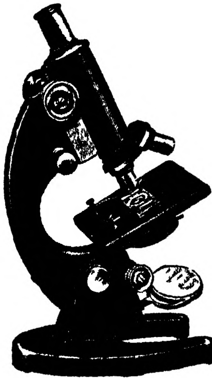
Birla Central Library
PILANI (Jaipur State)
Class No :- 578.6
Book No :- J63M
Accession No :- 35424



The B.L.M. Series of Microscopes

MODEL 3.E.S.

This Microscope is specially adapted for the student or amateur microscopist. For Medical, Educational or Industrial purposes it is unexcelled. Our instruments have a world-wide reputation for design, finish, mechanical excellence and optical performance.

MICROSCOPE IN CASE, with Divided Drawtube, Double Nosepiece, Abbe Condenser, Eyepieces x 5 and x 10, Objectives x 10 ($\frac{2}{3}$"), x 40 ($\frac{1}{6}$") £38 . 17 . 0

We can also offer Dissecting Microscopes, Microtomes, Mounting Tables, Magnifiers (Pocket and Dissecting), and all the necessary apparatus for preparing and mounting specimens.

SECONDHAND INSTRUMENTS

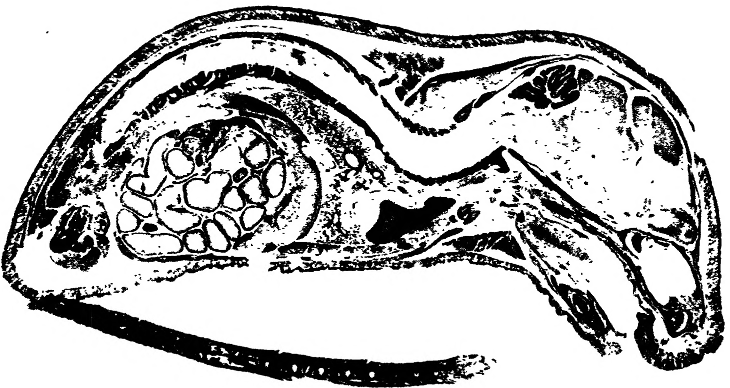
Let us know your requirements. We shall be pleased to quote what we can supply, and prices.
We buy, exchange, or sell on commission, microscopes and all kinds of scientific apparatus. Good prices given.

C. BAKER OF HOLBORN LTD.

244 HIGH HOLBORN, LONDON, W.C.I

MICROSCOPIC OBJECTS

HOW TO MOUNT THEM



NEWLY-BORN MOUSE, LONGITUDINAL MEDIAN SECTION.

Original slide from which photograph was taken was triple stained.

All blocks loaned and used by courtesy of W. Watson & Sons. Ltd.

Frontispiece]

MICROSCOPIC OBJECTS

HOW TO MOUNT THEM

By

JEAN C. JOHNSON

THE ENGLISH UNIVERSITIES PRESS LTD.
SAINT PAUL'S HOUSE, WARWICK SQUARE
LONDON, E.C.4

FIRST PRINTED . . . *September* 1935
REPRINTED . . . . *July* 1944
SECOND EDITION . . . *May* 1948

*The paper and binding
of this book conforms to the
authorized economy standard*

PRINTED AND BOUND IN ENGLAND BY
HAZELL WATSON & VINEY LTD
AYLESBURY AND LONDON

FOREWORD

THE object of this little book is to aid the beginner who is desirous of making his own mounts, and to give him some idea of the way to set about it. Much information concerning the preparation and mounting of microscopic objects has been published in the past, but the compiler believes there to be a very real need for a work which deals exclusively with this subject, and not forming only, as so often is the case, supplementary chapters in books written on the microscope as an instrument.

This book is not intended as an exhaustive treatise, and a very great deal more matter might have been included, but an endeavour has been made to assist the beginner in the art of mounting by dealing with simple methods and formulæ. There is undoubtedly a great diversity of opinion as to the best mode of proceeding, each mounter having his own particular ideas and methods, and there will probably be some among my readers who do not entirely agree with the details set down, but this is unavoidable.

Reference has in several cases been made to the work of well-known mounters, and due acknow-

ledgment of the source from which the information has been obtained is made. Should this, however, have been anywhere omitted, the compiler wishes to apologise for the oversight, and offers assurances that it was owing to ignorance.

The compiler also wishes to tender her thanks to Messrs. W. Watson & Sons, Ltd., who have very kindly loaned her all the blocks for illustrations which appear in this book. These blocks were made from photomicrographs of actual specimens from their cabinets.

JEAN C. JOHNSON.

ACKNOWLEDGMENTS

MY most sincere thanks are due to J. McCartney, Esq., M.D., Director of the L.C.C. Pathological Services, and to W. R. Dracass, Esq., of the Analytical Laboratory, Southwark, S.E., for the great interest they have taken in this small book and for the amendments suggested by them which have been incorporated in this reprint.

JEAN C. JOHNSON.

April 1948.

CONTENTS

PAGE

FOREWORD v

CHAPTER

I. REQUIREMENTS 1

II. INSECT MOUNTING 7

III. CLEANING AND MOUNTING DIATOMS . . 22

IV. CLEANING AND MOUNTING FORAMINIFERA, RADIOLARIA, AND SPICULES . . . 44

V. MOUNTING CHEMICAL CRYSTALS . . 49

VI. PREPARATION OF ROCKS AND METAL SECTIONS 58

VII. BLOOD SMEARS AND BACTERIA . . . 64

VIII. PREPARATION OF ANIMAL TISSUES . . 77

IX. EMBEDDING AND CUTTING . . . 90

X. STAINING FLUIDS AND STAINING . . 98

XI. PREPARATION AND MOUNTING OF VEGETABLE TISSUES 109

XII. MOUNTING IN GLYCERINE AND GLYCERINE JELLY 118

XIII. OPAQUE MOUNTING 129

XIV. FORMULÆ 133

WEIGHTS, MEASURES, AND CONVERSION TABLES 140

BIBLIOGRAPHY 143

INDEX 144

LIST OF ILLUSTRATIONS

NEWLY-BORN MOUSE, LONGITUDINAL MEDIAN SECTION . . . . . *Frontispiece*

FACING PAGE

HEAD OF MALE GNAT, *CULEX PIPIENS* . . . 22
PROBOSCIS OF BLOWFLY, *CALIPHORA VOMITORIA* . 22
GROUP OF SELECTED DIATOMS, FROM ST. PETER'S, HUNGARY 23
FORAMINIFERA, CLEANED AND MOUNTED AS AN OPAQUE OBJECT 23
EYE OF DRAGON-FLY, *AGRION PULCHELLUM*, HORIZONTAL SECTION 24
FUNGUS ON STEM OF WHEAT, *PUCCINIA GRAMMINIS*, TRANSVERSE SECTION 24
FLOWER BUD OF LILY, TRANSVERSE SECTION . 25
ANTENNA OF MOTH 25
CATHCART MICROTOME, FOR FREEZING AND EMBEDDING 90
CAMBRIDGE ROCKING MICROTOME, FOR PARAFFIN OR CELLOIDIN BLOCKS 90
ROTARY MICROTOME, "MINOT" PATTERN . . 90
HAND MICROTOME 91
SIMPLE HAND CENTRIFUGE 91
SIMPLE EMBEDDING BATH, FOR PARAFFIN WORK . 91
FRESH-WATER POLYZOA, *CRISTATELLA MUCEDO*, MOUNTED IN FLUID 118
CRYSTALS OF SALICINE, PHOTOGRAPHED BY POLARISED LIGHT 118
HUMAN FLEA, *PULEX IRRITANS* 119
SECTION THROUGH BASE OF LEAF STEM OF VIRGINIA CREEPER, SHOWING DEFOLIATION . . . 119

Chapter One

REQUIREMENTS

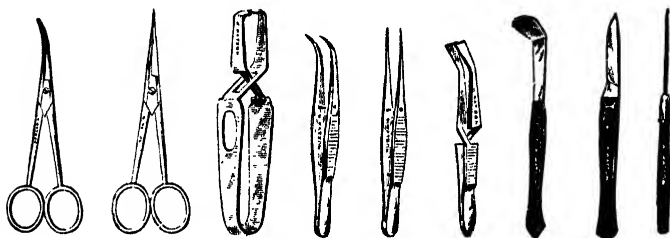
THE investigation of minute structures, whether organic or inorganic, can only be satisfactorily and exhaustively carried out when the specimens under consideration have been subjected to special processes and manipulation in order to render them suitable for microscopical examination. In the inorganic kingdom some minerals and chemical substances are found in Nature in such minute particles as to make them suitable for immediate examination, but in the majority of instances it is necessary to clean, grind down, or cut the material into the thinnest possible sections to facilitate the examination of the structure, while the more complex organisms require dissection and, in many cases, chemical treatment before they can serve any useful purpose.

To this end, many tools will be found indispensable. Nearly every microscopist prefers to work in his own way and with his own choice of instruments, and it would be waste of time to enumerate everything that could be turned to some use. There are some things, however, which are necessary to begin with, and the others can be added as the occasion demands.

The following list will prove useful to the student:

A good simple microscope with two eyepieces, × 6 and × 10, objectives 2 inch, 1 inch, and $\frac{1}{6}$ inch, and substage condenser.
Microtome and knife. A cheap and efficient instrument is to be found in the Cathcart-Darlaston, complete for freezing and embedding.
2 scalpels, blades 2 inches and 1$\frac{1}{4}$ inches.
1 eye knife.
1 pair 4$\frac{1}{2}$-inch straight fine-pointed scissors.
1 pair 4$\frac{1}{2}$-inch curved fine-pointed scissors.
1 pair 4$\frac{1}{2}$-inch straight fine-pointed forceps.
1 pair 4$\frac{1}{2}$-inch curved fine-pointed forceps.
1 metal section lifter.
6 dissecting needles in wooden handles, or 2 metal needle holders and a supply of needles.
6 sable-hair brushes, of varying thicknesses.
2 cork mats, backed with lead.
2 shallow dishes for dissecting under water.
3 flasks for boiling.
Set of test-tubes and rack.
A supply of short corked tubes for storing small sections.
$\frac{1}{2}$-oz., 1-oz., and 2-oz. bottles, corked for storing sections.
Washing bottle.
6 dropping bottles.

3 glass rods for stirring.
1 spirit lamp or Bunsen burner.
3 glass filters.
Supply of filter paper.
Quantity of flat-bottomed watch glasses.
2 graduated jars for measuring, 1 for 10 c.c., the other for 500 c.c.
1 gross 3 × 1-inch glass slides, students', greenish tint.
1 oz. each of glass cover circles, Nos. 1 and 2,



DISSECTING INSTRUMENTS.

½ inch, ¾ inch, and ⅞ inch, and some square or rectangular covers for very large sections.
Assortment of aluminium rings for cells, of varying thicknesses and diameters.
Brass heating table.
Retort stand, with sand bath and gauze.
Turntable for ringing.
1 bottle each of canada balsam neutral, canada balsam in xylol, canada balsam in benzole, gold size, black asphalt varnish,

marine glue, clove oil, pure glycerine and glycerine jelly, turpentine, industrial methylated spirit (99·24 per cent.), alcohol B.P., absolute alcohol, distilled water, purest obtainable.

The violet-tinted methylated spirit, which is obtainable from oil shops and chemists', is not suitable for microscope work, but industrial methylated spirit should be obtained instead. To do this it is necessary to obtain a licence, and this can be accomplished by application to the local excise officer, who will make a small charge for issuing this. Methylated spirit of this kind is not very expensive, can be purchased in quantity (after licence has been granted), and can be used in most instances instead of absolute alcohol.

As the worker progresses, a large number of other reagents and stains will be found necessary, but the foregoing is given as a starting-point.

To ensure satisfactory results, stress cannot be too strongly laid on the necessity for cleanliness and orderliness in manipulation. Each step taken should be noted, from the time the material is collected until the final mounting. Such notes could either be kept in a book put aside for the purpose, or on a series of cards, and the information so recorded will be found of untold value for future reference. All tools and glass-ware should be kept clean, and should be put carefully away

when finished with. Glass-ware, including 3 × 1-inch glass slides, can be washed in warm water with soap extract and dried on a soft rag. A most effective cleanser for all chemical apparatus is sodium metasilicate. It is sold in 1-lb. tins for this purpose, and should be used with agitation in a warm or hot 2 per cent. or 3 per cent. solution, and it will leave glass, earthenware, metal, and porcelain surfaces in a brilliant condition. It readily removes grease from the interiors of measuring glasses, funnels, etc., and is a most useful addition to a laboratory. It should be particularly noted that slides to be used for the attachment of paraffin and celloidin sections must be exceptionally clean, and great care must be taken in this respect.

Cleaning of Used Slides for Further Use

When mounting, there are often many slides which are failures and not worth keeping, and the economical user should clean them and use them again. To do this is quite easy, all that is necessary being to prepare two jars, each containing a strong, hot solution of soap powder, such as Hudson's extract or any of the soap flakes commonly sold. Take each slide to be cleaned, warm it over a spirit lamp or on a hot-plate to melt the balsam or whatever other medium has been used, and this enables the cover glass to be readily removed. The cover should then be pushed off

into one jar and the slide put into the other. Any number of slides can be cleaned off at one time, and both the slides and covers should be left in their respective jars over a considerable period, but during this time the water should be drained off and fresh supplies of hot water added to wash away the soapy solution. Drain off the final lot of water, and add a generous quantity of methylated spirit, which acts as a solvent for the balsam or other medium used and makes the slides clean. They can be left to soak in the spirit for as long as is convenient, and when wanted for use can be dried with a soft well-washed piece of linen.

Should they still seem not to be perfectly clean, a further immersion for a very short time in a mixture of hydrochloric acid and methylated spirit, followed by pure methylated spirit, will almost certainly complete the cleaning process.

In microscopic mounting the student must not be discouraged at the failure of the first attempts, but he must gradually try to acquire a knowledge of the principles with the manual dexterity necessary in their application.

Chapter Two

INSECT MOUNTING

TO obtain a satisfactory preparation, it is necessary to select insects which have been recently captured and killed. Insects can be killed either by subjecting them to chloroform vapour, with sulphuric ether, or by the cyanide of potassium bottle, which is often used. They can then be placed in methylated spirit, where they can be kept until wanted.

The procedure for mounting an insect in canada balsam is as follows:

1. From the methylated spirit, transfer the insect to ordinary water, and leave it there to soak for three or four hours to remove the spirit.

2. Then place the insect in liquor potassæ, a mixture of 10 per cent. caustic potash and distilled water, and let it remain in this fluid until it is soft. The length of time allowed for this to take place depends entirely on the size and density of the insect and strength of solution.

3. When soft enough, the insect must be transferred from the potash to a dish of water, which must be changed several times to ensure the removal of the potash. If this is not done, crystals are apt to form after mounting.

4. The water must be poured away, and concentrated acetic acid added. The insect can be left to soak in this until it is convenient to go on with the work.

5. Remove to a shallow dish of water, and with a sable-hair brush and a dissecting needle, spread out the wings and the other parts of the insect. Taking care not to detach the head or thorax, press with a gentle rolling motion in the direction of the anus, to expel the contents of the thorax and abdomen. A small roller of wood or cork might be used, or else the handle of the brush. When all the internal organs have been removed, place a clean 3 × 1-inch glass slide in the water under the insect, gradually lifting in such a manner that the insect is stranded on the slide, the excess of water being drained off. Then, very carefully, place all the limbs, wings, antennæ, etc., in the required position, and gently lower another clean glass slide on top of the other one, pressing it down until the insect is squeezed quite flat. Tie the slides together, and immerse in a jar of methylated spirit or rectified spirits of turpentine until wanted. They may remain in this for two or three months, if necessary, but the jar must be well stoppered.

6. Remove the slides from the jar, and wipe off as much of the fluid as possible. Then, exercising the greatest care, separate the slides. Float

the insect off the slide into a dish of spirit, and soak for half an hour.

7. Then very carefully lift the insect, using either the sable-hair brush or a section lifter, and place it in a saucer of clove oil to clear it. Leave it to soak in this until perfectly clear.

8. Remove from the clove oil, and leave it in turpentine for a few minutes.

9. Float the insect on to a clean 3 × 1-inch slip, removing as much turpentine as possible, and then, after carefully putting the various parts in the required position, place a very small drop of canada balsam on the insect. Then take up a cover glass with a pair of forceps, and bring its edge in contact with the slide, letting the balsam touch the cover, when it will be taken in between the surfaces by capillary attraction. With the points of the forceps press on the surface of the cover until the insect lies quite flat and the excess of balsam is squeezed out. After pressing the cover down, the slide may be left to dry. The drying process takes some time if no heat is applied, but the specimen is more transparent. To quicken the drying, the slide may be subjected to gentle heat from a spirit-lamp flame, taking care not to apply too much heat, as boiling of the balsam disarranges the object, and renders the mount so brittle that it is liable to break away from the slide if dropped or jarred in any way. The exuded balsam can be removed either by

using an old knife which has been made slightly hot, or by rubbing with an old rag wetted with xylol or methylated spirit. The final cleaning of the slide can be done with the aid of a little benzole, xylol, or turpentine, and the slide may be ringed and labelled.

Note.—Some mounters use silicic acid instead of acetic acid in step No. 4. After being washed in warm water it may be placed in silicic acid. This acid has an affinity for potash, turning it into silicate of potassium, which substance is very soluble in water. Remove the insect from the silicic acid, wash well in water, and continue as step No. 5.

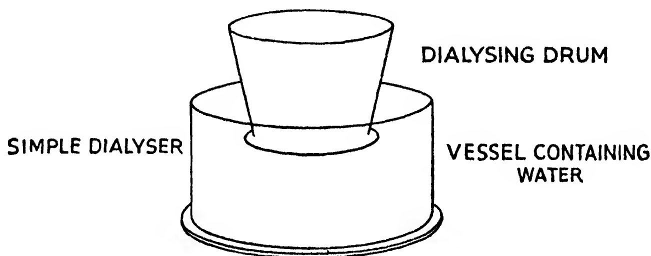
Formula:

$$\underset{\text{Potassium silicate}}{K_2SiO_3} + 2HCl = 2KCl + \underset{\text{Meta-silicic acid}}{H_2SiO_3} \rightarrow \underset{\text{Colloid silica}}{SiO_2 \cdot H_2O}$$

Take 3·5 c.c. potassium silicate and 55 c.c. water and mix them in a glass vessel, and gradually add hydrochloric acid in drops until a filmy appearance is obtained. Place the mixture in a dialysing drum, and put the drum in a basin of water. The potassium chloride thus formed by the action of the hydrochloric acid on the potassium will then pass into the water in the basin, leaving the silica in the form of colloid silica in the drum. It is better if the water in the basin is renewed every three or four hours.

This method of preparing and mounting insects

can be recommended as giving first-class results if due care is taken. It will be found that some small insects and many parts of insects need not be soaked in caustic potash. It is only necessary to soak them for a time in turpentine, and then mount them in the ordinary way. Soaking insects in oil of cloves or in turpentine has the effect of rendering them transparent, so that their



internal organs, muscles, etc., can be readily examined.

To Mount Whole Lepidoptera

Take specimens which have been perfectly set out and dried, and place them carefully in a dish of absolute alcohol. After about one hour pour off the alcohol and replace with a solution of 25 per cent. by weight of absolute phenol crystals dissolved in 75 per cent. by weight of pure xylol and filtered. Allow specimens to remain in this for about one hour. Pour off and replace with pure benzole. From this the specimens may be

mounted in canada balsam in benzole. The mounting is the most delicate part of the performance, and the procedure is as follows :

Very carefully lift the specimen from the benzole and place in position in the centre of a clean 3 × 1-inch slide. Put on 2 or 3 drops of the balsam in benzole, using this medium when it has a consistency of glycerine. Gently heat the slide, and place three small pieces of glass (which should be slightly less than the thickness of the object) so as to allow the cover glass to rest on the object as well as on the three small pieces of glass, to prevent the object from moving out of centre (see also p. 15). Now place the cover glass on the top of the three glass supports, fill up the space between the slip and cover glass with balsam by running in the balsam from the edge of the cover glass (put in plenty of balsam to allow for evaporation), and allow to stand for twenty-four hours. Add more balsam if necessary and bake the slide for ten hours at a heat not exceeding 175° F. Clean off superfluous balsam with a piece of soft cotton material and methylated spirit ; ring with shellac or some good cement, and after twenty-four hours finish off by ringing with black asphalt varnish.

