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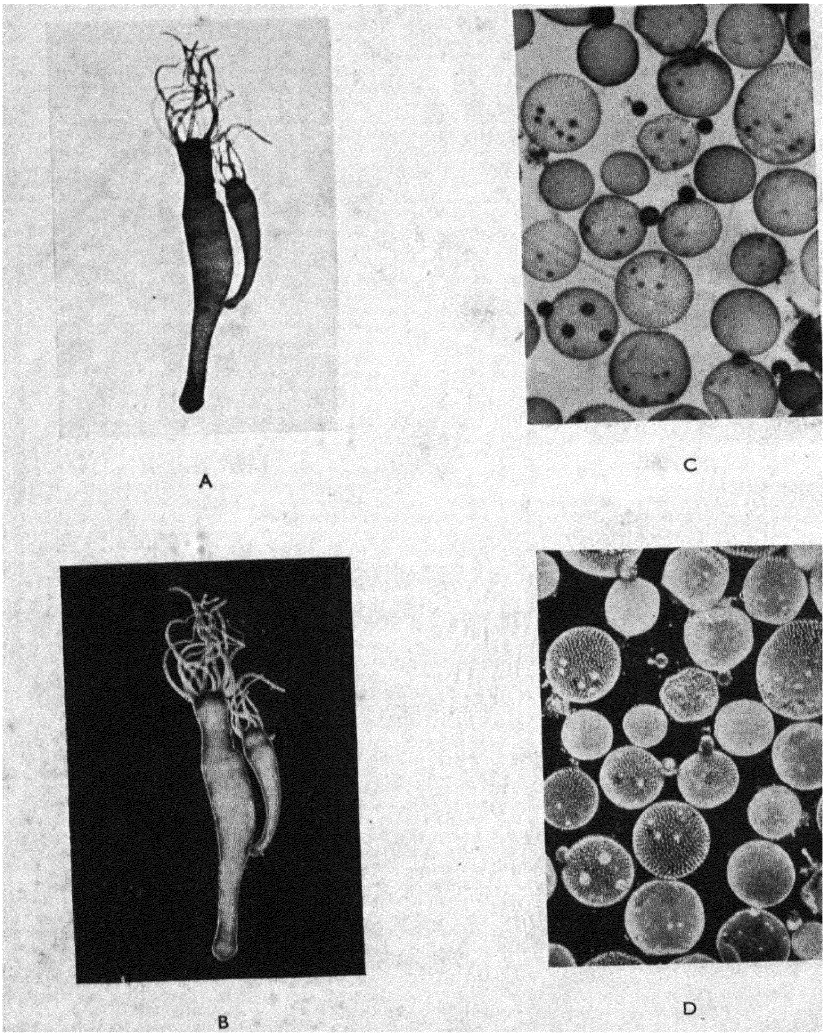
ZOOLOGICAL TECHNIQUE

PLATE I.



COMPARISON OF TRANSMITTED AND DARK GROUND ILLUMINATION.

The upper photographs by transmitted (direct) lighting.
The lower photographs by dark ground illumination.

A *and* B *Hydra* (×9)
C *and* D *Volvox* (×50)

(*Microphotographs by Messrs W. Watson and Sons, Ltd., London.*)

[*see page* 102.]

ZOOLOGICAL TECHNIQUE

FOR TEACHERS AND SENIOR STUDENTS

(ILLUSTRATED)

BY

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PREFACE

IN WRITING this book, an attempt has been made to fill a definite gap in educational books on Zoology. On many occasions it has been made clear that the teachers of Zoology, especially those newly appointed, and still more those who have to take up this subject as a second string, find themselves faced with a difficulty. Not only have they to possess a knowledge of the matter which they will teach, but they have now to do for themselves and their students much preparatory work which, while *they* were students was carried out by a laboratory steward. Thus they may be unaware where to buy material, how to kill, preserve and store it—they have no knowledge of that field which is comprised in the " hints and wrinkles " known to the lab-man.

The writer himself was in this predicament and as a result of the many difficulties he experienced the methods outlined later were acquired from many sources. The methods described will be found successful providing the teacher is prepared to give them all a fair trial. Some will doubtless feel that much has been neglected, but they should remember that what is given is primarily intended for those beginning almost *ab initio*.

T. L. GREEN.

The University, Bristol.

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To the Editor of the " School Science Review " for permission to embody material from articles which the author wrote for that Journal.

To Mr. W. B. Barker and the Editor of the " School Science Review " for permission to figure and describe the automatic aeration apparatus. (Fig. 9.)

To Messrs. Watsons & Sons who placed their advice at the author's disposal and who specially prepared the figures for the plates.

To The British Film Institute, The Central Information Bureau for Educational Films, G.B. Instructional Ltd., and Messrs. Kodak who provided data on sub-standard cinematography.

To many colleagues and teachers for information and ideas.

And finally to the publishers, Messrs. Allman & Son, Ltd., who have afforded much help and who have patiently suffered considerable delay.

FOREWORD

A GREAT deal has been written and said about the place of biology in education. In adding to this, the writer can plead that he has taught University students in their first year, and thus has formed some ideas about the good and bad points of their early teaching. Moreover, he has delivered series of lectures to prospective and practising teachers on the methods of teaching of zoology! He has paid visits to many different kinds of schools and seen what was done, and under what conditions.

As a result of this experience, slight as it is, several points have been made very obvious.

Many pupils reach a high standard of work, but others too often fail because their work shows lack of practice and lack of understanding of the principles involved. Time may be a limiting factor. But the teacher should always try to give reasons why things are done in a certain fashion. There must be a reason, and there must be a plan for each piece of work.

This tempts one to stray into the field of theory—and our demarcation of " theory " and " practical " is after all but a matter of convenience. The facts of zoology are organised into systems, there are principles or universals. Pupils no doubt all learn differently, but an effort should always be made to relate the details to the principle. Whether the learning process consists of fusing particulars into universals, or of analysing general principles into details is not a problem for a mere zoologist to discuss. The final aim, however, is to construct plans, principles, " laws "—what you will. Perhaps the final aim of the pupil (and too often impressed by the teacher) is to pass an examination. This, however, demands more and more not only mere memory and recall of facts, but the capacity to re-organise those facts—the capacity to make concepts. The pupil must be helped in doing so, and he must be given practice in doing so. Don't always set the standard question " Describe so-and-so." Make him compare things, get him to find out how things are related, how the same end may be achieved in different ways, or apparently similar things have different functions. Make him *think*.

That is the vital point. Those who would decry biology as a school subject generally do so because they say it is not an exact science, it has no " laws," it provides no training in method or technique, in fact it gives no training in " the scientific method." There is a moot point here about transference, but we must leave it alone, and confine our attention to the detailed charge against biology. Surely this results from a confusion, from an idea that " scientific method " is confined to the physical sciences, that it is a way of doing things. Is it not rather a way of thinking about things ? The habit of being critical, of thinking before speaking, of seeing relationships, and realising that a given situation has not only an actual but also a potential significance ? If this is true, then biology is a field for training in the technique of thought —but the teacher has to help make it so. It is far more difficult than merely dealing with simple numerical relations, because only a part of it is susceptible to practical proof.

Those who have discussed biology have begun to lay stress on the long neglected fact that it is said to be the study of life. Unfortunately, only by studying the dead animal can much of the field be approached. This should not mean that living things should never be seen. There are difficulties of course, but also there are ways and means of dealing with them. Among the most useful of these is the fact that children have an original interest in living things. And interest, the psychologist tells us, is the greatest incentive to effort; common sense provides a strong corroborative.

Only by dealing with living things shall we fulfil half the functions which some educationists seek in the teaching of biology. Is it at all likely that a knowledge of the structure of seeds or of the reproductive system of the earthworm will make any contribution to the solution of the sex problems which so many people attribute to childhood ? It appears much more probable that more will be learned by keeping animals, and in particular mammals, and by seeing them mate and reproduce. Even if this is so we have little reason to believe that knowledge alone will really solve any problems which do exist. But if it encourages a healthy habit of being able to understand and talk about such things as normal, then it may go a long way towards preventing such problems from ever arising.

For these reasons there are included some chapters which deal with the keeping of living animals. They may indeed be brief and incomplete, but any book which fails to go even as far as this, is not really in

line with the modern spirit and ideal in the teaching of biology. Few people are led to think about dead things, perhaps therefore our living animals will appeal to that natural interest of the child, and so give him problems to think about—the first function of education; it is hoped that he may then be taught how to think about them—education's chief task.

T. L. G.

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CHAPTER I

THE PREPARATION OF CLASS MATERIAL

THE various methods of dealing with class material, which are to be described in this chapter, are not the only ones available. They are, however, those which have always been found satisfactory, and they are those which are most suitable for a school laboratory.

AMOEBA

Purchase tubes of living material. Free living forms are collected from water samples. Intestinal forms can often be obtained from the host immediately after death. Culture is carried out by Sister Monica Taylor's method as follows. Make a food pabulum by boiling 20 grains of wheat in a litre of water. Cool and innoculate with amoebae. Further grains are added at monthly intervals. Sub-cultures should be prepared every term. The cultures are prepared in large glass jars with a raised glass cover to permit aeration, but to exclude dust.

It is essential that well stained permanent preparations are available. These may be purchased or prepared in the laboratory. Fixation is best carried out by steam or other heat, osmic acid vapour, absolute alcohol, formalin or Schaudin's fixative. For staining, Ehrlich's or Delafield's haematoxylin is used. Living amoebae can be stained with vital stains. (See page 38).

In addition to routine observations of general structure, the behaviour, reactions to heat, dilute acids, light, mode and speed of movement should be studied. Dark ground illumination (page 102), will be found very helpful for all Protozoan work. (See plate I, Frontispiece.)

PARAMOECIUM

Paramoecium is purchased living, collected, or cultured by an infusion method. Slowly heat to boiling some chopped hay, when cold, inoculate with paramoecium. This will not be a pure culture, and the faunal succession must be watched carefully, especially to get

stages in conjugation. The hay may be replaced by shreds of pickled cabbage leaf.

Stained preparations are essential. Fix as for Amoeba. Stain with Ehrlich, Delafield or Iron haematoxylin. Borax carmine often gives good results.

Observe living material. The rate of movement is reduced by adding dilute Epsom salts (about 0.5 per cent.), or a little glycerine, or by mechanical means, as with strands of cotton wool. Note mode of locomotion. Demonstrate endoplasmic streaming by placing animal in dilute suspension of carmine powder or Indian ink. Time the rate of discharge of contractile vacuole. Discharge of trichocysts may be effected by irritation with acetic acid, iodine or osmic vapour.

VOLVOX

Purchase living material or collect from stagnant green water in well lighted positions. Visits should be paid to a good collecting ground on several occasions during the year to obtain all phases in the life history. A culture may be successfully kept in filtered pond water from the original source. The methods described for *Euglena* may prove successful.

Permanent preparations are made by fixing in 70 per cent. alcohol, staining in Gentian violet or Methylene Blue, and mounting on a celled and ringed slide in equal parts of glycerine and formalin. Note the intracellular connections.

Observe reactions to light. The presence of beating cilia may be inferred from the formation of wavy diffraction lines when dilute glycerine is added.

EUGLENA

Purchase living material; collect from stagnant green ponds, from the surface of mud, etc. Culture by inoculating with Euglena either of the solutions given by Dakin ("Elements of General Zoology," p.27.)

Culture medium 1.

Peptone	0·5 grm.
Glucose	0·2 grm.
Citric acid	0·2 grm.
Magnesium sulphate	0·02 grm.
Potassium phosphate (KH_2PO_4) ...	0·05 grm.
Water	100 c.c.

Culture medium 2.

Ammonium sulphate	0·2 grm.
Potassium phosphate	0·2 grm.
Magnesium sulphate	0·1 grm.
Trace of iron sulphate	—
Tap water	200 c.c.

Permanent preparations of *Euglena* are only made with great difficulty. An attempt can be made to mount them unstained in glycerine jelly, glycerine and formol. Berlese's medium sometimes acts well.

MONOCYSTIS

Slides to show all stages in the life history must be purchased or prepared. Living material is obtained from the seminal vesicle of the earthworm. This is teased out in normal salt solution. Deal with each worm separately, otherwise a well infected mass will be very much diluted.

Permanent preparations are made as follows :—

1. On a clean cover glass make a thin smear from the seminal vesicle.

2. Allow this to *almost* dry, and then fix in 90 per cent. alcohol.

3. Stain in Ehrlich's haematoxylin. Over stain heavily and completely differentiate with attention focussed upon the trophozoites ; do not let sperm-mother cells affect the result.

4. Dehydrate and mount in balsam.

The whole operation is carried out in a watch glass with another one inverted above as a cover.

In addition to the common *M. agilis* specimens of the very large *M. magna* may be met with. These should be carefully mounted.

OPALINA

Opalina is obtained from the frog's rectum immediately after death. The rectum is opened and contents placed in a watch glass.

Fix in alcohol or Bouin's fluid. Stain with Ehrlich's haematoxylin.

Make observations upon living specimens.

HYDRA

Hydra fusca and *H. viridis* may be purchased, but are not difficult to collect and culture. The former (brown hydra) is usually found upon the hanging stems of duckweeds. The green hydra is frequently obtained in green pond water.

Culture of *H. fusca* is effected by keeping specimens in filtered pond water and feeding them upon *Daphnia*. *H. viridis* is kept in stagnant water in a well lighted position. (Culture *Daphnia* as for *Amoeba*.)

Nematocysts are discharged by irritation with iodine, or acetic acid. The nematocyst batteries are demonstrated by the following technique :—

1. Fix in hot saturated mercuric chloride in 70 per cent. alcohol.
2. Wash well in alcohol.
3. Stain in 10 per cent. methylene blue.

The nematocysts are blue and other cells unstained.

The detailed cellular structure can only be followed completely with the aid of macerated specimens prepared as follows :—

1. Kill in 1 per cent. acetic acid with a trace of osmium tetroxide.
2. Macerate in above solution for 5 minutes.
3. Wash in water—very carefully.
4. Gently tap the cells apart with a small brush.

In preparing specimens for making permanent preparations, note following points :—

1. Very gradual menthol narcotisation is generally necessary to get expanded specimens.
2. Sudden application of a hot-fixative (60° C.) may be successful in avoiding contraction.
3. Fixation is best effected with mercuric chloride.
4. Heidenhain's haematoxylin is the best stain.
5. Mount in formol glycerine or balsam—the latter alone in the case of sections.

OBELIA

Both hydroid and medusoid forms are usually bought from a dealer. Material may sometimes be obtained from a seashore collecting expedition. Menthol narcosis is usually required. Fix in alcohol or 10 per cent. formalin. Stain in Ehrlich's haematoxylin or Borax carmine. The medusa requires very careful handling in

mounting (see pages 31 and 32 for hints). Acid differentiation destroys the otoliths. For handling the medusa use a wide mouthed pipette.

ASCARIS

Material is purchased from dealers or an abattoir. Preserve in 5 per cent. formalin, preferably in shallow flat jars. Living specimens obtained direct from the host can be transported in normal saline kept at 37° C. in a thermos flask. They can then be kept for several days in an incubator, and thus provide developmental egg stages.

Fix in formalin, stain in Mallory's triple stain. To facilitate sectioning in wax soak the material in 5 per cent. potassium hydroxide overnight.

TAENIA

Sections and complete specimens are usually purchased but a careful watch should be kept on all laboratory dissections which may provide parasites. *Taenia serrata* and *Dipylidium caninum* are common in the dog. The "bladder worm" stage of the former is frequently present in the rabbit.

Fix in alcohol, aqueous mercuric chloride or Bouin's fluid. Sections should be stained in Mallory's stain. Whole mounts stain very well in Borax Carmine—several proglottides in different phases and a scolex should be mounted.

DISTOMUM

Purchase or collect from an infected sheep at an abbatoir. For fixation use alcohol and compress the specimen between two slides. The alimentary and excretory systems can be injected using a fine glass pipette. Whole mounts are stained in Borax Carmine or Ehrlich's haematoxylin. For sections use Mallory's Triple Stain.

An excellent technique for obtaining miricidia larvae has been described by Miss Jepps (Nature. 1933. Vol. 132, page 171). Gall bladders from infected sheep are washed out into glass basins. The sediment will contain eggs. After several washings these are spread over the bottom of a dish in tap water which must be changed daily if it becomes foul. Development of miricidia is completed in 6 to 8 weeks at 16° C. Hatching is brought about by placing a few eggs in a watch glass of cold water. The larvae may be used to infect the host snail, *Limnaea trunculata.*

LUMBRICUS

Worms may be purchased from dealers, but are easy to collect at dusk, particularly after rain. They can be driven to the surface by " watering " soil with a very dilute mustard solution. A winter culture can be made by keeping worms in a heap of loam and leaves covered by sacking.

For dissection kill with chloroform vapour, hot water, or hot 20 per cent. NaCl solution. The dissection should be made at once, in normal salt solution. Specimens are to be fixed in Gilson, Zenker or Bouin. For staining use Mallory or Delafield's Haematoxylin.

" Whole-mount transparencies " are useful, either stained in Borax carmine or unstained. They should be cleared and kept in a tube of cedar wood oil. The ovarian segment, complete, is well suited to this mode of treatment.

In preparing worms for the above processes the gut contents usually cause trouble. The best method of clearing the gut is to insert a pipette and wash the contents out with a gentle stream of normal salt. Fixatives can be injected afterwards, and by the use of ligatures the body can be kept distended with fixative, and of course immersed in a further supply of fixative. By these means the organs retain their real general relations.

The parts played by the nephridia and dorsal pores can be demonstrated by injecting into the body cavity, a dilute suspension of Indian Ink or carmine granules. The animal is subsequently fixed and sectioned.

To demonstrate the nephridia pour saturated aqueous mercuric chloride over a dissection. In mounting a nephridium dehydrate very completely, and use the diffusion gradient method for clearing in cedar wood oil. (page 31).

The blood vessels of a dissected specimen will sometimes show up black on a yellow ground if it is soaked for 2 or 3 hours in a mixture of 4 parts of nitric and 2 parts of hydrochloric acid.

The whole secret of dissecting the earthworm is to " know where you are." Thus, a prominent landmark of constant position, such as the openings of the vasa deferentia on segment 14 must be located and specially marked, and used as a landmark throughout the dissection. By such a simple expedient the process can be changed from a haphazard muddle to an orderly sequence of operations.

HIRUDO

Leeches are purchased from dealers or pharmaceutical chemists. *H. medicinalis* is sometimes collected from ponds and slow streams, but confusion with other species is likely to occur.

Living specimens should be kept in a covered jar of clean water. They should be fed by being allowed to suck blood from a piece of meat, though they are able to withstand starvation for long periods.

In the living animal note locomotory actions and also degree of colour change as a response to altered environment.

Leeches are killed for dissection by one of three methods :—

(*a*) Narcotise very slowly with menthol crystals which are dropped on the water.

(*b*) As above, but drop chloroform into the water.

(*c*) Remove the animal from water, and kill with chloroform vapour.

ASTACUS

The crayfish may be caught locally. It can be purchased and transported alive in damp moss in an aerated can. On arrival specimens should at once be placed in clean running water in a darkened place.

Demonstrations of the following actions on a living crayfish should always be made :—

(*a*) Walking and swimming.

(*b*) Respiratory current . . . use carmine granules, and watch the schaphognathite working when the edge of the branchiostegite has been cut away.

(*c*) Remove a piece of the dorsal carapace and watch the heart beat and action of the ostia.

For dissection kill the animal by plunging for a moment into boiling water, and then at once remove to cold water. Dissect at once. Preserve some in 70 per cent. alcohol for dissecting the nervous system.

The blood system can be injected in a living specimen using a fine pipette inserted into an ostium in the heart.

Exoskeletons are prepared by bisecting an animal longitudinally in the sagittal plane. Cut away as much of the flesh as possible. Soak in 2 per cent. KOH for 2 days, brush away all the remaining flesh and preserve the exoskeleton in 70 per cent. alcohol.

PERIPLANETA

Purchase, or collect living specimens from a bake-house. Examine a living specimen and watch the actions of the mouth parts through a hand lens. Note the rhythmic movements of expansion and contraction of the abdomen, which are probably largely respiratory in significance.

The heart beat and intestinal peristalsis may often be observed by examining living animals with a low power binocular and intense transmitted light. The observations must be focussed upon the unpigmented intersegmental membrane.

Kill the animal with chloroform vapour or by plunging into boiling water. Dissect immediately after death. The animal is best held for dissection by partial embedding in wax in a small dish.

Each student should make a mount of the mouth parts, the salivary glands and a piece of trachea. The glands must be dissected out, in water, floated on to a cover glass and fixed *in situ* thereon with 70 per cent. They are extremely difficult to completely dehydrate. Borax carmine is probably the best stain.

In mounting chitinous objects it is usual to boil in strong KOH, which removes flesh and effects a certain amount of depigmentation. Frequently it is better to soak in cold KOH overnight. Very complete depigmentation is achieved by boiling in some 10 per cent. HCL, with a little potassium chlorate, which liberates nascent chlorine, a powerful bleaching agent.

ANODON

Purchase living material. Specimens may often be collected with ease. They can be kept alive in slowly running water in a shady tank, which should be provided with a mud bottom.

Anodon can only be successfully dissected alive. If it is felt that this must be avoided at all costs then it should be killed in hot water and dissected at once.

Two specimens are required for each student. One is to be dissected. The other is hardened by immersion in 2 per cent. chromic acid with the valves wedged open. This requires about 10 days. The specimen is then washed in water for 24 hours, and subsequently cut into transverse slabs.

