NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 191

COURSE TITLE: General Biology Practical I

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MODULE 1

UNIT 1 WHAT PRATICAL BIOLOGY INVOLVES AND MAKING SLIDES

CONTENTS

1.0 INTRODUCTION

You are going to start this general practical biology course by gathering a general view of what practical biology involves. You must have, by now read the course guide which gives you all you need to know about the course. If you have not read it, you are advised to do so now. Reading the Course Guide is very important for your success in the

course work as a whole. Below are the objectives for this unit, which you must work hard to achieve.

2.0 OBJECTIVES

At the end this unit, you should be able to:

- state why practicals are important in biology
- give six hints on how to successfully do practical work in biology
- state at least six things involved in practical biology
- make good biological drawings
- highlight the points in good biological drawing.

3.0 MAIN CONTENT

3.1 Importance of Practical Biology

Biology is the study of living things. They (living things) perform various activities which biologist could study as they perform these activities. It is likely that if you see the organism you are studying physically and even in its natural location, you will remember and understand better than when you are taught only theoretically. Many facts about living things are continuously discovered in the laboratories and practical studies, most of these facts are discovered as biologists physically study the organisms. The large-scale destruction of natural flora and fauna upset the natural interdependence among living organisms. These facts also are evident in practical biological studies. Some of these interference which also have serious effects on man, can be better understood when the studies involve practical work. It is therefore important that practical, as part of the study of biology, should go hand in hand with theory. Once relationships and patterns existing among living things have been understood, biologists and other scientists can approach the problems in a logical orderly manner.

Throughout history, man has been challenged by the mysteries of their bodies and things that were around them. They wanted to know the causes of diseases and how to fight them; how life is produced and maintained and many other things. These are the knowledge that practical biology as part of biology will make available to us.

You may already have known these facts about the importance of practical biology. Take time to think of other important things not mentioned here.

3.2 Hints for Successful Practical Work

Even though these points look simple and commonplace, they will help you to succeed in your practical work. First, let us list here what you require to do a successful practical work. Without these you will experience difficulties in your practical work.

Requirements

You should get these things for yourself. They are essential for practicals:

l. Sharp pencils (H.B type)
2. Sharpener to resharpen when necessary
3. Sharp pen knife, razor-blade or scalpel
4. Dissecting pins
5. A flat piece of thick cardboard about l 10cm sq
6. Hand lens.
7. Pair of foolscaps
8. Watch glasses of various sizes
9. A small ruler
10. Microscopes and slides should be available to you at the study centre. Ask your tutor for these.

Pencils: It is necessary to specifically mention that unless your pencils are always sharp; your drawing will not be good. In an examination, you sharpen two or more pencils ready so that you don't loose time reshaping

Observations: You should always observe your specimens closely and very carefully. Look out for all details, size, texture, adaptive features etc. Some examiners even count number of part of the specimen you are supplied with. So your must draw exactly what you are given.

You must follow all the techniques for drawing in biology. They have been spelt out in details in the section on biological drawings.

Following Instructions: You must know that in practical biology, you must follow instruction that you are given to the letter. Sometimes, even for laboratory safety reasons, it is imperative. When carrying out experiments, your results could be wrong if you do not comply

Seek advice from your tutor when you are not sure of what to do in the laboratory. This is safer and important. You save your time as well by doing so.

3.3 Practical Biology - Involvements

We will now consider six things involved in biology Practical. Practical biology is part of the study of biology. It is ideally better if it goes side by side with the theory. It involves at least six things.

3.4 Field study

Sometimes, Biologists choose to learn about organisms in their natural habitats. In such places, you can see how they live and relate to others organisms and how they impact on their environment. A lot of what is done in ecology is done by field study.

However you as a student of biology could do a field trip to partly see where such organisms inhabit, then collect the organisms, take them to the laboratory for further studies.

SELF ASSESSMENT EXERCISE 1

If you go out of the room where you are now, work a distance of about 100 meters radius, you will surely find some plants and animals. Look closely to understand and describe where you find what. In the table below, put in the required information.

Serial/No.	
Name of Organism(s) (optional)	
Describe organism	
Where found.	
Describe location	

The items in the table can become your specimen(s). This takes us to the next involvement in practical biology work.

3.5 Collection of Specimens

In the activity above, you may need tins or empty clean bottles to put in your specimen. Let us imagine what you could have found. We could name them as follows:

Rats, leaves, seeds, fruits, flowers, flies, fish, birds, lizards, snakes, toads, frogs, butterflies, mosquitoes, grasshoppers, ants, snails, scorpion ants, worms, etc.

If you want to “catch” or collect these specimens, you will agree that the method of collection will vary with the type of organism and in which

state you want to have it and the kind of study you intend to do. You desire to study how the toad breaths; you will have to device means of collecting it without killing it. If you desire to study the structure of the skeleton in the snake, you must consider how you can “catch” the snake safely because of its nature, its land fragile skeleton. If however you wish to collect different types of butterflies to study the difference in their designs and colours, you will have to device or use an instrument to reach them as they fly. A net used in collecting butterfly may be different from the one which must necessarily be used for collecting fish from a pond.

There-fore various methods are used depending on the type of study, the type of organism, their habitats and how they live. Sometimes, the biologist has to collect his specimen. He may however seek the help of a laboratory assistance that may be specially trained to do so as well as many other things in the laboratory.

3.6 Preparing Specimens for Laboratory Studies

When you would have collected your specimens, you may find it necessary to bring them into the laboratory so that you could take time to study them. Often, you want to keep them for as long enough as necessary for sufficient studies to be made. For this reason, you need to know how best to keep what preservation. The same methods may not apply to all types of specimens. For example, bones will not be stored the same way as worms. The laboratory technologists may handle the details of these. It is however important for you to know that some storage and preservation may be necessary.

3.7 Preservation and Storage

Apart from preservation and storage, you might also need to prepare the specimen for the kind of study that is desired .For example, if you want to study the structure of a section through the root of a plant, you need a microscope to view the details of the sections. Besides, you need to make a slid. In Module 2, unit 5 you will learn about making slides

3.8 Making Sections and Slides

When you have to study the internal structures of (some parts of) some organisms you have to make sections.

These sections are described by the way they are made. A transverse section across a root for example is cut with a sharp blade-or penknife across the root. However, a longitudinal section is cut along the length

of the root. These two sections for example, will show different perspectives of the same structure.

Sections are most useful for biological studies when you cut them very thin so that the cells can be made easily visible either by the use of hand lens or microscopes.

You will need several practices to make good sections. So when you try and you do not get the whole section sufficiently thin, do not worry, try again you will soon be an expert. You may have to apply stains on sections to make them visible. Your course tutor will direct you on this matter. Remember that sections are best visible with magnifying glasses and microscopes. They may need to be mounted on the slides and kept well for viewing under the microscope.

3.9 Study of Specimens in the Laboratory

Identification is the first thing to do when you have brought your specimens, into the laboratory. Here you look out for characteristic features. In an examination or identification exercise, you state the features you have observed before concluding by naming the specimen. In other words, you are to give reasons first before saying what it is. You will look out for details in the external features. As you do this, you note the parts and their functions. You will look out for differences and similarities and why these exist. If the object of study is small, you may have to use the hand lens or the microscope.

3.10 Biological Drawings

Usually, the practical work includes drawings because it is a method of putting on record, what you did in the laboratory. When you have studied the details of an organism, be it the external or internal structures, you want to make a diagrammatic report of what you saw. It becomes very important for the biology student to be able make such diagram. Sometimes, you are specially told what to do and draw. It is very important to follow instructions and do exactly what you are told. You must also ensure that you understand the instructions you are given. You are advised not behave like some who choose to do what they like; they do not follow instructions they are given for practical. They even do not draw what they have seen in their practical work. They chose to draw from memory and such drawings may not be exactly what they have been given in the practical exercise. Examiners, they think will not be able to tell when they are not complying with instructions. But this is not true. Examiners are quick to know and they can interpret as dishonest on the part of the students who often have to loose marks for this reason. Your drawings should not be too small or too big your

drawings can be between a third or half a foolscap page. While drawing, you are also expected to study the specimen taking note of proportions. For example where the length of the mango is about twice its width, your drawing most also reflect this proportions. You are expected to record all curves. You are not expected to shade in biological drawings. Your pencil must be sharp. Most times you have to re-sharpen your pencil as you draw. The lines of your diagram must be thin and continuous. You should not allow the lines to go wavy or woolly. Drawing diagrams with wavy lines is often the problems with beginners. If you find that you do this in your practice, do not worry. With time you'll get used to drawing neat single lines. Fig. A shows you an egg drawn with wavy and woolly lines. Fig B shows clean clear drawing of the egg. Can you spot the woolly and wavy portions in the drawing 1a?

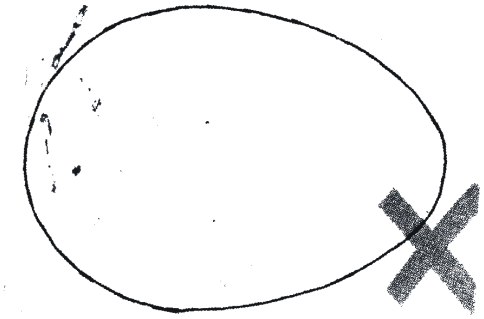


Fig. A A poor diagram of an egg – notice that the lines woolly (x1)

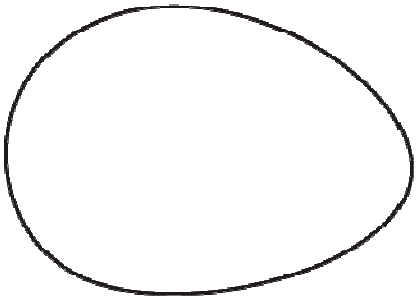


Fig. B A clean clear diagram of an egg

3.11 Views and Magnification

When you draw, you are expected to give some basic information about your specimen. You want to say how big the specimen you are presenting is in relation to your drawing. For example, you have drawn an egg and the size is exactly as it is physically. You will express this size by x l. If however you have drawn a bone. You have made your drawing half the actual size. You will express this by x1/2(or x.5). Suppose however you have drawn a bean seed which has been drawn five times bigger than it actually is, you will express this by x 5.

You are also expected to say what aspect or view of the organism you are drawing. Here the views refer to views of the organism as it exists naturally. For example you naturally see a dog standing on its legs. If you have placed the dog on its back and drawn it as you see it lying back down, the view you are seeing is the ventral view of the dog. If you draw the dog from the front i.e. as you see it looking at its face you have an anterior view. Fig.1.1 shows other perspective of organisms that you could have.

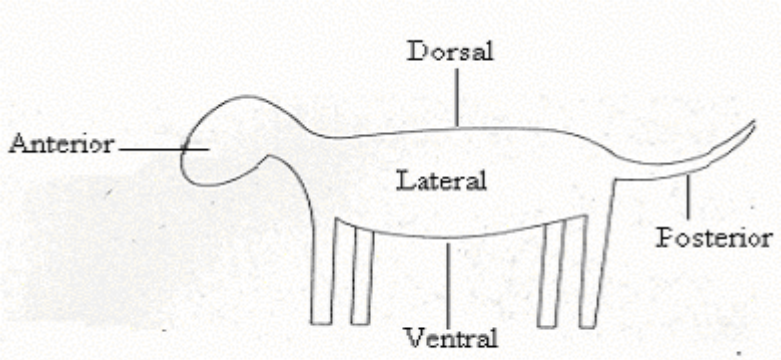


Fig 1.1 Aspects of a specimen

Sometimes, you draw what you see as you cut through your specimen to reveal internal structures. You call these sections. You may have to draw a longitudinal section through the mango fruit. This is a section you make by cutting the fruit from top to bottom along its natural length. (see figs 1.2 and 1.3)

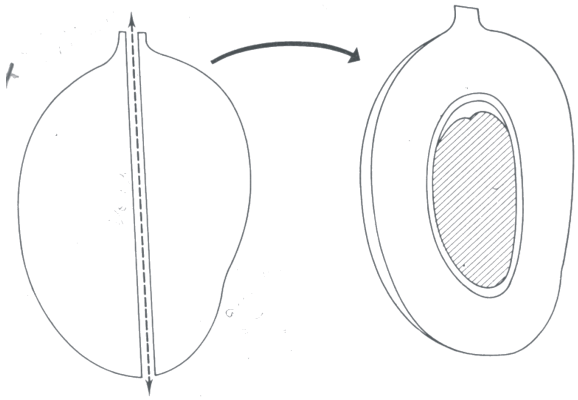


Fig. 1.2 Longitudinal section of a mango fruit(x 1).

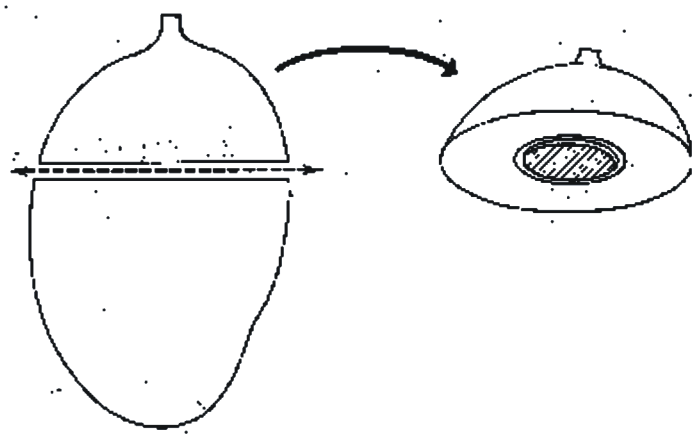


Fig 1.3 Transverse section of a mango fruit (x 1)

3.12 Labeling

An important aspect of biological drawings is the labeling lines indicating what you are labeling are called guidelines. The correct way to draw guideline is horizontally (i.e. running parallel to the top and bottom edges of your drawing paper). You should never allow them to cross each other.

They should be ruled not freely drawn with the hand. The guideline could be drawn on either side of the drawing if necessary, but you should not allow the lines to cross each other or the drawing itself. The shorter the guidelines, the better they are. You should write the labels with pencil. You should not put an arrow at the head of the guidelines because in biology arrows show movement at direction. Fig. 1.4 and 1.5 show correct way to label and the incorrect way respectively.

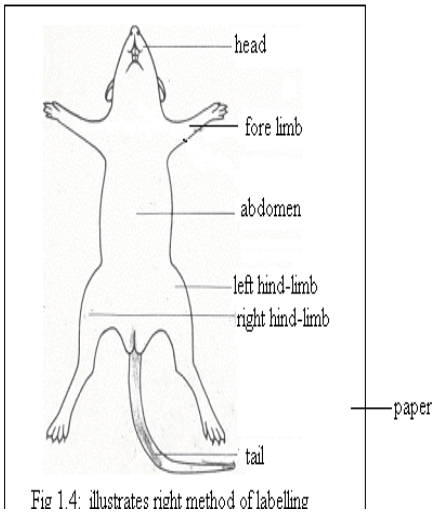


Fig 1.4: illustrates right method of labelling

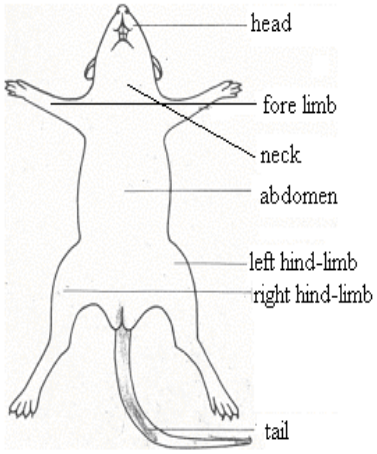
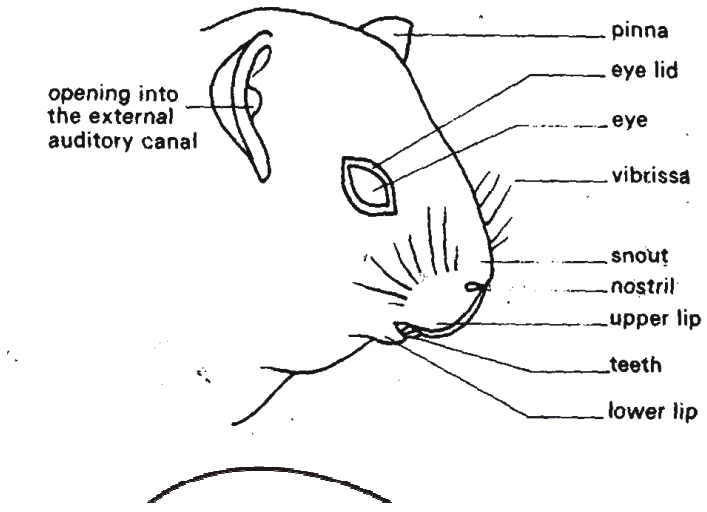


Fig 1.5: illustrates incorrect way of labelling



You will always remember to label your diagrams fully. (see fig 1.4)

3.13 Headings

You must put a heading for all diagrams that written above or below the diagram.

The heading contains three vital elements:

1. The identity of what you have drawn e.g. diagram of the head of a guinea pig.
2. The magnification or size.
3. The view or the organism, which you have drawn.

SELF ASSESSMENT EXERCISE 2

1 What should be incomplete labeling of fig. 1.6?
2 Identify two reasons why fig. 16 is described as wrongly labelled.
3. Identify good drawing in fig. 1.4 and state why they are good.
4. Identify bad drawing in fig. 1.5 and state why they are bad.

3.14 Experimentations

You have to perform certain experiments in biology practical. Scientists do experiments to find out facts, or you do experiments in biology to confirm them. You may have carried out experiments to show some enzyme actions, prove that some processes (e.g. respiration and transpiration) are taking place. You may also want to demonstrate certain confirmed happenings organisms. For example, you know that root grow downwards not upwards, or that plant grow towards light source, or the impact of auxins on growth, you may also have to determine the amount of water in a soil sample or amount of humus, or differences in amount of capillarity between two types of soil: You can-, continue to name other experiments you may have to carry out. The important issue here is that your practical work in biology can also involve doing experiments.

You are expected to report your experiment systematically.

The items of your report should include the following in the order

1. Date of experiment
2. Purpose-of experiment
3. Materials- A piece of raw potato - iodine Petri-dish

4. Procedure- Describe what you did e.g. place the piece of potato the Petri dish add two to three drops of iodine. Here state what happening e.g.
5. Result -A blue-black colour appear on the potatoes
6. Conclusion- Shows that starch is present (if necessary)

Sometimes you might want to make a diagrammatic representation of the steps in the proceed. This is so where there are various set-ups on the same experiment. You will number progressively or use arrow to show directions.

3.15 Reporting Practical Works

Like the practical proceedings. You will record or report accurately all the practical work that you have done.

4.0 CONCLUSION

In this unit you have learnt that practical work is an essential complement of theoretical study of biology.

You have learnt about the basic essentials and techniques in practical biology. You even learnt about the things you need for practical. We have noted that all through practical work you need to be careful, exact and follow instructions carefully.

5.0 SUMMARY

Now you are ready to apply these basic principles of practical biology to your practical lessons, in biology. In the unit that follows, we will learn about the structure and function of magnifying glasses. Remember that we need them in practical biology to properly study small organisms or small parts of large organisms.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly describe why practical work is important in the study of biology
2. What are the things you are likely to be doing in a practical session.
3. Describe with illustrations how to make good biological drawing.

7.0 REFERENCES/FURTHER READING

LT. 2 Laboratory Techniques Manual Biology 3.

UNIT 2 LABORATORY ORGANISATION 3

CONTENTS

1.0 INTRODUCTION

In principle a well designed, organised, and aesthetically appealing lab. is attractive to everyone. Besides, it’s convenient to use, easy to clean and maintain. It elevates the mood, increases the enthusiasm of students and teachers alike, and is conducive for working. Unfortunately, many of us tend to neglect this aspect and do not make an effort in this direction.

Whether you will be allowed to reorganize a lab. or not, through this exercise, you will become aware of the approach and guidelines used for organizing it. Besides, you may get a few tips for organizing other places such as - home, work place, factory etc. and one would like that whichever place you are allowed to implement your ideas you will take the opportunity and do so.

In most jobs a well-organised place is primary for efficient functioning. Organising a place is almost always a collective activity and a lab. is also organised by the joint efforts of people using it. Though, there are a set of guidelines common for most laboratories and dos' and don't are well-defined but each lab. is unique in its set-up and reflects the talents of the users. It is seen that many people are uncomfortable at the thought of rearranging a place because it is quite demanding and involves hassles. Therefore, they simply put up with any amount of disorder and

lack of cleanliness. This attitude is undesirable and we must strongly discourage it.

In this exercise you will be doing the following activities with regard to laboratory.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- itemise all the things available in the laboratory
- plan the situation of available items in the laboratory
- display general instructions for laboratory use
- label specimens, slides and reagents in the laboratory
- compare notes on laboratory organization.

3.0 MAIN CONTENT

3.1 Laboratory Organization

3.2 Activity 1: To Plan the Organisation of a Laboratory According to Items Available

It is important for a technician to participate in lab. organization since he/she is given the responsibility for its day to day management. He/she is expected to know about everything that is present in the lab. and its purpose. In this activity you will learn how to plan the organization of a lab.

First, prepare a comprehensive account of the items 1 to 10, listed below. Examine the lab in which you are conducting the practical and find out what is available. To save your time we have listed the things commonly available in most labs. Add the missing things to complete the list and strike off what is not available. With regard to each item, it is important to consider the size, number and suitable place for keeping them. You should classify small items and put them together.

1. Accommodation

No of main labs
Preparation room
Store
Office
Museum

Any other (animal house, green house, herbarium etc.)

2. Persons to be Accommodated

Number of Permanent Staff
Number of Teachers
Number of Supporting Staff
Number of Research Students
Number of Batches of Students
Number of Classes
Any other

3. Furniture

Teacher's table, chair
Work tables/ benches
Revolving stools Almirah for storage
Filing cabinet (s)
Slide cabinet
Open shelves
Office tables
Any other

4. Apparatus/Equipment

Dissecting Microscopes
Compound Microscopes
Microtomes
Spectrophotometer (s)
Incubators
Refrigerator (s)
Centrifuge (s)

Hot air oven
Hot plate
Water baths
Magnetic stirrer
Balances (physical and chemical)
Electrical balance

Distillation plant
Knife sharpener
Shaker
Autoclave
Pressure cooker
Safety cabinet

pH meter
Any other.

5. Glassware

Beakers
Test tubes
Conical flasks
Round bottom flasks
Volumetric flasks
Distillation flasks
Pipettes
Burettes
Measuring cylinders
Jars
Bell Jar
Desiccators
Troughs
Petri dishes
Watch glasses
Micro slides
Cover slips
Cavity blocks
Any other

It is important to know the quantity in each category to estimate the space requirement

6. Chemicals

Solid chemicals
Liquid chemicals
Stock solutions, stains and culture media

Try to prepare a list of the above and estimate the space required.

7. Specimens, Charts and Models

Write details of each and estimate the space requirement and appropriate place for each.

8. Miscellaneous items

Bunsen burners
Spirit lamps
Iron stands

Tripod stands
Wire gauges
Dissecting kits
Any other

Complete the list and try to categorise them.

9. Provision for disposal

Garbage cans
Incinerators

10. Files, -Records and Catalogues

Files for documents.
Records of activities/events.
Catalogues of classification for ease of reference.

3.3 Activity 2: To Plan the Placing of Items in the Laboratory Based on Available Space

Ensure that you estimate in details the space required for each of the items classified (I10) as already explained. Once your list is ready examine the organization of the lab. See if there is scope for improvement. It is also likely that you may come up with a completely new plan of organization. If in any case you cannot implement your ideas for this lab, then put down your suggestions in table 2.1

You must consider the following questions before your suggestions.

l. Is the present arrangement satisfactory?
2. Who will use the lab and for what purpose?
3. Is the furniture arranged in a manner that staff and students would be able to move around easily?
4. What items are to be used everyday?
5. Which of the experiments are routinely performed?
6. What precautions are required while placing electrical appliances?
7. Which is the best place for storing glassware?
8. Where could the different chemicals (acids/photosensitive/heat sensitive) be safely stored?
9. Is the provision for storage most appropriate?
10. Are the provisions for safety adequate?
11. Is the arrangement of chemicals and equipment adequate for independent student activity?
12. Are the provisions for good housekeeping adequate?

13. Is the arrangement of furniture, equipment, glassware etc. convenient and serviceable.
14. Is the area for conducting certain experiments identified?
15. Is it possible for the students to study the displayed charts, models and specimens?
16. Is there a convenient arrangement for frequent washing of the glassware by the students?
17. Is the arrangement for disposal satisfactory?

Now complete the table given below:

Table 2.1

List of Items	Placement		Suggestions
	Appropriate	Not Appropriate	

After completing this exercise discuss your suggestions with your peer group and Counsellor. Remember organizing a place is a collective effort.

3.4 To Display General Instructions for Laboratory Use

Materials Required

You require the following materials in order to write and display general instructions for laboratory use.

- Coloured chart paper - Scissors
- Coloured pens
- Adhesive tape

Procedure

Display instructions/rules is very important for housekeeping, convenience, ease and above all the safety of a lab. Preparing a display is partly a creative exercise since it requires a little bit of artistic ability. A display should be conspicuous, legible, neat, and attractive.

Here we have listed some laboratory safety general guidelines. Choose any 7 instructions that you consider more important that the rest for the display. Cut a chart paper of an appropriate size, write the instructions neatly using a colour pen that can highlight it and try to make it attractive without missing the purpose. When it ready, display it at an appropriate place, using the adhesive tape.

1. Keep the lab scrupulously clean and free of unnecessary things.
2. While entering the lab, place all books, notebooks and purses in the designated areas and not on the working benches.
3. Do not eat, drink or smoke in the laboratory.
4. Do not make a noise in the lab.
5. Do not wear short or loose clothes in the lab.
6. Always wear a lab coat.
7. Do not perform unauthorized experiments.
8. Do not use equipment without reading the instructions.
9. Report all spills and accidents to your instructor immediately.
10. Never leave heat sources unattended.
11. Keep containers of alcohol, acetone, and other inflammable liquids away from flame.
12. Leave the laboratory clean and organised for the next batch of students.
13. Wash your hands with powdered soap prior to leaving the laboratory.
14. Upon completion of laboratory exercises, place all discarded materials in the disposal area designated by your Counsellor.
15. Do not allow any liquid to come into contact with electrical cords. Handle electrical connectors with dry hands. Do not attempt to disconnect electrical equipment that cracks, snap, or smoke.
16. Wash skin immediately and thoroughly if exposed to chemicals or microorganisms.
17. Never pipette by mouth. Use mechanical pipetting devices.
18. Wear disposal gloves whenever necessary.
19. Use bandage on cuts or scrapes before attending the lab.
20. Do not taste the chemicals
21. Do not lick the labels.
22. Decontaminate work surfaces after any spill of potentially dangerous chemicals/micro-organisms

23. Wear safety glasses, gloves or other protective devices whenever necessary.
24. Turn off gas, electricity and water before leaving the lab.

3.5 Activity 4: To Label Specimens, Slides and Reagents

Specimens Labels

Biology laboratories have a collection of a variety of plants and animals. In units 6,7 and 8 you have learnt how to collect and preserve biological specimens. After the collection it is essential that such specimens are appropriately and attractively labeled. Likewise, certain slides of tissues, organs or micro-organisms prepared by a teacher or student may be essential or valuable for permanent record. These need to be appropriately labeled for proper identification.

Specimen labels should therefore carry the following essential information:

Example:

1. Common name of organism
2. Kingdom - planta
3. Phylum - Angiospermatophyta
4. Class - Angiosperms
5. Order – Moncotyledoneae
6. Family - poaceae
7. Genus - Zea
8. Species –zeamays
9. Place from where it was collected (optional)
10. Name of the collector, date.

Labels for Slides

They should carry the following information

1. The name of the organism - if the whole organism is mounted then the slide can be marked WM = whole amount or E = entire. Examples: *Paramecium* WM, frog Blastula WM.
2. The part of the organism used, e.g liver, root, examples liver of frog, onion root.
3. The type of preparation, e.g smear, squash: examples human blood /smear
4. Type of section e.g. TS=transverse section; VS =vertical section; LS = longitudinal section. Examples TS of kidney, VS of phloem.

5. Other specifications e.g type of cell division (mitosis, meiosis) stage of cell division (prophase/metaphase), sporulation.

   The following information is desirable but not essential.

6. Stain(s) used e.g. H.E = Haematoxylin Eosin.

   If the slide is prepared in house then it should be:

7. Dated

8. Installed.

It is common to use two labels, one on each end. Self - adhesive or gummed slide labels pre-printed with lines are available form lab. suppliers. Alternatively, use ordinary self-adhesive or gummed labels. You will find it easier to write the label before you stick it one the slide and remember if the label is gummed (rather than self adhesive) you must not lick it. Instead use a wet sponge.

Prepare at least one label and stick it to a slide.

Sample Label

- Onion root tip, squash
- Mitosis, prophase
- Vinita sharma,
  20th October, 1999

Labels for Reagents

We also need to label routinely prepared stock solutions, stains and culture media for identification. These should carry the following information.

- Name of the Chemical(s)
- Percentage / molarity
- Aqueous/any other solvent
- Date
- Initial

Sample Label
5% CuS04 (aqueous)
Lalit, 15/3/2000

3.6 Activity 5: To Compare Notes on Laboratory Organisation

In order to compare notes on all that you have done, you need to look at a demonstration on laboratory organization. You may use the supplementary video clips for this purpose. Ask your course tutor for help in making this available. Happy viewing as you compare notes

4.0 CONCLUSION

In this unit, you have learnt how to organise a laboratory. You will find the experiment with the exercises very useful to you in general and for laboratory work in particular. You have learnt that order and neatness enhance the success of anything you do. Laboratories are places where serious studies go on. Sometimes, a lot of care is necessary to keep safe either in handling equipment or in ensuring your personal safety.

5.0 SUMMARY

Whether you are involved in organizing your study laboratory, you will by this experience know why things are placed where they are, or why you must follow instructions as well. The next thing you would want to know is how you would handle the kind of equipment you will find in your study laboratory.

6.0 TUTOR-MARKED ASSIGNMENT

Suppose your father allowed you a room in your compound for your use and you want to make part of it into a laboratory corner for your practical studies, how would you set it up?

7.0 REFERENCES/FURTHER READING

A NOUN Video Demonstration on Laboratory Organisation (To be produced by NETC)

Berril, N.J. (1979) Biology in action. London Heineman Educational Books Ltd

Soper, R. (Ed) (1998) Biological Sciences. Cambridge UK, Cambridge University Press.

Stone, R.N., Cozens, A.B, Emia, F.I (1972) New Biology for Tropical Schools London, Longman Group Ltd.

UNIT 3 HANDLING COMMON LABORATORY EQUIPMENT

CONTENTS

1.0 INTRODUCTION

Each Science lab. i.e. Physics, Chemistry and Biology is equipped with equipment. In this unit, you will study about the equipment which are necessary for a biology lab. A lab technician should know about various types of equipment and their uses. As a lab. technician, you should also know about the working and maintenance of equipment. If you have not seen any of these equipment before, don't worry. You will soon get used to seeing and using them. You only need to follow the guidance of your course tutor and ask questions when you are not sure.

2.0 OBJECTIVES

After going through this unit, you should be able to:

- Use a pressure cooker and autoclave for sterilization,
- Operate hot air oven and water-bath,
- Handle a centrifuge,
- Operate laminar air flow system,
- Use microtome knives.

3.0 MAIN CONTENT

3.1 Handling Common Laboratory Equipment

3.2 Materials

Autoclave
Pressure cooker
Microtome knives
Dissecting kit
Hot air oven
Incubator, Water bath, Centrifuge
Laminar Airflow System

3.2.1 Pressure cooker

In this experiment you will study about the procedure to use a pressure cooker.

Procedure

l. Pour 300 ml water into a cooker
2. Place the container with materials to be sterilized inside the cooker and close the lid.
3. Lock the cooker making sure that it is sealed properly.
4. Place the cooker on a burning stove.
5. See that the air is expelled from inside the cooker and a clear and continuous steam comes out from the vent tube. Fit the vent weight on the vent tube immediately.
6. A hissing sound is heard in about 5 minutes and the vent weight (pressure regulating device) will produce a whistle. It there is no hissing sound after 5-7 minutes and the steam is seen escaping around the rim, it means the lid has not been properly fitted. In this condition take the cooker off the stove, adjust the lid with thumbs pressing down slightly.
7. Now the steam will lift the vent weight and will be expelled from the vent tube producing a loud hissing sound. This will indicate that the full cooking pressure (sterilizing pressure) has been reached.
8. Allow the cooker to remain on the stove for at least 15-20 minutes for sterilization.
9. Later, take the cooker off the stove and allow it to cool on its own for a sufficient period without removing the lid or vent weight (Fig 3.1)

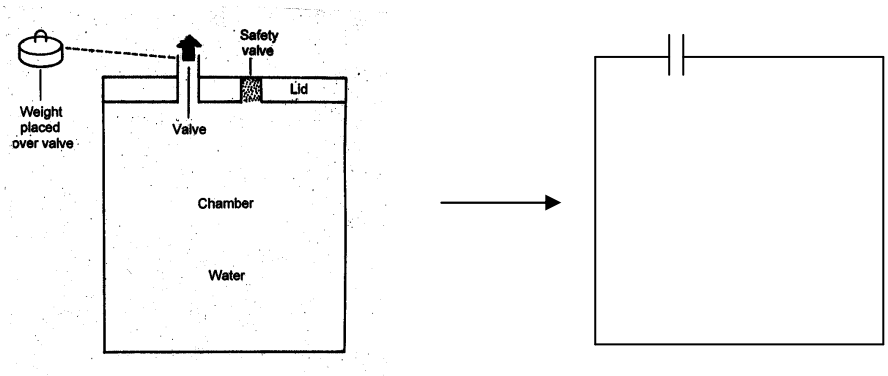


Fig. 3.1 Pressure Cooker.

3.2.2 Autoclave

These days sterilization is very conveniently done in the laboratories with the aid of an autoclave. These are of different types such as: (1) Simple autoclave (2) Steam jacketed autoclave and (3) Automatic autoclave.

In this unit, you will know how to use an autoclave.

Simple Autoclave

The following steps are to be carried out at the time of using an autoclave:

Procedure

1. Pour a sufficient amount of water into the autoclave.
2. Set the safety valves, put the articles to be sterilized in the container and load it inside the autoclave.
3. Open the steam outlet for passing it out.
4. Lock the door by tightening the bolts diagonally.
5. Set the autoclave pressure.
6. Place the autoclave on heat source ensuring that heat is maximum.
7. See that air is expelled from within the body of the autoclave and a continuous stream of steam comes out form the steam outlet. Now shut the steam outlet.
8. Allow the autoclave to come to the required pressure and adjust the amount of heat so that the needed pressure is maintained and then time it.
9. Let the materials remain at a necessary pressure for a definite time. Later cut the heat source and allow the autoclave to cool.

10. Open the steam outlet when the needle reaches zero in the pressure gauge.
11. Before opening the door of the autoclave and taking out materials/articles which were sterilized, let the autoclave cool down for a considerable time.

Precautions

l. Do not open the valve before zero is reached because the boiling liquid will dampen the cotton wool plugs and there will be chance of contamination by bacteria which may enter through the moisture film up to the medium.
2. Do not open the valve when a vacuum has developed within because the air will rush suddenly inside the autoclave and will carry loose fitting plugs into the autoclave.
3. Do not allow the vacuum to remain for a long time because it will take out moisture from the medium kept inside the autoclave for sterilization.

Steam-jacketed Autoclave

Procedure

1. Allow circulation of steam through jacket continuously at a required pressure.
2. Put the articles inside the autoclave when the jacket has attained a working temperature.
3. Shut the door, lock it in position and see that discharge outlet, provided at the bottom of barrel, is open.
4. Close the discharge outlet when required temperature is reached. (In automatic jacketed autoclaves he discharge outlet is thermostatically controlled which automatically closes when predetermined temperature is reached). Any drop in temperature due to condensation of steam during sterilization helps in opening of outlet. The condensed steam goes to waste allowing the fresh steam to enter inside the barrel and predetermined temperature is reached again.
5. Allow steam to enter from steam jacket through baffle fitted at the back of barrel.
6. Count the period needed for sterilization after required temperature has been reached and discharge outlet valve has closed (15-20 minutes are needed for sterilization of media).
7. Cut down the supply of steam to barrel after 15-20 minutes of sterilization and allow it to cool.
8. Open the door of autoclave when the pressure inside has become atmosphere and take out the sterilized articles (Fig 3.2).

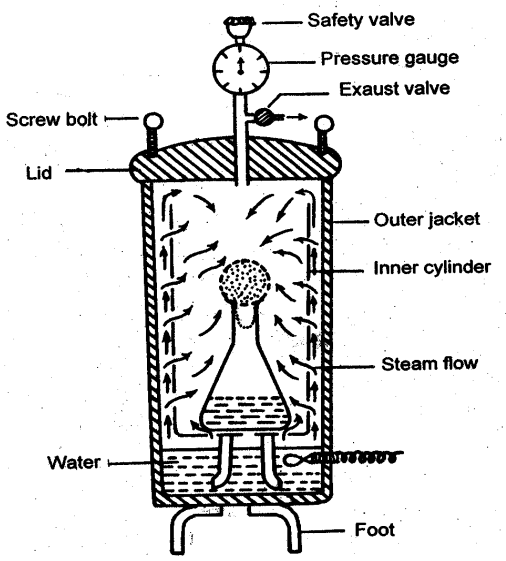


Fig. 3.2 Diagrammatic representation of an autoclave

3.2.3 Hot Air Oven

In this experiment you will learn how to use one.

Procedure

1. Sterilize only dry Petri-dishes singly or together in a metal can. If a metal can is not available, wrap the Petri-dishes with aluminium. Do not bring out the sterile Petri-dishes from can or unwrap them till they are used.
2. Flasks and tubes should be dried and plugged with cotton wool before sterilization. The tube should be placed together in iron, steel and heatresistant glassware e.g. enamel tray, test tube stand and beaker.
3. Pipettes should be plugged with cotton wool at the mouth end after air drying and placed in an oven in a closed metal cylinder.
4. Do not exceed the prescribed temperature.
5. Do not overload. These may prevent air circulation and the glassware may not be properly sterilized.
6. Allow the temperature to rise up to 160 degree centigrade and continue sterilization at this temperature for one hour.
7. Do not open the door of the oven immediately after the sterilization because glass may develop cracks due to sudden fall in temperature.
8. Use a towel to remove the glassware (Fig 3.3).

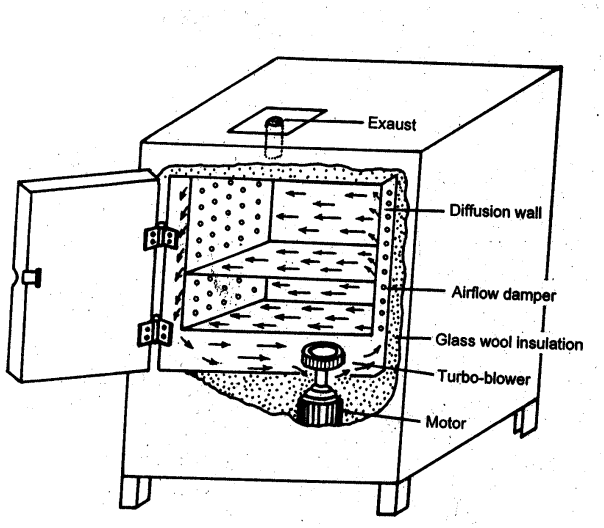


Fig 3.3: Diagrammatic representation of hot air oven.

3.2.4 Incubator

It is an electrically operated equipment designed to provide a controlled temperature for the growth and development of microorganisms in culture media. Its construction and operation are more or less the same as those of a hot-air oven.

Only the operational range of temperature is lower in an incubator which lies between room temperature to a temperature of 50 degree centigrade..

Incubators, hot air ovens and water baths require accurate temperature control. Required temperature in such apparatus is maintained by a thermostatic system.

3.2.5 Water-bath

The liquid contents of tubes or flasks kept in a water-bath are raised to the required temperature much more easily and much more rapidly than in an incubator. The difference in the level of water in the water-bath and that of the liquid in the tube or flask causes a convection current which makes the liquid in the tube to mix well and hasten reactions. A water-bath is equipped with thermostat, stirrers and cooling device. It is advisable to use distilled water in a water-bath to avoid chalky deposits on tubes.

3.2.6 Centrifuge

A centrifuge is an important equipment of the biology lab. In most biological experiments generally, low-temperature centrifuges should be

used. This can prevent metabolism and loss of viability or enzyme activity during the operation of the centrifuge.

Precautions during centrifugation

1. Tubes must be put in pairs to balance.
2. Be sure that rubber cushions are in position at the bottom of the bucket before inserting the tubes.
3. Be sure that cotton wool plug if used are not forced down during centrifugation,
4. Make sure that the metal buckets are properly sealed on the ring and are free to swing.
5. Secure the lid properly.
6. Bring the rheostat to zero before start. Gradually increase the speed and bring the speed to required rpm (resolution per minute).
7. After centrifugation, switch off the meter and then bring the rheostat to zero position allowing it to come to a stop. Do not apply hand to slow down the speed.
8. Take care to lubricate periodically.

3.2.7 Laminar airflow system

Handling of microorganisms under a bacteriological safety cabinet may not always be suitable for many practical reasons.

To keep such a room free of all microbes carrying particles, a new kind of technology has been developed. This technique is known as laminar airflow technique. In this technique, air of a closed room or cabinet is allowed to pass through a High Efficiency Particulate Air (HEPA) filter pack and the filtrate becomes free of all particles above 0.3 cm dimension. The technique involves sucking in room air and blowing it out through a bank of fitters with uniform velocity and in parallel flow line used in microbiological and pharmaceutical laboratories and in aerospace industries.

