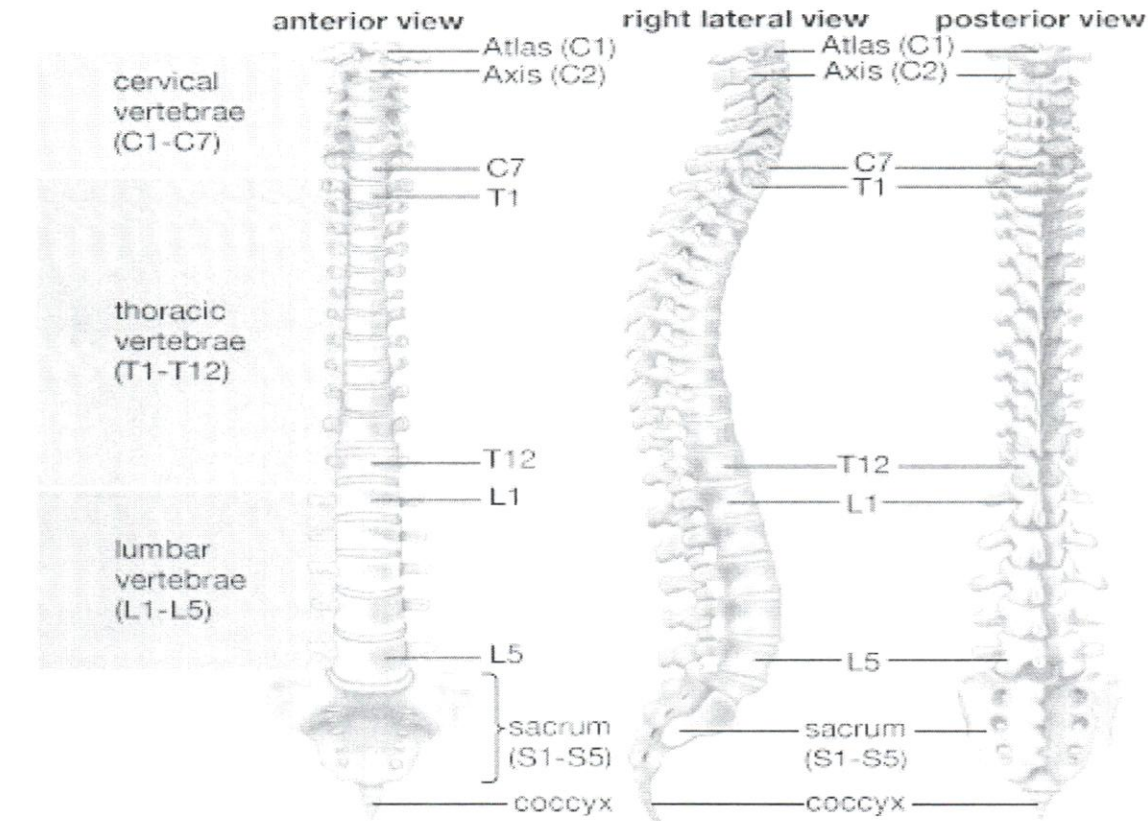
Figure 20.6 Diagrammatic view of human skull

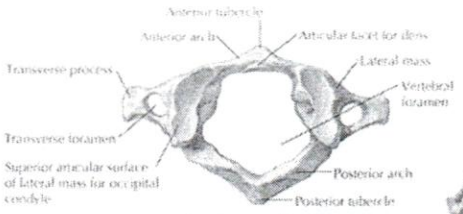
- Human skull is composed of 22 bones that are classified in two sets of bones cranial and facial.
- Cranial bones are 8 in number and forms hard protective outer covering i.e cranium for the brain.it includes pair of frontal bone, parietal bone, temporal bone and occipital bone.
- Facial regions are made up of 14 skeletal elements which form front part of skull. This includes pair of Sphenoid bone, Ethmoid bone, lacrimal bone, nasal bone, zygomatic bone, maxilla and mandible.
- A single U shaped bone hyoid is present at the base.
- Ear ossicle (Malleus, Incus and stapes is also part of skull).
- The skull articulates with vertebral column with two occipital condyles (dicondylic skull).

3. Vertebral Column:

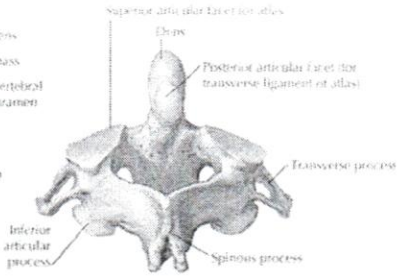
- Placed on dorsal side of body.

- Human vertebral column is formed of 26 serially arranged units called vertebrae. Prior to adolescence, the spine consists of 33 bones because the sacrum's five bones and the coccyx's four do not fuse together until adolescence.
- Each vertebrae has a central hollow canal called neural canal which is passage of spinal cord.
- First vertebrae is atlas which articulates with occipital condyle
- Vertebral column is differentiated into cervical (7), thoracic (12), lumbar (5), Sacral (1-fused), Coccygeal (1- fused).
- Vertebral formula of human :C7, T12, L5, (S5) Co4.

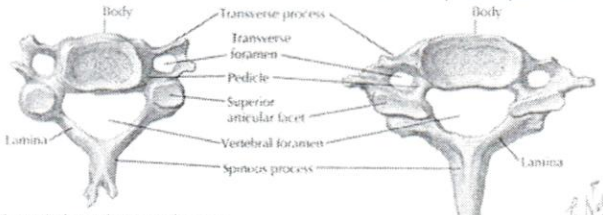




Atlas (C1): superior view



Axis (C2): posterosuperior view

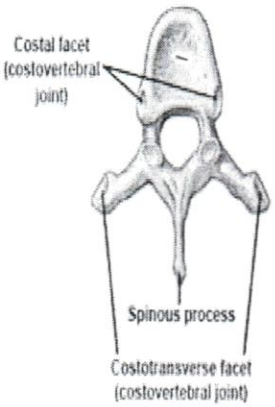


4th cervical vertebra: superior view

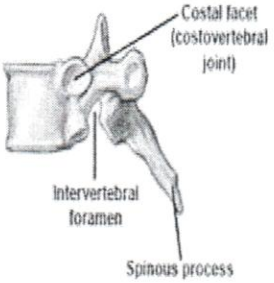
7th cervical vertebra: superior view

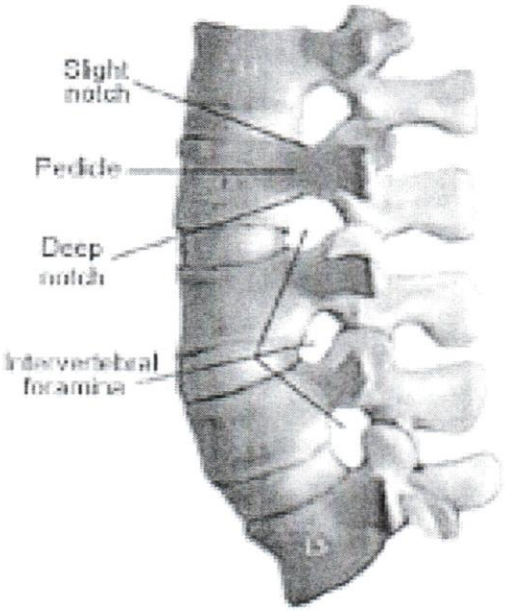
Thoracic Vertebrae

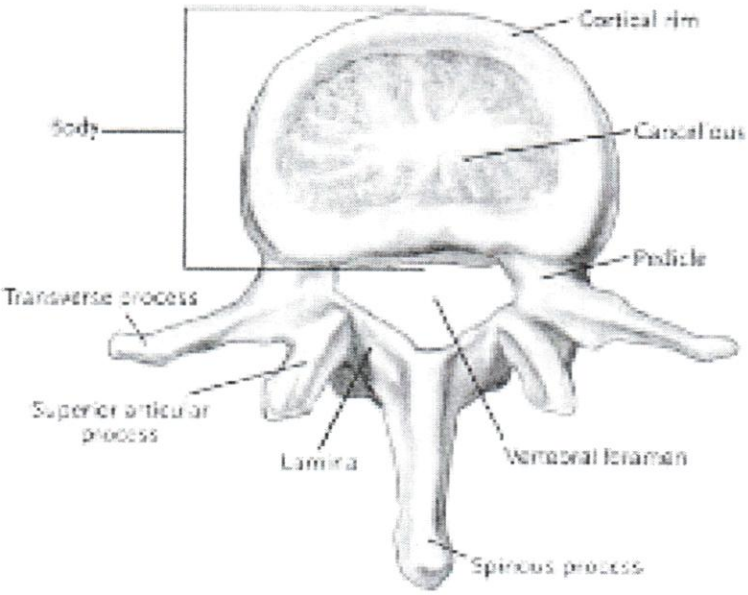
Axial (Overhead) View

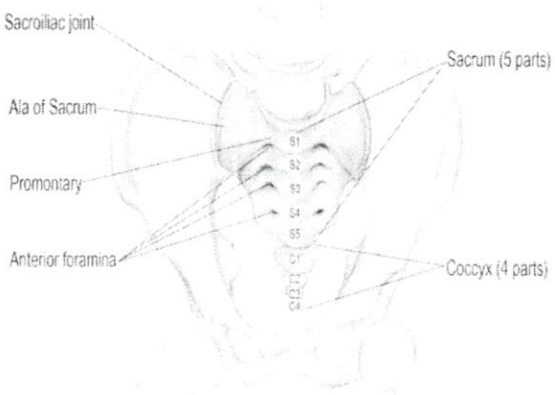


Lateral (Side) View

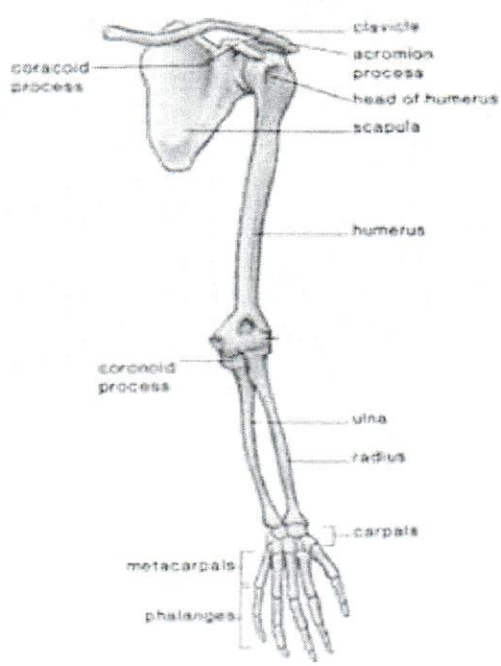


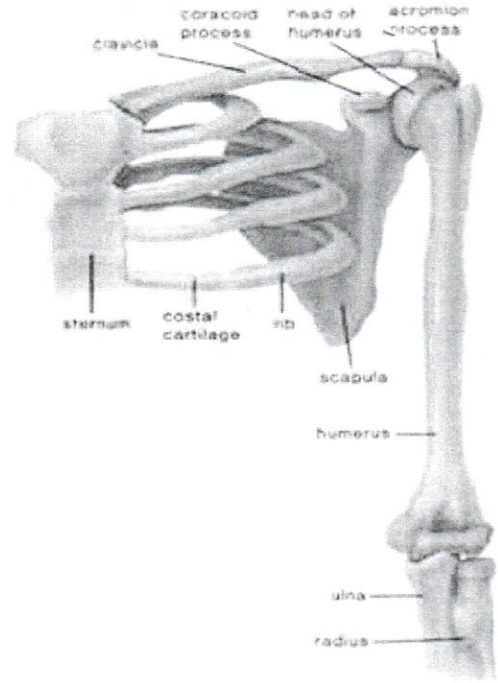






Pectoral Girdle





The shoulder, or pectoral, girdle is composed of the clavicles (collarbones) and the scapulae (shoulder blades).

