Modern microscopy : a handbook for beginners combining I. The microscope, and instructions for its use / by M.I. Cross ; II. Microscopic objects : how prepared and mounted / by Martin J. Cole.

MODERN MICROSCOPY

M. I. CROSS

AND

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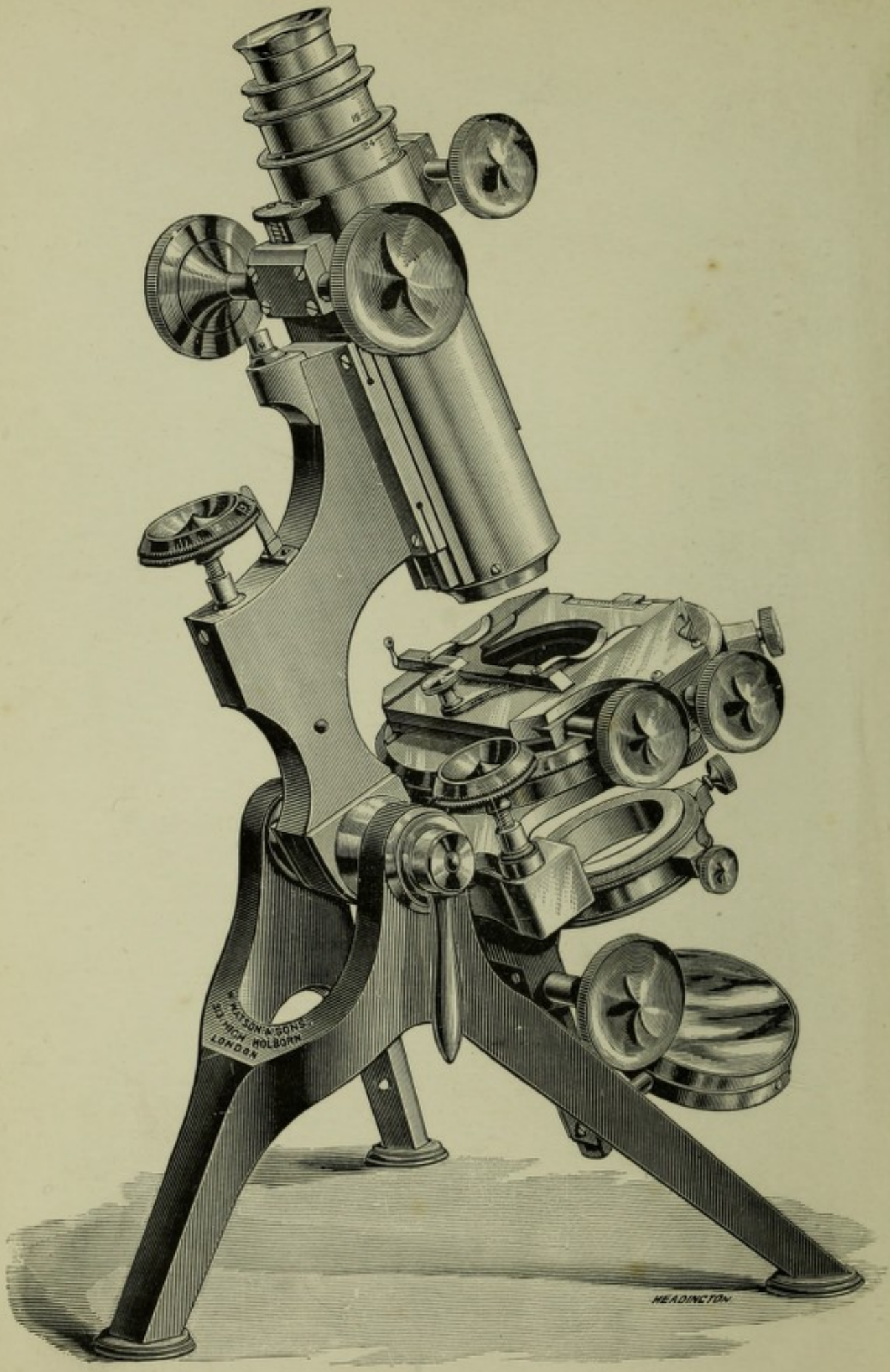

FIG. 1.—A Typical Modern Microscope.—Made by W. Watson and Sons to the specification of Dr. Henri Van Heurck, Antwerp, for Photo-Micrographic and High Power work.

MODERN MICROSCOPY.

A HANDBOOK FOR BEGINNERS,

COMBINING

I. THE MICROSCOPE, AND INSTRUCTIONS FOR ITS USE,

BY

M. I. CROSS.

II. MICROSCOPIC OBJECTS: HOW PREPARED AND MOUNTED,

BY

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SECOND EDITION.

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PREFACE TO SECOND EDITION.

THE very kind reception that was accorded to the first edition of this book has encouraged the authors to issue a second edition.

The subject-matter has been thoroughly revised, and additional information on methods of manipulation has been introduced. It is therefore hoped that the book may be found more than ever useful to the beginner, for whom it is especially intended.

LONDON,
October, 1895.

PREFACE TO FIRST EDITION.

THIS handbook is not intended to be an exhaustive treatise on the microscope, nor to give particulars of the various patterns of instruments that are made, of which details can be seen in the makers' catalogues, but to afford such information and advice as will assist the novice in choosing his microscope and accessories, and direct him in his initial acquaintance with the way to use it.

The directions for preparing microscopic objects by Mr. Martin J. Cole are the outcome of a very long experience as a preparer of Microscopic Objects of the highest class, and cannot fail to be of the greatest service to the working microscopist.

M. I. CROSS.

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MODERN MICROSCOPY.

INTRODUCTION.