The advantage of the system is that in operation involving inoculation, transfer of culture and in opening of lyophilized culture, no closed chamber is necessary, instead that operation can be done on a platform provided with the laminar airflow unit making it easier for handling (Fig 3.4)

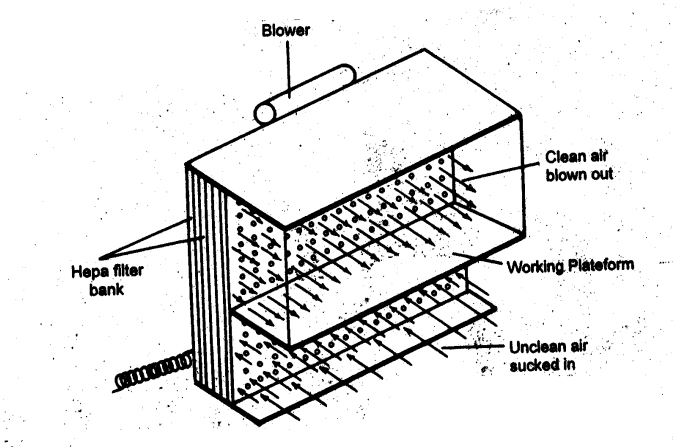


Fig. 3.4 Schematic diagram of a horizontal laminar airflow unit.

3.2.8 Microtome knives

There are three basic cross sectional shapes of knives, wedges, piano concave and double concave.

The best knives for the cutting of paraffin blocks are the double concave type, which should be of a heavy pattern cross-section to prevent vibration, i.e. the taper should be short and the base wide back down, to repeat the stroke. The knife is also moved laterally along it s length as the stroke is made.

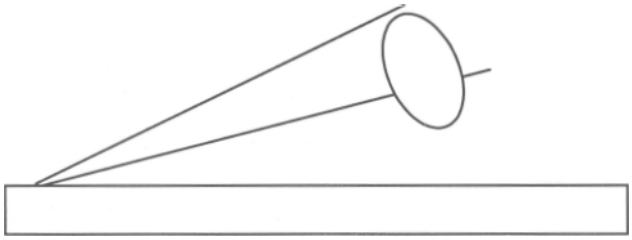
Strops are made of shell horsehide and require only a little light oil rubbed into the back of the leather occasionally to keep them in condition. There are strops which are fixed (hang) on a wood block to keep them rigid, and these require strop dressing applied sparingly to the front at intervals.

Hones are made of stone which is of an extremely fine texture and which is ground to a true flat face. Stones too are delicate and should be used over the full area of the face so that no inconsistencies develop in the glass-flat surface. Hones are generally dressed for sharpening with a little fine oil or water. Whether they are water or oil stones is stated on the manufacturer's instructions.

For honing, the action is exactly the same as that of stropping except that the knife edge is the other way round. Whereas the stroke for stropping is always made back first, the stroke for honing is always made edge first. Clearly, if the blade were stropped edge first, the strop would last about a microsecond! If the honing is done the same way as the stropping, a foil edge is produced by the drawing off of material from the edge of the knife which is being ground.

The blade gradually becomes blunt in use. The care of blade should be taken properly, even it they are not being used.

Ordinarily, all the blade needs between cutting is stropping, which involves rubbing it on a leather strap made for the purpose, but if it becomes nicked it must be honed on a stone or plate glass hone. For stropping and honing, a knife is generally, though not inevitably, fitted with a `back' which is a length of channel which slides over the thick part of the knife along its length, which increases the effective thickness of the back. The additional back determines the angle which the blade will make with a strop or hone when it is laid flat on either (Fig 3.5)



To strop a blade, it is placed over the strop and moved, back first from the near end to the far end of the strop whilst pressing down on the blade. The blade is then turned over, keeping the back on the leather, and the knife is drawn from the far end to the near end and then turned again keeping the back down to repeat the stroke. The knife is also moved laterally along its length as the strike is made. (Fig. 3.6).

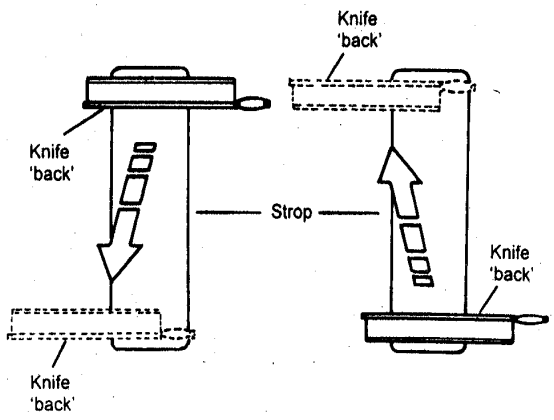


Fig. 3.6 Stropping the knife

3.2.9 Dissecting kit

Different components of dissecting kit are shown in the following diagram (Fig 3.7).

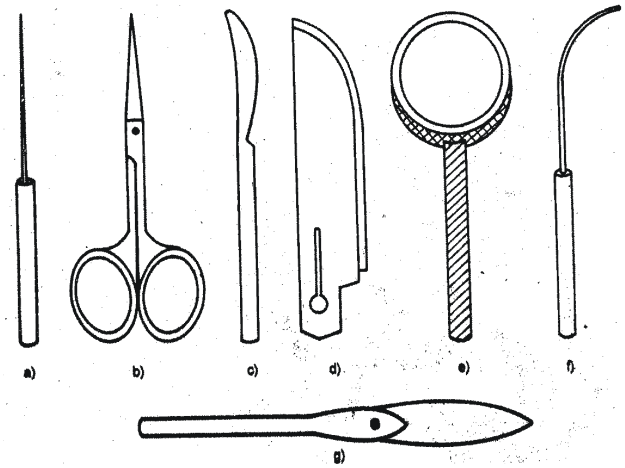


Fig 3.7 Dissecting kit equipment

(a) Dissecting needles: for teasing out specimens/holding skins/appendages on dissecting board
(b) Scissors: for cutting specimens/small bone.
(c) Scalpels: for cutting or making an incision on a specimen.
(d) Pen-knife: for making sections or cutting bones
(e) Hand lens: for magnifying the details of specimens.
(f) Seekers: for probing into the animal being dissected without causing displacement of the organs.
(g) Pen-knife opened: for making sections or cutting bones
(h) Mounted needle: for pining/lifting specimens

Though each student possesses his/her own dissecting box, some dissecting boxes should be available in the laboratory. One dissecting box should be compulsorily kept for the instructor. All the dissecting instruments should be clean and dry to use. Rusted instruments should be thrown off. After the dissection, instruments should be thoroughly washed and wiped and wiped clean to prevent rusting.

Dissecting trays: Dissecting trays should also be kept in a cabinet in the laboratory or in the store-room. It should be seen that wax is layered properly in the tray and there should be no leakage in the tray otherwise dissection cannot be done properly. Meanwhile, you may wish to look at a demonstration of the handling of common and improved laboratory equipment using the video clips (VC. 3.1). Let your course tutor know this. You will be handling them correctly after this participatory viewing.

You will study in detail about the preparation for dissection in Unit 15 of this course.

You will study details for microscope handling and maintenance in Unit 4 of this course and temporary slide preparation in Unit 8 of this course.

SELF ASSESSMENT QUESTION

1. How will you use a simple autoclave?

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2. Name the different components of the dissecting kits and give their uses.

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3. What precautions should be taken while doing centrifugation?

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4.0 CONCLUSION

You have learnt how to use some common laboratory equipment. There are yet a few you need to know about. Anything that is useful is worth caring for. When we care for our equipment, we make them last longer, we make them efficient and we save ourselves the possibility of having unpleasant experiences at work.

5.0 SUMMARY

In the next unit we shall learn about the usefulness of magnifying glasses in the laboratory study of biology.

6.0 TUTOR - MARKED ASSIGNMENT

1. In the absence of an autoclave, what would you use to sterilize non-glassware in the biology laboratory?
2. Describe how you would use the equipment you have chosen; including necessary precautions.

7.0 REFERENCES/FURTHER READINGS

Beril, N. J (1979): Biology in Action London, Heinemann Edicational Books Ltd

Soper, R. (Ed) Biological Sciences Cambridge U.K., Cambridge Press

Stone, R. N. Cozens, A. B. Emia, F. I. (1972), New Biology for Tropical Schools, Longman Group Ltd)

UNIT 4 GENERAL PRACTICAL BIOLOGY MICROSCOPE HANDLING

CONTENTS

1.0 INTRODUCTION

Optical or light microscopes are widely and routinely used in all kinds of biological laboratories, right from schools and colleges to hospital pathology and research labs. They are used to examine various kinds of cells, tissues, and micro-organisms and also to look at the chromosomes. In order to carry out the above tasks smoothly and effectively, it is essential that one should know how to handle the microscope. Handling the microscope involves familiarity with the instrument and its parts, the methods for its setting up, the focusing mechanism, and the use of instrument based on the nature of the material to be studied.

This exercise is a composite one that will enable you to have hands on experience in handling microscopes for specific purposes and in different situations, as well as in knowing the nitty-gritty of its care and maintenance.

2.0 OBJECTIVES

As you complete this experiment you should be able to:

- set the microscope's light source ensuring optimum direction and intensity of illumination by manipulating mirror and iris diaphragm;
- focus objective/eyepiece upon a slide;
- produce normal illumination by focusing the Abbe condenser upon the light source.

3.0 MAIN CONTENT

3.1 Microscope handling

3.1.1 Setting up a student microscope

In the first part of the exercise you will learn to set up a student microscope using a bench lamp for illumination, so that you can observe a cell preparation on a microscope slide. If you are not familiar with the use of this instrument, you should first understand and then practise the setting up technique following the steps (iI-x) given below. After this, you should practice from time to time without referring to the text.

Requirements

For this exercise you will need the following:

(a) A microscope, preferably one fitted with at least three eyepieces - 5x, 10x, and oil - immersion, a condenser and having a mirror for use with an external light source.
(b) A 40 W or 60 W lamp, preferably with a front cover-field stop, to restrict glare. The bulb should be frosted and ideally without any markings on the lower end.
(c) A well-stained, prepared slide for observation.

A typical student microscope is shown in Fig 4.1. You are already familiar with this figure, from your study of Unit 6 of this course. Before you begin to set up the microscope remember that a microscope is a delicate precision instrument, and you must handle it carefully. You must not touch its optical surfaces or allow them to come into contact with any liquid. You will spend quite some time with a microscope and it is therefore important that you know how to use it correctly. The following instructions should give you good results, so work through them step by step. The numbers given in parentheses in the following

instructions, refer to the parts of a microscope, that has been illustrated in Fig 4.1.

Method

(i) Put the bench lamp, with fitted iris to restrict the light, about 10 cm directly in front of the mirror (13). Point the lamp directly at the plane side of the mirror and switch the lamp on.

(ii) Check that the sub-stage iris (9) is open and check that the top lens of the condenser (8) is about 2 mm below the stage.

(iii) Rotate the nose-piece (6) to engage the 8x or 10x objective (7) depending on the objectives available on your microscope.

(iv) Remove the eyepiece and adjust the mirror until a bright spot of light is seen down the tube. Replace the eyepiece.

(v) Put the slide on the stage (18) so that the mounted object is under the objective lens. Use the stage clips (20) or mechanical stage to secure the slide.

(vi) View the microscope from one side and use the coarse focus control (16) to lower the 8x or 10x objective to within about 5 mm of the slide.

(vii) Look down the eyepiece and use the coarse focus control (16) to raise the objective slowly until the object on the slide comes into focus. Make final adjustments with the fine focus control (15) and, if necessary, readjust the mirror and condenser.

(viii) Move the slide to centralize the object to be observed.

(ix) If a higher objective magnification is required, first check that the object is in focus and centralized for the 8x or lOx objective. Then (without altering the focus controls) carefully rotate the nose-piece (6) to engage the 20x or 40x objective lens. Refocus using the fine focus control, (15) and move the slide as required. It may be necessary to readjust the mirror and/or condenser to give suitable lighting.

(x) You should note that some low power objective lenses such as the Biolam 3.7x objective have a long focal length (about 6.5 cm) and a wide field of and probably have to remove the condenser and use the concave side of the mirror to direct light onto the specimen. After using the 3.7x objective, replace the condenser and rotate the mirror back to the plane side. Refocus the 8x or 10x objective - see steps (vi) and (vii), before using the 20x or 40x.

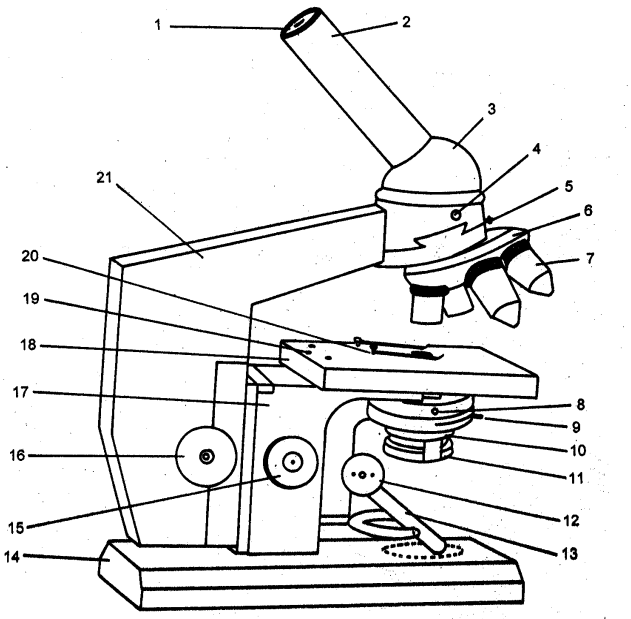

Fig. 4.1 Diagram of a typical student microscope

(1)	Eyepiece: 7x, 10, 15x	(11)	Accessory lens
(2)	Tube	(12)	Condenser focusing
(3)	Prism	(13)	Gimballed plano concave mirror
(4)	Clamp screw	(14)	Base plate
(5)	Nose-piece centring screw	(15)	Fine focus control
(6)	Nose-piece/turret	(16)	Coarse focus control
(7)	Objective 3.7x, 8x, 20x, 40	(17)	Stock (house focusing mechanism)
(8)	Condenser clamp screw	(18)	Stage (keep clean and dry!)
(9)	Condenser iris	(19)	Mechanical stage mount
(10)	Filter holder	(20)	Stage clip
(21)	Limp		

(xi) Many microscopes have three magnifications of eyepiece available - often 7x, 10x and 15x. It is therefore possible, using 3 eyepieces and 4 objectives, to obtain twelve different magnifications, for example, between 26x (3.7x objective and 7x eyepiece) and 600x (40x objective and 15x eyepiece). Oil immersion objectives of 90x magnification are available for examination of, for example, protozoa and blood. Generally, the magnification can be adjusted by (a) changing the eyepiece, or (b) rotating the nose-piece to bring different objective into position.

3.2 Trouble-shooting

Did you encounter any difficulty in setting up your microscope? Nevertheless, here are some tips for you.

(i) Not enough light? Check:

(a) that the iris (9) is open,
(b) that the condenser (8) is in position and properly focused,
(c) that the mirror (13) is properly angled,
(d) that the nose-piece (6) is properly located,
(e) that the sub-condenser filter holder (10) is not in the way.

(ii) Too much light?

Reduce the intensity by moving the lamp away from the mirror. Note that the iris should not be used to adjust light intensity.

(iii) Dirt in the field of view?

It may be on the eyepiece, objective or slide. Look down the microscope and see if the dirt moves when you:

(a) rotate the eyepiece, or
(b) move the slide.

If neither moves the dirt, then the dirt is on the objective. Clean the lenses very carefully using proper lens tissue paper only.

(iv) 'Cloudy' on high power?

The previous user (possibly you?) probably has allowed liquid onto the objective. Clean the objective lens (7) carefully - moisten the lens tissue if necessary.

(v) Dirty stage?

The previous user (surely not you?) has used a wet slide. So clean the stage carefully.

(vi) Lenses missing?

Take a different microscope and report about the missing part(s) of the instrument.

3.3 Routine Fault Finding

This section discusses the probable causes of some common problems encountered while setting up the microscope. Identification of the root cause of the problem enables one to take the appropriate corrective measures.

1. Inability to obtain a sharp image

There are two types of causes for it - optical causes and mechanical causes.

Optical causes

(i) Aperture of condenser too wide for objective in use. Reduce it.
(ii) If hard false `haloes' present - condenser aperture too narrow, increase it.
(iii) Field stop(s) set too wide (causing glare).
(iv) Use of eyepiece of too high a power for the quality of the objective.
(v) Grease, dust or old immersion oil on slide. Clean with lens tissue and polish with Selvyt cloth.
(vi) Lens surfaces not completely clean. Clean them. If the problem remains, try an objective from a different instrument. If this is satisfactory, the original objective is faulty.
(vii) Use of oil immersion objective without oil.

Mechanical causes

(i) Specimen slide upside down.
(ii) Stops wrongly set. The bottom stop should be set to prevent further movement of the coarse drive when the fine focus is at the middle of its range and the image is in focus. Even where stops are fitted, the fine movement may come to the end of its travel during focusing. This can usually be avoided if the fine adjustment is set at mid-range before the microscope is approximately focused with coarse drive.
(iii) Jamming of the lower part of a spring-loaded or retraceable objective in the body. If very gently attempts to be free the lower lens cell are unsuccessful, the objective should be returned.

2. Image moves slowly or suddenly goes out of focus

Its possible causes are:

(i) Body slip or slipping of the stage in stage focusing instruments, caused by poor adjustment of the focusing mechanism.
(ii) Hand pressure on flexible stage.

3. Condenser will not focus the surface of the lamp in the field of view

This may be caused by the following reasons:

(i) Condenser not pushed fully home in mount.
(ii) A stop (if present) set too low.

4. Field of view too bright

The probable causes for these are:

(i) Sub-stage condenser aperture or disc diaphragm aperture too wide.
(ii) Power of source too high.
(iii) (If variable) lamp intensity set too high.
(iv) Neutral density filters needed.

5. Field of view too dull

It may be due to one of the following causes:

(i) Sub-stage condenser aperture or disc diaphragm aperture too narrow.
(ii) Sub-stage condenser not properly focused.
(iii) Neutral density filters (if fitted) should be removed.
(iv) Power of bench lamp too low.
(v) (if variable) Internal lamp intensity set too low.

6. Specks in field of view

The possible causes of this may be:

(i) Specks ill defined and `floating' across field of view - in observer's eye.
(ii) Specks normally stationary but revolving with eyepiece - clean eyepiece lens, especially field lens.
(iii) Specks normally stationary and not revolving with eyepiece - clean (in order) condenser lenses, slide, any filters, objective.

7. Objective aperture will not fill with light

It may be due to any of the following reasons:

(i) Sub-stage condenser improperly focused.
(ii) Total condenser aperture inadequate for objective in use.
(iii) Oil immersion condenser (if used) not oiled to underside of slide.

8. Image moves sideways during focusing

This may result because of any of the following:

(i) Slide or specimen not perpendicular to optical axis.
(ii) Stage fitted `out of square'
(iii) (In a stage focusing instrument, if effect very small) poorly fitted dovetails, allowing stage to move under hand pressure.

SELF ASSESSMENT EXERCISE 1

Did you get a dull field of view in your microscope? List two probable causes for it.

i) .....

ii) .....

SELF ASSESSMENT EXERCISE 2

When setting up a microscope, if you find that you are unable to obtain a sharp image, what do you think will be wrong? Write down four possible causes.

i) .....

ii) .....

iii) .....

iv) .....

SELF ASSESSMENT EXERCISE 3

If you do not focus the substrate condenser of a microscope properly, what effect would this have? Tick the correct answer.

(i) Objective aperture would not fill with light.
(ii) Field of view would be too bright.
(iii) Field of view would be too dull.
(iv) Object would be out of focus.

3.4 Setting up Microscope with and without a Condenser

During the course of this practical you will set up microscope in different light conditions, and also learn about the utility of a condenser in this process. You can observe the differences brought about in different set-ups.

You require the following items for exercises 3.2.2 to 3.2.5

Requirements

Microscope
Illuminator or lamp
Slides
Coverslips
Specimens of materials relevant to the techniques to be studied Already prepared microscope mounts
Spatulas
Forceps

3.5 Illumination without a Condenser

There are two possible sources used for illumination, one an external light source (this is described in a), and the other is the use of daylight (discussed in b).

3.5.1 Setting up a Typical Microscope using an External Source

For optimum illumination and resolution the aperture of the objective should be filled (or nearly) with light. This is best achieved by having a condenser with a properly regulated iris diaphragm. In the absence of this, the apertures of medium and high power objectives may be-under filled and resolution will suffer. The vast majority of microscopes used at lower levels are not fitted with such condensers, but this is not serious as long as the limitations are recognized.

The difficulties experienced in using the simpler kind of microscope most often lie in obtaining optimal illumination with the simple controls available. The field may be difficult to illuminate evenly and there may be lack of contrast with excessive glare. The most common form of sub-

stage assembly for controlling illumination in simple microscopes, is the disc diaphragm. This is a movable metal or plastic disc with a series of circular holes of various diameters. Unlike the iris diaphragm of the more advanced instrument, which is imaged at the back lens of the objective and is therefore called an aperture stop, a hole in a sub-stage disc diaphragm is used mainly to limit the effective size of the source in order to reduce glare improve contrast. The holes in a disc diaphragm should be used to limit the area illuminated to the diameter of the field of view - they act as field stops. One should therefore usually select the smallest size of hole that will illuminate the field of view evenly. In fact, on many simpler instruments only the use of the 4x of 5x objective necessitates a change from the use of the smallest hole available.

Occasionally, it may prove difficult to illuminate the whole field of view with the 4x or 5x objective. In this case, the converging mirror may have to be used and the lamp moved nearer in order to produce an effectively larger source. This is acceptable for low power work but produces a distorted image of the source. On an ‘advanced’ instrument it may be necessary to remove the condenser. Even if it can be left in place, it may be difficult to focus.

3.5.2 The Use of Daylight Illumination

Ordinary daylight is often the most readily available of all sources of illumination; it is used a great deal for routine microscopy. However, there are limitations: daylight is a very diffuse source of virtually unlimited extent, so that light enters the microscope at almost all angles. It is, therefore difficult to know when the `source' is focused on the object and to restrict the illuminated area to the field of view. So, there is a greater tendency towards glare with daylight than with the more controllable artificial sources. Apart from this problem of glare, there are few serious objections to the use of daylight, provided care is exercised in properly illuminating the aperture of the objective.

Setting up the microscope in this way is quite straightforward

(i) Place the microscope near a large window, preferably one without too many glazing bars.

(ii) Focus the l0x (16mm) objective in the usual way using a well-stained slide.

(iii) Remove the eyepiece and inspect the back of the objective.

(iv) If fitted, the sub-stage condenser should be racked up and down until a fairly sharp image of the condenser iris diaphragm can be

seen at the back lens of the objective, and (when the diaphragm is fully opened), the back lens can be filled with light fairly evenly. With a simple instrument this step is omitted; use the sub-stage disc diaphragm to control glare.

(v) Replace the eyepiece. A reasonably good image should be seen and minor adjustments of the iris diaphragm can be made to reduce glare.

Obviously with daylight the source cannot actually be focused on the object. However, it can be helpful to hold a pointed article 150-200 mm from the mirror and to focus this in the field of view. This often ensures fairly good illumination of the back lens. Because the source is of almost unlimited size the exact position of the mirror is not critical. The imaging of glazing bars in the field of view can often be avoided by tilting the mirror slightly or by placing the microscope closer to the window.

To put it briefly, while daylight provides a very convenient and useful source, giving adequate illumination when specimens with high contrast are used, the more readily controllable artificial external sources are generally preferred.

3.6 Illumination Using an Abbe Condenser

Using an External Source to Obtain Correct `Nelson' or Source-Focused Illumination

Use a l0x (16 mm) objective and 10x eyepiece as follows:

(i) Place on the stage a specimen slide. Ensure that the coloured part (that is the material to be seen) of the slide is directly under the center of the objective.
(ii) Place the external lamp about 150-200 mm away from the instrument and level to it. Ideally, the lamp should have an opal bulb and should possess a circular field stop. Very often such lamps are not available and an ordinary bench lamp has to be employed. If this is the case, then draughtsman's tracing paper is fixed to one side of a hole cut in a piece of blackened cardboard or hardboard. This illuminated area then functions as a secondary light source.
(iii) Focus the slide and manipulate the plane mirror to center the illumination.
(iv) Focus the microscope condenser.The aim is to focus the image of the source in the object plane. If a field stop is fitted to the lamp, then the condenser may be focused on the edges of this. If there is

no field stop, or if, at its narrowest setting, it more than fills the field of view, the condenser can be focused on a pencil point or mounted needle held just in front of the lamp. The position of the condenser should be adjusted by means of its focusing mechanism until the image of the field stop or pointed object is in sharp focus at the same time as the image of the object on the microscope stage. An image of the surface of the bulb (possibly even of the manufacturer's name, etc.) may come into the field of view.

To avoid this, the condenser may slightly defocused.

(v) Change to the 40x (40mm) objective.

(vi) The aperture of the iris diaphragm should now be adjusted to suit the numerical aperture of the 40x objective. This is done by removing the eyepiece and inspecting the illuminated circle at the back of the objective lens. This can be done either by direct inspection or by using a centering telescope focused on the back lens of the objective. As the iris diaphragm is closed, the image of its edge will be seen at the periphery of the illuminated area.

The diaphragm should be closed until the illuminated area occupies two thirds to three-quarters of the diameter of the back lens of the objective. It is usually recommended that this aperture adjustment be carried out for each objective on each occasion it is brought into use. However, a single setting for the 40x lens will usually also serve adequately for the I0x objective and this saves time and trouble.

Readjustment of the iris aperture in the same way may well be needed when the low power is brought into use.

(vii) Replace the eyepiece. The microscope is now correctly adjusted and ready for use. If the light is too bright (not usual with a 60 or 100 W lamp) the intensity should be lessened by the use of neutral density filters, by changing to a lower power bulb, or by altering the field stop on the lamp if one is fitted. The iris diaphragm of the condenser should not be used to control the intensity of illumination, nor should the condenser focusing controls be adjusted. Slight adjustments of these controls may sometimes be necessary to control glare and obtain better contrast, but as a general rule, intensity of illumination should be adjusted at some point in the light path between the lamp and the iris diaphragm of the condenser.

3.7 Setting up for Kohler Brightfield Illumination

This is an important technique which is in a sense a foundation upon which other techniques rest. Here we refer to a simplified version, pertinent to a monocular compound microscope of a basic type with an Abbe condenser, using a mirror and an external source of illumination.

(i) Place the lamp about 20 cm in front of the microscope and switch it on. Ensure that the microscope is in a comfortable working position and is angled correctly for the user. Aim the light at the mirror, plane surface, and angle the mirror so that it reflects the light centrally on the base of the substage apparatus.
(ii) Close the iris.
(iii) If the lamp has a field iris, open it wide and focus the condenser so that the light falling on the iris overfills its area slightly. By closing and opening the field iris you can check the centricity of the lamp and the mirror.
(iv) Open the aperture iris and place a slide on the microscope stage.
(v) Focus on this slide using a low power objective. Ensure that the substage is as high as it will go, close down the field iris and look for its image in the microscope.
(vi) Having identified the field iris, focus its image by moving the substage condenser downward. If the field iris cannot be focused, move the lamp towards or away from the microscope until it can be focused; at this point the substage should be a little below the top of its travel. If the substage is not central, it is time to centre it using the image of the field iris as a guide.
(vii) Open the field iris until the whole field of view is just covered.
(viii) Remove the eyepiece and look down the tube. Open and close the iris so that you can identify its image. Now open it wide and then gradually close it until you just see it edges. Leave the aperture iris in this position and replace the eyepiece.
(ix) If the light is too bright the lamp may be dimmed by means of an electrical dimmer or by the use of neutral density filters. Attempting to change the light by moving either iris or by lowering the substage will destroy the Kohler illumination.

The illumination is set up for the critical lighting of the specimen. Changes in objective power will necessitate only the increase or decrease in intensity of light. The microscope nosepiece will ensure the centricity of the other objectives.

3.8 Setting Up for Phase Contrast Microscopy

For this exercise, you will additionally need a focusing telescope, also known as a Bertrand lens, for the following series of operations. A Bertrand lens is a supplementary lens system which may be introduced into the tube above the objective. Together with the eyepiece this forms a viewing telescope for examining the back focal plane of the objective. It is chiefly used when setting up the microscope.

(i) The correct substage annulus must be chosen to accompany the light ring of the objective required. Ensure that the objective turret, if used, is properly placed in its notch so that the objective is in the right place.
(ii) Open the iris diaphragm fully.
(iii) Place a slide of a thin section of animal tissue, e.g kidney, on the stage of the microscope and critically focus on an edge of the section.
(iv) Close down the field diaphragm and adjust the height of the substage condenser unit. A sharp image of the diaphragm can be seen.
(v) Open out the field diaphragm by means of the substage condenser centering screws.
(vi) Open the field diaphragm until it just goes beyond the edge of the field of view.
(vii) Take out the eyepiece and replace it with the focusing telescope. There should be an adjusting collar on the telescope to enable you to focus the telescope(you may have to undo a locking ring in order to achieve this). Focus the telescope on the image of the light ring on the phase ring.
(vii) The phase ring is axiomatically correctly placed; therefore it is necessary to centre the substage annulus so that the two images may be superimposed. This is achieved by means of a centering mechanism separate from that of the main substage condenser mount. The phase centering device often takes the form of a pair of spring loaded screws which only engage when pushed in. Find these screws and push them in until they engage and by screwing and unscrewing them centres the image of the light ring on the phase ring (see Fig. 4.2).

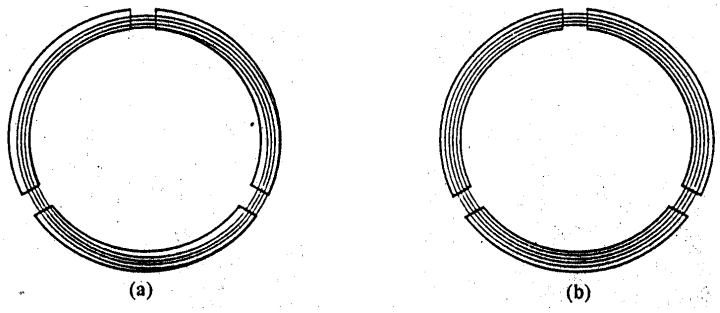

Fig. 4.2: Phase ring and light ring in different positions. (a) off, and (b) centred.

Each combination of annuli and objectives must be set up in this way, but unless the settings are interfered with they should remain set once and for all.

There is no written component for this practical upon which you could be directly assessed. You should make notes and diagrams of what you observed since these may be useful to you at a later date.

Ask your Counsellor to give you feedback on your techniques for the above set-ups of the microscope.

3.9 A Demonstration of Microscope Handling (Video)

You may need to watch a video demonstration of microscope handling. Let your course tutor help you have access to the video clips on this (VC. 4.1) Now go ahead and handle the microscope thereafter.

4.0 CONCLUSION

You are sure by now you know that the microscope is very important in biological studies. It is also an expensive and delicate laboratory equipment. In the next unit, you will learn how to care for the microscope as well as measure items under the microscope.

5.0 SUMMARY

In this unit, you have learnt about handling microscopes. You have seen how you can set up your study microscope under different conditions and the possible problems you might face under different conditions. Some possible solutions to these problems have also been suggested .

6.0 TUTOR MARKED ASSIGNMENT

1. Suppose you were using your microscope and PHCN took the light as can happen, what will you do?

7.0 REFERENCES/FURTHER READINGS

Berrit N.J. (1979) Biology in action London, Heinemann Educational Books Ltd.

Soper, R. (Ed) (1998) Biological Sciences Cambridge. UK Cambridge University Press.

Stone, R.N., Stone, A.B., Emia, F.1 (1972) New Biology for Tropical Schools London, Longman Group Ltd.

Noun Video clips (VC.4.1) of a demonstration of microscope handling. (To be produced by NETC)

UNIT 5 MICROSCOPICAL MEASUREMENTS AND MICROSCOPE CARE

CONTENTS

1.0 INTRODUCTION

Sometimes you want to know the size of the object you see under the microscope. There are devices by which this can be done. Here you will learn how to do so. You will also learn how to care for the microscope

2.0 OBJECTIVES

Sometimes when you have to work with very small biological specimen(s), you have to find ways of enhancing the efficiency of your microscope. The oil immersion technique is one of the ways we can do this. You will also learn doing this in this unit.

Since the microscope must be kept clean to be useful, you will also learn how to clean it after use especially the oil immersion objective. Doing simple maintenance methods and tasks for a compound microscope will also be part of this unit.

By the end of this practical session you should be able to:

- Calibrate an eyepiece graticule against a stage micrometer, and use it to measure various kinds of cells
- Demonstrate the setting up of an oil immersion objective

- Explain the need to clean off immersion oil after use.
- Describe the simple maintenance methods for a compound microscope.
- Perform simple maintenance tasks of a compound microscope such as cleaning lenses, changing bulbs and so on.

3.0 MAIN CONTENT

3.1 Microscopical Measurements

3.1.1 Making Microscopical Measurements

Objects on a slide cannot be measured directly. Instead, they are measured against a pre-calibrated graticule (see fig 5.1) which is placed inside the eyepiece of the microscope. A conversion factor is then applied to obtain the actual size of the object. This whole process is known as micrometry.

Requirements

Slide with an object to be measured. Microscope with a graticule in the eyepiece A lamp slide micrometer graduated in 0.1 mm or 0.01 mm units.

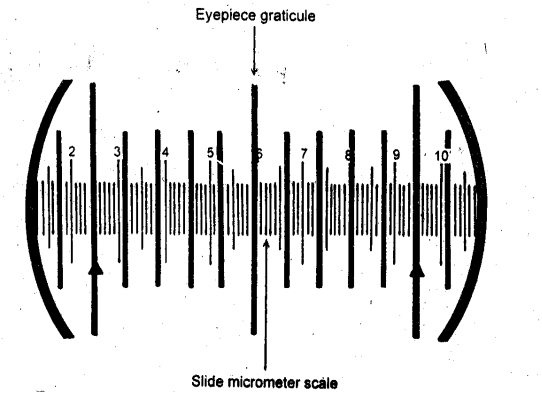

Fig. 5.1 Eyepiece graticule with stage micrometer graticule.

Method

(i) Focus the microscope onto the micrometer slide using the required combination of eyepiece and objective.

(ii) Align the two graticules; read off and note the eyepiece units (w) in fractions of a mm (x). Use at least half the field of view. i. Calculate the equivalence of one eyepiece unit by x/w. This is the conversion factor.

(iii) Remove the micrometer slide and focus on the slide carrying the object to be measured.
(iv) Align the object to be measured with the eyepiece graticule. Measure the object in graticule units and note the reading (z).
(v) Calculate the size of the object in mm by multiplying reading z by conversion factor.

A worked out example is being presented below.

Note that 70 eyepiece units (w) are equivalent to 10 micrometer units (x), i.e., 1 mm or 1000, um. Therefore:

1 eyepiece unit = 1000/70 = 14.3, um.

Suppose you are measuring a plant cell. Assume the cell measures 2.5 eyepiece units by 4 eyepiece units.

Its actual size, therefore, is (2.5 x 14.3), um by (4 x 14.3) um by 57.2, um.

3.2 The Oil Immersion Technique

When we have to examine small objects such as red blood cell (7,um) and bacteria (1-5,um), an objective lens of 90x or 100x is required. These lenses have a very short working distance and the diameter of the lower objective lens is very small. To maximize the light gathering ability of the lens, i.e. to raise its NA, medium of high refractive index is included between the objective and the coverglass. The medium normally used is immersion oil, or more rarely water.

Requirements

- Microscope fitted with Abbe condenser and usual objectives
- Oil immersion objective lens
- Good light source
- Immersion oil
- Lens tissue
- 1,2-dimethylbenzene (xylene) or 1,1,1 -dichloromethane
- Suitable stained permanent slide which must have a thin coverglass, recommended material - blood smear.

Method

(i) Check that the oil immersion lens is clean.
(ii) Polish the slide with a lint-free cloth.
(iii) Screw the oil immersion lens into the turret next to the 40x objective (or similar).
(iv) Set up the microscope. Pay particular attention to the condenser settings.
(v) Select an object of interest which is on the slide. Center it in the field of view and focus on it with the 40x objective. Use stage clips or mechanical stage clips, to fix the slide.
(vi) Using the coarse focus control, rack up or raise the nose piece and swing in the 90x or 100x oil immersion lens.
(vii) Put a drop of the immersion oil onto the slide.
(viii) Rack down until the oil immersion lens enters the oil and is very nearly touching the coverglass.
(ix) Now look down the microscope. Slowly rack up using the fine focus control. The object on the slide should come into view. If necessary, readjust the lamp, mirror and condenser.
(x) The slide can be carefully moved to examine adjacent objects - for example to compare the structure of red blood cells and white blood cells.
(xi) When you have finished, rack up and remove the slide and oil immersion objective.
(xii) The oil is removed from the objective lens with dry lens-tissue. Ordinary tissue is suitable for the slide. Slide and objective require more cleaning, use a tissue paper on which a few drops of xylene have been poured.

Caution! Avoid excessive use of the solvent as it is poisonous by skin contact and inhalation. Also it dissolves the content used to fix optical components in place. Now that you are familiar with the oil immersion technique, practice it and get the feedback from your counselor.

(xiii) Return the clean lens to its storage container and the slide to its tray.

SELF ASSESSMENT EXERCISE 1

Under what circumstances would you use the oil immersion techniques?

.....

.....

.....

SELF ASSESSMENT EXERCISE 2

Describe the procedure which you would use to correctly set up and focus an oil immersion objective so as to prevent damage to the objective and slide.

i) .....

ii) .....

iii) .....

iv) .....

v) .....

vi) .....

SELF ASSESSMENT EXERCISE 3

Give two reasons why it is important to use only the minimal amount of xylene (1, 2-dimethylbenzene) for cleaning the slides and the objectives?

i) .....

ii) .....

iii) .....

iv) .....

v) .....

3.3 Microscope Care and Maintenance

This section would acquaint you with the main elements of microscope care and maintenance. First we look up at some of the practices that we must follow, described as Do's and the ones that are not quite microscope - friendly also listed under the category of Don'ts in the subsection that begins now.

3.3.1 Some Do's and Don'ts

DO'S

- Handle it carefully.
- Focus correctly
- Keep it clean.
- Use the proper lens tissue.
- Report problems.

DON'TS

- Bang it down on the bench.
- Focus high power lenses with the coarse control.
- Use wet slides.
- Use ordinary cloth or tissue for cleaning lenses.
- Put it away dirty.
- Remove slide with a high powered lens in place.

3.3.2 Microscope Maintenance

In the subsection some descriptions and instructions are given that should enable you to tackle the routine servicing tasks, and also to keep the instruments in good order.

Give below are some of the operation which can readily be carried out.

(1) Cleaning of optics,
(2) Cleaning and degreasing of dovetails and nosepiece movement,
(3) Adjustment of some types of coarse focusing mechanism.

Caution: Work which should not be attempted includes anything requiring the dismantling of objectives, adjustments to fine focusing mechanisms or, in general, the removal of pinions or pinion bearings.

If a number of instruments are in constant use, or if instruments have complex focusing mechanisms, it may still be necessary to call in a service engineer periodically (from either the makers or a firm specializing in microscope servicing and repairs). However, regular attention to instruments, even if limited, can go a long way towards reducing the frequency of these visits.

3.4 Tools and Working Arrangements

Before starting operations, spread out a large, clean sheet of white paper on the bench to one side of the work station and lay out these tools and materials on the other side.

(1) Allen keys (Imperial and metric)
(2) A set of instrument (`watchmaker's) screwdrivers
(3) A range of larger flat-headed and a Philips screwdrivers (the former with wide but fine blades)
(4) A rubber hand bellows of the type used by photographers
(5) Camel hair brushes (different grades - finer - medium - coarse)
(6) A good quality hand lens
(7) Lens tissues
(8) Selvyt clothes and dogwood pegs
(9) Clean, lint-free rags
(10) 1,2-dimethylbenzene (xylene)
(11) 1,1,1 -trichloroethane
(12) Distilled water
(13) Lubricants for the main dovetail slides and the pinion bearing
(14) Cotton buds.

A few small dishes or boxes are useful for components which are removed. A well-shaded adjustable lamp is an asset, especially for the inspection of lens' surfaces.

When working on an instrument, give yourself plenty of room. Work well away from the edges of the bench so that screws and other small parts do not find their way to the floor.

If possible, work on the instrument should be completed in an unbroken, orderly sequence. If there are interruptions, it is not easy to remember just what has been completed. It is also advisable to work on one instrument at a time to rule out the possibility of parts getting mixed up. (This also has the advantage that if one does make a mistake or come to an impasse, there is only the embarrassment of one instrument in pieces, and not a whole set!)

3.5 Working Sequence

The sequence of operations in servicing a microscope can be broken down into a series of steps, these are illustrated in fig 5.2. The broken lines indicate the work sequence in routine maintenance of the optics when there isn't the time or the need to clean and regrease the mechanical components.

1. Removal of Optical Components

After removal, these should be placed on the sheet of clean paper to one side of the work station. Whenever screws are removed, take great care to use the correct size of screwdriver. If the blade is not an exact fit, place a piece of paper towel or cloth over the screw slot. If the instrument is relatively new and is being disassembled for the first time, some screws may be hard to loosen because of the presence of a locking compound.

Eyepiece

This should first be inspected to ascertain whether it is free or fixed. Some eyepieces make a snug, push fit in the tube; some are fixed by a grub screw which will require loosening with an Allen key or instrument screwdriver, some are screwed to the tube and must be unscrewed bodily.