Mounting Parts of Insects

Remove the eye from an insect, and brush away the dark pigment, using a sable or camel-hair

brush. Leave it in turpentine for several days, renewing the liquid several times, and then wash the eye well in clove oil just before it is to be mounted. As the eye is spherical, it must be snipped round the edges to flatten it, and then treat it with balsam as before described.

Cut the antennæ from the insect's head, and bleach by soaking in the following solution for a few days, to make them transparent:

Hydrochloric acid	10 drops
Potassium chlorate	1·94 grammes
Water	28 c.c.

Wash well, dry, and mount in canada balsam.

An alternative method is to soak the antennæ in a weak solution of chloride of lime, which will show the nerves well.

Many wings of insects, such as those of the Hemiptera and Coleoptera, cannot be satisfactorily treated with potash, and it is best to mount them by using chlorophenol or by simply washing in absolute alcohol, renewed several times, and immersing them in xylol to remove the air, and then mounting in balsam in the usual way.

Chlorophenol Method.—This can be used as an alternative method for either whole or parts of insects. The specimen should be soaked in chlorophenol warm (for several minutes) or cold (for several hours), the chlorophenol being

changed several times. Remove the specimen, place in xylol for a short time, and mount in xylol balsam.

The Scales from the Wings of the Lepidoptera (butterflies and moths) can be mounted as follows:

Place a very small drop of xylol on a clean 3 × 1-inch slip, and gently scrape the scales from the wings into the xylol. Let a small drop of balsam fall into the xylol, and gently lower a cover glass on the balsam as before described.

To Mount Insects in Canada Balsam without Pressure

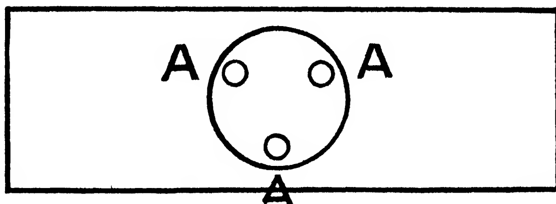
Method 1.—Soak the specimens in liquor potassæ until they are transparent. Wash well in distilled water, and transfer to 50 per cent. methylated spirit, then to a quantity of pure spirit, and allow them to stand for some hours. Then add oil of cloves, and allow the spirit to evaporate. By this method the formation of air bubbles in the interior of the specimens is generally avoided.

Method 2.—Wash the specimens well in distilled water, and then soak in pure spirit for some days. Transfer to carbolic acid until sufficiently transparent and then to oil of cloves. This method should be used in all cases where the

integument is not too opaque, and is especially useful in the study of the muscles.

Mounting.—If the specimen is not too deep it can be mounted as follows: Take a clean 3 × 1-inch slip having a central excavation, and just inside the edge of the excavation, equidistant from each other, cement three minute circles of glass, A A A.

Hardened balsam can be used for this. Put a small quantity of soft balsam in the centre of the



cell and gently warm it over a spirit lamp. Take the specimen, and place it upon the warm balsam, carefully arranging it in the required position. Now take a clean cover glass, and holding it between the points of a pair of forceps place a large drop of balsam in its centre and allow it to fall on the object. The edge of the cover glass will rest on the three small spots of glass. If the quantity of balsam under the cover glass is not sufficient to fill up the whole of the space between it and the slide, a little more must be allowed to run in, and if the object has become displaced, it

may be arranged by means of a fine blunt needle, introduced beneath the cover glass. A wire clip can now be placed on the slide to prevent displacement of the cover glass. The slide can now be set aside for the balsam to harden, when the surplus portion can be safely removed. A ring of black varnish can then be applied to finish the slide.

If the specimen is too deep to allow of the method detailed above being employed, a suitable cell must be made to take sufficient balsam to cover the object.

This can be done in several ways, the following two being the most usual:

1. By means of a turntable make a ring of gold size of a suitable diameter on a perfectly clean 3 × 1-inch slip. Put this aside to harden thoroughly. Then again using the turntable, apply a ring of a suitable cement, such as marine glue, Bell's cement, or Murrayite, on top of the ring of gold size. Allow this to dry thoroughly, and again apply a ring of gold size. This can be carried on indefinitely, the cements being alternately used and left to harden until the cell is sufficiently deep.

2. By using an aluminium ring specially made and sold for the purpose. The ring should be carefully rubbed on emery paper to ensure its surfaces being as smooth as possible, and ce-

mented to a clean 3 × 1-inch slip, by means of any of the cements mentioned above, including gold size. First clean the slide and warm over a spirit-lamp flame, and after having applied a coat of the cement to the metal ring and allowing it to nearly dry, bring the ring in contact with the slide on the warm side. Press down the ring until it adheres firmly to the slide, and put it away to dry. When ready to mount, fill the cell with canada balsam, allowing it to flow over the edge of the cell, so that it may serve as a cement to which to fasten the cover glass. Using a section lifter, gently place the specimen in the balsam in the cell, setting out the parts with needles, and put aside away from possible dust for twelve hours or so to harden. Then put a small drop of balsam on one side of the metal cell, and after having gently warmed a cover glass, bring its edge in contact with the drop of fresh balsam, lowering the cover gently on to the cell, taking care to avoid air bubbles. With a soft brush moistened with benzole, remove the surplus balsam and put away to dry. When quite hard, apply a ring of cement, and finish off with black varnish.

To Mount Insects in Glycerine

Glycerine can be advantageously used for the mounting of many of the smaller insects, parts of insects, or any similar type of specimen. The

object should first be cleaned with alcohol to get rid of all extraneous matter, and then, after soaking in glycerine, be mounted in it in much the same manner as described for balsam mounts. The larger and denser specimens must first be soaked in potash to make them more transparent. Make a cell of the required size and depth either by Method 1 or 2, apply a ring of cement to the edge of the cell and leave until nearly dry, and then fill the cell with glycerine. Put the specimen in it, spreading out the various parts with needles. Clean and warm a cover glass, and gently lower it on to the edge of the cell, making sure that it adheres to the cement. When quite dry apply another ring of cement, and when this is dry apply a coat of black varnish.

There is sometimes a difficulty in sealing a glycerine mount, and some mounters overcome this by making a ring of thick gum dammar on the edge of the cell before lowering the cover glass, in place of the cement mentioned above. Allow this to become sticky, and after having put in the glycerine and the specimen, lay on the cover and quickly wash off all the surplus glycerine. When perfectly well washed and dried, lay on two or three coats of gum dammar to finish it. Any glycerine on the slide can be cleaned away with a piece of damp linen.

The methods given so far in this chapter only refer to the mounting of external parts of insects,

as all the soft tissues and internal organs have either been pressed out or destroyed by the potash. If it is desired to mount these, they must be dissected out under water. Place the insect in a dissecting dish, and secure with pins in the desired position. If the abdominal or thoracic viscera are wanted, lay the insect on its back; if the nervous system, lay it on its undersurface. Fill the dish with water, and with a pair of fine-pointed scissors cut through the skin on each side of the abdomen, taking care not to cut and injure the internal organs. With a pair of forceps, remove the skin. The organs may now be removed and washed in distilled water, stained in borax carmine for a few minutes, washed in methylated spirit, immersed in acidulated alcohol for several minutes, dehydrated, cleared in clove oil, and mounted in canada balsam. If the specimen is very deep, it should be mounted in a cell, either in balsam or in glycerine as before described. Parts of large insects, such as the head, may be kept in the centre of the cell by the following method:

With a fine sewing-needle, threaded with a hair, run through the organ to be secured, and after unthreading the needle, take hold of each end of the hair, and with the object suspended stretch it across the cell, and embed the hair in the cement on each side. Apply a cover glass, and press it down until securely fixed, and if the specimen is

not in the middle of the cell, adjust it by pulling the hair to one side. Lay aside for a day or so to dry, cut off the ends of the hair as close as possible to the cell, wash away the superfluous mountant, allow to dry, and finish by ringing.

The alimentary system of insects and stings of bees and wasps may be treated as follows:

Place the specimen whole and while quite fresh in water, cover to keep out the dust, and let it soak for several days, until it smells somewhat offensive. Then wash in clean water, hold the insect in a pair of forceps, and with another pair of forceps carefully pull off the head, and this should bring with it the œsophagus, salivary glands, and stomach. The stings of bees and wasps can be extracted as follows: gently squeeze the abdomen of the insect, when the sting will protrude. Grip it gently with a pair of forceps and pull. This should bring out, not only the sting, but the duct and the poison sac. Then wash the specimen, and place it on a slide under a microscope. By careful use of needles, draw the stings from their sheaths. Stain in borax carmine, wash in alcohol, and then in acidulated alcohol, and lastly in water. After washing carefully, place it between two slides, tie them together with cotton, and immerse in methylated spirit for several hours. Remove from the spirit, detach one glass slide, clear in clove oil, and mount in balsam.

Many small parasitic and other insects can be mounted whole in a cell in glycerine without being treated in the liquid potash, but they should be cleared in the following manner. Melt 2 ozs. of solid carbolic acid and add ·03 oz. of glycerine, to keep the acid from becoming solid again, and soak the specimen in this until it is sufficiently transparent. The length of time allowed for this process must depend on the size and density of the insect. Prepare a cell as before described, and when dry run a ring of cement on its upper surface. Leave this until it is tacky, and then place the specimen in the cell and fill it up with glycerine. Gently lower the cover glass on to the tacky surface of the cell, and press down all round until it adheres to the cement. Water can then be applied to remove the excess of glycerine, and the slide put on one side for the water to evaporate. When dry, ring with cement, rub away any marks which appear on the slide with a very soft cloth, and ring the cell again to finish off the preparation.

Chapter Three

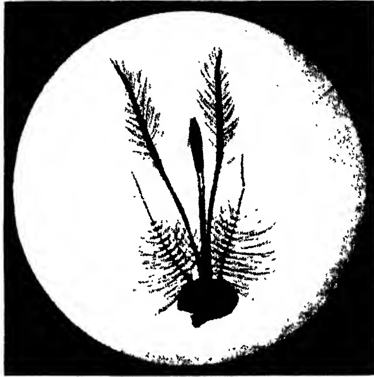
CLEANING AND MOUNTING DIATOMS

Mounting Media for Diatoms

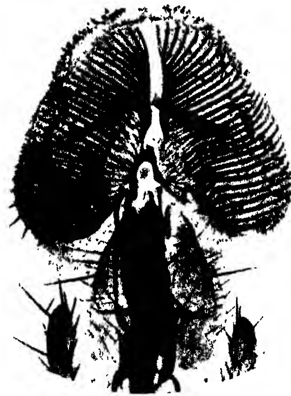
CANADA *Balsam* (R.I. 1·526).—Usually supplied dissolved in either benzine or xylol. Very easy to manipulate, but has too low refractive index for the resolving of the finer structure of diatoms. When purchased, if too thick add more spirit, if too thin evaporate spirit by leaving it exposed.

Styrax, either European (*Liquidambar orientalis,* R.I. 1·582) or American (*Liquidambar styraciflua,* R.I. 1·63).—Usually supplied dissolved in either benzine or benzole, sometimes in xylol. Chloroform is a much better solvent than any of these. Other solvents are xylol, methyl alcohol 60–40, xylol-ethyl-alcohol, or benzole alcohol. Easy to manipulate, makes permanent mounts, and has a sufficiently high refractive index to allow of the resolution of the majority of diatoms. It is a good all-round medium.

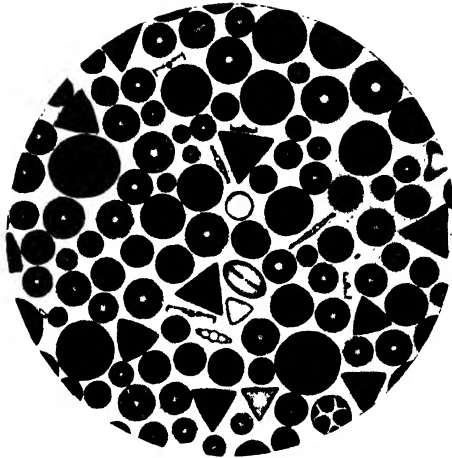
Hyrax.—A synthetic resin (R.I. 1·71) supplied dissolved in benzole. This is fairly easy to use, and has a sufficient increase in refractive index over styrax to make it eminently suitable for



HEAD OF MALE GNAT. *CULEX PIPIENS.*



PROBOSCIS OF BLOWFLY, *CALIPHORA VOMITORIA.*



Group of Selected Diatoms, from St. Peter's, Hungary.

Foraminifera, Cleaned and Mounted as an Opaque Object.

a medium in which to mount the finer diatoms. Mounts are permanent provided care is taken to see that no impurities are present anywhere.

Sirax (R.I. 1·8).—A synthetic resin. This medium is very suitable for diatom mounts, and is similar in every way to Hyrax, but owing to its tendency to give off air bubbles when heated, is more difficult for a novice to use than Hyrax or Styrax. It hardens quicker than Hyrax, and is slightly darker in colour, but not sufficiently dark to make any great difference in the finished slide. So far as is known it is quite stable, and slides made with this medium are permanent. Both Hyrax and Sirax mounts are liable to break away from the slide on cooling, especially if shaken or knocked in any way, and form colour effects which are known as Newton's rings. By very gently heating the slide from underneath, these rings can be made to disappear.

Piperine and Picric Piperine (R.I. 1·681).—These media are not so commonly used as those previously mentioned, as mounts are rarely permanent. There is always a tendency to crystallisation, which, however, can often be removed by further heating.

Realgar (R.I. 2·549).—A sulphide of arsenic, very difficult and dangerous to handle, but owing to its very high refractive index, of great value in

the resolution of diatoms having the very finest structure. If properly made, the mounts are permanent.

Cleaning and Mounting Diatoms

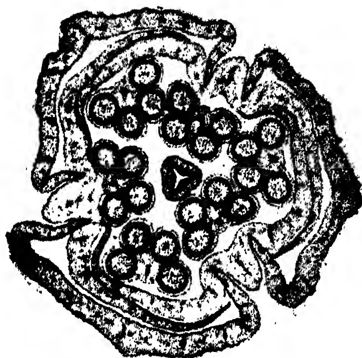
It is advisable, when about to clean either a gathering of fresh diatoms or a sample of a fossil deposit, to test the average of the specimens contained to see whether it is worth the trouble to be expended on it. This is best done by placing a small quantity of the matter containing the diatoms on a piece of platinum foil, or on a small piece of talc, and burning them over the flame of a spirit-lamp or a Bunsen burner, until the surrounding matter is burnt away and the diatoms are quite white. They can then be examined under the microscope and the forms noted.

To Remove Diatoms from Algæ and Unwanted Debris

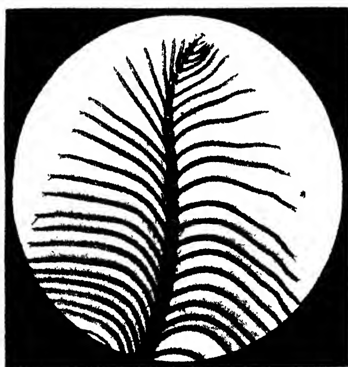
It is advisable to carry out this work in a deep sink in case of accidents. Pour the contents from the collecting bottle into an open evaporating dish, and boil in hydrochloric acid until the organic matter turns a dark colour, shaking gently while boiling. If the mass is seen to effervesce, it means that chalk or limestone is present, and this must be got rid of by adding more hydrochloric acid until the effervescence ceases. As the acid loosens the hold of the diatoms on the debris,

Eye of Dragon-fly, *Agrion pulchellum*, Horizontal Section.
Original slide from which photograph was taken was stained with iron hæmatoxylin.

Fungus on Stem of Wheat, *Puccinia graminis*, Transverse Section.



Flower Bud of Lily, Transverse Section.



Antenna of Moth.

they should be left macerating therein for at least twenty-four hours. After this, shake again, and strain into a large test-tube, using coarse muslin stretched over a funnel. If chalk or limestone has been suspected, every trace of the calcium chloride into which it has been converted must be washed away before the diatoms are treated with sulphuric acid. If this is not done, an insoluble precipitate of calcium sulphate will be formed, and this is not removable. The washing is done by filling the tube with water and leaving it to stand until the forms have settled. Then carefully pour off the liquid, leaving the deposit at the bottom. Again fill with water, and repeat the process about four or five times. Then leave the last settlement to stand overnight, so that the deposit may condense as much as possible, allowing the surplus water to be poured away, leaving a moist mass in the bottom of the tube. This mass now consists chiefly of diatoms and sand, and possibly a fair amount of organic material still remains. To this mass add a small quantity of sulphuric acid, pouring it in drop by drop down the side of the tube, and warm very gently over a flame. The strong acid soon causes the organic matter to char. Then add a very small piece of bichromate of potash, and this will entirely destroy the blackened organic material. When the reaction is complete, the residual fluid is greenish yellow. This fluid is extremely hot, and

the tube should be stood on one side until it is cool. When quite cold, pour it carefully into a large beaker of cold water. Then wash by the decantation method as before described, carrying out the process at least half a dozen times. If it is desirable to preserve the diatoms in this state before mounting, they may be placed in a small phial with a little distilled water.

To Clean Fossil Diatomaceous Deposits

Place a small quantity of the diatomaceous earth with four to six times its bulk of crystallised sodium acetate in a flask of 50–200 c.c. capacity. It is advisable to use a flask of pyrex or a similar glass to permit of its being plunged while still hot into cold water. Add a small quantity of water, approximately in proportion of 5 c.c. per 100 grammes of sodium acetate to the flask, and heat to the boiling-point until all crystals of sodium acetate are dissolved. Then allow the flask to cool to room temperature, either naturally or by placing in cold water. If the cold-water method is used, care must be taken not to allow any water to enter. Then drop a small crystal of sodium acetate into the flask, and this will cause the liquid to solidify. This solidification takes place very rapidly, and is accompanied by a considerable rise in temperature. The mass must now be allowed to get quite cold, when it will be noticed that shrinkage has taken place. Again heat the

flask, and repeat the process detailed above until the material is completely broken up. Should white anhydrous sodium acetate appear on the side of the flask, which is not readily dissolved in the hot solution, a few drops of water may be added from time to time.

When the material is completely broken up, fill the flask three-parts full of water, and shake vigorously, and then stand it aside and allow the contents to settle. Pour off the clear fluid, and repeat this process several times. If much iron or lime is still present, add dilute hydrochloric acid, and leave for awhile, when the impurities will be dissolved. Then wash several times as described above.

Pour off as much liquid as possible, and add concentrated sulphuric acid in quantity to about twice the volume of the sediment, by pouring very slowly down the side of the flask. Shake so that the acid mixes well with the sediment, and then heat the flask. Then add coarse crystals of potassium chlorate one at a time, until the liquid is practically colourless. It is important that the flask should be kept sufficiently hot so that each crystal when dropped in dissolves with a little hiss. If it splutters, it is because the liquid is not warm enough. When the material is bleached, the heating may be increased until white fumes are given off. Allow to cool, and then very carefully add distilled water, by pouring slowly down

the sides of the flask, in such a manner that the water flows gently on to the surface of the mass. Now shake the flask, and then allow it to stand so that the suspended particles may settle. Decant the liquid and wash several times in distilled water. Then to the washed residue add about 50 c.c. water, and 5–10 drops of 5 per cent. solution of caustic potash, and bring the whole to the boil as quickly as possible. As soon as the boiling-point is reached, allow the diatoms to settle, and pour off the liquid. Then fill the flask with water and shake vigorously for several minutes. In most cases this treatment is sufficient to break up the fine debris which remains suspended in the slightly alkaline liquid, while the diatoms, sand, etc., sink. Pour off the fluid, and pass the residue through a fine sieve of 150–300 mesh. This can be purchased ready made for the purpose, and separates the diatoms from the sand and fine pieces of other material.

If by any chance this treatment has not completely broken up the material, it should be re-crystallised several times with sodium acetate. In nearly all cases this should be adequate, but occasionally it may be found necessary to repeat the treatment with caustic potash.