To attempt to give a historical account of the development of the microscope, tracing it through its pre-achromatic days, and noting the successive improvements that have contributed to the high position it occupies to-day amongst instruments of precision, would alone fill a small volume. Suffice it to say that so recently as the year 1824 Tully constructed his first Achromatic Microscope, since which time every decade has added its tale to the march of progress. In 1883 the president of the American Society of Microscopists, in his annual address, remarked 'that lenses, which were believed to have so nearly reached the limit of perfection fifteen years ago, are antiquated now, and the limit of perfection has moved forward like the horizon, and is as far off as ever.' Since those words were uttered the optics of microscopy have been lifted to a far higher plane by the introduction of an entirely new system of lenses, termed the 'apochromatic objectives,' which have yielded results that our predecessors in matters microscopical would not in their loftiest flights of imagination have thought possible; and still further experiments are constantly being made with the view of affording yet greater facilities for the attainment of a higher degree of excellence in microscopical work. The mechanical design of microscopes has not been one whit behind the optical portion in the onward march. New models of instruments and improvements in detail,

enabling work to be done with increased accuracy, have from time to time been introduced ; and although it is impossible to prophesy in what direction progress will take place in either the optical or mechanical departments of the microscope, there is room for no doubt that an instrument on which so many branches of science are dependent will be developed to a still higher degree in the future.

One of the prime causes of the progress that has taken place in the microscope has been its adoption in many branches of education, and in manufacturing industries of every description.

Not many years since a hospital rarely possessed more than one or two microscopes, and these were usually kept under glass shades, and intended more for ornament than use. At the present time every hospital student has to provide himself with a microscope, and become practically acquainted with the ultimate structure of organs and tissues, both in health and disease. The microscope has been introduced into many ordinary schools, and teachers are slowly realizing that 'we think in pictures,' and that as sight aids the memory, so an ounce of ocular demonstration is worth a pound of oral description. The result is, that erstwhile dry-as-dust lectures in zoology and botany are now anticipated by students with keen interest on account of the pleasure derived by examination with the microscope of the actual subject of study. By means of the microscope the chemist is able to judge of the quality of his drugs ; the Medical Officer of Health uses it to detect adulterations, entozoa, and bacteria ; the brewer and the baker watch their ferments with it, and it is employed by seedsmen, dairymen, clothworkers, handwriting experts, and, in fact, in nearly every vocation it is becoming more and more an indispensable referee. It therefore is something more than a mere tool which magnifies, for it contributes in a vital degree to our well-being and comfort.

To the microscopical societies also is due in no small degree the evolution of the microscope, and especially does

this apply to the Royal Microscopical Society, and the Quekett Microscopical Club, both of which meet in London at 20, Hanover Square. Every improvement in the instrument and its accessories that takes place is presented to these societies for criticism, and in connection with both of them, as officers and members, are men who have attained the highest eminence in microscopical science and manipulation, whose judgments have influenced and moulded the character of microscopy, and who are ever willing to assist by advice and suggestion any who will avail themselves of their experience. It is most desirable that microscopists should become members of a good microscopical society, and those mentioned above enjoy the highest position in England.

The universal demand for the instrument has naturally given rise to considerable competition amongst opticians to produce thoroughly efficient and high-class microscopes at a low cost, with the result that a far superior outfit can be procured for a given sum to-day than was obtainable a few years ago at nearly double the cost, bringing that which was a luxury, and acquirable only by the wealthy, within the means of the slenderest purse.

To the amateur possessing scientific tendencies the microscope is an ideal means of recreation and culture. There are no external conditions such as weather, temperature, etc., essential to its employment, as demanded by many other semi-scientific pursuits, for it can be placed on a table by the fireside, and will weave its garlands of revelation in the mind of the observer, without causing him practically any physical exertion, while the mental vision will seem to be actuated in quite a new orbit, so grateful are the exquisite effects that it produces.

In order that the best results may be obtained, however, there must be a correct understanding of the methods of working the instrument. Facility in this respect can only be acquired as the result of experience and practice, and it is the object of this work to indicate in the plainest manner

the rules of manipulation that should be adopted in order to ensure success.

Perhaps some people may hesitate to attempt working with the microscope, not caring to use it merely as a means of amusement, and mistrusting their ability to employ it scientifically. They reflect that every department has its untiring, experienced workers, and available ground appears to have been gone over so repeatedly that it would seem hopeless for an amateur to attempt to add to existing knowledge on any subject. This idea is a mistaken one, and any microscopist who uses his instrument thoughtfully will be surprised at the manner in which the love for the work will grow upon him, and how gradually he will become master of some special department which he has adopted as his own. On this point we would echo the words of a well-known microscopist: 'It needs no marvellous intellect, no special brilliancy, to succeed in a scientific study; work at it ardently and perseveringly, and success will follow.'

We will now proceed to consider the microscope-stand, apart from its optical relations, which will be treated of in a succeeding chapter.

PART I.

CHAPTER I.

THE MICROSCOPE-STAND.

As one looks through the catalogues of the various dealers, and notices the microscope-stands varying in price from £2 to £40, a feeling of bewilderment arises as to what is essential and what can be dispensed with. We will, then, examine the parts, describe their uses and advantages, and state what is necessary for a beginner.

Here let us advise intending purchasers not to buy a microscope unless it bear the name of a manufacturer: a good workman is never ashamed of his handiwork. There are many very inferior instruments that look tempting, but a practical acquaintance with them soon discovers their weak points and inefficiency. Happening to attend the conversazione of a well-known microscopical society, at which there were exhibited over one hundred instruments, it was surprising to note the many makeshifts of microscopes belonging to some of the exhibitors—and many of them had probably cost a fair price, too. A manufacturer once remarked to the writer that he was some time ago in a provincial town, when an auctioneer asked him whether he could make him up a job lot of microscopes for sale by auction, as he was very successful in disposing of a certain class of pictures in that way. The disgust of the scientific workman can be better imagined than described. The microscopes often seen in the novice's possession seem to be of this genus, and but little satisfaction is derivable from working with them. If the user is at all progressive, an