Objectives

These can usually just be unscrewed carefully from the nosepiece. Small nonstandard objectives without milled collars can be gripped in a clothes peg type test-tube holder or, more simply, by sliding a short piece of rubber tubing over them. On a few instrument objectives have locking screws which must be loosened before the objective itself can be unscrewed.

Mirror and gimbal

These are usually a push fit, the gimbal peg being a split shaft which is housed in a hole at the base of the limb or on the base-top . However, this shaft may well be fixed by a grub screw which will have to be loosened before withdrawing the mirror gimbal. Illuminator disassembly is done in a similar way.

Condenser

Most focusing condensers are held in their mounting by screws. There may be one large clamping screw with a slotted head or several small screws requiring the use of an instrument screwdriver. If the latter arrangement is used, be careful to loosen only the actual clamping screws and not those holding the condenser components together. Some condenser mounts have a mirror or prism in the body tube. Do not remove this, as it is difficult to replace it in the correct position.

If the full sequence of servicing operations is not to be followed, but only the optics cleaned, follow the broken line in fig 5.2 to the Box no.6 (in the figure).

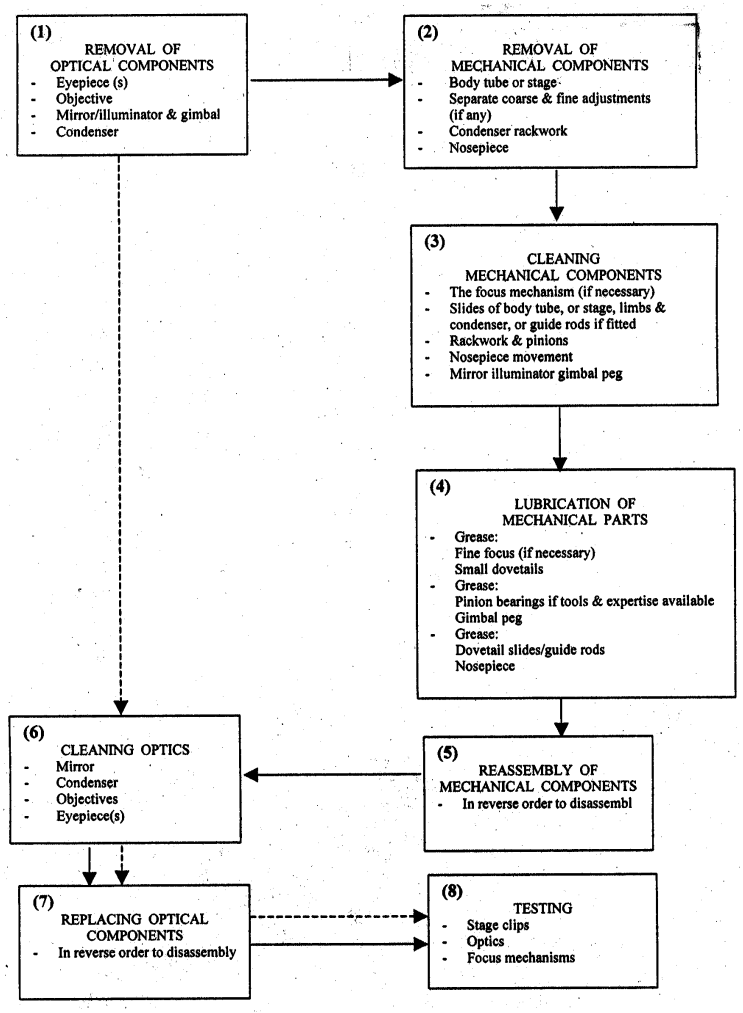

Fig. 5.2 A servicing schedule

2. Removal of Mechanical Components Body tube (traditional instruments)

The coarse focus mechanism should first be examined carefully to identify any stops or other features which limit its travel. This movement is almost always derived from a rack and pinion system with diagonally cut teeth

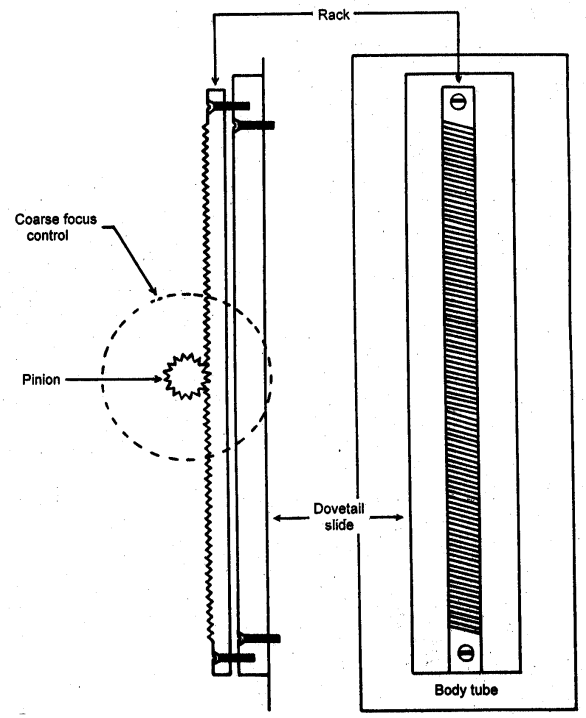

Fig 5.3 Coarse focus drive - rack and pinion: side (fig. on the left) and front (see the figure on the right) views.

Most instruments only have bottom stop, but fixed or adjustable stops may be present at either end of the movement. Any stops which limit the upward travel of the body tube should be removed. In extreme cases, it may even be necessary to remove the stage to take out a lower stop. The movement can then be run apart. This should be done carefully, especially when the end of the rack is nearing the pinion. Any small screws on the limb above the coarse focus spindle should not be removed. They are clamping screws affecting the tension on the pinion bearings and do not interfere with the removal of the body tube.

Stage focusing movements (modern fixed limb instruments and some junior microscopes)

In modern style instruments, the base and stage may have to be unbolted before the stage focusing mechanism can be removed. In a few cases, the removal of an upper stop will allow the stage to be racked off upwards.

Sub-stage assembly

Dismantle the sub-stage condenser scroll focusing assembly by loosening the mounting screws, or rack, if of the rack and pinion type. In some rack and pinion condenser movements it may be necessary to withdraw the pinion spindle; this may necessitate the removal of a screw from the plain end of the spindle.

Fine Focus Movement (if fitted)

The dovetail slides and other parts of a fine focusing movement are hardly ever exposed in use and usually do not require attention. Where the mechanism is a straightforward screw and lever (fig 5.4) or other simple type, it can be partly dismantled so that the condition of the grease may be inspected. If necessary, the dovetails are cleaned and regreased as described below. With more complicated movements, it may be wise to seek professional aid. Access is gained to a screw and lever type mechanism by removing the cover plate at the top of the fine dovetail slide. It is usually held in place by two screws. The plate should be held down while these are removed, otherwise the strong return spring underneath the plate and the plate itself will become airborne.

In most modern stage focusing microscopes the stage support carries the male part of the fine movement dovetail. It may be necessary to remove the stage (if this has not already been done) to gain access to the cover plate.

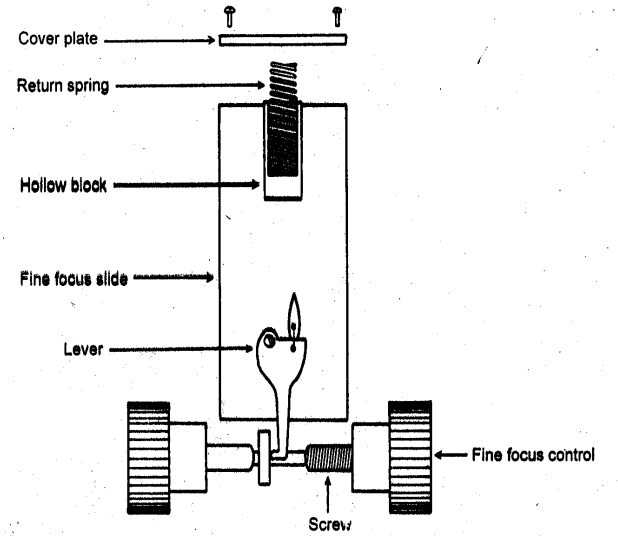

Fig. 5.4. Screw and lever fine focus movement

In traditional instruments, removal of the cover plate and spring will allow the coarse focus slide with the coarse focus knobs and pinion to be

removed upwards as a unit (fig 5.5a), exposing the fine focus movement in the head of the limb.

In many `modern' instruments the male half of the coarse dovetail, the coarse rack, the fine movement and the stage support are usually removed from the limb as a unit (fig 5.5b). In this case, removal of the cover plate and spring allows the stage support, with the male half of the fine dovetail, to be removed, thus leaving the fine focus as a separate unit with the screw and lever exposed.

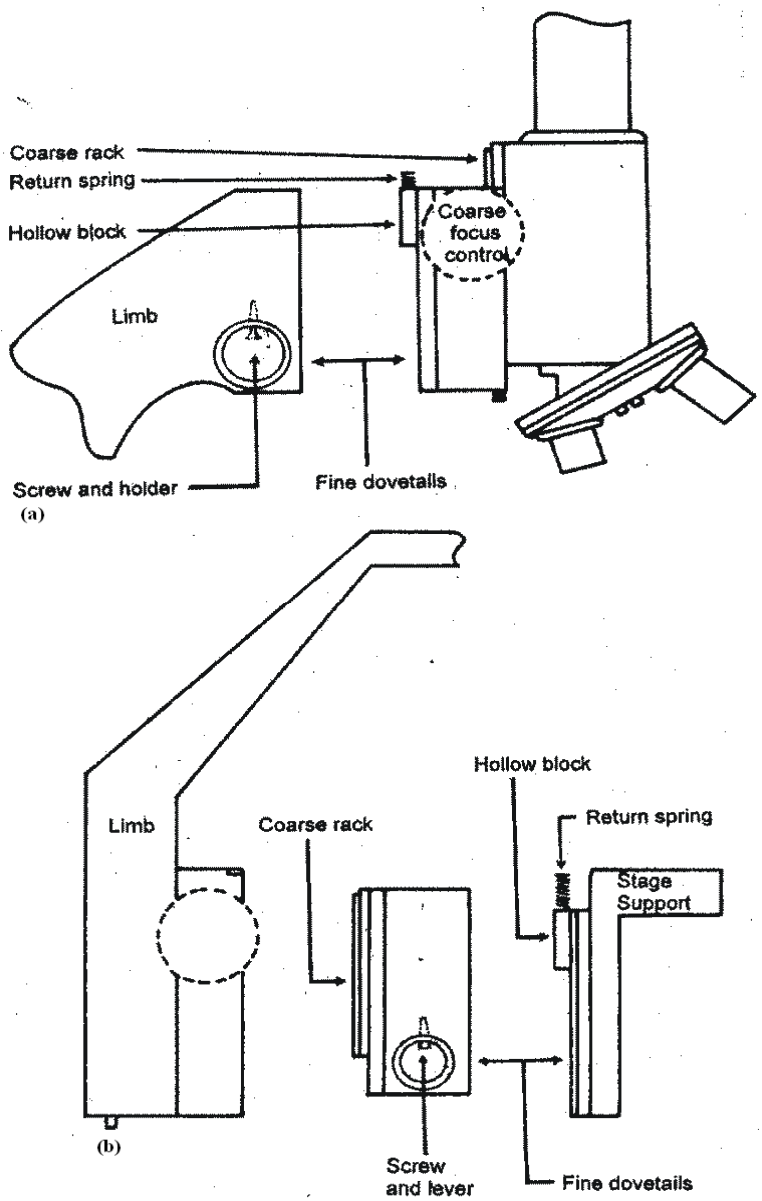

Fig. 5.5 Dismantled focusing mechanisms: (a) traditional stand; (b) 'modern' stand

Nosepiece

This can usually be removed by undoing a central holding screw (large headed with a narrow slot so that a wide fine bladed screwdriver is needed). For some instruments a special tool may have to be acquired or made before this screw can be removed. If there are no external indications of the type of nosepiece indexing mechanisms used (fig. 5.6b). They type of nosepiece should not be dismantled - or great care must be taken to avoid dislodging and losing the balls.

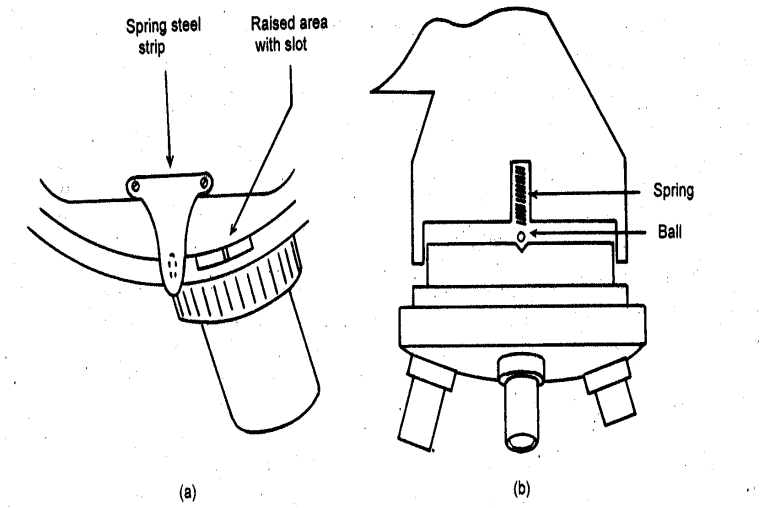

Fig. 5.6: (a, b) Nosepiece indexing mechanisms.

Coarse focusing pinion spindle

As a general rule, it is not necessary to remove the coarse focusing pinion. Indeed on some models, special tools and/or a little expertise are required. In some models, although the knobs are screwed on to the spindle in the usual way, locking nuts are used to prevent their relative movement. Special clamps are needed to hold the knobs while undoing the locknuts and again on reassembling. In some instruments, a side and a special C-spanner is required.

Unless these tools are available, the pinion spindle should not be removed from these instruments. Systems of tensioning the spindle and adjustment of the coarse focus mechanism are described in the point (5).

3. Cleaning mechanical components Fine focus mechanism

Normally, this requires no attention. Inspect the condition of the grease. It should be clean and soft without discoloration or lumps. If it is dirty and lumpy, carefully remove the worst portions with a clean rag over the end of a dogwood peg. Do not use a grease solvent as this might run into the fine focus pinion bearings and affect the grease there. Fresh grease

may be applied. Use the brand recommended by the manufacturer. However, if the original grease is in a very poor condition so that most of it needs removing, it is wise to seek professional aid. This applies especially where the movement is somewhat complicated.

Slides, Guide Rods, etc.

Dovetail slides, guide rods and the bearing surfaces of the nosepiece rims and spindle should have the old, dirty grease removed from them. A clean rag moistened with a solvent such as 1, 1, 1 - trichloroethane can be used for these accessible places such as corner in the dovetails.

Caution: Trichloroethane is less hazardous than the solvents formerly used, but the work area should still be well ventilated and the rag should not be saturated with solvent. Care should be taken not to allow solvent into the fine focus mechanism or on to the pinion, where it may run along the shaft and into the bearings. The bearing surfaces of pinion spindles are under very high loading and they must be kept separated by a continuous layer of grease, otherwise the bearing may seize.

Rack work and pinions

These should be brushed out with an old toothbrush and the teeth of the rack cleaned out with a sharpened dogwood peg. Similarly, treat any substage rackwork which may be fitted.

Mirror or illuminator gimbal peg

This can have the old grease cleaned off with the solvent moistened rag and its aperture in the limb or foot cleaned out with a rag over a dogwood peg.

Other ‘mechanical' parts (such as limb, foot and stage)

Theses can be wiped over with a moist rag and polished with Selvyt cloth. Gross staining or corrosion can be dealt with using appropriate cleaning agents. Frequent changing of the cloth used for a new one moistened with the cleaning material is advisable to minimise the risk of damage to other parts of the instrument.

4. Lubrication of Mechanical Parts

Greases can be applied as a smear with the finger tip, or worked in with a sharpened dogwood peg. All the larger dovetails and any scroll or helical movement of condenser mounted can be regreased. The spindle of the nosepiece and the touching surfaces of the rims should also have a

smear of the grease applied to them; for small dovetails and fine focus movements, use a thin grease developed for high loading. Oil should not be used on slides or most other moving parts of the microscope.

5. Reassembly of Mechanical Components

The mechanical parts of the stand can now be reassembled, in the reverse order to disassemble, ready to receive the optics, once these have been cleaned.

Take care while replacing rackwork. Slide the mating member down and very gently feed the rack onto the pinion. Do not bump movements together or serious damages may be done to the teeth. The mechanism should be worked a few times, remove any excess grease which appears. While replacing nosepiece movements, tighten the holding screw until the movement is firm without being harsh.

On many instruments, it is a relatively simple matter to adjust the coarse focus mechanism at this point. Usually there is some provision for increasing the “tension” or pressure on the pinion spindle at the bearing point. In some cases special tools and/or expertise may be required. However, there are a number of instruments where all that is required to effect an adjustment is simple contra-rotation of the knobs, this takes up the play by pulling the knobs against a pressure pad.

Where the bearings have to be moved to bring the spindle closer to the rack, trial and error will reveal just how hard to push while retightening screws. If the spindle bearings are clamped by screws on top of the limb these must be tightened alternatively by similar amounts until the system is properly adjusted.

Take care that you are adjusting the proper screws. Sometimes the actual adjusting screws lie at the bottom of holes in the top of the limb - the screws which can be seen merely serve to close the holes!

After adjustments have been made, any locking devices present are retightened and the movement is tested for “feel”. A smooth, but slightly stiff action is aimed for. A properly adjusted movement should be free from jerks even when operated very slowly.

If it is not obvious how adjustment is effected, consult the supplier. If a complex instrument has noticeable body slip or backlash, it is best to call in an engineer. Resist the temptation to effect a cure by making the teeth of rack and pinion engage more deeply by fitting packing pieces or by bending the rack. This will only make matters worse.

6. Cleaning the Optics

There are two distinct schools of thought on this subject. Some claim that frequent cleaning can do only harm, because the dust on being repeatedly rubbed across polished surfaces is bound to scratch them. Others claim that dust and grease must be removed regularly if instruments are to give of their best.

Certainly great harm can be done by heavy-handed cleaning and little is achieved by removing dust if a greasy fingerprint is left in its place. Cleaning optical glass needs a light touch and a certain amount of care to achieve best results and avoid damage. Compromise between too frequent cleaning and complete neglect.

If the only `dirt' present on the glass is dust or other particular matter, it can be removed by blowing air across the surface with hand bellows or by dusting with a soft brush. The ideal tool is a combination of the two - the photographer's blower brush'.

If there are greasy deposits, a gentle wipe with lens tissue may be required.

Heavy contamination with grease may mean the use of 1,2-dimenthylbenzene (xylene) as a solvent. However, this may attack the mounting compounds used to hold the objective components. Therefore, use enough only to moisten the lens tissue or Selvyt cloth.

Reminding once more

Another good reason for using only small amounts of xylene is its toxicity. It should be used only in a well-ventilated work area for short periods of time and should be kept off the skin. Objectives should never be immersed in solvents to soak off heavy deposits - they are likely to fall apart!

For contamination by `aqueous' materials such as sugar solution, blood, glycerine and copper sulphate, use lens tissues moistened with distilled water. Heavy contamination is best removed by repeated gentle rubbing, changing the tissue frequently. Never use a tool of any kind, and avoid the use of tap water (this may leave a deposit of salts after evaporation).

Immersion oil may be removed, using xylene instead of water, in the same way. If the old type of oil has been used and has dried up to a caked deposit, xylene may not remove it. In this case, fresh immersion oil may be an efficient solvent and remove most f the deposit. The reminder can then be leaned off with xylene.

It is wise to wear gloves, since some older types of immersion oil contain polychlorobiphenyls (PCBs) which, like xylene, are poisonous by skin absorption.

Use a hand bellows to blow off any loose fibres left behind by cloth or tissues.

Objectives

Objectives are cleaned using the techniques described above, choosing the actual cleaning methods according to the degree of contamination of the lens. After cleaning the front lens, hold the objective with this lens uppermost and puff air in strongly to remove any dust from inside. Hold it up to the light and examine the back lens with a magnifier to ensure that no dust remains. In persistent cases a camel hair brush may be used to clean the back lens gently. In no circumstances must you take the objectives apart. If components are displaced, the objectives will have to be returned to the manufacturer.

Eyepieces

Eyepieces are cleaned in much the same way as objectives, except that they can be dismantled for cleaning. If there is likely to be any confusion as to which way a lens should face, mark an edge with a grease pencil or make a sketch of the arrangement of the lenses as they are removed. Most eyepieces are opened up by unscrewing the top half. To minimise the amount of dust settling on cleaned surfaces, the top (eye) lens should be tackled first. While reassembling, take care not to cross the very pitched threads.

Condenser and mirror

Dirt on these components is relatively unimportant, though it may sometimes cause glare or loss of intensity. Cleaning is as for lenses, but there should be no need to use solvent. Some condensers can partly be dismantled by unscrewing components; and this adds to ease of cleaning. Mirror and condenser can be given a final polish with the Selvyt cloth before inspection with the magnifier for traces of dirt or grease.

Optical head of `modern' instruments

The prism or mirror in the angled head can be dusted with a blow-brush and gently polished with a lens tissue or Selvyt cloth.

7. Replacing Optical Components

These are refitted in the reverse order to disassembly - condenser, mirror, objectives, eyepiece. Before replacing the eyepiece, look down the eyepiece tube, using a centering telescope if available, and check the alignment of the components refitted. Small adjustments might be required to the mirror or condenser mounts or to the stage, to ensure that all the parts are correctly centred on the optical axis. Many of the threads used in the optical components are fine; care is needed to avoid cross-threading.

8. Testing

Once it has been reassembled, the instrument should be checked over to ensure that optical components have been thoroughly cleaned and that stops, stage clips and condensers are properly adjusted. The instrument should be set up appropriately.

SELF ASSESSMENT EXERCISE 4

When you inspect the fine focus mechanism on one of the lab microscopes, you notice that the grease is discoloured and you decide to replace it. By which one of the following ways would you remove the grease? Tick the correct answer.

1. Wiping the mechanism with a clean rag on a wooden peg;
2. Wiping the mechanism with a clean rag moistened with 1,1,1 - trichloroethane; or
3. Flooding the mechanism with a solvent such as that mentioned in (2) and then wiping it clean?

SELF ASSESSMENT EXERCISE 5

Based on your experience, write some points in addition to the ones mentioned in Section 3.3.1 that lists some Do's and Don'ts.

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SELF ASSESSMENT EXERCISE 6

What basic `working rules' should one keep in mind for the maintenance operations of a microscope?

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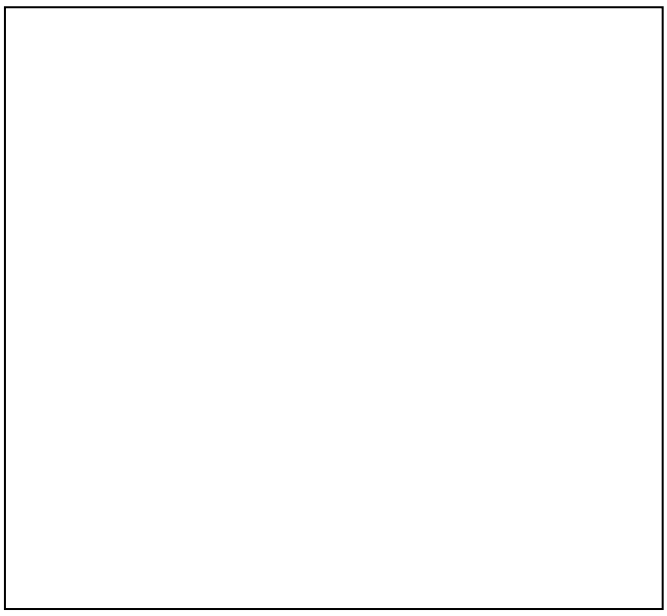
SELF ASSESSMENT EXERCISE 7

Pick any five microscopes from your lab., and observe them carefully to prepare an organizer for their servicing.



4.0 CONCLUSION

You have learnt to focus the microscope on the micrometer slide using the eyepiece and the objective combination you need. You have learnt to align the two graticules, read off and take note of a mm (x), using at least half of the field to view the object. You have also learnt how to calculate the equivalence of units measured.

You have also learnt how to enhance the efficiency of your microscope through the oil immersion technique. You have also learnt how to care and maintain your microscope.

5.0 SUMMARY

You have learnt in Unit 1 about what the work involved in the practical biology laboratory.

In unit 2 you learnt about laboratory organizations.
Unit 3 dealt with the handling of common laboratory equipment. Here you have learnt about the microscope.

Now its time to go to the specimens themselves.

In module 2 unit 1, we will learn how to procure animal materials for study.

6.0 TUTOR MARKED ASSIGNMENT

1. Describe the steps you would take to measure a cell under the microscope.

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biology in action London, Heineman Educational Books Ltd.

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Store, R.N., Cozens, A.B., Emia, F.I. (1972) New Biology for Tropical Schools London, Longman Group Ltd.

MODULE 2

PROCURING ANIMAL MATERIAL
IGNOU ADAPTED

Published by National Open University of Nigeria 2006. This edition has been adapted by the National Open University of Nigeria for use by the students of the National Open University of Nigeria from (Lt-2) (Laboratory Technique in Biology) designed and created by the Indira Ghandi National Open University by arrangement with the owner.

UNIT 1 PROCURING ANIMAL MATERIAL FOR LABORATORY WORK

CONTENTS

1.0 INTRODUCTION

One of the important duties of a lab technician is to collect, procure and preserve animal specimens for the use of the students. The live animals are collected with the use of accessories like nets, jars pans etc. From water bodies (aquatic animals) as well as from different land areas (terrestrial animals). The animals specimens are procured from the market. For this you have to place the orders with the dealers. The ways of procuring specimen and live animals and their preservation is dealt with in this unit. You will also learn how to identify male and female animals by studying the external features.

2.0 OBJECTIVES

After doing the exercises in this unit you will be able to:

- place the order with a dealer for purchase of specimen and live animals,
- collect live animals from their natural environments.

3.0 MAIN CONTENT

3.1 Procuring animal material

3.2 Placing order for the specimens and live animals

Specimens are to be procured from the market. For that you have to first invite quotations in sealed and marked envelopes for the articles to be purchased. The purchase committee in your institution will open all the quotations and decide from which dealer to purchase the specimens and/or live animals. You will normally expect that the purchase order is given to the lowest bidder. In some cases, you may realize that the company's stock is not the best, even though cheap. Some companies may fail to meet the deadline and you are forced to look up to the ones that can perform. However this must have been after they have really failed.

For this activity, two letters are to be written from your institution for inviting quotations and the second for placing the order. The letters are signed by the Head of the institutions or the person designated by the Head. Given below are examples of these letters. The first letter is an example of the format used for inviting quotations from the dealer and the second is an example of a letter for placing the order. There can be certain variations in the formats used in different institutions.

Letter No. 1

Dated:

Tel No ..... Fax No .....

The Manager,
Study Centre
National Open University
Coordinating Office
Jos.

M/s Vinay Chemicals
4, Ikpaja Road
Lagos.

Ref: EI/quotation/1999-2000

Dear Sir,

Quotations are invited for the supply of the apparatus/equipment as per the enclosed list quoting the price/discount (%) on the catalogue prices and the make of the apparatus/equipment.

It is requested that the following should be carefully observed in every detail while submitting quotations, otherwise they may not be considered.

Quotations should be sent under sealed cover addressed to the Manager, Study Center, National Open University, Coordinating Office, Jos so as to reach him not later than

The words "Quotations for apparatus" should be written prominently on the envelope.

Quotations should be for the supply of articles F.O.R Manager, Study Center, National Open University, Coordinating Office, Jos.

Kindly quote the price/discount (%) you will give on the listed price.

Thanking you,

Yours sincerely,
J.K. Babalola

Letter No. 2

Dated:

Tel. No ..... Fax. No .....

The Manager
Study Center
National Open University
Coordinating Office
Jos.

M/s Vinay Chemicals
4, Ikpaja Road
Lagos.

Ref: El/quotation/1999-2000/

Dear Sir,

Regarding your Quotation No ..... you are requested to kindly supply the following ..... immediately.

Items	**Number**
1.	
2.	
3.	
4.	

Bill may be sent in duplicate to the undersigned for payment.

Yours sincerely.

J.K. Babalola

3.3 Collection of live animals

Live animals are generally collected from aquatic and land areas. For collection visit a nearby aquatic body (e.g pond) and land area
(eg. Park/Field) along with your course tutor and other students of your group.

3.4 Collection of Aquatic Invertebrates Sources

The sources can be ponds, lakes, rivers for fresh water animals and ocean/sea coasts for marine animals. However, in this section, as an example we will study the collection of animals from ponds.

Materials Required

Nylon nets (Fine weave for small animals and coarse weave for large animals) Large clean jars or buckets
Shallow white pans or papers

Method

1. Take a clean bucket or a jar and fill it up to about half with the pond water from which you are going to collect the samples.
2. With a trowel, scoop a little amount of mud from the wet edge of the pond and put it in the bucket or jar having pond water.
3. Also put one or two small submerged branches of aquatic plants in the bucket or jar.
4. Take the suitable net and sweep through the water in the pond. You have to sweep more than once (see fig. 6.1 for different types of nets).
5. Take out the net. You will see the specimens trapped in the net. Transfer the specimen into the bucket or the jar.
6. Take some extra pond mud, submerged branches or aquatic plants along with some pond water and carry to the laboratory for subsequent use, if needed.
7. In the lab, transfer the live specimens into shallow white pans or place them on a large sheet of paper and spread them out for study.

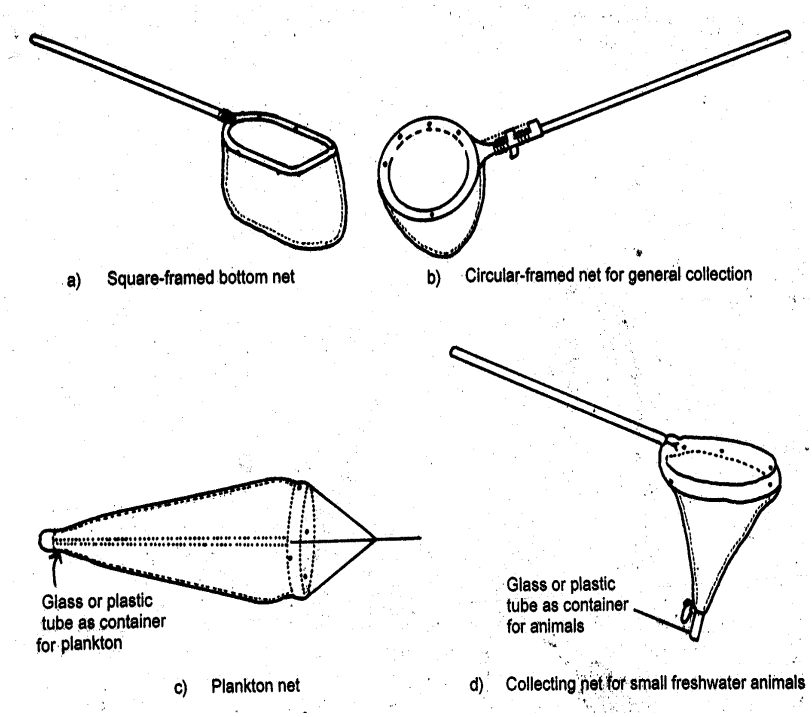

Fig. 6.1 Types of nets used for collection of various animals

3.5 Collection of Earthworms

These annelids can easily be collected from the soil having organic matter especially at night and after a rain, when they come out at the surface of the soil. In dry season earthworms are not easily available. So they are preserved whenever available for use in dry season

Sources

Rich garden soils, lawns, agricultural fields especially after a rainy day or night.

Material Required

A bucket
Flashlight torch (for night collection)
Blunt-end forceps

Method

Visit the collection site. Put some moist soil in the bucket. Pick up the worms with blunt-end forceps and put them in the bucket. Use flashlight torch if collection is to be done in the night. Take the worms to the laboratory.

3.6 Collection of Insects

Sources

Terrestrial insects are found in gardens especially during flowering seasons, in the fields and of course indoors. Aquatic insects can be collected from water bodies like ponds, lakes etc.

There are several methods of collecting insects but in this section you will collect terrestrial insects by three methods using:

a) net
b) light trap, and
c) aspiration

Methods

(a) Sweep Net Method

This method is suitable for collecting many insects.

Materials Required

Insect-collecting net and Killing jar

Steps

1. Go to the garden/field and identify the insects to be collected.
2. Approach the specimen(s) very quietly. You should try to avoid chasing the insects overtly as it would alert the insects and make them fly/run away.
3. Sweep the net (fig 6.1a) through the herbage over the specimen(s). You might have to sweep more than once.
4. When the insect(s) is trapped in the net, twist the net or your wrist so that net is closed (fig 6.1b) and the specimen is not able to escape.
5. Transfer the collected insects into the killing jar.

(b) Light Trap Method

In this method the collector is not required to be present. It is mainly used for nocturnal insects like moths, midges, some beetles and winged termites.

Materials Required

- Light sources such as an electric bulb (200 W) or a lantern lamp.
- Large shallow container such as a basin sauce pan
- White paper sheet
- Killing jar.

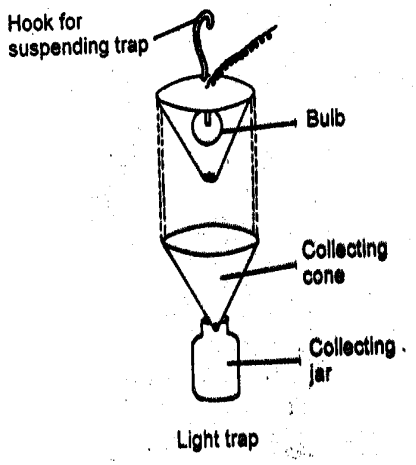

Fig. 6.2 Light trap for insect collection

Selects

1. Select an area where insects are abundance

2. Hang the light source with the help of a hook.

3. Put the white paper as lining in the shallow container and set the container below the light sources so that electric lamp is shining in the middle of the container.
   (In the absence of an electric light keep a lantern lamp in the middle of the container)

4. Soon the insects will be attracted by the light and fall into the container.
   (In case the shallow basin saucepan is not available you can keep a collecting jar fitted with a cone made of white sheet under the light source as shown in fig. 6.2. The most efficient light source for insect-trapping is a mercury vapour lamp)

5. Transfer the collected insects into the killing jar.

(c) Aspirator

An aspirator is a simple suction device used for collection of small insects such as mosquitoes, thrips, sandflies etc.

Materials Required

- A transparent vial made of glass or plastic (transparent plastic is preferably used). Rubber stopper with two holes
- Two glass tubes each with a bend Rubber tube
- Small piece of muslin cloth

Steps

The aspirator described her (fig. 6.3) is the one that is most commonly used.

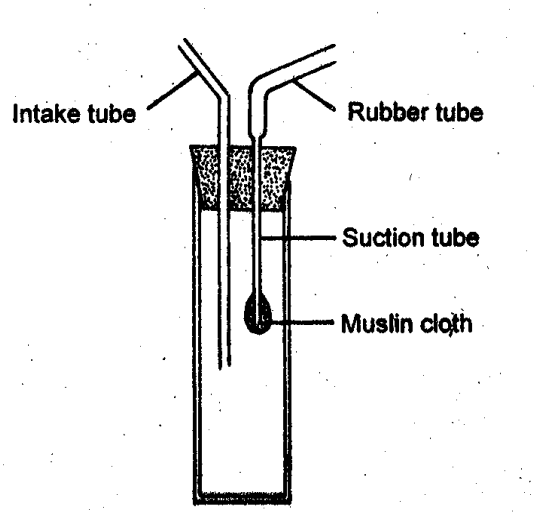

Fig. 6.3 Most commonly used Aspirator

1. Insert the two glass tubes (intake and suction tube) through the two holes in the stopper.
2 .At one end of one glass tube attach a rubber tube. Cover the other end of this tube by tying a piece of muslin cloth. This tube acts as a suction tube. The other tube is the intake tube.
3. To the open end of the vial fix the rubber stopper (with inserted tubes). The stopper should be tightly fixed in the vial. The end of the suction tube that is covered by muslin cloth should be inside the vial. The aspirator is now ready for use.
4. Place the aspirator with the outer end of its intake tube facing the insect(s) and suck through the rubber tube. The suction creates a partial vacuum in the vial there by drawing the insect through the intake tube. The muslin cloth tied on the inner end of suction tube will prevent the entry of insects into this tube.
5. Plug the outer end of the intake tube to prevent the escaping of the insects caught in the vial and then transfer the collected insects into the killing jar.

3.7 Collection of Live Animals Demonstrated

Now that you know what to do in order to collect live animals let us see it being done in real practice. Watch the video clips on the (take 6.1):

- Collection of some aquatic invertebrates (named).
- Collection of earthworms
- Collection of some insects (named)
  Now try collecting live animals too and see how many of these varieties you are able to collect.

Wishing you a pleasurable collecting experience.

Now you can do the following self assessment questions since you have learnt about them in this unit.

SELF ASSESSMENT QUESTIONS

1. Give some reason why you have to buy specimen animal from any higher bidder.
2. Write a sample letter to a dealer making orders for some rabbits and guinea pigs.
3. What type of light source is best for trapping insects?

4.0 CONCLUSION

In this unit you have learnt that you either buy or collect animal materials for laboratory work. Once they have been acquired, they must either be killed and preserved for studying or kept alive in suitable conditions for the same purpose of being studied.

5.0 SUMMARY

Having procured our animal materials in this unit, we will learn how to preserve them in the next unit.

6.0 TUTOR MARKED ASSIGNMENT

Describe how you will collect the following animal materials:

1. Aquatic Invertebrates
2. Earthworms and Insects

7.0 REFERENCES/FURTHER READINGS

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An NOU Video Demonstration on `Collection of Live animals.' (To be produced by NETC)

UNIT 2 PROCURING PLANT MATERIALS

CONTENTS

1.0 INTRODUCTION

Laboratory work is an integral part of learning science. In biological sciences living or preserved organisms have to be provided for the study of anatomy, Physiology, taxonomy etc. As lab. technical staff, you will have to provide various plant materials to the students for experiments. You have to collect lower and higher plants for study. In this unit we are going to describe all the techniques which will help in collection of plants only. For collecting the plants you should know what type of bottles, fixatives, solutions and equipment are needed. You can start by collecting the plants with the teacher who will help you identify them.

In due course of time you will also be able to perform this activity independently.

2.0 OBJECTIVES

After studying this unit you will be able to:

- Grow root tips of a plant species for squash preparation,
- Fix the root tip,
- Collect algae, bryophytes, pteridophytes and gymnosperms,

- Collect, press, dry mount, label, store, and preserve angiosperm specimens to make a herbarium.

3.0 MAIN CONTENT

3.1 Growing of material for squash preparation

The root tips may be obtained by allowing them to germinate on a wet blotting paper disc or/sand free from soil and debris.

Squash preparation is a technique of preparing slides of biological material which we will do in unit 10. In this unit you will only learn how to grow the roots for this technique.

Materials Required

Onion bulbs
Coplin jars or wide mouth bottles
100 ml beakers
Scalpel
Onion Bulbs

1. Take an onion and scrape off the dry roots from the bulbs to expose the disc.
2. Fill a coplin jar with tap water and place the onion bulb on it in such a way that the disc touches the water.
3. Place this near the window to get enough light for three to four days. Roots will start growing and the root tips can be clearly seen.

3.2 Collection of Lower Plants

The collection of plant material is a simple job but one should take much care of collected plants or their parts so that they are preserved without any damage. The plants may be collected in vasculum, polythene bags or in bottles. You will need a pair of secateurs for cutting hard material, a sharp knife for cutting soft parts, pick for digging out underground parts like roots and rhizomes, scalpel and forceps for separating those plants which grow attached to the barks of trees and rocks.

The stems and roots are cut into pieces of size about 3 cm long with the aid of sharp razor or knife so that the tissues at cut ends do not get macerated. Bryophytes are made free from soil particles and debris before storing in some preservative. The smaller leaves can be preserved as such and larger ones can be cut in pieces, and then preserved.

We will now discuss the collection of algae, bryophytes, pteridophytes and gymnosperms specimens one by one.

3.2.1 Algae

Sources: The algae occur widely in nature viz on the soil surface and below it, on the bark of trees, in fresh water, sea water, and a variety of other habitats.

Collection from Bark

i) In case of bark algae pick up the algae patches from the tree trunk with the help of iron spatula.
ii) Sterilize spatula by swirling it in spirit and then flaming it.
iii) Store various samples collected in separate sterilized bottles after fixing and labeling in their respective shelves.

Collection from Fresh Water

i) Collect the fresh water algae at the spot in sterilized specimen tubes containing some habitat water.
ii) Never fill the container more than a quarter so that the quantity of oxygen present in the water may be sufficient for respiration.
iii) Fix the material, label them and keep in respective shelves.

Collection from Sea Water

i) In Nigeria the southern coast is best for marine algae collection during low tides as during this period these are mostly in their reproduction stages.
ii) Collect marine algae in large bottles.
iii) Fix them and label them and keep them in their respective shelves,

3.2.2 Collection of Bryophytes

Now we will discuss the collection of bryophytes. Normally bryophytes occur in nature attached to wet soil, rocks, bark of living and dead trees, wood and humus rich in organic substances.

- Scrape the bryophytes from the place of occurrence with the help of sharp scapel or knife and keep them in polythene bags within which they remain alive to a number of days.
- Keep these bags loosely tied and in damp condition in laboratory.
- Wash the soil growing species with ordinary water to remove soil particles and dirt attached to plant.