Observe ciliary action on a small piece of gill filament removed from the living animal. The general direction of the currents on the gill

lamella is to be determined by dropping a suspension of carmine granules upon the gill surface.

Examine the gills for the presence of glochidia. A mite is frequently found living on the gills.

HELIX

It is usual to purchase *Helix pomatia*, the Roman or Apple Snail for dissection. The garden snail *H. aspersa* will do but is considerably smaller.

To arouse snails from hibernation, puncture or remove the " operculum " and keep in a warm damp place for a few days. Snails can be kept active all the year in a warm moist vivarium. Feed on lettuce or cabbage leaves.

In preparing snails for dissection the first precaution is to order more than are required for dissection because fully expanded specimens are necessary for dissection, but are difficult to guarantee.

Killing is best performed by placing the snails in a jar of air free water (boil and cool). The jar must be quite full and tightly stoppered so that air is completely excluded. The process may take as long as four days. There are several other possible methods, but this one is simple and reliable. Always avoid over-crowding as it leads to distortion instead of distention.

AMPHIOXUS

Specimens should be purchased fixed in formalin or Bouin's fluid. Living specimens would repay attention if they could be kept in an aquarium.

Every student should examine sections and also make a dissection of *Amphioxus*. The sections required are listed on page 108.

Whole mounts which have been stained, cleared and preserved in a small tube are very useful. They should be examined with a hand lens and a binocular microscope if possible.

To prepare *Amphioxus* for dissection soak the specimen in 20 per cent. nitric acid in 70 per cent. alcohol until the cuticle begins to disintegrate and the muscles can be easily picked away. This process may take two or three days at room temperature, or only a few hours at 30° C.

SCYLLIUM

Specimens are purchased from biological dealers and in this case the fish are usually in formalin. Fresh material can be obtained at far lower costs from fish dealers or a coast fisherman. In the latter case the proportion of the sexes is of course purely a matter of chance. On arrival fresh material must be dealt with at once. Slit open the belly, make a dorsal incision to expose the brain, and place in 10 per cent. formalin and then in 5 per cent., for say two days in each. The material is finally stored in 3 per cent. formalin. A flat dish should be used for storage rather than a deep tank, in which the fish are usually damaged in handling. A pair of cheap rubber gloves and long wooden forceps will be found useful.

An alternative method of preservation which can be used for short period (1-4 weeks) when a tank is not available is as follows. Cut off the tail. Inject 40 per cent. formaldehyde into the caudal artery until a rise in " turgour pressure " is very obvious. Plug the injection aperture with a match stick. Preserve the carcases upon a tray. The skin will dry fairly hard, and before dissection slit open the belly and soak in water for several hours.

Methods of preparing the skeleton are detailed elsewhere (page 55). The skull and vertebral column are to be dealt with as a single unit, as also are the fins and girdles. The visceral basket must be handled very carefully. The set of units are wired together later. Disarticulated sets are conpletely macerated and brushed free of flesh. Cartilaginous skeletal material is preserved in a mixture of 70 per cent. alcohol and glycerine in porportions of 7 to 3. Handle very gently.

RANA

When possible *Rana esculenta* should be purchased in preference to *R. temporaria* (the Common English frog) which is smaller, but possesses the advantage that it can be collected in the field.

On the living animal observe :—

(*a*) Buccal respiratory movement.

(*b*) Mode of leaping and swimming.

(*c*) Beating of posterior pair of lymph hearts, at the sides of the tip of the urostyle.

In a pithed frog observe :—

(*a*) Heart beat.

(*b*) Capilliary circulation in the web of the foot.

To pith a frog requires the confidence born of practice. To avoid inflicting pain (i.e., until skill is acquired) the first few frogs should be anaesthetised. Hold the body of the frog in the hand with head projecting over the thumb, dorsal side up. Insert a strong needle in the atlas depression, and pass it down the neural canal or into the brain as required.

Killing of frogs for dissection is carried out with chloroform vapour so that the animal is first anaesthetised and subsequently killed. Do not attempt to kill by placing several frogs in a jar and pouring chloroform upon them.

LEPUS

In purchasing rabbits for dissection, it is well to select young ones, as they are usually comparatively free from fat. A pair of adults are, however, necessary to demonstrate the mature sexual organs.

Kill with chloroform vapour. Anaesthetise first, and then increase the dose. Suffocation only results in unnecessary suffering for the animal, and often causes mutilation, such as rupture of the blood vessels. Some sort of lethal chamber is necessary—a large metal box is best. This should have two large holes in the lid. Place the animal in the box. Suspend a ball of cotton wool soaked in chloroform (or ether) through one hole. The animal now breathes air plus an anaesthetic, and will eventually be immobilised. When this happens, add more choloroform and close both holes until the animal is dead. This takes about 30 minutes.

COLUMBA

The pigeon should be killed in a lethal box with chloroform vapour, and care taken to avoid the lice with which they are usually infested.

For dissection it is convenient to have them plucked. This is sometimes rendered more easy by dipping into boiling water.

The pigeon must be dissected as soon after death as possible and is not so easy to handle after preserving in formalin.

CHAPTER II

HISTOLOGICAL TECHNIQUE

THE study of minute structure in small organisms, in tissues or in single cells demands a specialised preparatory technique. Though this may at first appear to be somewhat complicated it is actually dependent upon a few general principles. It is most important that the pupil understands these general principles, because although the methods may appear to be merely " rule-of-thumb " they are not so. A fact which is at once made obvious by the work of any pupil who has been taught only along such lines the moment he is faced by any result a little out of the ordinary. To make a success of an unexpected result, which looks at first like being a failure, is only possible for the pupil who can intelligently adapt the principles of his technique. Memorised facts cannot be called knowledge until they can be used by rearrangement and re-synthesis.

Making Microscopical Preparations

" Whole mounts " of small organisms, dissected-out organs, and microtome sections are all required for teaching purposes. The " mounting " of these demands a technique which is fundamentally the same for each class of work. The various stages of this technique and their purposes may be described as falling into the following stages :—

1. *Killing.*

The organism must be killed in some suitable manner which is usually dependent upon its size and kind. Some methods for special cases have already been indicated in Chapter I. Similar methods, with perhaps a few modifications may be used for other animals. In dealing with invertebrates, distortion frequently occurs. This can be overcome by preliminary narcosis with a trace of chloroform or ether ; or in the case of aquatic forms by dropping a few menthol crystals into the water containing the organism. Mechanical means can often be applied,

thus *Distomum* is pressed between slides, an insect larva can be placed in a small bore tube to prevent curling etc.

2. *Fixing.*

Fixing and killing are frequently synonymous as in the case of small invertebrates. Larger animals are first killed and pieces of their tissues are then fixed. Fixation is performed by using various solutions which carry out certain functions :—

(*a*) Fixation preserves the structural detail of the cells and tissues and resists post-mortem changes as far as possible.

(*b*) Fixation hardens tissues so that the risk of mechanical injury is lessened.

(*c*) By forming insoluble chemical compounds with many of the cell constituents fixatives not only preserve structural detail, but also bring about an optical differentiation between such constituents, thus rendering comparative observations more possible.

(*d*) Some fixatives act as mordants which are essential to certain special staining techniques.

Rules for the use of a Fixative.

(i) Always use plenty of fixing fluid compared with the volume of the material to be fixed. This overcomes the possibility that one constituent of the fixative shall be completely used up, leaving an incomplete fixative to carry on the process—unsuccessfully.

(ii) If necessary make up the solution anew for each time of fixing, some of the fluids are unstable and do not keep well.

(iii) Never use one volume of a fixing fluid twice.

(iv) Avoid under or over fixation, which both lead to poor results. This demands practice, experience and the keeping of careful records. The optimum time will be a function of several factors, such as :—

(*a*) Penetration capacity of the fixative ; osmic acid for instance has poor qualities in this way.

(*b*) Size of the piece of tissue, which should always be small, and always present as large a surface area as possible

compared with its volume. That is briefly that flat slices are preferable to cubical " chunks."

(*c*) Nature of the tissue to be fixed. A " uniform " tissue like liver tends to fix more quickly then a compound tissue in which one element may form an almost impenetrable sheath. The presence of chitin usually means a slow rate of penetration, which must be overcome by removing part of the chitin, making perforations in it, and so on.

(*d*) Temperature. An increase of temperature will result in an increased penetration rate, but at the same time, it tends to make the tissues brittle. Fixation with a hot fluid is, however, often of value in dealing with an animal which is likely to contract or curl up unless it is killed instantly.

(*e*) Pressure. Spongy tissues which are likely to be air filled, or insects with air filled trachea, often present a difficulty. Not only is penetration poor, but the subject cannot be completely submerged. This can often be overcome by fixation in a partial vacuum.

(v) After fixation it may be necessary to wash out the fixative from the tissues either to get rid of its colour or to prevent the subsequent formation of some kind of deposit.

Choice of a fixative.

The type of fixative to be used will be controlled by the tissue concerned, the subsequent staining method, and the purpose for which the preparation is to be made. Fixatives can be divided into three classes, which while they overlap considerably nevertheless possess certain characteristics.

(*a*) Morphological fixatives, which are used when gross anatomy alone is to be considered. Alcohol and formalin used alone are examples of this class.

(*b*) Histological fixatives, which are used for the study of tissues, and which include the vast majority of fixing fluids.

(*c*) Cytological fluids, which are used when attention is to be focused upon a single cell in order to study cell constituents, chromosome figures and other points in minute detail.

It will be obvious that the needs of the school laboratory fall chiefly within the first and second of these groups, although good results can be obtained in some fields of the third group.

A list of fixatives which are most likely to be of use is given later (page 34). In Table I is given information about the various tissues and the most suitable fixatives to use for them.

3. *Preservation of fixed material.*

It may be necessary to keep a stock of fixed tissues on hand for histological classes and for this purpose there are three suitable methods.

1. Material can be stored in 70 per cent. alcohol.

2. It may be kept in 3 or 5 per cent. formalin.

3. It may be dehydrated and cleared (see below) and kept in one of the clearing agents.

The same methods can be used for dealing with small complete animals, although method 3 is infrequently used for this purpose. The presence of chitin in the insects always presents special difficulties, and if they are to be stored for long periods the following fluid should be used :—

Glycerine	49 c.c.
95 per cent. alcohol	49 c.c.
40 per cent. formaldehyde	2 c.c.

In storing delicate or valuable material it should be placed in a small tube of fluid, the mouth of the tube plugged with cotton wool, and the tube itself placed in a larger vessel of the preservative. In all cases the outer container must be efficiently sealed with glycerine or paraffin wax to prevent evaporation. This is particularly necessary when only a single tube is used. It is only necessary to dip the corked end into melted wax, but a refinement is to also boil the cork in wax first of all.

4. *Staining.*

By the use of suitable staining methods, we are able to selectively distinguish between various elements in a tissue. Stains are considered as falling into two groups :—

1. General stains which act upon a whole cell or tissue.

2. Specific stains which only stain certain components of a cell or tissue.

TABLE I

STANDARD HISTOLOGICAL METHODS FOR COMMON TISSUES

Tissue.	Locality.	Fixative.	Stains. Major.	Stains. Minor.	Appearance.	Mountant.
Cartilage	Nasal or joint capsule of frog. Skull of dogfish	10% Formalin 1% acetic acid Bouin's fluid	Delafield and Eosin		Ground substance blue to purple	Temporary—Glycerine Permanent—Gurr's Neutral Mountant Canada balsam
Muscle—Striped	Leg muscle, etc.	5% Formalin in 1% NaCl	Heidenhain's Iron Haematoxylin		Shows up all detail well	Canada balsam
Unstriped	Coat of Intestine Bladder	Saturated aqueous Mercuric Chloride Bouin's fluid	Delafield and Eosin		Sarcoplasm pinkish Sarcolemma blueish Nuclei blue	
Ciliated epithelium	Lining of frog's mouth	5% Formalin 70% Alcohol	Delafield and Eosin Borax carmine		Nuclei blue. Cytoplasm red Nuclei red Cytoplasm pink	Glycerine or balsam
Nervous Tissue	T.S. spinal cord, pieces of nerve, etc.	10% Formalin	Methylene blue		General stain. Shows Nissl granules	Glycerine Balsam Gurr's Neutral Preferably one of the last two
		Sat. aq. $HgCl_2$	Heidenhain's Iron Haematoxylin		General stain. Nuclei black	
		90% alcohol Bouin's fluid	Borax Carmine		Carmine is good for non-nervous elements	
Tendon	Tendinous attachments of frog's muscle	70% alcohol	Mallory's Triple Stain		Elast. fibres reddish. Conn. tiss. blue. Conn. tiss. fibres blue. Nuclei red	Do.
Pigment Cells	Frog skin	Absolute alcohol 5% Formalin	*Mount some skin unstained in glycerine jelly*			Glycerine or Canada balsam
			Iron-haematoxylin and Orange G.		Cytoplasm orange Cytoplasm red	
			Delafield and Eosin		Connect. tissue and derivatives blue. Mucin and glands blue. Blood corpuscles yellow. Pigment (melanin) show black	
			Mallory's Triple Stain			

Another method is to divide stains into cytoplasmic and nuclear stains according to whether they stain cytoplasm or nucleus. Again they may be considered as basic or acidic depending upon the type of solution in which they are prepared. Actually this last mode of distinguishing is unreliable and often contradictory, and the other systems also have disadvantages.

Any stain may be used alone or in combination with a second counter-stain to provide a colour relief. This is very often done by using a specific stain followed by a general, cytoplasmic or counter stain.

The process of staining may be performed in one of two ways :—

1. Simple, direct or progressive staining, in which an object is merely kept in the stain until it acquires a desired depth of colour, after which it is at once mounted.

2. Indirect or regressive staining, in which the object is very much overstained and then differentiated so that a considerable degree of contrast between the various elements is obtained—depending upon their differential affinities for the stain in question.

The practice of staining.

In using and controlling any stain considerable practice is required to obtain consistent results. There is no way of marking an end-point. The process depends entirely upon experience. For this reason, careful preparation of materials and complete standardisation of technique is required.

The best results are obtained by regressive staining. The object must be in some fluid which is miscible with the stain in which it is kept for a period that can only be determined by experiment; and if the various experiments are to lead to experience, then careful notes of times of immersion, concentration of stain, temperature, etc., must be kept.

Having stained the object it is next subject to the process of differentiation whereby the stain is removed from some of the constituents but left in others. This process will be carried out by different means for the different stains. Information on suitable stains will be found later (page 35). The differentiation must always be carried out as slowly as possible (i.e., use dilute solutions) and must be watched with a microscope.

5. *Dehydration.*

The ultimate object of the complete technique of the microscopist is to preserve his material in some mountant. For permanent keeping qualities it is usually essential that all water in the tissues be completely removed. This process of dehydration is effected by passing the material through various grades of alcohol up to 100 per cent. As this last is very costly the process can be stopped at an earlier stage, providing that due compensation is made later.

In carrying out dehydration, always keep the material in closed or covered receptacles, and avoid all chance of contamination with water or water vapour—even breathing upon the specimen may be fatal. In transference from one solution to another always reduce the time of exposure to the air to a minimum. Finally, do not hurry the process. Incomplete dehydration will do more than anything else to ruin a preparation.

When dealing with very delicate objects, such as *Obelia* medusae, dehydration must be very gradual. If the changes of media are too rapid the diffusion currents set up are prone to cause mechanical injury. This difficulty can be overcome by making only slow changes in the concentration of the alcohols or by altering their make up by actual mixing of the grades, rather than by lifting the object straight from one solution to the next. The gradient-diffusion method has much to recommend it in this respect, for dehydration as well as for clearing.

6. *Clearing.*

When dehydration is completed the specimen must be cleared. Unfortunately, there is no index for this end point, but a couple of changes of absolute alcohol (using as much of the fluid as possible) should usually complete the work started by the lower grades. This finished, the object is placed in a fluid which will render it transparent, and which must also be miscible with alcohol and Canada balsam, or what ever mountant is used.

The chief reagents are :—

Cedarwood Oil
Clove Oil
Xylol
Benzol,

but other essential oils are frequently used also.

For all round work, cedarwood oil is probably the best, as the second often causes brittleness, and the third usually results in shrinkage. Clove oil has an advantage in not demanding such complete preliminary dehydration as the others do. If this advantage is to be pushed still further, Terpineol can be used, as it will clear straight from 90 or even 86 per cent. alcohol.

The rates of diffusion of alcohols and the clearing agents may differ considerably so that some compensation must be made to avoid producing artefracts. The diffusion-gradient method can be used to this end when handling delicate objects. The dehydrated object is placed in a tube of absolute alcohol. This is tilted to one side and the clearing agent is run, drop by drop, down the inclined side. It collects below the alcohol and the object will come to lie at the interface between the two fluids. In this region a slow mixing of the two fluids takes place. Through this diffusion zone the object will gradually fall into the pure clearing agent below. The alcohol can then be removed by a pipette and evaporation.

7. *Mounting.*

Having stained, dehydrated and cleared the object, it is finally mounted on a glass slide in some suitable mounting medium, and covered by a thin protective cover-glass. A well prepared slide should last indefinitely, especially if the edges of the cover-glass have been "ringed."

1. *Choice of a mounting medium.*—In general work, the choice of medium depends on whether it is desired to make the mount permanent or only temporary. In the latter case not only are the results temporary, but in addition, such slides have to be handled more carefully. In more detailed work it is well to consider the refractive index of the medium as compared with that of the object to be mounted. If they were identical many delicate unpigmented objects would be almost invisible.

2. *Using a mounting medium.*—Having selected the most suitable medium, there are certain general rules to be observed in making use of it.

(*a*) Prepare the medium with extreme care and accuracy to standard formula (see page 33).

(*b*) The mountant must be absolutely dust free and must be preserved in a suitable capped bottle to avoid contamination.

(*c*) It is advisable to prepare certain media (such as Canada Balsam) in three different degrees of viscosity for use with different types of object.

(*d*) For very soft or thick objects (e.g. *Obelia* medusa) a careful technique is essential to avoid crushing.

This can be based on the following methods :—

(i) Support the cover glass above the object by small slips of glass, a ring of paper or cardboard, a glass ring, a ring of cement or paint, etc.

(ii) Use a " hollow slide " in which a circular depression has been ground out.

(iii) Place a drop of thick medium on the slide, drop the object upon this, and add a little more of the mountant. Allow the surface to set slightly . . . it may even be allowed to air dry for several hours. Finally, add a very small amount of a thinner solution, and place the cover slip in position. This method is particularly useful when mounting in Canada balsam and will effectively prevent severe crushing and folding by the weight of the glass slide.

(*e*) Place the mounted slide on a flat surface to dry. The process is slow unless a hot oven or hot-plate is available. This of course, is not used for glycerine jelly and similar media which set hard on cooling.

(*f*) The dry slide should be carefully cleaned first by scraping, and then by wiping over with alcohol.

(*g*) If possible " ring " the edge of the cover-glass with a cement, gold size or a varnish. Formulae for these are given on pages 53 and 71.

Note.—An excellent make-shift drying oven can be made by mounting two carbon-filament lamps, or a small spirit wick lamp, inside a large tin box on top of which the slides are placed. Another method is to bend a long strip of sheet iron (3 inches wide) to the shape shown in text fig 1. This is supported by the foot *a*, on which a micro bunsen burner or small lamp stands. Slides can be moved along in series on the " shelves " *x*, *y* and *z*. Do not overheat.

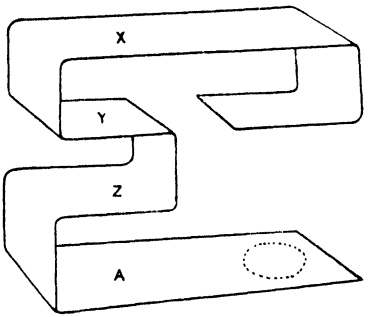


Fig. 1.—METAL STRIP FOR DRYING SLIDES.

Description given in the text (page 32). The circle on A *shows position of heating device.*

MOUNTING MEDIA

Fluid media are used in conjunction with a celled slide which must subsequently be ringed with a cement or varnish, or gold size, etc.

1. Formol and glycerine in equal parts are used for whole mounts of *Volvox*, etc.

2. Pure glycerine and water in equal parts are frequently used. Refractive Index about 1·4.

3. Glycerine Jelly, R.I. about 1·47. Soak 10 grams of best French gelatine in water for several hours. Place in 60 c.c. of water and heat to completely melt the gelatine. Add equal volume of pure glycerine and a few drops of carbolic acid. Melt by heating before use. The object to be mounted is transferred through water, dilute glycerine, pure glycerine, and finally to the medium itself.

4. Canada Balsam in Xylol. R.I. 1·524. Dissolve balsam in xylol to make a thin solution which is evaporated down to required thickness. It is well to have three solutions of varying viscosity for different objects.

5. Dammar in Xylol. R.I. 1·52. Prepare as balsam. Does not discolour with age.

6. Gurr's Neutral Mountant R.I. 1·51. Purchase from maker. Slides, etc., are placed in medium direct from alcohol.

7. Euparal. R.I. 1.483. The low R.I. makes this very useful. Objects can be mounted from xylol or alcohol so that the process is quite rapid. Purchase from dealer.

8. Hyrax. Purchase from maker.

FIXATIVES FOR ZOOLOGICAL WORK

1. Osmic Acid. (Osmium tetroxide). This most nearly approaches the ideal as it causes very little distortion or shrinkage and gives a picture most nearly that of the living cell. It has poor powers of penetration, and is expensive (about 15s. per gram for the solid). It is used for cytological work, and is unsuitable for general work.

There are several methods of use :—

(*a*) As a 1 or 2 per cent. solution alone.

(*b*) As Flemming's Fixative (without acetic acid).