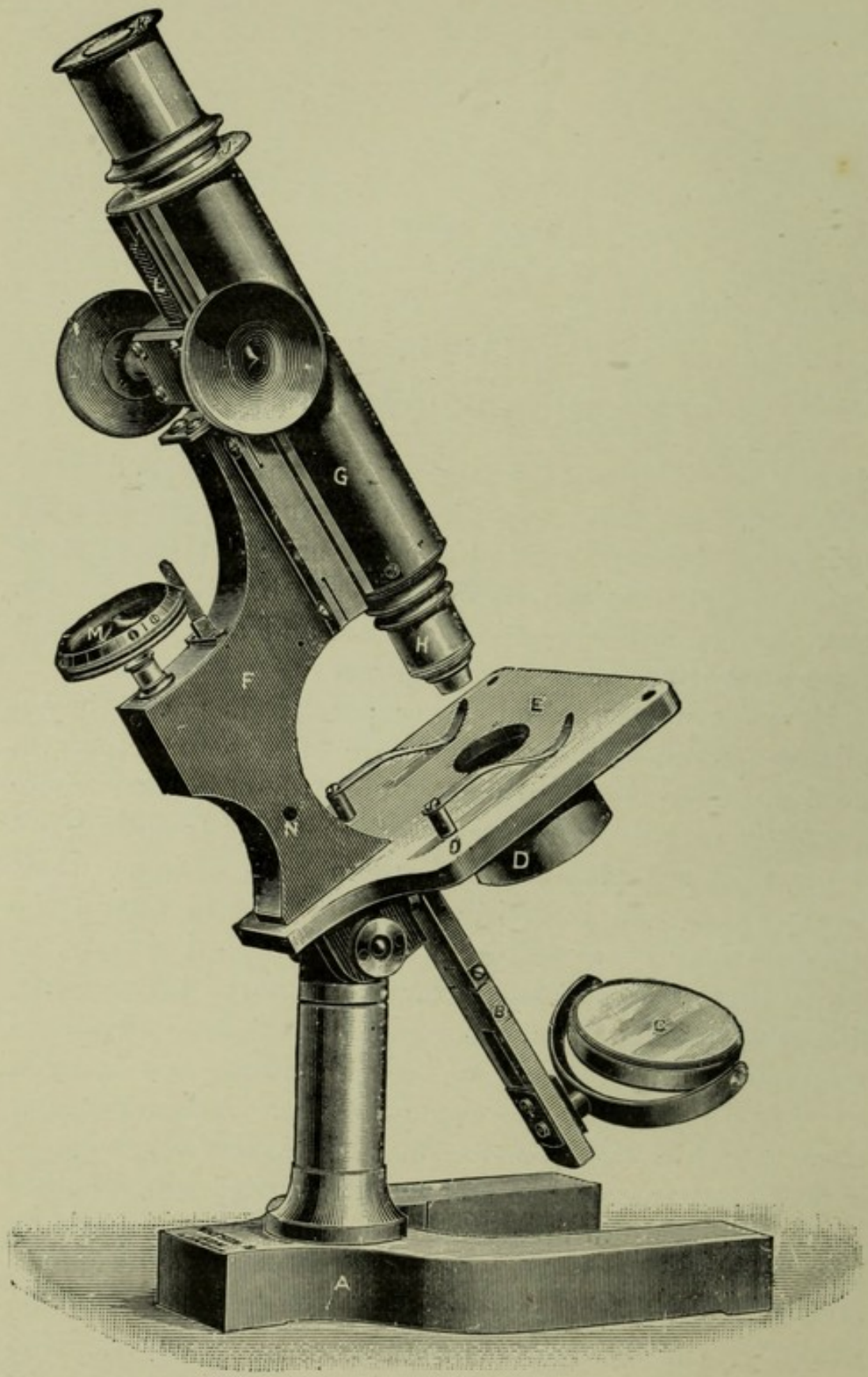

Fig. 2.—Edinburgh Students' Microscope, Stand B, by Watson and Sons.

instrument of this kind is speedily discarded in favour of a well-made one.

Although a good second-hand instrument may be occasionally met with, great discretion is required in purchasing, because improvements may have been introduced since its manufacture, or some damage may have occurred to the optical parts. If it be obtained from a respectable dealer who understands his business and will give a guarantee of condition, there is some inducement; but a friend who is up to date in microscopy is generally the best to advise. In all cases, before purchasing, a catalogue should be obtained from the maker whose name the instrument bears, so that it may be ascertained whether the pattern is still made or is antiquated and out of date. It is much better to buy a good stand capable and worthy of receiving additional apparatus from time to time, rather than an inferior instrument that is completely furnished with objectives and accessories. These latter rarely engender pride of ownership, and are often relegated to some obscure corner after a short acquaintance; whereas if a good instrument be purchased, with but one objective to start with, there is always a pleasure in working with it, and a peculiar fascination from its quality—a satisfaction in feeling that one has something superior.

There are several very cheap microscopes on the market, and although it is sometimes claimed that they foster the scientific spirit and educate the beginner, it is very doubtful whether they do not do more harm than good in consequence of the dissatisfaction with their performance which their owners must feel on comparison with those of superior make: possessors of such become disheartened, often wrongly attributing want of success to their own incapacity, and not to the instrument.

To make the different parts quite clear, on page 18 is figured a student's microscope, of which the following is a description:

Fig. 2.—A is the stand or foot.

B is the tailpiece carrying the mirror (C) with which light is thrown upon the object.

D is the under-fitting, into which are fitted the sub-stage condenser, polarizer, etc.

E is the stage on which the object is placed.

F is the limb carrying the body (G).

At the lower end of the body is a screw, into which the objective (H) is fitted.

At the upper end of the body is a sliding fitting called the draw-tube (J), by means of which additional magnification may be obtained, and into this draw-tube the eyepiece or ocular (K) fits.

L is a rackwork, by means of which the body (G) is raised and lowered in order to focus the objective (H) upon the object which is placed on the stage (E).