- Keep the bags under illumination at 0ºC - 5ºC to keep the plant alive for a longer duration.
- Fix the material, label and keep it in the cupboard.

3.2.3 Collection of Pterldophytes

Pteridophytes are commonly known as vascular cryptogams, these are spore-producing vascular plants. They possess the vascular tissues xylem and phloem. They grow in variable habitats. Most of the pteridophytes are terrestrial which thrive well in moisture and shade while others are found growing in xeric conditions. A few are epiphytes and some of them are found in aquatic habitats.

- Collect the pteridophytes from natural habitats in mature spore producing stage.
- Collect the plants with or without strobili or mature sporophyll in polythene bags loosely tied at mouth.
- If the material is large cut them into pieces, fix label and keep them in a cupboard.

3.2.4 Collection of Gymnosperms

Gymnosperm belong to seed plants but the seeds are naked with a very conspicuous and independent sporophyte which is the main plant and have very reduced gametophyte dependent on the sporophyte. They have xeric characteristics also.

i) Collect the root, stem, leaves, male and female gametophytes of the plant.
ii) Cut the material into small pieces of 3 cm, fix them, label them and keep them in a cupboard.
iii) You can collect the dry fruits an cones of gymnosperms and preserve them as such.

3.3 Collection of higher plants for making a herbarium

In this section you will study about preserving plants for a herbarium because as a technician you have to make and maintain a herbarium. It is a place where plant specimens are kept, often in a dry state for biological studies. Some plants are also grown in a botanical garden or specially maintained in green house for this purpose.

3.3 Collection of Plants

1. You should remember one important thing about collection - Do not pick plants haphazardly. Choose only those plants whose flowers are large and whose organs are easily seen.
2. If possible collect complete plants with flowers and fruits (sometimes they are necessary for recognizing species of some families such as Cruciferae, Umbelliferae, Compositae revised name Asteraceae).
3. The underground parts (root, bulbs, rhizomes) are often interesting. Try to collect them. If the plant is large take just a branch with leaves, flower and fruits.

3.4 Carrying the Specimen

1. Do not mix up or damage the selected plant with others.
2. Place the plant quite flat between newspaper and place in a cardboard folder and fastened in a strap or keep the plant vasculum.
3. Attach a label to each plant stating details of its dwelling place, date, name of place, flower colour and any other interesting features.

3.5 Examining the plant

1. Identify the name of the plant with the help of your course tutor. In due course of time you will be able to name the plant by yourself.
2. You can also keep a plant with some labeling with it and it could be identified with the of the flowers.
3. Make a few sketches to point out the characteristics which helped you in identification of the plant.

3.6 Drying

1. Spread each plant carefully in between blotting paper or newspaper.
2. Then place the plant in the plant press available with its label.
3. After a few days (the nest day, if the plants are very moist) check that the plants are properly laid out and change the paper.

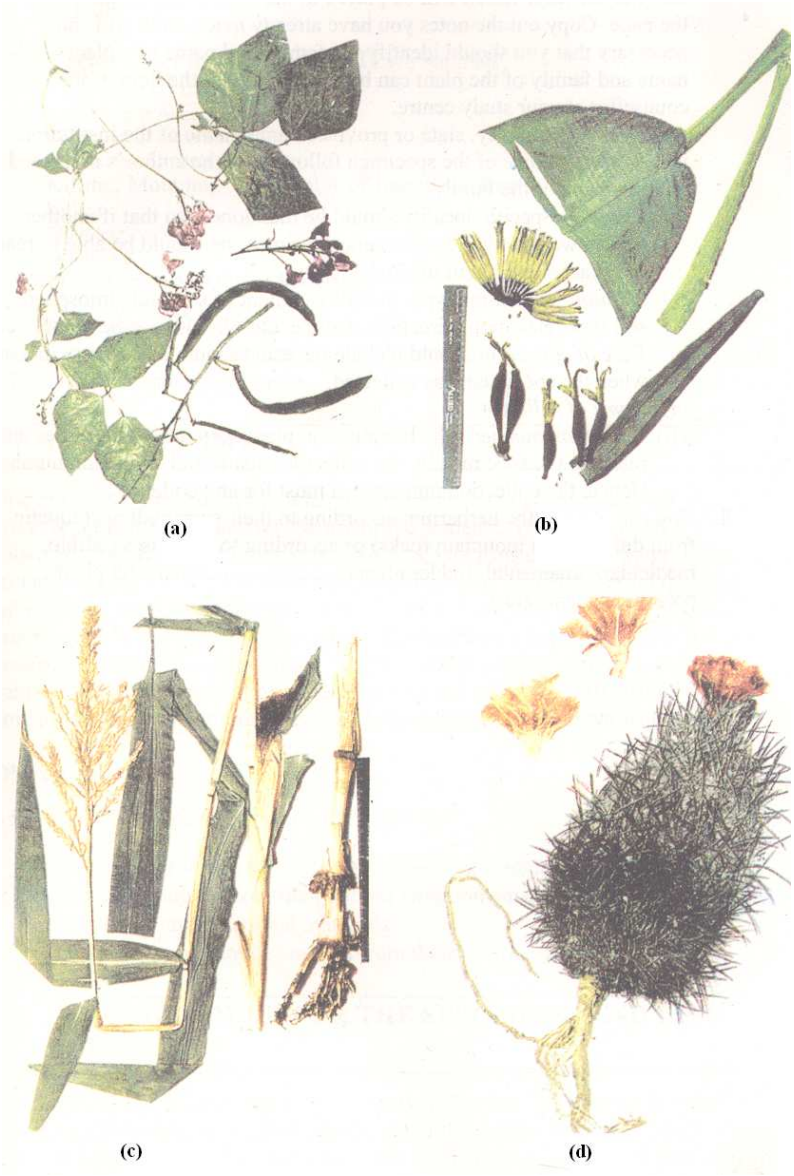

Fig. 7.1 Mounted specimens of (a) Vigna unguicalata, (b) Musa Sp. (c) Zea mays (maize) and (d) Bixa sp.

4. Check the specimens and if they are properly dried, place them in your herbarium.
5. Place the plant on herbarium sheet (a fairly large piece of special paper or on any fairly thick paper), fix the dried plants in their natural position with some cellotape.
6. If the plant is too big, break the stem cleanly or arrange fragments of the leafy stem, the floral stem and the fruits.
7. Rewrite the label which will be placed in the bottom right hand corner of the page. Copy out the notes you have already made on it. (It is not necessary that you should identify the family and name of a plant). The name and family of the plant can be identified with the help of the course tutor at your study centre.

(i) Heading: Country, state or provide name , name of the institution.
(ii) Scientific name of the specimen followed by the author's name and the name of the family.
(iii) Locality: Specific locality should be mentioned, so that if another person wants to collect the same specimen, he should be able to reach the exact site without much difficulty.
(iv) Habitat: Vegetation type, moisture content of soil and atmosphere, soil type, elevation, direction of slope, etc. should also be mentioned.
(v) Date of collection should include the exact month and year to indicate when the specimen was collected.
(vi) Name of collector.
(vii) Collection number: The literature on plant systematic identifies and refers to the specimen by the collector's name and collection number. Hence, the collection number is a must for any collector.

8. You can arrange the herbarium according to their surroundings (aquatic, from dunes, from mountain rocks) or according to their uses (edible, medicinal, ornamental, fodder plants .....; harmful plants, poisonous plants etc.).

3.7 Hints on Procuring Plant Material

Watch the video clips demonstrating "Plant Material Collection". `Take not of any similarities or differences in materials displayed in comparison with your own collections.

SELF ASSESSMENT EXERCISE

1. How will you procure growing roots for squash preparation?
2. From which places can you collect specimens of Algae?
3. What precautions will take when drying plants?

4.0 CONCLUSION

In this unit, you have learnt how to collect plants from algae to the flowering plants. You have learnt that you go to the natural habitat of these plants to find them. Most lower plants have to be fixed before storing away in labeled sterilized bottles. For most higher plants, their preservation process is drying and their storage place is the herbarium.

5.0 SUMMARY

Now we can assume that you have brought in all sorts of plant material to the laboratory. The purpose of doing so is to have them for studies. The next thing is to really start studying them. We will begin by studying their external features are trying to find the similarities and differences among them. This will be our focus in unit 9.

6.0 TUTOR MARKED ASSIGNMENT

1. Describe how to collect an alga plant from three (3) different habitats.

7.0 REFERENCES/FURTHER READINGS

Beril, N. J (1979): Biology in Action London, Heinemann Edicational Books Ltd

Soper, R. (Ed) Biological Sciences Cambridge U.K., Cambridge Press

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UNIT 3 KILLING PRESERVING AND MOUNTING ANIMAL MATERIALS

CONTENTS

1.0 INTRODUCTION

In unit1 of this module, you learnt how procure by buying or going to collect animal material from their habitat (unit 2). When you have brought them into the laboratory you will need to preserve all others you are not using immediately. Sometimes you even want to make a collection for display for the same purpose of studying and probably classifying. Sometimes you even want to distinguish male and female specimens. In this unit we learn how to kill, and preserve and display invertebrate specimens.

2.0 OBJECTIVES

To guide our study in this unit, you may like to set some targets. So we will say that by the end of this unit, you should be able to:

- Kill insect specimens
- Mount insect specimens
- Display insect specimen

- Preserve some other animal material
- Preserve some live animal material
- Identify male and female specimens.

3.0 MAIN CONTENT

3.1 Killing, mounting and display of insect specimens

For killing, the insects are transferred into a bottle, containing killing agent. Through various killing agents such as ethyl acetate, chloroform, ether, tetrachloroethane etc. can be used, the safe and most efficient agent is ethyl acetate. You can make a killing bottle as given below (fig. 8.1).

Materials Required

An empty glass bottle with an air-tight lid (you can take a jam or holicks bottle)
Ethyl acetate
Cotton
Blotting paper
Forceps

Steps

1. Soak a wad of cotton in ethyl acetate. You must hold this cotton wad with forceps and not with hands.
2. Place the soaked cotton at the bottom of the bottle and cover it with a piece of blotting paper. Blotting paper is used to avoid the direct contact of the specimens with the chemical because it will wet the specimens and
   spoil them. However, you can pour a few drops of killing agent over the blotting paper to make the bottle more effective. (Instead of cotton wool, plaster of paris can also be used).
3. Transfer the insects into the bottle. Close the bottle tightly. Take out the insects within 20 mins. Otherwise they will decolourise and get unduly hardened. Do not overcrowd the bottle with insects. Overcrowding of bottle with tough and fragile insects or large and small insects may cause damage to the insects. You should, therefore, use separate killing bottles for separate types of insects.
4. Label the killing bottle as `poison' and keep it away from the reach of the others. The bottles that are no longer able to kill should be buried or burned.

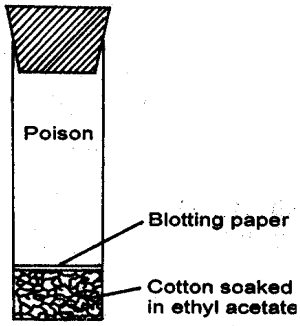

Fig. 8.1 Killing bottle containing soaked ethyl acetate and blotting paper.

3.2 General Information about Mounting

After being killed the insects are pinned with the help of entomological pins on the pinning board. These pins are made of steel and do not rust. Such pins are available in a variety of sizes and thickness. You can also prepare entomological pins with sewing needles and coloured beads. Take thin sewing needles, heat the eye of needle on a spirit lamp flame and insert the heated end into a coloured bead.

The bead forms the head of the needle. You can make many such entomological pins. Now you know that mounting means pinning the killed insect. It can be done by any one of the methods given below.

You should mount the insects immediately after killing them. If left for a long time in the killing bottle the insects become dry and stiff and one faces difficulty in pinning them. Under such circumstances insects must be first relaxed. For this you can make your own relaxing box by the procedure given below.

3.3 Relaxing animals in relaxing box before mounting.

Procedure for making a relaxing box are as follows:

1. Take a plastic container with a lid of about 2' x 1' x 1' size.
2. At the bottom of the container place a thin sheet of synthetic sponge or any other porous material of 2 to 4 cm thickness. Saturate this material with water.
3. In one corner of the container place a cotton wad soaked in ethyl acetate to prevent the growth of mold.

4. Place a sheet of blotting paper on the inside of the lid to absorb the moisture that may otherwise condense and fall over the specimen.
5. Put the insect in a Petridish or envelop and leave them in the relaxing box. Though relaxing time depends on the size and type of specimens, most of the specimens are relaxed satisfactorily when left for one night in the box. Too long a period in the relaxation box will cause damage and discolouration to the insects.

Now follow the procedure for mounting.

3.4 Mounting

3.4.1 Direct mounting

As you have read earlier mount the insects immediately after their death. Do direct mounting using the following steps:

1. The entomological pin is pushed through the thorax region of the insect.However, the exact point in the body of the insect through which the pin should pass differs in the different groups of insects. You can take the help of your course tutor in identifying that point in the insects you have collected.

2. Insert the pin vertically through the body or sloping in such a way that the front part of the body is raised very slightly.

3. Push the specimen up in the pin until its back is about 11/2 cm away from the top. This distance helps in holding the pin freely without having any contact with the back of the insect body.

4. Mount these pinned insects on the board or on a pinning block. Take care to mount the insects uniformly so that specimens can be examined and compared easily.

3.4.2 Point Mounting

This method is especially used for mounting small and dried insects.

Take the following steps

1. Take a stiff card paper and cut triangles from it. For a smaller insect the size of the triangle can be 6 mm long, 2 mm wide at base and 0.5 mm wide at the apex (tip). However, the size of the triangle varies depending upon the size of the insect.

2. Attach the dried specimens to the apical tip of the triangle with the help of a quick drying adhesive like quick-fix. The best places on the insect body for adhesion cab be at the sides of thorax below the wings, margin of the tergum and above or between the bases of the legs.
3. Insert the entomological pin in the broader end of the triangle and pin this triangle with mounted insect on the display board.

3.4.3 Spreading

To display the head, abdomen, wings and legs you have to spread the freshly killed insects on the spreading board. In the freshly killed insects the internal parts are soft to allow the pin in and appendages are pliable. The pin is pushed through the thorax region. A spreading board is available in the market but can also be made locally. A simple method is to take a thick sheet of cork or thermocol and cut a groove in it for the body of the insect.

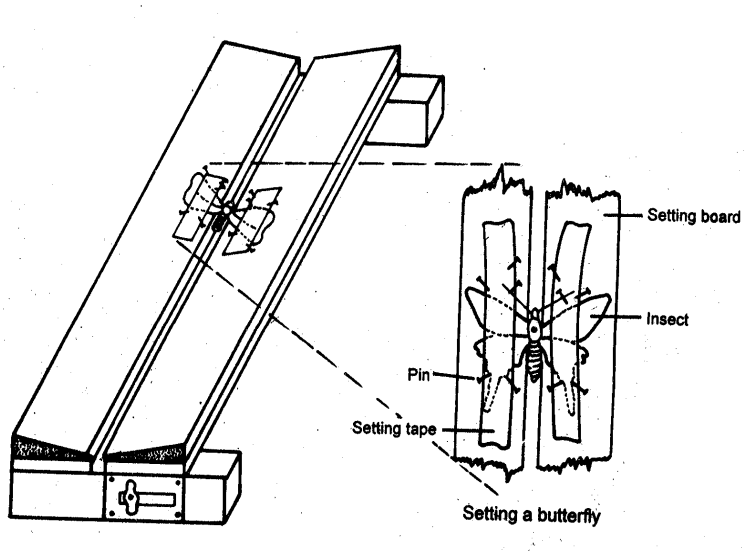

Fig. 8.2 Spreading of butterfly on the spreading board. The body-parts of the butterfly are properly spread and set on the board.

Steps

1. The insect is placed in such a way that the body and thorax of insect rest in the groove of the board.
2. One end of a narrow strip of setting paper is pinned at the front end on each side of the insect body.
3. The fore wings on the back are drawn forward and each pinned on either side with a fine pin inserted behind one of the strong veins in the wings.

4. The hind wings are also spread like this and pinned.
5. When the wings are correctly placed the paper strips can be taken over the wings and their other end is pinned on the back of the insect body so that both the wings are held by paper strips and setting pins.
6. The antennae are also spread symmetrically and pinned under the narrow strip.
7. Legs (appendages) are also spread and pinned on both sides under the strips. Care should be taken that while spreading, the joints and the shape of the appendages remain intact.
8. If the abdomen is inclined to fall into the groove it can be supported by crossed pins placed beneath it.

After the pinning and spreading the specimens are dried for few weeks in the open or in drying chambers and stored (see fig 8.2 for spreading the insect butterfly).

3.5 Displaying

Once the specimens are collected and spread, they should be given permanent labels. Proper mounting, spreading and displaying are necessary for taxonomic studies such as the identification and study of external morphology of the insects. These labels should be small and made of white card. The following information should be there on the label of each specimen:

1. Name of the insect.
2. Host plant, crop or the area from where it is found.
3. Locality from where it is found.
4. Date.
5. Collector's name (see fig 8.3)

The ink used for writing should be permanent and not spoiled when in contact with any type of liquid.

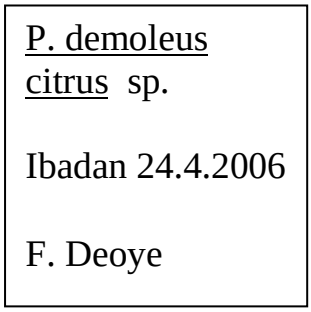

Fig 8.3: Label for display of insects

The spread board along with spread insects with labels should be displayed in wooden boxes with glass tops. The mounted insects should also be stored in closed boxes. You must keep naphthalene balls in the storing or displaying boxes used for insect specimens. In case these precautions are not taken the specimen insects can get spoiled or eaten by other insects/small animals.

3.6 Preservation of animal material

Once you have purchased the animals from the dealers or collected them yourselves, you might end up having material in surplus. In such a situation you will need to preserve these animals for further use at a later time. In this section you will study the way to preserve certain animals that are normally used in the school or college lab.

3.6.1 Preservation of Specimen Animals

Material Required

Animal specimens
40% formalin
Glass jars with lids
Big containers with lids

Steps

l. All the museum specimens are stored in 40% formalin in glass jars or containers with lids.
2. Earthworms, scolidon, rat and pigeon head, Mystus, prawns, Sepia, Pila Crab etc are also stored in 40% formalin in the containers.
3. For Pila Crab you need to make a hole in the shell before keeping it in the formalin so that formalin enters the mantle cavity and internal tissues are preserved.
4. Use different containers for different types of animals e.g, all earthworms in one container, scolidons in another container and so on.

All these preserved animals can stay fresh for 3-4 years.

3.6.2 Preservation of Live Animals

The live animals cannot be preserved for long. The methods for keeping animals alive differ for different animals and are as follows.

The material requirements for specific animals are written beside them.

- Leeches are kept in glass containers filled with fresh water. Water should be changed everyday or on alternate days. The water used should be clean and free of dirt.
- Cockroaches are kept in a plastic or metal jar. The must have many small holes so that air is available for their respiration. For feeding the insects small pieces of paper are put in the jar. It is advisable that fresh paper pieces are put in everyday.
- Rats are kept in rat cages. For feeding they need to be given bread pieces. If the rats are to be kept for a longer period i.e few days, they should be kept in separate cages, otherwise they will hurt each other and die. Prior to dissection the rats are killed with chloroform and treated with disinfectants like phenyl, dettol solution etc.
- Frogs are kept in a sink that is covered with a wooden or plastic plank having a few holes. The water tap should be adjusted to allow the water to fall into the sink drop by drop only. This keeps the skin of the frogs moist for respiration. (In some institutions a cemented tank that has water and certain aquatic plants is built. The cover of the tank has big holes for air circulation. The frogs are kept in this tank. Here they live and breed. Within the tank area a few cemented elevations of different heights and flat top surfaces are also made. So when the frogs need to be out of the water they can jump and sit on these elevations).

3.6.3 Preservation method for one live and one dead animal

l. Live leech can be preserved in clear clean fresh water. Fill the container with clear clean water and place the leech. Water should be changed at least every other day.

2. After killing a cockroach it could be preserved by mounting on a pinning board using entomological pins directly especially if is not dry. It is pinned by passed the needle through thorax and pushing up the cockroach up the needle thorax and pushing up the cockroach up the needle to about 2 cm away from the top of the needle. In this way the insect can be carried in the needle without being touched.

3.7 Identification of male and female animals

In the theory part of this course you have already read about the general characters of the animal phyla one generally handles in the lab. In this section you are going to identify and make a distinction between males and females of certain types of animals. You must be aware that lower invertebrates generally do not exhibit external variations in males and females. Also a few of them have both male and female sex organs. Let us now study some of the examples.

- **Earthworm and Leech:** Both are hermaphrodite i.e, the animal has both male and female sex organs.
- **Cockroach:** The female cockroach has two processes i.e, anal cerci at the abdominal end. The male has four processes at the abdominal end: two anal cerci and two anal styles.
- **Pila, Sepia and Prawn:** Males and females of Pila and Sepia cannot be distinguished externally. However, male prawns show appendix masculine in the pleopods (swimmerets i.e abdominal appendages).
- **Scolidon:** In males pelvic claspers are present. However, it is not easy to distinguish between male and female fish.
- **Rats:** The male rat has one external opening and possesses scrotal sacs. The female rat has two openings - vaginal and anal opening and no scrotal sacs.
- **Frogs:** Male and female frogs cannot be distinguished externally.

SELF ASSESSMENT EXERCISE

Now that you have performed various activities of this section, you will be confident in doing these activities as and when they are part of your assignment. Attempt the following SAQs to recapitulate your knowledge.

1. Differentiate between direct and point mounting procedure used for insect specimens.

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2. Take any two animals and write the distinguishing characteristic of the male and females of these animals.

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3. Write the preservation method used for one live and one dead animal.

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4.0 CONCLUSION

In this unit you have not only learnt how to kill animal material for laboratory use, you have also learnt how to preserve them. You have also learnt how to display and label, as well as to store them. You have learnt of the right containers to place each type and for how long they could be so usefully kept. You have further learnt of what precautions to take to ensure that they will continue to be useful for laboratory studies.

5.0 SUMMARY

Animal material surely will need a different treatment from plant material which you will also have to study in the biology laboratory. In the next unit, we will learn how to procure plant materials.

6.0 TUTOR MARKED ASSIGNMENT

1. What is Point Mounting?
2. Describe the steps involved in the process of Point Mounting.

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biology in action London, Heinemann Eductional Books Ltd.

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Stone, R.N. Cozens, A.B., Emia, F.I. (1972) New Biology for Tropical Schools London, Longman Group Ltd.

UNIT 4 EXTERNAL FEATURES OF PLANTS OBSERVING SIMILARITIES AND DIFFERENCES

CONTENTS

1.0 INTRODUCTION

You have learnt that plants are those organisms that have chlorophyll and a capacity to photosynthesize. The basic need of plant are water, mineral salts and light irrespective of their habitat. In this unit we will study the external features of a variety of plants. The features of organisms form part of the means of classification. They also are related to where they occupy, whether water, land or in-between. Let us study the external features of some plants across the various possible habitat.

We shall study the spirogyra, a fresh water filamentous alga, mucor (fungus) fern (pteridophyte), a pine (gymnosperm) and an angiosperm (a flowering plant). To achieve this goals, the following objectives are set for you.

2.0 OBJECTIVES

By the end of this unit you will be able to:

- Draw and label the following plants fully:- the external features of:- spirogyra, mucor, fern, a pine, a maize and the water leaf plant.

- Tabulate the differences and similarities between the following pairs
  (a) the spirogyra and the mucor.
  (b) The fern and the pine
  (c) The maize and the water-leaf plant.

3.0 MAIN CONTENT

3.1 Spirogyra and Mucor

3.1.1 Spirogyra

Visit a pond or a slow moving stream near your home. You are likely to see a netlike growth of green threads. You may also see air bubbles within this bright-green network. These are spirogyra threads kept afloat by the air bubbles.

Practical Activities

a. Collect some of these green threads with a cup or bowl, taking along some of the water.
b.. When you come back to the laboratory use your bare fingers to pick some of the green strands. Rub them gently with two fingers. Describe how it feels to this touch!
c. Put a drop of this water with the strand on a microscope slide and cover it with a cover slip.

Examine under the microscope

i Write down the magnification with which you are working.
ii Count the number of cells you can see in a thread of spirogyra.
iii Draw what you can see.
iv Using higher magnifications, make the drawing of one of the cells. Your course tutor will help you identify some of the structures.
v. Lift up the cover slip and put in a drop on iodine. The iodine will mix with the water on the slide.
vi. Examine the spirogyra after 5 minutes under high power magnifications.

Write down what you notice now to help you know how successful you are in your practical exercise, look at the following records of the same practical exercises as you did. Here are the answers that are correct for I to vi above.

Spirogyra feels slimy to touch.

i Magnification should not be more that x20
ii You can see between 4 and 5 spirogyra.

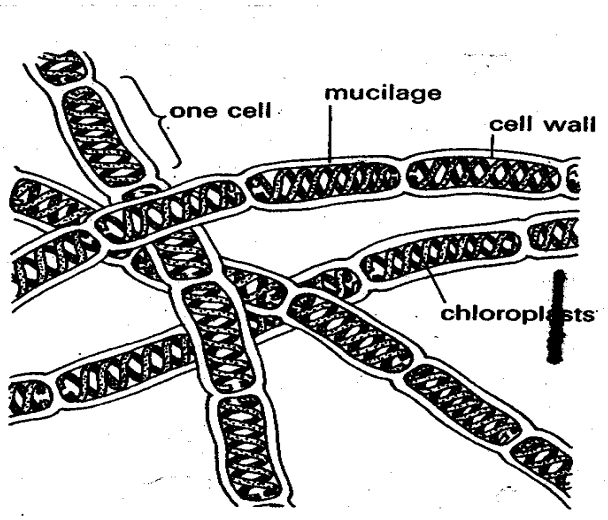


Fig. 9.1 Parts of four spirogyra filaments (x 20)

3.1.2 Mucor

Mucor is a fungus. Fungi are plants that do not posses chlorophll. They cannot make their food and so are either saprophytic or parasitic.

Practical Activities

i Wet a piece of bread or some garri, cover and leave on the table for about six days. You will notice white (or silver) threads. This mass of thread is the mycelium of the mucor.
ii Take some of this silver-like threads on the slide along with little water.
iii Spread `threads' out with your dissecting pin on the slide mount on a light microscope.
Iv Examine and draw three sporangia and sporangiosphores including their underlying mycelium under low magnification.

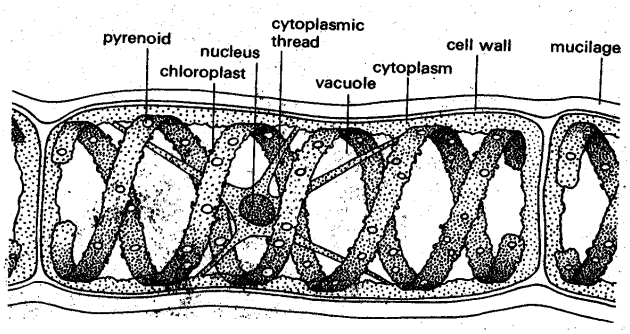


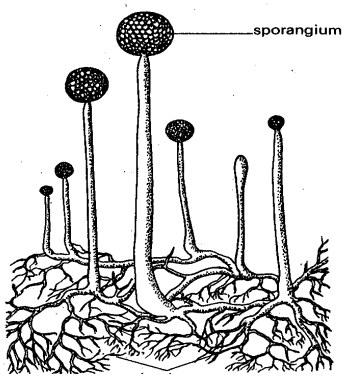
Fig. 9.2 Single spirogyra cells: Highly magnified

	Similarities	**Differences**
1	Both have nuclei	The nucleus in the spirogyra is bigger
2	There are cells	The cells are not separated in the mucor, but in the spirogyra they are separated.
3	Both have cell wall	There are no chloroplast in the mucor while the spirogyra has.

Can you notice other structural difference?

3.2 The Fern

The ferns have true roots, stems and leaves unlike the fungi. They live In a variety of habitats. Some live on other plants while others live on land. Visit the stream or pond nearest to you. You are likely to find ferns growing on other trees or in shady areas. The plant you are looking for is as shown in the picture (fig. 9.5).



hyphae

Fig. 9.3 Mycelium of Mucor

Young tree fern from Obudu Plateau, Nigeria showing aerial stem. Notice the thin soft stem. You will also notice that small swellings under the edge of the of the leaves. These are the reproductive structures. The sorus is covered with Gymnosperms with an inclusium.

1. Use your hand lens to study the structure of the sorus and the inclusium.
2. If you say it is a compound leaf you are correct.

3.3 The Seed Plants

3.3.1 Gymnosperms

Even though the stems of the ferns have vascular bundles, they are not as developed as in the seed plants. The seed plants belong to two groups, those that reproduce by spores stored in cones or strobili. For this reason they are called gymnosperms. The most common gymnosperms are called pines and are often seen as ornamental plants. They are not ordinarily natives of the tropical land. This means that:

1. The most likely place you should go to see the pine is in the city and the botanical garden where you can see many types of plants.
2. In the space provided, tabulate the similarities and differences between the fern and the pine.

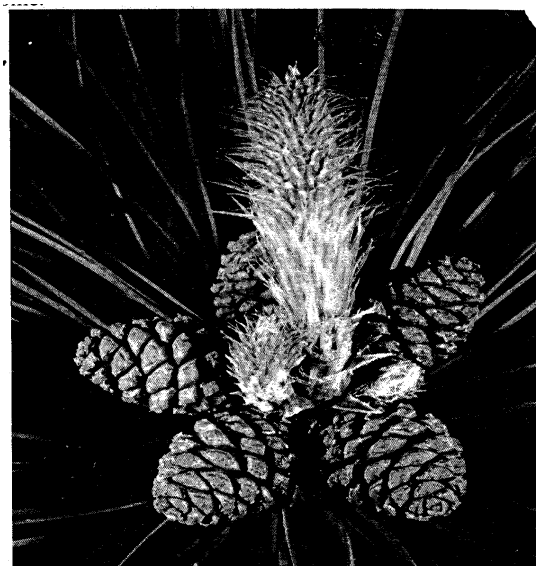


Fig. 9.4. Gymnosperm: Cones of the Austrian pine. Below are one-year-old cones which are pollinated the previous year. New cones, which will mature in another year, are seen as a central inflorescence. (Photograph by Anderson.)

	Similarities	Differences
1.	Both have stems, roots and leaves	The stem of the pine is aboreal while that of the fern and leaves is underground.
2.	Both bear spores	The pine grows into a big tree while the fern does not
		The stem of the pine is strong and woody. That of the fern is not. The leaves of the pine are needlelike or scale-like and covered with waxy cuticle.

You will remember that these are the things you can see. In the study of external features of any organisms only structures visible externally are considered.

Now we will study are the more common plants around us. These are the flowering plants called angiosperms

3.3.2 Angiosperms

Angiosperms are divided into two groups: The monocotyledonous and the dicotyledonous plants.

Here, let us study the maize plant as an example of monocotyledonous plant. Other plants like the coconut, oil palm, and grass belong to this group.

The maize plant is very commonly seen in most farms. Study it taking note of the following:

- The root hairs and prop roots
- Unbranched stem.
- The attachment of the leaves in a spiral form. Note the structure of the leaves
- In mature plant you may observe cobs. Study the cob in details.
- Learn to draw and label all the parts on your own.

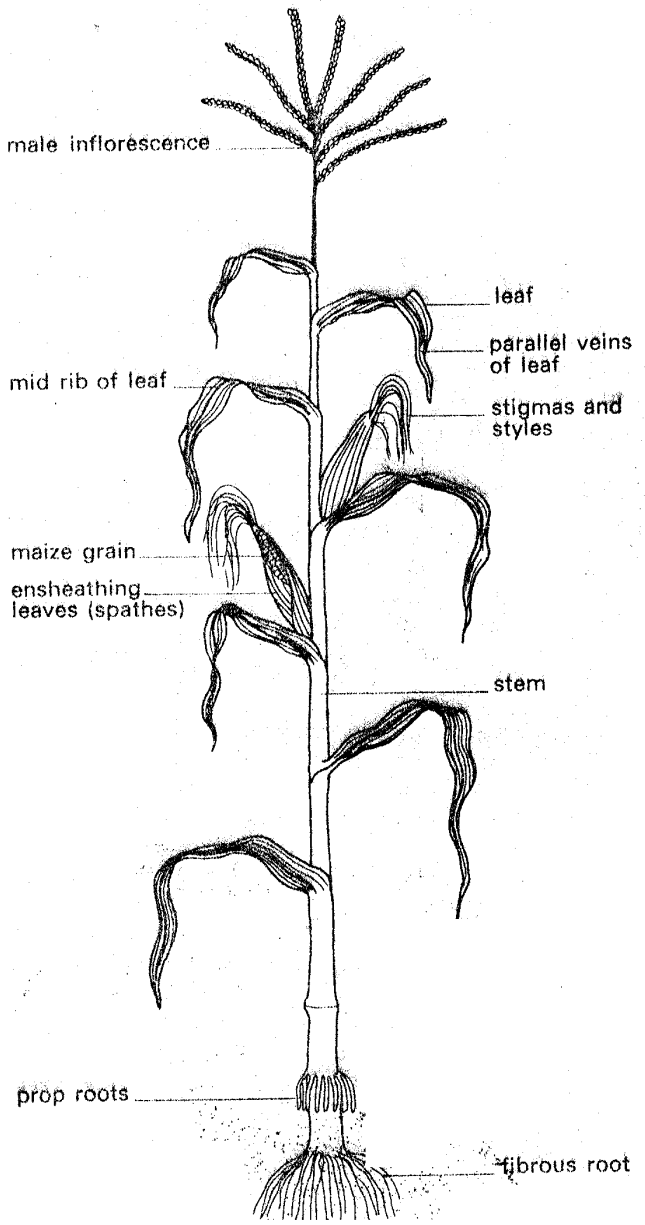


Fig. 9.5: Maize plant (x 1/6)

3.3.3 The Water - leaf Plant

As you have seen in unit 2 of this module (fig. 7.2b), the water leaf plant is an example of a dicotyledonous plant. You can get this plant easily in the garden around the house. The plant grows to develop stems, branches leaves, roots even flowers. To study this plant, carefully uproot it by first wetting the soil so that the root does not break as you pull it out.

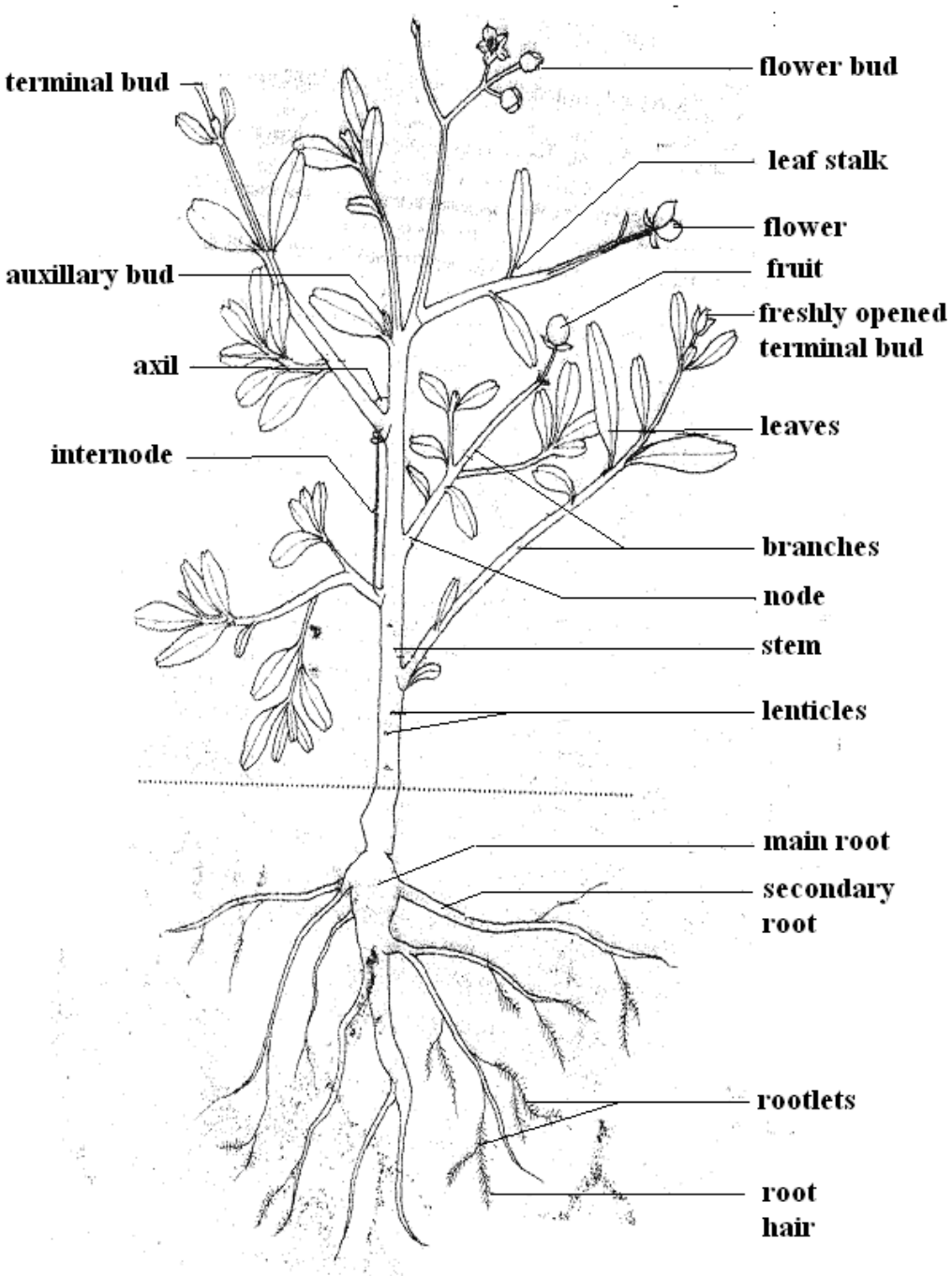


Fig. 9.6 Water Leaf Plant

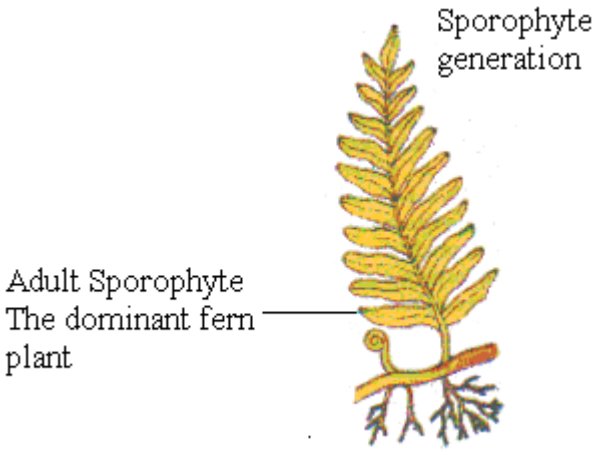


Fig. 9.7 Young tree fern from Obudu Plateau. Nigeria showing aerial stem

Note the following:-

- The tap root with root cap
- Secondary root, rootless and root hairs
- The epidermis, nodes, internodes, auxillary and terminal buds and lenticels.
- The thick fleshy nature of leaves and the reticulate venation.
- The upper and lower epidermis.
- The presence or absence of small hairs.
- The petiole, veins, apex, base and margins of leaf

Practice drawing and labeling the external features of the water leaf plant fully. Look at the diagrams in figs. 9.7 and figs. 9.8 will help you

4.0 CONCLUSION

You have done very well. You have visited the pond, stream and even the botanical garden. You have studied the external features of a variety of plants, ranging from small unicellular and cellular forms to multi cellular flowering plants. You need to note how these plants gradually became more and more complex in their external structures. You also need to remember how the earlier ones were very dependent on water and the later, the angiosperms, can completely live on land.

5.0 SUMMARY

What you have learnt in this unit concerns external structures only. Biologists also study internal structures and small parts of plants. Sometimes small plants especially microscopic ones have to be cultured during biological studies. For this purpose culture media are used. In unit 12 we will study how to make culture media. In unit 10 we will learn to prepare slides to study microscopic plants and plant parts.

6.0 TUTOR MARKED ASSIGNMENT

(a) Draw and label fully the external features of the maize and the water-leaf plants.
(b) In tabular form state the similarities and differences between the maize and the water-leaf plant.

Remember to submit your assignment before or on the due date.

7.0 REFERENCES/FURTHER READINGS

Berrill, NJ (1979) Biology in action London, Heinemann Educational Books

Soper, R. (Ed) (1998) Biological Sciences Cambridge U.K Cambridge University Press.

Stone, R.H Cozens, A.B, Emia, F.1 (1972) New Biology for London Schools.

UNIT 5 PREPARATION OF TEMPORARY SLIDES

CONTENTS

1.0 INTRODUCTION

You have learnt in Unit 1 of this course that to observe cells clearly under a microscope the cells need to be spread in a single layer. This single layer of cells is then fixed and stained and observed as a temporary preparation or a permanent slide is made which can be stored and observed later.