To Mount Diatoms Dry

It was stated above that when diatoms had been cleaned, they might be preserved in small

phials of distilled water. When required for mounting, shake the phial, and with a thin glass rod or tube take up a drop of the fluid and let it fall on a perfectly clean cover glass. This must be allowed to dry by the aid of a spirit-lamp or Bunsen-burner flame, without being shaken or interfered with, otherwise the uniformity of dispersion will suffer. As dry mounts of diatoms have often a tendency to develop moisture owing to insufficient drying of the specimens, they should be burnt upon the cover glass at a dull red heat. Diatoms burnt in this way can be used satisfactorily for dry, balsam, or any other type of mount, and the burning not only acts as an additional cleaning process, but effectually fixes the diatoms and prevents them falling off. The thinnest covers may be burnt without damage if they are placed upon a small piece of perfectly flat platinum foil, about $\frac{1}{100}$ inch thick. The flame of a spirit-lamp or a Bunsen burner may be used, and it is advisable to shade the cover glass from direct light, so that the action of the flame may be observed. Care must be taken to raise the temperature only to the dull-red heat beforementioned. The cover will then be ready for mounting as desired. Then take a 3 × 1-inch glass slide, and by means of a turntable draw a thin ring of an adhesive varnish, such as gold size, on the glass slip, and allow it to dry until tacky. Then warm both the cover glass and the

slide, and gently press the former upon the ring of varnish until the adhesion all round is complete. Put aside, and when quite cold, complete the slide by ringing with black asphalt varnish.

To Mount Diatoms in Canada Balsam

Shake the phial containing the cleaned diatoms, and with a thin glass rod or tube, take up a drop of the fluid and let it fall on the cover glass. It should then be dried slowly under a flame. When quite dry, place a small drop of canada balsam on the cover glass, and put aside out of reach of dust for twelve hours. Take a perfectly clean 3 × 1-inch slide, and place on a hot-plate, and apply gentle heat from a flame for about ten minutes. Allow it to cool somewhat, and then take up the cover glass on which are the diatoms, and bring its balsamed surface into contact with the warmed slide. The balsam will melt a little and run to the edge all round the cover. Should it not do so, warm the slide gently until it does. Put aside to harden, and when quite cold and hard finish with a ring of asphalt varnish.

Another method, differing in some degree from the above, is as follows: having burnt the diatoms on the cover glass as previously described, lay them flat with the diatoms upwards. Apply a drop of benzole to the diatoms on the cover so as to exclude the air from the valves and frustules.

Warm a clean 3 × 1-inch glass slide over a flame, and when warm place a fairly large drop of balsam on it, and heat it until it begins to steam. Hold the slide in a slightly inclined position, so that the drop of balsam becomes convex at its lower edge; then pick up the cover glass with a pair of forceps and bring it in contact with the balsam at this point. Gradually lower the cover glass, bringing it to a central position, and press down gently with the forceps. Put aside to cool, and finish as before.

To Mount Diatoms in Hyrax

Using a cover glass with the diatoms burnt on it in the manner previously described, place a drop of xylol on the diatoms, and allow it to penetrate thoroughly in order to remove any air from the interior of the diatoms. Meanwhile, place a drop of cold hyrax on the centre of a cold 3 × 1-inch slide. Invert the cover glass with the xylol over the cold hyrax, and gently lower it on the slide. Place the slide on a hot-plate, which has a surface temperature such that the hand can just bear to remain there a few seconds, and leave the slide on the plate for forty-eight hours or so. The length of time that the slide should be left on the hot-plate can best be judged by the fact that, when properly baked, any hyrax which has exuded from under the cover glass remains hard at the temperature of the plate. The slide should

now be allowed to cool, and the exuded mountant cleaned off carefully with cotton-wool moistened with benzole, and the slide returned to the hot-plate for a further twenty-four hours. At the end of this time the slide can be cleaned by placing in methylated spirit and dried off with a soft rag, and then placed on the hot-plate for as long as desired. There is no harm in leaving the slide for a week on the plate should it be convenient, before putting on the finishing ring of varnish.

Note.—A small electric hot-plate of very low current consumption will be found most satisfactory for this purpose.

Sirax mounts can be treated in exactly the same manner as described above for Hyrax.

To Mount Diatoms in Piperine (the late Mr. H. Chapman Jones's Method)

Piperine, owing to its high refractive index, is a distinct improvement on either balsam or styrax as a medium for diatom mounting, but when used as supplied commercially, causes the slides to deteriorate by going opaque, due to crystallisation of the medium. Such a slide, however, can be made usable for a considerable period by gently heating on a hot-plate or over a spirit-flame until the medium melts. This can be repeated over and over again. Fairly permanent mounts can be made by heating the piperine

to about 180° C. for about one hour. Then proceed as follows:

Using a cover glass with the diatoms burnt on it as before described, lay it, diatoms downwards, on a perfectly clean 3 × 1-inch slide, in the position in which it is to be mounted. Then put a small quantity of piperine at the edge of the cover glass. Place the slide on a heating stage or hot-plate which must previously have been warmed, and raise the temperature gradually until the medium melts. The liquid will be drawn in between the cover glass and the slide, completely filling up the space. Put aside until cold, and scrape away the exuded piperine. It is well to note, however, that a piperine mount should not be ringed with varnish, as doing so prevents remelting the mountant should it ever be desirable.

To Mount Diatoms in Picric Piperine (Dr. N. E. Brown's Method)

First arrange or strew the diatoms upon a clean cover glass, and see that they are perfectly dry. Then place a few crystals of piperine towards the end of a clean glass slide, and add to them about one-third of their bulk of picric-acid crystals. Hold the slide over the lamp flame until the crystals melt; then stir the fluid until it is well mixed. Keep it stirred for a short time, but do

not let it quite boil, and do not let it run to the central part where the cover glass is to go, as there are often some impurities or bubbles it is desirable to avoid. While the mixture is fluid, pick up the cover glass with the diatoms upon it, and slightly warm it over the lamp. Then with the diatoms upon its underside insert one edge in the marginal part of the fluid and *very gradually lower*, while inducing the fluid to flow under the cover glass, and get it at the centre of the slide, keeping the medium hot all the while. Apply a light pressure to secure an even thickness, and not too thick a layer of the medium, and put aside to cool, which will not take more than a few minutes, when the surplus medium can be scraped off and cleaned away with a rag *damped* (not soaked) with alcohol. It is best not to ring the mount, because the medium is apt to crystallise after a few months if (1) it has not been heated long enough to drive off the water of crystallisation, and (2) if ringed it has been found that the solvent of the ringing cement sets up some action causing crystals to form. The medium turns to a light or dark brown colour, which strangely seems in no way to detract from the resolution it gives, which is better than that of styrax. When melting the mixture, it is well to do it where the fumes can pass off up a chimney or otherwise, because the fumes of picric acid are injurious and hurtful to the throat.

Mounting Diatoms in Realgar (Dr. H. Van Heurck's Method)

The realgar used for mounting diatoms is not quite the same as the article known as realgar in commerce, which is a substance as brittle as glass, brownish yellow in colour, and quite opaque, but rather the realgar known to mineralogists, which is of a ruddy yellow colour and perfectly transparent.

Method of Preparation, No. 1.—Place 1 part of sulphur in 1·7 parts arsenious acid in a retort, and fuse the mass at red heat for several hours, raising the temperature to the point at which the product distils. The realgar is then dissolved by heat in a test-tube in some tribromide of arsenic, also obtained by distillation, and a syrupy liquid is thus produced of a yellow green colour, almost black when seen in bulk.

Method of Preparation, No. 2.—An alternative and perhaps a better method is as follows: In a flask or retort, 30 parts by weight of flowers of sulphur are dissolved in 10 parts of bromine, which gives a solution of sulphur in bromide of sulphur (S_2Br_2). Then add 13 parts of finely pulverised metallic arsenic, and heat the whole until the arsenic is completely dissolved. Turn the mixture into a porcelain evaporating dish, and heat in the direct flame, stirring all the while with a glass rod, until a drop of it when cool

becomes very brittle. The medium should then be turned out on to a cold plate, and after cooling can be broken up into pieces and preserved in a bottle fitted with a ground-glass stopper. When wanted for use, the quantity required should be heated until it becomes a thick liquid mass. This is very difficult to do, and in order to thin it for use, a very little bromide of arsenic can be added. It is not advisable to do this unless really necessary.

To Mount.—Fix the diatoms on the cover glass by burning them as described on p. 29, and cover them with a small drop of the liquid realgar. Turn the cover glass over on to a clean glass slide, which has already been warmed, and heat from the underside by means of a lamp flame or a Bunsen burner. It will probably be found that large air bubbles are formed, and the medium assumes a dark-red colour, while at the same time the bromide of arsenic evaporates. Continue heating until the air bubbles have ceased to form and no more evaporation takes place, and then gently press the cover glass down, and leave the slide to cool very slowly. In cooling, the medium loses its dark-red colour and finally assumes a pale-yellow tint.

The bromine mixture, as described by Dr. H. van Heurck, is fairly satisfactory, but one cannot always be sure of obtaining reliable results. The

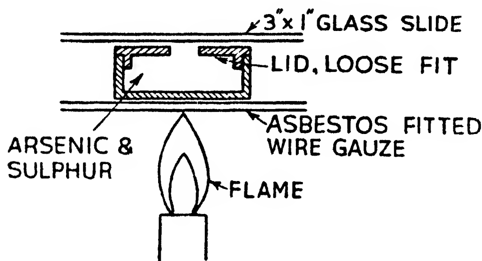
late H. Chapman Jones, Esq., found that by using the purest arsenic and sulphur obtainable, in the proportion of 7 parts of arsenic to 22½ of sulphur, he obtained lasting preparations. He used not to fill up the entire space under the cover glass with realgar, but ran in paraffin wax round the edges, allowing this to set hard. The slide was then ringed in the usual way. Another mounter, Mr. S. H. Meakin, uses the proportions of 7 parts of arsenic to 25 of sulphur, and his mounts are very good and quite permanent. It will be seen therefore that mounters vary the quantities used to suit their requirements.

Another method perfected by the late H. Grayson, of Australia, is to employ a shallow brass vessel, fitted with a loose-fitting lid. The lid should be pierced with a central hole 0·75 inch diameter. A small quantity of finely pulverised metallic arsenic should be placed in the vessel and heated by means of a Bunsen flame. Then drop in a few grains of "flowers of sulphur" into the mass in the vessel, and place a clean 3 × 1-inch glass slide over the hole in the metal lid. The glass becomes coated immediately with what must be practically pure sulphur in the first instance, and the coating presently melts into a clear uniform film. As the heating proceeds, however, the vapours from the arsenic dissolve in or combine with the sulphur, and no doubt the sulphur

coating itself tends to evaporate. The film becomes darker and thicker, and the action must be stopped before it runs together in drops. The film cools to a lemon yellow colour, closely resembling that in the Grayson test-plates.

Several points should be carefully noted:

1. *The medium itself is a deadly poison*, and the



dangerous vapours which are thrown off during the preparation should be most carefully avoided.

2. The preparation should be made in very dry weather, preferably in the open air and in the sun. If the work must be done indoors, it should be carried out in a fume cupboard. It cannot be repeated too many times that this is a very dangerous preparation to handle.

3. The arsenical medium should be heated until it cracks more or less on cooling.

4. The preparation should be made very quickly.

5. As soon as the preparation is completed and while it is still lukewarm, a thick layer of a mixture of gum and shellac should be applied round the edge of the cover glass.

6. The preparation should be kept in a dry place.

Note.—For further reference see *The Journal of the Royal Photographic Society*, January 1914, p. 14; *The Microscope*, by Dr. H. van Heurck; *The Microscope Record*, published by W. Watson & Sons, Ltd., Nos. 1–34.

To Mount Selected Diatoms in Balsam

Take an ounce of distilled water and add 6–8 drops of ordinary gum water to it, and filter.

Another and better way of making a colourless gum is to stir into 2 grammes of pure gelatine 75 grammes glacial acetic acid, using both substances cold. Still keeping the mixture cold, filter slowly. Then put 5 grammes of the above mixture into 3 c.c. absolute alcohol and 1–5 c.c. iso-butyl-alcohol, and preserve the mixture in either a black bottle or a black-coated bottle. This last is very important.

Place a drop of the gum mixture on a thoroughly clean cover glass, and put it aside out of reach of dust to dry. By means of a glass rod or a pipette place a drop of the fluid containing the

diatoms on a clean 3×1-inch slide. Hold over a spirit-lamp flame to dry. Select the desired forms and put them aside. Breathe upon the gummed cover glass, and carefully place the selected diatoms in the required position. Put aside to dry, and then add a drop of canada balsam to the cover glass and again put on one side for twelve hours out of the reach of dust. Take a 3 × 1-inch slide, and gently warm on a hot-plate, and then bring the gummed surface of the cover glass into contact with the warm slide, taking care not to move the diatoms. Gently press down and put aside to cool, then finish in the usual manner.

To Mount Selected Diatoms in Styrax or Hyrax

The simplest method is to place the cleaned diatoms on a clean 3 × 1-inch slide, driving off any moisture by holding it over a lamp flame. Then take a clean cover glass (a $\frac{3}{8}$ inch or at the most $\frac{1}{2}$ inch diameter will be found easy to handle), and carefully make a very small black ring about 2 mm. in diameter in the centre of the cover glass. Do this by breathing on a 3 × 1-inch slide, and place the cover glass to be used on it so that it will stick to the slide. Put the slide on a turntable, and make the ring with best waterproof indian ink, using a very fine (smallest procurable) sable-hair brush, or if preferred a fine mapping pen. When dry, smear the cover glass

with a slight film of the gum mixture before described, and put it aside to dry. When ready, place the cover glass, gummed side up, on a 3 × 1-inch slide (if not still on one).

For mounting, a Greenough Binocular Dissecting Microscope is the best, but failing this, an ordinary monocular microscope with a low-power objective will answer the purpose. Place the slide containing the diatoms and the slide holding the gummed cover glass side by side widthwise on the stage of the microscope. To commence, have the slide containing the diatoms under the objective, and examine the diatoms to be used. By means of a single badger hair or tiger's whisker lift the diatom to be mounted. The hair chosen for this purpose could be taken from a shaving-brush, and should be straight and clean. It should be mounted in a holder, preferably a thick one, and secured to this with a drop of shellac, balsam, glue, or any other suitable medium. Having lifted the diatom do not move the hand holding it, but with the free hand gently slide the two 3 × 1-inch slips forward until the one containing the gummed cover glass comes into view. Gently place the selected diatom in the desired position, and repeat the process until all the diatoms wanted are placed in order. When the diatoms are as perfectly placed as possible, remove the slide and breathe on the cover glass. This will soften the

fixative, and the diatoms will be fixed to the cover in a few moments. It is then advantageous to warm the cover over a flame, as this will further harden the fixative and render the diatoms less likely to slip. Before applying the medium (whether styrax or hyrax) flood the diatoms with the spirit (either benzole or xylol) used to dissolve the medium. One drop is sufficient, and before it evaporates put on the styrax or hyrax. Then place the cover glass on a heating table or hot-plate and warm. When the medium has set hard enough (a forcep prodding it does not leave an impression) bring forward the clean 3 × 1-inch slide, which is to be used for the finished slide, and place the cover glass, medium side downwards on the slide, as nearly as possible in a central position. Place the slide on the microscope and examine carefully. If the diatoms are not truly placed in the horizontal direction, gently turn the cover glass until satisfactorily placed. Remove the slide and return to the hot-plate, and heat gently until the medium is evenly spread. Then put aside to harden, and when ready it can be finished by ringing with black varnish.

Note.—Some mounters prefer to make the black ring on the 3 × 1-inch slide instead of on the cover. This is just as satisfactory, and is perhaps easier to do. Other mounters make a ring on either slide or cover by dissolving some methylene

blue in a solution of shellac in alcohol, and using a turntable as before. Use this mixture in place of the indian ink. Mucilage of tragacanth can also be used as a clear gum instead of the acetic gelatine before described.

As was noted on page 32, Sirax mounts can be made in exactly the same way as Hyrax mounts.

Chapter Four

CLEANING AND MOUNTING FORAMINIFERA, RADIOLARIA, AND SPICULES

To Clean Foraminifera

BREAK a lump of fresh chalk into pieces, not larger than an olive, and gently crush into a powder sufficiently fine to pass a somewhat wide-mesh sieve. Place the powder in a piece of stout calico, tie up like a pudding, drop it into a large basin of water, and knead well until the mass is reduced to one-third its original bulk. Pour off the milky fluid, fill up again with fresh water, and knead again. Again pour off the milky fluid, then untie the cloth and remove all the large pieces of chalk, stones, and other debris possible; retie the cloth, and again immerse in water. Continue the process until the water flowing from the mass is almost clear. When, on examination under a microscope, it is seen that all extraneous matter has been eliminated, transfer the mass to a wide-mouthed bottle and boil the whole in a strong solution of bicarbonate of soda for an hour or two. When clean, wash away the soda and bottle in distilled water.

To Mount Foraminifera in Canada Balsam

Take the forms from the phial in which they have been stored by means of a glass tube or pipette, and place them on a glass slide. Holding the slide with a pair of forceps, dry it over a spirit-lamp flame. Then scrape the forms off into a bottle of turpentine, in which they must soak until every trace of air has been removed. So difficult is it to rid the foraminifera of air that it is often necessary to submit them to an action of an air pump. Then place a small drop of canada balsam on a clean cover glass, and after pouring off the turpentine, take up some of the foraminifera and place them in the balsam, spreading them as evenly as possible. Then put the cover glass away out of reach of dust for twelve hours. Warm gently on a hot-plate for about ten minutes, apply a drop of fluid balsam, and after having warmed a clean 3 × 1-inch glass slide over a spirit-lamp flame, bring it in contact with the balsamed cover glass. Lower the cover glass carefully, and press down gently until it lies quite flat. If there is any difficulty about this, warm the glass slide from underneath and allow the cover to settle by its own weight. Put aside to cool, clean away exuded balsam with methylated spirit, and finish with a ring of black varnish.

To Clean Radiolaria

Break the lump of Barbadoes or other radiolarian earth into fairly small fragments. Put 3 or 4 ozs. of ordinary washing soda in a pint of water and boil it. Then throw the fragments of earth into this liquid and boil for half an hour. Pour off about nine-tenths of the liquid into a large glass vessel and gently crush the remaining mass. Add soda and water as before and again boil the liquid. Pour off the fluid as before into the glass vessel, and continue this process until the earth is broken up. Stir the liquid in the large vessel, and then leave it to stand so that the sediment settles. Then pour off the fluid, when the shells will be left, partially freed from extraneous matter. Put common washing soda in a fresh supply of water, bring to the boil as before, and throw the mass containing the radiolaria into the liquid and boil for an hour. Pour off the liquid as before into a large vessel, and continue the process several times. Again leave the large vessel to stand, so that the sediment may settle, and then gently pour off the liquid. Put the sediment in a jar in about ½ oz. of water, and add ½ teaspoonful of bicarbonate of soda. Let it dissolve, and then gently pour in 1 oz. of pure strong sulphuric acid. This liberates the shells and leaves them beautifully transparent. Wash well to remove all trace of the acid and soda, and place in a bottle in distilled water.

To Mount Radiolaria in Canada Balsam

Take the forms from the phial in which they have been stored by means of a glass tube or pipette, and place them on a glass 3 × 1-inch slide. Dry the slide over the flame of a spirit-lamp. Then place a small drop of canada balsam on a clean cover glass and scrape the radiolaria on to the balsam, spreading them as evenly as possible. Put the cover glass away out of reach of dust for twelve hours and then warm gently on a hot-plate for about ten minutes. Apply a drop of fluid balsam, and after having warmed a 3 × 1-inch slide, bring it in contact with the balsamed cover glass. Lower the cover glass carefully until it lies quite flat. If there is any difficulty about this, warm the glass slide from underneath, and allow the cover to settle by its own weight. Put aside to cool, clean away exuded balsam, and finish with a ring of black varnish.

To Clean and Mount Spiculæ

As some spicules are composed of silex and some are calcareous, there are two methods in use for cleaning these interesting specimens.