M is the milled head controlling the fine adjustment, which imparts a delicate motion to the body, in order that the objective may be more exactly adjusted than would be possible with the rackwork (L) when using high magnifying power.

N is a fitting for forceps, or side silver reflector; and O are the springs with which the object is held in position.

We have selected the instrument (Fig. 2) because, from practical acquaintance with it, we are able to strongly recommend it for a beginner's microscope, worthy of receiving additions from time to time as means may permit, especially as it may be had on a tripod form of foot similar to that shown in Fig. 1, instead of the horseshoe form. Still, it should only be considered as a typical one.

THE FOOT.

We would very strongly advise the beginner to purchase an instrument with a tripod foot, as shown in the microscope Fig. 1 (frontispiece). This is the most rigid form of foot that is made, and is in this respect a long way in advance of the horseshoe stand with a single pillar, so largely adopted on the Continent.

If the horseshoe foot be made in proper proportion, it is

not so condemnable; but unfortunately it rarely is, and at the best is not nearly so firm as the true tripod foot recommended. This is a most important feature; and though perhaps inappreciable to the novice, yet as he advances, all these minor details will become very significant, and are likely to prevent work being satisfactorily done, which with a more perfect stand he could have accomplished. If the foot of the microscope be shod with cork, so much the better, as the microscope is thereby insulated from vibration, and the annoyance caused by scratching tables with the feet of the instrument is avoided. It must be clearly understood, however, that even if this form of foot be not made in proper proportion its advantages will be annulled.

Next to this foot in point of convenience and rigidity we would place the Jackson model, as shown fitted to the instrument on page 35. This, if properly made, is but little inferior to the tripod, but it is heavier.

There are many other patterns made, mostly modifications of the Jackson model, and their rigidity can be tested by placing the instrument in a horizontal position, racking the body out, and then observing whether there is any tendency to topple over. If so, have nothing to do with them. The double-pillar form, with flat tripod foot, as shown on page 41, is a very good and convenient one. It is adopted by several firms for largest size instruments, and is certainly handsome.

Last on our list of feet we should place the horseshoe pattern, as shown on page 18. The great advantage this possesses over all others is its compactness for portability. It originated on the Continent, and is preferred both there and in English medical schools, etc., before any other. It is usual in laboratories to work with the microscope in a vertical position, and while the instrument is so placed the horseshoe foot is quite firm. If it be placed at an angle, however, as the amateur usually employs it, even with a well-made foot there is a tendency to side-falling. It would

be well, therefore, not to select this form of foot when choosing a microscope. The order of preference for the foot of the microscope would be:

1. The tripod foot, as shown fitted to instruments on page 37, and frontispiece.
2. The Jackson form of foot, as fitted to the instrument illustrated on page 35.
3. The flat tripod and upright pillars, as fitted to the instrument illustrated on page 41.
4. The Continental or horseshoe form.

THE STAGE.

The stage of the microscope on which the object is placed for examination may be divided into two classes —(1) mechanical, and (2) plain.

THE MECHANICAL STAGE.—The instruments figured on pages 35, 37, and 41 are provided with this type of stage, in which, by the turning of two milled heads which are attached to screws, plates are moved in dovetailed grooves one over the other, in rectangular directions, carrying the object with them. A first-class microscope should be provided with this form of stage; in fact, there is no means so suitable for systematically examining an object as is afforded by it. In addition to these mechanical movements, if a bar be fitted to slide in a vertical direction on the top plate the efficiency of the stage will be greatly increased. The mechanical stage lends itself to the adaptation of further important movements. A means of rotating the object is an essential in most classes of work. For this purpose the lowest plate of the stage is usually fitted to rotate on the fixed centre of the baseplate, the mechanical movements acting above it. It is then termed a concentric rotating stage, the object remaining in the field during the whole rotation of the stage. In mechanical stages of economical construction the rotating plate is occasionally fitted above the mechanical movements, and is carried by them, in which case it does not rotate concentrically. The object can not-

withstanding be kept in the centre of the field by constantly resetting it with the mechanical screws during the rotation of the plate. Some stages of the concentric form are arranged to rotate by rackwork and pinion; although this is not really an essential, it is often convenient: it also prevents the stage from rotating accidentally, especially in photography; when it is provided, it should have the pinion-wheel so arranged that it may be disengaged from the rack and replaced instantly.

Centring screws to the concentric rotating stage, by means of which the axis of the stage may be made true with any objective, will be found a useful addition, especially if petrological work is to be done. Divisions to the periphery of the stage for reading the angle through which the stage is rotated are not advantageous for ordinary purposes, but for chemical and petrological work they are a necessity.

Finders to Mechanical Stages.—Divided scales, reading to parts of an inch or millimetre, fitted to the plates of the mechanical stage, will be found of great utility. By means of such an arrangement, important parts of an object can be noted and subsequently refound. For instance, supposing a specimen were being examined, and an important feature were observed to which future reference would be desirable, it would only be needful to take the reading of the divisions on the stage, and record them on the slide—say, horizontal, 24; vertical, 20. On future occasions, on setting the stage readings at the same points and placing the object in the same position on the stage (for which purpose nearly all mechanical stages have a stop-pin against which the slide can be set), the special feature would be at once in the field of view. These divisions can also be used for roughly measuring objects, the *modus operandi* of which is given in the chapter on the measurement of objects, page 98.

If a mechanical stage be selected, it should be a good one, for if badly made it is far less convenient than a plain stage; also the frictional parts should be sprung, and

fitted with adjusting screws, so that compensation may be made for wear and tear.

PLAIN STAGES.—The stage of the microscope shown on page 18 has two flat springs only, to hold the object in position on the surface, and the movement of the object is effected by the fingers. For cursory examinations this answers every purpose, but where systematic work is to be done something more is needed, and this, when a mechanical stage is not provided, should take the form of a bar reaching completely across the stage and sliding in a vertical direction. If properly fitted and sprung, it will travel freely when gently pressed with one hand only. The object is carried by it, and can be moved in a horizontal direction upon this bar. With a little practice the fingers become educated to the work, enabling examinations to be conducted with the highest powers almost as rapidly and systematically as with the mechanical screws. This sliding-bar should further be provided with two flat springs, so fitted that they may be turned inwards to rest on the bar when not required. It is often necessary to set an object at an angle across the stage during observation, in order that some special feature may appear vertically in the centre of the field. If the springs be not provided this cannot be done.