Scapula is a large, triangular , flat bone.

Scapula consist of a spine like ridge called acromion process. It provides articulation to clavicle.

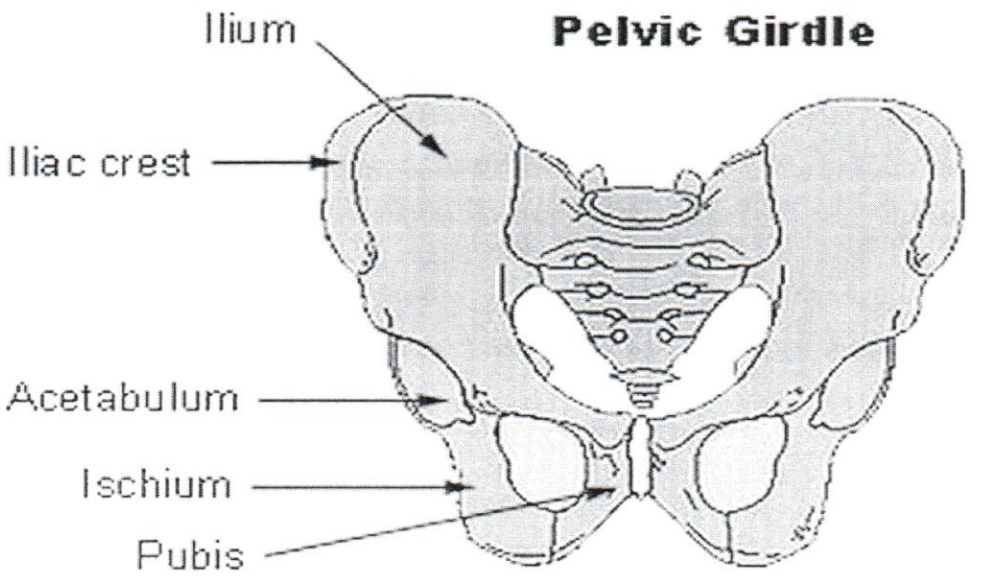
This girdle consist of a dipression called glenoid cavity which articulates with head of humerus to form shoulder joint.

Each clavicle is long slender bone called collar bone.

Functions

The pectoral girdle connects the upper limbs to the axial skeleton and serves as the attachment site for the muscles of the upper back, chest and neck.

Pelvic Girdle



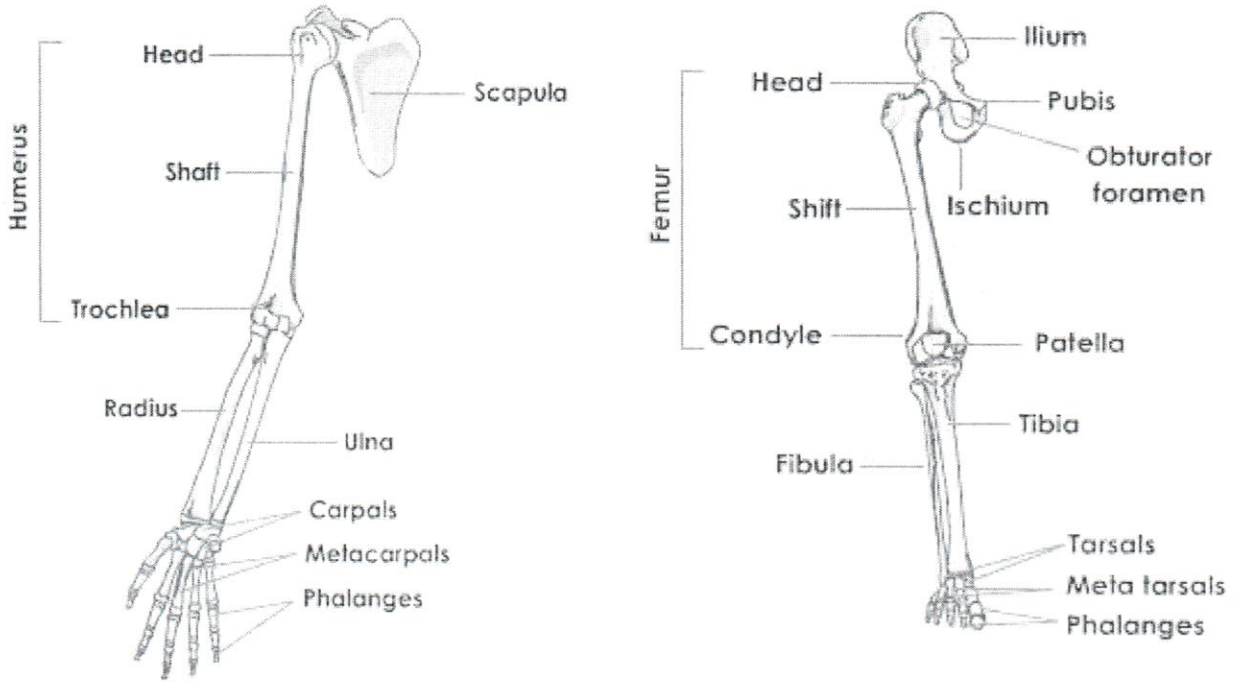
Pelvic girdle consist of two halves each of them is called innominate.
Each innominate is formed by fussion of three bones Illium, ischium and pubis.
At the point of fusion of this three bones is a cavity called acetabulum which provides articulation to thigh bone.
Two halves of the girdle meet ventrally to form pubic symphysis.

Functions

The strong and rigid pelvis is adapted to serve a number of roles in the human body. The main functions being: –

- **Transfer of weight** from the upper axial skeleton to the lower appendicular components of the skeleton, especially during movement.
- **Provides attachment** for a number of muscles and ligaments used in locomotion.=
- **Contains and protects** the abdominopelvic and pelvic visera.

Limb Bones



The limbs in human beings are designed on the same basic plan, as that of the pentadactyl limb, because each limb ends in five digits.

The limb bones support the arms (upper limbs) and legs (lower limbs). The bones of the legs are stronger than those of the arms.

Fore limb bones

- The humerus is the single bone of the upper arm.
- ulna (medially) and the radius (laterally) are the paired bones of the forearm.
- The base of the hand contains eight bones, each called a carpal bone,
- The palm of the hand is formed by five bones, each called a metacarpal bone.
- The fingers and thumb contain a total of 14 bones, each of which is a phalanx bone of the hand.

Hind limb bones

- Hind limb possess Femur, tibio fibula, tarsals , metatarsels and phalanges
- Femur is the longest bone of the body. It articulates with acetabulum of innominate bone of pelvic girdle to form hip joint. Posteriorly it inclines to the knee where it articulates with the tibia.

- The tibia or shine bone lies medial to the fibula. It is long bone with a shaftand two extremeties.
- The fibula is the lateral bone of the leg.
- Foot bones include seven tarsals, five metatarsal bones and phalanges

Questions:

1. Which bone is called shoulder bone?
2. What is vertebral formula of human?
3. What is the name of first cervical vertebra?
4. Total number of bones in human body.

Aim: To study parts of a compound microscope

Principle: Magnified and real images of minute objects are obtained using combination of lenses. A compound microscope is a complex assemblage of such lenses enabling highly magnified images of the microscopic living organisms and intricate details of cells and tissues. A monocular mono-objective compound microscope is normally used in a biology laboratory.

Requirement: A compound microscope, silk cloth, lens cleaning fluid and lens cleaning paper.

Procedure: Place the microscope on the working table and remove dust by wiping the body with a silk cloth. Clean the lenses with lens cleaning fluid and lens cleaning paper.

Identify the various parts of the microscope (Fig. 1.1) Draw a diagram of the microscope and label is various parts.

Take a permanent slide preparation or a temporary preparation made by you, keep it on the stage, fix the clips after focusing and view. Learn how the microscope can be tilted or inclined by moving the arm of the microscope. Note, how focusing is done by moving the coarse adjustment and fine adjustment knobs

Observation:

i) The microscope has a strong basal foot and a vertical arm joined by an inclination joint.

ii) The stage of the microscope is round/rectangle/square shaped and is fixed to the arm. In the centre of the stage is a small circular hole covered with glass for passage of light.

iii) The stage is provided with two clips or mechanical device to fix and hold the slide firmly in position. The material to be observed is brought into view of moving the slide and then fixed with the help of clips at desired position.

iv) A movable (rack and pinion mechanism) or a fixed substage is provided with an iris diaphragm and condenser. The condenser is a system of two or more lenses to receive parallel light rays and to converge these

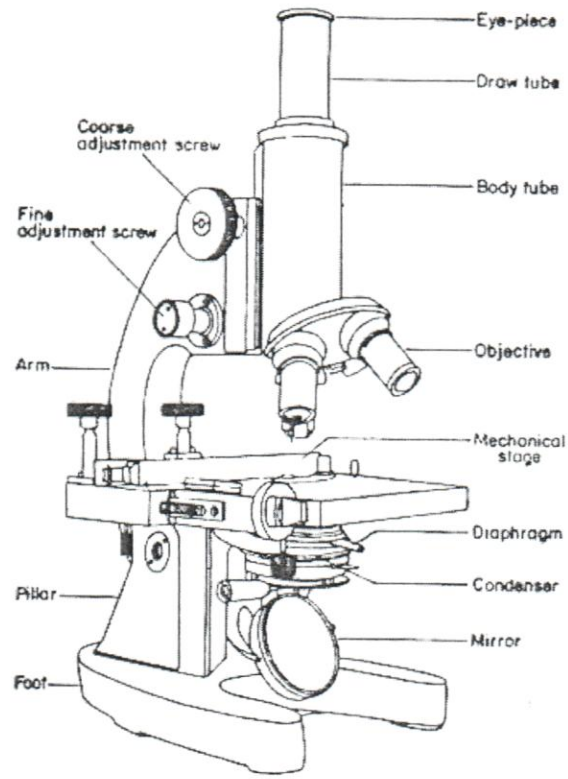


Fig.1.1 A compound microscope

on the object through the iris and the hole present in the stage. The diaphragm helps in regulating the aperture size and thereby controls the amount of light that passes through the slide (Fig. 1.2). The needle/ pin is used to increased or decrease the aperture size. Some microscopes do not have the condenser.