Those which are composed of silex should be boiled in strong nitric acid, which removes the animal substance adhering to them. Those which are calcareous must, however, be boiled

in liquor potassæ until all the material has been broken up.

In either case they must be freed from any trace of acid or potash with repeated washing in water, allowing the spicules to settle to the bottom of the container between each washing. When cleaned preserve in a bottle of dilute spirit. Proceed with the mounting in exactly the same way as directed for transparent preparations of strewn radiolaria.

Chapter Five

MOUNTING CHEMICAL CRYSTALS

MOST objects intended for examination with the micro-polariscope may be mounted in canada balsam, but many salts are soluble in this medium, or their forms are injured by it, and they must then be mounted in glycerine, gum water, or in castor oil, whilst a few other sorts should be mounted dry.

Method 1.—Make a saturated solution of any salt and drop it into alcohol (where it is not soluble in alcohol), and crystals will be instantaneously produced. By means of a pipette take up some of the crystals and drop them upon a cover glass. Allow them to dry slowly or evaporate over a lamp flame, and when dry add a drop or two of balsam, and mount in the usual way by slightly warming the 3 × 1-inch slide and lowering the cover glass.

Method 2.—Make a strong solution of the salt in distilled water, and add a few drops of gum water or a small piece of gelatine. Mix well and filter. By means of a pipette take up some of the solution and drop it on a cover glass, putting it

aside to dry slowly. When dry add a drop of balsam, and proceed as given in Method No. 1.

Method 3.—Place a small piece of the salt upon a slide, and heat over the flame of a spirit-lamp until fused. With a *hot* needle spread the salt over the required surface and put it aside to cool. As the slide cools the formation of the crystals takes place, and when ready for finishing, a drop of balsam should be added and a cover glass lowered and gently pressed, and then put on one side to harden. If it is found necessary to mount the salt in castor oil or glycerine, proceed as follows:

By means of a turntable make a thin ring with some shellac cement on a perfectly clean 3 × 1-inch slide, and allow it to become quite dry. Then make another ring of cement on top of the first ring, and when this is nearly dry fill the cell with castor oil. Crystallise the specimen on the cover glass as described in Method No. 1 or No. 2, and with a pair of forceps bring the crystallised surface in contact with the oil, taking care to avoid air bubbles. Lower the cover glass very gently on to the shellac ring and press it down with the forceps, squeezing out the excess of oil, and embed the edge of the cover in the shellac ring. Put aside to dry, and when ready to handle wash off the exuded oil with some turpentine, and ring with another coat of shellac cement.

Salts crystallise in a different manner according to the temperature used to effect this. Santonine, for instance, should be fused on the slide and treated as described in Method No. 3. This salt is slightly soluble in ordinary balsam, and should therefore be mounted in castor oil. If the fused salt is very hot, the crystals run in straight lines from a common centre, whereas if it is subjected to a medium degree of heat, the crystals show concentric waves of a very decided form, and if the slide is cool, the crystals, still concentric, are very minute. The most beautiful crystals are formed at a temperature between the medium and cool degrees. A simpler method of treating santonine is to dissolve a few grains of the salt in a drachm of chloroform and drop the solution on a glass slide. Allow the liquid to evaporate, and mount in castor oil.

Another salt, sodium tartrate, can be prepared by neutralising a strong solution of tartaric acid by the addition of carbonate of soda and by placing a drop of the solution on the slide. Warm the slide to evaporate the liquid, and put aside in a dry place for the crystals to form. This may take some time, probably a week or two, and when ready should be mounted in castor oil.

Hippuric acid should be prepared by making a saturated solution in absolute alcohol, and by dropping it whilst warm on a warm slide. The form of the crystals may be modified by the

atmosphere in which they are allowed to grow; thus, a moist atmosphere or the reverse, an atmosphere of vapour of ammonia or spirit benzole, will each produce a different result. These crystals are best mounted in castor oil or in pure balsam.

A method of preparing fine specimens of crystals is to mix a small quantity of two salts, such as calcium chloride and zinc sulphate. About 80 drops of a solution of zinc sulphate should be mixed in a teaspoonful of gum water, and about 80 drops of a solution of calcium chloride should be mixed in another teaspoonful of gum water, and the two should be gradually mixed and filtered. A drop of mixture can be placed on a cover glass and evaporated, when in a short time fine needle-like and radiating clusters of calcium sulphate will be formed. They can then be mounted in the usual way.

Glycerine, albumen, or a solution of gelatine instead of gum water can be used as desired.

To Mount Various other Specimens suitable for Examination by Polarised Light

Starches are to be found in the cellular tissue of almost every plant, and the best method of obtaining them is to grind the material into very small pieces, using a mortar if one is available. Then place the mass of matter in a wide test-tube

of water, and shake well, afterwards straining through a piece of fine muslin, so that the starch grains will pass and the debris be retained. Allow the liquid containing the starch to stand, when the grains will fall to the bottom, and the water can be drained off. Repeat the process of washing and draining until no trace of debris is seen among the starch.

When quite clean, take up a small quantity, spread on a cover glass, and put aside to dry. Then add as small a portion as possible of balsam diluted with turpentine, allowing it to spread as evenly as possible over the grains, and gently lower the cover glass on to a 3 × 1-inch glass slide and mount in the usual way.

Raphides or Plant Crystals.—These are to be found in the tissues of many plants, the commonest being obtained from the cacti, orchid, geranium, hyacinth, rhubarb, and onion. If it is desired to mount the raphides separately, a piece of the plant containing them should be bruised, so that the juice may flow upon a slide, which should be warmed to evaporate the moisture, and the raphides mounted in balsam in the usual way.

If, however, it is desired to mount the raphides *in situ*, strip off the cuticle from a fresh specimen, such as an onion, and soak it for some time in turpentine or benzole to render it transparent,

and mount in balsam as before. An alternative method is to dehydrate in methylated spirit, clear in clove oil, and mount in canada balsam.

Scales or Hairs of Plants.—These can be detached from the leaf or other part on which they are found by gently scraping with a knife, and should then be dropped in a bottle of turpentine and soaked for a long time to remove all traces of air. Then take up a few scales and mount in canada balsam. There is a little danger, when placing the cover glass on the balsam, of forcing out the scales on the wave of matter which is always ejected. This may be overcome by applying to the slide, previously to placing the scales upon it, an extremely thin layer of balsam diluted with turpentine, then arranging the scales in the required position, and putting aside to dry somewhat; then, after adding a little fresh balsam, gently lower the cover glass on top, pressing down carefully.

Fish Scales.—The scales of various fish when mounted in balsam make beautiful polarising objects. Scrape the scales from the fish and wash them thoroughly to clean them; dry them, soak for a day in turpentine, and mount in balsam in the usual way. An alternative method is to clean them in water, soak in liquor potassæ for an hour or two, wash away the potash, dehydrate in

methylated spirit, clear in clove oil, and mount in balsam.

Palates of Molluscs.—In the case of very small molluscs, such as limpets, whelks, and garden snails, remove the bodies from the shells, and boil them in liquor potassæ, when the bodies will be entirely dissolved, leaving the palates intact. Then wash well to remove the potash, and place the plate on a clean 3 × 1-inch glass slide, put another 3 × 1-inch glass slide on top of it, and tie the two firmly together with cotton and place in a jar of methylated spirit for a few hours. Remove the palate from between the glass slides, place in clove oil to clear, and then mount in balsam as usual.

Various Objects.—Many other specimens, such as bones, hoofs, horns, quills, etc., give beautiful results with polarised light if suitably cut in either transverse or longitudinal sections. To do this it is necessary to soften them either by soaking for a few days in methylated spirit in the case of the softer objects, or by soaking in liquor potassæ for a few days in the case of harder objects. When soft enough, wash in water, and place in methylated spirit, in which they may be preserved until required. Then make the required section, preferably in a microtome, dehydrate in methylated spirit, clear in clove oil, and mount in balsam.

Muscular fibres, such as are found in the tongue of a cat or similar animal, can be hardened in methylated spirit, embedded, and longitudinal or transverse sections cut in a microtome, dehydrated, cleared in clove oil, and mounted in canada balsam.

To Mount Fibres and Hairs

Hairs of animals, such as wool, should be well washed in warm water and then dried. Then wash in benzole or methylated spirit to remove the water and any natural grease, soak in turpentine or clove oil, put a few hairs on the slide, and place on the top of them a little canada balsam and mount in the usual manner.

Fibres of cotton, linen, and other textiles can be treated as for animal hairs, except that generally there is no need to soak them in benzole, as in the pure state there is rarely any grease.

The very coarse hair taken from a horse, if carefully plaited and mounted in balsam, makes a very pretty polarising object.

To make Transverse Sections of Hairs and Fibres.—Take a small bundle of the specimens, tie them together, and then wash in warm water. Then prepare a very strong solution of gelatine in water, and immerse the bundle in it, leaving it to soak for a long time until the gelatine has penetrated the bundle. Lift the bundle from the

gelatine on the point of a dissecting needle, and place the needle in such a place that the bundle is exposed to the air, and leave until quite cold. When cold, place in a quantity of methylated spirit for some hours, and then cut sections in a microtome in the usual manner. Dehydrate in methylated spirit, clear in clove oil, mount in balsam, and finish in the usual manner.

Chapter Six

PREPARATION OF ROCKS AND METAL SECTIONS

BY means of a hammer and chisel, detach a small chip of rock, approximately 1 × ¼ inch or less in thickness, and then take a hone (Water of Ayr) stone, moisten it with water, and rub one side of the specimen until it is quite smooth. An alternative method is to use a thick iron plate or a very flat stone, and cover this with a little coarse emery powder. Moisten with water, and with a circular motion rub the specimen round in the emery paste until it is flat and quite smooth. Then wash the specimen in water, and set it aside to dry. Take a clean 3 × 1-inch slide, and place a drop of canada balsam in the centre, heat gently, taking care that bubbles do not form through too rapid heating, and set aside until it is a little cool. Then press the smooth side of the section in it and set the whole aside until quite cold. The rough surface of the specimen should now be ground with coarse emery and water, on the stone or iron plate, or with the hone if preferred until it is very thin, and then rubbed down still thinner by using some fine flour emery and water. For this last purpose, a thick glass plate,

such as an old negative, will be found useful. When thin enough, wash well in water and dry. It may now be transferred to a clean 3 × 1-inch glass slide by warming so as to melt the balsam, and sliding it on to the clean slide, which has already been prepared by placing a drop of balsam in the centre and gently heated in the usual way. A cover glass must now be added, and the best method is to place a clean cover glass over the section and a drop of canada balsam at its edge. Heat the slide gently from underneath, and the balsam will be drawn under the cover glass by capillary attraction. The slide must bc put aside to dry, which process takes some days.

Sections of bones, teeth, stones of fruit, and similar hard substances can be made in the same way, but when the grinding is finished the section should be passed through alcohol into clove oil, and mounted in canada balsam in the usual way.

Very soft and easily broken substances, such as limestones, coal, and other sedimentary rocks, should be cut with a very fine fret-saw into workable pieces, and soaked in benzole for some hours. Then transfer the specimen to an ordinary solution of balsam in benzole, and leave it in this until the balsam has absolutely penetrated the tissues. Then place the specimen on a 3 × 1-inch slide, cover with canada balsam, and put on

one side out of the dust until the benzole has evaporated from the balsam. Then place on a hot-plate, and gently heat and bake until the balsam is quite hard. Grind down to the required thinness as before described, wash well in water, and mount in balsam in benzole.

Preparation of Metal Specimens

When preparing pieces of metal for microscopical examination, it is advisable to keep the specimens from ¾ inch to 1 inch square or in diameter, as they are easier to handle and polish. The specimen should, if possible, be cut out by means of a hack-saw, the blade of which should be lubricated with a soap solution, applied at intervals with a brush. For large material, other methods of cutting can be employed, but care should be taken to avoid any violent method likely to cause distortion of the material. It is also well to remember that no heat must be applied in any way, as this causes an alteration in the structure of the metal.

Method.—Cut the metal to a convenient size, and level one surface by rubbing it on a fine file, taking care not to cut or in any way make deep marks in this surface. This levelling may also be carried out by means of a grindstone, the surface of the stone being kept wet. When satisfied

that the level face is as smooth as it can be, the specimen should then be rubbed steadily, face downwards, on coarse emery paper, until the whole surface is covered with fine parallel lines. It should then be rubbed in the same steady way on No. 1 emery paper, but in such a way that the scratches now formed cut across the old scratches at right angles. Polishing should be continued until the first lot of scratches are replaced by a second and final lot at right angles to the first. The specimen should then be steadily rubbed on Nos. 0, 00, and 000 emery paper successively, each transfer being accompanied by a rotation of the specimen through a right angle. After having been rubbed on No. 000 emery paper, the metal should be washed in water and polished on a pad covered with a suitable polishing powder, such as rouge or alumina (diamantine). The pad, which could be made by stretching a piece of selvyt or wash leather over a hard wooden disc, should be flooded with water, and the diamantine applied and rubbed over smoothly. The pad should be kept in rotation and well supplied with water, and the specimen held against its surface and moved slowly in a reverse direction to the rotation of the pad. To obtain satisfactory results, it should be borne in mind that the metal must be kept wet so that the diamantine does not become embedded in the surface. If the surface appears free from scratches,

it should be washed in tap water and dried, being now ready for microscopical examination.

Rouge, as is mentioned above, is often used instead of diamantine for polishing, and is quite satisfactory, the same routine being observed as is outlined above. It should, however, never be used dry, as it adheres to the surface and cannot be removed by subsequent polishing.

When the metal to be polished is very soft, such as copper or aluminium, special care should be taken, and the pressure exerted should be of the lightest. Polishing is carried out in the usual manner under slight pressure of a slow-running disc, fed with alumina. Final polishing is often carried out by means of metal polish (liquid or paste) applied on a piece of chamois leather. One objection to this is that the polish leaves a greasy surface on the metal, but this can be removed by gently rubbing with a soft piece of chamois or selvyt moistened with benzine. Specimens thus polished are suitable for microscopical examination, but to reveal the finest structures, surfaces of such specimens are often etched. Several types of reagents are in use, such as acid solutions and salt solutions, but of these we will only consider the simplest. Very dilute nitric or sulphuric acid, and weak solutions of ammonia or caustic potash, can be used for many metals, and sometimes an infusion of liquorice root and

tincture of iodine are used to develop the structure of steel.

After the polishing has been done, the simplest method is to coat the piece of metal on all sides except the one to be etched with some protective varnish, and immerse the whole piece in a bath containing the reagent to be used. The reagent should be allowed to react for about twenty seconds, and the metal should then be removed and carefully washed in alcohol or methylated spirit. It should then be washed in water and dried with a very soft rag. It should now be examined under the microscope, and if the etching is insufficient, the process detailed above should be repeated for another twenty seconds, and so on until completed.

Metal specimens, owing to the action of the air on them, should be stored in a vessel containing calcium chloride, which absorbs the moisture in the air.

Chapter Seven

BLOOD SMEARS AND BACTERIA

Blood

THE two main methods of blood examination are (*a*) the examination in the fresh fluid condition, and (*b*) as films, which are allowed to dry and are subsequently fixed and stained in various ways. The first method need not be considered here, but the preparation of permanent films is important. A drop of blood should be placed on the surface of a clean 3 × 1-inch slide near one end. The edge of another slide should be brought in contact with this drop, which then spreads out so as to fill the angle between the two slides. By pushing the upper slide towards the end of the lower slide a film of blood will be left behind. The thickness of the film can be regulated by the angle between the two slides, but an angle of about 45° will be found to give a very useful thickness. The film should then be dried by waving it rapidly about in the air. Heat should not be applied, as this leads to distortion of the corpuscles. It is not absolutely necessary, but some hæmatologists further fix blood films by immersing the slide in absolute alcohol, or preferably a mixture of equal

parts of absolute alcohol and ether for ten minutes, and then drying the film in air. When fixed and dried, the film can be stained. The number of stains that can be employed is very large, but of the methods applicable, three only will be considered:

1. ***Hæmatoxylin and Eosin.****—Formula:*

Hæmatin	2·5 grms.
Absolute alcohol	50 c.c.
Alum	50 grms. to saturation
Water, distilled	1,000 c.c.

The hæmatin is dissolved in the alcohol and added to the solution of alum in water, and the vessel containing the mixture should be left loosely corked and exposed to the light in order to hasten the maturing of the stain.

(*a*) Cover the film with hæmatoxylin and leave from seven to ten minutes. The film should not be moved during this process.

(*b*) *Flush* off stain with tap water.

(*c*) Place film in tap water for five minutes to blue.

(*d*) Stain for half-minute with 1 per cent. aqueous solution of yellow eosin.

(*e*) Wash and dry in air.

It is important that the hæmatoxylin should be *flushed* off and not just poured off.

(*f*) Put a drop of balsam in xylol or Gurr's neutral mounting medium on the slide, and cover with a cover glass in the usual manner.

2. ***Leishman's Stain.***—When using this stain the film must be *unfixed* (see note above), and before staining it is advisable to put a broad grease pencil mark across the slide about ½ inch from the end. This prevents the stain from spreading all over the slide and so soiling the fingers. With a pipette drop 5 or 6 drops of the stain on the film and allow them to stand on it for from half to one minute. To these drops of stain distilled water should then be added drop by drop. Care should be taken to see that any distilled water used is not acid, as this is fatal to the stain. It would be better to use water giving an alkaline reaction to litmus. The slide should be moved in a see-sawing motion, so that the watery stain mixes as much as possible. The amount of water required is about double that of the stain, and should be added until the dark-blue colour of the stain is replaced by a pinkish colour in the mixture, whilst the precipitated stain can be seen floating in the fluid. The stain should then be flushed off with distilled water, and a drop of the distilled water should be allowed to remain on the film for about a minute. The film should then be blotted and allowed to dry. Canada balsam is not a good medium in which to

mount films stained with Leishman's stain, and it is best to mount them in some neutral medium, such as Euparal or Gurr's neutral medium.

3. ***Giemsa's Stain.***—The mixture should be made as follows, but is best bought ready made.

Azur II, eosin	3 grms.
Azur II	0·8 grm.
Glycerine, pure	125 grms.
Methyl alcohol	375 grms.

When ready for use, the stain should be diluted with distilled water, in proportion of 1 of stain to 15 of distilled water. The film should be made in the manner before described, and fixed in methyl alcohol for two or three minutes, or in absolute alcohol for five minutes and dried. The diluted stain should be poured on the film and left for some thirty minutes or longer as required, and the stain should then be flushed off with ordinary water and dried in air. It should be mounted as described for Leishman's stain.

Film Preparations of Bacteria

To make these films, a platinum loop is of great value, and such should be obtained from any laboratory outfitter. If a fluid containing bacteria is to be used, the platinum wire should

be placed in the fluid, and a loopful taken up and dropped on to a clean 3 × 1-inch slide, and spread over the surface with a needle. When a culture on a solid medium is to be used, a loopful of water should be placed on the slide, and a small portion of the growth rubbed in it, and spread out so as to form a thin film. Great care should be taken not to use too much of the growth. The film should then be dried in room temperature, or by very gentle heating over a Bunsen flame. The film should be further fixed to the slide by heating the underside of the slide until it is too hot to be borne on the palm of the hand. Care should be taken to prevent charring.

In the case of urine, the fluid should be allowed to stand and films made from any deposit, or the urine could be centrifuged and the films made from the deposit. When dried films are made from urine they should have a drop of distilled water placed on them, and be heated gently to dissolve the deposit of salt; then washed in water and dried.

Staining Bacteria

There are a large number of stains which can be used for bacteriological preparations, but the best for the purpose are basic aniline dyes. Those most commonly used are: crystal violet,

methyl violet, and gentian violet, methylene blue, thionin, toluidine blue, basic fuchsin, safranin, Bismarck brown, and chrysoidin. Of those mentioned, the violets and reds are most intense in action, and when using care must be taken not to overstain. On the contrary, it is very difficult to overstain with methylene blue, and films can be left in this stain for a considerable period without harm. It is best to keep saturated alcoholic solutions of stains ready for use, and when using they should be diluted with ten times the bulk of stain to be used with distilled water. This mixture should be filtered before use. A few drops of stain should be placed on the surface of the film, and left for a short period, and flushed off with tap or distilled water. The film should be dried by drawing off the surplus moisture with filter paper, and then by gentle heating over a flame. It should be mounted in neutral canada balsam or Gurr's neutral medium, covering as usual with a cover glass thin enough to enable the highest powers of the microscope to be used without any difficulty.