In this practical unit you will learn to make temporary slide preparations of cell suspension using the smear technique and of soft tissue using the squash technique. You will also learn to make temporary as well as permanent slides of whole mounts of protozoans and phytoplankton

2.0 OBJECTIVES

After completing this practical exercise you should be able to prepare a

- temporary slide of a given cell suspension using smear techique,
- temporary slide of soft tissue using squash technique,
- whole mounts of *Paramecium, Chlamydomonas, Volvox.*

3.0 MAIN CONTENT

3.1 Preparation of temporary slides

3.2 General method of making a temporary preparation

A temporary preparation of cells is usually observed as a wet mount. This is prepared by placing a cell suspension in liquid on t he slide or if the material to be studied is dry, by placing it directly on the slide and adding water, glycerin or stain to it. The material is then covered by a cover slip. You have to be careful not to trap air bubbles while lowering the cover slip over the material at it will cause interference in observing the material. The procedure is shown in fig. 10.1

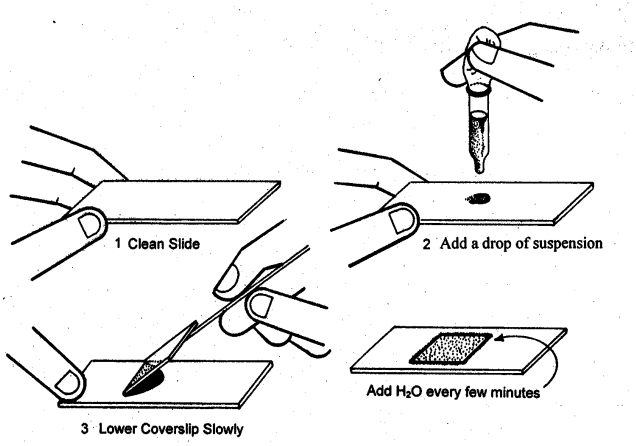


Fig 10.1 The technique for lowering the cover slip over the slide

3.2.1 Smear technique for cheek scrapings

In this exercise you will learn to make a temporary mount of cheek epithelial cells by following the given steps.

Materials Required

1. Cover slips, slides, slide labels
2. Disposable spatula or tooth pick
3. 9% NaCl
4. Methylene blue stain
5. Filter paper

Procedure

1. Rinse your mouth well with water.
2. Gently scrape the inside of your cheek with the broad end of a clean tooth prick or a sterilized / disposable spatula.
3. Spread the cells on a clean slide. Add a drop of 0.9% NaCl or physiological saline and a drop of methylene blue.

4. Cover with a cover slip and gently press it to flatten the cells. Alternatively you can introduce the stain by irrigation method (see fig. 10.2).
5. Put the slide under high power in a microscope. Make sure that your course tutor sees the slide. He/She would wish to assess its quality to award marks for it.

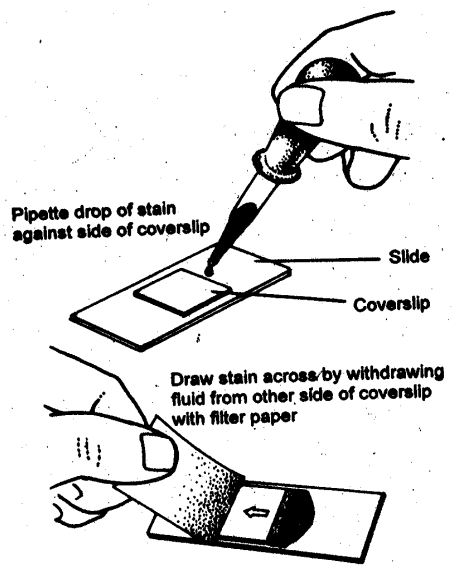


Fig. 10.2 Technique of Irrigation.

Observations

Locate a single cell under high power. Many of the cells will be crumpled and irregular in outline because the cell membrane is extremely thin and delicate. The nucleus will be stained dark blue in the centre of each cell.

If you don't let in too much light through the microscope you will be able to observe the cell structures better. Compare what you see with fig. 10.3. In epithelial cells obtained from females a distinct darkly staining body attached to the nuclear membrane can be seen. This is known as Barr body.

CAUTION: Certain infectious diseases can be transmitted through saliva. Avoid any contact with another person's saliva. Do not share a spatula with anyone else.

3.2.2 Squash Technique for Onion Root Tip

Squash technique is a simple method widely used for the study of chromosomes. This technique consists of applying gentle pressure on a small piece of stained tissue to spread the chromosomes in the cells. This technique is used to study dividing cells showing either mitosis or

meiosis and suitable tissues for this are onion root tip, grasshopper testis or another buds.

In this exercise you will learn to prepare a temporary slide of onion root tip showing mitosis, which can later be made in a permanent slide.

The onion root should remain in the acetic alcohol solutions for 12 - 24 hours. After fixation the tissue can be transferred to 70% alcohol.

Materials Required

1. Onion root tips
2. 70% alcohol
3. Forceps and dissecting needles
4. Pitette, glass dropper watch glass beaker
5. 2N Hydrochloric acid
6. Acetocarmine/aceto-orcein stain
7. Slides, coverslips,
8. Acetic acid
9. Filter paper
10. Nail polish

Procedure

1. Transfer the roots from the fixative or storage solution (70%alcohol) in a watch glass.
2. Wash in water until the roots sink.
3. Drain off the water using a pipette and add a few drops of 2N HCl and leave for 10 minutes at room temperature or for a minute over a spirit lamp. While hydrolyzing over the flame be careful not to overheat by moving the watch glass. Alternately you can warm the watch glass over a moving the beaker of boiling water till the liquid steams.
4. After hydrolysis drain off the HCl and wash root tips in water.
5. Remove water and add 1 % acctocarmine or aceto - orcein to the root tips for staining. The staining requires 10 - 15 minutes.
6. Transfer 2 - 3 root tips on a slide, cut above 2 mm from the pointed end. Discard the rest.
7. Place a drop of 45% acetic acid on the root tip and carefully place a cover slip. Remove excess acetic acid by the edge of a filter paper.
8. Place the slide between 2 layers of filter paper and gently tap the cover slip by the back of a pencil to get an even spread of chromosomes.
9. If air bubbles get trapped in the cover slip add a few drops of glycerol or acetic acid to the edge of the cover slip.

10. Seal the edges of the cover slip by applying nail polish so that the fluid evaporation is minimum and observe the slide under a microscope.
11. Label the slide appropriately and show to your counsellor.

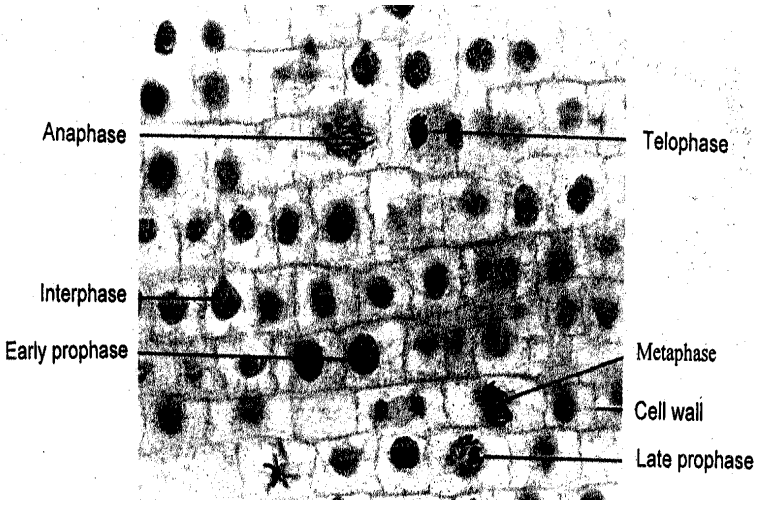


Fig. 10.4 Stages of mitosis from an onion (Allium) root tip

Observations

In a squash preparation of the onion root tip you will find that a majority of cells will be in inter phase (non - dividing cells). The inter phase nucleus will appear as a darkly stained body with acetomarmine or acetoorcein. The nuclear membrane is intact and the chromosomes form a fine network. You will have to look for dividing cells if you use acetoorcein as only the nucleus and chromosomes will be stained. Acetocarmine will stain the whole cell, use a textbook or the illustration of fig. 10.4 to identify different phases of division. You could also consult your course tutor more information.

3.3 Whole mounts of unicellular organisms

A unicellular organism has to carry out within one cell all the functions that are in a multi-cellular organism are performed by different cells and tissues. Accordingly it possesses a high degree of internal organization, which can be observed by making a whole mount of the organism. In this exercise you will learn to make whole mounts of three protistans. *Paramecium* an animal like protistan, chlamydomonas an autotropic protistan and, *Volvox* an example of a colonial protistan.

Material Required

l. *Paramecium* culture, *Volvox, Chlamydomonas*
2. Methyl cellulose
3. Methyl green in ethanoic acid/acetocarmine

4. Acetic acid
5. Albumin glycerin
6. Petridishes, Beakers, Slides, Coverslips, Slide labels
7. Alcohol series for dehydration
8. Xylene
9. DPX mountant
10. Noland's solution
11. Iodine solution

Procedure

A Temporary whole mount of *Paramecium*

Put a drop of *Paramecium* culture on a clean slide and cover with a cover slip. Since paramecia move very rapidly their movement can be slowed down by adding an equal amount of methyl cellulose to a drop of the culture. Irrigate your slide with either 1% methyl green in ethanoic acid or acetocarmine. Both fix the organism and stain the nuclei green in case of the first and red in the case of the second. Then observe under high power in the microscope. Label and show the slide to your counselor.

B Preparation of Permanent Mount

It is quite possible that some of you may be expected to make a permanent preparation of some protozoan like *Paramecium*. It is a fairly simple procedure and good results can be obtained if done carefully. The procedure is given below:

1. Take a clean dry slide, put a small drop of albumin glycerin in the centre of the slide and with the tip of your forefinger spread it in the form of a thin film.
2. With a glass dropper add 1-2 drops of *Paramecium* culture on the slide and observe under low power of the microscope. You will see a large number of paramecia moving rapidly in the culture medium.
3. Let the culture become dry you can put the slide under an electric lamp.
4. Open a bottle of acetic acid and quickly pass the slide right side down over the mouth of the bottle. This will fix the protozoan.
5. Keep this slide in a petridish of 6" diameter with the right side up. Add a few drops of the stain acetocarmine so as to fully cover the culture.
6. Stain the slide for about 5 - 7 minutes.
7. Drain off the excess stain by a blotting paper.

8. Wash the slide with 30%, 50%, 70%, 90% alcohol in ascending order. Always keep the petridish covered by another petridish. This will gradually remove the water from the culture.
9. Now wash the slide twice with absolute alcohol. This completes the dehydration process. Be careful that the petridish is covered tightly so that no atmosphere moisture gets in.
10. Remove the alcohol and add a few drops of xylene on the slide. This will clear the protozoan so that it is visible more clearly under the microscope.

    Any turbidity with the addition of xylene shows that the dehydration process is not complete. Repeat the step with absolute alcohol and then xylene again.

11. Put a drop of DPX mountant over the culture, and lower a cover slip carefully over it so as to trap no air bubbles.
12. Label the slide and keep the slide overnight in an incubator. Your permanent mount is ready.

C Whole mount of *Chlamydomonas* and *Volvox*

Chlamydomonas and *Volvox* are phytoflagellates that are found in freshwater ponds and ditches. Sometimes they are so abundant as to produce a green scum on ponds. *Chlamydomonas* are single-celled green flagellates while *Volvox* is an example of colonial phytoflagellate that forms a hollow spheroid with several thousand cells embedded in its mucilaginous wall. You would be supplied with specimens of these phytoplagellates collected from ponds and ditches or cultured in the laboratory. The method of preparing a temporary mount is the same for both species.

1. Place a drop of culture or a drop of water containing the flagellates on a clean slide and add a drop of 10 per cent methyl cellulose which will restrict the movement of the organism and allow you to study the organism.

2. Place a cover slip on the drop and observe under a microscope. By cutting down the light you will be able to observe the flagellar movement better.

3. You could stain the slide by the irrigation method using acetiocarmine to see the nucleus, Noland's solution to see the flagella and iodine solution to see the starch grains.

4. Show your slides to your course tutor.

Observation

Under high power of the microscope try and observe as much of the internal organs. In *Paramecium* you will find it difficult to see the micro nucleus as it is generally covered by the macro nucleus. You may be able to see the contractile vacuole discharging before you stain and fix the mount. Use the help of the given diagra (fig. 10.5) to identify the structures in the *Paramecium*.

Under high power you will observe that *Chlamydomonas* has two flagella, cellulos cell wall, two small contractile vacuoles, cytoplasm and cup shaped chloroplast, pigment spot and pyrenoid. You can compare your slide with fig. 10.6

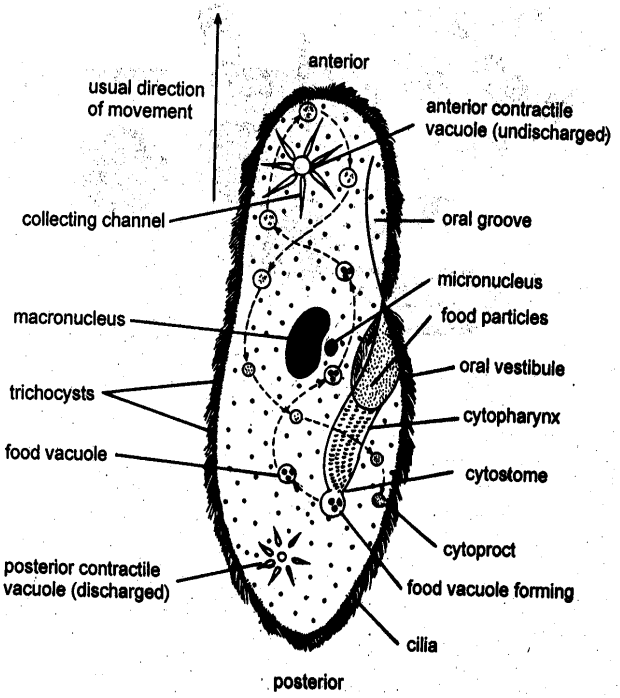


Fig. 10.5 *Paramecium*. By adjusting the light in the microscope all the structure can be seen

In colonial flagellate *Volvox*, notice that the flagellar beat of the individual cells is coordinated with each other so that the colony moves in an oriented fashion. Compare your slide with fig 10.7. You may also observe daughter colonies inside individual colonies.

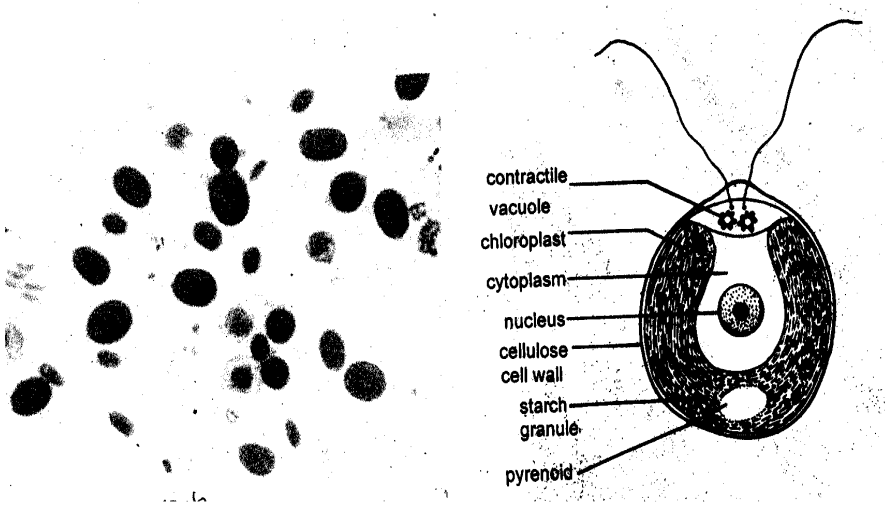


Fig. 10.6 *Chlamydomonas*, a common green unicellular algae

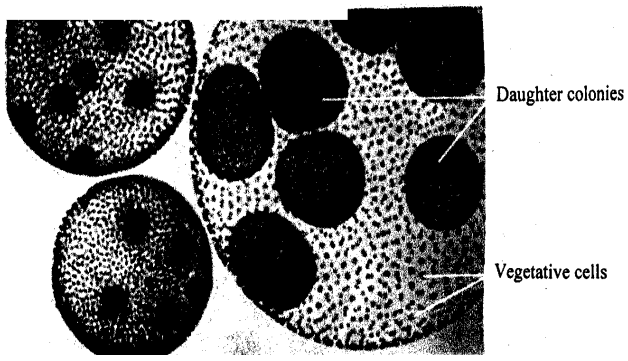


Fig. 10.7 *Volvox* with daughter coloniew. Each cell of the colony looks very much like the single cell of *Chlamydomonas*.

SELF ASSESSMENT EXERCISE

l. Name the methods for making temporary slides.
2. Suppose you work in a hospital laboratory and have to study a blood sample for the cause of fever, which of the methods would you use to prepare the slide for this sample?
3. What precaution will you take.
4. You are correct to say that the possible methods are:

    1. General method
    2. Smear technique
    3. Squash technique
    4. whole mounts technique
    5. You will be correct if you chose to use the smear technique to make a temporary slide for identifying the cause of a fever. You must take precautions to ensure that you do not come physically in touch with the sample

especially if you have any open wound. Such a wound should not come in contact with any such sample. It could be a source of infection.

4.0 CONCLUSION

In this unit you have learnt how to prepare slides of samples that will be studied under the microscope. You have seen that the method you choose will depend on the type of sample and the purpose of study. However, the general reason for making slides is to make the specimen more visible or render the details more clearly.

5.0 SUMMARY

Sometimes, when slides of the sample is produced, they are still not very visible unless they are rendered so by adding colour to create a contrast. This process is called staining in the biology practical class. In the next unit you will learn how to prepare stains and reagents for practical work in biology.

6.0 TUTOR MARKED ASSIGNMENT

How would you prepare a slide for the study of the various stages in the process of mitosis. What particular sample will you choose to use and why?

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biology in Action London, Heinemann Educational Books Ltd.

Soper, R. (ED) (1998) Biological Sciences Cambridge U.K Cambridge University Press

Stone, R.N.Cozens, A.B, Emia, F.1 (1972) New Biology for Tropical Schools London, Longman Group Ltd.

MODULE 3

UNIT 1 PREPARATION OF REAGENTS AND STAINS

CONTENTS

1.0 INTRODUCTION

Biological structures are usually transparent. Staining is a means of obtaining contrast between the different structures.

Table 9.1 shows some common stains used in light microscopy.

In unit 5 of module 2, you will remember we used some stains for preparing temporary slides. In this unit however we will expect you to prepare and remember the method of preparations of a few of the

important reagents and stains that are commonly used in the biology laboratories.

2.0 OBJECTIVES

After this laboratory experiment you will be able to prepare:

- Normal physiological saline for vertebrates Frog Ringer's Solution
- Alcohol series or grades of the following strength - 30% - 50% - 70% - 90%
- The plant fixative - Formalin Acetic Acid and the animal fixative - Aqueous Bouin's Fluid.
- Stains - Aceto-orcein, Aceto-carmine, Noland's solution, Methylene blue
- Benedict's solution
- Millions solution

3.0 MAIN CONTENT

3.1 Preparation of reagents and stains

3.2 General rules for preparing and handling solution

1. All solutions should be kept in clearly and correctly labeled containers.
2. When stock solutions are provided nothing should be poured back to stock bottle. If you take too much solution it is better to discard the excess rather than risk contamination of the entire supply by attempting to conserve a small amount.
3. Distilled water should be used in preparing solutions unless otherwise specified.
4. When diluting acids always add the acid to the diluent, never the diluent to the acid. The acid should be added slowly.
5. Use clean containers for preparing solutions
6. Strong acids and bases must be handled with care.
7. Avoid inhalation of acid and other fumes, particularly osmic acid or mercuri chloride powder. The laboratory should be well ventilated and volatile fluids such as xylol, tuluol, dioxane, chloroform, ether, etc. should be kept covered as much of the time as possible.
8. Flammable solutions should be kept away from open flames and sparks. Be particularly careful with ether and with ether-alcoho-celloidin mixtures.

9. Always wash your hands before smoking or eating after working in the laboratory.
10. Many fixatives and other solutions are poisonous.

3.3 Accuracy Required in Preparing Solutions

It should be noted that formulas have been presented with what may appear to be an inconsistent degree of accuracy. Thus, 0.5 ml of acid may be combined with 100 ml (not 100.0 or 99.5) of alcohol. This has been done intentionally, since it indicates the degree of accuracy required in the measurements. The standard practice is to write formulas to the degree of accuracy of the smallest component. Thus, 0.5 ml are combined with 100.00 ml in one formula, 1 ml combined with 100 ml in a second formula; and 100.00 ml combined with 0.24 gram in a third. Although this may appear more consistent in print, it is not realistic. If a volume is given as 100.00 ml it should indicate that the measurement is to be made in a volumetric flask. For most technique purposes (such as preparing fixatives and staining solutions) ordinary graduated cylinder provide the necessary degree of accuracy. Volumes of 1 ml and less should be measured in graduated pipettes. It is well to use a small graduated cylinder for small quantities (thus use a graduated cylinder o f10 ml, rather than a 1000 ml for measurements from 1 to 10 ml, and so on). Most weights have been given to tenths of a gram and the balance used should be accurate to one hundredth of a gram. A triple beam balance reading to one-hundredths of a gram is ideal for the technique laboratory. Unless milligrams are specified, an analytical balance is not required for routine preparations in microscope technique.

3.4 Use of chemicals

3.4.1 Acids

The acids most commonly employed in technique procedures are acetic and nitric acids in fixatives, and hydrochloric acid in decalcifying and destaining solutions. When percentages are indicated they are based upon dilutions of glacial acetic acid (99 percent); concentrated nitric acid (about 70 percent), and concentrated hydrochloric acid (about 39 percent). Thus, 1 percent hydrochloric acid for destaining refers to a solution prepared by adding 1 cc of concentrated hydrochloric acid to 99 ml of alcohol or water as specified. The exact percentages of the concentrated acids vary slightly with different suppliers and grades and this must be taken into account when a “normal” solution is specified.

3.4.2 Alcohol

References to "alcohol" in technique procedures mean ethyl alcohol unless otherwise specified. Isopropyl alcohol may be substituted for ethyl alcohol in the dehydration and hydration series. Methyl alcohol is preferred for the fixation of smears before staining in Giemsa's

WARNING: METHYL ALCOHOL IS HIGHLY POISONOUS ON DRINKING.

3.5 Preparation of normal physiological saline for vertebrates

Physiological saline is used for rinsing blood and debris from tissues before placing them in the fixative. Physiological saline for invertebrates and cold blooded vertebrates is different from that of warm blooded vertebrates because of the proportion of sodium chloride. The physiological saline for various groups within the warm blooded animal vertebrates also vary. In this exercise you will learn to prepare the physiological normal saline for mammals by following the given steps.

Materials Required

Analytical sodium chloride (NaCI) - 0.9 g;
Distilled water - 100 ml
Graduated measuring cylinder of 100 ml
Round flask of more than 100 ml capacity
Analytical balance.
Distilled water – 100ml
Graduated measuring cylinder of 100 ml
Round flask of more than 100 ml capacity
Analytical balance

Procedure

1. Weigh 0.9gms of analytical sodium chloride and put it in the round flask.
2. Measure 100 ml of distilled water in the graduated cylinder and pour it into the round flask gradually, shaking the flask in order to dissolve and mix the NaCl in the water.

3.6 Preparation of Frog Ringer's Solution

Ringer's solution is used for moistening tissues which must be kept for some period of time before fixation or for small animals or tissue study which need to be observed in the living state. The Ringer solution for

invertebrate and cold blooded vertebrate is different from that of the warm blooded vertebrates. In this exercise you will learn to prepare the Ringer's solution for frog by following the given steps.

Material Required

Analytical sodium chloride (NaCI) - 0.65 gms;
Potassium chloride (KC1) - 0.025 gms
Calcium chloride ($CaCl_2$) -0.03 g
Sodium hydrogen carbonate (NaHC03) - 0.2 g
Distilled water (a little more than 100 ml),
Analytical balance
100 ml graduated cylinder
Round flask with more than 100 ml capacity

Procedure

1. Weigh each of the following salts separately: 0.65 g of Nacl; 0.025 g of KCI, 0.03 g of $CaC1_2$ and 0.02 g of sodium hydrogen carbonate and put into the round flask.
2. Measure 100 ml of distilled water in the graduated cylinder.
3. Pour the 100 ml of distilled water into the flask containing the weighed salts and gradually dissolve the salts.
4. While pouring the distilled water into the flask, keep shaking the flask in order to mix and dissolve the salts in the water.

3.7 Preparation of alcohol series or grades 30%, 50%, 70%, 90%

Alcohol is used in various dilutions - 30%-50%-70%-80%-90%-100% (absolute alcohol) for dehydration of tissues and in the reverse (100%-90%-80%-70%-50%-30%) for rehydration of tissues. It is also used as a simple fixative primarily before certain histochemical tests.

Dilutions of alcohols or alcohol grades are usually prepared from rectified spirit which contains approximately 95% or 96 percent of alcohol and never from the much more expensive absolute alcohol. A simple method of calculating the dilution is to take the number of cubic centimeters of 95 percent alcohol as the percentage required (thus use 70 ml for 70 percent) and add enough distilled water to bring the final volume to 95 ml. For 50 percent alcohol use 50ml of 95 percent alcohol and dilute to prepare 95ml of 50 percent alcohol. Larger or smaller volumes are prepared on a proportional basis. For example: 700 ml of 95 percent will provide 950 ml of 70 percent; 35 ml of 95 percent will provide 47.5 ml of 70 percent. For technique purposes the latter dilution may be 47 or 48 ml since the exact percentage in the graded series of

solutions for hydration and dehydration is not critical. In this exercise you will learn to prepare the alcohol grades of 30%, 50%, 70%, 80%, 90% by following the given steps.

Materials Required

96% Alcohol (Ethanol or Methanol)
Distilled water
Graduated measuring cylinder of 100 ml,
5- 100 ml bottles with stoppers

Procedure

1. Take a graduated cylinder of 100 ml for preparing the alcohol dilution series.

(a) 90% Alcohol

(i) Pour 96% of alcohol into the measuring cylinder, upto 90 ml measure mark.

(ii) Add distilled water to the cylinder containing the measured amount of alcohol to make the volume up to 96 ml. Keep the 90% alcohol in a tightly stoppered bottle.

(b) 70% Alcohol

(i) Pour 96% of alcohol into the measuring cylinder, upto 70 ml measure mark.

(ii) Add distilled water into the cylinder containing the measured amount of alcohol to make the volume upto 96 ml. Keep the 70% alcohol in a tightly stoppered bottle.

(c) 50% of Alcohol

(i) Pour 96% of alcohol into the measuring cylinder upto 50 ml measure mark

(ii) Add distilled water into the cylinder containing the measured amount of alcohol to make the volume up to 96 ml. Keep the 50% alcohol in a tightly stoppered bottle.

(d) 30% Alcohol

(i) Pour 96% of alcohol into the measuring cylinder upto 30 ml measure mark.

(ii) Add distilled water into the cylinder containing the measured amount of alcohol to make the volume upto 96m1. Keep the 30% alcohol in a tightly stoppered bottle.

3.8 Preparation of Fixatives

The collected plant material or the animal material is stored in glass-stopped wide mouth jars containing any general fixative. The purpose of the fixative is to kill the material initially and to preserve the cells and their contents in natural condition as far as possible. Although there are several fixatives in use the best is that which changes the chemistry of the cell to the minimum and which preserves the cells best. In this exercise you will learn to prepare

(a) Plant fixative - FAA and
(b) Animal fixative - Bouins fluid

Materials Required

70% ethyl alcohol
Glacial acetic acid
40% formalin
Picric acid (saturated aqueous solution)
Graduated measuring cylinders of 50 ml, 100 ml
Round flask of 150 ml capacity
Bottles with stoppers

Procedure

(a) Plant Fixative - Formalin (Formol) Acetic Alcohol (FAA or AFA). It is a general fixative for plant material and nemtodes.

1. Measure 85 ml of 70% alcohol in the graduated, measuring cylinder and pour into the round flask.
2. Also measure 5.0 ml glacial acetic acid in the graduated measuring cylinder and add to alcohol present in the round flask.
3. Again measure 10 ml of 40% formalin in the graduated measuring cylinder and add to the round flask containing the measured amounts of alcohol and acetic acid.
4. Shake the round flask containing all the measured chemicals in order to mix the chemicals.
5. Pour the solution with all the ingredients in a bottle and stopper it.

WARNING: THE VARIOUS INGREDIENTS OF SOLUTIONS SHOULD BE COMBINED ONLY JUST BEFORE USE.

(b) Animal fixative – Aqueous Bouin's Fluid

1. Measure 75ml of saturated aqueous solution of picric acid in the graduated measuring cylinder or pour into a round flask.

2. Also measure 40% of 25ml formalin in the graduated measuring cylinder and add to the round flask containing the saturated aqueous solution of picric acid.
3. Also measure 5ml of glacial acetic acid in the measuring cylinder and add to the round flask containing the picric acid and formalin solution.
4. Mix all the ingredients by shaking the flask gently.
5. Pour the mixture solution into a bottle and stopper. This solution will keep indefinitely.

WARNING: PICRIC ACID MAY DRY ON THE CORK AND BOTTLE RIM AND BECOME UNSTABLE DURING STORAGE: WHEN REPLENISHING THE WATER IN THE BOTTLE ENSURE THAT NO SOLUTION REMAINS IN THESE AREAS.

3.9 Preparation Of Stains

Staining techniques are most difficult for the beginner to master because they depend both on method of preparation of stain and the fact that staining effects vary with different tissues. Staining also gets affected by the use of different fixatives, different period of storage and on different types of dyes used.

In this exercise you will learn to prepare some stains. The stains which you will learn to prepare are:

(a) Aceto-orcein
(b) Aceto-carmine
(c) Noland's solution
(d) 1% Aqueous Methylene blue.

Materials Required

Orcein stain - 0 5 - 1.5 g
Glacial acetic acid - 90-100 ml
Dry Carmine stain - 0.5 - 1.0 g Methylene blue - 1.0 gm
Genetian violet (crystal violet) - 0.20 g
Phenol crystals - 60 g
40% Formalin - 20 ml
Glycerol (glycerine) - 4 ml
Distilled water - 250 ml
Round flask of 100 ml capacity
Round Corning Flask of more than 100 ml capacity
Graduated measuring cylinder of 100 ml measure capacity
Analytical balances
Burner

Filter paper
4 Stain bottles.

Procedure

a) Aceto-Carmine: This is one of the most commonly used stains for chromosomal studies.

1. Weigh 0.5 g of dry carmine stain
2. Measure 45 ml of glacial acetic acid in a graduated measuring cylinder and pour into a round, corning flask.
3. Measure 55 ml of distilled water in the measuring cylinder and also add the round corning flask and mix the two.
4. Heat to boiling the corning flask containing distilled water and glacial acetic acid.
5. Add the weighed amount of 0.5 g of carmine stain to the boiling mixture and shake well to mix.
6. Cool the mixture and filter.

(b) Aceto-Orcein

1. Weigh 1.0 g of orcein stain and put in a round, corning flask.
2. Measure 45 ml of glacial acetic acid in the graduated measuring cylinder and pour it into the round flask containing the weighed stain powder.
3. Stopper the round flask with cotton wool and heat the mixture to boiling.
4. Cool the mixture in the round flask and add distilled water to it.
5. Filter the prepared stain before use.

(c) Aqueous Methylene Blue

1. Weigh 1 g of methylene blue powder and put into a round flask of 100 ml capacity.
2. Measure 100 ml of distilled water in a measuring cylinder and add gradually to the flask containing the methylene blue stain.
3. Shake the flask gently while adding water, in order to dissolve the methylene blue stain
4. Filter and pour the mixture into bottle for use.

(d) Noland's Solution

1. First prepare saturated solution of phenol in distilled water. Weigh 6 gm phenol crystals and put into a round flask. Measure 90 ml of distilled water and add to the flask. Dissolve the crystal

in the water by shaking the flask. Pour into a container which is well closed and protected from light.

2. Weigh 0.2 g of genetian violet and put into a clean round flask.
3. Measure 20 ml of 40% formalin in a measuring cylinder of 100 ml capacity and add to the round flask containing the genetian violet.
4. Also measure 4 ml glycerol in a measuring cylinder and add to the flask.
5. Also measure 80 ml of saturated solution of phenol that has been prepared earlier and add into the flask.
6. Shake the flask gently to dissolve the ingredients.

WARNING: DO NOT HANDLE PHENOL CRYSTALS WITH BARE HANDS. DEATH HAS RESULTED FROM AS LITTLE AS 1.5 GRAM ABSORPTION. NEVER APPLY TO LARGE PORTIONS OF BODY SURFACE.

SELF ASSESSMENT EXERCISE

1. Name seven stains and reagents commonly used in the biology laboratory and state their uses.
2. Suppose you need to study the frog as a living thing, state what reagent would you use.
3. Why are stains necessary in biology laboratories?

4.0 CONCLUSION

In this unit, you have learnt how to prepare different solutions, and the precautions you must take. You have also learnt the general rules for handling solutions.

5.0 SUMMARY

Apart from such preparations for studies, it is sometimes necessary to grow organisms under specified conditions in the laboratory. Such cultures enhance certain type of studies. In the next unit, you will learn the techniques of making cultures as well.

6.0 TUTOR MARKED ASSIGNMENT

1. What general rules will you give to anyone preparing and handling solutions in the biology laboratory?
2. How will you prepare the Benedicts solution?

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biology in action London, Heinemann Educational Books Ltd.

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UNIT 2 TECHNIQUES FOR MICROBIAL CULTURE AND GRAM'S STAINING

CONTENTS

1.0 INTRODUCTION

Cultivation of micro-organism involves the provision of the correct nutrients and physical conditions in the laboratory so as to enable the organism to grow in an environment similar to their natural one. In the theory part of this course we have discussed the nutritional requirements of the micro-organisms in general. In this practical you will practise growing micro-organisms in the laboratory.

On the basis of their response to Gram's stain, bacteria can be categorized as Gram positive or Gram negative bacteria. You will use Gram's staining technique to identify the type of bacteria. Wear gloves while carrying out these experiments.

2.0 OBJECTIVES

After going through this practical session you should be able to:

- prepare the medium required for culturing the microbes,
- sterilize the medium and glassware in order to minimize the contamination,
- describe the ways of streaking the inoculum to get the isolated colonies, list the stains, used in Gram's staining method, and
- prepare and stain the bacterial smear for categorization as Gram positive or Gram negative bacteria.

3.0 MAIN CONTENT

3.1 Microbial cultures and gram's staining

3.2 Fungal culture

Most of the fungi can be grown on various kinds of media that are also used for culturing the bacteria. Culture media can be of two types: synthetic and nonsynthetic. Synthetic media i.e. Defined media are those whose constituents and the chemical composition of the constituents are known. Czapek's Dox medium, Richard's solution, Sabouroid agar, Starch Casein agar are some of the synthetic media that are used for growing fungi in the lab. Non synthetic media i.e. Complex media consist of complex natural products whose exact composition is not known. These media are of plant or animal origin and include extracts of fruits, vegetables, milk, egg, blood, meat, yeast, malt etc. and some known chemical substances. Since these media are of complex nature, they can support large varieties of microbes especially fungi and are useful for routine laboratory cultures. In this practical we will use non-synthetic solid medium made from potatoes for fungal culture. You can always use the specific medium requirements for the specific micro-organisms whenever you need to cultivate them in your lab.

Materials Required

500 ml Beaker
500 ml Erlenmyer flask
Petriplates
Test tubes
Metal loop
PH meter/Ph paper
HCl 1 Normal
NaOH 1 Normal
Electronic Balance
Media components

Pealed potatoes	-	50 gms
Dextrose	-	4 gms
Agar	-	3.75 gms

Distilled water

Procedure

1. Cut the pealed potatoes into small pieces, boil in 100 ml distilled water in the beaker to make a thick paste.
2. Add some water (about 50 ml) to the paste and filter through muslin cloth. Put the filterate in 500 ml flask.
3. Weigh required amount (4 gms) of destrose and add to the filterate.

4. Add distilled water to make the volume upto 250 ml.
5. Adjust the pH of the medium to 6 - 6.2 (Check the pH of the medium be using pH meter or pH paper. If need arises adjust the pH by using 0.1N HCl or IN NaOH as the requirement may be.
6. Weigh required amount of agar (3.75 gms - 1.5%) and add it to the medium.
7. Cover the flask with cap or cotton plug, wrap the foil around the cap and autoclave the medium for 15 min at 15 lb pressure.
8. When the pressure of the autoclave drops to zero, sterilization is complete. Wear the gloves, take out the medium and place in the water bath set at 50oC. This temperature will enable the medium to cool but prevent it from setting.(you can also keep the medium outside and let it cool till you can hold it in your hands. Medium kept in the flask beyond this time will solidify)
9. Pour 30 ml of medium in each petriplate. For this, first pour the medium from the flask into a graduated test tube and then from the tube into the petriplate (fig. 12.1).
10. Let the petriplates be cooled and medium be set.

11. Take into the loop the spores from the fungal stock culture and streak on the medium surface as shown in fig 12.2. You can streak several plates from the same stock culture. Streaking is done to gradually dilute the concentration of the inoculum spore so that isolated colonies growing from a single spore can be obtained. You can do the streaking in the following way:

    (a) As shown in fig. 12.2 (a) and (b), take and spread a loopful of culture over area A without touching the sides of the plate. (Before this inoculation process, ensure that the agar surface is completely set).

    (b) Area A will contain the highest concentration of bacteria and is referred to as the pool. As you can see the directions in the fig 12.2(b) streak the culture over area B,C,D and E subsequently one after the other. Before each step sterilize the loop over a hot flame and cool it prior to use. This streaking method effectively dilutes the concentration of spores in a stepwise manner and it becomes possible to produce isolated colonies. You can streak in different patterns as shown in fig. 12.3. You must streak by moving the loop in the forward direction only. Never make backward movements with the loop otherwise you will not be able to get isolated colonies, as mixing of spores will occur.

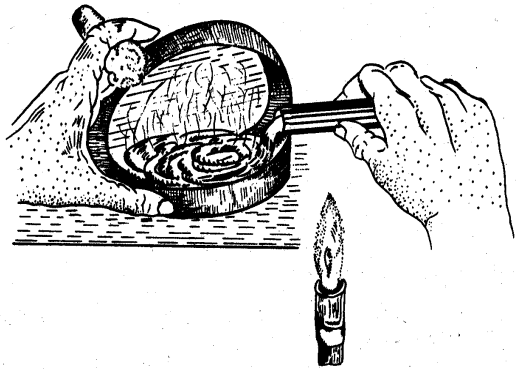

Fig. 12.1 Pouring medium into petriplates.

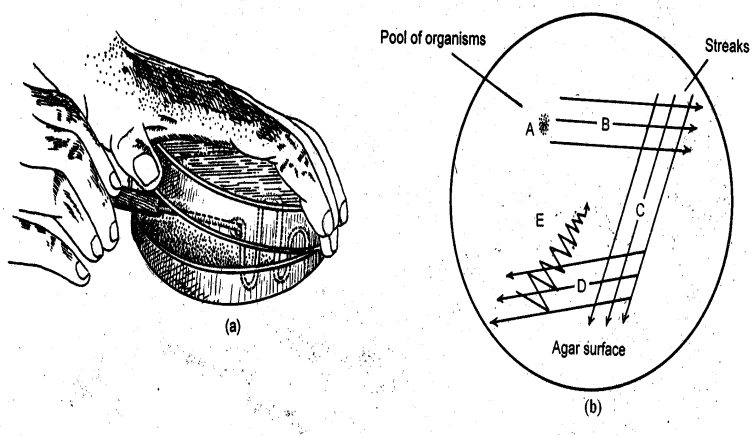

Fig. 12.2 Streaking method for culturing microbes on the solid medium: (a) use of metal loop for streaking the inculum (fungi spores). (b) streaking directions on agar plate.

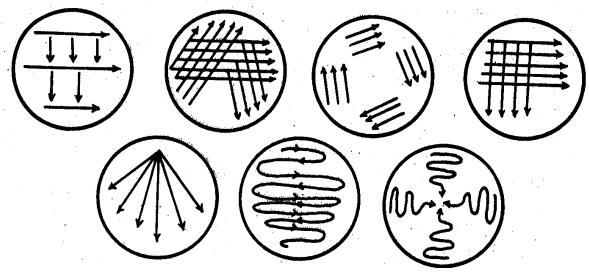

Fig. 12.3 Different ways of streaking on solid medium

12. Keep the plates in an inverted position in the incubator at 37oC for 3 to 4 days.
13. Observe the plates after 48 hours and see the separated colonies growing. These are isolated colonies and each of them has grown

from a single spore. You can reculture these isolated colonies on other solid medium plates and store as stock culture.

Precautions

1. All the glassware and the loop must be thoroughly washed, dried and sterilized before use.
2. The medium should be poured and culture should be streaked on the laminar flow bench. You have already read about how to operate the laminar flow in the earlier practicals. In case you don't have the laminar flow bench in your lab, perform these activities on a normal bench. The bench should be first cleaned by spirit or any other disinfectant. Light the spirit lamp and keep it on the bench before you start your work. (Preferably use two spirit lamps, one on each side on the bench). A spirit lamp is used to sterilize the surrounding air.
3. Operate the autoclave, oven and incubator as you have already learnt in earlier experiments.
4. Thoroughly wash your hands with soap and clean with disinfectant before and after doing the experiment.
5. Clean the bench with disinfectant after finishing your experiment.
6. Mark the plates on the back of the petriplate for the culture identification. To mark, write the name of the fungus cultured and the date of the culturing. Preferably use a permanent marker pen for writing.
7. Dispose off the culture material and sterilize the glassware as you have read in Unit 3.

3.2 *Paramecium* Culture (hay culture)

Material Required

500 ml Erlenmyer flask
Petriplate — Heat resistant glassware
Wheat grains Hay
Tap water

Procedure

1. Collect some water from a pond having submerged leaves and dead organic matter and *Paramecium*.
2. In the flask take 450 ml of tap water, add 20-25 grains of wheat and some hay.
3. Boil this mixture for 5 to 10 mins, and allow it to cool down. This is the culture solution.