2 per cent. osmic acid, 4 parts.
1 per cent. chromic acid, 15 parts.

(*c*) As a vapour from a 2 per cent. solution. This is excellent for dealing with minute organisms, particularly Protozoa.

Fixation with osmic acid is slow, and must be followed by very complete washing with distilled wtaer. It stains fat black and thus has a useful purpose.

2. Alcohol used at strengths from 70 per cent. to absolute. Acts rapidly but causes shrinkage. A morphological reagent.

3. Formalin. Used as a morphological reagent at 10 per cent. or below. Particularly useful, in combination with special staining methods, for studying the histology of the nervous system. Preserve fixed material in 3 per cent. formalin.

4. Mercuric chloride. Made up as an aqueous solution, saturated at about 15° C. Can be used hot (60° C.) for small animals. Tissues must be afterwards treated with a solution of iodine in alcohol until this solution retains its colour when the fixed tissues are left therein for several hours. If this is not done a precipitate of mercury salts is likely to occur.

5. Bouin's fluid. Probably the most universally used and best all round fixative. After fixation wash the tissue in 70 per cent. alcohol until the yellow colour has been removed as completely as possible.

Picric acid (saturated aqueous solution) ...	75 parts.
Formol (40 per cent. formaldehyde) ...	25 parts.
Acetic acid	5 parts.

6. Gilson's fluid.

Glacial acetic acid	4 c.c.
Corrosive sublimate	20 grms.
60 per cent. alcohol	100 c.c.
Nitric acid 80 per cent. (sp. gr. 1·456) ...	15 c.c.
Distilled water	880 c.c.

Penetrates well and objects may be left in for long periods. Wash out with water containing a little iodine.

7. Zenker's Fluid.

Mercuric chloride	5 grms.
Potassium bichromate	2·5 grms.
Sodium sulphate	1 grm.
Glacial acetic acid	5 c.c.
Distilled water	100 c.c.

The glacial acetic acid should be added immediately before use. Otherwise the solution does not keep. Wash in water after fixation and transfer up to 70 per cent. alcohol and add drops of iodine until the alcohol remains brown coloured when all the bichromate has been dissolved. Failure to do this will result in the precipitation of mercuric salts.

8. Schaudinn's Fluid.

Mercuric chloride (saturated aqueous)	2 parts.
Absolute alcohol	1 part.

Used for Protozoa. Can be applied cold or warmed to 60° C.

STAINS FOR HISTOLOGY, ETC.

The following list gives details of composition and mode of use of some of the more common stains suitable for school use. Many stains are made up to several different formulae, those quoted are varieties which have been proved suitable for student use.

1. Stains which may be used alone or with a counter-stain.

(*a*) Delafield's Haematoxylin.

(i) Dissolve 4 grm. of haematoxylin crystals in 25 c.c. of 90 per cent. alcohol.

(ii) 400 c.c. of saturated aqueous ammonia alum.

Mix equal parts of (i) and (ii), leave standing in an open bottle for 2 or 3 days. Add 100 c.c. of glycerine and 100 c.c. of Methyl alcohol. Leave standing. Filter and " ripen " for a month. Dilute with water before use (1 : 5).

Use as a regressive stain—that is overstain the object and differentiate with acid 70 per cent. alcohol. Wash in 70 per cent. and 90 per cent. alcohol and " blue " in ammonia vapour or alkaline 90 per cent. alcohol. If this is done before washing, i.e., immediately after the acid bath a white flocculent precipitate will form. Dehydrate and mount.

If desired a counter stain may be used according to the directions given later.

(*b*) Ehrlich's Haematoxylin.

Haematoxylin crystals	2 grm.
Glacial acetic acid	10 c.c.
Glycerine	100 c.c.
Absolute alcohol	100 c.c.
Ammonia alum	to saturate.
Water	100 c.c.

Keep exposed to light and air until a dark red colour. Use as Delafield's haematoxylin.

(*c*) Borax Carmine.

Aqueous borax 4 per cent.	100 c.c.
Carmine	1 grm.
Alcohol 70 per cent.	100 c.c.

Boil the carmine in the borax until completely dissolved and then add the alcohol. Cool and filter. Use as a regressive stain differentiating with acid alcohol, washing, dehydrating and mounting. A counter-stain is not frequently used with borax carmine. An excellent stain for whole mount work.

(*d*) Heidenhain's Iron-haematoxylin.

One of the finest and most critical stains available, but requiring experience and rather a long process to carry out. Details of a quick process are given.

(*a*) 5 per cent. aqueous iron ammonium alum (use only crystals of pale amethyst colour).

(*b*) Haematoxylin ... ... 0·5 grm.
Absolute alcohol ... ... 10 c.c.
Water (distilled) ... ... 90 c.c.

Do not add water until all the stain is dissolved in water. Keep for 2 weeks before use.

(*c*) As (*a*), but 2 per cent. strength.

Mordant Sections in (*a*). Stain in (*b*) and differentiate with constant watching in (*c*). Experience is the only guide, but in (*a*) about 1 hour will be followed by about 2 hours in (*b*).

Shortt's Rapid Method.

Boil 1 grm. of haematoxylin in 95 c.c. of water and add 5 c.c. of carbolic acid. Cool and filter. Mordant in (*a*) above for a few minutes, stain for a few minutes in Shortt's preparation and differentiate in (*c*) above.

2. Combination or Polychrome Stains.

This includes a very large number of stains, but for school work the only one cited will be found very good. The stain requires a mordant, but by combining the mordant in the stain a separate process is avoided.

Mallory's Triple Stain.

(*a*) 0·2 per cent. Acid Fuchsin (in water).

(*b*) Analine blue ... ... 0·5 grm.
Orange G. ... ... 2·0 grms.
1 per cent. Phosphomolybdic acid ... 100 c.c.

Stain in (*a*), wash in water and stain in (*b*). The relative times vary, but 1 minute in (*a*) and 6 to 10 in (*b*) can be tried at first.

3. Counter Stains.

Counter stains are usually general cytoplasmic stains with a general action in all the tissues. They are used after the primary process is completed and are introduced at any convenient point in the dehydration or clearing technique. Thus they are frequently made up in 90 per cent. alcohol and sometimes in the clearing agent.

Suitable forms are those given below :—

Eosin (alcohol soluble) ...	1%	in	90%	or absolute alcohol.
Orange G.	0·5%	„		„
Light green	1%	„		„
Gentian violet	0·5%	„		„

Orange G. is easily handled in the clearing process, but in the others, the clearing process usually takes so long that the staining is carried too far.

Borax carmine (prepared as detailed above) is often used as a counter-stain.

4. Vital Stains.

Certain stains are able to stain living tissues and are useful for dealing with Protozoa for demonstration work. Among such stains we may mention :—

Neutral red.
Nile blue.
Congo red.
Bismarck brown.
Janus Green B.
Janus Blue.

These stains are all used in dilute solution, about 1 in 2,000 to 1 in 10,000 parts of water. Success is a matter of chance and a good deal of dissension exists regarding the results.

5. Blood Stains.

Making stained preparations of blood requires considerable practice and a rigorous technique. Three points must be emphasized. The need for absolutely clean glass ware. The need for making a thin blood *film* and not a thick smear. And the need for a standardised repeatable technique.

The making of the film requires further comment. The blood should be placed as a small drop on a clean slide or cover glass. A similar glass sheet is then used to make the film by drawing out the droplet as the one glass is drawn slowly across the other, as shown in text—fig 2.

The fixation of the blood is an important point in the whole procedure for failure here can never be rectified whereas one can usually restain an unsatisfactory but well fixed slide. Fixation of blood is frequently carried out after air drying. Experience alone will tell when drying has been carried far enough. The slide may be gently heated or merely waved about in the air, but the drying must be rapid. If it is too slow the corpuscles shrink and shrivel and undergo crenation and then appear to have radiating points and mounds on their surface—showing particularly around the edge of course. If carried too far they also

show resultant artefacts. The film should appear to be nearly dry. If the film is well distributed, the area is large enough for there to be a differential rate of drying so that some parts reach the desired state. As the film will be examined with high power magnification the successful area may be comparatively small.

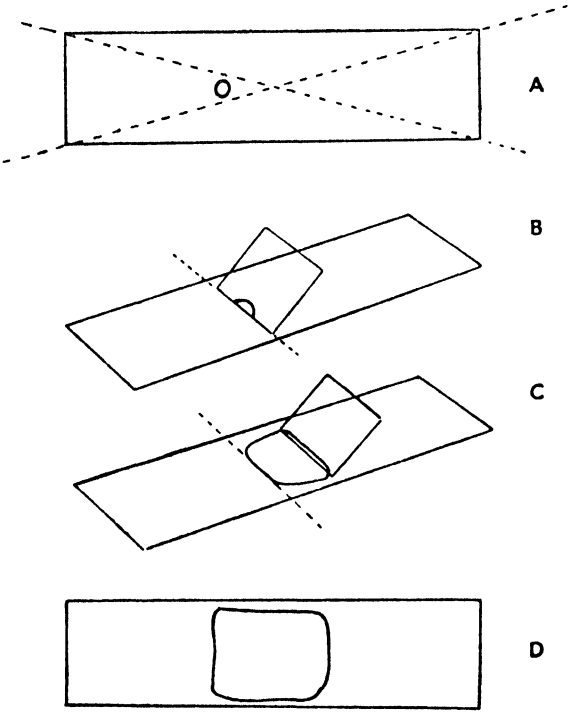


Fig. 2.—MAKING A BLOOD FILM.

Details given in text (page 38). The dotted lines in A are drawn on a sheet of paper on which the slide is placed, thus helping to place the blood drop in correct position. B and C show stages in making the film which is completed in D.

After drying the film is fixed, though some stains are used without previous treatment beyond drying. The fixatives used are usually alcohol and formalin. The period will be quite short, from one to ten minutes will usually be ample.

Stains for blood work are of many types, and are usually all of the regressive type. Polychrome, specific and counter-stains are nearly always adopted. For the use of pupils themselves haematoxylin and eosin, or a simple methylene-blue-eosin technique should be adopted.

TABLE II

METHODS FOR BLOOD FILMS

Technique		Results			
Fixative	Staining Method	Erythrocytes	Leucocytes	Platelets	Nuclei
Vapour of 40% Formalin	Delafield's Haematoxylin and Eosin	Red	Blue	Pink	Blue
(i) 10 parts Formol, 90 parts 100% Alcohol (ii) Absolute Alcohol (iii) Equal parts of Ether and 100% Alcohol	Methylene-blue and Eosin	Red-pink	Blue-purple	Pinkish	Bluish
Do not fix at all	Leishman's Stain (Methylene-blue, Methylene Azur and Eosin)	Orange-pink	Blue	Purple	Blue
Absolute alcohol	Giemsa. (As Leishman, but acts more slowly)	Bluish	Deep blue	Purple	Purple
Dry in air, fix in absolute alcohol	Laveran's Eosin and Methylene-blue	Pink	Blue-purple	Pink-purple	Bluish

The teacher can be more ambitious and try other more advanced methods.

Table II gives brief data for fixation and staining and shows what the results should be.

The various stains and modes of preparation are described below. Most of the stains can be purchased ready for use.

Finally, it may be noted that blood films are often stored dry, that is, they are washed after staining, blotted (with fluff-free blotting paper), air dried, and stored in this state without a cover-glass.

Stains for blood films.

1. Haematoxylin (Delafield or Ehrlich) and Eosin.

Prepare as described on page 36. Use the eosin in solution in 90 per cent. alcohol. Stain in the haematoxylin for about twice as long as used for sections. This method does not give such good results as more specialised techniques.

2. Methylene blue and Eosin (Laveran's method).

Various firms market proprietory stains of this type, which can be used as a single solution. Special forms of methylene-blue are also obtainable (e.g., Borrel's) and are superior to that usually used for other work.

Two solutions are prepared :—

(i) Borrel's methylene-blue made up as makers direct and diluted with about 10 volumes of distilled water.

(ii) Aqueous eosin, about 1 in 10,000 which is prepared by diluting a 10 per cent. stock solution.

The slides are stained in a mixture of (i) and (ii). Only experience will show how this must be constituted and it is well to make two or three mxitures, one of equal parts, one with one part of (i) and two parts of (ii), etc., and then select the best results obtained.

3. Leishmann's Stain.

The stain is purchased as a powder and dissolved in absolute methyl-alcohol to make a 0·1 per cent. solution. Store in a dropping bottle.

Stain unfixed films as follows :—

(i) Add a few drops of the stain to the slide.

(ii) One minute later add the same amount of distilled water, and leave for 10 minutes.

(iii) Finally wash, dehydrate, clear and mount.

4. Giemsa's Stain.

Purchase dry stain and prepare according to makers' directions to give stock solution. For use take stain diluted considerably, e.g., 1 drop of stain to 1 c.c. of water, or a 1 per cent. solution in distilled water. Staining requires about 30 minutes, wash in water and examine dry or mount in balsam.

Mounting small objects, unstained.

For small larvæ, insects and the like Berlese's medium may be used. The object may be dropped direct into medium or killed in 10% acetic acid. If it is in alcohol, wash first in 10% acetic acid. The medium consists of :—

H_{2O}	...	...	20 ccs.
Chloral hydrate	...	...	16 gm.
Gum arabic	...	...	15 „
Glucose syrup	...	...	10 „
Acetic acid	...	...	5 „

The slide must be ringed with a cement or varnish.

CHAPTER III

MICROTOMY

THE actual cutting of thin sections with the microtome is not at all difficult, but too few schools seem prepared to undertake it. As it permits the assembly of a really fine collection of teaching slides it is well worth consideration. A comparison of the cost of the apparatus and the cost of purchasing the slides which could be made shows that it is almost definitely of financial advantage to any school teaching biology regularly.

The sections are cut with an instrument known as a microtome, of which many forms exist. The Cambridge Rocking Microtome is to be recommended as being simple and reliable. The details of construction and mode of working must be obtained more fully from a catalogue or the makers' instructions.

Essentially the process depends upon cutting thin serial sections from a specimen which has been embedded in wax to provide mechanical support. The razor or microtome knife is fixed and the block moves up and down across its edge, being moved forward for every stroke. As a result of the impact of the wax block on the razor edge the wax becomes slightly softened and the series of sections thus stick together and form a long ribbon. In this are the sections of the object; it is suitably prepared and eventually stained and mounted.

The complete sequence of events is thus as follows :—

1. Fixation of the tissue.
2. Dehydration of the tissue.
3. Clearing of the tissue.
4. Embedding in the wax block.

(*a*) The object is soaked in a paraffin wax solvent, such as xylol or benzol.

(*b*) Paraffin wax is slowly added to this solvent until the object is impregnated with a solution of p. wax.

(*c*) The object is next placed in a bath of pure melted wax in a hot oven where it is eventually impregnated with pure wax.

5. The melted wax is poured into a mould and solidifies into a mass which contains the object to be dealt with.

6. The mass is trimmed to a solid rectangular shape with the object suitably orientated.

7. Sections are cut with the microtome.

8. The ribbon is mounted on slides :

(*a*) Clean the slides and smear with a thin layer of Meyer's albumen.

(*b*) Place lengths of ribbon on slide.

(*c*) Add distilled water and stretch the ribbon by gentle warmth. (An alterantive method is to *first* stretch the ribbon on the surface of a bowl of warm water and then place on the slide).

(*d*) Dry throughly.

9. The final process is to stain after first dissolving away the paraffin wax in xylol and passing the sections through alcohol down to the required grade, which depends upon the stain. This may be dissolved in water, or 50 per cent. alcohol, etc.

10. The stained slides are differentiated, dehydrated, cleared and mounted in balsam.

This process is best learned from a practical demonstration followed by considerable amounts of practice. The following points require special care.

1. *Care of the Microtome Razor.*

Microtome knives may have two flat surfaces, or be hollow ground to a slight extent on the front surface. No other form is very satisfactory. After use, clean the blade by wiping it over with alcohol and when dry place in a rack, preferably in a box containing a small rack.

At long intervals it is wise to have it ground and set by a cutler. Otherwise it can be kept in good condition as follows :—

(*a*) Hone it with great care upon a very smooth water or oil stone, which must be used for razor work only, and always cleaned and covered after use. The knife edge is pushed along the stone and slightly drawn across the stone at the same time. This results in the blade following a sweep which is an arc of a large circle.

(*b*) Strop the razor on a soft pliable strop made of fine quality leather. The razor is carried along the strop back first, that is trailing the cutting edge. As many as three or four hundred strokes may be required.

(*c*) A third process, seldom attempted by the amateur, is to polish the cutting edge on a sheet of fine optically ground plate glass covered with rouge powder.

2. *The Embedding Process.*

Use only pure high grade paraffin wax. The waxes usually used have melting points ranging betwen about 46° C and 58° C. The harder wax is used in summer or in a warm room, and a softer wax under cold conditions.

Wax is purchased in blocks, and before being used for embedding is treated as follows. Hold pieces of wax in forceps and melt them into a pot by playing a bunsen flame on them. In this way, having reached melting point the wax at once falls into the pot. Whereas when wax chips are heated in a pot it can easily be overheated, with damage to the properties of the wax. Mere melting in the oven is of little use as it does not drive off the gases contained in the wax. If this is not done the wax blocks which are made contain many bubbles and have a crystalline appearance.

After use all wax chips should be saved and used again. Contamination with dust particles and mixing up waxes with different melting points must be avoided.

3. *Making the Wax Block.*

The molten wax plus the object has eventually to be poured into a mould. The most successful mould is a solid watch glass. Paper moulds, watch glasses, brass block moulds and other forms are available. The mould is always to be used cold.

Pour the wax and the object in steadily or continuously. The wax which first touches the mould solidifies and forms a layer which prevents the object falling right to the bottom of the mould. When enough wax is poured in, allow it to cool for a minute or two, until a surface skin forms (this is accentuated by fanning) and then slowly lower below the surface of a bowl of cold water.

DON'TS.

(i) Never transfer the object from oven to mould in a pair of forceps. Pour it out with the wax, otherwise air bells, surface crystallisation and other troubles may arise.

(ii) Never plunge the mould below water too soon—this results in a " wax-spout."

(iii) Never cool the mould very slowly—this results in crystallisation of the wax. A fine homogeneous structure is required.

(iv) Never attempt to remove the block from the mould until it is hard all through, otherwise the orientation of the object may be upset, or it may suffer considerable distortion from pressure.

4. *Trimming the Wax Block.*

From the large block taken from the mould a small block is cut which must contain the object and must also always have two parallel faces which will form the adjacent edges of the sections in the ribbon. Thus there are two conditions which must be watched with care.

(i) *Orientation of the Object*

The plane in which sections are to be cut must be decided before embedding. Sections may be :—

(*a*) Transverse : in the vertical plane and at right angles to the long axis of the object.

(*b*) Longitudinal : parallel to the long axis of the object and thus either in the vertical or horizontal planes ; the former are sagittal sections. Actually there can only be one such section, those on either side of the median section being para-sagittal sections. Longitudinal horizontal sections are known as frontal sections.

When the desired conditions are known, the block is so made that there is enough wax behind the object to effect union with the block holder, and enough ahead of the object to allow of a few preliminary trials to test the razor edge, etc.

Large or pigmented objects present little difficulty. Transparent objects should always be slightly stained so that they can be seen in the melted wax and suitably arranged. Very minute objects should be mounted beside a piece of hair so that this can be seen and used as a guide for the position and orientation of the object whilst trimming the block.

Some workers stick small objects, in a known plane, to a small piece of liver, the whole is then embedded and for orientation is treated as a large object.

While the object is in the still molten wax in the mould it may be handled and orientated with a pair of forceps. These must be warmed slightly, but care must be taken to never overheat them as they may then damage the tissues.

(ii) *Trimming the block faces*

The block containing the object is cut roughly to shape, using a sharp scalpel and shaving wax off the block in thin slices. To attempt to cut large pieces off a small block usually results in fracture of both block and specimen.

Two parallel faces are required which will be the top and bottom surfaces of the block when mounted on the block holder. If the faces are truly parallel then a straight ribbon should result. If not, the ribbon will twist or curve. However the block must also be mounted so that its front lower edge is also parallel with the razor edge. If this is not so, a curved ribbon may result from an apparently perfect block.

5. *Mounting the block*

The wax block has to be mounted on the block holder. The block holder is arranged so that it may be fastened to the microtome arm. It is necessary to be able to exactly orientate the block on the block holder. The one end of the block is slightly warmed by touching with a warm knife and then placed in contact with the block holder face. This is wax covered, and must also be warmed. The block must be quickly moved into the correct position before the wax hardens. An adjustable block holder may be used instead in which the block can be adjusted by a ball and socket joint. Whichever type is used, always note the following conditions :—

(i) Harden the wax joint in cold water before any attempt at sectioning is made.

(ii) Be sure that the basal wax on the block holder is rigid. Otherwise it may fall off the holder and so ruin the block, or it will result in the sections being alternately thick and thin.

(iii) Leave enough wax behind the object to protect the razor edge from striking the block holder.

6. *Electrification of the Wax Ribbon.*

Electrification of the wax ribbon is frequent and annoying, and its cause not at all easy to find. It is to some extent related with atmospheric temperature and humidity, and also appears to depend upon the relative areas of the object and the wax in each section. Finally, it is correlated with the nature of the tissues, being usually pronounced in a " mixed tissue," as in cross sections of an insect containing some hard chitinous parts.

As palliatives the following alternatives are possible :—

(i) Work in a most warm atmosphere—keep a pan of water boiling nearby.

(ii) Sometimes conditions are impoved by keeping one or two electric lamps burning nearby. Whether this is a function of temperature or the properties of an electrical field, are unknown.