Most bacteria will stain easily if treated as described above, but there are a few exceptions —tubercle, leprosy, and smegma bacillus being outstanding examples. Ziehl-Neelsen has perfected a stain for such bacteria, the formula being as follows:

Ziehl-Neelsen Carbol Fuchsin

Basic fuchsin	1 grm.
Alcohol	10 c.c.
Carbolic acid crystals	5 grms.
Water	100 c.c.

The fuchsin should be well powdered and mixed with the alcohol. Then add the carbolic acid and finally the water in small portions. For general purposes, i.e. not for Ziehl-Neelsen, it is diluted with 10–15 times its volume of water, and takes from ten to fifteen seconds to stain. To use, filter a few drops of the carbol fuchsin on to the film, heat until steam rises, and repeat this several times, allowing the stain to act for five minutes. Wash well with water, then contrast with a saturated watery solution of methylene blue for half a minute. Wash in water, dry, and mount.

If, when the film has been in the stain it appears to be deeply red, it is probably necessary to remove some of this excess colour. This should be done by immersing the film in a solution of either strong nitric acid, hydrochloric acid, or sulphuric acid in water. Then wash in water, but if the film still shows distinctly red, the film must be returned to the acid. Wash again, and continue until the right tint is reached.

Both films and tissues containing bacteria which have already been embedded and cut into sections can be stained by the Ziehl-Neelsen method.

Method of Staining Bacteria to show Flagella

***Silver Precipitation Method* (Method A).**—Solutions required:

Fixing Solution	c.c.
Absolute alcohol	60
Chloroform	30
Formaldehyde (40 per cent.)	10

Mordant	c.c.
Ferric chloride (5 per cent. solution)	20
Tannic acid (20 per cent. solution) (dissolved by heat and allowed to cool)	60

Before use dilute with an equal volume of water.

Silver Stock Solution.—Place 10 grammes of silver sulphate in a clear glass bottle, and add 200 c.c. of distilled water. Incubate at 37° C. for twenty-four hours, shaking occasionally. The solution improves by leaving it exposed to daylight, and keeps indefinitely.

Silver Staining Solution.—Rinse a clean 100-c.c. flask with distilled water. Place 40 c.c. of filtered silver stock solution in the flask, and add quickly 0·6 c.c. ethylamine 33 per cent. W.V. A precipitate forms, which is immediately redissolved. With a clean pipette add filtered silver stock solution, until a permanent slightly opalescence results. Finally, add 10 c.c. distilled water.

(1) On a clean slide place 1 platinum loopful

of bacteria, either film or growth, and spread evenly, drawing excess of fluid to one end of the film.

(2) Place slide in the fixing solution for one to three minutes.

(3) Rinse in spirit, then wash thoroughly in water.

(4) Treat with mordant for one to five minutes.

(5) Wash well in water, dry the underside of the slide.

(6) Lay the slide on a staining rack over the sink, filter on silver staining solution. Heat the slide by holding it over a flame, keeping it moving, until it becomes a dark brown colour and a metallic scum forms on the edge of the fluid. Cease holding over the flame, and allow the heated silver solution to act for some thirty to forty-five seconds.

(7) Wash off the staining solution in running water (the staining solution must not be poured off or deposits will form).

(8) Dry the film and mount in benzole balsam.

A well-stained film shows the organisms uniformly black, the flagella being clearly defined and of a light brown-black or grey colour. Flagella showing a granular appearance may be caused by the staining solution containing excess of ethylamine, by overheating while staining, or

by over-treatment in mordant. A crystalline deposit in the preparation is caused either by excessive heating while staining or insufficient ethylamine in the staining solution. A silver staining solution which contains excess of ethylamine may be corrected by the addition of a few drops of the stock silver solution. One which contains too little ethylamine had better be rejected.

Method of Staining Flagella without Heat

Method B.—Reagents:

Fixative

Fixative as in Method A.

Mordant

As in Method A, used undiluted.

Stock Staining Solution

	c.c.
Basic fuchsin, saturated alcoholic solution	45
Shunk's mordant (aniline 1 part, 95 per cent. alcohol 4 parts)	18
Distilled water	100

Mix Shunk's mordant with the alcoholic fuchsin, then add water. All glass-ware used should be rinsed out with alcohol before use.

Staining Fluid

	c.c.
Stock staining solution..	50
Potassium hydrate (2 per cent. solution)	1

Mix and filter. The stain is usable so long as no precipitation occurs.

(1) Make the film as described before.
(2) Place film in fixing solution for one to three minutes.
(3) Rinse in spirit, then wash thoroughly in water.
(4) Treat with mordant for three to five minutes.
(5) Wash well in water, and place slide in staining fluid for five to ten minutes.
(6) Wash in water, dry, and mount in benzole balsam.

The longer treatment in the mordant and staining fluid in Method B gives more intense staining of the flagella, and is very delicate, compared with the silver precipitation method.

To Mount and Stain Epithelium

Squamous epithelium can be obtained by scraping the tongue of the frog or any other animal; columnar epithelium can be taken from the internal lining of the stomach, and ciliated epithelium from the back part of the roof of the mouth. In each case make a smear on the slide of the epithelial cells, and dry in air. Stain with a strong solution of picrocarmine for about five minutes, wash with water, and allow to dry.

Apply a drop of canada balsam, and finish in the usual manner.

Teasing out Tissues

Place the tissue on a 3 × 1-inch slide and, using two dissecting needles, separate the fibres from each other. It is best to do this by placing a few drops of distilled water on top of the specimen, and when it is sufficiently teased, drain off the surplus water. Apply a drop of the mounting fluid, balsam, or Farrants' medium, and mount as usual.

Striated muscle should be hardened in a 2 per cent. solution of potassium bichromate before being teased, and yellow elastic tissue from the ear in chromic acid and spirit for some days. White fibrous tissue should be hardened in methylated spirit, and nerve fibres in a 1 per cent. solution of osmic acid; in the case of the former for about a week and of the latter for about one hour.

To Mount Endothelium

This specimen can be obtained from the omentum of any animal, and should first be washed in distilled water to thoroughly clean, and then stained in a ¼ per cent. solution of silver nitrate until it shows a milky appearance. Then wash in tap water, and leave in bright sunlight until it goes a brown colour. Then mount in balsam or Farrants' medium.

Endothelium from the mesentery can be first washed in distilled water, and then stained with hæmatoxylin for some minutes, washed in distilled water, cleared in clove oil, and mounted in canada balsam.

Chapter Eight

PREPARATION OF ANIMAL TISSUES

MOST animal tissues require hardening before sections can be cut from them, and to secure this, they are immersed for varying periods in suitable reagents. Hardening agents can be divided into two types: (*a*) those—e.g. alcohol, nitric and picric acids, etc.—which do not interfere with the subsequent processes of staining the tissues and sections, and (*b*) those—e.g. osmic and chromic acids, etc.—which do more or less affect the action of the staining reagents. In hardening tissues, it is most important to be sure that the specimens are thoroughly penetrated by the liquid, and to this end it is necessary to immerse them in an abundance of the liquid. If small pieces are used, and placed in about 100 times their bulk, this should lead to satisfactory results. The tissues must be as fresh as possible; no tissue can be placed too early in the fixing medium, and so soon as the medium has become fouled, it should be changed. Frequent changes are advisable. Material may be suspended in the fixative by a thread, or some workers prefer to wrap them in pieces of linen, with a label bearing its name and other data attached to each separate parcel. In this way many specimens can be hardened

together in the same fluid, and much waste of space and fluid can be avoided without risk of confusion. No hard-and-fast rule can be laid down in respect of the time it takes each specimen to harden, and it is only by careful experiment and experience that this can be determined. Also it is not possible to say that a given tissue must be fixed in a given medium, as each tissue requires fixing by a different process according to the structure it is desired to show. For instance, to display the corpuscles and nerves of the cornea of the eye, it must be treated with gold chloride, whereas to show the cell spaces silver nitrate must be used.

Alcohol

Alcohol is not very suitable as a fixing agent, but is sometimes used, as its action is very rapid. When employed as the sole hardening agent, it should be strong, i.e. of 95 per cent. The specimens should be immersed in an abundance of spirit, which must be changed frequently, particularly for the first few days. Some tissues will be found to be sufficiently hardened in a few days, whilst others, especially large specimens, will require weeks.

Industrial Methylated Spirit (99·24 per cent.)

This may be used universally and is very inexpensive. It has a tendency to shrink specimens

too much. It hardens material in about ten days. The specimens should be immersed in it and the fluid should be changed very frequently.

Chromic Acid and Alcohol

Mix 1 part of a $\frac{1}{6}$ per cent. solution of chromic acid with 2 parts methylated spirit. This should only be made just before it is wanted for use. All tissues placed in this fluid should be daily examined, after the third day, and when they have become tough, transferred to spirit. Moreover, the fluid should be changed after the first twenty-four hours and at intervals of a few days afterwards.

Potassium Bichromate

Make a 2 per cent. solution of potassium bichromate with ordinary water. Tissues placed in this will harden in about three weeks. They should then be transferred to methylated spirit and the spirit changed every day until no colour comes away from the tissues.

Bouin's Fluid

Make a mixture of 75 c.c. of a saturated aqueous solution of picric acid, 25 c.c. of 40 per cent. formalin, and 5 c.c. glacial acetic acid. Place the tissue cut in small pieces in this mixture for twelve to twenty-four hours and then wash in 70 per cent. alcohol, until fluid is colourless.

This is a generally good fixing agent for ordinary histological structure.

Ammonium Bichromate

Make a 2 per cent. solution in water. Immerse specimen in it for several weeks. Use as given for Potassium Bichromate.

Müller's Fluid

This is made by dissolving 25 grammes potassium bichromate and 10 grammes sodium sulphate in 1,000 c.c. water. Müller's fluid has great penetrating power and hardens slowly, taking often from five to seven weeks. It is useful as a commencing fixing agent, but is often followed by another of greater shrinking power, such as the chromic acid and alcohol solution, or alcohol. For instance, a tissue may be placed in Müller's fluid for two days, then for a week in the chromic acid and alcohol solution, then in a weak methylated spirit solution, and later in pure spirit.

Zenker's Fluid

Make up 1,000 c.c. of Müller's fluid, and add to it 50 grammes mercuric chloride, and just before using this mixture, as a fixing fluid, add 50 c.c. glacial acetic acid. According to size, allow material to remain in this fluid from six to

twenty-four hours, then wash in running water for twelve hours, place in 50 per cent. alcohol for two to three hours, and then remove to 70 per cent. alcohol, to which has been added a few drops of a saturated solution of iodine in 95 per cent. alcohol until it becomes a sherry colour. This colour must be maintained by the addition of fresh iodine solution, then remove the specimens, and wash in 70 per cent. alcohol.

Formalin (Formaldehyde), 40 per cent.

This can be used universally as a fixing agent, but is not generally recommended. For fixative, use a 5 per cent. solution in 0·9 per cent. saline solution. Leave the material in it for twenty-four to forty-eight hours, according to the size of the specimen. Then wash specimen in 50 per cent. alcohol.

Carnoy's Fluid

Make a mixture of 10 c.c. glacial acetic acid, 30 c.c. chloroform, and 60 c.c. absolute alcohol. Specimens need only be left in this fluid for a comparatively short time, from twelve to sixty minutes according to the size of the specimen. Then wash material with 90 per cent. alcohol until all traces of acetic acid have been removed. This fluid forms an exceedingly penetrating mixture, and is suitable for cytological work.

Flemming's Fluid

Another fixative for work on cell structure is made by mixing 15 c.c. aqueous solution of 1 per cent. chromic acid, 1 c.c. glacial acetic acid, and 4 c.c. of 2 per cent. osmic acid. With this fluid, again, long immersion is not necessary, twelve to twenty-four hours being found adequate for small pieces of tissue. Then wash specimen in running water for twenty-four hours, remove to 50 per cent. alcohol for six hours, and then into 70 and 75 per cent. alcohol for a short time.

Picric Acid

Make a cold saturated aqueous solution, and leave small pieces of tissue to harden in this from twelve to forty-eight hours. It is especially suitable for decalcifying fœtal bones, and is also suitable for cartilage. After the specimen has been in the fluid for a sufficient period, wash in repeated changes of methylated spirit until no colour is given off, and preserve in alcohol until required.

Corrosive Sublimate

Tissues may be fixed very quickly in corrosive sublimate. Make a saturated solution in 5 per cent. glacial acetic acid and place the tissues to be fixed in this solution. They should be removed from the solution as soon as they are opaque throughout. Then wash in repeated

changes of 70 per cent. alcohol to which a few drops of tincture of iodine have been added.

Osmic Acid

This acid can be purchased as a 1 per cent. solution, and should be diluted with distilled water to a ½ or ¼ per cent. solution as required. It is a hardening agent which also stains fatty matter, and is useful in blackening the medullary nerve fibres. It is well to mention that it is costly, poisonous, and deteriorates on exposure to light. It should be kept in a well-stoppered bottle, which should be of black glass or covered with black paper, so that no light is admitted. Substances placed in it harden in from four to thirty-six hours, and should then be transferred to a mixture of glycerine and water. It is best to mount the specimens fixed in osmic acid in either glycerine, glycerine jelly, or Farrants' medium. The vapour of a 2 per cent. solution of osmic acid is used as a fixative for films, smears, and similar very delicate specimens.

Chromic Acid and Nitric Acid Solution

Make a ⅙ per cent. aqueous solution of chromic acid, and to every ounce add 5 drops of nitric acid. This is suitable for bones, teeth, and similar hard specimens, and softens them for cutting. The specimens should be immersed in a generous quantity of the liquid, and the

liquid should be changed several times. When soft enough, transfer specimens to water, soak for some time, pour off water, and add a 10 per cent. solution of sodium bicarbonate to remove all trace of the acid. Wash again in water, and place in methylated spirit until required.

Most of the Fluids detailed herein are not only fixing but hardening reagents, but sometimes it will be found that even after immersion in them for a considerable period the tissue has not reached the requisite hardening for cutting. By further immersing in strong alcohol, this can be remedied, but it must be borne in mind that material cannot be placed from water or other media straight into strong alcohol, but must be passed successively through various grades from about 50 per cent. alcohol through 60 to 70 per cent., etc., up to absolute alcohol, remaining in each grade for some considerable time. If the material is to be embedded in celloidin it must not be taken so far as the absolute stage, as celloidin is soluble in absolute alcohol. The 95 per cent. stage must be the last.

Miscellaneous Preparations

Silver Nitrate

Make a 1 per cent. solution of nitrate of silver in distilled water, and keep in a bottle carefully covered all over with black paper. This solution

can be further diluted to a $\frac{1}{2}$ or $\frac{1}{4}$ per cent. solution if required. Place specimen in the solution for a few minutes, wash in ordinary water thoroughly, transfer to a dish containing water, and expose to diffused daylight until slightly brown. Then stain and mount as desired.

Chloride of Gold

Make a 2 per cent. solution with pure distilled water, and keep in a black bottle. To use this fluid, some formic acid and a fresh lemon are also required. Place a very fine thin piece of the specimen, such as the cornea from an eye, in a watch glass, and squeeze the juice from a fresh lemon over it, and let it remain immersed therein for five to ten minutes. Then wash in distilled water to remove all the juice, and steep for half-hour in a 1 per cent. solution of the gold chloride. Wash away the surplus gold chloride, and then place the specimen in a mixture of 1 part formic acid and 3 parts water for twenty-four hours, in a cool place, away from the light. Then wash out the acid and mount as desired.

Decalcifying

There are many substances, such as teeth, bone, horn, etc., which are too hard for cutting without first being subjected to a softening process. One method of doing this is to add 4–6 drops of nitric acid to a $\frac{1}{8}$ per cent. aqueous solution of chromic

acid. Hard substances can be left to soften in this fluid for a considerable period, the time varying according to the size and hardness of the specimen. It is advisable to change the fluid several times during the process, and an easy method of testing for softness is to run a needle into the specimen. If it goes in easily and cleanly, the softening process is complete; if not, leave it to soak for a longer period. Then take the specimen out of the acid, and soak it well in water, pour off the water, and further soak in a 10 per cent. solution of sodium bicarbonate for about ten to fifteen hours, so that all trace of the acid is removed. Further wash in fresh water, and the specimen is then ready for embedding. If it is not required immediately, it can be stored in methylated spirit. A good number of specimens can be decalcified in the same jar, provided a generous quantity of fluid is used.

Clearing

Material which is to be embedded in celloidin does not require clearing, but if the material is to be infiltrated with paraffin wax, it must be cleared of all traces of the alcohol in which it has been dehydrated or stored. Clearing agents also are used to render the specimen transparent.

Clearing Sections

After the material has been embedded and cut, the resultant sections will require clearing before

being mounted in canada balsam or any other medium. This is done by replacing the alcohol with another liquid which has a refractive index about the same as that of the medium in which it is to be mounted, and which will mix readily with it.

There are several fluids which are used for this purpose:

Natural Cedar-wood Oil.—The material should be placed in a quantity of this oil, and left in it until it has sunk to the bottom. This oil is not expensive, but is very slow in its action. It clears perfectly, and does not greatly affect aniline dyes, nor does it shrink the specimens. It is particularly useful for clearing vegetable tissues, and celloidin sections may be cleared in it, although it takes several hours to render them transparent.

Oil of Bergamot.—This oil is a most useful although costly agent. It is rapid in its action, and clears well. It clears celloidin-embedded sections, and does not affect aniline dyes to any great extent.

Oil of Cloves.—This oil is very extensively used, as it combines well with both alcohol and balsam, and does not shrink the specimen. After material has been cleared sections may be mounted in balsam direct from clove oil, but it is best to remove the oil of cloves from the material

before mounting in canada balsam. This is done by placing the specimen in turpentine after it has been cleared. All specimens or sections which have been placed in turpentine should be mounted in balsam which has been hardened and redissolved in turpentine and carefully filtered.

Xylol and Absolute Phenol.—This consists of equal parts of xylol and absolute phenol, and is a most rapid clearing agent. It is easy to use, and has the advantage that it is readily miscible with canada balsam, which should be used dissolved in xylol. Place the fluid in a saucer to about ¼ inch depth, and place the sections in it for about five minutes. They will almost instantly fall to the bottom. Take a clean cover glass, and with a section lifter float the specimen on to the cover glass, and spread it perfectly flat. Drain off the excess fluid on to a piece of blotting-paper by holding the cover glass in forceps and placing its edge upon the blotting-paper. Then lay the cover glass section upwards on the blotting-paper to remove any fluid from the underside, and before the clearing fluid has entirely evaporated from the section apply a drop of balsam dissolved in xylol. The balsam being miscible with the clearing fluid, there is nothing antagonistic or greasy to remove, which is the case when oil or turpentine is employed as a clearing agent. This medium is the best that can

be used for clearing sections cut from specimens which have been embedded in celloidin, upon which it has no solvent effect. It is usual, when material has been embedded in celloidin, after cutting the sections, to retain the film of celloidin surrounding the specimen and mount it with the specimen. Such sections may be stained by all ordinary staining fluids and mounted in balsam, glycerine, glycerine jelly, or Farrants' medium.

When, however, it is not desired to mount the section with its surrounding celloidin, turpentine should be used as a clearing agent, as it is a solvent of both celloidin and paraffin. It should not, however, be used for specimens which have been hardened or preserved in alcohol or methylated spirit, as it has great shrinking power. Turpentine is a good clearing agent for vegetable tissues of a woody nature. All sections cleared by turpentine should be mounted in canada balsam which has been hardened, redissolved in turpentine, and carefully filtered.