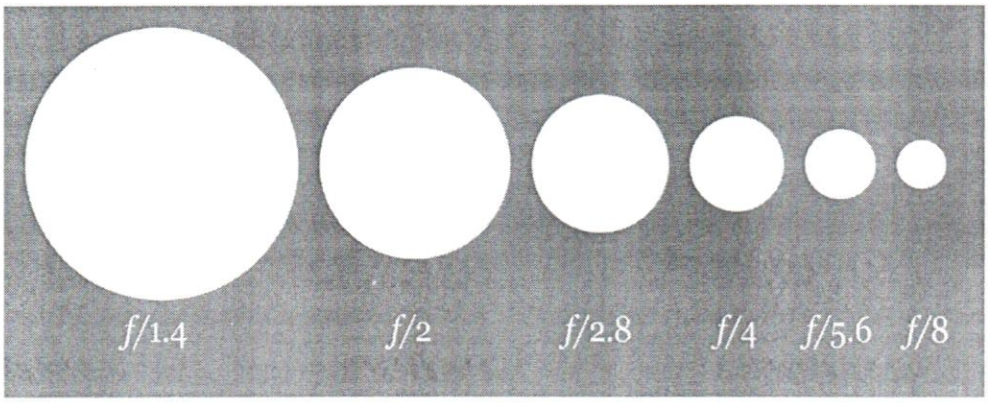


Fig. 1.2 Iris diaphragm showing different aperture sizes

v) An adjustable mirror is fitted below the condenser. It has plano-concave surfaces to focus the converging rays of light on the object through iris diaphragm and condenser in order to obtain a brightly illuminated image of the object.

vi) The body of the microscope consists of a movable tubular body tube raised on rack and pinion mechanism. The tube has an ocular or eye piece of specific magnification (which can be changed for lower or higher magnification, i.e., 5X, 10X or 15X. Eye piece with pointer are also available. Two objective lenses 10X, 40X or 45X are mounted on a revolving nose piece at the lower end.

Exercise 1: Some microscopes may also have a third objective lens (100X) called oil immersion lens.

The tube with eye end objective lenses can be moved up or down to focus the object sharply with the help of coarse adjustment knob and fine adjustment knob.

The object is first viewed under lower magnification using the coarse adjustment and then under higher magnification by rotating the revolving nose piece on which the objective lenses are mounted. While viewing at higher magnification, only fine adjustment knob is used for fine focus tuning.

Magnification: Magnification by a microscope is a multiple of the X value of the lenses of the eye piece and objective. For example, a 5X eye piece and a 40X objective will magnify the image $5 \times 40 = 200$ times the size of the object. Similarly, when a 10X eye piece and a 40X objective are used, magnifying power would be $10 \times 40 = 400$X. Generally, in a compound microscope, the eye pieces lenses are 10X or 15X and the objective lenses are 10X as well as 40X.

Precaution:

(i) Always clean the lenses before and after using the microscope.
(ii) For cleaning lenses, always use lens cleaning fluid and lens cleaning paper.
(iii) While observing, the objective lens should be carefully adjustment so as to avoid touching the slide lest it breaks the slide.
(iv) Always put back the microscope in its case after use.

Questions:

1. What is the magnification achieved when a 15X eye piece and a 40X objective are used together?
2. What should happen if direct sun rays are focused by the mirror into the body tube?
3. What is the difference between a dissecting and a compound microscope?
4. Which of the following parts provides support and supports the weight of a microscope?
   (a) Arm (b) Stage (c) Body tube (d) Foot
5. To which part of a microscope is the objectives fitted.
   (a) Nose piece (b) Diaphragm (c) Stage (d) Arm
6. Magnification power of a microscope is calculated by
   (a) magnifying power of eye piece × magnifying power of mirror.
   (b) magnifying power of eye piece × magnifying power of objective
   (c) magnifying power of objective × magnifying power of plane mirror.

Urine Test for the Presence of Urea, Sugar, Albumin and Bile Salts

Introduction:

1. A large number of volatile and non-volatile waste products are produced in various metabolisms in the body.
2. The elimination of these waste products from the body is called excretion.
3. Urea is the principal nitrogenous waste product produced in the human body. It is eliminated from the body in the form of urine along with several other waste products.
4. The urine of a normal person in pale yellow in colour and contains water, urea, other nitrogenous waste products (ammonia, uric acid, creatinine and hippuric acid), mineral salts and some non-nitrogenous organic substances.

Characteristics of Urine

1. **Colour.** Yellow (amber) due to presence of urochrome, urobilin a degraded product of bilirubin, Haemoglobin.
2. **Odoour.** Aromatic when fresh. But later turn ammonical due to degradation of urea by bacteria.
3. **Volume.** (a) Less in summer or in warm climate than in winter or cold climate. (b) normally 1½ lit or more is produced per day.
4. **Specific gravity.** 1-010-1.40 *(i.e.,* more than plasma).
5. **pH.** 6.0 (acidic)

Composition of Urine

1. **Water.** 95-96%
2. **Urea.** 2% (30 mg.)
3. **Dissolved substances.** 2%
   (a) Nitrogenous substances – uric acid-0.5%, Ammonia-0.04%, creatine-0.075-1.
   (b) Organic substances – oxalic acid, lactic acid (non-protein substances)

Class XII Unit VI Chapter 2

Aim: To observe pollen tube growth in style of a pistil

Principle: Pollination involves landing of pollen grains on the stigma. If the stigma and pollen grains are compatible (pollen –pistil interaction) the germination of pollen grain takes place on the papillae/ surface of stigmatic lobes. The pollen tube emerges out of a pollen grain through a germ pore. The pollen tube reaches the micropylar-end of the ovules through stylar tissues. It passes through style either by dissolving the cells of style (solid style) or along the margins of inner layer of style (hollow styles). The emergence of a pollen-tube from a pollen grain, pollen-tube passage through the styles can be observed through suitable preparations. The cell walls of pollen-tube are cellulosic, the pollen-tube possess a polysaccharide, callose and a vegetative cell nucleus and a generative cell (or two male gamete cells).

Requirement:

- Freshly collected pistil (distal regions of a pistil) of any flower. The suggested ones are: ray floret pistils of *Tagestes*; pistils of *Petunia*; Lilies; China-rose;
- Test-tube, distilled water; spirit-lamp;
- Watch glass/Cavity block, Macro slides and micro coverslips; needles, forceps; microscope.
- Cotton blue (aniline blue dissolved in lactophenol); glacial acetic acid.

Procedure:

- Collect by picking the styles along with stigma from, freshly opened flowers/from flowers not later than 24 hrs of anthesis (opening of flower) of a desired flower.
- Immediately transfer them to a test tube containing distilled water.
- Boil it for 5-10 minutes.
- Transfer them to a watch glass/cavity block containing 1-2 ml of glacial acetic acid
- Keep it overnight
- Transfer the cut pistils to watch glass/cavity block containing 1-2 ml of cotton blue.
- After 1-2 hrs; pick-up the pistils, put them on a macroslide, add a drop of lactophenol, cover with microcoverslip. Press with the tip of the needle; soak the excess mounting medium with the help of blotting paper. (If the styles are still rigid, then split them open vertically with the help of needles). Press the coverslip gently, if required till the stylar tissues are spread out. (Do not press too hard).

Observation: You shall observe the well spread stigmatic tissue and stylar tissue. The pollen grains, pollen tubes shall take bluish stain, and can be observed piercing through along with the papillar tissue of sigma and the cells of styles.

Draw the Following: Pollen grains, germ-pore, emerging/just emerged pollen tube, pollen-tube-papillae; pollen-tube in the stylar tissue; callose in pollen tube (dark-blue patches).

Conclusion: Following pollination, pollen tube emerges out of a pollen grain through a germ pore. Not all the pollen-grains germinate. Pollen tube grows at tip travel through style. Proximal regions of a pollen-tube are blocked by callose plug (you may or may not observe it in your preparation).

Questions:

- How does a pistil recognize the pollen grain of its species?
- What governs the pollen germination on stigma?
- What in the function of a pollen-tube?
- Why does a pollen tube release two male gametes in an embryo-sac?
- In which region of an embryo-sac the male gametes are released?
- What is double fertilization?

Class XI Unit III Chapter 8

Aim: To study the structure and contents in plant cells

Principle: A parenchyma cell is often taken as an example to study the structure of a typical plant cell. However, not all of the parenchyma cells present in a plant is exactly similar. They vary in size, cell wall composition, and protoplasmic contents. Let us, study the composite structure by observing parenchyma cells for various plant sources.

Requirement: Plant Materials:

(a) Cell wall: Scale leaves of Onion bulb (peel mount), epicarp of tomato/capsicum fruit (peel/ mount)

(b) Protoplast: A Rotten tomato (squeezed and filtered through a muslin cloth). The filtered obtained is to be observed under the microscope. (Remember mount in water, avoid mounting in glycerin).

(c) Nucleus: Epidermis peel of scale leaves of onion bulb.

(d) Plastids:

i. Leucoplast: Smear of potato tuber cells containing starch
ii. Chloroplast: W.M. of Hydrilla leaf
iii. Chromoplast: Mount peel of epidermis of red tomato/ capsicum/ colored petals
iv. Vacuole: Isolated protoplast of tomato/scale leaf whole mount of onion bulb

Chemicals:

1% safranine (for cell walls)
1% acetocarmine (for nucleus)

Glass ware apparatus: Compound microscope, macroslides, microcoverslips, watch glasses; petridishes; scalpel; needles; brushes; forceps; dropper

Procedure: Prepare suitable strained and unstained preparations (for comparison) for each of the plant material listed. (You may choose any other plant material(s) as per the availability). Observe under the microscope.

Observation:

- Present your observations by making suitable labeled sketches. Take care observe and record the relative dimension of various components of the cells.
- Except for the preparation of rotten tomato fruit filtrate, in the other preparations, you shall observe a group of cells. All the adjacent, contiguous cells are cemented/separated by pectin rich middle lamella. Observe it. Can you observe openings/discontinuities in the cell walls on either side of middle lamella? These are sites of the plasmodesmata (you shall not be able to observe this). However, in the peel mounts of epicarp of tomato/ capsicum, the cell wall are very thick. Here, the discontinuities are very distinct.