4. In a petriplate pour 20-25 ml of culture solution and add 5 to 10 ml of water taken from the pond and cover this petriplate.
5. Keep this petriplate in the incubator. The optimum temperature for culturing *Paramecium* is 22oC - 25oC.
6. You will see that *Paramecium* has appeared in the culture within 4 to 5 days. However, you may also observe the growth of certain bacteria, when you make a slide of the culture and see it under the microscope. These bacteria come along with pond water.

Observation

Study the structure of *Paramecium* by making a temporary mount of this organism. Put a drop of culture on a slide, cover it with the cover slip and see the slide under the microscope.

3.3 Gram's Staining Of Bacteria

Material Required

Reagents for Gram's Staining:
Crystal violet, Gram's iodine, Ethyl alcohol or Acetone, Safranin
Slides
Distilled water
Wash bottle
Pasteur Pipette
Inoculating loop (metal loop)
Cultures of nonpathogenic bacteria like pseudomonas, E.coli etc.

Procedure

1. Transfer a loopful of bacteria culture on a clean slide. Before it is used, the inoculating loop is sterilized by heating in the flame (Bunsen burner) and cooled. The loop should again be sterilized before it is set down after use. (Ask for the bacteria culture from your course tutor).
2. To make the smear, spread the bacteria culture on the slide with the help of another slide and allow it to air-dry. This dry smear is passed through the flame 4 to 6 times to heat fix the bacteria. Because of heat fixing the bacteria enzymes are denatured and autolysis is prevented. Also the bacterial cells adhere to the slide because of heat exposure.
3. To the fixed smear apply crystal violet (called primary stain) solution by Pasteur pipette. All bacteria are stained purple.
4. Next apply iodine solution (Gram's iodine also called mordant). This intensifies the ionic bond between the crystal violet stain and the bacteria.

5. Apply ethyl alcohol or acetone (decolourizing agent). The primary stain i.e. crystal violet can be washed out of bacterial smear (decolorization) or the smear can remain unaffected.

   Note: The bacteria are gram positive bacteria if the smear is not decolourized and retains the deep violet colour due to crystal violet stain. The decolourized smear contains gram negative bacteria and should be processed as follows:

6. Apply to the decolourized smear safranin stain (secondary stain or counter stain).

   Note: The decolourised bacterial smear will be stained red due to counter staining.

SELF ASSESSMENT EXERCISE

1. What is the difference between synthetic and non-synthetic media?

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2. How can you obtain isolated colonies of fungal culture on solid medium?

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3. List four important precautions you should take while working with microbes.

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4. On the basis of staining, how can you differentiate between gram positive and gram negative bacteria?

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4.0 CONCLUSION

You have successfully learnt how to make culture materials and how to do the Gram staining of bacteria. As you must have noted that while working with bacteria you need to be very careful. Always wear your gloves and wash your hand before and after you finish.

5.0 SUMMARY

Whether natural or artificial, culture media are important in biology. They enable us to grow organisms for study. They also facilitate the isolation of organisms of particular interest. However, this is only one of the many ways in which biological studies are studied. Sometimes physiological experiments have to be carried out to estimate the extent of a reaction or a process. In the next unit, we will see how you can set up some of such experiments.

6.0 TUTOR MARKED ASSIGNMENTS

1. Why do you sometimes have to cultivate organisms.
2. If you had to culture *Paramecium* in a natural culture medium, how would you do it?

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biology in action London, Heinemann Eductional Books Ltd

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UNIT 3 SETTING OF DEMONSTRATIONS OF PHYSIOLOGICAL PROCESS IN PLANTS

CONTENTS

1.0 INTRODUCTION

Certain experiments require special equipment and are tedious to set up. Instead of asking students to perform such experiment individually, demonstrations are set up for the class in such a way that each student can record, interpret and analyse the readings and present the results. The job of demonstrating experiments is assigned to a technician. In this exercise, you will learn to set up demonstrations for the following three physiological processes.

1. Transpiration
2. Respiration
3. Photosynthesis

2.0 OBJECTIVES

After doing this exercise you should be able to:

- List and procure equipment and materials required to set up a given demonstration.
- Set up the demonstrations for transpiration, respiration and photosynthesis, improvise suitable alternative equipment and material for these demonstrations, if need be,
- Help students in recording the readings.

3.0 MAIN CONTENT

3.1 Setting Up Physiological Demonstrations

3.2 To Set a Potometer

The loss of water by a plant through stomata or other pores is called transpiration. The instrument used to show the process and measure its rate is called a potometer. In this exercise you will set up a potometer.

Materials Required

Potometer
250 ml beaker
Iron stand with a clamp
2 ml graduated pipette
Razor, sharp knife or scissors for cutting a branch
Rubber bung
A piece of rubber tubing (about 6 cm)
Stop watch
Eosin or any other soluble dye

Method

Potometer is generally available in most labs. If it isn't, you can assemble one as shown in fig. 13.1. To set up the equipment you should follow the steps given below:

1. Bring a small branch of a soft-leafed plant from your compound it is important to see that the plant material used is in healthy condition; therefore while you cut the branch minimize the damage done. It is best to cut the part to be used for the experiment under water. So keep a water container ready with you and place the cut end of the twig quickly in it. Also remember to make a second cut above 2 cm or more from the end of the twig, while under water before you fix it in the rubber bung.

2. Fit the lower end of the branch into a non-flexible rubber tube and insert it tightly through the hole in the rubber bung. If you need to drill a hole use a cork-borer. Both the bung and the cork-borer must be kept wet all the time. You can drill better if water plus a trace of surfactant is used. A surfactant is a detergent-like chemical that lower surface tensions.

3. To set up a potometer choose a place on a clean bench close to a window, clamp it to the stand and assemble it carefully. The

essential part of a potometer is a graduated capillary tube which shows very small losses of water by transpiring leaves.

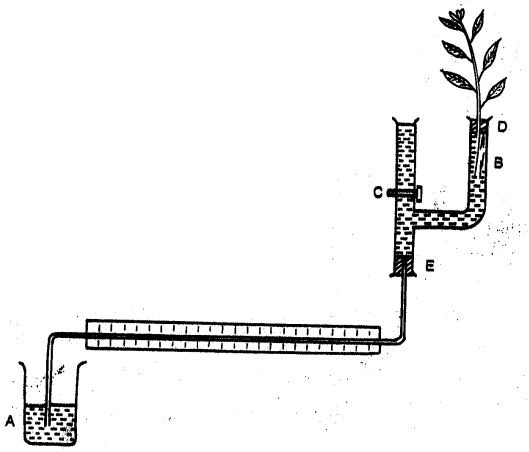

Fig. 13.1 A Potometer

Pour eosin coloured water into the beaker (A)
You must ensure that when the capillary is dipped in the beaker containing colored water there isn't a trapped air bubble. Fill the tube B with tap water and then close the tap C. Also make sure that the two rubber bungs (D and E) are sealed well.

Now lift the capillary end out of the beaker carefully and hold it till a bubble of air is introduced. Then re-immerse it so that the bubble of air is caught. Since the twig is transpiring the bubble will gradually travel along the capillary followed by coloured water. When the bubble proceeds beyond the marked area it may be driven back by opening the tap C.

Now your potometer is ready, and you can demonstrate to the students transpiration of water from leaves, and they can measure its rate. The rate of transpiration is measured as distance moved by the bubble per unit time. To compare the rates under different environmental conditions you should place the apparatus under the fan, in light and dark.

3.3 To set a Respirometer

Respiration involves gaseous exchange of 0_2 and $C0_2$. Warburg devised a manometer (a manometer is a device for measuring gas pressure) to measure this exchange, therefore the instrument available commercially is called Warburg manometer. Since it is an expensive instrument, a simple manometer also called respirometer is assembled in most labs for routine demonstrations to the students. In this exercise you will learn to assemble a respirometer to measure respiration rates.

Materials Required

500 ml conical flask
25 ml test tube
T - tube
Pinch clip
A rubber bung
A piece of rubber tube
1 mm diameter graduated pipette
KOH pellets
Stop-clock
Wire gauze
Thermometer
Sprouted mungbean

Method

1 You can assemble a simple respirometer as shown in figure 13.2
2. Take a T - tube, fix its one end in the rubber bung and slip a piece of rubber tubing and pinch clip to the other end.2 Now fix the end with the rubber bung to a test tube.
3 Wrap 2 pellets of KOH in a piece of wire gauze and place them at the bottom of tube. Add a few drops of water.
4 Now insert carefully a wad of cotton to partition KOH from the specimen (see fig. 13.2). Make sure that the cotton is well above the wire gauze.
5 Weigh 20 germinating mung bean and place them on the cotton wad.
6 Join a 2 ml graduated pipette to the central arm of the T with a piece of rubber tube.
7. Assemble the apparatus and place it in a 500 ml conical flask containing water at room temperature or any other suitable water bath. Place thermometer in it to record the temperature. Observe the marking on the 2 ml graduated pipette of manometer and calculate in ml the minimum volume change it indicates. If 2 ml is divided into 20 fractions each fraction will be equal to 100 µl (1 ml = 1000 µl).
8. Loosen the pinch clip and leave it to equilibrate for 5 to 10 minutes. Then tighten the pinch clip.
9. Insert a drop of colour solution (eosin) in the pipette. It will start moving to the left. Adjust it to extreme right up to a certain mark by carefully loosening the pinch clip.
10. Now the student can start the stop-watch and take the reading at zero and after an interval of 5, 10, 15, 20 and 25 minutes and they can calculate the volume changes (distance moved by the colour drop) in µl /minute.

The unit should be air-tight. You must ensure that when you place an organism (seeds or insects) it must not be in contact with KOH as it is corrosive.

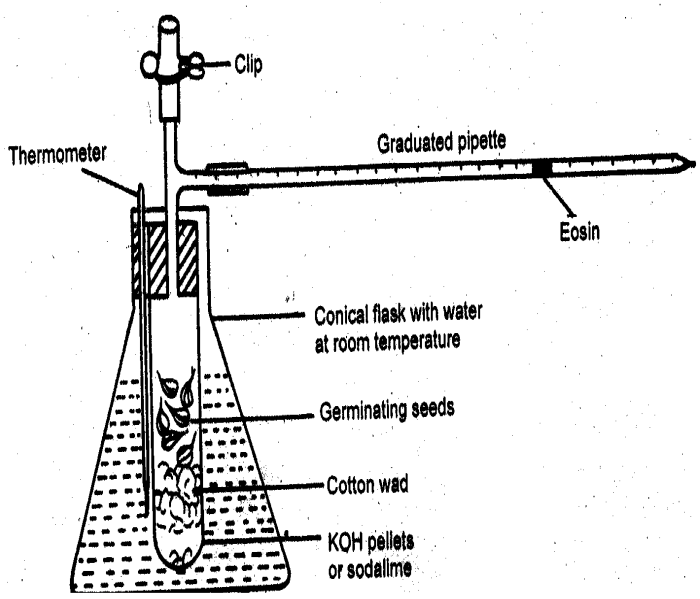

Fig. 13.2 A simple pipette manometer.

3.4 To Set up Demonstration Experiments for Photosynthesis

Photosynthesis is a process by which green plants make carbohydrates, sugar and starch from C02 and H2O using light energy absorbed by chlorophyll. The process can be demonstrated by evolution of gas bubbles from a leaf or the water plant *Hydrilla* in the presence of sunlight. You may have seen this demonstration in your school. In this exercise you will set up this demonstration and also learn how to improvise it for measuring the effect of quantity of light.

Materials Required

250 - 500 ml beaker
15 - 25 ml test tube
Leaves
Cork borer
Table lamp with 100 W bulb
Iron stand with a clamp
$NaHC0_3$
Green, blue and red cellophane paper.

Method

The simple pipette manometer used for measuring the rate of respiration can be used for the demonstration of the following experiments on photosynthesis:

A. Measurement of Rate of Photosynthesis

1. Clean the manometer thoroughly and dry it before use.
2. Using a dropper pour 2 ml of 1 % sodium bicarbonate solution into the tube. Make sure that it does not touch the sides of the test tube. Now insert a cotton wad into the tube to partition bicarbonate solution.
3. Cut several discs of leaves with 1 cm diameter cork borer and keep them moist in a petridish in the dark. Insert one piece in the tube and place it vertically on the cotton wad.
4. Assemble the apparatus and place it in a 500 ml flask containing water at room temperature. Cover the flask with black paper thoroughly. Let it equilibrate for 5 to 10 minutes.
5. Introduce a drop of eosin dye in the pipette and adjust it towards a mark to extreme right.
6. Start the stop-watch and note the readings on the pipette immediately and after an interval of 5 to 10 minutes.
7. Now adjust the black paper to make a vertical slit so that the leaf can be exposed to the light source. Keep the light source 10 cm away from the apparatus.
8. Again adjust the marker drop to the extreme left up to a mark.
9. Readings can be taken at zero time and after an interval of 5, 10, 15, 20 and 25 minutes**.**

B. Effect of Light Quality on the Rate of Photosynthesis

1. Cover the light source with green, blue or red cellophane paper. Readings are taken as in the previous experiment.

SELF ASSESSMENT EXERCISE

1. Why are KOH pellets kept in the boiling tube of a pipette manometer?

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2. Why is a drop of coloured solution introduced in the pipette?

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(3) What is the purpose of a pinch clip?

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(4) What would happened if the rubber tube is sealed at the top?

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4.0 CONCLUSION

In these experiments you have learnt to set up demonstrations for respiration, transpiration and photosynthesis. You have also known how to improvise alternative materials where necessary. You have also learnt how to make readings from the experiments.

5.0 SUMMARY

Experiments like the ones in this unit are part of the things that are involved in learning biology in the practical sessions. There are still more other activities that biologists are involved in, in the practical work. Dissection is another aspect which will learn about in the next unit.

6.0 TUTOR MARKED ASSIGNMENT

Describe how you set up an experiment to determine the rate of photosynthesis.

7.0 REFERENCES/FURTHER READINGS

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UNIT 4 SETTING UP APPARATUS FOR DEMONSTRATING PHYSIOLOGICAL ACTIVITY IN ANIMALS

CONTENTS

1.0 INTRODUCTION

In the earlier experiment you learnt how to set up an apparatus to show physiological processes in plants. In this experiment you will learn how to set up two apparatuses to demonstrate physiological activity in animals. You will set up the kymorgraph apparatus to show physiological activity in the muscle and heart of a frog, and set up an experiment to observe the capillary blood flow in the webbed foot of a frog. These experiments are usually performed at undergraduate as well as graduate level and it is the lab technical staff who are expected to arrange all the equipment required for them. The students are expected to do the dissection and make the actual recording. The supervisor/school in charge of the lab usually demonstrate these experiments to the students but it is the technical staff present who give assistance where the students require it.

2.0 OBJECTIVES

After doing this experiment you will be able to:

- set up a kymograph apparatus to measure the heart rate and muscle activity in frog.
- Set up an experiment to show the capillary blood flow in a frog, and
- immobilize a frog by pithing.

3.0 MAIN CONTENT

3.1 Apparatuses for Animal Physiology

3.2 Setting up a Kymograph Apparatus

A kymograph apparatus consists of a vertical cylindrical drum which can be made to rotate at a variety of speeds by an electric motor. It is used for recording movements such as contraction of muscle, contraction of heart, etc.The speed of rotation can be adjusted as required. Movement can be recorded by either a pen filled with suitable ink, writing on white paper, or a writing stylus marking on a writing paper which has been blackened evenly. This paper is pasted on the drum.

If the kymograph recordings are to be of any use, it is important to provide a time scale. In most kymographs, the speed at which the drum rotates is given and a time scale is worked out from this information.

An electrical stimulator is usually made by attaching wires on each side of a 1.5 volt dry cell. The ends of these wires are fitted with simple electrodes.

Materials Required

Kymograph Recording System Consisting of:
Kymograph apparatus
1.5 volt dry cell (stimulator) with electrodes attached to it
Muscle lever
Double hook
Femur Clamp and Stand
5 gm weight
Recording stylus
Dissection tray, dissection instruments
Bone cutter
Frog Ringer solution, 20% urethane
50 ml beaker
Cotton, Thread
Injection syringe and needle
Live Frog

Procedure

Blackening of Kymograph Paper.

1. Cut a strip of recording paper to the height of the drum and paste it on the drum. Blacken it evenly by holding it over a kerosene / zylene flame.

2. When the drum is ready for recording, mount the drum on the instrument taking care not to smudge the blackening.

Setting up the instrument for recording the muscle twitch

1. Fit the femur clamp to the stand. This will hold the nerve muscle preparation vertically directly above the point where it will be attached to the muscle lever (dissected muscle preparation will be provided by the course tutor Fig 14.1
2. Hook the thread to the lever with a double hook and to the other end of the hook tie a 5 g weight. This arrangement keeps the lever in a horizontal position and the muscle and thread in a vertical position .
3. Clamp the muscle in such a way that the distance between the fulcrum and the tendon attachment raises the lever by about 2 inches for every contraction of the muscle. Keep the preparation moist by using a cotton soaked in frogs Ringer solution.
4. Attach the writing stylus to the horizontal lever in such a way that it just touches the drum without affecting its speed. Set the motor at a speed of 32 cm/sec. Now the kymograph apparatus is ready for recording the muscle twitch.

Setting up the instrument for recording heart beat in situation

1. Cover the revolving drum with sooted writing paper.
2. Attach the thread to the lever by means of a small piece of plasticine. Position the lever on the stand and counter balance it with plasticine so that the heart is fairly stretched and the lever is horizontal.
3. You will be provided with a frog with heart exposed. Attach a hook with thread to the muscle of the ventricle. The writing point should move up a centimeter with convulsion of the heart.
4. Be careful to adjust the writing lever in such a way that there is hardly any friction between the writing stylus and paper.

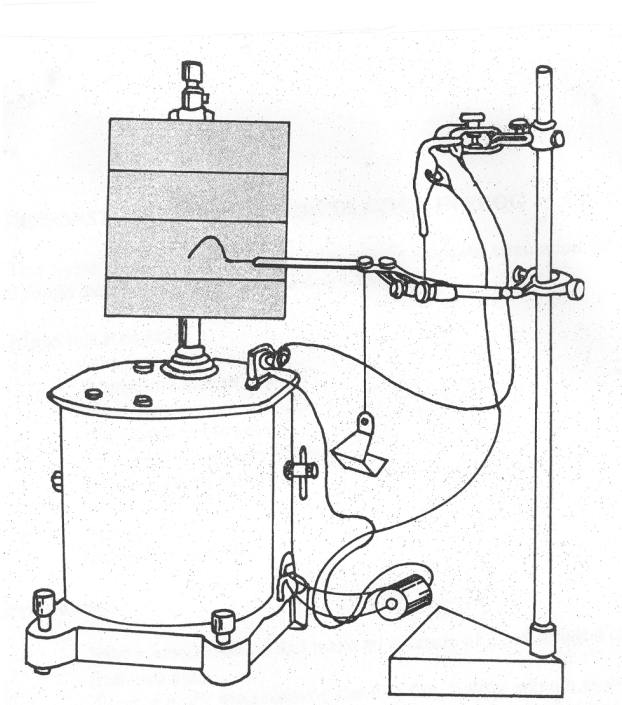

Fig. 14.1 Kymograph instrument arranged to record muscle twitched in frog.

3.3 Demonstrating Microcirculation In Frog

This experiment is set up to demonstrate to students circulation of blood through capillaries in the web of a frog's foot.

Materials Required

1. Live frog
2. Compound microscope
3. Medicine dropper
4. Absorbent wet towel
5. Isotonic frog's Ringer solution
6. Board made of soft wood
7. Pins
8. String
9. Adrenaline solution

Procedure

1. Take a wooden board and make an aperture of 2 mm about 4 cm away from one end.
2. Wrap a moist towel around the live frog tightly enough to prevent it from moving but leave one foot exposed. Tie up the frog on the wooden board placing the web of the foot over the aperture.

3. Pin the web of the foot over the aperture. You will not hurt the frog as long as you don't pin it by the foot. The web does not have any nerves in it, therefore it does not feel any pain on being pinned.
4. Support the plank on the stage of the microscope as shown in fig 14.2 and adjust the foot under the low power of the microscope. The demonstration is now ready to be observed by the students.

3.3.1 How to Pith a Frog

When using live animals in the laboratory it is important to understand the purpose for which they are to be used. They must always be treated in a humane way. Never cause them unnecessary injury or irritation. Accordingly, if any tissue or organ damage may results from experimentation, first put the animal under an anesthetic or treat the nervous system to make it insensitive to pain. Avoid injuring the animal's tissue or making it bleed; such damage makes the animal less capable of normal reactions.

A spinal frog, one in which the entire brain is destroyed, is prepared by a procedure known as pithing.

1. Hold the frog as shown in fig. 14.3, using the thumb and finger to secure the limbs.
2. With the index finger, press the snout down so that the head is at a sharp angle to the body.
3. Run the dissecting needle down the midline of the head. 2-3mm behind the posterior border of the eardrums, until a depression is felt at the rear of the skull. This indicates the location of the foramen magnum, the opening through which the spinal cord emerges out of the skull.
4. Using the dissecting needle, with a sharp movement pierce the skin at this point and insert the needle into the brain through the foremen magnum. Twist and turn the needle to destroy the brain. Halt any bleeding that may result and use the animal.

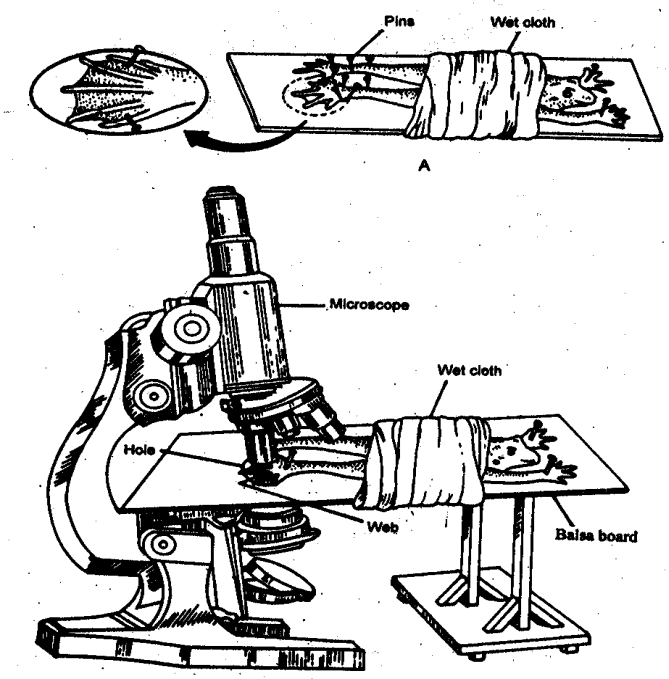

Fig. 14.2: Demonstrating microcirculation in the web of frog

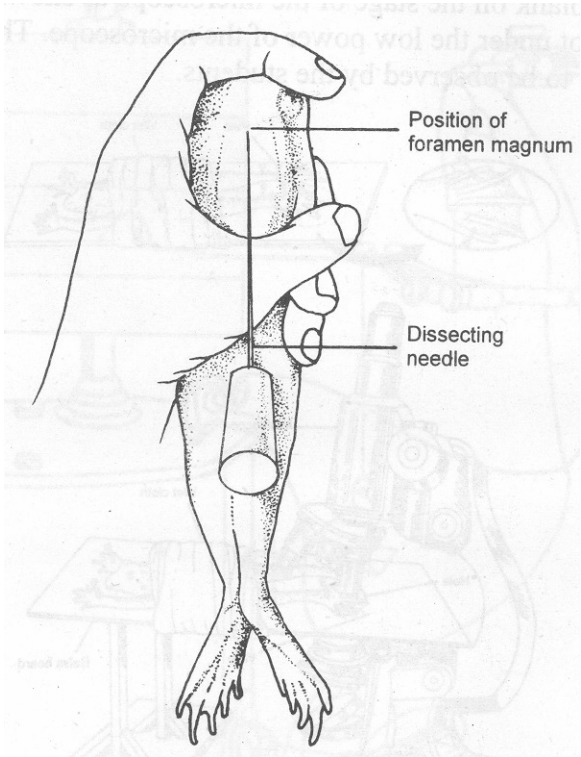

Fig. 14.3: Pithing the brain of a frog

SELF ASSESSMENT EXERCISE

Practically demonstrate how you will immobilize a frog by pithing.

You may not find this easy. Don't worry; you will not have to do this too often.

4.0 CONCLUSION

You have learnt that with some apparatuses you can study animals alive. The advantage in such device is that the real happening in the organisms can be clarified. Using only dead organisms may not be as explicit. So you can see that biologist involve various means in their study of life.

5.0 SUMMARY

In this unit we saw how animals can be studied while still alive. In the next unit we will see how they are studied as dead. We will be able to discern those aspects of studies that can still be meaningful even with dead animals.

6.0 TUTOR MARKED ASSIGNMENT

1. How will you demonstrate microcirculation in a frog?

7.0 REFERENCES/FURTHER READINGS

Berril, N.J. (1979) Biological in action London, Heinemann Educational Books Ltd.

Soper, R. (Ed) (1998) Biological Sciences Cambridge U.K. Cambridge University Press.

Stone, R.N., Cozens, A. B, Emia, F.I. (1972) New Biology for Tropical Schools London, Long man Group Ltd.

UNIT 5 PREPARATION REQUIRED FOR DISSECTIONS

CONTENTS

1.0 INTRODUCTION

Dissection is a major component of the biology practical. The meaning of dissection is to cut open the animal in order to ascertain the structure of its parts. The object of dissection is to separate the several parts from one another, so as to define their boundaries and display clearly their mutual relations. Dissection consists mainly in removing the connective tissue which binds the several parts together. Dissection requires lots of preparations before hand.

In this unit you are also provided with some dissecting guide to help you through some dissections.

2.0 OBJECTIVES

After doing this experiment you should be able to:

- prepare dissection trays/petriplates,
- use the dissection guides provided
- anaesthesize the animals correctly,
- dispose dissected animals safely.

3.0 MAIN CONTNET

3.1 Preparation for Dissection

Materials Required

Dissection trays
Petridishes
Dissection kit
Chloroform
Ethane
Formalin
Urethane
Microscope
Table lamp
Animals

Procedure

In schools, colleges and universities, dissection of chordates/non-chordates is one of the major practical. A lab technician has to perform several tasks before and after dissection.

3.2 Procuring Animals

Orders for animals should be placed with an animal supplier according to the number of students in a class. This exercise should be done two-three days before dissection is to be performed.

3.3 Anaesthesizing the Animals

Before dissection animals are given anaesthesia. Chloroform and either are used as anaesthesing agents. Rats, frogs, and pigeons are generally freshly chloroformed for the dissection, though for some of the dissections e.g. cranial nerves in rat and internal ear in frog, preserved specimens are required. Similarly scoliodon is also preserved after anaesthesizing them. Preservatives commonly used are formalin (5%, 8% or 10%) and 70% alcohol.

3.4 Theoretical Knowledge

Keep a well-labeled diagram of the dissection to be done in the classroom. You should have some theoretical knowledge of the dissection to be performed e.g if students have to dissect male or female reproductive organs of rat, frog or other animals, then you should be able to differentiate them morphologically, just by looking at them externally.

3.5 Setting up of dissection trays

Large animals like frog, rat, fish, pila, leech and other animals are dissected in dissection trays whereas small animals such as cockroach and small insects can be dissected in petriplates.

It should be seen that wax is spread over trays uniformly and water does not drip from the trays. The trays should be half filled with water so that the animal can be fully immersed in it. The trays should not be completely filled with water.

One set of dissection kit (about which you have studied in detail in Experiment 1), dissection tray, a microscope and a table lamp should be arranged for the teacher/instructor. Similar sets should be arranged for students. It should be seen that dissection instruments are clean and sharp.

Water should be kept clean in the dissection tray during dissection by changing it whenever it is stain with blood etc.; so that visibility remains the same. If any animal is bleeding profusely, alcohol dipped cotton should be applied to the affected organ blood vessel. See that every waste of dissection is put in the petridish. It should not be thrown carelessly on the top of table.

3.6 Pithing

Some of the experiments like muscle twitch and heart profusion in frog do not require chloroform-anaesthetized animals. Before dissection is started, you have to anesthetise the frog by injecting 2.5 ml of 20% urethane intramuscularly. This would quieten the frog. Alternatively you could immobilize the frog by pithing. You have learnt how to pith a frog in Unit 4 module 3, of this course.

3.7 Flag Labeling

Frequently, the students are asked to dissect some organs, blood vessels or nerves etc. and flag label them. For flag labeling, small pieces of paper (2.5 x 0.7 cm) are prepared and a needle is passed through each close to one end.

The names of organs, blood vessels or nerves are written on these flags and each flag is inserted in the dissecting trays close to the organ, blood vessel or nerve bearing the name on the flag.
Some of the dissections require black-preparing. You have to cut black paper into small and thin strips which can be inserted underneath blood vessels and nerves.

3.8 Safe Disposal of Dissected Animals

You have already studied in Unit 14 and Exercise 13 of LT - 1 course that the dissected animals should be buried deep in the soil. Simply placing the material in a plastic bag and putting it in the dustbin is not good enough.

SELF ASSESSMENT EXERCISE

1. Which are the commonly used anaesthesing agents?

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2 How do you pith animals?

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3. How will you display the dissected specimens?

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4. Which is the safest way to dispose off a dissected animal?

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.....

3.9 General Rules for Dissection.

As you have learnt in section 3.2.2, you need to give some anaesthesia to animals before you dissect them. The common anaesthesia used in the biology laboratory are chloroform and ether. For rats, frogs and pigeons, it is better to dissect them immediately after anaesthesia.

However, certain studies for example, of the nervous system are better done with preserved animals. Formalin (5%, 8% or 10%) and 70% alcohol are the common laboratory preservatives. It is usually better to do dissection after you have done the theoretical studies.

Opening Up

Invertebrates are better opened up from their dorsal side while vertebrates from their ventral side. In both cases, you pick the skin up with a pair of forceps (if the animal is big), if it is small, you will have to hold the animal with your left hand while with a pair of scissors you make your incision with the right hand on the mid-line of the animal with the scissors pointing upwards to avoid damage to lower internal structures. This incision should go along the line of the animal as much as possible. Then make side slits in the same manner, with the scissors pointing upwards to avoid damage to underlying tissues.

With the scapel, gently scrape the inside layer of the skin to separate skin from the underlying tissues by cutting through subcutenous tissue. Once the skin has been removed the animal must be laid down (even if it is small) and pinned securely to the dissecting board. It is important to pin specimen securely down so that both hands will be free for use. Seekers could then be used to probe parts. When other walls have to be opened up to reach internal organs, the same cautions as we took for opening up the skin will apply.

You have to be very gently in dissecting so as to avoid damage to internal organs. If the dissection involves cutting through tissues (especially blood vessels) then you must take steps to ensure that the blood flow or the contents of the cut tissues or organs do not interfere with your studies. Usually, you may have to wash, and soak away blood with cotton wool or blotting paper.

Appendixes are laboratory

Mammal you could use for dissecting the earthworm,
Grasshopper,
The bony fish,
Cartilaginous fish,
Amphibia,
Reptile,
Fish,
Birds,
Mammals.

3.10 Dissection of Earthworms - General Anatomy

General Remarks

Earthworms are usually killed with chloroform soaked on cotton wool. Avoid the liquid coming into direct contact with the worm as this will make it contract violently and rupture. This contraction will cause rigor which makes the worm stout and difficult to pin. An alternative method is to kill the worm by immersion in very hot water and removed as quickly as possible. If left for too long they become flabby and decolorized. The dead earthworm should be allowed to relax for about 15 minutes.

Procedure

Examine the worm externally and note the prostomium, chaetae and the position of the genital and clitellum. This is important in order to ascertain the species of *Lumbricus* because the local species is definitely not *Leterrestris* generally described in most text books.

Distinguish the dorsal and ventral sides of the worm and then proceed to dissect.

i Hold the worm in the left hand between the thumb, forefinger and the third finger with the dorsal side up.

ii With a pair of fine scissors make a slit on the body wall along the middorsal line in the region of the clitellum. Do not cut too deeply, keep the points of the scissors up and cut forwards as far as to the prostomium.

iii Then proceed to pin the worm in the waxed plates as follows:

a. Place a pin through the prostomium and another through the mid posterior end such that the worm is fully stretched.

b. Starting from the anterior end, place pins in pairs as nearly opposite as possible.

c. Use pins to tear off the septa and free the body wall between each pair of pins.

Note: Each pin should be loped at an angle of about 45º so as to allow free movement of instruments. It is absolutely important that the worm be fully stretched as you work. Later adjustments by removing pins will result in poor and unsatisfactory dissection.

Now that you have opened the viscera of the worm, proceed to identify the various organs and systems without necessarily displacing them.

Nervous System

i Having pinned the gut to the side, display the nerve chord which runs through the entire length of the animal and also the segmental ganglia.
ii identify the circumpharyngeal nerve ring.
iii Display the ring to one side.

Excretory System

Identify the segmentally arranged paired nepthridia. Draw your own dissection and label fully.

4.0 CONCLUSION

In this unit, you have learnt how to prepare different solutions, and the precautions you must take,. You have also learnt the general rules for handling solutions.

5.0 SUMMARY

The dissection of different parts of lower vertebrates, higher vertebrates, and invertebrates has really shown us the complexity and linkages in the body system of animals.

6.0 TUTOR MARKED ASSIGNMENT

1. Describe the process of setting up a dissection tray in the laboratory.
2. What are the rules one should observe in the process of carrying out dissection?

7.0 REFERENCES/FURTHER READINGS

Beril, N. J (1979): Biology in Action London, Heinemann Edicational Books Ltd

Soper, R. (Ed) Biological Sciences Cambridge U.K., Cambridge Press

Stone, R. N. Cozens, A. B. Emia, F. I. (1972), New Biology for Tropical Schools, Longman Group Ltd)

MODULE 4

UNIT 1 DISSECTION OF INSECTS - GENERAL ANATOMY

General Remarks

The essence of the dissection is to show as clearly as possible, the various internal organs / systems of the animal. To this end, the organs / systems should be displayed properly.

Materials

Freshly killed grasshoppers e.g Zonocerus variegatus or cockroaches.

Procedure

Pin down the insect to your waxed dissecting dish with the dorsal side facing upwards. You may hold up the insect with your left hand as an alternative approach. Then make longitudinal cut along the dorso-ventral line (i.e. laterally preferably on the hand side, progressing from the posterior tip of the abdomen to the anterior end of the animal (Fig 16.1).

If you held your insect while making the lateral cut, now pin down on your dissecting dish. Carefully lift the roof of the abdomen (tergites) intact from the right and turn it in such a way that the undersurface of the roof faces upwards. If this is done properly, the hearts and aorta circulatory system can be seen (Fig. 16.2)

Pin down the roof and cover the specimen with water. Then identify:

i The Alimentary System

Pull this out to the right and pin down and take note of the following: Salivary glands, and ducts, oesophagus, crop, gizzard, caeare, midgut, malphigian, tubules, lundgut and rectum.

ii The Reproductive System

A Female: ovarioles, a pair of calyx, a pair of lateral oviduct, vagina, spermatheca, spermathecal duct.

B Male: Testes, follicles, testis, vas deferens, sEmial vesicles, accessory gland, ejaculatory duct.

iii The Nervous System

Brain (lies on the dorsal side of the head, sub-oesophageal ganlion) (lies under the oesophagus), circum-oesophageal commissures, three thoracic ganglia, five abdomen ganglia, the connectives.

v Having dissected the insect to show the features listed above, make a large drawing of your own dissection and label fully.

Questions

Why are invertebrates generally dissected from the dorsal surface?

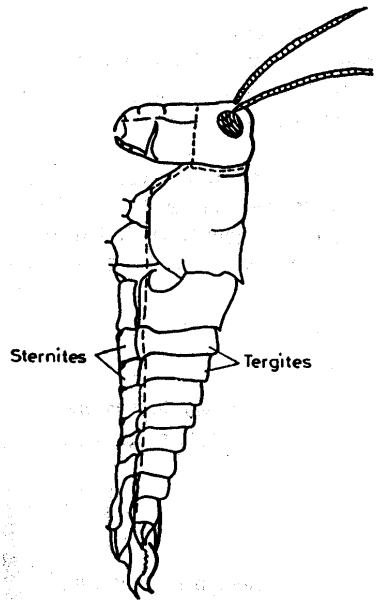


Fig. 16. 1Cutting open the insect (Variegated grasshopper, along the broken line)

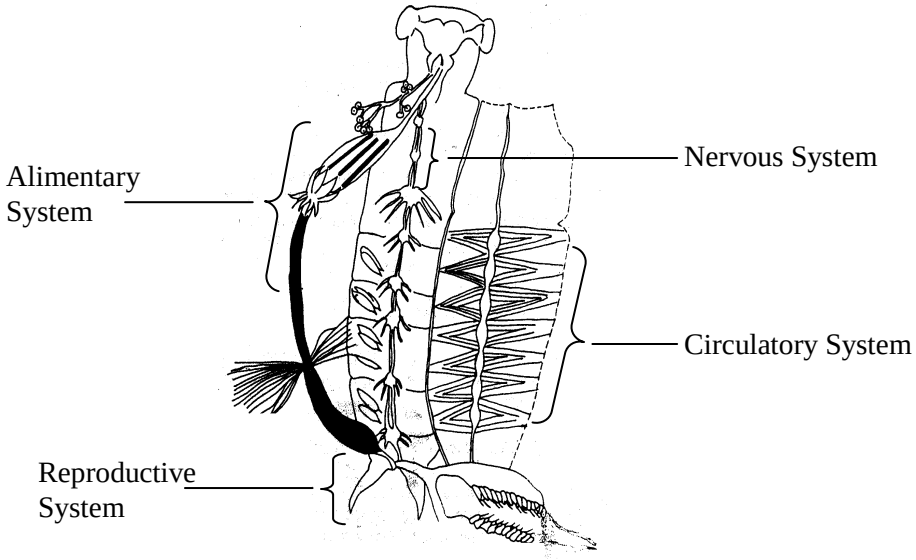


Fig. 16.2 Reproductive, alimentary, nervous and circulatory systems of the variegated grasshopper

UNIT 2 MORPHOLOGY OF LOWER VERTEBRATES - FISH, AMPHIBIANS AND REPTILES

General Remarks

A Fish

Fishes are the largest group of vertebrates. There are two classes of fish, namely Bony fish or Osteichthyes and Cartilaginous fishes or Chondricthyes

1. Bony and Cartilagenous Fishes

(a) **Bony fish** possess mainly bony endo-and exo-skeleton. They constitute the largest number of living vertebrates. Tilapia is a tropical and subtropical bony fish which live in fresh water and estuarine waters of Africa. *Tilapia zillii* is here described as a typical example.

Based on Nelso (1976) "Fishes of the World", *Tilapia zillii* can be classified as follows:

Phylum: Chrodata

Subphylum: Vertebrata

Subclass: Actinoptergii

Infraclas: Teleostei

Superorder: Acanthopterygii

Order: Perciformes

Family: Cichlidae

Genus: Tilapia

Species: Tilapia zillii

Many species of *Tilapia* abound in Africa and are very similar to each other, differing only in minor details. *Tilapia* species are substrate spawner while identical species which brood their egg and frys in the mouth are called Sarotherodon.

Head

The head is roughly triangular when viewed from the side (Fig 17.1). The mouth is terminal. One nostril is located on each side of the head just above the mouth in front of the eyes.

The round eyes lack nictitating membrane. The upper and lower lips are armed with teeth - the bicuspid outer series and the tricuspid inner series of teeth. The operculum (gill cover) is found on each side of the head. The first gill arch has 8 - 10 gill rakers on its lower part.

Trunk

The trunk bears two pectoral fins situated high up on the sides just behind the posterior edge of the operculum and a pair of pelvic fin which is ventral but thoracic in position just posterior to the pectoral fins (fig. 17.1). There is a single dorsal fin with 14 - 16 anterior bony spines and 11 - 13 posterior bony rays (that is DXIV - XVI, 11 - 13). There is an anal fin with three bony spines and 7 - 10 bony rays (that is A 11, 7 - 10). The anal and urinogenital orifice (Fig 17.2) is towards the posterior to the urinogenital orifice. The genital papilla is more pointed in males than in females. The dorsal and anal fins are often with yellow spots and the edges with yellow outline.

Tail

The tail is the part of the body posterior to the anal fin. It is laterally compressed and ends in symmetrical (homocercal) caudal fin (fig. 17.1). The caudal fin often has yellow spots.