(iii) Stick a long label one inch wide by one narrow end just below the razor edge, and let the sections slide off down the paper. This effects some amount of insulation. Electrification may be the result of friction between knife and wax block.

(iv) The possibility of using static charges, of earthing, or of counteracting the one by inducing another, have never been explored very fully, but may well repay attention.

7. *General Instructions.*

Continual practice soon results in confidence and will produce good results. As a further guide, a list of defects and suggested cures is added below :—

Fault 1. Sections fail to stick together to make a ribbon, and instead lie one above the other at the knife edge.

Causes (*a*) Knife blunt.
(*b*) Section too thick.
(*c*) Wax too hard for room temperature at that time.
(*d*) Acute electrification.

Cures. These will be obvious so far as (*a*), (*b*) and (*c*) are concerned. To avoid complete re-embedding, and if a warmer room or local heating fail (*c*) can often be overcome by putting a thin layer of very soft wax on the top of the block. As far as (*d*) is concerned see above.

Fault 2. Ribbon curved instead of straight.

Causes (*a*) Edges of knife and block not parallel.
(*b*) One part of knife sharp and one part blunt.
(*c*) Block badly trimmed so that top and bottom surfaces are not parallel.

Cures. These are obvious, but sometimes the fault persists, or is instead evinced as a twisting of what should be a straight ribbon. Probably due to very pronounced electrification.

Fault 3. Sections crumble or object falls out.

Causes (*a*) Incomplete infiltration of wax.
(*b*) Wax much too soft.
(*c*) Knife blunt.

Cures. Before deciding to re-embed, try dipping block in very cold water and cut at once. An external layer of hard wax does not act as a cure.

Fault 4. Sections roll up singly or serially.

Causes (*a*) Knife blunt.
(*b*) Sections too thick.
(*c*) Wax too hard.
(*d*) Knife edge makes too acute angle with block face.

Cures. Try the obvious ones first, starting by sharpening the knife, and then cutting thinner sections. All microtomes do not provide means of combating (*d*), but then the knife is fixed almost vertically to start with—with just enough tilt to prevent the block dragging on the knife on the up-stroke. If no knife adjustment is available, and if other efforts have failed, try altering the angle by using packing pieces behind the knife.

Fault 5. Longitudinal scratch on ribbon.

Causes (*a*) Notch in knife edge.
(*b*) Hard particle in block causing (*a*).

Cures. Examine block face with lens for (*b*). Resharpen or use different part of knife edge.

Fault 6. Sections alternately thick and thin.

Causes (*a*) Block loose in holder.
(*b*) Holder loose on microtome arm.
(*c*) Knife loose in knife slots.
(*d*) Forward feed of block uneven because of fault in the feed mechanism.
(*e*) Failure to make complete strokes.

Cures. Be sure there are no loose screws, etc. Examine pawl and ratchet of feed for stiffness or loss of teeth on the ratchet disc. Be sure that (in the rocker type of instrument) the lever is moved the complete distance each stroke.

Fault 7. Ribbon flies to and adheres to anything in the vicinity, particularly getting mixed up with the apparatus or sticking to operator's hand where it usually melts—excessive electrification.

Causes. Not at all clear, seem to be correlated with temperature and humidity and undoubtedly with electrical state of the atmosphere. (See above, page 47).

Fixing the ribbon on the slide.

This is achieved by using Mayer's albumen, a little of which is smeared on the slide, the ribbon placed in position, and then flattened. Use only a little albumen. It is prepared to the following formula :—

White of egg	...	...	50 cc.
Glycerine	...	...	50 „
Sodium salycalate	...	...	1 grm.

Filter before use.

CHAPTER IV

THE SCHOOL MUSEUM

THE function of a museum so far as a school is concerned, should be to display an index collection illustrating in detail the points made by the teacher, and also to house a collection of material designed to expand some of these details into broad general principles.

In starting to make a school museum, these and other points must be kept clearly in mind the whole time.

The collection must have a purpose, it must be organised with a definite plan so that every exhibit plays a part. If the crude collecting and acquisitive habits of children are allowed to run riot, the museum becomes a mere mausoleum of unwanted ancestral relics.

The material must be well arranged, well exhibited, clearly labelled, easily accessible and well catalogued. These conditions apply to large or small collections and are easy to fulfil providing a good standard of work and an obvious general method is set up from the very start.

The general points in the technique demanded will be dealt with in the following sections.

Storage and Exhibition.

Material which is to be on constant exhibition, must be easily visible from every aspect. Material used for reference only makes less exacting demands as it probably is used to display one special point. In either case, the nature of the specimen dictates the style to be adopted. Many specimens will require glass containers. Skeletons are articulated and mounted on a base-board, or kept as isolated bones in boxes.

Museum jars are unfortunately very expensive. A good deal can be done with makeshifts of domestic origin—if air-tight lids are available. Tin boxes with glass lids are very useful. A plentiful supply of strong cardboard, plywood and suitable tools should be kept at hand. A sealed jar is more permanent and safer than one with a loose removable top. The disadvantage is that the object cannot be removed for minute examination. Duplicate specimens, one of which may have to be renewed at long intervals, overcomes this difficulty.

Specimens kept in spirit or formalin are usually fixed on to a supporting sheet which just slides into the jar and affords much protection to the specimen. Mica and sheet glass are usually used, and the latter is preferable. The object can be stuck into position with an adhesive (which must withstand the preservative fluid) or still better sewn into place. The technique for cutting and drilling glass is described later (page 64). Whilst opal, blue or green glass often " shows up " the specimen very well, it means that only one side can be examined. Thus clear glass is often preferable.

Many problems arise in connection with the labelling of museum preparations, especially those which are kept in a fluid. A supply of specimens which bear no identification is necessary for revision purposes—even though the very shape of the jar seems to become a specific character in the student mind! Other specimens must be clearly labelled, and wherever any confusion could arise there should be some sort of label inside as well as outside the jar. The inner need only be in the form of an identification disc with reference to a catalogue card, and is written in pencil.

Among the possible means for preparing and applying labels the following will be found useful.

(*a*) External labels.

(i) The name is written on the glass jar with glass ink (various proprietary brands) and this painted over with cellulose, shellac, or gold size.

(ii) The name is written in indian ink or printed upon an adhesive paper label attached to the jar and painted with one of previous media.

Preserving media.

Fluids for preservative purposes are almost legion. For most general work one of the two following is generally used :—

(*a*) 5 per cent. Formalin. Commercial formalin is a 40 per cent. solution of formaldehyde. This must be taken into account when making up solutions. Although nothing but a very specialised technique will preserve natural colour certain precautions can be taken to avoid complete loss of colour differentiation in specimens. In particular, it is preferable to use neutralised formalin for all preservation work.

(*b*) 70 per cent. Alcohol. Probably most generally used because its vapour does not attack the mucous membrances or eyes of the worker, and the fluid does not have quite such a drastic effect upon the hands.

There are many specialist fluids but only one is likely to find a use in school, that is an alcohol-glycerine mixture for preserving cartilaginous skeletons. It consists of 70 per cent. alcohol and pure glycerine in the proportions 7 to 3.

Sealing the jar.

For temporary closure use a jar with a stout rubber washer, glass drop-on lid, and metal pressure rim which screws on. The washer and the inside surface of the rim should be lightly smeared with vaseline before closing. Screw down enough to make an airtight joint, but not so that it can never be opened again.

Permanent closure is effected by the use of a cement. The jar for this work usually has the top edges ground square with a drop-on plate. See that the plate makes contact all round the edge. The joint is made with some form of cement. A list of suitable types is given below. Finally, a piece of wet bladder can be stretched over the top, allowed to dry then trimmed neatly and coated with shellac, gold size or cellulose paint.

Cements for sealing museum jars.

Various dealers have put on the market a series of proprietary cements for this purpose. Details of these can be found in catalogues.

Most of the cements detailed for aquarium work can also be used and will usually answer the purpose well.

Gold size, shellac dissolved in methylated spirit, celluloid in acetone, first class copal varnish, marine glue and such media can also be used.

Any cement for this work may have to withstand the action of the preserving fluid, especially if the jar is to be frequently handled. Thus allowance must be made for this.

Three cements in common use are prepared as follows :—

1. Resin and boiled linseed oil are warmed together to make a viscous preparation which hardens when cold. Apply hot and cement the faces together when " tacky."

2. Asphaltum and guttapercha are warmed together and used as above. This cement resists both alcohol and formalin.

3. White beeswax and chicle gum are prepared as above and must be used while hot.

Models and Casts.

Certain rare specimens can only be represented by reproductions. These are usually expensive to buy, but either teacher or pupil should be capable of carrying out the work.

For materials, plasticine, artists modelling clay, plaster-of-Paris, beeswax, paraffin wax, etc., can be used, aided by the use of suitable colouring matters.

Probably only diagrammatic models are possible unless much time and effort is to be expended. These, however are of great value in teaching, but selection of the objects to be modelled must be controlled by one important point, their capacity to be really useful. It is waste of time, effort and material to model what the pupil can easily observe on the real thing. On the other hand a model of the ovarian segment of the earthworm, even if only in bas-relief, may prove of great use in teaching how to dissect out the ovary. In the same way a model of a pair of the thoracic pillars in the endophragmal skeleton of *Astacus* will help the pupil to a better conception of how to get at the nerve cord. But in both cases suitable preparations of the real thing should be available too. The model merely has the advantage of larger size which is so useful in demonstration to a class. The ultimate work, however, has to be done on the life-scale.

The type of model usually made falls into one of two classes. It may be a " sketch " model of diagrammatic kind, or it may be a large scale reproduction prepared with exact precision.

In the first class the mode of modelling will depend upon the object, but always a large drawing should first be made in which some of the major dimensions must be defined. The model must give the correct general effect, and must thus be of the correct proportions. In all cases too much detail is to be avoided.

Models of the second class are built up of layers of material, such as sheets of cardboard cut to shape by projecting from microscope sections on to them and drawing in the appropriate outlines. The linear magnification must be adjusted to suit the thickness of the material available for building the model.

The card is then cut out, the pieces placed in correct relative order and position and fixed together with pins and glue. The surface is

cleaned up with care, and the model painted with various colours to represent the vraious elements. The method is laborious, but useful.

Preparation of Skeletons.

Skeletons are obtained from the carcase by one of the following methods of maceration :—

(*a*) Stand in cold water until the rotting flesh can easily be removed.

(*b*) Simmer in hot water until the flesh can be easily scraped and brushed away.

(*c*) Simmer until tender in Holden's chemical macerator, consisting of ½oz. of crescylic acid (cresol in commercial ammonia) in 6 galls. of water. Clean by scraping and brushing.

(*d*) Simmer gently in Rowley's fluid :—
1 table spoonful of sodium sulphide ;
2 table spoonfuls of pancreatin ;
1 gallon of water.

The usual dissecting instruments are used for cleaning the bones, aided by a few small brushes with fairly stiff bristles. Old toothbrushes are useful.

A fine jet of boiling water or steam is useful for working on localised spots. The latter is obtained by leading steam through a rubber tube to a glass jet from a flask of boiling water. A blow off safety valve should be provided, such as a Bunsen valve made from rubber tube (*c.f.* text fig. 9, page 74).

In all the above methods, first clean away as much flesh as possible before commencing maceration.

Always treat the carcase in separate units. Remove the skull and limbs from the trunk and prepare each part in separate vessels. If the bones are to be set up as an articulated skeleton, do not carry maceration too far, particularly in the case of the limbs and feet.

When the bones have all been cleaned as much as possible they should be soaked for a short time in a fat solvent to remove grease. Chloroform or xylol will do this. Next they are soaked in alcohol which completes this process and starts dehydration which is finally completed by air-drying. If the bones do not appear to be white enough they can be placed in a dilute solution of hydrogen peroxide before the alcohol treatment. None of these treatments should be prolonged. The times necessary are a matter of experiment.

The fitting together of the bones to set up an articulated skeleton is moderately simple. The work is carried out with wire of various thicknesses, some form of adhesive (Croid glue or Durofix will be found very suitable) and some patience. For drilling holes in bone an Archimedes drill, as used by fret-wood workers, is essential. (Messrs. Hobbies, Ltd., make a suitable form which is effective and inexpensive).

As a guide for anyone wishing to mount skeletons of the usual type specimens, the following notes on the method should be useful.

1. *Rabbit Skeleton.*

The complete set of bones should have been prepared and dried, and if possible, be in the following units.

(*a*) Skull and jaws.

(*b*) Vertebral column :

(i) Cervical vertebrae which are probably separated from each other.

(ii) Thoracic vertebrae all united together and bearing the ribs and sternum—only effected by reducing maceration to the minimum.

(iii) Lumbar vertebrae, which generally fall apart.

(iv) Sacral vertebra usually united to the first four or five caudal vertebrae, the rest being separate.

Limb girdles.

(i) Scapulae and cartilaginous clavicle separated.

(ii) Pelvis separated from the sacrum but with the ventral symphyses complete.

Elements of Fore-limb.

(i) Humerus, radius and ulna, all separate.

(ii) Fore-foot, very lightly macerated and hence present as a complete unit.

Elements of Hind-limb.

In condition similar to above.

It will be seen advantageous to keep some parts of the skeleton articulated, whilst others are in separate elements. Thus it is essential to deal with the various regions separately.

Articulation is carried out as follows :—

1. *Vertebral Column.*

(*a*) Place a cork, which will fit into the foramen magnum, on a stiff wire which must be a few inches longer than the vertebral column, and should have been bent to a suitable curve.

(*b*) Thread the vertebrae on the wire in the correct order with articulatory surfaces in contact. The sacral and a few post sacral units will be found a tight fit to the wire and can be jammed on so holding the anterior vertebrae in position.

(*c*) Thread the caudal vertebrae on a finer wire and jamb this into the last post sacral on the large wire.

Better still, use a single wire for the whole series and enlarge the spinal aperture of the last few caudal vertebrae.

2. *Girdles.*

(i) Place the pelvic girdle in position, bore a small hole through each iliac wing and sacral surface. Thread a wire through and twist the ends together.

(ii) Support the scapulae in position by wire fastenings.

3. *Limbs.*

Articulate the limb elements by drilling and wiring together. Drill holes in the head of the humerus and femur so that the holes abut on the centre of the respective articular surfaces.

4. *Ribs.*

Professionals fasten the thoracic skeleton together with wires running longitudinally on each side and twisted around each rib. This is tedious and not easy. Support may be provided by cutting a narrow strip of stout Bristol board about $\frac{1}{4}$ in. wide, placing it inside the ribs, and bending to shape and glueing to each rib. Use one strip each side.

5. *Articulation of Limbs and Girdles.*

Drill a hole in the two articular facets of each girdle and push in a short length of stiff wire. This will form a peg on which each limb can be hung and removed when required. Permanent wiring can be used instead.

6. *Skull and Jaws.*

Fasten the mandibles together by wiring the symphysis. Hang the jaws from the skull by drilling and wiring. The skull connects with the vertebral column by fitting on to the anterior cork.

7. *Attachment to baseboard.*

Make a suitable wooden baseboard. This must bear two stiff vertical wires at such a distance apart that one of them will make contact with the sacrum and the other with the cork in the skull. Drill through the basi-sphenoid and into the cork for the front wire. Drill a hole in the ventral surface of the sacrum for the posterior wire which will lie in front of the public symphysis.

Anchor the feet down by temporary or permanent wire loops, according to whether the limbs are removable or fixed in position.

2. *Frog Skeleton*

Being small and delicate the frog skeleton must be prepared very carefully and not macerated too strongly, otherwise the skull and feet will give trouble. It must be dealt with in units, as the rabbit, and mounted on a small baseboard.

For the amateur, this skeleton is best articulated with glue, and a shaved down match stick used for threading the vertebrae.

The bones are prepared. The vertebrae and skull mounted on the axis with the aid of adhesive. The girdles, urostyle and limbs are then placed in position and glued.

Finally, it is well to build a small glass case with thin sheet glass (old photographic plates) and narrow passe-partout binding.

3. *Dog-fish skeleton.*

A cartilaginous skeleton demands careful handling. Macerate gently and brush with very great care. As soon as possible dissect away the girdles and fins and branchial basket, so that the following units are handled separately :—

(i) Skull and jaws.

(ii) Branchial apparatus.

(iii) Vertebral column with tail and axial fins.

(iv) Pectoral girdle and fins.

(v) Pelvic girdle and fins.

The gill apparatus must not be disarticulated. Prolonged patient work with gentle maceration by steam and hot water is necessary.

The fins have the skin cleared away at the bases to show the basal pterygia, but the skin must be left to support the delicate fin rays.

Articulation is carried out by sewing the element into position on a glass plate with strong thread. The jaws and branchial bars can be afforded a little extra support with very thin wire.

The completed skeleton is preserved in a jar into which the supporting plate makes a good fit to prevent rocking about and subsequent damage.

Demonstration skulls.

It is useful to prepare a few special skulls for teaching purposes. In particular a skull with the sutures outlined in India ink, or painted in different colours is useful. The internal structure is seen best in a bisected specimen which can be sawn through sagittally with a fret or tenon saw.

The presence of the permanent dentition developing below the milk teeth is shown by cutting away the jaw surface. Dental wax or Durofix is used to fix loose teeth.

Skeletal Transparency Methods.

Small animals, such as fish, embryos, aquatic larvae, etc., can be dealt with by a transparency method in which the skeleton is coloured and viewed through the transparent body tissues. This type of technique also gives excellent preparations of the frog skeleton and is of great value for demonstrating the anatomy of the carpal and tarsal regions.

The two most useful methods are those given below. In either case a certain amount of experiment and experience is necessary.

Van Wijhe methylene blue method for cartilaginous elements.

Excellent preparations of embryos and larval vertebrates can be obtained by this method. The object is stained for about one week in a 0·25 per cent. solution of methylene blue in 70 per cent. alcohol containing 1 per cent. of hydrochloric acid. It is then washed in acid 70 per cent. alcohol until further colour ceases to wash out. Dehydrate and clear and preserve in cedarwood oil or mount in balsam. The cartilage should be stained blue, and all other tissues colourless.

Dawson Alizarin method for ossified elements.

(*a*) Fix material in alcohol and harden in 90 per cent. alcohol for some time, at least 48 hours.

(*b*) Place in 1 per cent. potassium hydroxide until the bones become visible through the tissues. Considerable experience required to estimate this point.

(*c*) Stain in potash-alizarin (1 part of alizarin in 10,000 parts of 1 per cent. KOH) until the bones are obviously deeply stained.

(*d*) Clear in the following solution:

water, 79 parts.
glycerin, 20 parts.
potash 1 part.

(*e*) Pass through successively stronger grades of glycerin and finally store in pure glycerin.

Injections.

Dissections of injected specimens are necessary for demonstration work. In particular the vascular system in *Astacus*, *Rana*, *Scyllium*, *Lepus* and *Columba* should be prepared in this way.

(i) Apparatus.

Injections of small animals may be made with a hard glass pipette and rubber teat, a hypodermic syringe, or a pipette to which pressure is supplied from some suitable device. In the latter case, it is well to have a calibrated apparatus so that a known amount of material is injected. A good method is that of merely keeping the injection mass at a higher level and getting a head of pressure by gravity. In using such methods it is best to work at a low pressure, and to increase the time period until the desired amount of medium is introduced. Too high a pressure usually leads to rupture and contusion. (text—fig. 3).

The actual pipette is made from glass, about $\frac{1}{4}$ in. or $\frac{3}{16}$ in. in diameter. This is drawn out to about $\frac{1}{8}$ in. or less, and beyond this, the actual injection tip made. (text—fig. 4).

Injection Media.

(*a*) Gelatine media.

These can be purchased ready made or prepared in the laboratory. In either case only deal with small stocks. Once opened such media do not keep well.

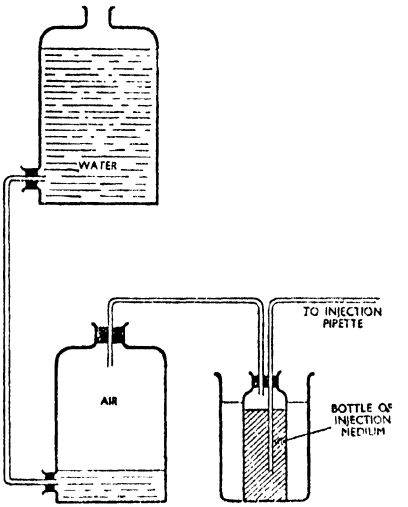


Fig. 3.—CONSTANT PRESSURE INJECTION APPARATUS.
Water from the upper reservoir is drained into the lower jar from which the air is driven under pressure to the bottle containing the injection medium. This will be forced through the outlet tube and is led to the injection tip. The injection mass is warmed by placing the bottle in a beaker of hot water. By placing a length of rubber tube with a screw clip between the two reservoirs the rate of flow can be controlled.

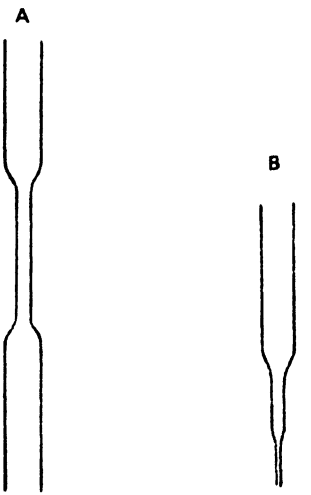


Fig. 4.—MAKING AN INJECTION PIPETTE.
Details given in text (page 60).

Among many formula for laboratory preparations, Guyer's will be found good and simple.

Five grams of carmine is ground up in 10 c.c. of water to which ammonium hydrate is added until the solution is transparent. Fifty grams of sheet gelatine is melted in water and the carmine added. While this is cooling, acetic acid (25 per cent. strength) is added drop by drop until the mass becomes opaque and smells faintly acidic. For a blue matrix Berlin blue is used instead of carmine.