Conclusion: A eukaryotic parenchyma cell of a plant is made up of a firm outline and a given dimension. The exterior of the cell is delimited by a cell wall. It is basically then, a primary. The cell walls of the adjacent cells are cemented by a middle lamella. The protoplast localized within the cell walls of a cell is connected to the protoplast of neighboring cells through minute plasmodesmatal connection. The nucleus within the protoplast is very conspicuous organelle. It stains brightly with acetocarmine as well as safranine. The remainder of the protoplast is termed cytoplasm. One or more vacuoles are/can be observed. They look empty under the microscope. They, however, contain plant cell sap. Plastids can also be observed dispersed in the cytoplasm. Their number per cell may vary. They may be colorless (leucoplast), green (chloroplast) or colored (chromoplast). The presence of the cell walls the vacuole and the plastids together distinguish a eukaryote plant cell from a eukaryote animal cell.

Questions:

- How can you distinguish a plant cell from an animal cell?
- Do all plant cells have only primary, cellulosic cell walls? Mention any 3 variations.
- Does a primary all wall is only composed of cellulose? Mention any 2 additional components.
- What is the chemical composition of a middle lamella?
- Mention at least 2 other plants where you can demonstrate the presence of cell wall, nucleus, leucoplast, chloroplast, chromoplasts, vacuoles, to the students.
- Name the cell/cell/tissues where following are not present vacuole; plastids; nucleus; plasmodesmata
- Name the cells/tissues/where the following are present:

  -Thick primary cell-wall; Thick-lignified cell wall; suberized cell wall; chitinous cell wall.

Class XI Unit II Chapter 5

Aim: To survey the morphology of vegetative plant parts of flowering plants.

Principle: A flowering plant (both dicotyledonous and monocotyledonous) posesses root and shoot systems. The root system mostly is underground while the shoot system is aerial. However, underground shoots and aerial roots are observed in some plants. The underground roots system could be tap root or adventitious. The shoot system consists of axis, the stem and the laterals, the leaves. The distal regions of root and stem axes are regions of primary meristems, the apical meristem. In addition, axil of every leaf bears an axillary bud. The older stem and roots axes may have lateral meristems, the vascular and cork cambia. The branching pattern, the phyllotaxy, the morphology of leaves is characteristics of every plant species.

Requirements: Hand lens, paper, pencil

Procedure: Students are to work in pairs. They shall prepare a table in the format as given. They may even modify the same. Students should visit various sites within premises of their school or nearby open fields e.g. school garden, neighboring park(s); natural open spaces, etc. They shall observe a plant and record the morphological characteristics and fill in the columns in the table. The recordings are to be done for as many plants as possible. For e.g.: The table to include the characters as under:

Habit : herb/shrub/tree

Aerial & subterranean

Shoot: Branched/unbranched:

If branched: Monopodial / Sympodial / other

Leaf: Sessile / petiolate

Simple / compound

Palmate / pinnate

Reticulate / parallel venation

Glabrous / Hairy

Modification of any kind

Phyllotaxy: Alternate / spiral / whorled / any other

Roots: Do not uproot all the plants; uproot only those plants which are herbaceous (only after taking permission from the concerned authorities)

- Tap root / adventitious
- Subterranean / aerial
- Modifications of any kind

Any other character of your choice, you may even delete some character after consulting your teacher.

Conclusion:

- A pair of students should describe the morphology of at least two plants. However as a class 20 plants should be tabulated and described.
- Group the plants with similar characters as well as contrasting characters.
- Prepare a comparative report.

Aim: To isolate DNA from the given plant material

Principle: DNA is the hereditary material in all living organisms including plants (except some viruses). DNA is present in the nucleus of the cell in the form of chromatin material. It can be separated from tissues by treatment with certain chemicals/enzymes e.g. EDTA. Plant cells are enclosed by cell wall. Thus for isolation of chromatin material (DNA) plant material should be chopped & homogenized well by blending it thoroughly.

Material required: Any suitable plant material (e.g. onion) cauliflower/ spinach / banana), beakers, salt (Nacl), petridish, strainer, spirit lamp, blender, water, chilled ethanol, EDTA (shampoo / liquid dish washer can be used)

Procedure:

1. Chop the plant material into fine pieces (peeled onion/ cauliflower / papaya / spinach etc. may be used)
2. Take about 30 ml water in a beaker
3. Add one teaspoon salt in a beaker & stir it.
4. Add two spoons washing liquid (shampoo/dishwasher containing EDTA) to the solution in a beaker.
5. Stir to mix the contents. Avoid bubble formation.
6. Put/mix chopped onion/plant material in a blender & blend it.
7. Add blended plant material & solution containing EDTA in a beaker.
8. Blend it again to get a smooth mixture.
9. Pour mixture in a beaker & heat it for about 1-2 minutes. Keep stirring the mixture with the help of a strainer.
10. Pour filtrate into chilled ethanol.
11. The DNA precipitates on the surface of the beaker. It can be removed by spooling

Conclusion: Chromatin material (DNA) is seen floating on the surface of chilled ethanol which can be removed by spooling. It can further be stained by acetocarmine & observe under microscope.

Class XI Unit IV Chapter 11

Aim: To demonstrate upward transport of water through xylem

Principle: All terrestrial land plants take water from the soil. The soil water, along with the nutrients dissolved in it, is carried to all the aerial parts of the shoot through xylem. The water is lost to the environment by transpiration from the surface of the leaves, through the stomatal openings. The dead, empty, thick-walled tracheary elements of the xylem are used to transport water from base to the apex of the plant body. Xylem occupies the core of the stem. Phloem is in periphery of the stem.

Requirements:

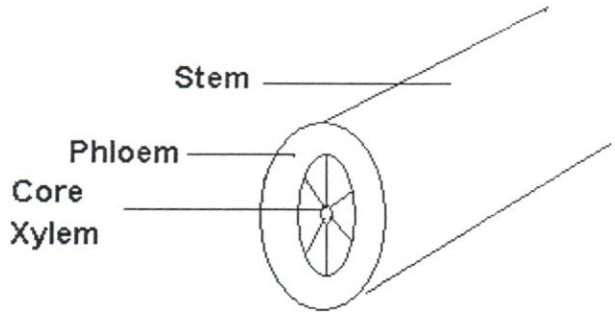
- Fresh plant specimen of Vinca/Impatiens/Balsam
- Beaker (1000 ml)
- Distilled water
- Any dye (Safranine, I_2KI, etc....)
- Sharp razor blade

Procedure:

- Dissolve the dye in the water
- Wash the roots of freshly plucked plant under a tap
- Place the roots of the plant in coloured water
- Keep the set up in Sun for 2 hours.
- Remove the plant
- Cut the stem of the plant horizontally (T.S.) with the help of a sharp razor blade.
- Observe the upper-cut surface of the stem for the dye distribution.

Observation:

- The central core of the stem consisting of xylary tissues shall have the color of the dye
- The peripheral phloemic regions do not exhibit the dye color.



Conclusion: The upward movement of dye along with the solvent water moved from roots through the stem. The central-core xylary tissue was the route of water transport. The peripheral phloem tissue is not involved in the upward transport of water.

Questions:

- Name the treachery elements of xylem.
- Describe the structure of these cells.
- Are they living or non-living?
- Why are their cell-lumen empty?
- Why are their cell-walls lignified?
- What is the physiological principle associated with the upward transport of water?
- Is the rate of transport of water uniform through out the day? What are the factors which affect it?
- Does this transport require expenditure of metabolic energy?

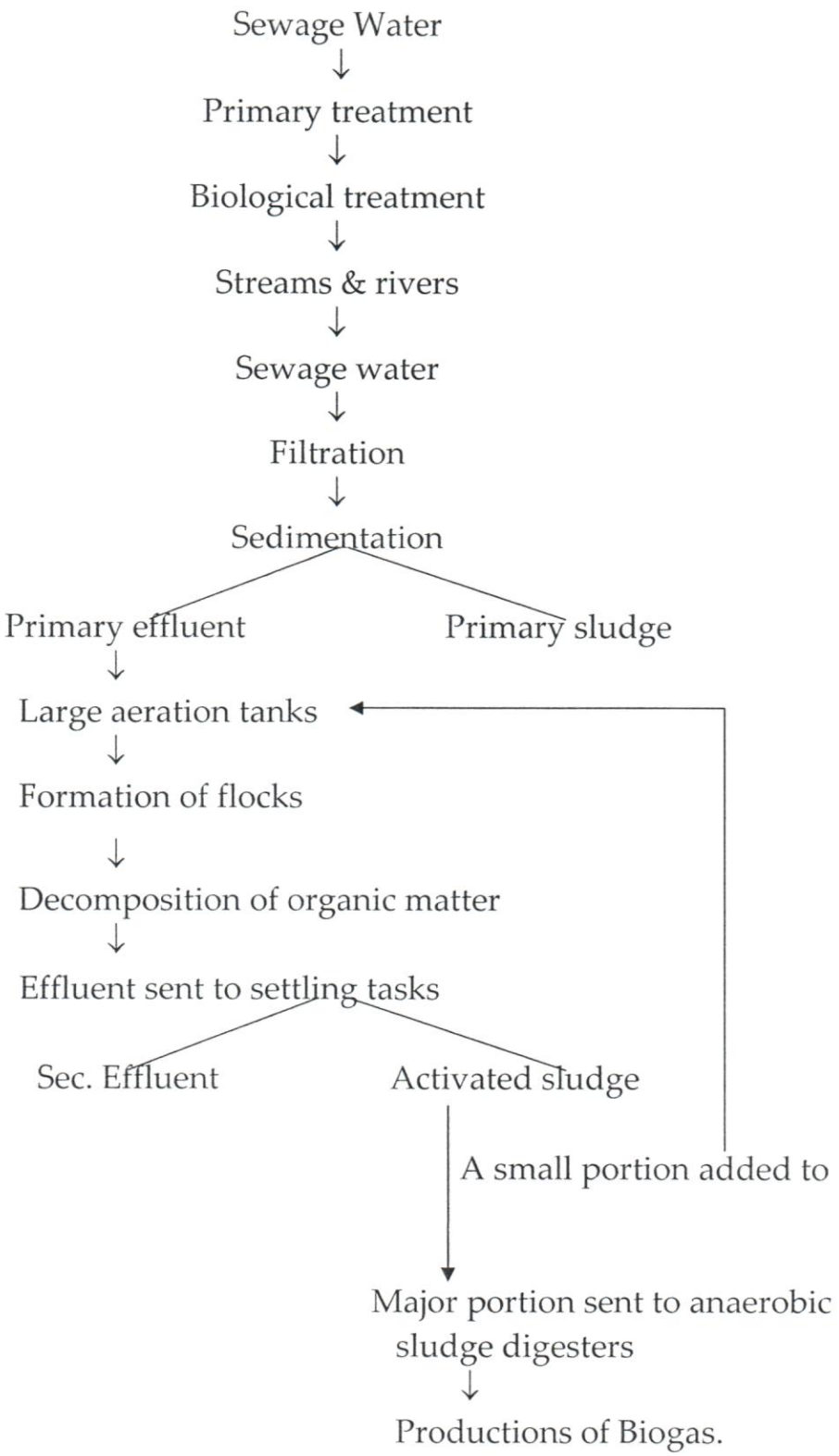
Class XII Unit VIII Chapter 10

Microbes in Human welfare

Aim: To visit a sewage treatment plant.