Chapter Nine

EMBEDDING AND CUTTING

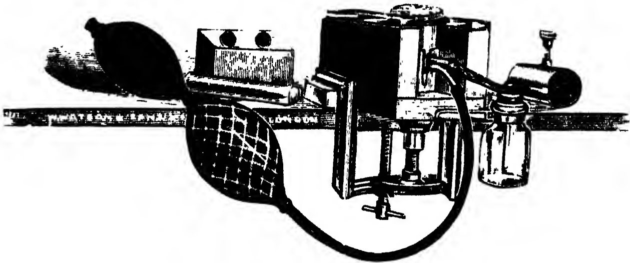
AFTER tissues have been suitably hardened and cleared, they may be cut into sections, and this can be done in several ways. The simplest is to support the material in some substance such as elder pith or carrot, and by means of a sharp knife or razor, cut the thinnest possible section from the material, cutting across both the material and the supporting substance, letting the resultant sections fall into a dish containing some methylated spirit, and then staining them, clearing them, and mounting them as desired. This is a very rough-and-ready way of doing the work, and much more satisfactory sections are obtained by using a microtome. It is always necessary to further support the tissues against being torn by the section knife, and this is usually done in one of three ways:

(1) By freezing in gum mucilage;
(2) By impregnation with paraffin; or
(3) By embedding in celloidin.

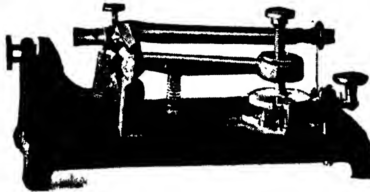
1. By Freezing

Pieces of tissue already hardened should first of all have all the alcohol removed from them by

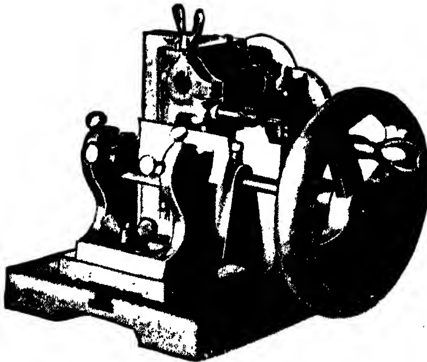
MICROTOMES.



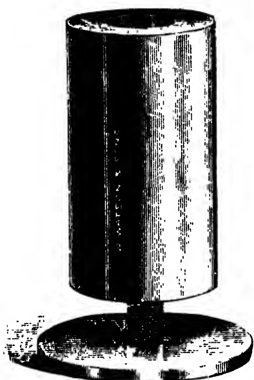
Cathcart Microtome, for Freezing and Embedding.



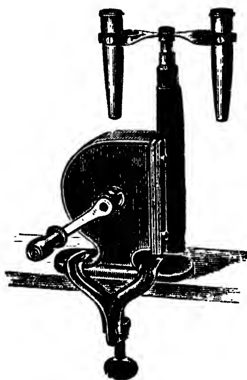
Cambridge Rocking Microtome, for Paraffin or Celloidin Blocks.



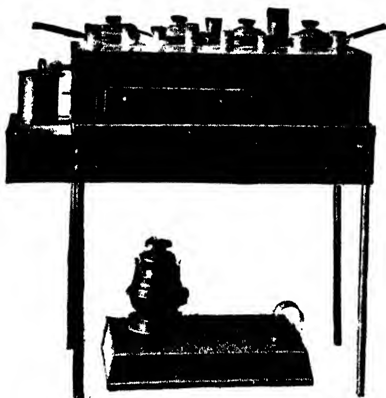
Rotary Microtome, "Minot" Pattern.



Hand Microtome.



Simple Hand Centrifuge.



Simple Embedding Bath, for Paraffin Work.

washing in running water for twelve to twenty-four hours. They should then be placed for about the same period, according to size, in a thick syrupy solution containing 2 parts mucilage acacia and 1 part syrup. If a few drops of carbolic acid are added to the mixture, tissues can be left in it for months.

For cutting frozen sections, a microtome specially made for the purpose, such as the Cathcart-Darlaston, should be employed. Clamp the microtome to the table, fill the bottle with methylated ether or pure ether, and attach the bellows in the place provided. Then cover the top plate of the fitting supplied for it with a coat of the gum and syrup mixture, and transfer the tissue to be cut into the middle of this plate. Work the bellows, and as the mixture freezes add more gum and syrup mixture, and freeze again, and so on until the tissue is well covered and frozen into a solid mass. Then drawing the razor backwards and forwards over the mass, cut off the sections as thinly as possible. If they roll up or fly off, the tissue is either frozen too hard or there is not enough syrup in the gum. This can be easily remedied by allowing the mass to thaw a little, or in the second case, by adding more syrup to the gum mixture, and allowing the tissue to soak again. The sections should be placed in a bowl of water, which should be changed often, so that the gum and syrup may dissolve out. They may

either be stained immediately or stored in methylated spirit.

2. Paraffin Embedding

To embed in paraffin it is necessary to have an oven in which the wax can be kept at a constant temperature just above its melting-point. This oven can either be purchased from laboratory outfitters, or, if a little ingenuity is used, be manufactured by the reader himself. A steamer could be used such as cooks employ, and so arranged that it can be heated by a spirit-lamp or a Bunsen burner from inside the lower half of the steamer, and the top half should be fitted with a flat metal lid, bored with holes to take several test-tubes, and a thermometer. The lid, however, must be soldered on to prevent the escape of water vapour, as on no account must this be allowed to reach the melting wax. The paraffin can be obtained to melt at various temperatures, but that melting at about 54–56° C. is most generally used.

After the material has been thoroughly hardened and dehydrated, it should, as has been previously mentioned, be cleared. When it is to be embedded in paraffin it is advisable to permeate it with some solvent of paraffin, which will expel any dehydrating fluid, and prepare for the entrance of the paraffin. There are several solvents suitable for this purpose: chloroform or

xylol are most generally used. From the absolute alcohol used for hardening and dehydrating the material should be transferred to a mixture of equal parts of absolute alcohol and chloroform, and left in it for twenty-four hours. Then transfer to pure chloroform for a further twenty-four hours, or longer if necessary. At the end of this time the material should sink or float heavily. Then transfer to a mixture of equal parts of chloroform and paraffin, and place on the oven for about twenty-four hours. Then transfer to pure paraffin, using sufficient paraffin to be in considerable excess over the bulk of the tissue to thoroughly expel the chloroform, otherwise the resulting block will not be sufficiently hard. With experience, the persistence of the slightest trace of chloroform can be recognised by the smell.

The material should then be cast in blocks of paraffin, which is carried out by means of two L-shaped metal castings, made for the purpose. These can be obtained from W. Watson & Sons, Baird & Tatlock, and other laboratory outfitters. By laying these two L-shaped pieces together, preferably on a glass plate, a rectangular trough is formed. By means of a pair of forceps, lift the material out of the paraffin, and place it in this trough. Fill up the trough with warm paraffin. The direction in which the sections are to be cut should be noted before the paraffin sets hard.

The glass plate should be put in a very cold place, or cold water may be run over it to hasten the cooling of the paraffin, which should be as rapid as possible. When the block is quite cold and hard, the metal L's should be detached, and the edges of the block can be pared or rounded as desired. The block can then be attached to the holder of the microtome by using a little melted paraffin, and the thinnest possible sections cut from it. When cut, the sections should be floated on the surface of a dish of water, kept at a temperature about 10° C. below the melting-point of the paraffin. The sections must next be fixed to the 3 × 1-inch glass slides, and the simplest way to do this is to take a clean slide and thrust it obliquely in the water containing the sections, and then withdraw it, bringing with it one of the sections to be mounted. With a needle gently arrange the section in the middle of the slide, and draw off the surplus water with filter paper. Then place the slide in some sort of support, section downwards over the paraffin oven, keeping it at a low temperature, and leave for about twelve hours. It will then be sufficiently fixed to the slide and should be able to stand all the necessary handling during the staining and mounting processes. Some sections, however, will be found to stick badly, and this is particularly so in the case of those fixed in alkaline fluids. It is then necessary to coat the 3 × 1-inch slides with a thin layer

of albumen. This can be prepared by mixing the white of a fresh egg with 10 parts of distilled water and filtering, adding a few drops of thymol to keep it fresh. Then dip an absolutely clean slide into the mixture, and draw it over the surface of another clean slide, so that both slides have a very thin film of the albumen mixture. All the slides to be used can be treated in this way, and put aside to dry. They can then be stored in a wide-mouthed bottle until needed. When they are to be used, take out a slide thus prepared, and on it place a small drop of water, and on this the section. Arrange the section in the desired position and place the slide on the warm oven, at a low temperature. This will melt the paraffin, and the excess of water can then be drawn away. To further fix the section to the slide, gently heat over a Bunsen burner or spirit flame for a short time. The slide may then be allowed to become absolutely dry, and can be stored until it is convenient to proceed.

If it is necessary to remove the paraffin from the section so that aqueous and other stains can be used, the slide carrying the section should be placed in xylol and agitated in it for two or three minutes. This dissolves the paraffin. To remove the xylol, place in strong spirit or absolute alcohol, and again agitate the slide. The slide can then be placed in water to remove the spirit and stained as considered advisable. If the

tissue has been hardened in corrosive sublimate, it should be rinsed in iodine solution and again washed before staining. After staining, dehydrate in alcohol, clear in oil of cloves, wash in xylol if aniline stains are used, and mount in xylol balsam.

3. Embedding in Celloidin

Celloidin can be purchased, and is usually supplied either in shreds or in flat slabs. If slabs are bought, they must be cut in small pieces before being used. The celloidin should be made up in two solutions: one a 6 per cent., the other a 3 per cent. These are made by placing about 6 grammes of shredded celloidin in a wide-mouthed bottle and dissolving them in a 40 c.c. absolute ethyl alcohol, adding about 40 c.c. ether some twenty-four hours later. The quantities just given are for the 6 per cent. solution, and it is an easy matter to calculate the amounts necessary for the thin solution. To embed in celloidin, the general principle is the same as for paraffin, the procedure being as follows:

(*a*) Place the material in absolute alcohol and leave it there for twenty-four hours.

(*b*) Then soak in a mixture of equal parts of ether and absolute alcohol for a further twenty-four hours.

(*c*) Transfer to the thin celloidin solution (3 per cent.), and leave for from twenty-four to forty-eight hours.

(*d*) Again transfer to the 6 per cent. celloidin solution, and leave in it several days.

(*e*) Place the specimen on a small block of wood on which a small quantity of thick celloidin has already been placed, and leave exposed to the air for a few minutes. Then pour a little thick celloidin over the specimen, expose to air a few minutes, and so on, until the specimen is well covered. Expose to air, and when a whitish film appears on the surface, place in 60 per cent. alcohol, which hardens the celloidin block. The block can be stored in 60 per cent. alcohol until required for cutting.

Section Cutting

Sections can be cut in a microtome, preferably one in which the knife can be set obliquely, and should be as thin as possible. The microtome knife should be kept moistened with 60 per cent. alcohol while cutting, and the section should be floated into alcohol of the same strength. Sections must never be allowed to dry. Slides coated with albumen as before described should be used, and before being mounted the sections should be soaked in 95 per cent. alcohol for a few minutes. Then float the section off on to the slide, arrange in the desired position, and draw off the surplus fluid with filter paper.

Chapter Ten

STAINING FLUIDS AND STAINING

STAINING is an art requiring much experience and great delicacy of manipulation. A great deal might be written on this subject, but the object of this book is merely to indicate the way to set about this branch of the work and to deal with some of the more common methods. It is one thing to colour a section and quite another to stain, and especially to double stain and differentiate it. In double and triple staining the great desideratum is to secure a thoroughly good nuclear stain and to supplement this with other stains, which will prove good contrasts to the first, differentiating the tissues. The stains most generally in use are: hæmatoxylin, carmine, picro-carmine, and a large number of aniline dyes. The brilliancy of carmine has its advantages in many cases, whilst the delicacy of the beautiful graduation of tints from the deepest violet to the palest blue, which by careful treatment are produced by a good hæmatoxylin, make them very suitable for general work. Attractive stainings can be produced by a prolonged immersion, for say twelve hours, of the section in a solution of hæmatoxylin (see formula below)

which has been diluted to a light violet colour, and then by leaving the sections so stained in tap water for twenty-four hours, which will result in a blue ground-colour with the nuclei of darker blue. If the worker would like to triple stain the section, carry out the staining just described, then place the section in spirit for half an hour, then in a stain made from 2 parts of a saturated alcoholic solution of rubin and 1 part of a saturated alcoholic solution of yellowish eosin diluted with alcohol until it becomes a delicate pink, in which the sections may be kept until they are cleared in xylol-phenol, and mounted in xylol-balsam.

Hæmatoxylin Staining Fluid

Hæmatoxylin crystals	1 grm.
Alum, powdered..	10 grms.
Distilled water	100 c.c.
Alcohol, absolute	5 c.c.
A small piece of camphor.	

This fluid does not become active until from two to three months after it is made, and improves by keeping, remaining perfectly good for two or three years. It must be filtered each time it is used, and may be used over and over again. Its full strength is too strong for use, and for rapid staining should be diluted by adding an equal volume of distilled water to the stain and leaving the tissue in it for five to ten minutes. For slow

staining, dilute until its colour becomes a light violet, and leave the section in the stain thus diluted for six to twelve hours. Overstained sections can be decolorised to any desired tint in distilled water 5 parts, acetic acid 1 part. After staining place in tap water for twenty-four hours, which will turn the dye blue, dehydrate in methylated spirit, clear in clove oil, and mount in xylol balsam.

Ehrlich's Formula

(*a*)	Hæmatoxylin	1½–2 grms.
	Absolute alcohol	100 c.c.
(*b*)	Glycerine	100 c.c.
	Distilled water	100 c.c.
	Glacial acetic acid	5 c.c.
	Ammonia alum, powdered (to saturation).	

Dissolve the hæmatoxylin in the alcohol, and the alum in the water, and mix the two solutions; add the glycerine and glacial acetic acid. Allow to stand for about six weeks, exposed to light, shaking occasionally, and then filter and keep in a stoppered bottle. If the section has been hardened in any of the chromic solutions, place it in a 1 per cent. watery solution of bicarbonate of soda for about five minutes, and then thoroughly wash in distilled water. If, however, it was hardened in spirit, only wash in distilled water, as all sections should be washed in distilled water before they go into a stain.

(*a*) Place the section in the stain, adding a few drops to 1 oz. distilled water, leaving it for some time, up to three hours if necessary.

(*b*) Wash in distilled water, then in ordinary tap water.

(*c*) If necessary decolorise as advised for hæmatoxylin, dehydrate in methylated spirit, clear in clove oil, and mount in xylol balsam.

If its action becomes too rapid or diffuse, add acetic acid. It may be used over and over again.

Weigert's Formula

(*a*)	Hæmatoxylin crystals	1 grm.
	Absolute alcohol	10 c.c.
	Distilled water	100 c.c.
(*b*)	Potassium ferricyanide	2½ grms.
	Borax (powdered)	2 grms.
	Distilled water	100 c.c.

Make up these two solutions and keep them separately. First place sections in absolute alcohol and then in solution (*a*) until they become quite black, then place them in distilled water for a few minutes, decolorise in solution (*b*) until they are a dark bluish brown. Wash in distilled water, dehydrate in methylated spirit, clear in oil of Bergamot, and mount in xylol balsam.

Double Staining in Hæmatoxylin and Eosin

Stain in hæmatoxylin as directed before, and after washing in tap water, leave in spirit for half

an hour, place the section in an alcoholic solution of eosin—·06 grm. eosin dissolved in 28 c.c. methylated spirit—and let it stay there for about five minutes; wash well in methylated spirit, clear in xylol-phenol, and mount in xylol-balsam.

Carmine and Picric Carmine

These also are invaluable nuclear stains, whilst for many tissues, particularly vegetable, they are preferable to hæmatoxylin.

Grenacher's Alcoholic Borax Carmine

Carmine	3 grms.
Borax	4 grms.
Alcohol (70 per cent.)	100 c.c.

Dissolve the borax in water, add the carmine, and heat with constant stirring until the liquid nearly boils. Set aside to cool, and when cold add 100 c.c. of 70 per cent. alcohol, shake thoroughly, let it stand for from thirty-six to forty-eight hours, filter, and keep in a stoppered bottle. When using this stain, also make up a solution of 5 parts methylated spirit mixed well with hydrochloric acid 1 part.

To stain section, wash it in distilled water, and place in a little carmine for four or five minutes. Wash it in methylated spirit, and then soak in the hydrochloric acid solution just mentioned for from five to ten minutes, or, if section is over-

stained, until the excess has been removed. Wash the section in methylated spirit to remove all traces of the acid, and place it in a fresh supply of strong methylated spirit to dehydrate it from ten to fifteen minutes. Clear in clove oil, and mount in xylol balsam.

Picric-Carmine Solution

Take of carmine and of picric acid equal parts by weight. Dissolve the picric acid in 100 times its weight of distilled water. Heat may be used if necessary. Dissolve the carmine in 50 times its own weight in liquid ammonia fort. Mix the two solutions, filter thoroughly, evaporate the filtered liquid to dryness, and dissolve the filtrate in 100 times its weight of distilled water. Filter until a perfectly clear solution results. Then add to each 100 c.c. of the solution 25 c.c. glycerine and 10 c.c. absolute alcohol. This will keep perfectly well, but should be filtered at intervals of a few months. Wash the section in distilled water and lay it out flat on a clean 3 × 1-inch slide. Drain off surplus water, pour on the section several drops of the picric-carmine solution, and warm the slide over a lamp flame to a heat that can be borne by the hand. Keep it at this temperature for about ten minutes, remove excess of stain, add a few drops of Farrants' medium, and apply a cover glass. This is an effective double stain, the nuclei taking a red stain and the remainder of

the tissue yellow. Tissues stained with picric-carmine may be mounted in xylol balsam, but Farrants' medium is better.

Heidenhain's Iron-Hæmatoxylin

This stain is especially suitable for staining the chromosomes in root tips and similar sections showing mitotic structure, and for staining the nuclei in the protozoa.

(*a*) Make a dilute solution of iron alum, about 3·5 per cent., in distilled water.

(*b*) Make a 0·5 per cent. solution of hæmatoxylin in distilled water. Allow to ripen by standing it in sunlight for some weeks, until the reddish solution becomes deep drown. Shake occasionally.

(*c*) Place the slide containing the section or protozoal film in the iron-alum solution, and leave for from five to ten hours.

Note.—Slides should have been washed in distilled water before being placed in the iron alum.

(*d*) Rinse in distilled water.

(*e*) Place in hæmatoxylin solution for from five to ten hours, leaving it therein until the preparation is black.

(*f*) Rinse in distilled water.

(*g*) Wash out the surplus stain by leaving the slide in the iron-alum solution, which can be

diluted if necessary, examining the preparation under the microscope from time to time to determine when the requisite depth of staining has been reached.

(*h*) Rinse in distilled water, and then in running tap water for at least half an hour.

(*i*) In the case of films, dry in air, apply a drop of Gurr's neutral medium or balsam, and finish in the usual manner. In the case of sections, dehydate in methylated spirit, clear in clove oil, and mount in canada balsam.

Aniline-Blue

Take some soluble aniline-blue, and make a 1 per cent. solution in distilled water and filter.

(*a*) Place the section in the stain for about ten minutes, according to the depth of colour required.

(*b*) Wash in distilled water.

(*c*) Place in methylated spirit, to remove surplus stain, if necessary.

(*d*) Clear in clove oil or xylol-phenol.

(*e*) Mount in canada balsam.

A very great deal more could be written on the subject of stains and staining, but enough has been said in the foregoing to give some idea of the methods employed.

There are a number of very good and compre-

hensive books, written for the student of histology, and anyone desiring further information is advised to consult them.

To Stain and Mount Sections of Worms

Such specimens as Acaris, Lumbricus, Tænia, etc., can be first hardened in alcohol for about ten days, and then should be cut up into pieces about ¼ inch long. They should be soaked in equal parts of alcohol and ether for twelve hours, and then transferred to a thin celloidin solution (see p. 96); they must remain in this until perfectly infiltrated. Again transfer them to a thick solution of celloidin, and leave them to soak for a further twelve hours. Remove from the celloidin on the point of a needle, and hold exposed to the air for a few minutes so that the surface of the celloidin may harden. Then place the specimen in methylated spirit, and this will have the effect of thoroughly hardening the interior of the celloidin. Place in a microtome and cut thin sections, and stain in borax carmine for five minutes. Wash in methylated spirit, and then place in acidulated spirit—1 part hydrochloric acid in 5 parts methylated spirit—for about three minutes to remove excess of stain, and then wash again in ordinary methylated spirit to remove the acid; place for one or two minutes in absolute alcohol, clear in oil of cloves, and mount in canada balsam.