A useful medium is made by colouring rubber latex as desired, and injecting it with a pressure apparatus. The animal, after injection, is placed in a bath of acid formalin or dilute acid which causes the latex to solidify. An excellent feature of this method is the elasticity conferred upon the parts injected, which can thus be stretched aside for dissection of underlying structures. Latex can be purchased from a rubber firm or macintosh maker.

A dilute preparation of plaster-of-Paris is frequently advised for injection. The resulting work becomes very brittle and difficulties are met with sometimes in keeping the fluid preparation free from lumps.

The practice of injection.

The operation of injection should always be performed as soon as possible after death. After moderate delay a simple washing out of the vessels with warm normal salt solution is useful. After longer delays some form of " blood solvent " should be injected first to overcome the effects of clotting. For this purpose use a solution of 0·5 per cent. warm sodium chloride or 0·5 per cent. warm sodium citrate.

(i) Dogfish.

Specimens must be sent from the coast to the Laboratory as quickly as possible, but injection should be successful in fish which have been dead three, or even four days. If the delay has been long, use a preliminary wash-out treatment.

For the ventral arterial system inject through the ventricle with the nozzle inserted into the conus.

The dorsal arterial system (efferents) and general visceral arteries are injected through the caudal artery after cutting off the tail; plug the artery with a match stick.

The visceral veins may be injected through the haemal (caudal) vein.

The venous sinuses are injected from the sinus venosus or the openings into the cuvierian ducts.

(ii) Frog.

Inject the arterial system via the ventricle and the venous system via the sinus venosus. Excellent results may be obtained with a hypodermic syringe. The injection must be made very slowly using very gentle pressure.

(iii) Rabbit.

The comparatively large volume of blood in the rabbit necessitates drainage of the system before injection. This is performed by severing some suitable vessel, generally in the hind leg. A preliminary wash with warm saline should at once follow this. The arteries are then injected through the base of the aortic arch, and the veins through the sinus venosus.

(iv) Pigeon.

Prepare the animal with great care. If the sternum is completely removed, vascular ligatures will be required, unless a very careful dissection is made. It is possible to operate through the sternum *in situ*, and to inject through parts of the heart.

Control of the injection nozzle.

In all cases the greatest care must be exercised in inserting and handling the injection nozzle. The use of a sharp tip is best avoided so that a hypodermic needle must be ground down before use. If this is not done the cutting edge will probably lacerate the vessel wall beyond the real point of insertion and result in leakage. The incision for inserting the instrument must be made with a very sharp instrument. The jet must lie as nearly as possible in the axis of the vessel, if the angle is acute it will pierce the opposite wall. A ligature is placed round the needle tip to prevent the injection medium leaking backwards.

Double Injection.

A preparation with the arteries and veins injected in different colours is very useful. The process is a little more difficult and perhaps demands greater skill in dissection to display all the structures.

The two injections are made through suitable places as soon after each other as possible. Using the gravity or similar type of apparatus

makes it possible to perform them simultaneously—but a constant watch must be kept on the progress of the injection.

The two media are usually effectively prevented from mixing by the large terminal pressure set up by the capilliaries. In some cases, as experience shows, it may be necessary to supplement this by suitably disposed ligatures.

Combined Injection and Transparency Methods.

Experiment has shown that it is possible to combine the injection technique with the alizarin transparency method. If it can be successfully completed the results are of considerable value for demonstrating the relations of vascular and skeletal systems.

Glass Cutting.

One of the tasks which sooner or later falls upon the museum technician is that of cutting glass. And this frequently frightens the amateur. There is no need for this to be so. It is a job which anyone can acquire some skill in, even though he may never be able to face very heavy plate glass or skew curves with any ease of mind. The essential point is to get plenty of practice so that confidence is acquired.

Glass is cut with a diamond mounted in a " pencil " or with a small hard steel disc mounted in a suitable holder. The former cuts a scratch on the glass, but the latter appears to roll a groove into the glass rather than scratch it.

Mark out the position of the cut with two dabs of ink or paint. Place a straight edge between them and run the cutter straight down the sheet along the rule. One cannot describe the technique. Practice will soon show what angle and pressure are required. The aim is to make one attempt only and produce a straight clean cut. Second attempts and joined-up " runs " seldom give a clean edge.

Having prepared the way one has to " part " the glass. The professional just lifts up one edge of the sheet, grips with a finger and thumb close to each side of the cut, and gives a little jerk. This seems to be half an effort to break the glass by forcing the two sides at an angle to each other, and half an effort to pull the pieces apart. The result is a clean fracture.

The amateur is advised to tap the underside of the glass below the cut until he can see a fracture carried right across the sheet. When

this is done the glass can be parted professionally or by supporting the fracture along the edge of a bench and forcing the one side downwards.

Far the best way of learning is to watch a glazier and then put in plenty of practice on old sheet glass.

Cutting Circles.

Discs of glass are cut with a diamond mounted at the end of an arm with a central pivot. The arm is a square metal bar, the diamond

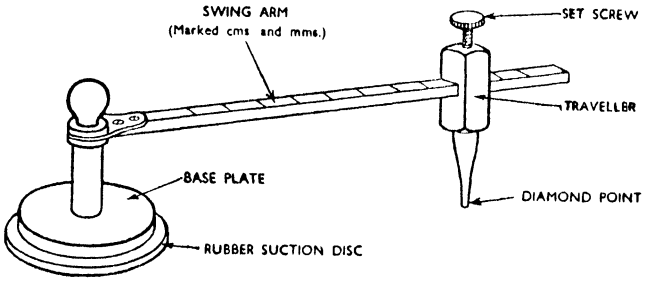


Fig. 5.—GLASS CIRCLE CUTTER.

Description given in text (on this page). In use, the circle must be scribed with one complete rotation.

holder can slide along this and be locked to any desired position for different diameters. The pivot is a rubber suction disc. The instrument is shown in text fig. 5 (above).

With this a circular scratch is made. From this take a diamond pencil and run out three or four cuts from the circumference to the edge of the plate. Tap round the circle and the unwanted glass will fall away in pieces.

Trimming glass edges.

Slight corrections can be made to a badly cut edge by breaking pieces away with a pair of blunt nosed pliers. Small fragments are removed with a coarse file or by grinding with a small emery disc.

The glazier, if a first class workman, does not need such aids. Should he make a mistake he usually cuts a new sheet, rather than try to patch up bad work.

Boring glass.

For sewing specimens to glass sheets, small holes are required. These are bored with a hard steel or diamond bit mounted in an Archimedes drill which is operated as shown below.

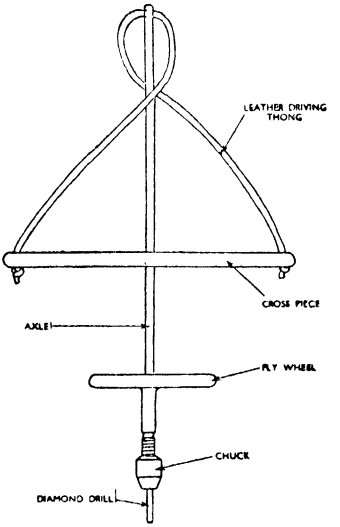


Fig. 6.—GLASS DRILL.

The cross piece is moved up and down the axle which is thus rotated by the leather thongs which are alternately wound and unwound round its upper end.

CHAPTER V

AQUARIUM METHODS

Introduction.

IF school biology is to be the study of life an aquarium will be of obvious value, moreover it will also help to reduce the costs of the biology class by supplying specimens. Books dealing with aquarium keeping are legion, but the majority of them tackle the subject as a sort of ornamental hobby. This section will attempt to provide in a brief space, details of the more important points in the general technique.

Types of Aquaria.

Aquaria are of many kinds, and fall into the following classes :—

(*a*) 1. Fresh water aquaria.
2. Salt water aquaria.

Each of these types may be again divided into two minor types :—

(*b*) 1. Micro-aquaria.
2. Macro-aquaria.

Again we may have any type of aquarium arranged as one of the two following :—

(*c*) 1. Pure-culture aquaria.
2. Mixed-culture aquaria.

Finally, we may arrange any of the previous types in one of two ways :—

(*d*) 1. Cold water aquaria.
2. Warm water aquaria.

The first division (*a*) requires no further comment at this point. The differentiation under (*b*) is based upon the organisms to be cultured, not upon the size of the aquarium—for work on a small scale as in a school, it does, however, happen to be more convenient and economical to rear *Paramoecium* in a jam jar rather than in a 100 gallon tank.

Micro-aquaria may be based upon jam jars, water tumblers, pie-dishes, etc. Macro-aquaria, in which larger animals, such as fish, are kept require larger tanks.

Division C is important. It may be said that in nature, C1, the pure culture type almost never occurs. It is an artificial aid for the biologist. In this type we have some particular species which is reared in large quantities, the culture being fed with a food pabulum, or with organisms of another species. Thus *Daphnia* is reared as a pure culture (feed on wheat-pabulum) and is used as food to support pure cultures of *Hydra*.

C2 reflects the conditions found in nature. A series of organisms living together closely inter-connected by organic needs, their relationships being frequently based upon food-chains.

Sub-division (*d*) reflects natural conditions inasmuch as it may be imposed by the special conditions originating from warm or cold climates. It may also, however be derived from the fact that metabolic rate and temperature are inter-connected. We may need to speed up or slow down the hatching of eggs, etc., and can impose some control by altering the temperature of the water in which they are reared.

Water Supplies for Aquarium use.

1. Fresh Water.

There is plenty of opportunity to exert control over the medium in fresh water tanks and every effort should be made to select water most suited to the organism—which usually means bringing a supply from the original environment. It is well to filter water to remove predaceous or other undesirable organisms whilst leaving its physico-chemical properties unaltered. Water above chalk, limestone, clay, etc., usually has a characteristic fauna and flora. To reproduce these conditions it is practically essential to take water from the original source.

Many organisms are susceptible to variations in acidity and it will often repay trouble to keep some control of this factor. For this purpose the B.D.H. Comparator or Capillator pH sets will be found very useful. These are marketed by British Drug Houses, Ltd.

Oxygen content is another factor of obvious importance to which attention must be made. Technical methods for aeration are described later.

For transporting water supplies from the field to the school, glass vessels should be used if possible. Failing this, perfectly clean rust free metal cans may be used. If any form of transport is impossible then tap water must be used, although it may often prove uncongenial.

Running water may sometimes be essential. Its chief character in a natural stream is a high oxygen content, so that well aerated still water may be used instead. Some species are, however, adapted to life in swift water and unless this can be provided artificially they do not thrive in captivity.

2. Sea Water.

The keeping of sea water aquaria is all too seldom attempted. Schools near the sea obviously have the best conditions, but inland schools can try " artificial sea-water " as a medium. There are several available recipes among which the following can be tried :—

1. Lulham's Method.

$46\frac{1}{2}$ oz. salt (kitchen block salt).
$3\frac{1}{2}$ oz. magnesium sulphate.
$5\frac{1}{4}$ oz. magnesium chloride.
2 oz. potassium sulphate.
$13\frac{1}{4}$ gallons fresh or tap water.

Dissolve each of the constituents in a separate portion of the water, and finally mix and stir well. Some days later add some living seaweed, and stand the solution in a good light for about six weeks. Before adding any considerable stock of animals test the water with a single specimen such as a prawn.

2. Tiedemann's Sea Salt.

A very convenient method is to purchase Tiedemann's Sea Salt concentrate from a pharmaceutical chemist and dilute it according to the instructions provided. This gives a solution containing the salts normal to sea water, and has been frequently used with success for marine aquaria.

Marine forms from the upper limits of the tidal zone are most easy to keep in captivity, such as the green shore crab, the sand hoppers, the sea slater, etc. These really only require a moist vivarium to be perfectly happy. Of the truly aquatic marine forms, some of the sea-anemones and the mussel can be reared successfully. The chief problem is to prevent the carnivores eating the rest!

Tanks.

The size of the tank will be controlled by the specimens it is desired to keep. Micro-aquaria can be no more than small dishes or jam-jars. For larger forms real tanks are required. Old battery (accumulator)

jars are often used. Next come specially made glass tanks of clear glass in one piece. Finally, and usually restricted to the larger sizes, are the built up tanks with heavy plate glass sides and slate bottoms with angle iron frames.

Lack of a real aquarium jar or tank should not prevent effort. Almost any watertight container or can can be pressed into service. Whatever is used, there is one golden rule—have a large water surface exposed to the air, compared with the volume of water. Of tanks of identical volume that with the largest surface area is to be chosen. The traditional " gold-fish bowl." is the worst possible type as it presents a relatively small surface for aeration.

Tanks should be kept covered as much as possible to avoid contamination with dust, but free access of air must not be prevented. A glass sheet raised on cork supports may be used. A sheet of muslin weighted down with lead at the corners is frequently adopted. The muslin may also be fixed in position by thoroughly damping it, spreading it over the tank, and then smoothing the turned down edges into close contact with the glass. This will dry into position.

Tanks with angle iron frames should be filled with water and left standing for a couple of weeks before being used. Finally, wash them well, and set up as aquaria. By this simple expedient, any deleterious action caused by the fresh paint is usually avoided.

These composite tanks often give trouble by developing leaks. To minimise this, the tank should not be moved about when full, because this strains the frame. To cure a leak several methods may be tried, but if possible, never adopt the commonly used and seldom successful method of putting a layer of cement all over the bottom and up the edges of the frames. If the leakage is serious enough to require large scale treatment, it is wiser to unframe the glass and set it up anew using an aquarium cement. For a small leak the seam can be painted with red or white lead, gold size or have soft putty pressed into it. Better still would be a real cement. There are several methods of preparation among which the following may be tried—it must be noted that some variation in the recipes must be allowed for. The amounts stated are a guide and must be confirmed by experiment and experience.

(*a*) linseed oil ... 3 oz.
tar ... ... 4 oz.
resin ... 16 oz.

Melt together by heating gently. It is well to start with only 2 oz. linseed oil and add more as required. Too much prevents the mixture from solidifying. This mixture has the great virtue of remaining " mastic," it does not become hard and brittle, and hence permits of a certain amount of twisting of the frame without a leak breaking through.

(*b*) litharge
plaster-of-Paris
dry silver sand
} equal weights.

powdered resin—about one-tenth of the above amounts.

These are mixed together to form a putty with boiled linseed oil. This cement will stick to almost anything, and resists the action of salt water.

(*c*) white lead ground in oil
red lead dry
litharge dry
} equal weights.

Mix thoroughly with much working. Hardens quickly and resists water for a very long time.

(*d*) gutta percha
stockholm tar
pitch
} equal weights.

Mix by gentle heating. Can be painted on to a leaking seam while still warm.

AQUARIUM APPARATUS

The more common apparatus required may be listed as follows :—

(i) Glass pipettes of various sizes and lengths.

(ii) A pair of long forceps—home-made wooden ones will do.

(iii) Lengths of glass tube for catching specimens, removal of debris, etc. These are used by closing one end of the tube with the thumb and lowering the other end into the water till it is very close to, and directly above the desired object. When the thumb is removed water-plus-object rushes into the tube. By replacing the thumb, and holding the tube vertical, the object can then be lifted out of the tank.

(iv) A small net with fine mesh. This is easily made by bending a stout wire frame and lashing it to a wooden handle and making a muslin net.

(v) Some small dippers—a can or cup on a long handle.

(vi) A siphon tube (rubber) with a glass funnel at one end. The mouth of the funnel is covered with muslin and so acts as a trap to prevent clogging of the tube.

Aeration Devices.

It may be necessary to artificially aerate the water, though with suitable plants present and a large surface to which the air has free access this should not be a frequent need.

Among the many possible devices, the following may be mentioned.

1. Where a continual supply of water is available, a tank can be aerated by leading the water through a very small glass jet which sprays on to the water-surface. Each of the water droplets is not only well aerated itself, but it also carries water bubbles down with it.

2. *Lulham's aeration device.*

This consists of a glass tube about 1 in. in diameter and about 12 in. long (text fig. 7 A). In the middle is bored a small hole (*x*) about $\frac{1}{8}$ in. diameter. This tube is closed by corks through which two

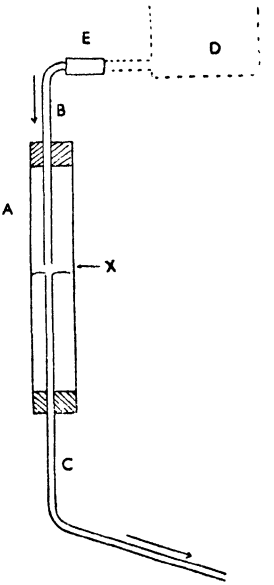


Fig. 7.—LULHAM'S AERATION APPARATUS.

Details given in text above.

smaller tubes enter. One of these (*b*) leads from a reservoir (*d*). The other (*c*) leads to the aquarium. Water from (*d*) siphons through (*b*), and provision of a rubber insertion with a screw clip (*e*) permits the rate of flow to be controlled. Air is drawn into (*a*) through (*x*) and a column of air and water passes through (*c*) to the aquarium. If (*d*) is large and the rate of flow small, this will carry air to the tank for a long time.

This device is simple to set up and easy to work. The tank must be large enough to accommodate the water from (*d*), which can be siphoned out of the aquarium and replaced in D. Provision of two reservoirs and a constant level device leading water to the second reservoir would be a useful addition. When (*d*) is empty, it ~~the~~ becomes a question of replacing it by the now filled second jar.

This apparatus could be so controlled that (*d*) emptied overnight, thus aerating the tank during a perod when the plants present were not producing oxygen.

3. *Aspirator Air Bottle.* (Text fig. 8 below).

A large aspirator bottle (*a*) is filled with a double bored bung, carrying an aspirating pump (*b*), and and air outlet (*c*). The outlet

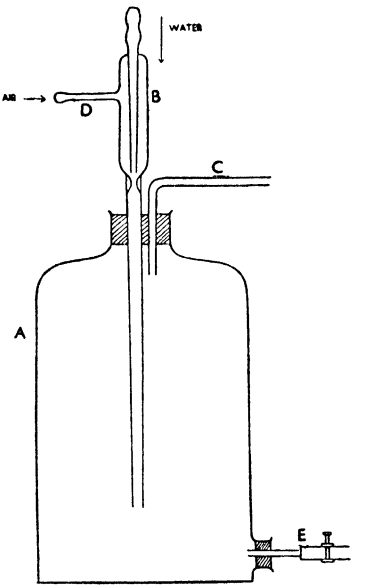


Fig. 8.—ASPIRATOR AERATION BOTTLE.
Details given in text above.

(*e*) is a tap or tube with rubber and clamp to control rate of outlet. The passage of water through (*b*) draws in air through the side arm (*d*), so that (*a*) tends to fill with a mixture of air and water. Air under pressure collects in the top of (*a*) and is led through (*c*) to the aquarium. By a suitable arrangement of the various rates of flow this makes an excellent aeration device, but it demands a source of running water.

4. *Automatic Aerator.*

An excellent device was described by W. B. Barker (*School Science Review*, Vol. XVI, No. 61, page 119, October, 1934) in which a siphon is introduced so that the apparatus is automatic and may be kept functioning continually. The arrangement is shown in text fig. 9 (below). (*a*) has a capacity of about 1 pint. This is filled by a quick succession of drops of water so that air is displaced and driven over to

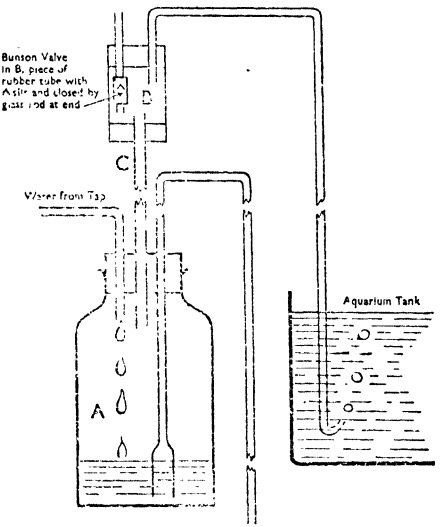


Fig. 9.—Barker's Aeration Apparatus.
Details in text (above).

the aquarium. When (*a*) is filled it automatically empties by the siphon and air is drawn in through the Bunsen valve (*b*). The instructions expressly state :—

(i) The siphon tube must not exceed ¼ in. in diameter. Its outer limb must be as long as possible.

(ii) At the inner end of the siphon a short piece of wide tube must be sealed on, otherwise the siphon column will not break when the bottle is emptied.

(iii) The height of (*c*) and the siphon must be an inch or so more than the depth of the tube immersed in the aquarium.

(iv) The V slit in the rubber of the Busnen valve is to be cut with a razor blade, and the cut faces dusted with French chalk to avoid sticking.

Mr. Barker writes (in a private communication) that the rubber used for the valve must be of moderate thickness and not too pliable. His apparatus has been in use for 3 years practically without attention.

5. *Electrically Driven Air Compressors.*

Various dealers market small air pumps which are driven by low power motors. Where many tanks are to be aerated such means are very useful, but they are comparatively expensive and hence seldom used except in large scale work. If they are used, and if they are to be kept in continuous operation over long periods, it is essential to select a well built machine with adequate bearing surfaces and efficient means of lubrication.

Constant Level Controlling Devices.

In tanks in which running water is used, or to which water is added during the process of aeration, some device for controlling the water level is essential.

(i) A simple, but usually risky, method is to provide an outlet pipe of large size and adjust this with regard to the inflow rate so that the level remains as constant as possible. A wire gauze or other trap must be provided to prevent clogging up of the outflow and consequent flooding.