Principle: Huge quantities of municipal waste water called sewage are generated everyday in cities & towns. Sewage contains large amount of organic matter & microbes which may also be pathogenic. Thus, it cannot be drained out into streams & rivers directly. Before disposal it has to be treated is Sewage Treatment Plants (STPs) which have been set up in almost every city.

Exercise: The students shall visit a nearby Sewage Treatment Plant with their teacher and observe the treatment of Sewage water at various stages. They shall note down the steps of Sewage treatment and answer the questions that follow.


Sewage Water
Primary treatment
Biological treatment
Streams & rivers
Sewage water
Filtration
Sedimentation
Primary effluent
Primary sludge
Large aeration tanks
Formation of flocks
Decomposition of organic matter
Effluent sent to settling tasks
Sec. Effluent
Activated sludge
A small portion added to
Major portion sent to anaerobic sludge digesters
Productions of Biogas.

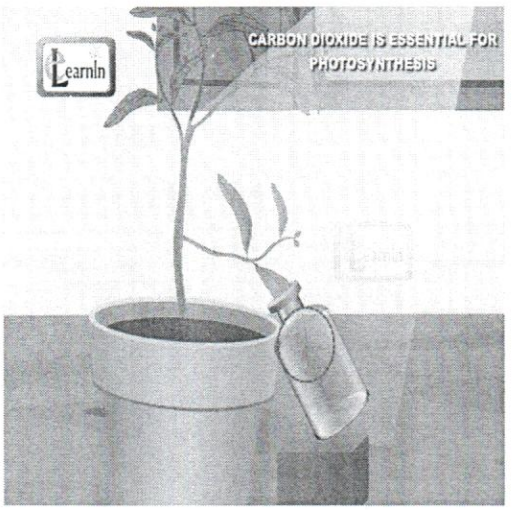
Conclusion: Prepare a report on the visit.

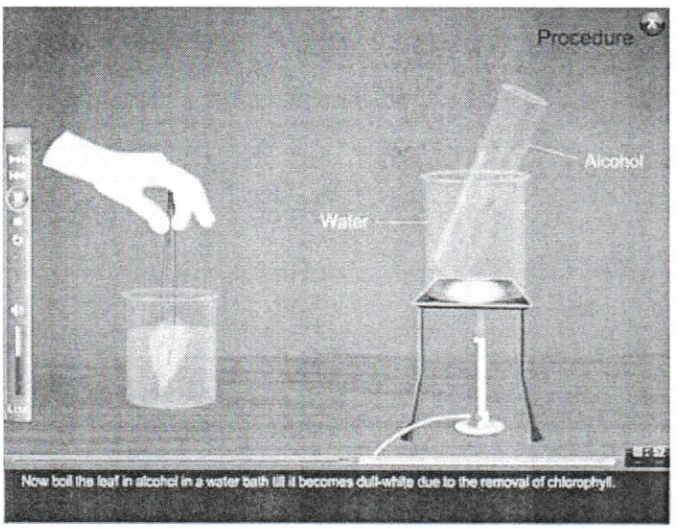
Questions:

1. What is primary treatment of sewage water?
2. What steps are taken in secondary treatment of sewage water?
3. What is activated sludge? Why a small portion of it is pumped back into aeration tanks?
4. What is the composition & function of flocks?
5. What is the role of anaerobic sludge digesters in the STPs.

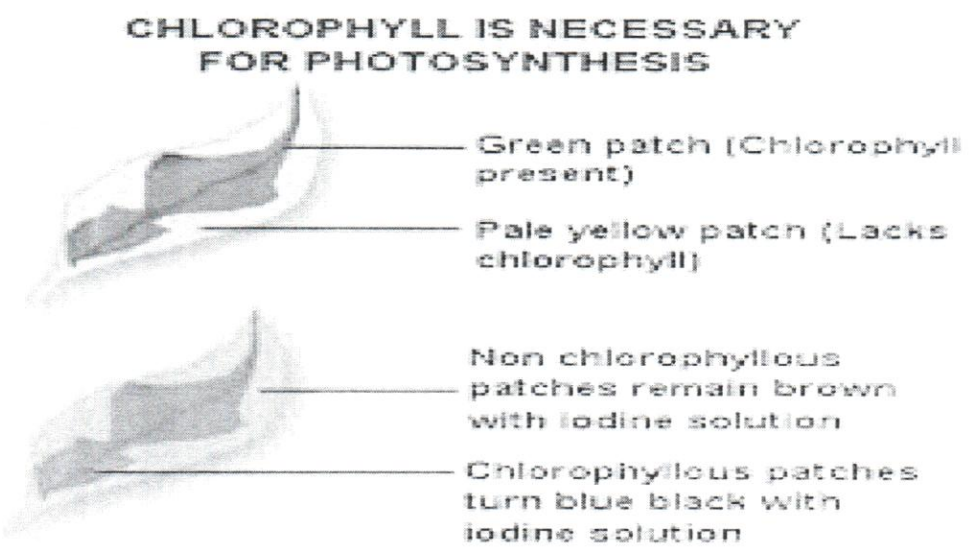
Class XI Unit IV Chapter 13

Aim: To demonstrate that both light and carbon-dioxide are essential for photosynthesis through Moll's half leaf experiment
Requirement: Potted plant, KOH solution, split cork, stand.
Procedure: Take a well watered potted plant will long leaf blades and keep it in dark for 3 days to de-starch the plant. Select a leaf and fix it as shown in the figure (with help of a split cork, boiling tube containing KOH solution and a burette stand) with part of leaf out side the test tube, part in side the test tube and a portion in between split cork
Keep the set up undisturbed in light for 4-6 hours and allow it to do photosynthesize.





After 6 hrs, pluck the leaf; detach it from the boiling tube.
Boil the leaf in alcohol over a water bath to bleach (remote the pigments).
The leaf becomes brittle. Soften it by boiling in water.
Now add IKI to the leaf to test for presence of starch, and record your observations.



Positive test for presence of starch can be observed only in the part of the leaf exposed to atmosphere where light and CO_2 are available for photosynthesis. The part of the leaf between the split cork does not show starch as light is not available (Though CO_2 is available), and the part of the leaf present inside the test tube also does not show presence of starch, though light is available. There is no CO_2 in the test tube as it was absorbed by KOH.

$$KOH + CO_2 \rightarrow KHCO_3$$

Aim : To show Light is essential for Photosynthesis.

Theory: Photosynthesis is a photobiological reaction during which light energy is harvested and chemical energy is produced in the form of carbohydrate.

$$6\,CO_2 + 12\,H_2O \xrightarrow[\text{Chlorophyll Pigments}]{\text{Light}} C_6H_{12}O_6 + 6\,H_2O + 6\,O_2\uparrow$$

The entire process is located in chloroplast which is known as photosynthesis process is divided in to light and dark phase.

During the light reactions (In the grana thylakoids, pigments mediated, light dependent) ATP and $NADPH_2$ are produced.

In 1937 R. Hill demonstrated/ gave the 1st biochemical proof showing that chloroplast is the site of photosynthesis. He demonstrated that isolated chloroplasts have the ability to bring about photolysis of water, liberation of oxygen and reduction of an artificial electron accepted (using the H^+ relaxed during photolysis of water, provided light is given to the chloroplast.

$$H2O + \underset{\text{(oxidized)}}{\text{Artificial electron acceptor}} \xrightarrow[\text{Light}]{\text{Isolated chloroplast}} \underset{\text{(Reduced)}}{\text{Artificial electron acceptor}} + O2\uparrow$$

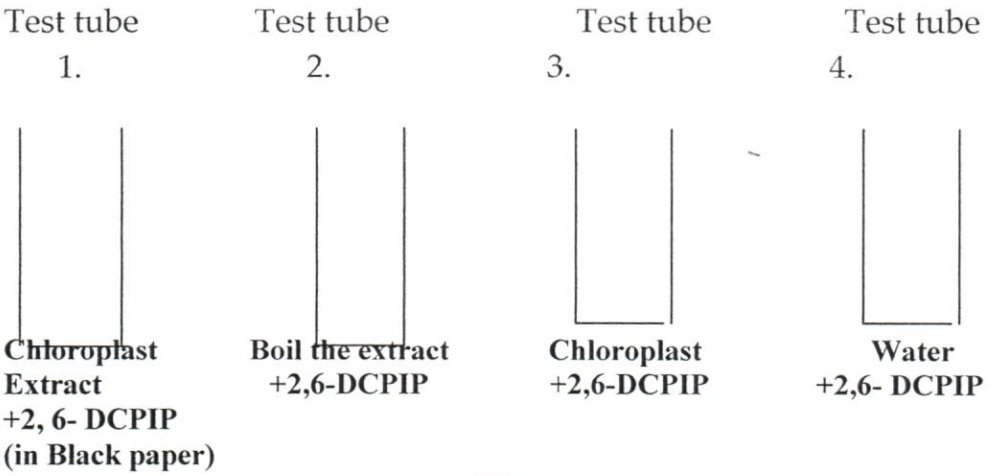
In the nature it is NADP which gets reduced (Ochao & Vishaniac 1951)

This reaction, popularly known as Hills reaction can be demonstrated in the laboratory using 2, 6 DEPIP (2, 6- dicholorophenol indophenols) which is blue in oxidized state and colorless in the reduced state.

Take 25-30 gms of palak (spinach) leaves, wash them in distilled water, cut them is to small pieces and grind manually/with help of mixie using 0.3 M sucrose. (We use 0.3 M sucrose to maintain chloroplast structure intact).

2. Filter the extract through a cheese cloth(muslin) and use the filtrate in 0.3M sucrose as Chloroplast suspension

3. Take 4 test tubes in a test tube stand, number them 1, 2, 3 and 4 as shown in figure. Add the chloroplast extract and 2, 6, DCPIP as shown in figure.



Diagram

4. Shake the contents of the test tubes, and leaves them in light for 5-10 minutes. At the end of the experiment observe for the color change (Blue to colorless) of 2, 6 DCPIP only test tube 3 will show color change.