FINDERS FOR PLAIN STAGES.—The form of finder suggested by Mr. Lewis Wright for plain stages is the most efficient for practical purposes. Many proposals have been made, but none equal this one for simplicity. On the right-hand side of the central aperture, one inch of the stage is divided into 50 parts in vertical and horizontal directions. A special feature of interest in an object having been discovered, the slide being maintained in a horizontal position across the stage by means of the sliding-bar, it is only necessary to read from the top right-hand corner of the slide the lines against which it lies. A note of same is made on the labels of the object, and the specimen can subsequently be placed in exactly the same position, and the point reproduced. Without the sliding-bar it is somewhat

difficult to keep the object exactly straight across the stage, but with care, on observing an important feature, the slide can be gently turned until it is in a correct position for taking readings.

The great saving of time that is afforded by such a device as this should establish its claim to be placed on every student's microscope. Several makers have already adopted the arrangement, and it would be a great gain to microscopists in general if a uniform position for the divisions were agreed upon between them, so that a person noting a special point with his microscope could send the specimen, with the readings marked upon it, to a brother worker, and he, having the same kind of finder on his stage, would at once be able to find the desired spot.

The following method would be suitable for the average size of stage: A piece of metal the same size as an ordinary glass slip (3 × 1 inches) should be adopted as a tool, and $\frac{3}{4}$ inch from one end and $\frac{1}{32}$nd inch from the edge a minute spot should be made with a small drill. The metal slide should be placed on the stage with the spot towards the front, and the $\frac{3}{4}$-inch space to the right of the centre of the stage. The drilled spot should then be placed central in the field of a one-inch objective, and the outer margin of the square of divisions marked off from the right-hand end of the metal slide.

The Wright's finder is obviously unsuitable for any other than a stage whose upper surface does not travel.

In selecting a stage for a microscope our choice would therefore be as follows:

For a first-class microscope: Mechanical movements; concentric rotation; screws to make the rotation quite true with any objective; sliding-bar to top plate and stop-pin for object to go against; divisions to plates of stage reading to parts of millimetre or inch; rackwork rotation to stage; and (optional) divisions to periphery of stage.

For a second-class microscope: Mechanical movements; sliding-bar to top plate; non-concentric rotation.

For a third-class microscope: Plain stage, with springs to hold object in position; if provided with sliding-bar or plate as object-carrier, so much the better.

THE SUB-STAGE OR FITTING UNDER THE STAGE TO CARRY CONDENSER, ETC.

THE SUB-STAGE.—This consists of a tube which should be 1½ inches full, internal diameter—termed the 'society size.' It carries illuminating apparatus for condensing the light on the object, the polarizing prism, and other apparatus, referred to on a later page. It is adjusted in a vertical direction to and from the under surface of the stage, by means of a rack and pinion, and the ring carrying the apparatus is mounted in an outer collar provided with screws, by means of which the condenser, or other apparatus, can be made exactly central with the objective with which it is working. This central fitting is made to rotate by rack and pinion in some instances for using the polarizer, etc., but this is so rarely needed that it is hardly necessary except for special work. It is essential that the sub-stage should be substantially made, as it is a most important fitting, often too little appreciated. For the sake of economy, some makers fit the sub-stage without a rackwork, it merely being designed to slide up and down in the dovetailed fitting; this is neither desirable nor convenient. A fine adjustment, to permit of the condenser being focussed in the most exact manner, is often provided with the best stands, and it is exceedingly convenient and of very great importance where high-power work is intended to be done. Often it is wished just to alter the focus of the sub-stage condenser very slightly. In attempting so to do the tension on the sub-stage pinion milled head is apt to cause vibration, so that the best point of adjustment cannot be at once observed. By communicating this small amount of motion with the fine adjustment the focus is obtained to a nicety. It is also specially convenient where a number of specimens have to be examined. The varied thicknesses of the slips

necessitate a slight readjustment of the condenser in each instance, and this can be very quickly done if a fine adjustment be fitted to the sub-stage.

Where a microscope is provided with a sub-stage it is necessary to ascertain if it will centre with the objective by means of its screws; this should be done in the same manner as described below for the 'under-fitting,' and the centring screws turned. Also, it is very important that when the sub-stage is racked up or down it should maintain its centre with the optical axis; but few instruments will stand this test; for some reason or another the sub-stage goes out of centre—slightly in some cases, considerably in others. There ought to be absolute truth if everything is square, and any great deviation in this respect should call for rectification. If a fine adjustment be fitted to the sub-stage, it may be tested by using the upper surface of the fitting as a stage and placing the object on it; this may be made to adhere with a little tallow or grease. A medium power of objective will probably not focus, the sub-stage being too far away. The nosepiece end of the microscope must therefore be lengthened; for this purpose remove the prism from an analyzer fitting, and use this fitting as a lengthening adapter. The object is then viewed in the usual way. All the microscopes that are herein figured, have a rackwork sub-stage, with the exception of that on page 18.