To Mount Flukes and Segments of Worms Whole

Heads of tape-worms, flukes, and segments of tape-worms can all be mounted whole, by being allowed to harden in methylated spirit for a few days, and then stained for from one to twenty-four hours in borax carmine. Wash in methylated spirit, soak in acidulated spirit to remove excess of stain, and then place for a short time in water to soften the tissues. Place the specimen on a slide and put another slide on top; press down and tie the two together. Place in a jar of methylated spirit, and soak for at least twenty-four hours. Then untie, separate the slides, and place the specimen in alcohol for ten minutes to dehydrate. Clear in clove oil for one hour, and mount in canada balsam.

Trichina Spiralis

Take a piece of muscle containing the Trichina, and place it in methylated spirit to harden. Then embed in celloidin, cut the requisite sections, stain in borax carmine, pass through acidulated spirit, wash in water, and mount in glycerine jelly.

It is also possible to isolate these worms from the surrounding muscle, and this is done by placing the material on a glass slide, and with the aid of a dissecting needle, under a microscope or pocket magnifier, separate the capsule containing

the worm from the surrounding tissuc. Place it in some dilute hydrochloric acid, which dissolves the capsule and sets the worm free. Wash in water, and mount in canada balsam or, better still, in glycerine jelly.

Chapter Eleven

PREPARATION AND MOUNTING OF VEGETABLE TISSUES

IT is advisable, whenever possible, to use the freshest material obtainable. Material such as stems, leaves, ovaries, roots, etc., must be hardened before being cut, and this should be done by leaving them in spirit for a week or two, changing the spirit every twenty-four hours for the first three days. There are other fixing fluids, such as: Bouin's Fluid (see p. 79) for nuclear work, or Carnoy's Fluid (see p. 81) for similar material; a 2–4 per cent. solution of formaldehyde (see p. 81) for Algæ and Fungi; and chromo-acetic acid (2 grammes chromic acid, 3 c.c. glacial acetic acid, and 300 c.c. distilled water) for cell contents; but a solution of 90 per cent. alcohol forms a convenient fixing agent for general work.

After the material has been hardened and washed, it may be stored in a mixture of equal parts of alcohol, glycerine, and water. When, however, material is to be embedded in either paraffin wax or celloidin, it is necessary to dehydrate it thoroughly after it has been in the above-mentioned mixture, so that all traces of the glycerine may be removed. Nearly all vegetable

material needs to be bleached before being stained. In the case of delicate tissues, this can be done by leaving them immersed in alcohol, but hard and deeply coloured stems and woods require something more effectual. To 1 pint of water add 2 ozs. fresh chloride of lime, and after shaking thoroughly, leave it to stand until the chloride of lime settles. Make a saturated solution of common washing soda, pour off the clear fluid from the chloride of lime, and by degrees add it to the soda solution, shake well, and wait until all precipitation ceases. Filter the solution, and keep in a stoppered bottle in a dark place. No fixed time can be given for the bleaching process, the colour and density of the tissues being so variable. The specimens to be bleached must be thoroughly washed with distilled water, and then placed in the bleaching fluid for from ten to twelve hours. Wash material well in water, and be sure that all traces of soda have been removed before being infiltrated, otherwise they will fall to pieces during subsequent handling.

Vegetable material can sometimes be cut to the requisite thinness by hand, but it is more general to embed it in some supporting material and place in a microtome, by means of which instrument thin, even sections can be cut. For simple work, such as stem and root sections, the material may be embedded in carrot or elder pith, placed in the well of a Cathcart-Darlaston

microtome, and cut into suitable sections. It is better, however, to infiltrate with either paraffin wax or celloidin, and this is carried out in exactly the manner described for animal tissues. Flower and leaf buds should be tied before being infiltrated, otherwise they will fall to pieces during subsequent handling. When the sections have been cut, soak them in water, to be changed several times, for twenty-four hours. Then for twelve hours place them in the following:

(1) ***Mordant Solution.***

(*a*)	Sulphate of alumina	10 grms.
	Dissolved in distilled water	200 c.c.
(*b*)	Acetate of lead	30 grms.
	Dissolved in distilled water	600 c.c.

Add (*a*) to (*b*) until the precipitate ceases, and allow the whole to settle. Siphon off the clear liquid, filter it, and store in a stoppered bottle. When required for use, dilute a portion with 4 equivalents of water and filter afresh.

(2) Stain in borax-carmine solution for one or two hours.

(3) Wash in acidulated water, 1 drachm nitric acid to 1 pint water (= 1 in 160).

(4) Wash rapidly in pure water.

(5) Place in alcohol for one hour.

(6) Place in green stain for one to three hours.

Acid green	2 grms.
Distilled water	75 c.c.
Glycerine	28 c.c.

Mix glycerine and water well together, and dissolve the green crystals in the mixture, filter, and keep in a stoppered bottle.

(7) Wash in absolute alcohol.

(8) Clear in xylol-phenol.

(9) Mount in xylol-balsam.

Picric Carmine Double-staining

Carmine	2 grms.
Liquid ammonia	½ drachm
Distilled water	28 c.c.

Dissolve the carmine in the ammonia by means of gentle heat, add the water.

Picric acid	8 grms.
Alcohol	28 c.c.

Dissolve the picric acid in the alcohol by means of gentle heat, and mix with the carmine solution.

(1) Place section in alcohol for one hour.

(2) Immerse in recently filtered staining solution, for from half to three hours, according to depth of stain required.

(3) Wash in alcohol.

(4) Immerse in an alcoholic solution of picrate of ammonia for one hour.

(5) Take out of this solution, and immerse in a similar solution for another hour.

(6) Place in alcohol for a few minutes.

(7) Clear in xylol-phenol.

(8) Mount in xylol-balsam.

Another Process

(1) Place the section in an alcoholic solution of iodine green (3 grains to 28 c.c. alcohol) for one or two hours.

(2) Soak in alcohol for ten minutes.

(3) Remove to water for one minute.

(4) Immerse for two hours in carmine fluid made as follows:

Carmine	1 grm.
Ammonia (·880)	1 c.c.
Distilled water	100 c.c.

Dissolve the carmine in the ammonia by means of gentle heat, add the water and filter.

(5) Wash thoroughly in distilled water.

(6) Place in alcohol for ten minutes.

(7) Clear in xylol.

(8) Mount in xylol balsam.

Double-staining Method

(*a*) Having cut the required sections, wash them in water, and put them into safranin which has been dissolved in spirit, for about five minutes.

(*b*) Wash well in water.

(*c*) Wash well in spirit.

(*d*) Put into absolute alcohol for a few minutes.

(*e*) Place the section on a slide which has been prepared to receive it.

(*f*) Cover the specimen on the slide with a

solution of light green in clove oil, and leave for from five to ten minutes.

(*g*) Wash off the clove oil with xylol.

(*h*) Hold the slide over a dark surface, and examine to see whether it is transparent.

(*g*) Take a clean cover slip, and drop a small spot of canada balsam on it.

(*h*) Lower it, balsamed side downwards, on to the section.

(*i*) Press down gently, and apply a little heat to spread the balsam.

(*j*) Set aside to dry and finish in the usual manner.

Note.—Instead of safranin, methylene blue can be used, and instead of green, erythrosin.

Staining Cuticles and Leaf Hairs

Take a small piece of the leaf or article to be mounted, and bleach in chlorinated soda in the manner before described. Wash thoroughly in water, and stain in a 2 per cent. aqueous solution of soluble aniline blue. Leave in the stain for about twelve hours, then wash well in water, and then leave soaking in methylated spirit until nearly all the colour is removed. Clear in clove oil for several hours and mount in balsam.

Note.—If the specimen is not fresh, but has been preserved in spirit, it must be very well washed in water to remove the spirit before being bleached.

Staining and Mounting Algæ in Balsam

Mounts of any of the filamentous algæ, such as Vaucheria, Spirogyra, etc., can be made as follows:

(*a*) Fix the material in 10 per cent. formaldehyde for some hours.

(*b*) Wash thoroughly in distilled water.

(*c*) Stain in Delafield's hæmatoxylin, diluted with 10 times its bulk in 30 per cent. alcohol.

(*d*) Wash in 40 per cent. alcohol, and take the material up through the various grades until it reaches 75 per cent. alcohol.

(*e*) Counter-stain in eosin, diluted with 75 per cent. alcohol.

(*f*) Dehydrate in several grades of alcohol.

(*g*) Clear in xylol.

(*h*) Mount in xylol balsam.

Mounting Pollen

Place the anthers containing the pollen in some small receptacle such as a pill-box, and put aside until thoroughly dry. Shake well so that the pollen is set free, and by means of a pair of forceps remove the unwanted anthers. Place the pollen in a bottle or test-tube containing turpentine, and leave it there for several days to remove all trace of air. Put a small drop of canada balsam on a clean cover glass, and after pouring off the turpentine from the tube containing the pollen,

place a small quantity of the pollen in the canada balsam. Spread the pollen over fairly evenly with a needle, and put the cover away until the balsam has set hard. Take a clean 3 × 1-inch slide, warm it over a flame, and place on it a small quantity of fresh balsam. Warm until fairly fluid, and lower the cover glass containing the pollen balsam side downwards into the warm balsam. Press together with a pair of forceps, and put aside to dry. When dry, ring and finish in the usual manner.

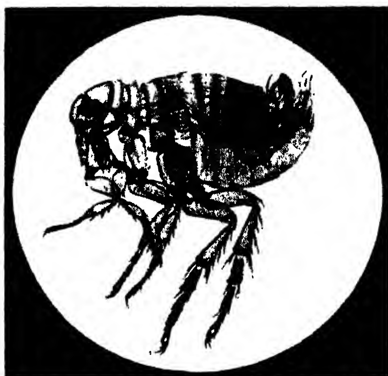
Small seeds, spores, and powdered drugs and other specimens of a similar nature may be mounted in the manner just described.

Cellosolve (described by Mr. H. F. Frost. Watson's Microscope Record. No. 34)

A reagent which has only recently been used in microscopical technique is ethylene glycol monoethyl ether, sold commercially as cellosolve. This fluid can be used in the place of alcohol as a dehydrating agent. It is a solvent for water, alcohol and xylol, and specimens can be taken direct from the stain, whether single or double, placed in cellosolve for half a minute, and mounted in canada balsam in xylol. Fresh material may be completely dehydrated by immersion for about one minute in cellosolve, and can then be placed in an alcoholic stain, cleared in clove oil, and mounted in balsam. Cellosolve

Fresh-water Polyzoa, *Cristatella mucedo* Mounted in Fluid.
Photographed with dark ground illumination.

Crystals of Salicine, Photographed by Polarised Light.



HUMAN FLEA, *PULEX IRRITANS*.

SECTION THROUGH BASE OF LEAF STEM OF VIRGINIA CREEPER, SHOWING DEFOLIATION.

water. The jelly, in an airtight jar, should be kept in a cool place until required.

There are two ways of mounting in glycerine jelly—it may be liquefied by placing the jar containing the jelly in hot water and then dropping the liquid jelly on the slide, or a small piece of solid jelly may be cut from the mass in the bottle and put on the specimen, which should have already been placed on the slide. The slide should be warmed gently from underneath, when the jelly will diffuse itself through the object. If the first method is to be used, warm the slide gently, take up a little liquid jelly in a dipping tube, and place it in the centre of the slide. Take up the specimen to be mounted and, avoiding making air bubbles, place it in the jelly. Take a clean cover glass, and place it on the surface of the jelly, pushing it down with the point of a needle, until it is quite flat, and set aside to cool. Remove any excess of jelly round the cover glass by washing carefully in water, dry, and ring first with two or three layers of gum dammar, and then add several coats of cement and varnish.

Should the second method, however, be decided on, some sort of clip should be used to hold the cover glass in position when heating over the flame. When heating, the jelly will first begin to bubble from the centre outwards, and if the slide be carefully watched, a very perceptible crack

may be seen and heard. At this moment without any delay the slide must bc withdrawn from the heat and placed on a cold surface (an iron slab, for instance), when the jelly will set rapidly. The slide must then be washed, and sealed as before described.

Glycerine jelly is a very suitable mounting medium for a large number of specimens. Corallines whose tissues, hard and opaque, may be cleaned by soaking for a short time in a weak solution of hydrochloric acid and washed well in water, marine algæ which should be washed well in fresh water to remove the salt prior to mounting, volvox, desmids, moss plants, mycetozoa, spiral and scalariferous vessels, starches, raphides, and many such which there is not space to mention.

Glycerine, Pure and Undiluted

This forms an admirable medium in which to mount many specimens, but the hermetical sealing of such mounts presents difficulties. Place the slide on to a turntable, and run on it a ring of cement, gold-size, Bell's cement, or some such suitable varnish. The ring should of course be the same size as the cover glass which is to be used. When the ring is half dry but still tacky, place the specimen on the centre of the ring, and on it put a drop of glycerine. Then lower the cover glass in the usual way, and gently press the

edge of the cover all round, so as to make it adhere to the varnish. Should it not do so at any point, gently warm the varnish by holding the slide over a flame of a lamp, and then press the cover glass home. The slide can now be dipped in water, and any glycerine which has overflowed or been pressed out can be washed off with a camel-hair brush. It can then be ringed with two or three layers of gum dammar, and when dry with a shellac varnish. The depth of the shellac ring made must of course depend on the depth of the specimen, and the mounter's own judgment must be exercised to determine this. If the specimen is deep, a suitable cell must be built up with alternate layers of cements as described on page 16, allowing each ring to dry before applying the next. If the specimen is still too deep for this method, a specially made cell must be constructed. Aluminium rings of varying diameters and thicknesses can be obtained from any laboratory outfitters at a small cost. A 3 × 1-inch glass slide must be thoroughly cleaned and a ring of some good cement placed in the centre of it by means of a turntable, set aside, and left to dry until tacky. Rub the aluminium ring down carefully on each surface to obliterate any roughness by using a fine glass or emery paper, and then place the ring on top of the tacky cement ring on the slide. Press firmly down all round, and put aside to thoroughly harden. When

in the centre, fill the space with pure glycerine and put a layer of cement on the top surface of the metal ring. When just tacky lay the cover glass on the top, pressing down so that it is firmly in contact with the ring. Put aside to thoroughly dry. When dry, clean off excess of glycerine, and finish with black asphalt varnish (see also chapter on Insect Mounting).

It must be remembered that *all* tissues, sections, or specimens to be mounted in pure glycerine should be subjected to a prolonged immersion in glycerine and distilled water, the proportion of the glycerine to that of water being gradually increased every day or two until pure has been substituted for diluted glycerine. Great care should be taken that the specimen is thoroughly saturated with the glycerine before being mounted therein. The soaking may be done either in watch glasses or very small staining saucers, and many specimens or sections can thus be immersed together. In order to prevent the glycerine from absorbing moisture from the atmosphere, and to ensure cleanliness, the saucers should be placed under bell jars while the soaking is in progress. If not previously saturated in glycerine, specimens mounted therein often shrink seriously. Tissues, sections, delicate marine animals, insects, etc., which are to be mounted in glycerine may be preserved therein until required. Glycerine affects logwood stain-

ings adversely, but if slightly acidulated with acetic acid, it does not attack carmine or picric carmine.

Mounting Volvox Globator in Glycerine (Dr. T. Stephanides' Method)

The volvox should be passed through varying strengths and finally into pure glycerine; 10 per cent., 25 per cent., 50 per cent., and 75 per cent. are sufficient to prevent distortion, provided that the material is left at least twelve hours in each change. To make a permanent mount, place a drop of the pure glycerine containing the volvox on a slide, and apply the cover glass, which can if necessary be supported on a cement cell as already described, or on three equidistant fragments of fairly thick cover glass (see chapter on Insect Mounting) so as not to crush the material. The size of the drop should be such that a comparatively large margin is left all round when the cover glass is in position. With the aid of gentle heat, melted paraffin can be run into this space and left to harden. When ready, finally seal with good varnish.

Glycerine 20 per cent. diluted with 5 per cent. formalin is frequently used as a medium for mounting the fresh-water polyzoa, such as *Lophopus* and *Cristatella*, the *Marine Bryozoa, Hydrozoa*, and a very large number of similar specimens, and preserves them in a marvel-

lous manner. Such specimens should be killed in such a manner that their tentacles are fully extended. The material after being gathered should be emptied into a dish of clean water, the volume of the water being very greatly in excess of the bulk of the material. Then lift out the specimens to be mounted and transfer to a saucer or shallow dish containing clean water. In order to fix the creatures in such a way that the tentacles are extended in a life-like manner, it is necessary to narcotise them before killing them. Several substances are used for this purpose, one very generally used being a 2 per cent. solution of hydrochlorate of cocaine, 3 parts of which should be put in 1 part alcohol or methylated spirit and 6 parts water. Some people use a 1 per cent. watery solution of hydrochloride of eucaine (specially recommended by Mr. G. T. Harris). A few drops of the narcotising fluid should be put into the saucer containing the organism. If on being gently touched on the end with a pipette or a needle the tentacles do not contract or show any movement, the narcotic has been effective, and the tentacles will remain permanently extended. The organism must then be killed and fixed, and this is best done by very gently flooding them with a quantity of 20 per cent. formaldehyde, leaving them in this for about a quarter of an hour. They must then be lifted out of the fluid and transferred to a saucer of clean water,

so that the fixing fluid is washed out. They can then be either mounted or stored in a mixture of 5 per cent. formalin in 20 per cent. glycerine. They should be mounted in a deep cell in glycerine or a mixture of formalin and glycerine, in the manner already described.

The narcotising fluid should be used in very small quantities at first to accustom the creature to the influence of the narcotic, the quantity being increased at intervals.

Narcotising (Mr. E. R. Newmarch's Method) Menthol Crystals

A very small quantity will last for a very long time if kept in a well-stoppered bottle. *Cristatella mucedo*, when found, is generally in large colonies, and it is advisable to cut off the pieces to be mounted immediately upon gathering it. *Lophopus crystallinus*, *Plumatella*, *Fredericella*, and other Polyzoa are most often found in smaller pieces, and should be collected while still contracted. Place each specimen in a shallow dish of clean water by the aid of a section lifter, making sure, in the case of *Cristatella*, that it is resting on the natural foot. Allow the specimens to remain undisturbed in the water for half an hour or so, and then take up a few crystals of menthol on the point of a knife, sprinkle them on the surface of the water, and then cover the dish with a piece of glass to keep the narcotising fumes in. An

improvement on this method is to moisten the piece of glass, and to spread the crystals thereon, instead of on the water, and this keeps the surface of the water clean. Allow them to remain in the menthol for from eight to ten hours, and then kill them with from about 10 per cent. to 20 per cent. formaldehyde. It is advisable to make sure first that they will not contract by touching with the point of a needle as before mentioned. The killing and fixing is best effected by gently flooding the specimens with a pipette with as little disturbance as possible, taking care that the point of the pipette is put under the surface of the water. Allow them to stay in this for about ten minutes, and then transfer each specimen separately into a dish of clean water. In doing this take care not to break or injure the specimen, as during the foregoing process the creature will have attached itself to the bottom of the dish by foot suction. Change the water two or three times, and then they can be placed in about 20 per cent. formaldehyde for from about twenty-four hours to harden. Then transfer them into a mixture of 5 per cent. formalin and 20 per cent. glycerine. The specimens will at first float on the surface, but if left in the fluid for about six to eight hours they will sink to the bottom. For greater convenience the specimens can be transferred, each individual creature being placed in a separate test-tube, with just sufficient to cover

them, keeping the tubes upright. They can remain in these tubes indefinitely until wanted for mounting.

Just before mounting they should be allowed to float about in a vessel, containing the glycerine and formalin mixture, and one which has a large surface area, for about a week, covered over to keep out the dust. They will become very transparent and retain this transparency for many years.