By comparing

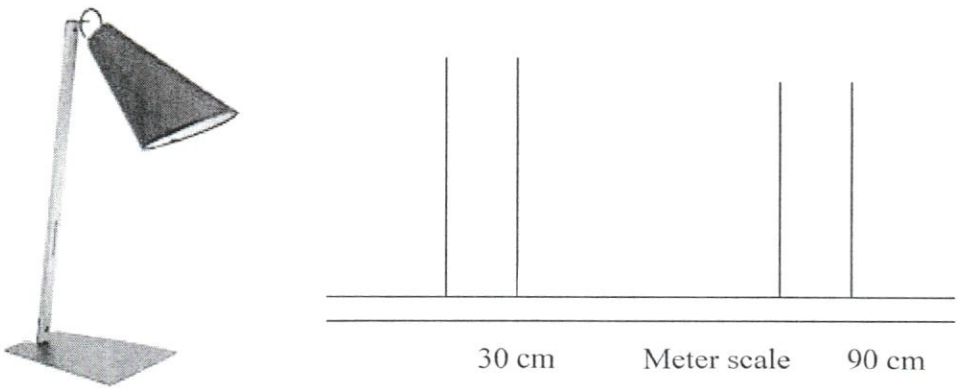
Test tube no 3 with 1 shows that light is essential for dye reduction (Hills reaction)

Test tube No. 3 with 2 shows that live chloroplasts is essential for Hill's reaction

Test tube No. 3 with 4 shows that chloroplast is essential for Hill's reaction to occur.

This experimental set up can be used to demonstrate

(1) Intensity of light affects the rate of photosynthesis.



Diagram

5. Shake the contents well and place test tube 3 and 4 at 1 feet and 3 feet distance respectively. Hove a table lamp (see figure) with help of test tube stands.

After 10 minutes, compare the color change (Blue to color less) due to dye reduction.

Test Tube No. 1 will not show any color change and remain blue as light was not available.

Test Tube No. 2 will remain bluish as the dye was not reduced since chloroplasts were denatured.

Test tube No. 3 & 4 will show dye reduction or color change from blue to colorless.

The intensity of color change will be more in test tube no.3 than test tube no. 4 indicting that intensity of light plays a role in dye reduction.

Aim: Estimation of the particulate matter deposited on the leaves of various plant species by gravimetric method.

Requirements - Leaves of different plants, a drawing brush, Graph paper, pencil, electronic digital balance and Polythene bags with lock system.

Theory - The plants growing at various localities are subjected to various types of pollutants which may be gaseous, particulate or in the form of bacterial and fungal spores, pollen grains and trichomes. Vehicular traffic is mostly responsible for the deposition of these entities.

Plants in general and trees in particular act as pollutant sinks, and highly reduce the atmospheric pollutants from reaching the animals and human beings. The amount of the particulate matter deposited on the leaves of various species of plants differs at any given time and largely depends on their texture. There is comparatively more deposition on the leaves which are rough and provided with tomentum, compared to the leaves which are smooth.

Learning objective - The polluted air contains a variety of both solid and gaseous material which originate from various sources. With the help of this experiment one learns the magnitude of the particulate matter at a given location and time as well as its contents such as dust, pollens, fungal hyphae, trichomes and bacteria and their endospores etc. For the persons allergic to a particular pollutant may be advised not to venture the area, when it is heavily loaded with allergenic substances. The amount and nature of the particulate matter deposited differs from time to time.

Method - At least 10 leaves of almost identical size are carefully removed from the trees/shrubs preferably growing on the roadsides with heavy vehicular traffic. Each leaf is transferred to a preweighed polythene bag and brought to the laboratory. Now the leaves are taken out one by one and with the help of the brush, the particulate matter is removed from the leaf surface and collected in a preweighed Petri dish with the help of a drawing brush . Now two or three leaves are put on a graph paper, their outline is drawn with the help of a pencil. From the drawing thus made, the area of the leaves is calculated. From the areas of the leaves, the mean area is calculated.

In another method, the leaves are gently washed under running tap water and washings are collected in a large beaker. The washings are then filtered and particulate matter collected on the filter paper is dried and weighed.

Observations: By visual observations it shall be found that the leaves are loaded with a very high amount of particulate matter of diverse type. After removal of the deposited material the leaves become more or less clean and exhibit a natural look. We come across with dust particles, fungal hyphae, spores, pollen grains and even bacteria in the deposited material, if observed under a microscope.

Calculations: (i) The area of the three leaves taken for measurement is summed up and divided by 3. It gives the average area of a single leaf. It is then multiplied by the number of leaves taken.

(ii) The increase in the weight of Petridish containing the particulate matter gives the total amount of the deposited matter.

(iii) The area of one leaf is multiplied by the number of leaves taken.

(iv)Now the total weight of the deposited matter is divided by the total area of the leaves to get the amount of particulate pollutants deposited per cm2 at any given time.

Result: The result is expressed in terms of mgs of the particulate matter deposited per sq. cm at any given time.

Questions

1. Is there same degree of deposition on the leaves of different species of plants growing side by side?
2. Which leaf surface shall retain more particulate matter on its surface (A) Tomentose (B) Glossy?
3. What type of plants you shall recommended to be grown as avenue trees to combat air pollution?
4. Will there be any variation in the amount of deposition of the particulate matter at different times?

Aim: Extraction of natural dyes from roots, stem bark, leaves, flowers, seeds and fruits. Dyeing of cotton fabric using different mordants.

Requirements - The above plant parts, corning beakers of 250 ml capacity, water, heating device, mordants (Alum, salts of aluminium, chromium copper, iron etc.)

Theory - Natural dyes are obtained from the flowring plants, lichens and fungi, and also from some invertebrates. Most of the plant dyes are anthocyanin and carotenoid pigments which impart colour to the flower petals and fruit. The mordant is usually an inorganic compound that helps in fixing the natural dye on the fabric. There may be different colour development using different mordants. Aluminium sulphate, Copper sulphate, Chromium chloride, Ferrous sulphate and even Sodium chloride etc. are used as mordants.

Learning objective - Apart from the synthesis of carbohydrates, proteins, organic acids and lipids, the plants produce a number of secondary metabolites such as alkaloids, tannins, terpenoids, flavonoids, carotenoids and various shades of anthocyanin pigments, which are specific for a particular species. Natural dyes may come from roots (*Rubia tinctorum*) , rhizome (*Curcuma longa*), bark (*Punica granatum*), leaves (*Indigofera tinctoria*), flowers (*Carthamus tinctorius*), fruits (*Terminalia catappa*) and seeds (*Bixa orellana*) etc. These dyes are capable of imparting colour to the cotton fabric which may be fixed with the help of a mordant.

Method - The natural dyes present in various plant parts may be extracted through:
(i) Soaking the dried/fresh and crushed plant material overnight or for several days.
(ii) By boiling and filtering it for dyeing fabric.
(iii) By Soxhlet extraction using water or Methanol as solvent.
After the dye is obtained, the fabric, usually cotton is subjected to dyeing by submersing it for varying durations.
The dyed fabric is treated with any mordant solution, stated above for fixing the dye to the fabric. Sometimes the fabric is first immersed in a mordant, dried and then dyed. For better dyeing combine one part of vinegar with four parts of water and boil the mixture for one hour, then immerse the fabric in the desired dye solution.
For dyeing synthetic fibres (Rayon, nylon, a terene, acrylic etc.) with natural dyes the fabric is pretreated with plasma, ozone or alkali such as NaOH.

Observation: As extraction of the dye progresses we observe an increase in the intensity of the colorant collected with the passing of time. Most of the natural dyes are water soluble and diffuse in water slowly. While extracting the dye through Soxhlet extractor, small amount of dye is siphoned in each cycle. For the complete extraction several cycles are required.

Result: After the extraction is over, we are left with a colorant or dye in the flask which is ready to be used as colouring agent with or without mordant.

Questions
1. What are different methods for the extraction of natural dyes?
2. What is the chemical nature of the natural dyes.
3. What are mordants? what is their function ? Name a few mordants which are commonly employed for dying with natural dyes.
4. Name the agents which are used as a pretreatment for dyeing synthetic fibres

Aim: Extraction of oil from the seeds of *Sesamum indicum* (Sesame), *Brassica campestris* (Mustard), and *Prunus amygdalus* (Almond) using Soxhlet apparatus.

Requirements - Soxhlet apparatus, reflux condenser, Rubber/PVC tubing, Round bottomed flask, heating mantle, ctton, Market samples of the aforesaid oils mortar & pestle and Petroleum ether.

Learning objective - We come across with several commercial brands of the

aforesaid seed oils. Whether particular oil is adulterated or pure can be found out by comparing the saponification value, Iodine value and Iodine number of the market samples and the one extracted in the laboratory.

Theory - A Soxhlet extractor is a laboratory apparatus for the extraction of lipids and other molecules from a solid sample. A Soxhlet extraction apparatus is composed of a condenser, an extractor, and round bottomed flask. It was invented in 1879 by Franz von Soxhlet Typically a Soxhlet extraction is used when the desired compound has limited solubility in a solvent and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

Procedure:

1. The source material containing the component to be extracted (*e.g.* oil) is placed inside the thimble after crushing it using mortar & pestle.
2. A small amount of cotton is placed at the bottom of the thimble before keeping the material.
3. The extraction solvent to be used is placed in a round bottomed flask.
4. The flask is placed on the heating mantle.
5. The Soxhlet extractor is placed atop the flask containing the requisite solvent.
6. A reflux condenser is placed atop the extractor.
7. The condenser is connected to the running water tap with the help of rubber/PVC tubing.

Operation: The solvent is heated in the range of its boiling point to reflux.

The solvent vapour travels up the distillation arm and after condensation floods into the chamber housing the solid.

Observation: The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound dissolves in the solvent. When the Soxhlet chamber is almost full the chamber is emptied by the siphon into the flask containing the solvent. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the flask. During each cycle, a portion of the non volatile compound dissolves in the solvent. After many cycles, the concentrated desired item is collected in the round bottomed flask. The material (say oil) is recovered by subjecting to reflux condensation. For doing so, the contents of the thimble are emptied. The empty thimble is fitted as in the beginning. The solvent accumulates in the thimble and we are left with the desired material in the round bottomed flask.

Note: The extraction is complete when the siphon is filled with only solvent (no colour).

Result: The material from the flask is collected and subjected to the desired physical/Chemical studies. The solvent thus recovered can be re used.

Questions:

1. Who invented this extraction apparatus?
2. Can we extract all the constituents using a particular solvent only?
3. How do we come to know that the extraction of a particular constituent is complete?
4. Based on polarity, what is the sequence of the following solvents for the extraction of various constituents of the given plant material?
5. Can we use the same plant material for the extraction of all the plant constituents?