THE UNDER-FITTING.—In the cheaper instruments, instead of the sub-stage as above described, an ordinary plain tube, termed the under-fitting, is screwed into the under side of the stage; and in this tube the condenser or other apparatus is moved up and down to focus. It is shown fitted to the microscope figured on page 18. This must be truly centred with the optical tube, and it is well to test it by placing a small diaphragm in the under-fitting, and with an objective in the body to focus the diaphragm. If it be not central its practical importance is annulled. The additional convenience and necessity of the centring

sub-stage cannot be too fully impressed upon the beginner who contemplates doing thorough work. Swift and Son, of Tottenham Court Road, in their new series of 'Students'

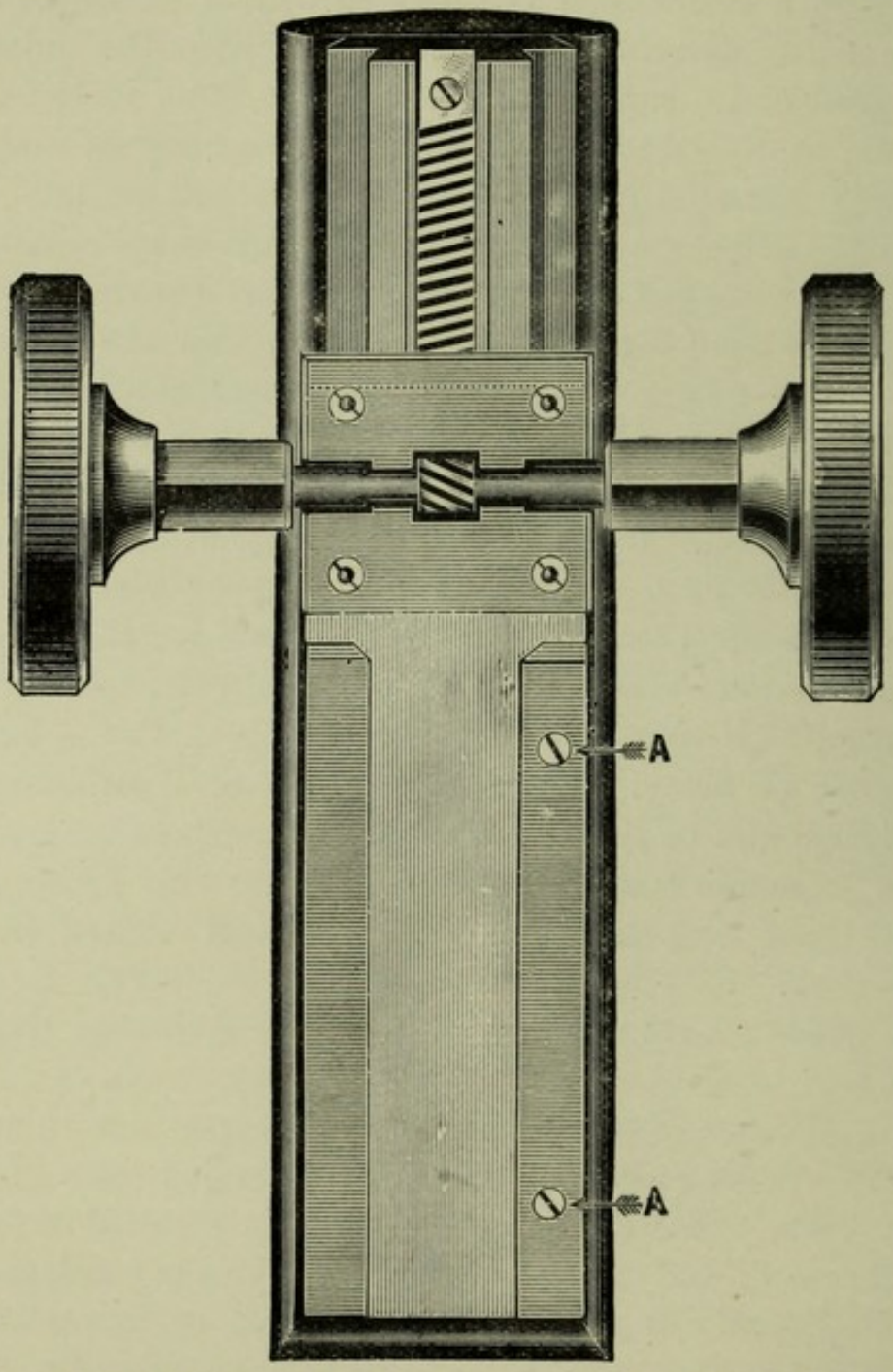

FIG. 3.—View of diagonal Rackwork attached to one of Watson and Sons' Microscopes, showing Dovetailed Fittings to receive Fine Adjustment Slide and Adjusting Screws (marked A).

Microscopes,' and Watson and Sons, of High Holborn, in their 'Edinburgh Students' Instruments,' make many of the fittings interchangeable, so that a person who starts with an

under-fitting, and subsequently experiences the necessity of having a sub-stage, can himself remove one fitting and replace it by the other. W. Watson and Sons have lately adopted a form of centring under-fitting; it is inexpensive and very considerably increases the efficiency of a student's microscope. It can be fitted to almost any microscope.

The great convenience will be found in many instruments of being able to swing the sub-stage aside out of the optical axis of the instrument on a hinge-joint fitting. It saves much time to students, especially where two or three powers are constantly being interchanged, and the condenser may not be required for all of them. Where this arrangement exists, it should be adapted in a workmanlike and substantial manner, and a proper support given to the fitting when in the optical axis to make it perfectly rigid.

The choice with regard to a sub-stage would therefore be—

In a first-class microscope: Sub-stage, having rackwork and fine adjustment for focusing, and provided with facilities for centring; rackwork rotation, if for examination of crystals or for petrology.

Second-class instrument: Sub-stage having rackwork and centring adjustments, and means of lifting aside out of the optical axis.

Student's instrument: The same as the second-class or with the plain under-fitting; the latter, preferably with centring arrangement. In any case it is imperative that it shall be of the 'Universal' size.

ARRANGEMENTS FOR FOCUSING.