Mounting

Take a thoroughly clean 3 × 1-inch slide of the type which can be purchased with an excavation in the centre, and round this excavation run a light ring of cement, and put it aside until it becomes tacky. Then fill the cavity with the glycerine and formalin mixture, and with a section lifter take up the specimen to be mounted, and gently place it in the cell, adding a drop of the mixture on the top to thoroughly immerse it. Take a clean cover glass, hold with forceps, and carefully place on the top of the cell. A valuable hint given by Mr. Newmarch, who was the first to make use of it, that in order to counteract the tremendous and inevitable rush of fluid, with the consequent risk of damaging and altogether losing the specimen, is, instead of placing the cover on the cell edge direct, touch the centre of the globule of fluid with the edge of the cover glass,

and when the surface film is broken, gently raise it so far as the elasticity of the film will allow, sliding it (still in contact) towards the cell edge, and then lower in the usual way. Very gently press the cover down until it adheres to the cement, and apply a wire spring clip to keep the cover in position. Drain off surplus fluid, wash the slide carefully to remove the glycerine, and without removing the clip give a first ringing coat of cement. In about half an hour remove the clip, and give the slide a second ringing with cement. Set aside to dry, then ring again, and afterwards finish in the usual manner. It is important to remove all traces of the glycerine, as it will render ringing difficult and unsatisfactory.

Chapter Thirteen

OPAQUE MOUNTING

TO obtain permanent and satisfactory mounts, two things are of paramount importance: (*a*) that the specimen be perfectly clean, all dirt and other foreign matter being eliminated as much as is possible; and (*b*) it is absolutely necessary that the specimen be perfectly dry, as objects mounted in a damp state often become covered in a few months with a growth of fungi, or the cover glass becomes obscured to such an extent that the object is seen as through a fog. Where the object is not naturally dry or where it is impossible to apply heat for drying purposes, it is advisable to make use of a desiccator. This consists of a special type of glass jar, containing chloride of calcium, carbonate of potash, or some other moisture-absorbing substance in the bottom half, with a space above in which the specimens requiring desiccation can be placed and can remain for a period. Such a desiccator can be purchased from a laboratory outfitter, or could be constructed by the reader, using a dish to contain the desiccating agent, above which must be placed a shelf to hold the specimens, the whole being covered with a bell jar, care being taken to ensure

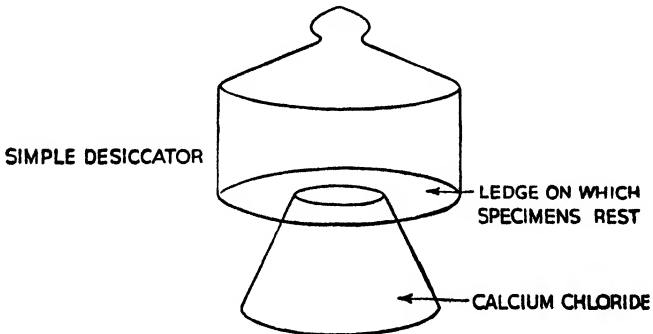
that no air can enter under the rim of the jar. When ready for mounting proceed as follows:

Using a turntable, make a disc in the centre of a 3 × 1-inch slide of some black varnish, and put it aside to dry. From a piece of dead-black paper cut out a disc exactly the same size as the one on the slide, and attach it to the slide inside the painted disc, using a little gum. Take an aluminium cell ring of the same size, grind down each edge, and with gold size, Bell's cement, or any other equally good cement, fasten it to the slide exactly surrounding the paper disc. When such cells are being made, it is a good plan to do a quantity at once, as by the time several have been done the hand acquires greater skill and the work becomes easier. Sometimes a mounter desires to mount some of his specimens in balsam, but wants at the same time to have a black background.

It is obvious that the paper disc mentioned in the foregoing would be spoilt if balsam were used directly on it, so the following is the method employed:

Using a turntable, make a disc of black varnish in the centre of a 3 × 1-inch slide, and put aside to dry. When thoroughly dry, make a ring of gum dammar in benzole round the disc. Let that dry, and then cover the black ground with a heated cover glass which will soften the dammar and adhere. Ring the edge of the cover with alcoholic shellac (brown cement), and let it dry.

Give another coat in a week's time and a thicker one a week later. When that is dry, objects may be mounted on the cover glass in balsam which, however, must be allowed to harden without the application of any heat. When finishing, scrape the exuded balsam, ring first with dammar in benzole, and finally with black asphalt varnish. When quite dry, put a very small quantity of gum



in the exact centre of the paper disc, and place the specimen in it, taking great care not to extend the gum beyond the object, otherwise the appearance of the slide will be spoilt. When the gum is quite dry, put a layer of cement round the edge of the metal ring, and when tacky lower a clean cover glass on to it, pressing gently but firmly down until it adheres all round. When quite dry finish with several coats of black varnish.

A very large number of specimens can be seen at great advantage mounted in this way, and

when viewed under the microscope with a low-power objective are very striking. To mention a few: small whole insects, particularly diamond beetles, insect eggs, seeds, crushed precious stones and minerals, foraminifera, polycystina, and other similar subjects are eminently suitable.

Chapter Fourteen

FORMULÆ

THE following formulæ are given so that the reader can, should he so desire, make up the media to be used, for himself. If only small quantities are wanted, it is cheaper to purchase them ready made, but if the media are to be used extensively, it is better to make them in quantity.

Liquor Potassæ Solution

Caustic potash	50 grms.
Water	1 litre

Caustic soda may be substituted for the potash, for nearly all purposes.

Iodine Solution

Iodine	3 grms.
Iodide of potassium	4 grms.
Water	0·56 litre

Dissolve the two substances in 4 ozs. water, and then add the remainder of the water.

Gum Water

Gum arabic	113 grms.
Glycerine	7 grms.
Weak carbolic-acid water	113 c.c.

Allow to stand in a cold place until dissolved. This will be found an excellent medium for attaching labels to glass.

Glycerine and Acetic Acid

Glycerine	50 c.c.
Glacial acetic acid	15 c.c.

Glycerine and Gum (Farrants' Medium)

Glycerine	100 c.c.
Arsenious acid	1 grm.
Distilled water	200 c.c.
Gum arabic (or gum acacia)	130 grms.

Dissolve the arsenious acid in the distilled water, and in this mixture dissolve the gum arabic at ordinary room temperature, frequently stirring. Add the glycerine and mix well. Filter. This reagent is recommended, because tissues mounted in it preserve their natural appearance, and also because as it dries at the edges it fixes the cover glass in position, and is therefore easier to seal than is a glycerine mount. Tissues may be mounted in this direct from water, but even so, it is better to first soak them for a short time before mounting in dilute glycerine. If it is desired not to use arsenious acid, chloroform water may be used instead, and the gum arabic should be dissolved in it.

Deane's Medium

Nelson's gelatine	28 grms.
Honey	141·5 grms.
Creosote	6 drops
Alcohol or spirit of wine	15 c.c.
Water	140 c.c.

Soak the gelatine in 110 c.c. of the water for twelve hours until soft. Heat the honey to nearly boiling-point in a separate vessel, add it to the gelatine mixture, and boil the whole together. When partly cooled, add the spirit and remainder of the water in which the creosote has been dissolved. Allow to cool, and filter through a fine funnel.

Deane's medium can be used in place of glycerine jelly, and must be warmed when it is to be used.

Glycerine Jelly.—For formula see p. 118.

Mounting Fluid for Algæ, of which it preserves the colour, and for fresh gatherings of the diatomaceæ and desmidiaceæ.

Acetate of copper	1 grm.
Camphor water	110 c.c.
Distilled water	110 c.c.
Glacial acetic acid	1·2 c.c.

Mix all together and add glycerine 250 c.c., mercuric chloride 0·65 grm. Mix thoroughly and filter very carefully.

Gold Size

Linseed oil	140 c.c.
Redlead..	28 grms.
Powdered whitelead	28 grms. } Use quantity as needed
Yellow ochre	28 grms. } Use quantity as needed

Boil the oil and redlead together for about three hours, taking care it does not burn or boil over; pour off the clear fluid, and boil again with a mixture of equal parts of the whitelead and yellow ochre, added in small successive portions; pour off clear fluid, and bottle it for use.

Black Varnish (Davies)

Indiarubber shreds	30 grms.
Egyptum asphaltum	110 c.c.
Solvent naphtha (mineral)	300 c.c.

Dissolve the indiarubber in the naphtha, add the asphaltum, using heat if necessary.

Brown Varnish

Indiarubber shreds	20 grms.
Bisulphide of carbon	quantity as required
Shellac	55 grms.
Methylated spirit	225 c.c.

Dissolve the indiarubber in the smallest possible quantity of carbon bisulphide, and to this add the methylated spirit in such a manner that the whole is mixed without the formation of clots. Then add the shellac, and place the jar containing

the mixture in boiling water until the whole of the shellac is dissolved and the smell of the bisulphide has disappeared.

Black Varnish

Gum dammar	25 c.c.
Gum mastic	25 c.c.
Benzole	170 c.c.
Lamp-black as required.	

Mix together. If the benzole evaporates, the mixture can be used simply by moistening with benzole.

Black Varnish

"Robbialac" cellulose enamel forms a very good ringing varnish. It is obtainable from all motor or cycle accessory stores.

Naylor's Brushing Belco, a cellulose varnish, is another product, sold commercially, which is very suitable for ringing. It does not run into or dissolve in immersion oil.

Cement

Resin	70 c.c.
Beeswax..	14 grms.
Canada balsam	3·5 c.c.

Dissolve with heat and mix. When wanted for use it must also be rendered mouldable by the action of heat. It is a strong cement, and may be used for fixing cells to glass.

Shellac Cement or Varnish

Break shellac into very small flakes, and put these into a bottle of methylated spirit. Shake it up frequently until there results a solution sufficiently thick to be used easily with the brush. If it should become thick with keeping, add more spirit.

Marine Glue (*Caoutchouc Cement*)

Indiarubber shreds	55 grms.
Shellac	55 c.c.

Dissolve the indiarubber in solvent mineral naphtha, add the shellac in powder and heat until liquefied, well mixing the whole together. It produces solid marine glue. Marine glue dissolved in benzole forms a very good sealing varnish, is a safe material to use, and quite free from any tendency to run in, which is sometimes a failing of gold size. When quite dry it may be covered over for finishing with black varnish.

White Zinc Cement (*Walmsley*)

Obtain a large tube of artist's zinc white, and empty it in a large bottle. Remove as much of the oil as possible by adding a considerable quantity of benzole, shaking well, and leaving to settle. When the zinc oxide has settled in the bottom of the bottle pour off the fluid. In another bottle make

a saturated solution of gum dammar in benzole, and heat it in a water bath until the gum is dissolved. When this is accomplished pour the gum solution upon the zinc white, shake the mixture thoroughly, and strain through fine muslin. Then add about ½ drachm gold size to each 1 oz. of the solution. If too thin, allow the benzole to evaporate until the cement becomes of a consistency to flow smoothly and readily from the brush, and if too thick add benzole. This cement should be kept in a wide-mouthed stoppered bottle, and stirred, not merely shaken, each time it is used.

Euparal (*R.I.* 1·438)

This medium can be used for mounting instead of canada balsam, and owing to its neutrality has little, if any, effect on stain. Euparal essence can be used with advantage as an accessory clearing agent. The formula for Euparal is privately owned, and this medium must be purchased ready made up.

Yucatan Elemi

Dissolved in xylol, is sometimes used to replace canada balsam, and is said to be less acid and less destructive to stains. It is generally purchased dry and dissolved as required by the user.

WEIGHTS, MEASURES, AND CONVERSION TABLES

AVOIRDUPOIS WEIGHT

1 dram	= 0·0625 ounce	=	1·772 grammes.
16 drams	= 1 ounce	=	437·5 grains.
16 ounces	= 1 pound	=	7,000 grains.

APOTHECARIES' WEIGHT

1 grain	= 0·0648 gramme	=	6·48 centigrammes.
1 scruple	= 20 grains	=	1·29598 grammes.
1 drachm	= 60 grains	=	3·88794 grammes.
1 ounce	= 480 grains	=	31·1035 grammes = 3·11035 decagrammes.

APOTHECARIES' MEASURE

1 minim = 0·05919 cubic centimetre or millilitre = 5·9192 centimils.

1 fluid drachm = 3·5515 cubic centimetre or millilitre.

1 fluid ounce = 8 fluid drachms = 28·4123 cubic centimetres = 2·8412 centilitres.

1 gill = 142·061 cubic centimetres = 1·42061 decilitre.

COMPARISON—BRITISH AND METRIC

1 dram	=	1·772 grammes.
1 ounce	=	28·350 grammes.
1 gramme	=	15·432 grains = 0·035 ounce.
1 centilitre	=	0·3519 fluid ounce.
1 decilitre	=	3·5196 fluid ounces.
1 cubic centimetre	=	0·061 cubic inch = 16·894 minims.

3·5 cubic centimetre = 1 drachm.

28·4 cubic centimetre = 1 ounce.

COMPARISON—BRITISH AND METRIC (*continued*)

Millilitre	= cubic centimetre	= 0·007039 imp. gill.
Centilitre	= 10 cubic centimetres	= 0·07039 imp. gill.
Milligramme	= $\frac{1}{10}$ centigramme	= 0·01543 grain.
Centigramme	= $\frac{1}{10}$ decigramme	= 0·15432 grain.
Decigramme	= $\frac{1}{10}$ gramme	= 1·54324 grains.
Gramme	= unit	= 15·432356 grains.

CONVERSION TABLE

Decigrammes to Grains × $1\frac{1}{2}$
Grammes to Grains × $15\frac{1}{2}$
Grammes to Ounces × $3\frac{1}{2}$ and ÷ 100
Grains to Centigrammes × $6\frac{1}{2}$
Drachms to Grammes × 4

	Grammes to Grains.	Grains to Grammes.
1.	15·432	0·06480
2.	30·865	0·12960
3.	46·297	0·19440
4.	61·729	0·25920
5.	77·162	0·32400

	Grammes to Ounces.	Ounces to Grammes.
1.	0·03527	28·394
2.	0·07055	56·699
3.	0·10582	85·049
4.	0·14110	113·398
5.	0·17637	141·748

REFRACTIVE INDICES

Air	1·0003
Absolute alcohol	1·364
Albumen	1·350
Canada balsam	1·526
Castor oil	1·49
Cedar-wood oil	1·52
Chloroform	1·45

REFRACTIVE INDICES (*continued*)

Substance	Index
Creosote	1·538
Diatom silex	1·434
Ethyl alcohol	1·36
Ethyl ether	1·354
Ether (60° Fahr.)	1·357
Glycerine	1·47
Glass (Flint)	1·619
Glass (Crown)	1·534
Gum arabic	1·512
Gum dammar	1·520
Human blood	1·354
Hyrax (Synthetic Resin)	1·710
Iceland spar	1·654
Oil of cloves	1·533
Oil of linseed	1·485
Oil of turpentine	1·474
Monobromide of naphthalene	1·658
Piperine and balsam	1·657
Piperine and picric piperine	1·681
Realgar ($AsS7\frac{1}{2}$)	2·549
Salt	1·375
Sea water	1·343
Sirax	1·80
Sulphur (melted)	2·148
Styrax (*Liquidambar orientalis*)	1·582
Styrax (*Liquidambar styraciflua*)	1·63
Water	1·33

BIBLIOGRAPHY

Still in Print

Elementary Histological Technique, by J. T. Holder.
Microtomist's Vade Mecum, by A. Bolles Lee, edited by J. Brontë Gatenby, D.Sc., F.R.M.S., and E. V. Cowdry, M.A., Ph.D.
Introduction to the Technique of Section Cutting, by Frances Ballantyne.
Histological Technique. A Guide for the Use in a Laboratory Course of Histology, by B. F. Kingsbury and D. A. Johannsen.
Methods in Plant Histology, by C. J. Chamberlain, Ph.D.
Histological Technique, by Dr. H. M. Carleton, M.A., and F. Haynes, B.A.
Histology for Medical Students, by Hartridge and Haynes.
Practical Chemistry by Micro-Methods, by E. C. Grey.
Metallographers' Handbook of Etching, by Torkel Berglund.
Modern Microscopy, by Cross and Cole.
Turtox News, published monthly by the General Biological Supply House, Chicago.
Bulletin of Staining and Stains, published by the Vector Manufacturing Co.

Out of Print

The Microscope and its Revelations, by Carpenter and Dallinger.
On Mounting Microscopic Objects, by Davies.
Methods and Formulæ in the Preparation of Animal Tissues, by Squire.
Microscopic Objects, by Dr. Martin.
The Microscope, by Dr. Henri van Heurck.
The Microscope Record, published by W. Watson & Sons, Ltd., Nos. 1–33.

Free use has been made of these works. For further information the student would do well to consult them.

INDEX

Algæ, 115, 135
Animal Tissues, 77

Bacteria, Preparation of, 67
Blood Smears, 64
Bones, 59
Bouin's Fluid, 79

Canada Balsam, 14, 30, 45
Carbol Fuchsin, Ziehl Neelsen, 70
Carmine, 102
Carnoy's Fluid, 81
Cathcart-Darlaston Microtome, 2
Celloidin, Embedding, 96
Cellosolve, 116
Cements, 16, 136, 137
Chemical Crystals, 49
Chlorophenol Method, 13
Cleaning Glassware, 5
Clearing Fluids, 86–89
Coleoptera, 13
Crystals, 49–53

Decalcifying, 82, 85
Desiccator, 131
Dialyser, 11
Diatomaceous Deposits, 26
Diatoms, 22–43

Ehrlich's Formula, 100
Embedding, 90
Endothelium, 75
Eosin, 65, 101
Epithelium, 74

Fibres, 56
Fish Scales, 54
Fixing Reagents, 77–84
Flagellæ, 73
Flukes, 107
Foraminifera, 44, 45
Formulæ, 133
Freezing, 90

Giemsa's Stain, 67
Glycerine, 17, 118
Greenough Binocular Dissecting Microscope, 41
Grenacher's Carmine, 102

Hæmatoxylin, 65, 99, 101
Hæmatoxylin, Heidenhain's, 104
Hairs, Animal, 56
Hairs, Plant, 54, 114
Harris, G. T., 124
Heidenhain's Iron-Hæmatoxylin, 104
Hemiptera, 13
Hyrax, 22, 31, 40

Insect Mounting, 7
Insect Parts, 19
Instruments, 2

Leishman's Stain, 66
Lepidoptera, 11

Metal Sections, 60
Methylated Spirit, 4, 78
Mordant Solution, 111
Müller's Fluid, 80

Narcotising Polyzoa, 125

Opaque Mounting, 129
Osmic Acid, 83

Palates of Molluscs, 55
Paraffin Embedding, 92
Piperine, 23, 32
Plant Crystals, 53
Pollen, 115

Radiolaria, 46, 47
Raphides, 53
Realgar, 23, 35
Rocks, 58

Silicic Acid, 10
Silver Precipitation Method, 71
Sirax, 23
Spicules, 47
Staining Fluids, 98
Starches, 52
Styrax, 22, 40

Teasing Out Tissues, 75
Teeth, 59
Trichina Spiralis, 107

Van Heurck's Method, 35, 39
Vegetable Tissues, 109

Weigert's Formula, 101
Weights and Measures, 140
Worms, 106

Xylol and absolute Pheno :,88

Zenker's Fluid ,80

SECONDHAND MICROSCOPES AND ACCESSORIES

Thousands of Secondhand MICROSCOPE SLIDES from **6d.** each.

• • • •

NEW AND SECONDHAND TELESCOPES, BINOCULARS, MONOCULARS AND OPERA-GLASSES

• • • •

Repairs to all kinds of Optical Instruments by Expert Craftsmen.

INSTRUMENTS BOUGHT FOR CASH!

NOTE NAME AND ADDRESS

BROADHURST, CLARKSON & Co., Ltd.

TELESCOPE HOUSE

63 Farringdon ROAD, LONDON, E.C.1

(3 minutes from Farringdon Street Metro. Rly. Station)

Also at 69 Fenchurch Street, London, E.C.3

DATE OF ISSUE

This book must be returned within 3, 7, 14 days of its issue. A fine of ONE ANNA per day will be charged if the book is overdue.