Aim: To analyse living organisms in water samples.

Principle: The productivity and the trophic status of a water body is determined by assessing the number and type of organisms (micro as well as macro) present in the water body. Water body with very high density of phytoplankton per unit area is a productive water body. Such water bodies are usually turbid and have high amounts of nutrients and dissolved oxygen. These water bodies support fairly large number of organisms of different trophic levels. This is in contrast to non-productive water bodies, which have very low density of organisms per unit area, fairly transparent waters with low mineral concentration and dissolved oxygen and also fewer trophic levels. The status of health of a water body can be determined by analysing water samples for the number and type of organisms present in it at a given time. Such assays also help us to find out whether a water body is polluted as some of the organisms are strong indicators of water pollution.

Requirement: Water samples from different water bodies (lake, pond, river etc.), beakers, a few vials or small test tubes, slides and cover slips, watch glasses, dropper, compound microscope and 5% FAA (5:5:90,Formalin: Acetic acid: Ethanol) as preservative.

Procedure:

1. Collect about a liter of water sample from nearby water body (pond, lake, Reservoir, river etc).
2. Add about 5 ml of FAA to fix and preserve the living organisms present in Each sample at the place of collection.
3. In the laboratory, transfer the water sample into a measuring cylinder of one litre capacity.
4. Label each water sample to indicate the site from which the water sample has been collected.
5. Leave the water samples undisturbed for 48-72 hours.
6. Decant off the clear water, leaving concentrated sediment at the bottom.
7. Transfer the sediment into a vial or a small test tube. Cork and label each vial for future use.
8. With the help of a dropper, transfer a few drops of sediment liquid from a vial into a watch glass. Dilute the sediment with water if the sediment is Highly concentrated.
9. With the help of a dropper transfer a drop of water from the watch glass on the center of a slide and mount it. Blot the excess water using blotting Paper.
10. Prepare a few more slides of each water sample in the same way.
11. Observe each slide, first under lower magnification and then under higher magnification.

Observations:

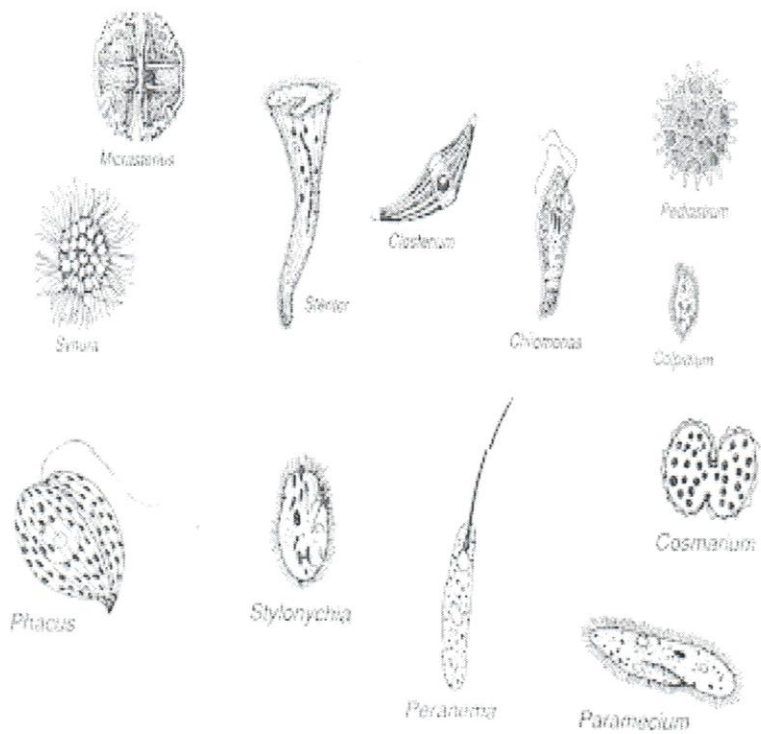
1. Record the different types of organisms present.
2. Count the number of organisms under microscope.

3. Some of the commonly found organisms of water bodies are- Euglena, Paramecium, Ceratium etc.

Conclusion: Prepare a list of organisms observed in each water sample and make an assessment of type and density of different organisms in each water sample. Polluted waters may contain very few types of organisms but in very high density. The non-polluted waters will have large variety of organisms in low density.

Questions:

1. Why do you find few organisms in polluted water? Explain.
2. Why is FAA (Formaline, Acetic acid and Alcohol) added after collecting the water sample?
3. Name at least one phytoplankton and zooplankton commonly found in polluted water.



Aim: To identify the presence of Barr body in the female Buccal cavity.

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

Materials required: Pre-cleaned slides, Round coverslips, Leishman stain, Microscope, Epithelial cells (sample)

Procedure :

1. Gently rub the inside of the cheek with a flat rounded piece of wood or round cover slip and transfer the scraping over a clean glass slide.
2. Then, made a thin film of cells on the slide and keep them for air-drying.
3. Add few drops of Leishman stain on the smear and incubate for 5-10. minutes.
4. After staining, the slide was washed with distilled water to remove the excess stain.
5. Finally, the slide was kept for air-drying and then observed under the microscope.

Observation: In female epithelial cells dark stained heterochromatin (Barr body) is observed. Usually in the periphery of the nucleus, clearly under high power microscope. While it is absent in male epithelial cells.

Conclusion: Cells are found scattered in the smear. Barr bodies are found inside the nucleus or on the membrane of the nucleus.Barr body is an inactivated(heterochromatinized) X chromosome. It was first observed by Murray Barr in1949 therefore, named barr bodies. It is found only in female cells, because one X chromosome is sufficient for metabolic activity however it is absent in male somatic cells, because only one X chromosome is present in them which is in an active form.

Precautions: The smear or film should be uniform and thin over the glass slide so that the cells will not overlap each other.

Questions:

1. Why is barr body found in female epithelial cells only?
2. How is barr body named?
3. Can we find more than one barr body in humans. Explain why?

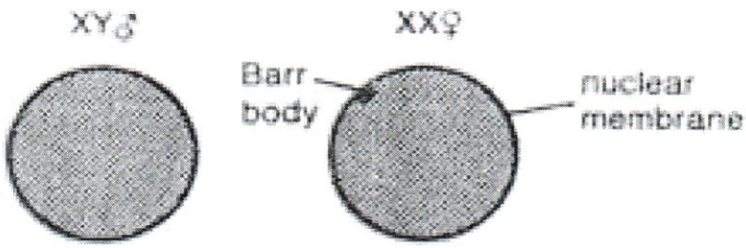
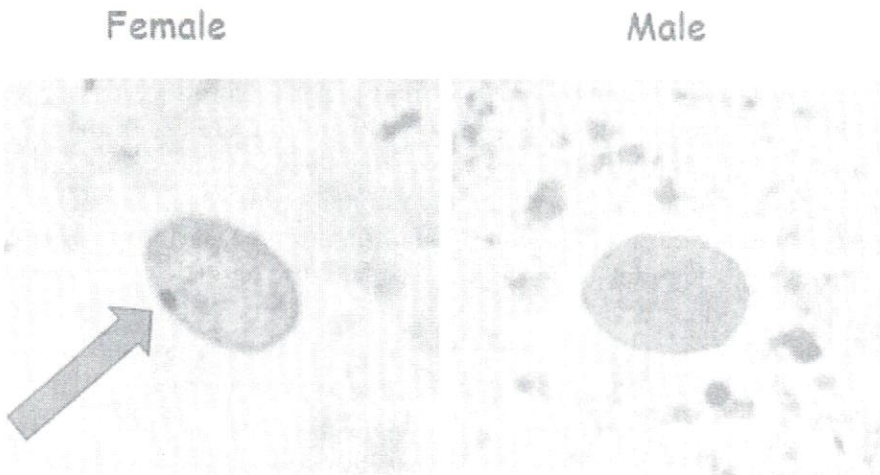


Fig. 61 **Barr body**. Sex chromatin in nuclei from human male and female buccal epithelial cells.



Barr body, an inactivated X chromosome

Aim: Separation of pigments by Paper Chromatography and chemical extraction

Theory: The chlorophylls are the essential components for photosynthesis and occur in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. Chlorophylls a and b occur in higher plants. Carotenoids are ubiquitous in plants. They are accessory pigments in photosynthetic systems and give characteristic color to plant parts.

Principles of chemical extraction

The total chlorophyll and carotenoids are extracted and partitioned in organic solvents on the basis of their solubility. Chlorophyll and carotenoids are extracted in 80% acetone for the initial extraction of the pigments of chloroplasts. Further separation is based on differences in solubility of the pigments; as chlorophyll a is soluble in petroleum ether, while chlorophyll b is more soluble in methyl alcohol.

Principles of chromatography

Slightly different solubilities of like compounds permit compounds to be separated in water and an organic solvent. If a strip of Whatman #1 filter paper is introduced as a wick into a small sample of such a mixture or into a closed atmosphere saturated with the mixture, the compounds will migrate up or across the paper at different rates. The factors affecting the separation of pigments along the filter paper are a combination of adsorption, ion exchange, and partition. Thus, unknown compounds can be identified by measuring the relative speed at which they migrate up the filter paper. The distances traveled by any compound are dependent on the rate of migration of molecules along a solvent front that is produced by the rise of the solvent along the length of the filter paper. As these compounds move along the filter paper, they distribute themselves between the solvent and the cellulose fibers of the paper. In general, the more soluble a compound is in water compared with the organic solvent, the slower it travels; it moves even more slowly if the pigment also absorbed by the fibers of the paper. Since the fastest molecules will travel the greatest distance, or to the highest point along the strip in ascending chromatography, the relative distances can be measured, and the rate of flow , or migration, of the molecules (R_f) can be calculated.

$$R_f \text{ (rate of flow or migration of the molecules)} = \frac{\text{distance solute (pigments) travelled}}{\text{distance solvent travelled}}$$

Expt. #1 **Demonstration on identifying pigments of chloroplasts**

Materials:

Sources of leaves:

1. Leaves of spinach (*Spinacia oleracia*)
2. Thin leaves of certain Shrubs (*Ocimum sanctum*)
3. Tender Leaves of *Bougainvillea* Sp.
4. 7-10 day or 14-21 day seedling of bean

Chemicals : 1. Acetone 2. Petroleum ether

Glasswares: 1. Test tube with stopper

2. Petridish

3. Micro Pipette or capillary tube

Other Material : 1. Whatman filter paper

2. Mortar and pestle

Methods of separation of pigments:

A) **Extraction of chloroplast pigments**

- Weigh 15 g of fresh spinach leaves and grind them in 15 ml of 80 percent acetone.
- Allow them to stand for 10 min and then filter to obtain a deep green colour filtrate. (This filtrate contains an acetone extract of the chlorophylls, carotenoids and other breakdown products of chloroplasts.)