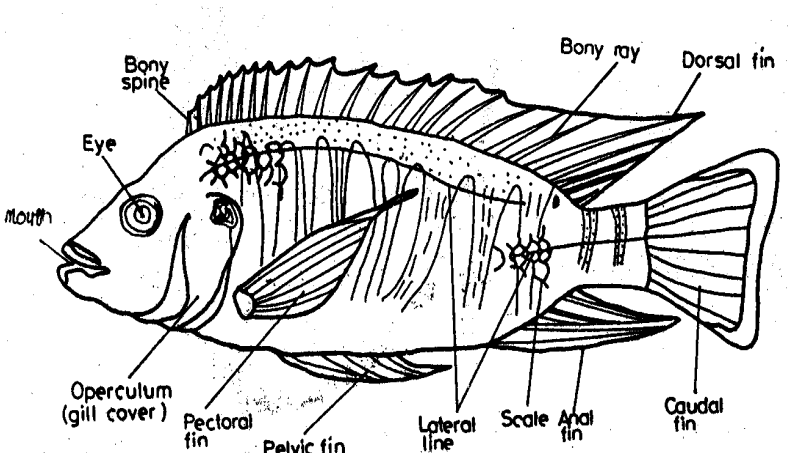


Fig. 17.1 ***Tilapia zilli***

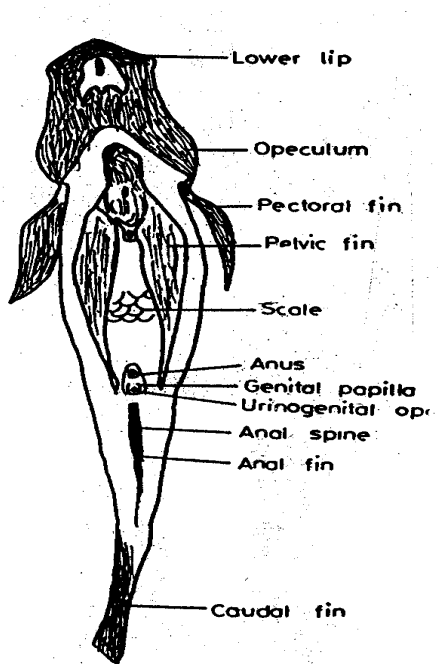


Fig 17.2 *Tilapia zilli* – Ventral view

b. Cartilagenous Fish

The West African sharp nosed or requiem shark (*Scolidon terrae-novae*) possesses cartilaginous skeleton. Fine placoid scales or dermal denticles cover the body. They are caught off the coast of West Africa. It is carnivorous and grows up to over three metres in length. It is dorsoventrally flattened, dark-greyish coloured dorsally and lighter coloured ventrally. Based on Nelson (1976) "Fishes of the World". It is classified as follows:

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Gnathostomata

Grade: Pisces

Class: Chondrichthyes

Subclass: Elasmobranchii

Order: Lamniformes (Galeoidea)

Family: Carcharhinidae

Genus: Scoliodon

Species: *Scoliodon terrae-novae*

The head is that part of the body from the tip of the snout to the beginning of the front of the pectoral fin. It is roughly flat with a pointed muzzle. The mouth is ventrally located about half-way from the tip of the snout to the anterior attachment of the pectoral fin (Fig. 17.3). A pair of nostrils which admit water tool factory epithelium is found on the ventral side of the muzzle, a short distance from the tip of the snout (Fig. 17.3). The eyes with vertical pupil are on each lateral part of the head, about half-way from the snout to the gill slits. The eyes have small upper and lower eyelids. A third nictitating membrane is present in each eye

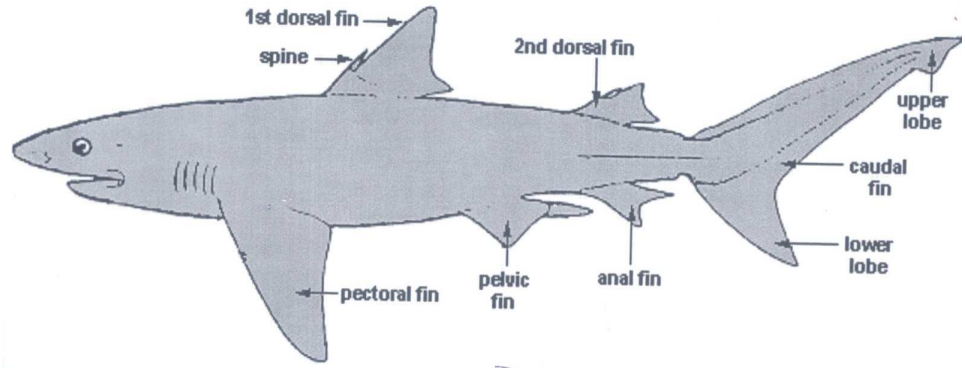


Fig. 17.3

Five gill slits open each lateral part of the head; four of these in front of pectoral fins and the last one over or behind the beginning of the pectoral fin. The anterior edge of each gill projects slightly backwards to close the gill slits watertight during intake of water.

Left and right sides of the head have a series of canals which are series of branches of the lateral line canal in the head (Fig. 17.3). Small pit organs or Ampullae of Lorezini are connected to the lateral line canal of the head. These are said to detect temperature changes.

2 Amphibia (e.g. Toad)

The common African toad (*Bufo regularis*) is found on land in damp humid areas throughout tropical Africa. It reverts to water to breed as do most Amphibia. When fully grown, it measures about 8 - 9cm from tip of mouth to cloaca. The skin is dry and warty but secretes milky fluid upon handling.

Toad can be classified as follows:

Phylum: Chordata

Subphylum: Vertebrata

Class: Amphibia

Subclass: Aspidospondyli

Order: Salienta (Anura, Batrachia)

Family: Bufonidae

Genus: Bufo

Species: *Bufo regularis*

Head

The head has a rounded snout bearing a terminal mouth with wide gape. Two very bulbous eyes sit on upper part of the head. The eye has eyelids that open the eye by up and down movement. Inside the eyelid is a third eyelid, the nictitating membrane. A pair of external nares open just above the mouth. A tympanic membrane or ear drum is seen as oval patch of skin on the angle of the jaw just behind the eye. A little posterior to the tympanum is oval patch marking the opening of poison glands. These glands excrete milky fluid upon handling the toad (Fig.17.4).

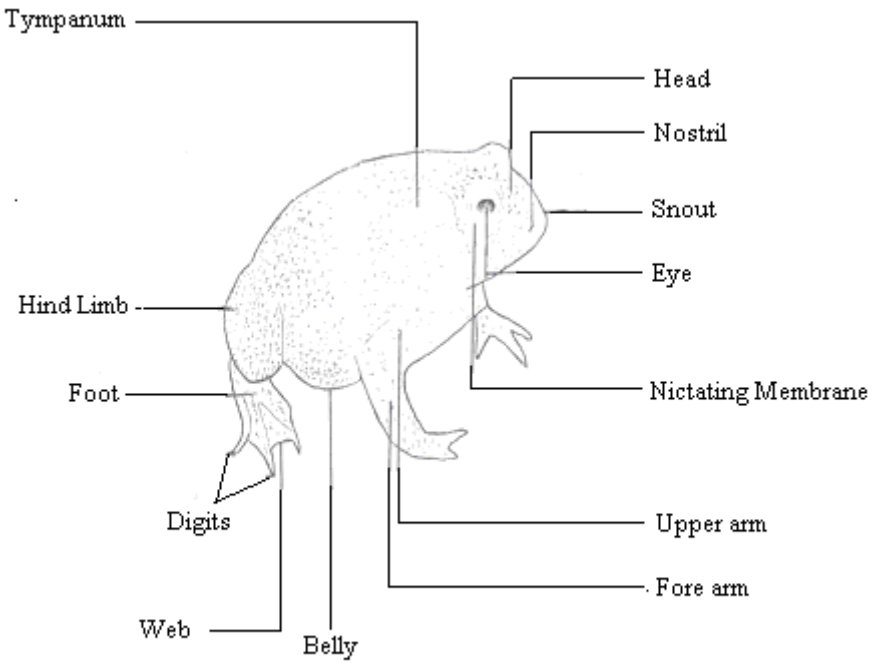


Fig. 17.4 External Morphology of a Toad

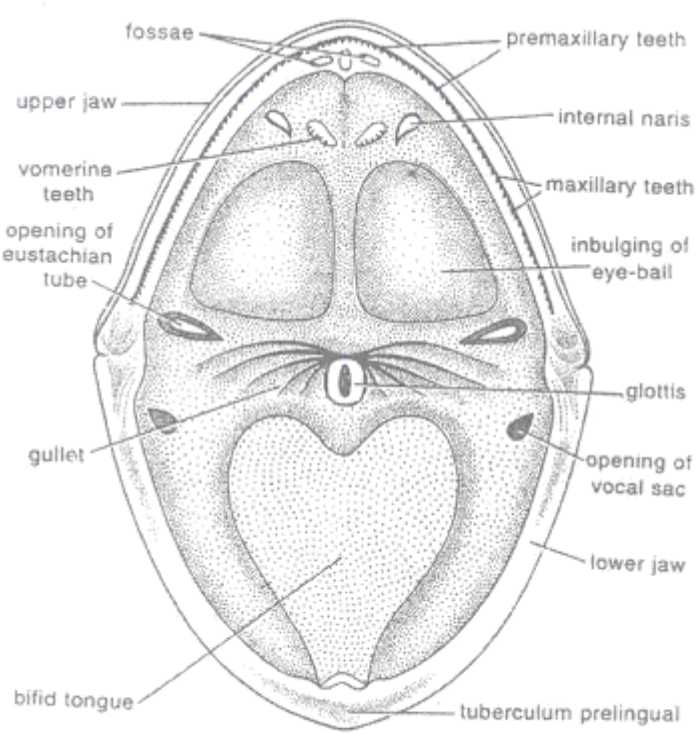


Fig 17.5 Bucco-pharyngeal cavity of a male Toad

The mouth bears no teeth. The tongue is attached to the anterior floor of the lower jaw. At the roof of the mouth are two bulged rounded structures corresponding t the position of the eye balls. Near these and anterior to them are internal openings of the nasal cavity (Fig. 17.4). Female toad has creamy chin with narrow pigmented border under the lower jaw, male toad has darker chin.

The toad has no neck. The head merges insensibly into the trunk. At the beginning of the trunk are two short forelimbs bearing four free digits ending in pads or tubercles. In male toads the first digit is enlarged. It is used to hold the female during mating. The hind limbs are long and muscular with five webbed digits. At the posterior end of the trunk and slightly to the dorsal side is the opening of the cloaca.

The belly is often disteneded and bigger in females than in males.

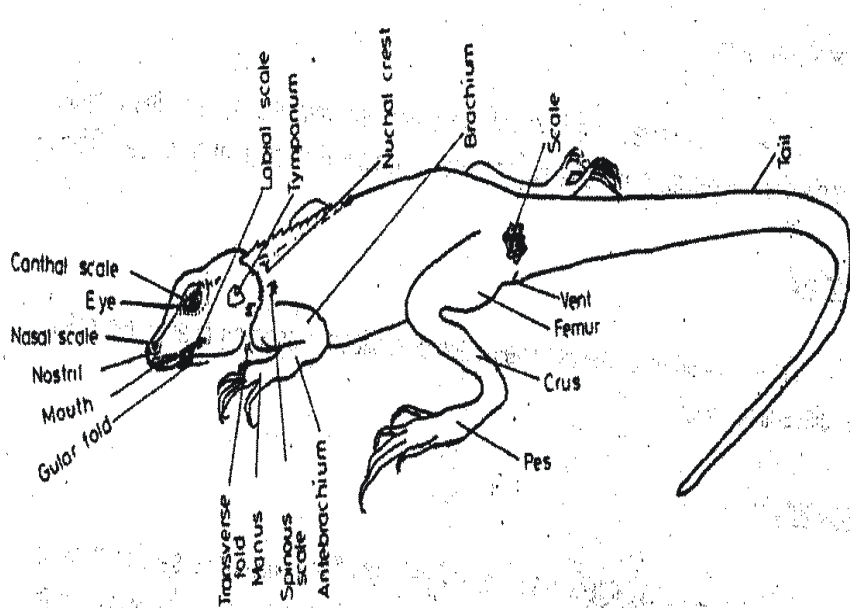


Fig. 17.6Rainbow lizard – *Agama agama* (external features)

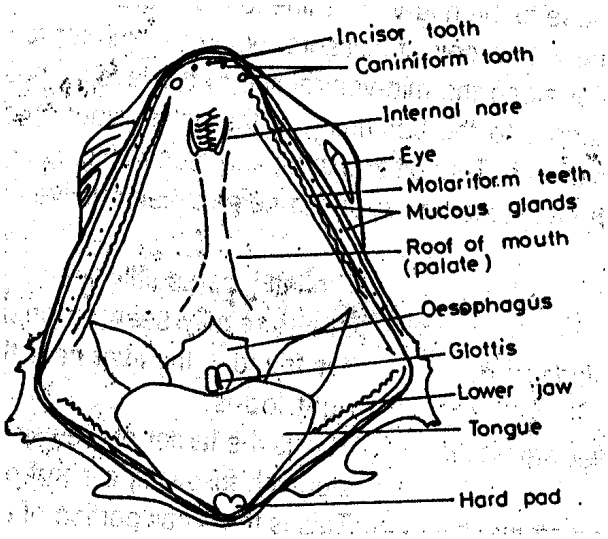


Fig. 17.7 Rainbow lizard – *Agama agama* (buccal cavity)

3. Reptile (e.g Lizard)

The rainbow lizard (*Agama agama* Lizard) is a common reptile occasionally seen basking in the sun on walls of buildings and barks of trees in tropical African. They are brilliantly coloured and capable of changing their colour. A mature male lizard has red head neck with yellowish spinous scales on the neck and behind the tympanum while the body is blue-black fading gradually from the base of the hind limbs to the middle of the tail. The tail is red in the male being black towards the tip and at the tip. The mature female lizard has a dark green head with light green patches while the body is brownish green with yellow patches on the belly.

Classification

Phylum: Chordata

Subphylum: Vertebrata

Class: Reptilia

Order: Squamata

Family: Agamidae

Genus: Agama

Species: *Agama agama*

The body of the lizard is divisible into three distinct parts, namely: the head, the trunk and the tail. The head is joined to the trunk by a narrow neck and the body is covered with scales (fig. 17.5).

Head

The head is triangular and separated from the trunk by a short neck. It bears a terminal mouth with a wide gape (fig. 17.5). Near the tip of the snout are two external nares each of which opens through a nasal scale. Each of the two eyes is located about one-third of the way from the tip of the smout to the beginning of the neck. The laterally directed eyes bulge slightly above the head and are protected dorsally by canthal scales. Eyelids are joined in front and behind the eyes. There is a nictitating membrane which is difficult to observe in undissected orbit.

A tympanum is found laterally on each side of the head as far behind the eyes. It is covered by tympanic scales. A large median scale, the pineal scale, is found on top of the head beneath which is the pineal foramen. Below the chin is a fold of skin, the jugular fold. Nuchal crest is found on the dorsal side of the neck.

Trunk

The trunk is about two and a half times the length of the head. It is roughly flattened dorso-ventrally. It bears two pairs of limbs with five clawed digits on each limb. The fore-limbs are shorter than the hind-limbs and fold close to the chest with digits pointening forwards. Each fore-limb consists of brachium (upper arm), antibrachium of forearm, and manus (hand). The hind-limbs has femur (thigh), crus (shank), and pes (foot). The number of joints on digits starting from nearest the body

outwards and including the claws are 2.3.4.5.3 for manus and 2.3.4.5.4 for pes. Transverse vent occurs behind the hind-limbs and ventral to them. A row of 8 or 10 callous pre-anal pads, border the bent in males.

Tail

The tail is about two times the body length from the snout to the vent. It tapers gradually from the region of the vent to the most posterior tip. Tails of adult males are thicker than those of adult females. They may be stumpy due to frequent shedding (automy).

Dissection of the Fish, Toad and Lizard

Materials

A Fish

Tilapia, *Scoliodon*, measuring boards, meter rules, forceps, petri dishes, dissecting microscopes or hand lens, dividers, scalpel, quinaldine (1% solution).

B Amphibia

Freshly killed toad

C Reptile

Freshly killed or preserved lizards, hand lens, meter rule, weighting balance.

Procedure

Fish

You can kill the fish 1% quinaldine solution or head blow or by applying a pointed seeker into the brain. Use a measuring board or ruler, measure the standard length, (from tip of snout to base of tail), total length and width of the fish. You can use a pair of dividers to transcribe the width of the fish onto a ruler. Then weigh the specimen.

Examine the fish starting from the head to the tail. Take note of the position and shape of the mouth and the eyes. Note also the shape of the head, the number and position of the nostrils, the operculum and the colour of spots on it.

Open the mouth with a pair of foreceps and examine the teeth. Note the number of cusps. Lift the operculum on one side of the head and count the number of lower gill rakers on the first gill. Write down all the observations you have made. Take note of the colour of body, the number and position of the lateral line. Count the number of scales along the upper lateral line and record. Count also the number of spines and fin rays on the dorsal fin, and on the anal fin, and record.

Draw the fish to show as many external features as possible.

Examine the ventral side of the fish with a magnifying lens or dissecting microscope, taking note of the position of the anus, the urogenital opening, and shape of urogenital papilla. Draw and identify the sex of the fish. With a scalpel scrape out some scales and examine with a magnifying lens and draw.

Toad

Examine the entire animal and then draw. Take note of its adaptations to life in water and on land. Open the mouth of the animal and take note of the point of attachment of the tongue, the glottis, the opening of the Eustachian tubes etc (fig 17.5).

Lizard

Examine the entire animal for shape (fig. 17.6) Make a close examination of

i. the head
ii. the mouth opened (fig. 17.7)
iii. the region of the vent.

Draw

Measure the length from the snout to the vent and from the vent to the tip of the tail, and compare the relative lengths of the regions measured. Weigh the animal and record the weight.

UNIT 3 DISSECTION OF DOGFISH - GENERAL ANATOMY

General Remarks

Dissection of vertebrate is usually done from the ventral surface as the vertebral column which houses the spinal cord poses an obstacle if one dissects from the dorsal surface.

Materials

Freshly killed or preserved specimens of *Scolidon*, dissecting boards, dissecting kits, dissecting awls.

Procedure

With the aid of a large scissors or scalpel, cut off the anterior dorsal fins close to the body. Lay the fish on its back on the dissecting board and nail down with awls or nails on the snout, and on the tail. With a scalpel, remove the skin on the pectoral region passing from close to the mid-ventral line to the lateral line on each side. Make a slit at the mid-ventral line with a pair of scissors, being careful not destroy the internal organs. Continue the cut along the mid-ventral line to the level of posterior pectoral fins. Make a similar cut posterior to the beginning of the pelvic sides of the body (fig. 18.1) and pin aside the flaps of skin. Trim off to expose the rectum (fig. 18.2). Identify, display, draw, and label the structures listed below, taking note of their relative positions, colour, and texture.

i **Liver:** The left lobe usually lies beneath the intestine, the right lobe Is readily seen on opening the coelomic cavity.

ii **Gall bladder**: It is found in the liver near the junction of the left and right lobes.

iii **Cardiac Stomach:** This is the larger of the two portions of the u-shaped stomach following a short oesophagus.

iv **Pyloric stomach:** This is the small portion of the stomach.

v. **Intestine:** The short tube following the pyloric stomach.

vi **Spleen:** A long grey structure along the medium edge of pyloric stomach.

vii **Pancreas:** The light coloured structure between the two portions of the stomach.

viii **Rectum**: The short portion of the gut following the intestine and ending in the cloaca.

ix **Rectal gland:** A cylindrical organ about 1.5 - 2cm long and on the dorsal surface of the rectum.

Make a longitudinal cut about 5cm long in the intestine, open up and examine intestinal valves (fig. 18.2) Draw.

Make similar cuts on the pyloric and cardiac stomach, and compare the relative thickness and folds of muscles in each section of the gut. Why are the walls and foldings of the different section of the gut of different thickness? Identify the following blood vessels (fig. 18.3 and 18.4).

(a) Hepatic portal vein - outside the right lobe liver;
(b) Posterior intestinal vein - from rectal gland;
(c) Lienogastric vein - between the spleen and pyloric stomach;
(d) Posterior gastric vein - from ventral surface of cardiac stomach
(e) Anterior gastric vein - from dorsal surface of cardiac stomach
(f) Coeliaco-mesenteric artery - from the dorsal aorta.

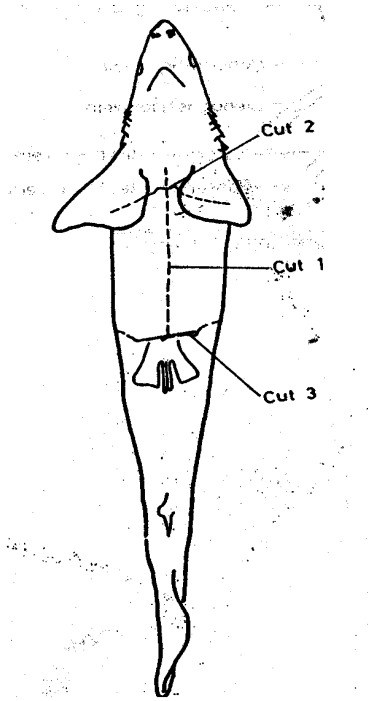


Fig. 18.1 Positions to cut when opening the abdomen of S*coliodon*

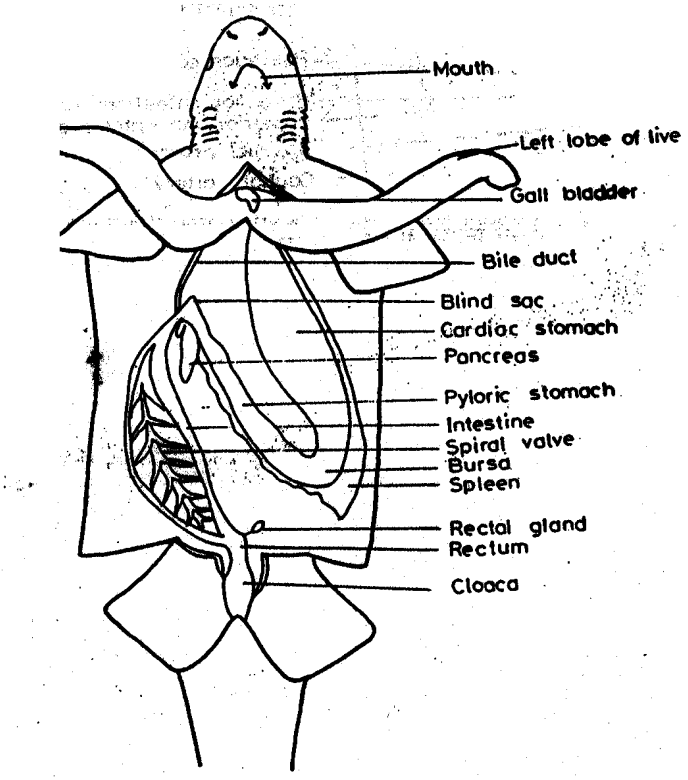


Fig. 18.2 ***Scoliodon terrae-novae*** **– alimentary canal and associated organs**

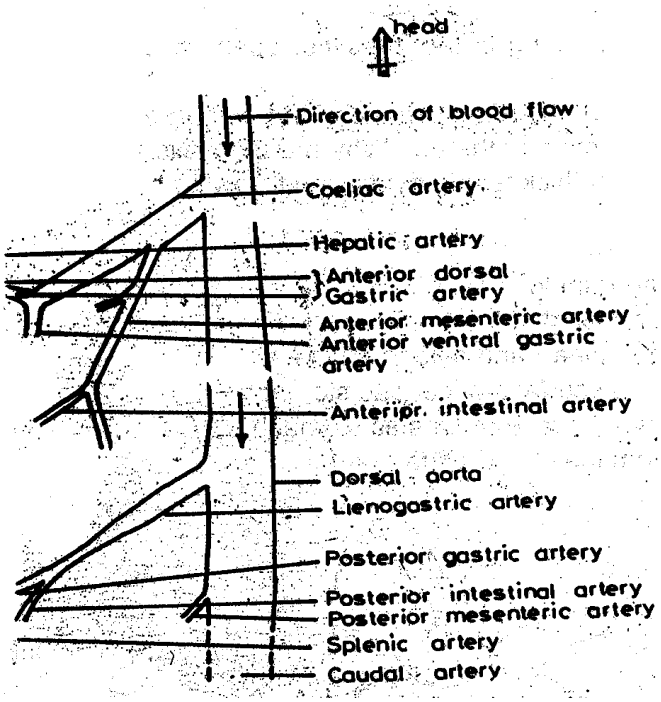


Fig. 18.3 ***Scolidion terrae-novae*** **– a schematic diagram of arteries supplying the digestive system**

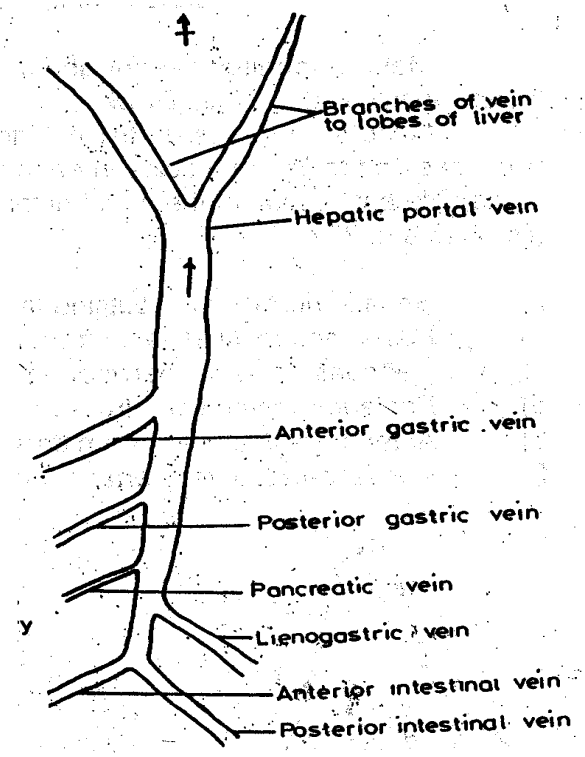


Fig. 18.4 *Scoliodon terrae navae* – schematic diagram of hepatic portal system

UNIT 4 DISSECTION OF TOAD - MUSCULATURE, ALIMENTARY CANAL AND URINOGENITAL SYSTEM

Materials

Freshly killed common African toads (*Bufo regularis*), dissecting boards or waxed dishes, dissecting kit, thread.

Procedure

1. Musculature of body wall

With the ventral side of the toad facing up, extend the limbs and pin each limb down on a dissecting board with an awl or 2.5cm thin nail. Using a fine foreceps, lift the loose skin from the belly and make a small cut on the midventral line making sure not to cut underlying muscles and organs (Fig. 19.1)

While holding up the skin with a pair of foreceps, continue the cut along the mid-ventral line to just below the tip of the lower jaw. Make a similar cut from the first small cut to the cloacal opening. Continue the cut from the midventral line to the knees, and from the mid-ventral line to the elbows.

Free the flap of skin from the underlying musculature by cutting the loose connective tissue with a scalpel. Identify the underlying muscles (Fig. 19.2)

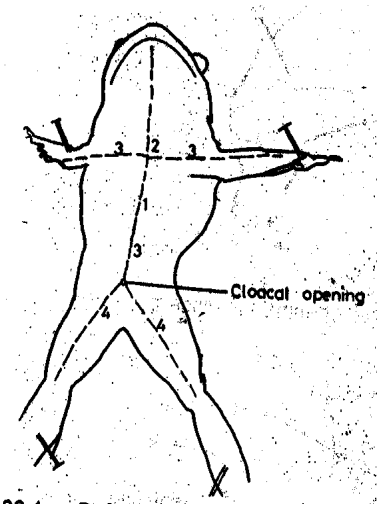


Fig. 19.1 *Bufo regularis* – position of cuts for opening the skin numbered in orders of cuts

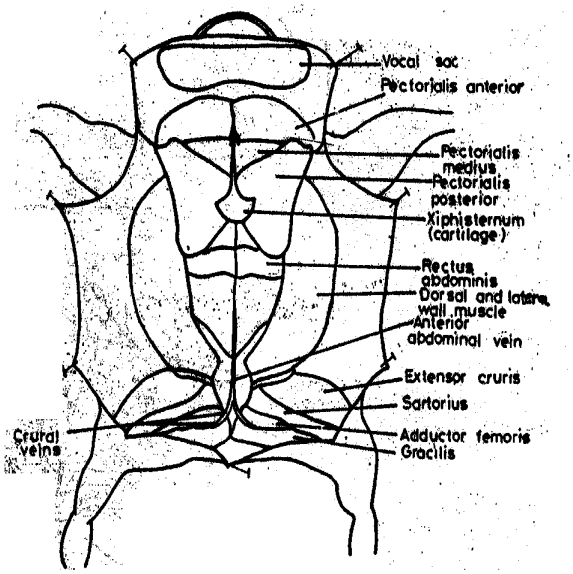


Fig. 19.2 *Bufo regularis* – musculature of body wall of male; vocal sac is seen only in males

2. Alimentary canal

Lift the abdominal muscle near the anterior abdominal vein and make an incision to the right of the vein with care, so as not to damage the underlying organs. Make similar cut to the left of the vein (Fig. 19.3). Continue each cut into the pectoral girdle and cut through the bones of the girdle the coracoid, and the clavicle, with a big scissors.

From the point of first incisions near the anterior abdominal vein, cut backwards to beyond the cloacal opening. Cut this central strip of muscle just anterior to the pectoral girdle and free same from the underlying connective tissue and the anterior abdominal vein. Be careful not to hold the blood vessel with foreceps when freeing it from the strip of tissue as this will damage the vessel. Cut the muscular body wall laterally towards the posterior pectoral girdle and to the posterior end of the body. Pin aside the two flaps of muscles on each side of the body.

Make a cut with a large scapel or scissors through the middle part of the pelvic girdle to expose the rectum. Pull the legs apart by removing the awls or nails from the dissecting board and refasten them in the new extended positions. Trim of any muscles obstructing the rectum from view. Identify the following:

i the heart covered by pericardium and located in the pectoral girdle area;
ii the liver which is a dark red mass posterior to the heart;
iii the gall bladder which is a greenish sac under the right lobe of the liver
iv the lungs are pink sacs dorsal to the liver

v. the intestine and rectum are on the right side of the liver;
vi. the intestine and rectum are on the right side of the liver;
vii. the kidneys are located in the posterior region of the cavity;
viii. the testes or ovaries are located in the posterior part of the body cavity;
ix. the truncus arteriosus lies on top of the auricles and branches into the two major arteries (Fig. 19.5)

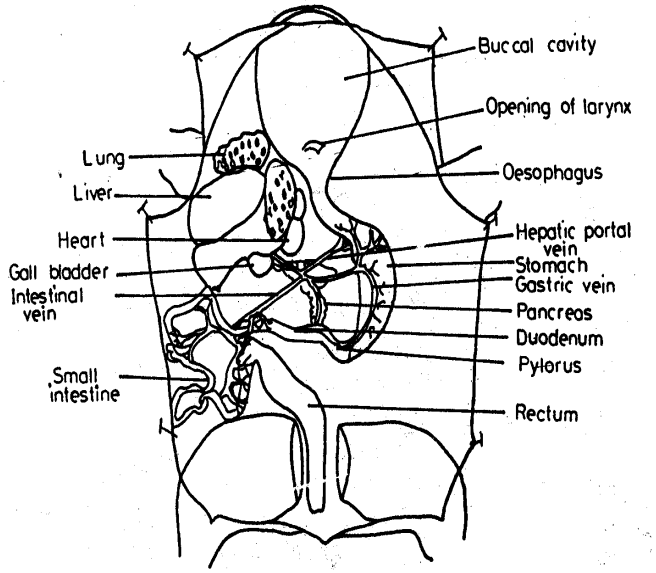


Fig. 19.3 *Bufo regularis* – alimentary canal and associated structures

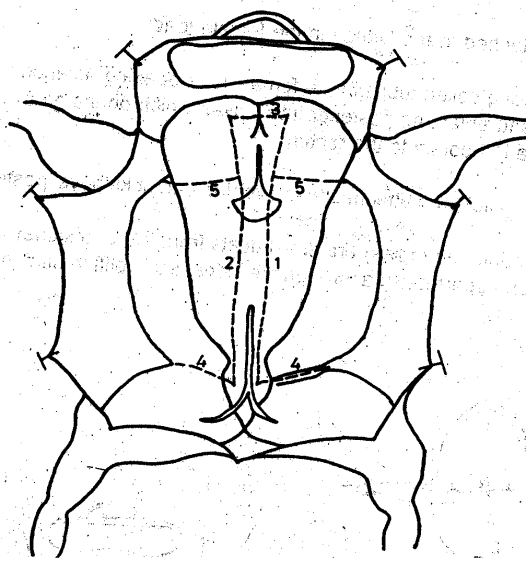


Fig. 19.4 *Bufo regularis* – position of cut for opening the viscera

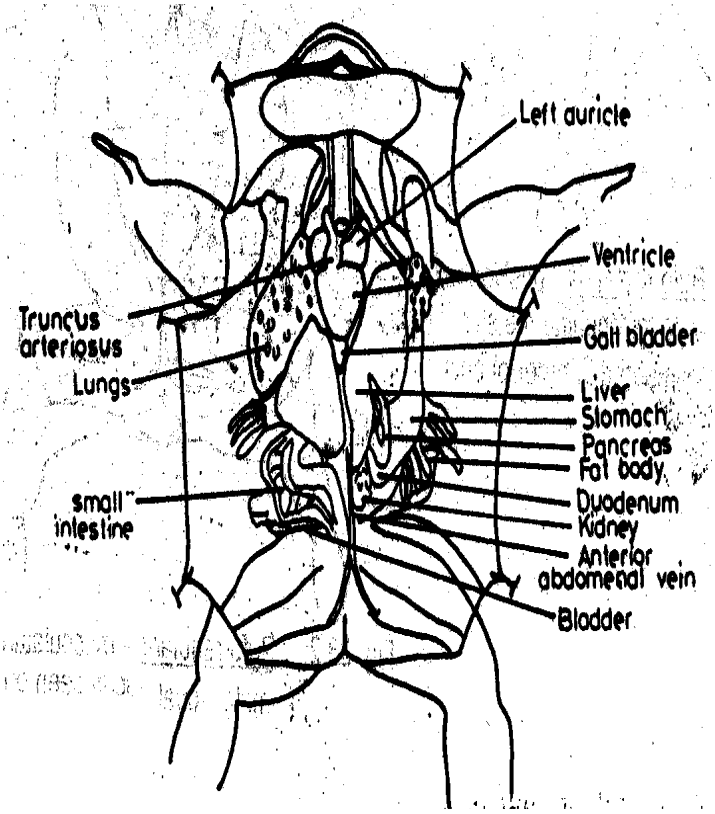


Fig. 19.5 *Bufo regularis* – the viscera in situ

3. Urinogenital System

Ligate the anterior abdominal vein in two adjacent positions and cut the vessel between the knots. Cut through the rectum and oesophagus to remove the gut. With a scalpel and forceps. Free the bladder from the wall. Lay the left lobe of the bladder over on the right lobe and dissect away the rectum from underlying urinary and genital duct. Display parts of the urinogenital system.

If your specimen is a female toad, cut through the mesovarium (connecting tissue from ovary to dorsal body wall) on one side and remove one of the very ovaries. Identify the following:

i kidney on the dorsal side of body cavity;
ii adrenal body - a strip of tissue of lighter colour on the ventral side of the kidneys;
iii wolffian duct runs from the kidney to the dorsal side of cloaks;
iv vesicular semialis the expanded posterior part of wolffian duct; v bladder, abilobed sac on the ventral side of the cloaca;
vi ovaries attached to the kidney in the female;
vii oviducts long coiled tubes in the female toad opening anteriorly near the base of the lung and enlarging posterior into ovisac which opens on the ventral side of the cloaca but dorsal to the rectum;

viii testes (in males) are attached to the ventral part of kidneys, posterior to fat bodies;

Vasa efferentia (in males) are small ducts from testis to kidney. These carry spermatozoa through the kidneys to wolffian duct (Figs 19.6 and 19.7)

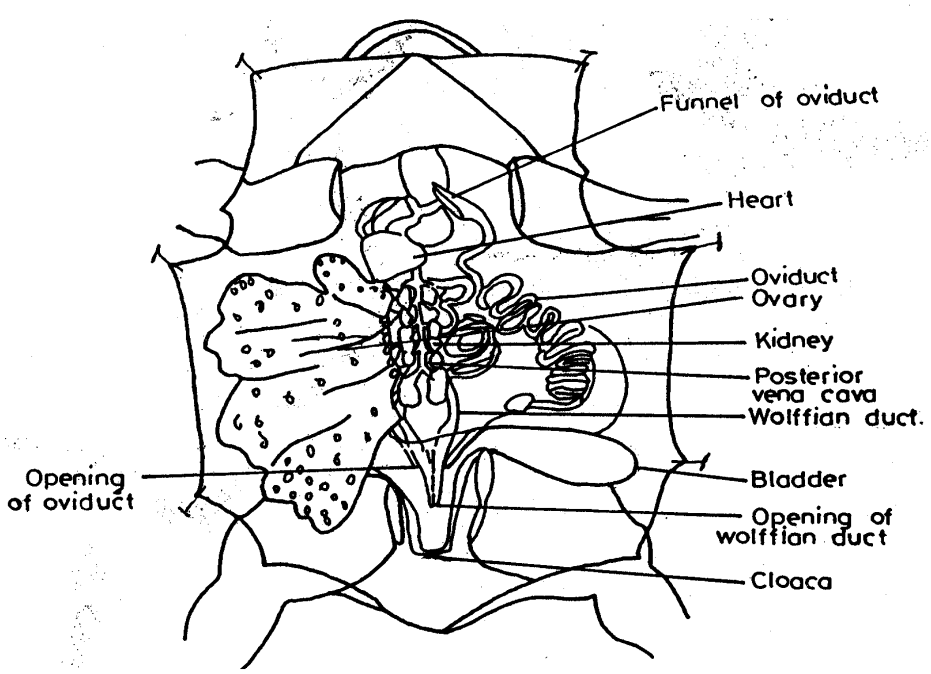


Fig 19.6 *Bufo regularis* – female urino-genetal organs with left ovary removed

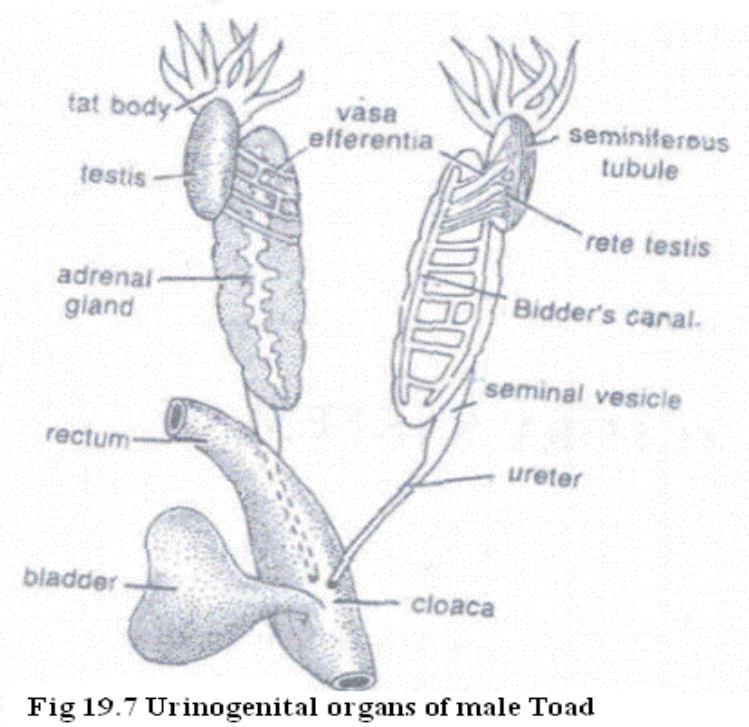


Fig 19.7 Urinogenital organs of male Toad

UNIT 5 DISSECTION OF TOAD - ARTERIAL SYSTEM

General Remarks

The blood vascular system comprises the fluid and the structural elements. The structural elements are the heart and the blood vessels (veins, arteries and capillaries).

The heart of a toad is an s-shaped tube consisting of two parts: the receiving thin walled anterior portion and the thick walled propelling posterior section. The receiving portion is provided with a sinus venosus which joins the ducts of cuvieri and merges anteriorly into the atrium. The anterior portion is completely demarcated into two thin-walled atria or auricles. The propelling section, the ventricle, is not so demarcated but is adequately provided with strands which attempt at keeping apart the blood received from the left and right auricles. Blood leaves the heart into the general circulation through the ventral aorta or truncus arteriousus. This is typically regarded as part of the heart since it has the basic substance of the heart.

The blood vessels are simply conduits leading blood to and from the heart. The thin walled superficial veins lead blood to the heart whereas the deeper seated muscular arteries lead blood away from the heart.

Oxygenated blood from the lungs and skin is squeezed from the left auricle into the left side of the ventricle. On the other hand, the oxygen deficient blood is forced from the right auricle into the right side of the ventricle. The oxygenated and oxygen deficient blood are intricately held apart by muscular strands in the ventricle.

The oxygenated blood from the ventricle is driven into the truncus arteriosus, from which arise three aortic arches on each side. The carotid arch is the most anterior and gives rise to external and internal carotids all directed anteriorly.

The middle systematic arch passes obliquely round the oesophagus on to the dorsal side, to unite with its counterpart just in front of the paired kidney, to form the dorsal aorta. Arising from this arch are buccall, occipito-vertebral, the sub-clavian and oesophageal arteries. Lower down on the dorsal aorta, the coeliaco-mesenteric, urinogenital and iliac arcteries branch off.

The most posterior arch, the pulmo-cutoneous arch, breaks up into the cutoneous and pulmonary arteries.

Materials

Freshly killed toads, dissecting board, dissecting kit, etc

Procedure

Open up the animal as described in unit 4 of this module. The arteries lie dorsal to the veins and are more deeply seated. It is therefore essential to litigate the major veins for good display of the arteries. The two major veins normally affected are anterior abdominal vein and the anterior vena cava. Ligature is achieved by tying two knots (Smm apart) round the vein and cutting through the region between them (fig. 20.1). This reduces blood loss and collapse of the vessels. Locate the following arteries.

Truncus arteriosus and branches

Display the heart to expose the truncus arteriosus. This runs deep into the anterior region. Soon after leaving the truncus arteriosu it bifurcates to give rise to the external carootid, supplying blood to the base of the muscle, of the tongue and the internal carotid that extends deep into muscles, passing beneath and forward to enter the skull at the side of the pro-otic bone.