The next point for consideration is the means of focusing the object-glass. This is done by two adjustments, called the coarse and the fine movements. The former consists of a rack and pinion actuating the body in a very true-fitting dovetailed bearing, as per Fig. 3, shown on page 28. In the illustration it will be seen that the rack is cut diagonally, and this, although rather troublesome in the

making, is undoubtedly the best kind, a softer motion being obtainable than with the horizontal form. In order that it may work at its best, each tooth of the rack has to be carefully 'ground in'—that is, fitted to a leaf in the pinion—and so that the fitted tooth of the rack may always engage the correct leaf of the pinion, it is necessary so to fix the body that, when racked up as high as possible, it may not be withdrawn from its bearings and rackwork, it being, in fact, provided with a 'stop' screw. It has been recommended that microscopists should take their instruments to pieces in order that they may judge of their workmanship; but in reality a well-made microscope requires to be as carefully put together as a watch, and for a novice to attempt to undo the parts means very probable detriment to the instrument. The name of a first-class maker on an instrument may generally be considered a guarantee of good workmanship, otherwise he could not possibly maintain his reputation.

Now as to the **Fine Adjustment.** A great diversity of opinion exists with regard to the qualities of the different forms, most writers having their preferences and prejudices. It is a most important movement, and it is absolutely necessary that it be quite perfect if high powers are to be used. It must impart a very slow motion and be quite free from lateral movement. After some years of practical acquaintance with Messrs. Watson and Sons' instruments, the writer is able to state that with high powers of large aperture he has found their form of fine adjustment perfectly satisfactory. It is shown in position on the instruments (Figs. 1 and 2). The body is raised or lowered in a dovetailed fitting by means of a lever contained within the limb of the instrument, and a pin passing through it horizontally acts as a fulcrum. By turning a milled head attached to a micrometer screw, force is applied to the lever at one end against a pointed rod, attached to the body and entwined by a coil spring, at the other extremity. As the body moves upwards, the spring is compressed

against a brass plate, and on the micrometer screw being released this spring produces the reactionary power. One arm of the lever is four and a half times longer than the other, consequently the weight of the body at the milled-head end of the lever and the motion imparted are reduced in this ratio. Thus the makers give the weight of a body as 17 oz., and this divided by $4\frac{1}{2}$ reduces the resistance to $3\frac{7}{9}$ oz. This system has the advantage that the position on the limb is convenient for manipulation, and is not altered when the body is racked up—that is, it is not carried by the rackwork, as in many forms, so that its attachment to a focusing rod of a camera for photo-micrography is easy and convenient. There is also a very simple means of adjustment provided for taking up any slackness through wear. The slide in which the fine adjustment is fitted has sprung slots, to which are fitted screws (shown in Fig. 3, page 28, marked A). By turning these screws slightly, the spring-fitting grips the bearing more tightly, and so takes up any wear caused by friction. Any microscopist can thereby adjust his own instrument.

Another very good and rigid form is that fitted by Messrs. Swift and Son. It consists of a lever placed in a vertical direction by means of which the body-tube is raised and lowered. The actuating milled head is shown in position on the instrument on page 35, marked 'patent,' and is carried by the coarse adjustment when focusing. This is rather a disadvantage, especially in photo-micrography, as it necessitates an alteration in the position of the connecting arrangements of the camera for different powers; also, the milled head being on the side of the instrument renders it additionally awkward, as only one hand can be applied to it. For their working qualities both of the fine adjustments mentioned are very good—in fact, as perfect as can be desired—but for convenience Watsons' is preferable. Powell and Lealand's instruments are also provided with a fine adjustment having special merit, consisting of a lever actuating a long tube sliding up and down

inside the body. This, again, has the disadvantage of being carried by the rackwork when focusing for different powers. The differential screw fine adjustment, as made by Mr. Charles Baker, has been well spoken of, but the writer has had very little experience with it.

When testing the performance of the fine adjustment, a central cone of light must be used; if the light be thrown obliquely, there will be of necessity an apparent movement in the direction from which the light comes. With central illumination, there should be no shake or displacement whatever in the object when it is focused.

Nearly every maker has his own system or systems of fine adjustment, possessing features more or less desirable, but they are mostly modifications of those mentioned here. Some firms adopt a most excellent form of fine adjustment for a superior class of microscope, while in the students' patterns the method employed is dissimilar and oftentimes useless for high-class work. It would be far better that efficiency were not sacrificed in such a manner for the small saving in cost involved.

Above all things eschew the form of fine adjustment which carries the *whole* weight of the body of the instrument, or depresses it against a spring, as in the Continental instruments and cheap students' forms: these are almost worse than no fine adjustment at all, as they invariably soon work loose in the fittings and cause great annoyance.

There is also another form, now almost non-existent, which is fitted to the microscope at the nosepiece end, and consists of a milled head attached to the body, by means of which a tube which carries the objective is raised and lowered inside the body-tube against a spring. This can never work thoroughly well, for if two round tubes are perfectly fitted one inside the other, they clutch, and to overcome this, one of the tubes has to be rendered a little eccentric, and consequently a lateral shake arises.

In the choice of a fine adjustment, therefore, reject the direct acting and the nosepiece forms.

THE LIMB.

The limb of the instrument is another important detail. It will be noted by the illustration (Fig. 5) that there is no support for the body-tube from the bar to the top of the eyepiece, and unless this bar be most perfectly fitted, and every detail of construction considered, with such an amount of leverage as there is at the eyepiece end, a very apparent shake will soon be noticed. The limb fitted to the other instruments shown, known as the Jackson form, is therefore recommended, as it supports the body from one end to the other, and at the same time is as firmly connected with the other working parts of the instrument as the bar form.

THE BODY-TUBE.