(ii) If the inflow is merely a slow drip an outflow siphon can be provided made from glass tube and arranged as shown in text fig. 10.

(iii) A well known and exceedingly reliable apparatus is described by Findlay (" Practical Physical Chemistry ") and shown in text fig. 11. It consists of an air valve (*e*) which is filled with water, and has a rubber tube with clip at the top and a water valve (*b*) into which water enters. Water leaves (*b*) through (*d*) and passes through the air trap (*e*), and on through (*f*) to the tank. Excess water from (*a*), or water siphoned back to (*b*) from the tank escapes through the waste-pipe

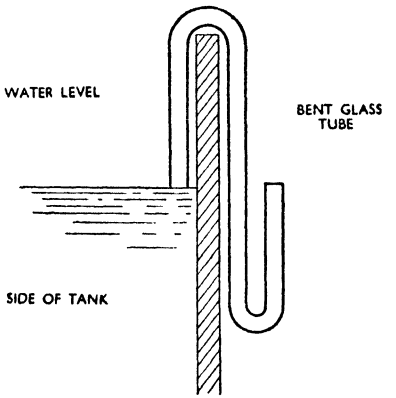


Fig. 10.—Simple Constant Level Device.
The bent glass tube should be about ¼ in. internal diameter.

(*c*); this pipe must be of larger diameter than (*a*). The level of (*c*) will be the level of the water in the tank.

Warm-Water aquaria.

In general the aquarium is allowed to assume room temperature. It can be kept cooler by being provided with a constant stream of

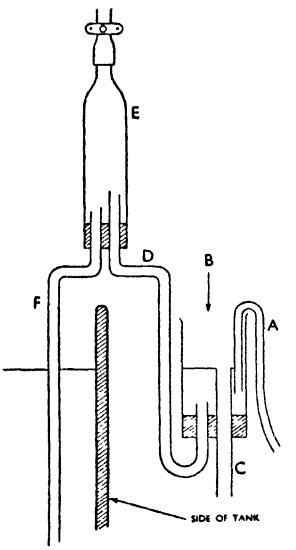


Fig. 11.—Constant Level Device.
Details given in text (page 75).

tap water. To keep a warm tank for sub-tropical plants or animals, is not difficult, though to keep an accurate constant temperature requires thermostatic control.

The simplest method of warming a tank is to raise it on a suitable stand, such as a flat biscuit tin, and keep a carbon filament lamp burning below it. A small spirit lamp, or even a night light will also do. The source of heat must be kept clear of the glass.

In schools with suitable radiator or water pipe heating, the tank may be supported above, but not in direct contact with such a source of heat. In many ways this is preferable to any sort of flame heating, which not only may blow out, but may easily lead to accident.

Setting up the Aquarium.

While the actual details to be described in this section will vary when different species are under consideration, it will be found that the general principles remain constant.

The tank should be washed with warm soap and water and then swilled out with fresh (or salt) water. It should next be placed in its permanent position which must not be too much exposed to sunlight, though shields can be made from plywood or cardboard if necessary. It is particularly desirable to avoid frequently moving the tank about, especially when full, as this causes considerable strain and is a potent source of leaks especially in angle-iron frame models.

The bottom of the tank is covered with pebbles, stones or sand. All these must be washed absolutely free of debris before use. In order to support plant life a layer of mud is frequently introduced. It is much better to place each plant in a small pot which stands on the floor of the tank. These can be moved about or completely removed without resulting in a general accumulation of dirt everywhere, and a thick suspension of small particles in the water.

When adding water to the tank this should be directed in a jet against a large flat stone to avoid washing away the sand, etc., from some particular spot.

The " balanced " Aquarium.

Under natural conditions during long periods of time the population of a natural body of water becomes adjusted to conditions which are largely settled round the available food supply. During the complete year there will be successive floral and faunal changes, but always in an

orderly sequence. Under such conditions a pond may be said to be in natural equilibrium. The aquarist aims at getting such a similar equilibrium or balance in his tanks, but most frequently the community is not self supporting and for this reason food and oxygen have to be supplied and an artificial control exercised over the rate of growth and increase of the flora and fauna.

Except in pure-culture work where only one form is bred it is well to try to get a self-supporting community. It ultimately saves time and effort. Thus some particular habitat must be well studied under natural conditions and reproduced as closely as possible. The problem usually falls into three parts :—

(*a*) To supply oxygen to the water.

(*b*) To supply food to the larger animals.

(*c*) To avoid contamination by any form of debris.

(*a*) The first problem has been partially dealt with so far as artificial aeration is concerned. The use of suitable aquatic plants is also possible, and is the most usual means adopted to supply oxygen to the water. There is a large variety of plants which may be used, and among them the following may be specially mentioned.

(i) The Canadian water-weed, *Elodea canadensis.*

This is easy to collect, is very hardy, grows rapidly and at first the question of actual " planting " need not arise because the plant will grow while merely floating in the water.

(ii) The Italian water-weed, *Vallisnaria spiralis.*

This is purchased from aquarium dealers. It is dicœcious and also increases by producing runners. If possible, plant in a pot 3 or 4 inches deep with loam at the bottom covered by sand or fine gravel. The plant grows best in warm water or in an aquarium kept indoors. It requires plenty of light and must always be given a good growing start before animals are added to the tank, as the tender shoots are frequently eaten.

(iii) The Frog-bit, *Hydrocharis morsus-ranae.*

A hardy plant which grows while floating in water. Liable to attack by some water snails.

(iv) The Arrow-head. *Sagittaria sagittifolia.*

Common and hardy. Plant in pot with some loam. Requires rather a large tank.

It is wise to keep a tank or two solely for cultivating these and other water plants.

(*Note*, that the terminology is that usual in dealers' catalogues and so probably not in accord with modern systematics.)

(*b*) The problem of food supply is more complicated. Many of the larger animal forms, such as fish and newts, are carnivorous to a great extent, and prey upon smaller organisms. Hence it is essential that such organisms (e.g., *Daphnia*, *Cyclops*, small worms) are present, and that the general relations are such that they can reproduce rapidly enough to keep up the food supply. Actually, this is seldom realised, and a subsidiary pure culture of suitable form is kept for feeding purposes.

When the artificial mode of feeding is adopted several precautions are necessary. Of these there are two universal rules :—

(i) Don't overfeed—fish seem to have little self control!

(ii) Don't use a constant monotonous diet—few animals are such specific feeders that they refuse variety.

Feeding should take place at regular intervals. Fish are fed about three times a week. All uneaten food (unless actually living) must be removed before decay sets in. For details of suitable food for specific animals the reader should consult the list given later (page 80). As far as the actual foods are concerned, a few points may be mentioned in general guidance.

Meat.—This is presented in fine shreds unless a very large piece is used, which none of the fish could possibly swallow. In aquaria with mixed species this is to be avoided, among a set of minnows no goldfish ever gets a look in. The method is good for frog tadpoles which collect in a bunch around a large piece of meat, which can be easily removed the day after presentation. Raw lean meat is used.

Vermicelli.—Always soak in water until soft, before giving to fish.

Ant Eggs.—Usually found to be pupæ, not eggs. Avoid a constant course of this diet.

" Gentles."—Small larvæ of blow fly are excellent for such forms as will only take living active food, e.g., newts.

Worms.—Very small earthworms, or large worms well chopped, are good.

" Blood-worms."—The active larval form of *Chironomus* which is aquatic is excellent for fish and newts. *Daphnia*, *Cyclops* and other small organisms may be mentioned here too.

Dog-biscuit.—Various kinds of dog biscuit which contain meat derivatives make good food when powdered and soaked.

Dried *Daphnia*.—Many dealers sell dried *Daphnia* which form a good food, but does not supply the exercise provided by the living form.

Proprietary foods.—Many brands of patent foods are on the market which all possess the one characteristic of convenience and ease of preparation—most have other good features, but many possess the disadvantages common to the patent foods made for the human market. Among these is a very common absence of food value.

(*c*) Scavenging is dealt with by several methods. Dead animals and unused food materials are to be removed at once. For this work a glass suction tube, long forceps and a small hand net are required. Smaller debris is dealt with by some of the inmates of the aquarium. The water louse, *Asellus aquaticus* does a good deal of this work very well. Some of the snails, particularly *Planorbis* and *Limnæa* keep down confervoid growths—but don't have too many snails, or they soon present a problem themselves by the accumulation of fæces.

If the stonework is well washed, the plants kept in small pots, and the above ideas put into practice, the tank should remain clean for long periods.

Special Cultures.

For supplying large numbers of specimens for class use, special pure-cultures are made. Protozoa, small crustacea, insect larvæ and other forms can thus be provided.

The essential points in the technique are that one species shall be preponderant, and that it shall feed at the expense of some other species or on a special food pabulum. Under such conditions a normal " natural balance " obviously does not exist and such cultures require looking after with a good deal of care. Details for several species will be found in Chapter One.

Species which can be reared in the Aquarium.

Fish

Almost any of the small common " coarse " fish will thrive, but some prefer running or artificially aerated water. The following can be easily dealt with :—

Perch.—Carnivorous and will attack smaller fish, feed on meat, worms, etc.

Roach.—Feed with artificial foods, small worms, etc.

Tench.—As roach, provide plenty of weedy shelter and a mud bottom.

Carp.—As for Tench.

Gudgeon.—As for Tench, provide a pebbly bottom, will take small gentles.

Minnows.—Require a mixed diet, feed with finely chopped meat once a week.

Stickleback.—As for Minnow, the males fight during breeding season.

If a warm-water aquarium is available, some of the sub-tropical forms can be kept. Particularly the Paradise fish and the Siamese Fighting fish. For both, provide plenty of shelter, a mixed diet including various small organisms. Keep the tanks covered. The fighting fish are best kept as pairs, and must not share a tank with other fish.

Amphibia

Palmated Newt, Smooth Newt and *Warty or Great Newt.*

These three common English forms are easily reared, especially in an outdoor pond with access to land. Provide plenty of shelter, a mixed diet and suitable plant life. The arrangements must always take into account the fact that the animal is a true amphibian. Larval forms require a living diet, small crustacea, tiny worms, small fly larvæ (" gentles "), etc.

Frog and Toad.

A pair of frogs *in copula* can be brought in to deposit spawn, or kept in an outdoor pond. The difference in the egg habit should be noted. Feed tadpoles on meat when large and free swimming. The early stages are vegetarian or feed on microscopic life on leaf surfaces. Make provision for metamorphosis.

Among invertebrates, the crayfish may live in running water in captivity, it is carnivorous ; the water-mussel is hardy and requires a deep mud bottom—it feeds on microscopic organisms. Other forms, such as snails, lice, etc., are kept in a well stocked aquarium and left to forage for themselves.

CHAPTER VI

VIVARIUM METHODS

A VIVARIUM is just as essential as an aquarium for keeping and observing the habits of the living organism. The methods and apparatus are both simple, and possession of a vivarium opens up new and important fields of work.

Types of Vivaria.

Various types of vivaria can be adopted—indoor or outdoor, heated or unheated, or, depending upon the size they can be classed as micro- or macro-vivaria. The type selected will depend upon the teacher's needs and opportunities.

Indoor Vivaria.

These are generally of small size. If purchased, they are usually constructed of zinc sheet, with a glass front or top. Such a type can also be made from plywood. A sketch for a convenient form is given

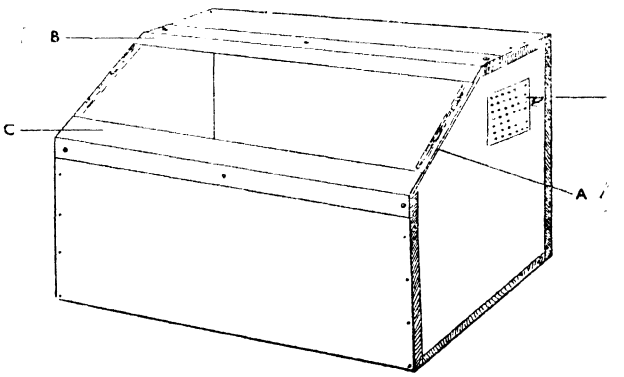


Fig. 12.—A SIMPLE VIVARIUM BOX.

The box is made of thin wood (plywood is excellent) and painted inside and outside. Air is provided through the perforated zinc sheet D. A *is a sheet of thick glass which is held in place and slides between the two metal strips* B *and* C. *These are best made by buying lengths of right-angled brass strip and opening out as required.*

in text fig. 12. Boxes large and small can be pressed into service, their lids being replaced by glass, perforated sheet zinc or muslin. They should be lined with bark or cork to provide hiding places and if possible, be divided into two halves, one of which is darkened. Provision for a water supply is essential—a porcelain pan will do well. Finally, it is an advantage in some cases to have duplicate removable floors so that one can be removed and cleaned while the other is in use.

For very small forms, such as insect larvæ, round tin boxes with glass lids are excellent. These can be purchased from dealers but often exist as bye-products from domestic sources.

Temperature Control.

It is more difficult to control the temperature of the vivarium than of the aquarium. A good way is to place the case in a stream of warm air above hot water pipes or a radiator. It should never be in actual contact with the heater. An electric lamp of the carbon filament type provides an excellent source of heat, but provision of a dark chamber is essential—all animals do not like continuous sunlight. While there are many other possible means of heating these two will no doubt be sufficient. Most of the remaining methods are complicated.

To keep a constant temperature demands thermostat control with an air circulating fan. The method proposed above is no doubt somewhat rough, and uncertain, and liable to a wide range of fluctuation. If the cages are large enough this will not matter, because a temperature-gradient will be set up and the animals will select their own environment. In any case few animals, other than internal parasites of warm blooded vertebrates, live in an environment with constant temperature.

If a heated chamber is used a thermometer should be present, which should be read daily, and which should record the maximum heat developed.

Humidity Control.

Many terrestrial animals are affected by the relative humidity of the atmosphere. Thus it may be necessary to have some control of this factor. The physical problem of the relation between temperature and humidity is simple, and it will be obvious that it is the evaporation capacity of the air which is the important factor, rather than the actual amount of water-vapour present. This fact is well known from work on plant ecology.

Accurate control of humidity is difficult, but a cage can be kept moist by always having a large water surface exposed therein. In a completely closed cage, such as a chemical desiccator, more accurate control can be effected, but only when the temperature is also controlled. The problem thus has two aspects, the control of the vapour pressure in the air, and the control of the air temperature.

Humidity can be controlled in a closed space by exposing a surface of sulphuric acid or potassium hydroxide solutions. The former is probably most suitable for school use. The acid is used in solutions of varying strengths. The following data (Table 3) of the proportions

TABLE III

DATA FOR PREPARING SULPHURIC ACID SOLUTIONS TO CONTROL RELATIVE HUMIDITY.

N.B.—The values given are only approximate. The Relative Humidity will vary little over a moderate temperature range, but the Saturation Deficiency varies considerably.

Specific Gravity of Solution.	Percentage of Acid present.	Relative Humidity.
1.0	0	100
1.1	13	95
1.5	20	90
1.2	28	80
1.25	33.5	70
1.3	38	60
1.34	44	50
1.44	54	30
1.52	62	15

of water and acid and the specific gravity of the solution to obtain various degrees of relative humidity. The value of the relative humidity above these solutions will remain constant over a fairly wide temperature range, but the evaporation capacity, which is expressed as a saturation deficiency, will vary. It is this factor which is of biological significance. Further detail about this point must be sought in a suitable text book of physics.

Ventilation.

It is essential that the cage be as well ventilated as possible, though this obviously affects the problems of temperature and humidity

control. It is, however, unlikely that these factors will be controlled in any but very small spaces, such as small tins and jars. In these cages ventilation is to be looked upon as a necessary evil. Oxygen must be provided and carbon dioxide removed—without upsetting the water-vapour pressure in the box. A compromise must be made by opening such boxes at intervals.

Large cages must be provided with a wire screen or an area of perforated zinc through which air can pass. If these areas are not too large it is possible to provide plenty of ventilation and also to keep a humid atmosphere where necessary.

Cleaning.

All cages and live boxes must be kept quite clean. It is usual to paint the walls with a hard gloss paint in wooden and metal boxes. Glass cages are more easily dealt with. Cleaning is facilitated by having several slide-out floors as already suggested. Another useful feature is a method of shutting off one part of the cage containing the animals, whilst the rest is cleaned, and then changing them over to the clean side. By being able to darken one part of the box by a slide or cover, and having a vertical partition with a sliding trap, one is often more easily able to segregate the animals.

Out-of-doors Vivaria.

These provide more adequate chances for producing natural conditions for various animals, but never forget that some animals can burrow a long way!

The safest method is to make a vivarium with concrete walls which slope inwards. This can either be an excavation or be built up above ground level. A small central hill, some large stones and climbing sticks, holes for shelter, etc., are provided.

If such a scheme appears too ambitious, an enclosure can be made by using fine mesh wire netting on a wooden framework. The wire should be sunk a little way below ground level.

Combined Aquaria and Vivaria.

Under out-door conditions a small pond and a suitable enclosure built up around it, form an excellent feature. The pond must always have one side at least which shelves very slowly so that such animals as newts or newly metamorphosed frogs can easily get from the water on to the land.

Special examples for the Vivarium.

At the end of this chapter will be found a list of various animals which can be successfully reared in a vivarium. There are one or two special forms which are of much general interest and which demand more attention. The following brief notes should make some degree of success possible.

It is obviously impossible to provide a long list of animals for the vivarium. So much depends upon the opportunity, time at disposal, and initiative of the worker. There are, however, many species of common animals of the countryside which can be reared, in addition to those which the dealer can supply. On the whole, the writer is averse to keeping mammals and birds in captivity as it is difficult to provide them with enough freedom. The keeping of mammals, however, has its uses in practical illustrations for sex-education purposes.

In all cases, study the natural habits of the animal at wild, and try to reproduce the correct environment. If the pupils can go out and " see the real thing " don't keep a poor caricature of it in the school classroom!

Mammals.

Tame mice and rats.
The guinea pig.
The hedge hog—provide outside accommodation as well as a cage.

Reptiles.

Slow-worm.
Grass snake.
Lizard.
Green lizard—purchased. Requires plenty of warmth.

1. *The Stick Insect.*

Eggs or young nymphal stages are purchased from dealers. Feed on privet or euonymus leaves. A few stalks should be passed through a hole in a cork into a small jar of water, thus the material remains fresh longer. The eggs must be kept in a humid atmosphere until hatched. The insect moults several times and reaches a length of about 3 in. when adult. It is parthenogenetic and lays many eggs over a long period.

2. *The Slow Worm.*

Although snake-like this is a lizard devoid of limbs. Provide warmth and plenty of shelter. Feed on small garden slugs and worms. The slow worm is of course quite harmless, though it frequently dies the death as a " poisonous snake." Make provision for hibernation.

3. *The Tortoise.*

The tortoise may not provide much excitement, but is hardy and able to withstand hard treatment—and even neglect. Purchase from a reliable dealer and so get a form which is suitable. Feed on cabbage and lettuce leaves. Provide an outdoor " run " opening to a suitable cage which provides warmth and shelter.

Amphibia.

Toad
Frog
Newt (3 English species.)

} Require combined aquarium and vivarium for breeding.

Arthropods.

No list would suffice for this group. It may be pointed out, that the wood-lice, millipedes, spiders, etc. are too much neglected in favour of insects. See special note on keeping an ant colony.

Molluscs.

Among many forms, the common garden snail (*Helix aspersa*), and the Roman or apple snail (*H. pomatia*) can be kept with ease. Provide a damp atmosphere. Plenty of cover. Feed on succulent vegetables.

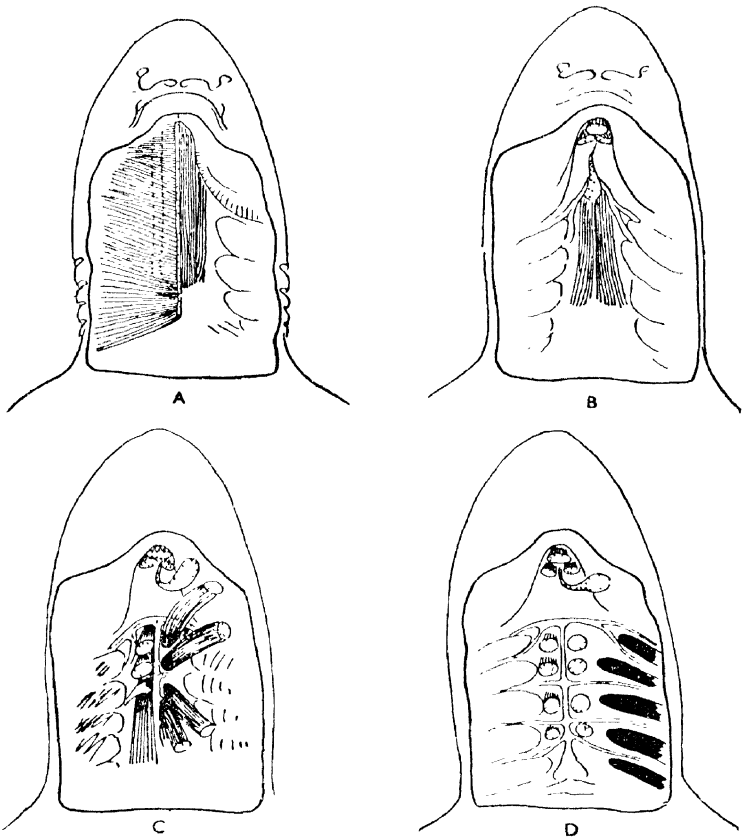
How to keep a Colony of Ants.