B) **Prepare 1 inch wide strip of whatman filter paper**

C) Each student may take 1 inch wide & 150 mm (15cm) long strip of Whatman filter Paper. Cut the strip such that it tapers at one end (fig.). Let the students apply a drop of the extract of chloroplasts with a micropipette (or capillary tube) a few millimeters from the end of the strip and allow this dry. Apply a second time and after drying, a third time so on for 5-6 times so that the drop becomes quite green as the pigment is concentrated on the strip.

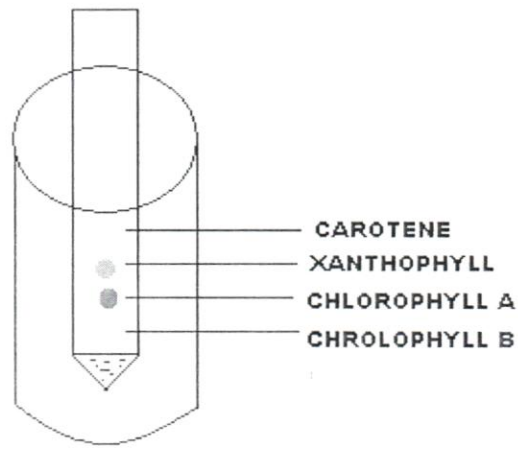


Fig . Strip filter method

Allow the students to place the strip loaded with pigments as a wick into a test tube containing 1 inch or so of a mixture of petroleum ether and acetone (caution: both chemicals poisnous) in 9:1 (9 ml petroleum ether plus 1 ml acetone). Use the stopper to hold the strip so that the tip of the filter paper wick dips into the solvent. Observe that as the solvent rises by capillarity along with which the pigments travel along with the advancing front of the solvent. Let students see the bands of different colours within 10 min. Remove the filter paper from the solvent when the solute i.e. pigments reach within an inch of the edge of the paper. With a ruler and a pencil student can mark the position of the center of the dot of the chloroplast pigments on the strip. Measure the distance of the edge of the advancing front (solvent) from the starting point (center where pigments were loaded) on the filter paper. Also measure the distance travelled by the different solutes or pigments or use the following formula for R_f.

$$R_f \text{ (rate of flow or migration of the molecules)} = \frac{\text{distance solute (pigments) travelled}}{\text{distance solvent travelled}}$$

Using the solvents mixture of petroleum ether and acetone as mentioned above, **students may find that carotene (bright yellow colour) travells fastest, followed by xanthophylls (dark orange colour), chlorophyll a (dark green colour) and then by slow moving chlorophyll b (yellow green colour).**

Expt.#2: **Chemical extraction of chloroplast pigments**

Materials required:

i) Chemicals: 80% Acetone, Petroleum ether, ethyl ether, 92% methanol, 30% methyl alcoholic KOH (30 g KOH + methanol till 100 ml)

ii) Glass wares: Separatory funnel, beaker, iii) Distilled water: 500 ml, iv) Fresh spinach leaves

Procedure:

A. **Preparation of leaf extract**

- 20 g leaf(green) is taken, homogenize in mortar and pestle with 80% Acetone, make total volume 100 ml
- solution is allowed to stand in separating funnel for some time(10 min), lower layer is discarded and the supernatant is treated as **pigment extract or green acetone solution**

B. **Separation of pigments**

Into the separatory funnel pour 40 ml of green acetone solution + 60 ml petroleum ether + 80 ml of distilled water down the side of the funnel, carefully rotate the separatory funnel; a separation of two layers should be visible-the upper layer, a deep green color, is soluble in petroleum ether

↓

Draw off and discard the bottom layer (acetone-water layer), Wash the petroleum ether layer with 40 ml of distilled water, rotate the funnel, note the separation of two layers

↓

Draw off and discard the lower water layer, repeat this procedure for 3-4 times to **retain a clean green petroleum ether solution**

↓

To this petroleum ether solution, add 50 ml of 92% methyl alcohol solution, shake well the solution (rotate the funnel), pour the two layers into separate beakers/bottles

↓ ↓

A-Upper petroleum ether layer contains carotene and chlorophyll a

B- The lower layer, the methyl alcohol layer contains xanthophyll and chlorophyll b

A. (Upper layer) Into a separatory funnel, pour 30 ml of petroleum ether solution (containing carotene and chlorophyll a) and 15 ml of freshly prepared 30% methanolic KOH (30% methyl alcohol-potassium hydroxide solution), shake the

funnel, add 30 ml of distilled water and rotate the funnel well until the two layers separate in the funnel.

i) Upper layer (a) contains **carotene** and **chlorophyll a**

ii) Lower layer (b) contains

the solution appears **greenish yellow** in color

and the solution color is **dark green**

B. (lower layer) Into a separatory funnel, pour 50 ml of the methyl alcohol solution (containing xanthophyll and chlorophyll b) add 50 ml of ethyl ether solution, mix slowly by rotating the funnel , add 5 ml of distilled water along the side of the funnel and rotate, until two layers separate out, discard the lower, methyl alcohol layer. Pour 30 ml of the ethyl ether solution into the test tube, add 15 ml of freshly prepared 30% methanolic KOH (30% methyl alcohol-potassium hydroxide solution) ,shake the tube, now add 30 ml of distilled water and shake until the two layers separate in the tube

i) Upper layer(c) contains **xanthophyll, chlorophyll b,**

ii) Lower layer (d) contains

solution is **brownish** in color

the solution is **yellowish green** in color

Students may compare the rate of development of pigments in growing leaves of seedlings or compare the development of pgments in leaves of buds of any plant with the pigment content of full-grown leaves as they develop from over a period of weeks.Students also find out that which solvents are best for carotenoids or chlorophyll a or chlorophyll b? Students also examine each solution of pigments in reflected and transmitted light to observe evidence of fluorescence.

Aim: To study human blood groups (ABO) and Rh group

ABO Blood groups: ABO blood groups in man consists of four blood types A, B, AB and O. The ABO system provides is with one example of multiple alleles. Phenotypic expression of A, B, and AB and O result from three allows in the population.

Material required: Glass slides, Pasteur pipettes, ethyl alcohol, anti A and anti B serum (blue and yellow coloured solutions, strived needless, anti serum D, sterile toothpicks, cotton.

Procedure:

1. Take a clean, grease free glass slide and put it on the working table.
2. Put one drop of antiserum A (blue coloured solution) on the slide at one end of it, and one drop of antiserum B (yellow colored solution) on middle and anti serum D on the other and of the same slide.
3. Take a piece of cotton (soaked in alcohol) and clean the middle finger tip of the subject (the person whose blood is to be tested).
4. Prick the middle finger of the subject; whipe out the first drop of blood that oozes out, with cotton.
5. Take the very next drop (marked 1) of free flooring blood on the slide besides the drop of antiserum A, and another drop (marked 2) besides the drop of antiserum B and antiserum D.
6. Immediately afterwards, mix the blood samples with respective antiserum with two separate sterile toothpicks. Following through mixing, fill the slide gentle for a while and observe as directed.

Result: There is No coagulation in Anti A and anti B, but coagulation is observed in Anti D. Hence the blood group is O^{+ve}

Aim: Staining of mitochondria in check epidermal cells by Janus green stain.

Materials required: Round cover slip, distilled water, Janus green stain, microscope, slide.

Principle: Mitochondria are formed in all eukaryotic. It is sausage shape forming outer and inner membrane. Inner membrane projects with matrix in the form of folds called cristae. The space between outer and inner Mitochondria membrane is called perimitochondrial space. The cristae bears several F1 particle. Mitochondria is said to be originated by endoplasmic reticulum. The membranes are chamber of Mitochondria bears several respiratory enzymes. It is called the power house of the cell as it released energy. By the oxidation of food stuff stored in it in the form of ATP.

Janus Green Stain: It is discovered by Leonor Michaelis in 1900. It is a basic dye used in histology. It changes the colour according to oxygen present.

When oxygen is present, the indicator ixides to blue green color. In the absence of oxygen, the indicator reduces to pink color.

Procedure:

1. Take all the required material as above
2. Wash the mouth and scrap the buccal epithelial cells with the edge of cover slip which is cleaned by absolute alcohol.
3. Spread the scrapping on the slide and dry it so that it can form a thin layer of cells.
4. Now add few drop of Janus green stain in the cellular layer.
5. Spread it over the slide & put it to dry.
6. Now observe it under compound microscope.

Precaution:

1. Scrapping of buckle cells should be done carefully.
2. The cellular material stain should be droid properly.

Observation: Many dot-like structures, dark green in color are observed in each cell which indicates the presence of Mitochondria in light or colourless cytoplasm in check epithelial cell.

Aim: To prepare a Karyotype from handout.

Material required: Plane sheet of paper, Karyotype handout, pair of scissors, glue, ruler, cello tape, pencil, and eraser.

Principle: Karyotype is a valuable research tool and to determine the chromosome complement within cultured cell. Different technical procedures are used to produce banding patter n an metaphase chromosome. A band is defined as the part of the chromosomes that is clearly distinguishable from it. Adjacent segments by appearing darker or lighter. The chromosome is visualized as consisting of a continuous series of dark and light bands. A.G. straining method resulting in G-band uses a Giemsa or Leishman dye mixture as the staining agent.

Procedure:

1. Firstly, the size of chromosome were measure and recorded in the handout for tentative estimation of homologues chromosome based on their length
2. Each individual chromosome was measure their cut out and arranged on plane sheet chromosome where then ordered by their length, the position of centromere, the position of chromosome bands and the relative hands and size and distribution
3. Chromosome are then secured with glue, chromosome were paired closely together and aligned the chromosome for easier hand. Comparison and checking for structural chromosome aberrations.

Observations:

Group – A: It consists of chromosome no. 1, 2, 3. The chromosome are large metacentric chromosome. In chromosome 1, 5 bands could be seen. In the chromosome 3, three bands are distinguishable. These are larges in size.

Group – B: It consists of chromosome no. 4 and 5. These are large submetacentric chromosomes.

Group – C: It consists of chromosome no. 4, 7, 8, 9, 10, 11, 12. These are also large (but smaller than Group-B) submetacentric chromosome. In chromosome no. 6, 9 two bands could be seen. In chromosome 8, 10 three bands could be seen.

Group – D: It consists of chromosome no. 13, 14, 15. These are acrocentric chromosome.

Group – E: It consists of chromosome no. 16, 17, 18. These are small metachentric chromosome.

Group – F: It consists of chromosome no. 19, 20. These are also small metachentric chromosome.

Group – G: It consists of chromosome no. 21 & 22. These are small acrocentric chromosome.

Sex chromosome: Sex chromosome X and Y are obtained Y is smallest in size.