Systemic arches

The left and right systemic arches bend backwards to join just beneath the position of the stomach to form the dorsal aorta. Before uniting into the dorsal aorta, each gives the following.

(a) the buccal artery supplying blood to the buccal cavity;
(b) the subclavian artery running to the muscles of the fore limb; and
(c) the occipito-vertebral artery emanting from the junction of the subclavian and the main arch to supply blood to the pharynx.

Dorsal aorta and branches:

The dorsal aorta gives off the following arteries posteriorly.

i. the coeliaco-mesenteric artery - this further divided into two, the celiac artery to the stomach, the pancrease, and the intestinal artery which further breaks up into anterior mesenteric to the upper intestine, posterior mesenteric to the lower intestine and rectum, and splenic artery to the spleens.

ii. Three pairs of urinogenital arteries supplying blood to the kidneys, gonads,and fat bodies. In the male they arise as three main branches from the dorsal aorta before bifurcating to the left and right branches arise independently from the main aorta.

iii. Terminally, in the region of the pelvic girdle, the dorsal aorta bifurcates to supply each leg as the iliac arteries. The iliac artery continues downwards to curve under the leg muscles of the femur as the sciatic artery. Before then, however, it branches into the hypogastric artery just at the upper part of the girdle before dividing into the vesical artery to the bladder and epigastric to the lateral body wall.

Pulmo-cutaneous: The most posterior arch, the pulmo-cutaneous arch, is rather short and must be traced with care. It breaks up into two, the cutaneous artery supplying blood to the skin of the head and body and the pulmonary artery supplying blood to the lungs, (Fig. 20.2)

Draw your own dissection and label fully.

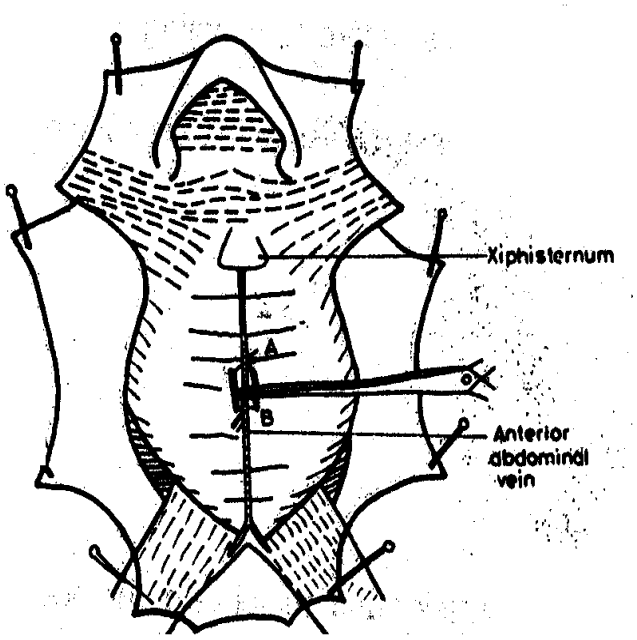


Fig. 20.1 Dissection of toad

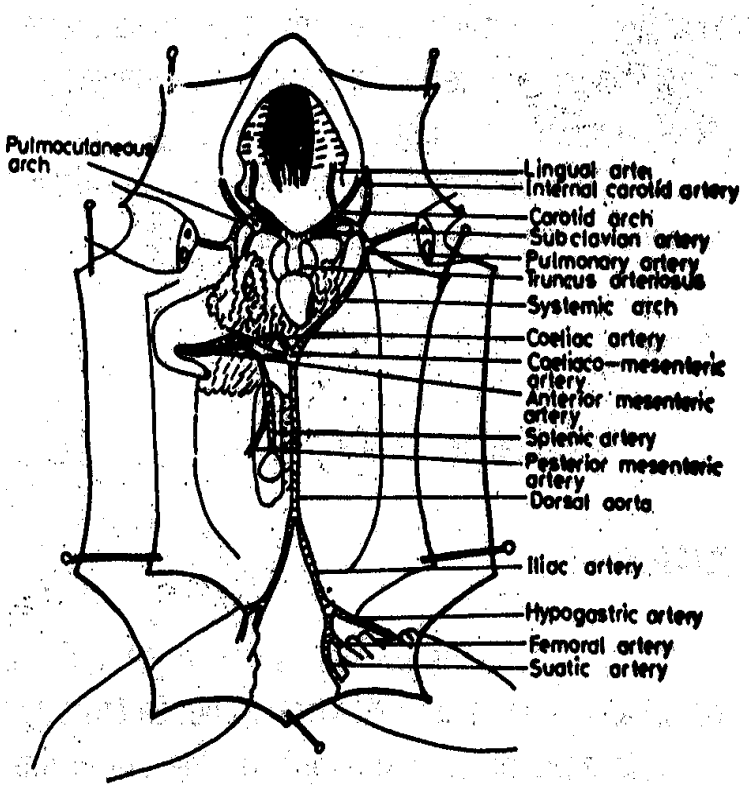


Fig. 20.2 Toad – arterial system

MODULE 5

Unit 1 Dissection of Toad – Venous System
Unit 2 Dissection of Toad – Nervous System
Unit 3 Dissection of Lizard – General Anatomy
Unit 4 Morphology of Higher Vertebrates – Birds and Mammals
Unit 5 Dissection of the Mammal – Rat, Guinea Pig, Rabbit

UNIT 1 DISSECTION OF TOAD - VENOUS SYSTEM

General Remarks

Two major vessels return blood to the heart. They are the venae cavae and pulmonary veins. These are helped by two portal systems to ensure complete and successful drainage of all blood to the heart.

The anterior vena cava is formed from the merging of three major veins with six branches draining blood from the anterior region of the toad. The most anterior, the external jugular, is composed of the lingual and mandibular veins. The middle branch the innominate veins, comprises the internal jugular and the subscapular veins. The posterior branch, the subclavian, is made up of the branchial and the musculocutaneous veins.

The posterior vena cava is a large vessel and begins posteriorly in between the kidneys. As it travels anteriorly, it receives the renal veins and either the spermatic or the spermatic or the ovarian veins. Anterior to the liver, it receives the hepatic veins before terminating in the triangular sinus vernosus.

The typically short pulmonary veins drain from the lungs and pass to the left auricle to unite before entering the heart.

Material

As listed in module 4 unit 5

Procedure

Open up the animal as described in Module 4 unit 4. The anterior abdominal vein can now be clearly seen in the mid-ventral line. Cut through the abdominal wall on either side of the anterior abdominal vein. Continue the cut in anterior and posterior directions to the pectoral girdle and leg muscles, respectively. In the anterior region, cut through the coracoid and clavicle of the pectoral girdle. Pick the mid-ventral strip just below the buccal region and cut to free it from the surrounding

body wall. Using a scalpel, detach the anterior abdominal vein from the strip. The heart can be seen lying beneath the pectoral girdle covered by the pericardium. With the aid of a pair of forceps and a pair of fine scissor, cut open and remove the pericardium to expose the thin walled atria and the thick muscular ventricle. Turn the heart to one side to expose the bulbous sinus venosus with the venae cavae emptying into it.

Slowly and gently remove the mesenteries and cut adhering muscles to the veins in the anterior venae cavea. Warning : Do not pick up the veins with forceps, instead pick cut the surrounding tissues. Locate the following:

(a) The lower branch of the left vena cava, the subclavian vein, draining blood from the fore limb through the branchial vein, and the skin and muscular body wall, through the muscuao-cutaneous vein;
(b) The mid-branch of the left vena cava, the innominate vein, which returns blood from the skull and the fore limb through the internal jugular and subscapular veins, respectively;
(c) Finally the most anterior, the external jugular vein, draining blood from the tongue and the lower jaw through the lingual and mandibular veins, respectively. Starting from the sinus venous in the posterior direction, locate the following:
(e) The posterior vena cava, emptying directly into sinus venosus. Immediately at the base of the sinus venosus the posterior vena cava receives the left and right hepatic veins draining blood from the liver.
(f) The posterior vena cava continues downward to terminate near the base of the paired kidneys. The renal veins drain blood from the kidneys into the posterior vena cava. This large also drains blood from either the testis or ovary through the spermatic or ovarian veins.

Turn the lungs to expose the pulmonary veins that unite by the left auricle. They are short and usually obscured by the anterior vena cava. Trace the terminal end of the anterior abdominal vein from where it drains into the left and right liver. From here, advance to the pelvic region and carefully tease out the muscles to show the pelvic veins that unite to give rise to the anterior abdominal vein. Immediately after the junction, the vescical vein from the bladder and the rectal vein join the anterior abdominal-vein.

Locate the hepatic portal vein that drains blood into the liver near the point of bifurcation of the anterior abdominal vein. It has two main branches:

(a) The intestinal vein from the rectum, intestines, spleen, and oesophagus,and
(b) The gastric vein from the pancreas and the stomach.

The renal portal veins drain blood into the lateral sides of the kidneys.

They transport blood from the sciatic and femoral veins of the legs and the dorso-lumbar veins of the back muscles. Draw your own dissection and label fully (fig. 21.1).

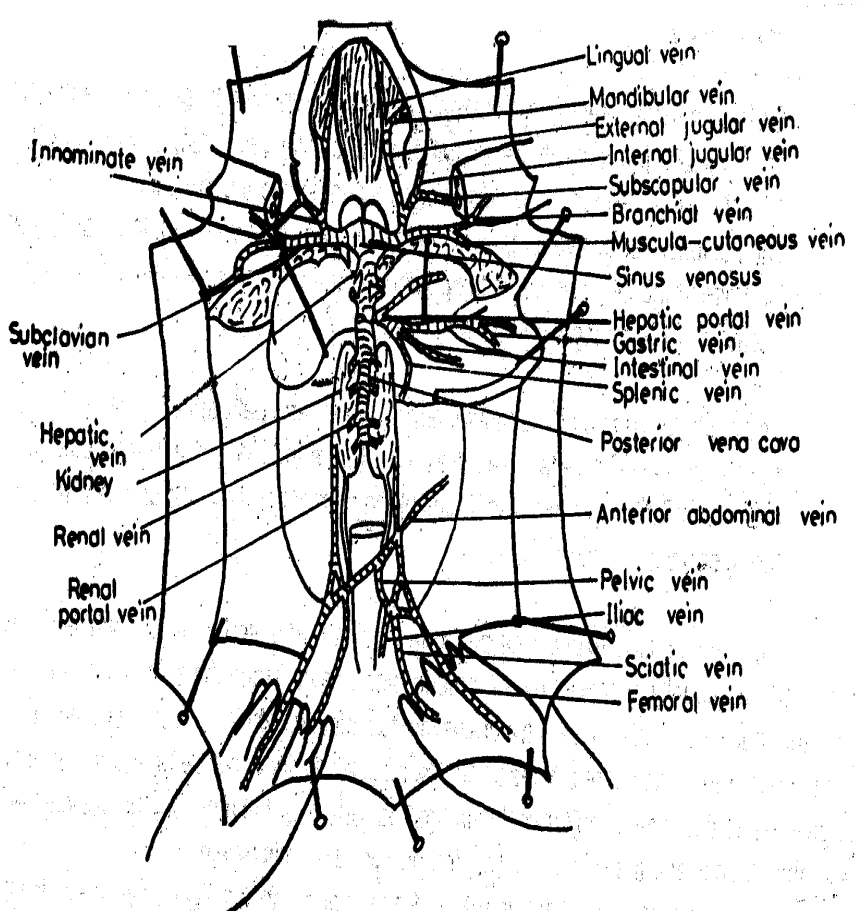


Fig. 21.1 Toad – venous system

UNIT 2 DISSECTION OF TOAD - NERVOUS SYSTEM

General Remarks

The nervous system is made up of three main components:

1. The central nervous system made up of the brain and the spinal cord:
2. The peripheral nervous system comprising all the verve tissues outside the brain and the spinal cord and serves in the inter-connection of sense organs, sense cells on the surface of the body and muscles of the body wall and limbs to the central nervous system;

    (a) The autonomic nervous system made up of these nerves arising from the brain and spinal cord and passing to the viscera. Functional peculiarities separate this system into two: the sympathetic comprising nerves from the spinal cord in the neck, thoracic and lumbar regions, and the parasympathetic, comprising nerves from the brain and the spinal cord in the sacral region.

Materials

The specimens used for Module 4 unit 5 and Module 5 unit 1 but preserved in 5% formalin, dissecting boards, dissecting kit etc.

Procedure

Brain and Spinal Cord: Dissection of the brain and the spinal cord is done usually from the dorsal aspect and preferably on specimens previously preserved for a week in 5% formain or 70% alcohol.

Place the toad on the dissecting board with the dorsal side upwards. Cut away the skin of the head completely. Using a sharp scalpel in a horizontal position cut carefully to the sides and raise up the roof of the cranium leaving the brain intact.

The dorsal brain shows that most anterior left and right olfactory lobes with tiny projections at the tip, the olfactory nerves. Just beneath them are the larger and more elongated cerebral hemishpheres. Behind these is the thalamencephalon in which is situated the pineal body. Two almost circular bodies, the optic lobes, come next and joint with the disc shaped cerebellum attached to the medulla oblongata. This medulla oblongata narrows to the spinal cord to terminate finally in the tiny but long fillium terminate.

To expose the spinal cord, continue the cut backwards after removing the overlying muscles. Cut through each of the neural arches and remove the roof of the neural canal/neural spines. Next, remove the darkly pigmented membrane, the pia mater, to expose the different parts of the brain and the spinal cord.

If a ventral view is required, then gently ease out the brain and the spinal cord from their casket to a display the anterior olfactory lobes with the fine flask shaped olfactory nerves cutting out at the anterior end with the narrow ends. This is followed by the cerebral hemispheres with the thalamencephalon located in the posterior middle, anchored by the optic chiasma with their extended optic nerves. Under the curvature of the optic chiasma in the posterior position are the two circular more anterior infindibulum and posterior hypophysis. Both make up the pituitary body. This leads down the spinal cord to the fillium terminale (Fig. 22.1 and 22.2).

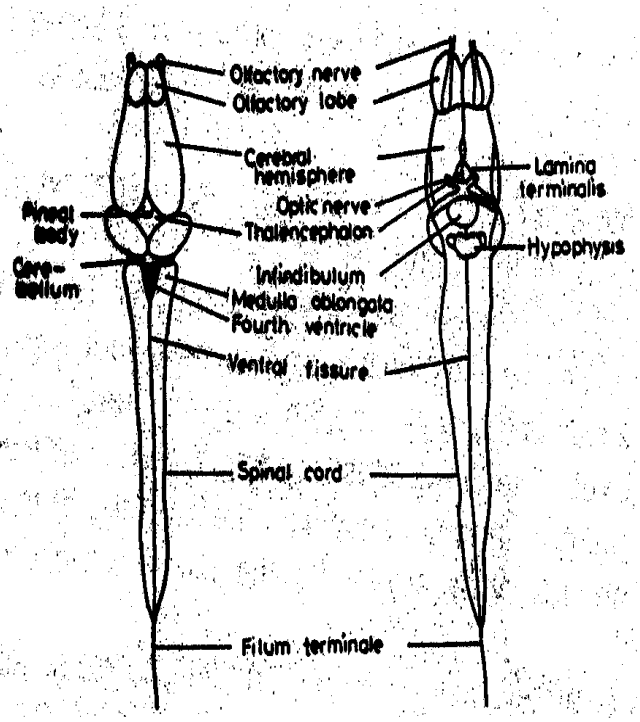


Fig. 22.1 and 22.2 Toad – dorsal and ventral view of the brain

Cranial nerves

Unless the specimen was previously preserved in 5% formation or 70 % alcohol, flush the brain and the cranial nerves with 70% alcohol as soon as they are exposed. This gives the nerves a whiter colour and are easily demonstrated.

The cranial nerves are ten in number:

1. The olfactory nerves extend anteriorly from the olfactory lobes as single strands that arborise slightly to supply the olfactory gland.
2. From the side of the brain ventral to the lobes arise the optic nerves. They partially cross over to form the optic chiasma before entering the eye.
3. Four muscles move the eye up and down, from side to side, or in an oblique direction. These muscles are supplied by the oculomotor nerve located on the ventral surface of the mid brain.
4. Another small nerve, the pathetic nerve arises from the dorsal side of the brain to pass to the oblique muscle of the eye.
5. The trigEmial nerve arises at the side of the brain at the anterior end of the medulla and breaks up in the region of the auditory capsule with two branches: the ophthalmic nerve supplying the skin to the fore part of the head and the maxillo-mandibular which immediately splits into the maxillary and mandibular nerves supplying the upper and lower jaws, respectively.
6. The abducens nerves arises from the ventral surface of the medulla to enter the rectus muscle of the eye.
7. Just behind the trigEmial arises the facial nerve. It passes forward in the anterior direction with the trigEmial before breaking up into two: the anterior palatine supplying the roof of the mouth and the posterior hyomandibular. This receive a branch from the 9th cranial nerve to give rise to a small mandibular just above the angle of the jaw and runs forward as the hyoidean nerve to supply the floor of the mouth.
8. The auditory nerve arises from the side of the medulla by the facial nerve to enter angle of the jaw and runs forward as the hyoidean nerve to supply the floor of the mouth.
9. The glossopharyngeal nerve arises from the side of the medulla next to the auditory nerve. It is normally closely associated with the 10`h cranial nerve. It has two branches: the anterior branch joins the facial nerve while the main branch continues outwards giving off a small branch to the buccal cavity. The main branch continues to the muscles of the tongue.
10. Finally, behind the glossopharyngeal lies the vagus or pneumogastric nerve. The main nerve gives off initially a posterior branch to the muscles of the black and further on an anterior branch to the musculature of the lateral body wall. The main nerve moves round the body to break up to send two branches to the gut, one to the lung and yet another to the heart.

Spinal Nerves

The vertebrae and the urostyle are each associated with one pair of spinal nerves. Hence there are ten spinal nerves.

1. The first or hypoglossal can easily be traced to the floor of the mouth where the pair run side by side through a circuitous journey to end in the tongue.
2. The second or branchial nerves arise from between the 2nd and 3rd vertebrae and pass straight to the muscles of the shoulder and arm and is the major component of the branchial plexus.
3. The third spinal nerves arise from between the 3rd and 4th vertebrate, moves forward and outward and just after receiving a small branch, the branchial nerves passes backward to the skin and muscles of the body wall.

4-6 These arise from 4th and 5th, 5th and 6th, 6th and 7th vertebrae, respectively, to supply the musculature and the skin of the body wall.

7-9 These arise from the 7th and 8th, 8th and 9th and urostyle, respectively. The 7th nerve sends a small nerve to the crural nerve and then becomes the ilic-hypogastric, supplying the musculature and the skin of the belly. The 8th and 911 h usually anastomose to form the sciatic plexus. The 8th divides, sending a branch to the 9th the major arm continues as the crural nerve, supplying the muscles of the thigh. The nerve emerging from the plexus is really the 9th the sciatic nerve and supplies the musculature of the leg.

11. The tenth or coccygeal nerves are rather small nerves contributing to the sciatic plexus before running to the dorsal body wall just above the cloaca (fig. 22.3)

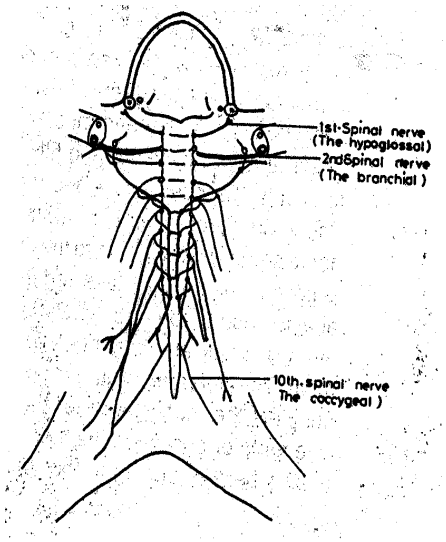


Fig. 22.3 Toad – spinal nerve

The Autonomic Nervous System

On either side of the vertebral column lie two strands of nervous tissue, the autonomic nervous system. The sympathetic trunk possesses segmental ganglia from which short branches, rami communicantes, connect the trucks with the corresponding spinal nerves. Sympathetic nerves are seen to arise from the ganglia and pass to the viscera and the blood vessels.

The first sympathetic ganglion has a trunk adjacent to the hypoglossal nerve. This trunk breaks up and rejoins it mate to form a ganglion in association with the 10th cranial nerve. Sympathetic nerves from ganglia 4 and 5 form the solar plexus in the region of the coelicomesenteric artery. From this plexus, nerves pass on to the viscera. Sympathetic nerves from ganglia 6, 7, 8 and 9 are located mainly in the kidneys, gonads and blood vessels. The 101h cranial nerve is connected to the sympathetic chain by a branch from the 9th sympathetic ganglion.

Having displayed the structures listed above, draw the dorsal and ventral views of the brain and relate the sizes of the various parts to the functions they perform.

Draw the brain and the spinal cord and show clearly the cranial and spinal nerves.

UNIT 3 DISSECTION OF LIZARD - GENERAL ANATOMY

General Remarks

The aim of this exercise is to display as many internal organs and systems of the animal as possible and observe the relationships that exist.

Materials

Freshly killed lizard (*Agama agama*) dissecting board, dissecting kit, pins, etc

Procedure

Pin the specimen with its back down onto the board. Lift the skin at the mid-ventral surface near the cloaca with a pair of fine forceps and make an incision on the skin. Extend the incision anteriority to the tip of the lower jaw and along the fore and hind limbs (Fig. 23.1). Separate the skin carefully from the musculature on the ventro-laterial surface head and limbs and pin it down to the board. Notice the anterior abdominal vein beneath the abdominal muscles. Cut open the abdominal cavity up to the pectoral girdle and transversely and then pin the body wall muscle onto the board. Ligate the anterior abdominal vein as usual. Insert one arm of the scissors through the cloaca and cut the pelvic girdle at the pubic symphysis to expose the rectum and cloaca. Some of the contents of the body cavity would have now been exposed. Then cut through the sternum and the pectoral girdle and remove as much tissue as possible to expose the heart and lungs. The heart is enclosed by the pericardium. Remove the pericardium from the heart and around the great vessels of the heart. The contents of the thoracic and abdominal cavities would have now been exposed. Notice the absence of a partition between the abdominal and thoracic cavities.

Draw the organs in situ. Push the gut to one side and display it carefully (using pins as necessary) but do not break or destroy the associated blood vessels. This will enable you to see other internal organs.

Examine the digestive system and the associated blood vessels. Identify the lobed liver at the anterior part of the body cavity, with the gall bladder on the right lobe, the lungs on either side of the heart, the narrow oesophagus, the tubular stomach (push the liver one side or cut part of it if hidden by the liver), the duodenum, the coiled small intestine, the short rectum (large intestine) which opens into the cloaca. Note the small caecum between the small and large intestine. Identify

the pancreas located between the stomach and the duodenum, the spleen held by mesentery connecting the stomach and the body wall.

Trace and clean the major blood vessels associated with the gut, the hepatic portal vein, the coeliaco-mesenteric artery (from dorsal aorta to the stomach, spleen, and intestine), the posterior intestinal and rectal arteries. Draw your dissection (Fig. 23.2).

Display of the Urinogenital System

It may be necessary to cut off the rectum in order to expose the urinogenital system clearly. Identify the paired gonads (testis or ovary on either side of rectum, the gonadic ducts (epididymis and vas deferences in the male, and the oviduct in the female). Trace the gonadic ducts to the cloaca. Note the positioning of the right and left gonads,

Identify the paired kidneys on the dorsal body wall at the base of the hind limbs and the ureter. You may have to remove the peritoneum covering the kidneys to see the ureters clearly. Trace them to the cloaca (Fig. 23.3). Note the blood vessels associated with the system. Have a close look at the dissection of a specimen different in sex from your own. Draw.

Display of the blood vascular system

Note the dorsal aorta along the mid-dorsal body wall with its branches to the gut and gonads (already seen) and to the body wall. The inferior (posterior) vena cava seems to pass through the liver and divides into the right and the left auricles, the ventricle and the sinus venosus. Raise the right and left anterior vena cava. Identify the three arterial branches on the anterior-ventral surface of the heart-the pulmonary arch and the right and left systemic arches. The right systemic arch is slightly to the left side. The right and left systemic arches join in the body cavity to form the dorsal aorta (already seen) (Fig. 23.2)

Other Structures

Other structures worthy of note are the trachea (lungs had already been identified), the vertebral column along the mid-dorsal line and the fat bodies.

Draw your dissection.

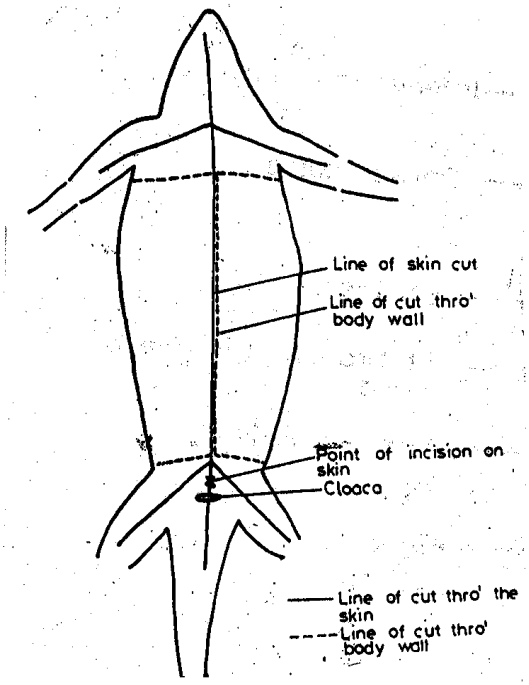


Fig. 23.1 Dissection of *Agama*

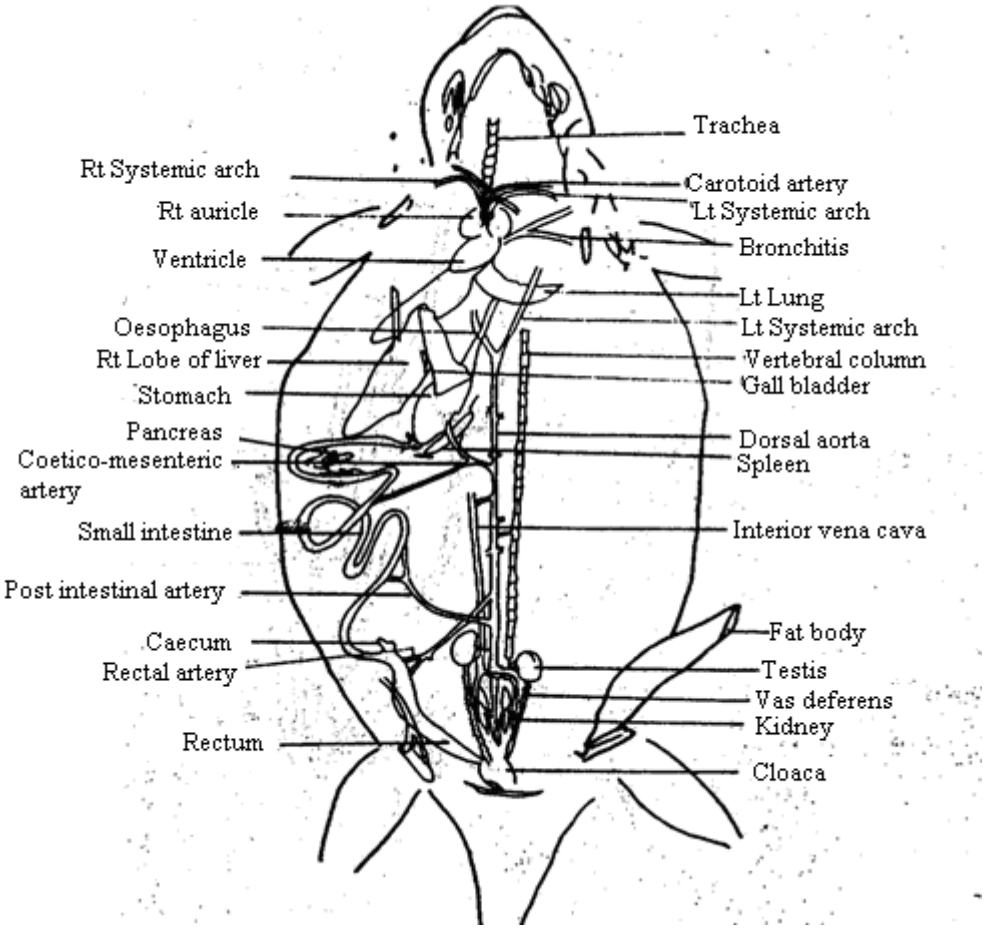


Fig. 23.2 Internal anatomy of *Agama*

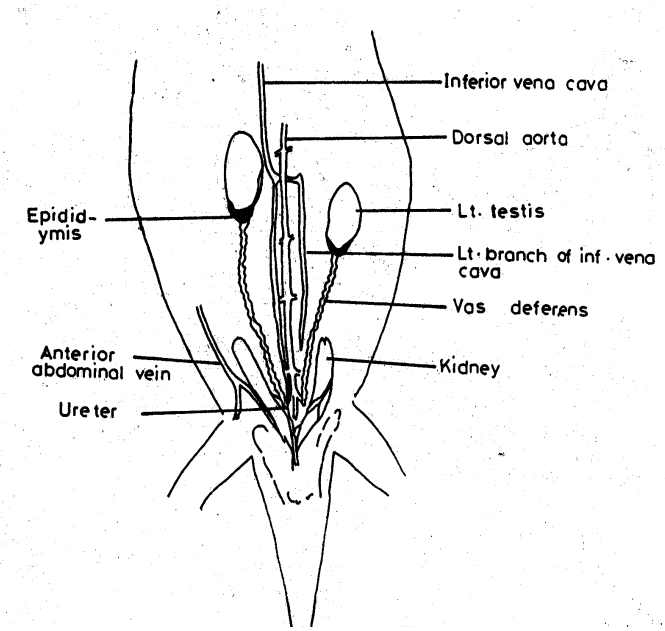
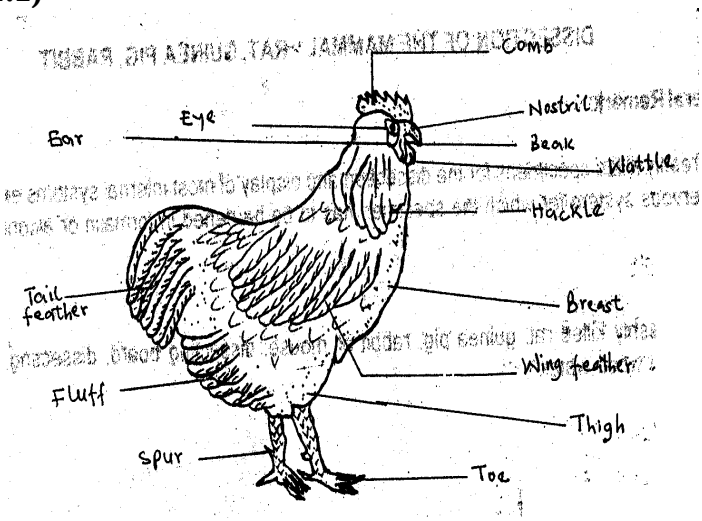


Fig. 23.3 Urinogenital system of male *Agama*

UNIT 4 MORPHOLOGY OF HIGHER VERTEBRATES - BIRDS AND MAMMALS

General Remarks

Birds (Fig 24.1)



Birds (Fig. 24.1)

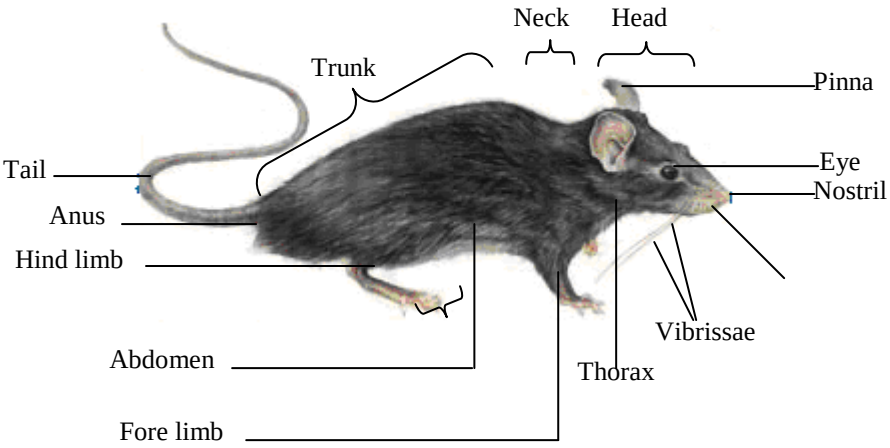


Fig 24.2 External features of *Rattus rattus*

Birds (Aves) resemble reptiles in that they have a shelled egg and possess scales, but are different in possessing feathers and wings. They are warm blooded. The brain is large and so they show elaborate behaviour patterns especially in connection with their reproduction. Nesting and feeding of the young are aspects of the parental care which is very highly developed. In birds, the fore limbs are modified as wings thus making them bipedal. The centre of gravity lies over the hind legs.

Both auricles and ventricles are completely divided. The body is covered by feathers. The body can be divided into three parts:

Head

Generally birds have small heads and prominent beaks. Beaks are modified according to the food habits of birds. On each side of the base of the beak is a swollen area of soft skin - the cere, surrounding the nostril. The eyes are very large and each is guarded by upper lower eye lids and a transparent nictitating membrane. Some distance behind the eye is located the auditory aperture concealed by auricular feathers. This leads to the external auditory meatus, closed below by the tympanic membrane.

Trunk

The trunk bears the fore limbs (wings) and hind limbs (legs) (fig. 24.1). The wings of birds snow the three typical division of the fore limb: upper arm, fore arm, and hand. The parts of the hand are closely bound together by skin and only three imperfectly marked digits, with the second being much larger than the first and third. During rest, the three divisions of the fore limb are bent upon one another in the form of the letter z. During flight, they are straightened out at a right angle to the trunk. The alar membrane (prepapatagium) is a fold of skin between the upper arm and the fore arm. The postpatagium lies between the proximal portion of the upper arm and the trunk.

In the hind limb, there is a short thigh which is closely bound to the trunk. There is also the long shank which extends to the knee. The foot is divided into the tarso-metatarsus and four digits of which one, the hallux, is directed backwards.

Tail

The true tail is short and is called the uropygium. This structure gives origin to the series of large feathers (rectrices) which are usually called the tail. On the dorsal surface of the urogygium is a pailla with the opening of the oil gland.

Mammals (fig 24.2)

Mammals are the dominant animals on land and are also successful in the sea (e.g. Whales, seals, and dolphins), and in the air (e.g. bats). They are warm-blooded and are covered with hair which provides insulation. They have four chambered hearts and the efficient gaseous exchange is made possible by the presence of a diaphram.

Characteristic features

All mammals possess dermal milk glands. Only mammals possess this structure from which the name mammal is derived. They are generally viviparous and show parental care. The body of all mammals comprise the head, neck trunk, and tail (fig. 24.2). The head has a pointed snout, nostrils, a narrow mouth with short lower jaw, two small eyes that can look diagonally, forwards, or side ways, except in the primates. There is also the ear lobe (pinna) and numerous whiskers (vibrissae) around the mouth. On the jaws of mammals are different types of teeth.

Materials

Live or mounted birds, assorted beaks adapted for seed cracking, insect catching, fish and frog catching and for tearing flesh; feet adapted for perching, walking, swimming, wading, scratching, tearing, and clinging; rats, rabbits, or mice.

Procedure

Draw the range of beaks and feet of birds provided, indicating (with reasons) what each of them is adapted for:

Name the bird or group of birds that possesses each of the structures. Draw the mammal (s) provided and list the main characteristics of the group as found in the specimen (s). Which of these characteristics have contributed to the success of the group?

UNIT 5 DISSECTION OF THE MAMMAL - RAT, GUINEA PIG, RABBIT

General Remark

Use freshly killed specimens for the dissection and display of most internal systems except the nervous system for which the specimen has to be hardened in formalin or alcohol.

Materials

Freshly killed rat, guinea pi, rabbit or mouse; dissecting board, dissecting set, pins, cotton wool, water.

Procedure

Opening up of the Animal

Place the animal in the position for dissection i.e. with the ventral side facing up, and pin down to the board. Make a small slit on the mid-ventral line of the abdomen, avoiding cutting through the underlying muscles. Pull the skin away from the body wall using your fingers. Extend the cut up to the lower jaw and down to the anal region. Free the skin from the fore limbs by turning it inside out and pulling on the elbow. Pull it as far down as the paw. Cut it away leaving a small portion of the skin to the paw area. Repeat the process for the hind limbs. While freeing the skin in the neck region, be careful so as to avoid rupturing the blood vessels, especially the jugular veins.

Notice the limits of the thorax and the abdomen and the parts of the alimentary canal showing through the thin abdominal wall. Lift the abdominal wall with forceps and make a slit in the mid-line, but be careful so that you do not puncture the underlying intestine. Cut upwards to the sternum and downward to the anal area. Cut sideways along the boundary of the abdomen and thorax very near the diaphragm.

Display of the alimentary System

Pin down the wall to expose the alimentary canals. The alimentary canals is much coiled in situ. Examine and draw the alimentary system in situ. Then display the duodenum and parts of the hepatic portal system. The intestine should be displayed away from the animal on the board (with important blood vessels intact) but not severed from the animal.

Lay the caecum over to the animals left side. Push back the lobes of the liver to expose the stomach. Pull the duodenum to the right. Display the duodenum in such a way that the pancreas becomes visible. Adjust the stomach and the liver lobes to expose the gall bladder and bile duct.

Draw the alimentary canal as exposed (Label fully)

The diaphragm can be displayed by pulling the liver away, allowing it to suspend in the falciform ligament holding it. Hold up the xiphoid cartilage and observe the outline of the lungs and heart. Also notice the posterior vena cava close to the falciform ligament.

Display of Unrinogenital systems

The urinary and reproductive systems are generally considered together. Sometimes the alimentary canal is cut off but if properly displayed way from

the rest of the body, it can be retained while examining the structures of the two systems.

Hold the liver away from the kidneys with a pin. Cut through the public symphysis. For a male specimen cut also through the wall of the scrotum. Remove the flap of muscle and bones to expose the bladder and urethra. Pin the bladder aside. Loosen the connective tissue in the pelvic region to display the ureters, vagina in female, and rectum; or the vasa deferentia, urethra and rectum in the males. Clear away any available fat from the ureters and kidneys Draw.

Display of heart, lungs, and anterior blood vessels

For the neck and thorax, place pins through the cheeks to hold the head steady and to arch the neck. Insert one blade of a pair of scissors through the lower part of the wall of the thorax and cut along one side of the thorax up to the clavicle. Turn the cut portion asides so that the lungs and heart can be seen. Take care so as to avoid puncturing any of them. Repeat the cutting exercise on other side of the thorax and remove the ventral par to the thorax. Tie a thread round the xiphoid cartilage and attach the other end of this thread to a pin near the tail. Then observe the structures within. After observation, loosen the large pectoral muscles carefully and cut away. Further, loosen and cut the small tare muscles. Clear the superficial connective tissue away from the external jugular vein and the anterior and posterior facial veins. Also loosen and cut away the mastoid muscle of the neck very close to the skull. Repeat all the processes on the other side.

Lay aside the sub maxillary salivary glands to expose the salivary ducts. Loosen the sterno-hyoid muscles from under the jugular anaestomosis. Cut away the stylo-hyoid and mandibular muscles and trim the edges of the cut thorax to the level of the anterior vanae cavae.

Slit and remove the entire pericarduium up to the aortic-heart junction. Pin the heart to the right. Loosen the connective tissue around the blood vessels and identify them.

Display of posterior blood vessels

Cut one ureter and one uterus in the female animal or one ureter and one vas deferens as well as the gubernaculums of the corresponding testis laying aside the testis in the male. Pin aside the vagina and rectum (or just the rectum in the male) then clear the connective tissue from the blood vessels. This exposes the abdominal blood vessels identify them and draw.

Note

It is possible to expose all or most of these structures in one animal and a comprehensive drawing made of the total dissection. The nervous system is best studied with preserved specimens.

Questions

1 List the blood vessels that make up the arterial system.
2. What are the organs associated with the alimentary system and what are their functions?
3. By means of annotated drawings compare and contrast the male and female reproductive organs.
4. What is the difference between urethra and ureter?

Examining the content of the abdomen of the Guinea Pig in Situ'

Whenever you are told to examine `in situ' it means that you should study the organs in their natural position without displacing any part. In the rat for example, you will see in Situ' the thoracic cage, and immediately below it, the lobes of the liver. Under the liver, you will see a large sac lying across the abdominal cavity. This is the stomach. Below the stomach on the left hand side is the spleen. The rest of the abdominal cavity is predominantly coils of intestine, the largest part of which is the caecum lying across the lowest part of the cavity.

The alimentary canal

Now you can use your hands, weaving your gloves to gently spread out the organs, especially the intestines to enable you see all parts of the alimentary canal. The oesophagus will appear below the diaphragm to join the stomach. Your course tutor will help you trace the rest of the alimentary canal and displace the organs for you to see them clearly.

The Excretory and Reproductive System

To reach this, you will have to remove the alimentary canal. Observe the position of the two kidneys on the wall of the abdominal cavity and note which of the kidneys is placed a little higher than the other. You may want to slice open a kidney to see the structure.

DISSECTION OF THE RAT TO SHOW THE FEMALE URINO GENITAL SYSTEM

The Respiratory System

Your course tutor will show you how to open the thoracic cage. You will have to cut the ribs. The two main organs in this cage are the heart and the lungs. You can also cut open the neck to observe the different structures - the larynx, trachea, bronchi, lungs and diaphragm.