The social organistion of insects is a fascinating study. It can be studied to some extent in the open, but such work can be supplemented by classroom or laboratory studies. For such work the ant makes excellent material. An artificial cage or formicarium is built up using a wooden tray about 2 in. deep, and about 20 in. by 12 in. overall. A heavy sheet of glass which makes good contact with the edges forms a lid or cover. It is preferable to use a slightly deeper box, and line it with Plaster-of-Paris. One part of the box must now be made considerably shallower by placing thick pieces of wood in the bottom, so that there is about $\frac{1}{2}$ in. between the top of this wood and the glass cover. A porcelain pan of water should be provided which must reach up above

the wooden platform, but not reach the glass. Now fill up the tray with suitable light loamy soil until there is a $\frac{1}{2}$ in. gap between the soil surface and the glass lid. The wooden platform should now be covered with a very thin layer of soil till the general surface is level with the artificial pond. Finally, add a few bits of stick, pine needles and plant debris. An ants nest is raided and the captives liberated in the formicarium. Efforts must be made to capture a queen. Keep one half of the nest covered with a cloth which can be moved to watch the nest activities. Foraging ants will visit the uncovered part which must contain the pond. A little sugar and water or honey should be provided. This scheme can be elaborated, and if felt necessary, a " moat " can be made round the edge of the tray and kept filled with water to prevent escapes when the lid is removed. There are several common ant species, and some are more easily kept in captivity than others. Other arthropods such as woodlice and millipedes can be kept in a similar box.

A suitably placed school should keep bees using a hive which permits observation of the work of the insects.

PLATE II.



LABORATORY NOTE BOOK SKETCHES OF A DISSECTION OF THE AFFERENT BRANCHIAL ARTERIES OF THE DOG-FISH.

A. *Ventral surface skinned. Superficial muscles removed on one side to expose longitudinal muscle bands.*

B. *Superficial muscles removed entirely. Longitudinal muscle bands severed close to attachment. Thyroid body exposed with anterior branchial arteries showing immediately dorsal (i.e., topographically ventral). The first pair of "pillar muscles" lying in median line covering succeeding pairs.*

C. *The four "pillar muscles" (coraco-branciales) of one side severed at their origins and lifted up to show relations. The first two of the other side severed showing third with extra-branchial cartilage lying on it.*

D. *All the pillar-muscles severed near their attachments. The branchial arteries traced out partially on one side and completely upon the other on which the gill pouches have also been slit open.*

CHAPTER VII

THE TEACHING OF DISSECTION

WHILE the First School Certificate Examinations do not, in general, demand that the candidate shall himself have performed dissections, it is necessary that he shall have seen them. In the Higher Examinations capacity to dissect is essential. It may be said that a knowledge of animal biology is impossible without dissection.

Thus the teacher must always dissect, and in some cases teach his pupils to do so too. Now no one ever gives a pupil a burette and tells him to titrate without instruction—or expects him to weigh without instruction in the use of the balance. All too often, however, teachers neglect to show how to dissect; they merely hope that possession of a practical text-book and a box of instruments is all that is required. Far from it.

The teacher should make a demonstration dissection of every type studied. He should deal with each system in turn, describe what he does, why and how, and if possible, build up a sketch drawing *en passant*. Only by these means is the student helped to bridge the gap between the skin of the animal and the text-book illustration. Such illustrations show the ultimate aim. They give no help to the student who is floundering about, in three planes, and through unknown and unexpected structures. The ideal method could be arranged as follows :—

1. Show a diagram of the system concerned and also a finished dissection of it.

2. Step by step pursue a dissection and make rough drawings for each stage.

3. Start the student off on his own dissection, but supply him with serial drawings or photographs, or even actual preserved dissections as a guide.

4. Ensure that the student follows the exact method detailed. Experiment can come after experience.

By these means, the student is never left guessing, or tempted to " take a chance and cut it."

Why is such an obvious method so seldom followed? Possible reasons are not difficult to seek, and among them is the fact that a good deal of biological teaching is undertaken by staff members who have had to make it an " extra." It is very difficult to get from summer schools, or revision courses, the same training as is provided by a complete biology course.

To make a good dissection quickly requires skill and confidence. It demands a careful programme of work—and it is here that much school work fails. The pupil has had no clearly cut plan in which there are a series of significant and mutually inter-dependent stages.

This at once raises the question of whether there is a best way for any particular dissection to be carried out. Probably not, because individuals have personal views. The point is to have a clear cut technique and insist that all beginners learn it.

The dissection of the various types is described in the text books. But few of them give real instructions for dissection, nor is it easy to do so. What the books give is the destination. What the pupils wants is a map of the way there. The first steps in filling this gap have already been mentioned. The next step is to design the technique for each dissection.

In doing this, reference must be made to the total time available, to the duration of the periods available, to the material, to the detail required, to the period for which the carcase can be preserved, and so on.

Ideally every student should completely dissect one specimen of one sex, and observe the work of another student on the opposite sex. He should then make a revision dissection of the second sex. As far as possible, each dissection or stage in a dissection should be completed and drawn at a sitting. We all know the mutilated products of overnight storage in the tank, which too frequently gives rise to that well known excuse, " that it got broken in the tank, sir."

For the benefit of those with slender biological training the following section will describe the order of dealing with selected types, and will also illustrate on a single example (dogfish, ventral arterial system), the application of the suggested method of demonstration. Dissection of other vertebrate types should proceed along similar suitably modified lines.

SUGGESTED SERIAL PLANS FOR DISSECTION

1. ANODON

Dissection 1.

(*a*) Examine external characters.
(*b*) Remove left valve, examine and draw inner surface.
(*c*) Examine and draw animal *in situ*.
(*d*) Reflect left mantle lobe : draw.
(*e*) Reflect gill plates : draw.
(*f*) Cut attachment of left inner gill to foot, indentify excretory pore and gonopore.
(*g*) Remove animal from right valve. Pin out mantle flaps so that animal is held upright, dorsal side up. Open cloaca to pericardium. Identify and draw.
(*h*) Turn animal over. Split foot in median plane to disclose pedal ganglia.
(*i*) Open epi-branchial chamber, cut through roof to expose visceral commissures, trace to visceral ganglia.
(*j*) Dissect away labial palp and expose cerebro-plueral ganglion.
(*k*) Open up mouth to stomach and trace intestinal coils by splitting one wall of gut.

Dissection 2.

Take a chromic hardened specimen and cut into transverse slabs $\frac{1}{4}$ in. thick.

Examine and draw each section from posterior surface. Work out relations of epibranchial spaces, gill lamellae, foot, etc.

2. ASTACUS

Externals.

(*a*) Examine and draw external features.
(*b*) Remove a complete set of appendages, draw selected series to illustrate general principle.
(*c*) Remove branchiostegite and examine gills.

Dissection.

1. *Viscera.*

(*a*) Remove exoskeleton in a broad band along whole dorsal length. Identify and draw organs exposed.

(*b*) Remove carapace laterally. Draw organs *in situ.*

(*c*) Sever the sternal artery, œsophagus, gonoducts and muscles of the gastric mill. Sever gut near anus. Reflect gut and slowly lift out entire visceral complex. Examine, identify, draw.

2. *Nervous System.*

(*a*) Identify ventral nerve cord at thoraco-abdominal junction and expose completely, starting with thorax. (A prepared exo-skeleton is essential at this point to demonstrate method of dealing with endophragmal skeleton).

(*b*) Draw C.N.S.

3. Lumbricus

Externals.

(*a*) Examine, identify and draw external features.

Dissection 1.

(*a*) Open along dorsal surface. Mark segment 14 with special pins to act as a reference point. Draw.

(*b*) Sever the gut in the region of segment 30, and reflect it forwards segment by segment. With segment 14 as an index, identify the parts of the reproductive system.

(*c*) Complete the removal of the gut and examine and draw the nervous system.

Dissection 2.

(*a*) Repeat dissection 1, (*a*) and (*b*).

(*b*) Identify and remove the ovaries. Mount and draw.

(*c*) Remove, mount and draw a nephridium.

(*d*) Repeat 1, (*s*) if necessary.

(*Note.*—When dissecting the worm, it is well to always make a smear of the vesicula seminalis and examine for *Monocystis.*

4. Helix

Externals.

(*a*) Examine living and dead expanded specimens.

(*b*) Examine empty complete shell and also a shell cut in half in the vertical plane.

Dissection 1.

(*a*) Remove shell.
(*b*) Examine complete animal.
(*c*) Open branchial space, reflect the roof and draw contents of mantle chamber.

Dissection 2.

(*a*) Open the foot by dorsal longitudinal incision, reaching from anterior end to posterior part of branchial cavity.
(*b*) Sever the excretory duct.
(*c*) Carry the incision right round all the coils of the visceral mass.
(*d*) Expose and spread out the reproductive organs.
(*e*) Work out the relations of the alimentary system.

Dissection 3.

(*a*) Identify the nerve collar commissures round the buccal mass.
(*b*) Trace the courses of the various nerves.

All the above operations are carried out on a single specimen. Finally remove and mount (unstained) the radula.

5. Scyllium

The Skeleton.

The skeleton must be studied from specially prepared material, and thus must be done before making the dissection. While making the dissection the pupil should identify all skeletal structures with which he comes into contact. The same is true for all vertebrate dissections. The method of study can be based on the notes given later dealing with the skeleton of the frog.

Externals.

(*a*) Examine and draw in side view.
(*b*) Examine and draw ventral surface.
Pay particular attention to pores opening to cloaca.

Dissection 1. Abdominal Cavity. General Relations.

(*a*) Open abdominal cavity and examine relations of organs *in situ*. Make careful drawings.
(*b*) Lay the animal on its left side and draw the organs out of the cavity so that the dorsal wall of the abdominal cavity is exposed.

Draw. Note particularly the arteries in the suspensory mesenteries and the falciform ligament supporting the liver.

Dissection 2. Alimentary System.

(*a*) Open the intestine and examine the spiral valve.
(*b*) Build up a schematic drawing of the relations of all the parts of the alimentary canal and its associated glands.

Dissection 3 Urino-genital system. Male.

(*a*) Remove gut and liver.
(*b*) Examine mesentery at anterior end of the testis, for vasa efferentia.
(*c*) Dissect away dorsal periotoneal epithelium to expose kidney and associated ducts.
(*d*) Expose kidney completely, paying particular attention to the posterior end.
(*e*) Work out and diagrammatise relations of the various organs. ducts—and their openings to cloaca.

Dissection 4. Urino-genital system. Female.

(*a*) Remove gut and liver.
(*b*) Examine ovary, oviduct, common median opening of the oviducts to coelom.
(*c*), (*d*), (*e*) Proceed as for male.
(*f*) The oviduct of one side is to be removed completely posteriorly to permit examination of urinary sinus.

Dissection 5. Ventral arterial system. (Afferents).

(*a*) Skin ventral surface and remove underlying muscle sheets.
(*b*) Remove longitudinal muscles.
(*c*) Remove pillar muscles.
(*d*) Trace out ventral arteries to gill arches.

Dissection 6. Heart and Sinus venosus, etc.

(*a*) Trace conus arteriosus to the heart.
(*b*) Examine heart and pericardial cavity.
(*c*) Examine sinus venosus. Identify openings of hepatic sinuses.
(*d*) Identify anterior and posterior cardinal sinuses and ducti cuvieri.

Dissection 7. Dorsal arterial system. (Efferents).

(*a*) Remove lower jaw by incision from its posterior angle carried back through pharyngeal walls.
(*b*) Identify dorsal aorta below œsophagus (which was severed in Dissection 3 (*a*)).
(*c*) Cut away œsophagus and skin roof of buccal cavity to trace aorta forwards.
(*d*) Examine efferent arches and pectoral (brachial) artery.
(*e*) Trace out anterior relations of carotid system which will necessitate opening base of cranium and base of orbit.

Dissection 8. Nervous System and Sense Organs.

A.—The Orbit.

(*a*) Skin round the eye and pull the eye as far outwards as possible. Examine and draw from dorsal surface.
(*b*) Sever the optic nerve and six eye ball muscles, and draw contents of the orbit.
(*c*) Section the eye and examine the major structure.

B.—The Cranial Nerves.

First Part.

(*a*) Expose the brain in the dorsal mid-region.
(*b*) Trace the ophthalmic branches of fifth and seventh nerves forwards to snout.
(*c*) Expose anterior part of brain and open the nasal capsules.

Second Part.

(*a*) Trace the ophthalmic branches to their origin by cutting away the posterior walls of the orbit on one side only.
(*b*) Identify eighth nerve.
(*c*) Examine the roots and branches of fifth, seventh and eighth.

Third Part (Ear).

(*a*) Dissect the cartilage away so that the semi-circular canals are exposed and left standing.

Fourth Part.

(*a*) Remove the skin at the side of the head behind the orbit, but leave the rim of the spiracle intact.
(*b*) Identify the hyomandibular branch of fifth, and trace its peripheral elements.
(*c*) Trace this branch back behind the spiracle to the brain.

Fifth Part.

(*a*) Expose below the auditory capsule the roots of ninth, and trace outwards to the first gill pouch.

(*b*) Expose posterior to ninth, the roots of tenth and trace outwards.

(*c*) Expose the peripheral parts of tenth by cutting away a thick slab of muscles so that the vertebrae are exposed. On the outer edge of this, just inside the gill slits, is a thin membrane. Split this by a longitudinal cut and by pressing apart the walls of the chamber exposed the branches of tenth are seen.

(*d*) Identify and trace out the branchial, lateralis and visceral components of tenth.

C.—The Spinal Nerves.

(*a*) Expose the spinal cord and identify the roots of the spinal nerves.

(*b*) Examine the pectoral plexus of spinal nerves to the pectoral fin.

(*c*) Examine the pelvic plexus.

D.—The Brain.

(*a*) Sever the roots of the cranial nerves inside the cranium and as far away from the brain as possible.

(*b*) Dissect away the cartilage and make such incisions as are required to leave the nasal organs free from the head, but attached to the olfactory lobes of the brain.

(*c*) Lift out the brain and examine in lateral and ventral aspect.

Type Demonstration Dissection

Ventral Arterial System of the Dogfish

Required :—

(*a*) Dogfish preserved in 3-5 per cent. formalin. Wash well before dissection.

(*b*) Injected dissected museum preparation to demonstrate final state of dissection.

(*c*) A series of photographs or drawings or unfinished dissections to show each stage in the whole process. See plate II.

Method :—

1. Pin the fish with the ventral side up on dissecting board. Expand pharyngeal region by inserting test tube into mouth and pushing it back into œsophagus.

2. Skin lower surface of head back to pectoral region. This exposes a sheet of transverse muscle fibres divisible into three regions.

3. Remove the above muscle sheets with care, they are very thin. This exposes the lateral gill pouches and in the median line a longitudinal muscle originating from the pectoral bar and inserted on the lower jaw.

4. Remove the above muscle. This exposes paired muscles arranged longitudinally.

5. Remove the above paired muscles. This exposes the thyroid gland in the median line and the anterior, topographically uppermost pair of a series of 4 pairs of pillar muscles.

8. Remove the thyroid gland, or reflect it forwards. This exposes immediately below (morphologically dorsal) the thyroid the anterior end of the ventral aorta which bifurcates into two branches.

7. Remove the first and second pairs of pillar muscles. It is essential that each muscle be cleaned and carefully defined before cutting. When its limits are clearly visible it is severed at both ends and lifted out complete. Always leave a short piece of the dorsal end (which is topographically ventral while dissecting) to act as a landmark. When the two pairs of muscles have been removed the base of the second afferent vessel is visible.

8. Remove the third muscle pillar on each side. Identify afferent artery three on each side.

9. Remove the fourth pair of muscle pillars. Identify the next pair of afferent vessels.

Note.—Throughout the demonstration of the method it is well to place each of the muscles, as removed, in serial order upon the bench. This emphasises the need for dealing with them as complete units instead of cutting them out in haphazard fashion which provides no check upon position.

Trace each branchial arch as far as possible along the gill. To facilitate this, remove the morphologically ventral half of each gill pouch.

Open the pericardium by cutting away its ventral walls. Trace the conus arteriosus forwards to the ventral aorta.

The completion of the dissection of the heart should now be followed out on similar lines.

Throughout the dissection the teacher should make rough diagrams of the various phases, and so should the student. These are the only

real means of providing for revising a dissection, without actually doing it again.

RANA

A.—The Skeleton.

1. *Skull and jaws.*

Draw carefully from three positions :—

(*a*) Dorsal view.
(*b*) Ventral view (without lower jaw).
(*c*) Lateral view (with lower jaw in position).

2. *Vertebral Column.*

(*a*) Draw a single vertebra from the following aspects :—
  (i) anterior.
  (ii) posterior.
  (iii) dorsal.
  (iv) ventral.
  (v) lateral.
(*b*) Examine and draw relations of column to the pelvic girdle.
(*c*) Examine and draw the urostyle.

3. *Pelvic Girdle.*

(*a*) Draw the pelvic girdle :—
  (i) dorsal view with sacral vertebra and urostyle in position.
  (ii) lateral view to show limits of the three elements.

4. *Pectoral girdle and sternum.*

Draw dorsal and ventral views.

5. *Fore limb.*

(*a*) Make a diagram of the general relations of the parts of the fore limb.
(*b*) Make a large scale drawing of the relations of carpals, metacarpals and phalanges. For this a well prepared skeleton may be examined with a hand lens, or an alizarin transparency used.

6. *Hind limb.*

(*a*) Make a diagram of the general relations.
(*b*) Make a large scale drawing of the foot.

B.—External features.

(*a*) Examine and draw to show general features.

(*b*) Open mouth and draw.

C.—Anatomy.

Dissection 1. Superficial muscles.

(*a*) Skin ventral surface starting with a median longitudinal incision and reflecting the skin. Be careful not to cut the cutaneous vein below and just posterior to the arm.

(*b*) Examine and draw body muscles.

Dissection 2. Mechanism of respiration.

(*a*) Remove the mylo-hyoid muscle.

(*b*) Note and draw the genio-hyoid muscle.

(*c*) Note the sterno-hyoid (better seen later on).

(*d*) Identify the petro-hyoid.

(*e*) Make serial diagrams of action of this mechanism.

Dissection 3. Abdominal Cavity. General Relations.

(*a*) Make a longitudinal incision from pubes to xiphoid along each side of anterior abdominal vein.

(*b*) Place two ligatures $\frac{1}{8}$ in. apart on the abdominal vein, the most anterior being immediately behind the xiphoid cartilage.

(*c*) Sever the abdominal vein between the ligatures and reflect the posterior portion. Anterior to the ligature and below (really dorsal) the xiphoid, this vein turns at right angles and runs vertical to its former course. Thus in front of this there is no fear of a median incision cutting the vein.

(*d*) Carry the two longitudinal incisions (made at 3*a*) around the xiphoid cartilage, thus leaving a small island.

(*e*) Carry a single median incision through the sternum.

(*f*) Reflect the abdominal walls—and draw the organs exposed *in situ.*

(*g*) Spread out the organs exposed and make diagrams of general relations of alimentary, reproductive and excretory systems. Examine visceral blood supply.

Dissection 4. Urino-genital System.

A.—Male.

(*a*) Remove the gut by severing rectum and œsophagus.

(*b*) Identify testes and vasa efferentia.
(*c*) Identify kidney, supra renal, fat bodies, ureter and vesicula seminalis.
(*d*) Draw.

B.—Female.
Repeat with suitable variation.

Dissection 5. Posterior Vascular System.
(*a*) Identify blood vessels in legs.
(*b*) Examine vascular relations of the kidney.
(*c*) Construct diagram of this.

Dissection 6. Venous System (anterior).
(*a*) Open pericardium and reflect heart forwards by cutting its suspensory ligament.
(*b*) Trace relations of the superior venae cavae.
(*c*) Trace posterior vena cava forwards.
(*d*) Relate results to diagram 5 (*c*).

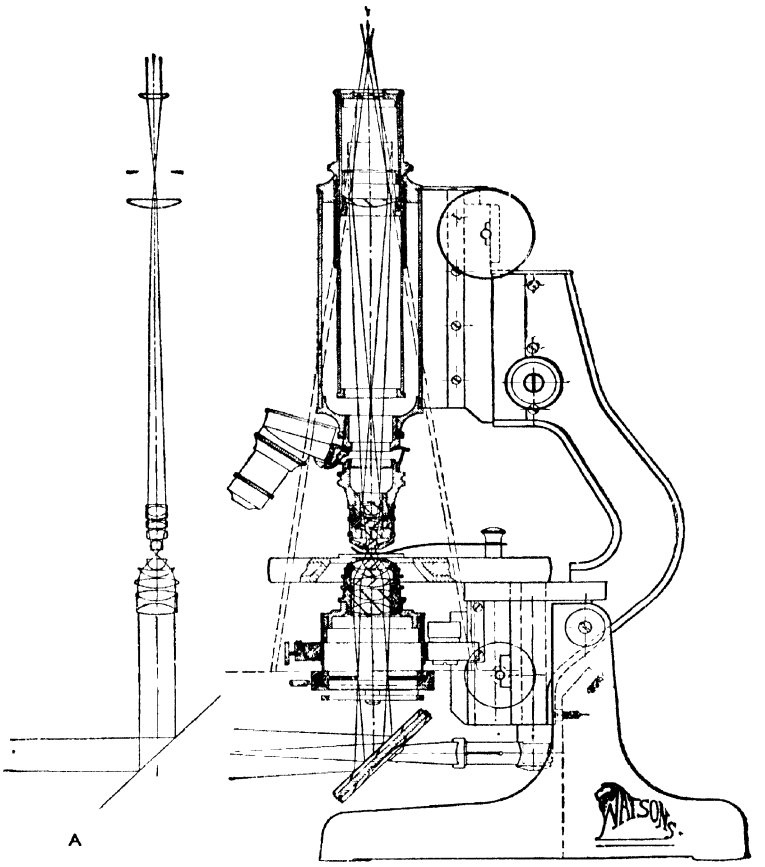
Dissection 7. Arterial System (anterior).
(*a*) Remove as much of venous system as is necessary.
(*b*) Trace in turn from the conus arteriosus :—
  (i) carotid arches.
  (ii) systemic arches.
  (iii) pulmo-cutaneous arches.
(*c*) Diagrammatise and show relation with 5 (*c*).

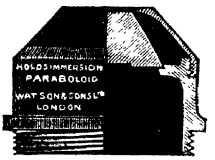
Dissection 8. Spinal Nerves.
(*a*) From ventral surface examine spinal nerves.
(*b*) Examine sympathetic nerve chain.
(*c*) Identify rami communicantes.
(*d*) Make a diagram of these nerves.

Dissection 9. Cranial Nerves.
(*a*) Remove one eye and examine orbit.
(*b*) Open cranium dorsally.
(*c*) Trace out cranial nerves.
(*d*) Draw.

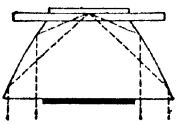
Dissection 10. Brain.
(*a*) Remove brain from cranium.
(*b*) Draw dorsal, lateral and ventral views.

PLATE III.





C



D

A. *Diagram of optical principle of the compound microscope.*

B. *Sectional view of compound microscope.*

C. *The "Holos" immersion paraboloid.*

D. *Optical principle of immersion paraboloid for dark ground illumination.*

CHAPTER VIII

SOME HINTS ON THE MICROSCOPE

THE study of minute detail, either as pure morphology or from the functional aspect, is impossible without artificial optical aids. The means adopted can be arranged at three levels :—

1. The hand lens.
2. The dissecting microscope.
3. The compound microscope.

Such an arrangement is artificial, but convenient.

1. *The Hand Lens.*

Although the hand lens is an instrument of great possibility, it is too frequently neglected. This often results from a false economy which has purchased practically useless forms. The available types are many, ranging from a shilling in price, up to thirty shillings or so, and they show a corresponding variation in mode of mounting.

Each student should possess a hand lens of some kind. A folding model with two powers, X6 and X10, which can be combined, is very useful, but rather than buy a cheap model of this sort, it is better to buy a single lens at the same price. The lens should be handled carefully and kept clean. In use it is placed close to the eye and focused by moving head and lens up and down. A watch-maker's model is useful as it leaves both hands free.

Some makers have attained the same end by attaching the lens to a needle or other instrument. By this means very delicate dissection is possible, as the needle tip can always be kept in focus.

For examining opaque objects, surface structure and cavities, it is useful to have a lens mounted in conjunction with a hand lamp. Several small forms are on the market.

In the purchase of a hand lens, attention must be paid to its colour correction, its spherical aberration, and its focal length. The ideal is a large flat field of observation with a long working distance below the lens.

2. *The Dissecting Microscope.*

In its simplest form this is merely a hand lens arranged to slide up and down on a vertical rod. A makeshift can easily be devised, and models are produced from about fifteen shillings upwards. Here too a good lens on a firm stand is the ideal. A pair of arm rests and a reflector for illumination of the object are useful.

For demonstration work, such an instrument is a great asset, for example, in showing the ovaries of the earthworm *in situ.*

If funds permit, a binocular microscope is of great value in demonstration as it permits of a fairly high power stereoscopic view.

3. *The Compound Microscope.*

In selecting a microscope attention must be paid to the mechanical efficiency of the stand and tube adjustments, and to the optical equipment. For school use, sturdiness and simplicity and capacity to withstand much consistent oll-use are essential.

Each microscope should possess a $\frac{2}{3}$ in. and a $\frac{1}{6}$ in. objective, preferably mounted on a revolving nose-piece. Eye-pieces X2 and X5 are required. An oil-immersion for demonstration work is useful.

Dark ground illumination. (See plate I) (frontispiece).

The use of dark ground illumination is greatly neglected, but for some work (e.g., observing living Protozoa) it may provide results superior to those obtained with transmitted light. A special dark ground condenser is required which is so designed, that no direct light rays enter the objective, but only rays, which have been diffracted, refracted or reflected. The observed field would thus appear to be dark and empty, but any particles therein are lighted up indirectly. Some condensers are designed for use with an oil film and a slide of definite thickness, and as a variety of patterns exists, this makes it necessary to follow the maker's instructions with great care.

The " Holos " Immersion Paraboloid may be used for dark ground illumination with high power objectives providing the Numerical Aperture does not exceed 1.0. Recently Messrs. Watson of London have placed on the market a one-seventh inch Oil Immersion Objective with N.A. less than 1.0. This forms a convenient way of reducing costs as it combines the functions of the one-sixth inch and the one-twelfth inch oil immersion lens.

Microscope Drill.

Pupils frequently possess a detailed knowledge of the optics of the microscope, and yet fail to apply it to their work. The optical facts which they have learned carry no significance for them in practice, so that they fail to get the best out of their instrument.

For this reason, each student should be well trained in how to use the microscope. He should understand its mechanical and optical structure, at least in outline, and be instructed in its use. For this end, a series of standard experiments and tests should be made by the student. Particular attention should be paid to the technique of illumination. It must be made clear that the light must be reflected along the optical axis of the microscope. This is tested with a two-thirds inch objective by racking the tube up and down ; any apparent movement of the object across the field means that the light is not axial and must be corrected by moving the mirror. Finally every effort is necessary to achieve critical illumination by using the iris diaphragm.

The Micro-Projector.

There are now on the market several forms of micro-projector for school use. Such an instrument makes demonstration possible, whereby a group of students can make simultaneous observations. It is also valuable for projecting from living specimens and can be used in preparing large scale diagrams. The most useful type of apparatus is one in which the instrument can be used, both vertically and horizontally, and with which a live box and tank are available for dealing with living forms. A shield or daylight box, or a ground glass screen must be provided for working in comparatively light rooms. Finally, interchangeable optical equipment with standard R.M.S. threads is essential.

CHAPTER IX

THE CINEMA AS AN AID TO ZOOLOGY TEACHING

THE film technique has received different receptions from various workers in the educational field. Probably all would agree that it possesses considerable value when properly used. The difficulty arises in attempting to decide what is, and what is not, proper to the needs of the educationist. Fortunately, this difficulty is not too pronounced in the question of the teaching of biology.

The advantages which the film offers to the biologist are several and important, and it is well to consider a few of the major points.

Above all things the cinematograph gives us a method of controlling the time-factor in many processes. Some things take place too quickly for the eye to watch, others are imperceptibly slow. " Slow motion " and " speeding up " however, overcome these difficulties. On the screen we are able to watch the complete sequence in locomotory movements or the complicated actions of feeding mechanisms in small animals. Similarly we can see an embryo developing, or a plant growing, at such a rate that the whole process takes but a few moments. This control of the time factor is of great value, but it may have dangers, and every effort should be made to give some idea of the relative scale of all the factors concerned. Not only must we explain that the pictures are magnified, say 500 times, but also that the time of one second in the film is in reality a time of perhaps hours.

The application of cinephotography to microscopic work permits subsequent observation upon large scale pictures. Detail visible only with a lens and patient observation is easily accessible—though someone has had to be patient to get such results.

This factor of size makes it possible to demonstrate to a large class of pupils, which is of obvious importance. It would be of interest to investigate the initial and upkeep expenses of a single cine-projector, and of a set of microscopes ; perhaps the latter would appear to be much cheaper from some points of view. The possible differences to the effectiveness of the teaching would also have to be taken into account.

The film also gives us some idea of the behaviour of organisms. Necessity dictates that we are unable to show the dogfish or jelly fish

swimming about, and many other such things are too often unavailable for pupils' observation. Biology is, however, the study of life, and life is characterised by behaviour. To many students the dogfish must consist of only a head and trunk, and even pictures of the complete animal give no conception of its swimming powers.

Here then is a factor of importance, the film may help us to see how animals live and behave. This is of even greater value when we turn to really inaccessible forms, which are rare, very large, or for other reasons cannot be obtained for school work and demonstration.

There remain two other aspects of the film as an adjunct to biological teaching which so far do not appear to have been explored.

The first of these is in demonstration of how to dissect. The teacher, as a demonstrator, is faced with the fact that only a few pupils can effectively watch the operation. Some ways of overcoming this difficulty have been outlined in Chapter VII. Obviously, films of the process would be ideal. A whole class could watch each step, and control of the time factor adds still greater value. Parts of the film could be " cut," perhaps a little trick work would be necessary, but with slow and speeded work, and the aid of the continuity artist a very useful result could be obtained. It is strange that this aspect of teaching technique has not received more attention, especially as the field of surgery has already been explored for demonstration purposes.

A second line awaiting progress is the making of good diagram films. The text book diagram is useful because it gives nothing but essentials ; these, however, are static in form. Most of the processes which we, and our pupils, find it difficult to follow, are dynamic. Thus there is a chance for the application of Mr. Disney's methods to zoological diagrams. An excellent field for such a method would be in the demonstration of the early embryology of the frog, in showing the general features in the functional aspects of a circulatory system, and in making moving pictures of nuclear phenomena. Many pupils find chromosome movements difficult to follow, and it would not be too difficult to devise serial pictures showing the principal points. Those pupils who spend their time in making serial sketch figures of their form master at the top corners of book pages, and then entertain the back row with primitive moving pictures, might be induced to have a shot at dealing with some of these points !

An immediate problem which arises in connection with the use of cine-films is the question of cost. It is not possible to enter into details

of this here. It should be noted that there must be an initial cost for a projector and screen, and that films can then be purchased outright, or hired. If certain of the demonstration type already suggested were available these would form an excellent start for a film library. They are the type which would be regularly used in teaching the principles of biology. Others, especially of the natural history type, could be hired as required.

Standard film is 38 mm. wide. The sub-standard sizes are 16 mm. and 9.5 mm. The smaller sizes are cheaper to purchase, but of course, usually entail showing a smaller picture. In each size a very wide selection of material is available.

Until fairly recently films in the biological field were very largely of the natural history type. Now there are signs that a more technical kind is being produced with a definite reference to educational demands, and not merely a sale in the " general interest " field.

A further advance is the development of the " talkie " technique, especially in sub-standard size. Again this is of great value, but not till lately has this been well explored. It has a great potential value in the demonstration type of film as proposed for teaching dissection, etc.

Many new ideas are hailed with enthusiasm and almost become a cult with their supporters who push them far past their legitimate confines. The biological film is faced with this danger. There should be two golden rules for those who propose using this technique :—

1. Do not let the demonstration become a " show " in which the pupil is not expected to be an active agent. It is to help him to think, to show him problems and to demonstrate principles—and the moment he becomes passive, the moment he looks upon it as no more than a diversion, failure commences.

2. Do not use films for demonstrating what the pupil, with a little effort, could find out for himself. In a normally equipped school laboratory he can do a good deal of work of a high order and can observe living things himself. If this is so, why go to the expense and waste of time of showing him a film of it, which however good, does have some disadvantages ? It is the same with the film as with the school aquaria, etc.—when possible, let him see the animals under natural conditions, not poor caricatures of them in captivity. In the same way examine the real thing rather than moving pictures of it. The pictures may move and simulate life—but the animal responds and behaves, and the films do not.

TABULAR DATA

This section will endeavour to provide a guide as to what material will be required for class teaching, what its approximate cost will be, and how much time should be devoted to the study of various specimens.

1. *Costs of Specimens.*

Table 1 provides a list of the specimens required, while it may appear ambitious, it is in reality but a minimum for effective teaching. The prices quoted are to be considered as only approximate, they will be controlled by locality, laboratory facilities, and the amount of time the teacher can spend upon preparatory work. Postage and carriage is of course an extra.

A.—Microscopic Preparations.

Discretion and available funds will decide whether each pupil is to be provided with a slide, or whether only a demonstration slide be available for common use. Whenever possible, each student should make some of his preparations, but even here, a professional slide will act as a useful index of the pupil's success.

A. 1. General

	s.	d.	
Amoeba	2	6	per slide
Paramoecium	1	6	„
„ conjugation phase	2	0	„
Volvox (various phases if possible)	1	9	„
Euglena	1	3	„
Monocystis (all phases)	1	9	„
Opalina	1	9	„
Hydra, whole mount	1	6	„
„ „ „ showing budding	2	6	„
„ „ „ „ gonads	2	6	„
„ transverse section	1	3	„
„ longitudinal section	1	9	„
Obelia, hydroid stage	1	6	„
„ medusoid stage	1	6	„

	s.	d.	
Ascaris. T.S. various regions of both sexes	1	9	per slide
Taenia scolex	3	0	„
„ proglottide to show sexual organs	1	9	„
„ ripe proglottide	1	6	„
Distomum, whole mount stained	2	6	„
„ „ „ gut injected	3	0	„
„ „ „ excretory system injected ...	3	0	„
„ transverse section	1	3	„
„ miracidia, rediae (for demonstration) ...	3	6	„
Lumbricus, T.S. typical segment	1	3	„
„ L.S. anterior region	2	6	„
„ whole mount of ovary	2	6	„
„ „ „ nephridium	2	6	„
Periplaneta, mouth parts	1	6	„
„ salivary gland	1	9	„
„ membranous wings	1	6	„
Anodon, T.S. gills	1	3	„
„ glochidia	1	6	„
„ T.S. young specimen cut in 3 regions to show relations of gills, foot and " body "	3	0	„
Amphioxus, complete whole mount	15	0	„
„ T.S. pharynx	1	3	„
„ „ and liver	1	3	„
„ gut and atrium	1	3	„
„ posterior to atrium	1	3	„
„ through olfactory pit	2	0	„
Scyllium, vertical section of skin	1	6	„
Rana „ „	1	6	„
Lepus „ „	1	6	„
Columba „ „	1	6	„

A.x2. General History

These slides are usually all of mammalian tissues.

Skin	1	6	„
Cartilage	1	0	„
Bone	2	0	„
Ossification in cartilage	2	6	„

	s.	d.	
Teeth	1	6	per slide
Tongue	1	0	,,
Oesophagus	1	0	,,
Stomach (pyloric and cardiac)	1	0	,,
Small intestine	1	0	,,
Large intestine	1	0	,,
Artery	1	0	,,
Vein	1	0	,,
Blood (mammal, and non-mammalian)	1	3	,,
Simple tissues (areolar, pavement epithelium, etc.) ...	1	0	,,
Nervous tissues, spinal cord, T.S.	1	0	,,
nerve, T.S.	1	0	,,
Glandular tissues, liver, spleen, salivary, etc. ...	1	0	,,
Muscle, T.S. and L.S. striped	1	0	,,
,, ,, unstriped	1	0	,,
,, ,, cardiac	1	0	,,

Note.—It will be found that a single slide of each of the above will be very useful, but for many purposes, a smaller list would do. But a good pupil of scholarship standard ought to have the chance of seeing all these.

B. Material for Dissection

Specimen.	Amount.	Cost. s.	d.	
Protozoa	tubes for 12 students	1	6	or more
Hydra	... ...	2	6	
Obelia hydroids ...	... ...	2	0	
,, medusae*	2 specimens each	0	3	each
Ascaris	1 of each sex per student ...	0	3	each
Taenia	(tube of material without head)	1	6	
Distomum ...	1 per student (slides will do instead)	0	9	each
Lumbricus* ...	4 per student	1	0	
Astacus*	2 per student	1	2	
Periplaneta* ...	2 per student	0	4	
Helix*	1 per student	0	6	

* Order excess to allow for death, accident and failure.

Specimen.	Amount.	Cost. s. d.
Amphioxus ...	1 per student	2 6
Scyllium	1 of each sex per student :	
	(*a*) preserved	2 6
	(*b*) fresh	0 9
Rana	1 of each sex per student ...	1 0 each
Lepus	1 per student	3 0
Columba ...	1 per student	3 0

2. Time Allocations for Practical Work

Subject.	Total Time required.
Amoeba	3 hours
Paramoecium	3 „
Volvox	3 „
Euglena	3 „
Monocystis	3 „
Opalina	3 „
Hydra	5 „
Obelia	7 „
Ascaris	7 „
Taenia	5 „
Distomum	5 „
Lumbricus	12 „
Astacus	12 „
Periplaneta	7 „
Anodon	10 „
Helix	7 „
Amphioxus	12 „
Scyllium	22 „
Rana	22 „
Lepus	24 „
Columba	22 „

APPENDIX

THESE lists are very incomplete, but they serve to indicate the extent of the available material. From nearly all the sources which are cited further references can be obtained.

JOURNALS

THE AQUARIUM REVIEW (Editor, R. C. Cook, 89 Charterhouse Street, London).
SCHOOL NATURE STUDY (School Nature Study Union).
BIOLOGY (British Social Hygiene Council).
SCHOOL SCIENCE REVIEW (Science Masters' Association).
DISCOVERY.
THE MUSEUMS JOURNAL.
SCIENCE PROGRESS.
NATURE.
JOURNAL OF THE LINNEAN SOCIETY.
ANNALS AND MAGAZINE OF NATURAL HISTORY.

The last-named Journals are of an advanced technical nature, but often contain articles on technique, reviews, abstracts, etc. which would be valuable to teachers of zoology. They can usually be consulted in Public Libraries.

REFERENCE BOOKS

THE MICROTOMIST'S VADE MECUM (Lee, 9th Ed., Gatenby & Cowdry, Churchill & Co.).
HISTOLOGICAL TECHNIQUE (Carleton). Oxford University Press.
ANIMAL MICROLOGY (Guyer). Chicago and Cambridge University Press.
ELEMENTARY MICROTECHNIQUE (Peacock). Arnold.
THE SCIENCE MASTER'S BOOK, produced by the Science Masters' Association

BOOKS ON HABITS, REARING, ETC.

INTRODUCTION TO ZOOLOGY THROUGH NATURE STUDY (Lulham). Macmillan & Co.
FRESHWATER AQUARIA (Bateman). Bazaar, Exchange and Mart.
THE VIVARIUM (Bateman). Bazaar, Exchange and Mart.
LIVING CREATURES (Von Wyss). Black & Co.
HANDBOOK TO THE MARINE AQUARIA at the HORNIMAN MUSEUM, Forest Hill, S.E.(King).
HANDBOOK TO THE VIVARIA AND FRESH WATER AQUARIA at the HORNIMAN MUSEUM, Forest Hill, S.E. (King).

BOOKS ON HABITS, REARING, ETC.—*contd.*

LIFE IN INLAND WATERS (Carpenter). Methuen & Co.

PAMPHLETS OF THE SCHOOL NATURE STUDY UNION. (An excellent series dealing with every aspect of natural history.)

MODERN SCIENCE MEMOIRS. (Reprints from the " School Science Review ")

BOOKS ON BIOLOGY TEACHING.

(*These deal chiefly with Junior School Work.*)

THE TEACHING OF NATURE STUDY (Von Wyss). Black & Co.

THE AIMS AND METHODS OF NATURE STUDY (Rennie). University Tutorial Press.

THE STUDY OF NATURE WITH CHILDREN (Carter). Methuen & Co.

THE TEACHING OF BIOLOGY (Poulton). Methuen & Co.

BIOLOGY AND EDUCATION (Edited by Crowther). British Social Hygiene Council.

SCHEMES SUGGESTED FOR CONSIDERATION AND DISCUSSION. British Social Hygiene Council.

EDUCATION AND BIOLOGY (Lauwerys). Sands & Co.

EDUCATIONAL FILMS, ETC.

The activities of the organisations cited below are too extensive to describe more fully. Full information can be obtained on application to the respective secretaries. The writer is indebted to these organisations for their help in placing information at his disposal.

THE BRITISH FILM INSTITUTE, 4 Great Russell Street, London, W.C.1. Publications include :—

(*a*) " Sight and Sound " (quarterly).

(*b*) " Monthly Film Bulletin " (issued to members only).

(*c*) A series of leaflets describing various aspects of the Institute's work and also dealing with the available non-theatrical cinematograph apparatus and films.

(*d*) Annual Report.

CENTRAL INFORMATION BUREAU FOR EDUCATIONAL FILMS, 103, Kingsway, London, W.C.2. Publications include :—

(*a*) A Guide to Instructional and Educational Films (a very complete catalogue of the field).

(*b*) Pamphlets dealing with all aspects of educational cinematography (by annual subscription).

(*c*) The International Review of Educational Cinematography.

G.B. INSTRUCTIONAL, LIMITED, 12, D'Arblay Street, Oxford Street, London, W.1. This firm is producing a special series of sound films dealing with biological topics.

EDUCATIONAL FILMS, ETC.—*contd.*

KODAK, LIMITED, Kingsway, London, W.C.2. Complete information of the Kodak service can be obtained on application.

Space forbids further extension, but information upon this newly developed educational field should be obtained by all teachers.

ADDRESSES

The well-known firms advertise widely in Journals and periodicals. One difficulty which seems to confront teachers is to obtain supplies of fresh dogfish straight from the coast. Providing that at least one dozen are purchased, and and the cost of packing and transit are paid, they can be obtained at a very reasonable price depending on size and season from :—

Mr. Ted Williams,
4, Britannia Row,
Ilfracombe,
North Devon.

Addresses of organisations cited in the text :—

British Social Hygiene Council,
Carteret House,
Carteret Street,
London.
(Mr. P. F. Lee, Education Officer.)

Science Masters' Association,
Membership Secretary,
Mr. B. M. Neville,
42, Constantine Road,
London, N.W.3.

School Nature Study Union,
Hon. General Secretary,
Mr. H. E. Turner,
45, Cheviot Road,
West Norwood,
London, S.E.27.

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