It has always been the custom, in the construction of the full-sized English microscopes, to make the body of fairly large diameter; while on the Continent the reverse is the case, and it is made as small as possible. Owing to the extended use of Continental oculars and objectives, English makers have in recent years adopted the Continental diameter of body to a considerable extent. For photographic purposes it is held by some workers that a fairly large body should be used, but for ordinary visual work we do not consider there is any advantage in the large over the small tube. It may here be said that Continental instruments have their bodies constructed very much shorter than the English forms, the rule being to employ with them objectives adjusted for a tube 160 millimetres long, while English opticians usually adjust theirs to one of 250 millimetres. The microscopist who enters enthusiastically into his work invariably has objectives of both Continental and English make, and he therefore requires the convenience of being able to use both perfectly. One or two English opticians make microscopes with a body of 160 millimetres, and a draw-tube sliding inside it, by means of which a length of 250 millimetres can be obtained; but a greater range is often found convenient, and Watson and Sons in their

Van Heurck microscope (shown in the frontispiece), and Mr. Baker in his Nelson model microscope, supply two draw-tubes giving a range of body from 140 millimetres (5½ inches) to 310 millimetres (12 inches). One of these draw-tubes works by a rack and pinion: the object of this being to afford facility for adjusting the objective for thickness of cover-glass, as described on page 59. This form of body is coming more and more into use, and will be found a very great convenience to the all-round worker. No precise advice can be given without knowing the work intended to be done, but, generally speaking, the six-inch body with the two draw-tubes is much to be preferred to any other.

The draw-tube usually has a scale of divisions engraved upon it to parts of a metre or inch. The object of these divisions is to enable a record to be kept of magnifications at different points of extension, or a note to be made of the lengths of tube that give the most perfect corrections for certain objects and objectives.

In all microscopes of medium or high class, the universal thread should be fitted to the lower end of the draw-tube; where there are two draw-tubes it should be supplied to the outer one. The advantages of this adapter are numerous. A low-power objective can be used in it which it is often impossible to focus on many stands, owing to the compactness of the build and shortness of the movement of the coarse adjustment. With the two draw-tubes, if the outer one have this adapter fitted, nearly 10 inches of separation can be obtained between the eyepiece and the objective. It is further useful for carrying the apertometer objective and the analyzer, described respectively on pages 56 and 87; also the Bertrand's lens for examining the 'brushes' of crystals, and for many other purposes.

It has occurred within the experience of the writer that results obtained on a microscope having a large tube could not be reproduced with the same objective on an instrument having a small tube. This was traced to be due to

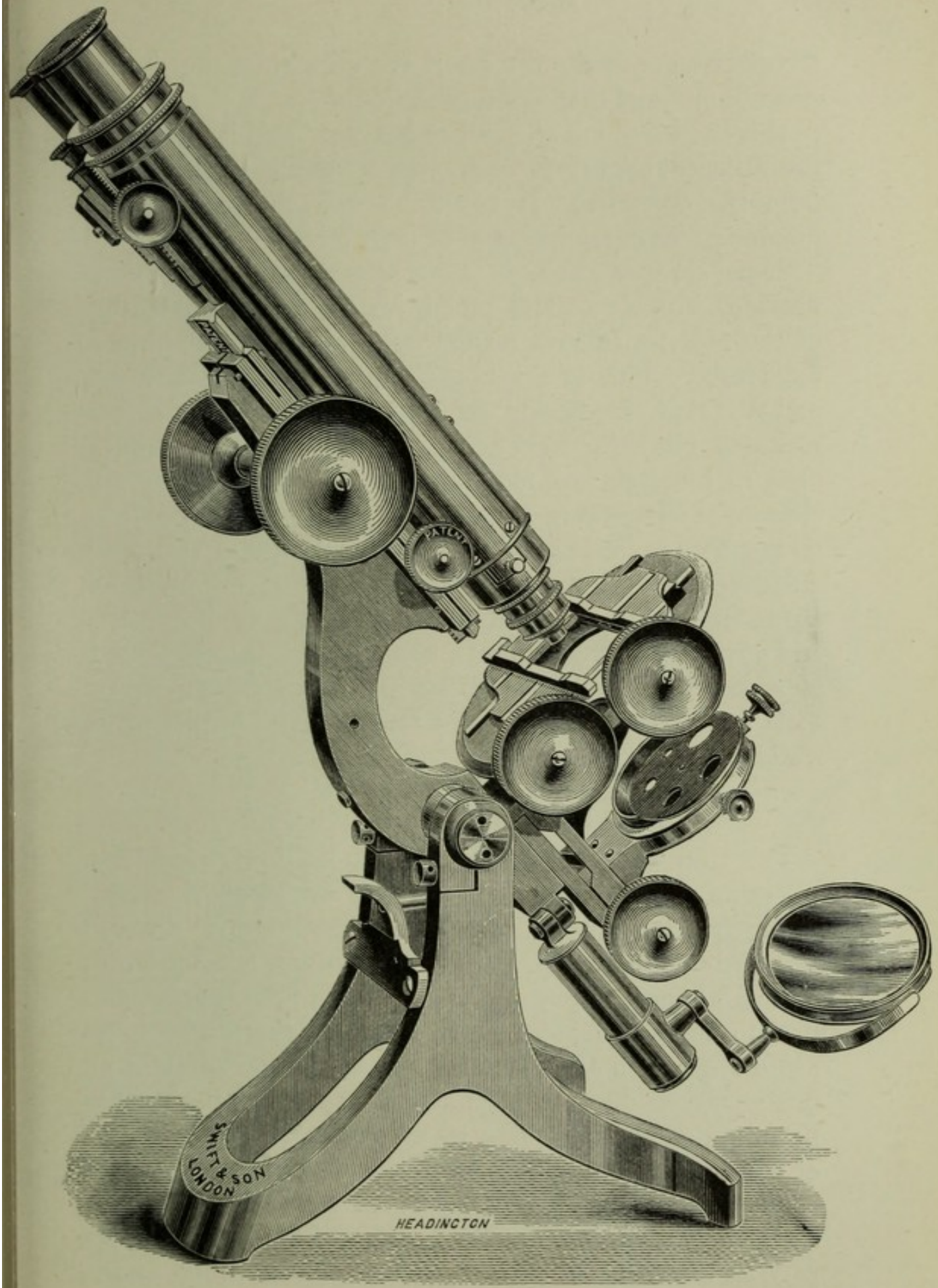

FIG. 4.—Swift and Son's Binocular Challenge Microscope C, with Jackson Form of Foot. A typical amateur's microscope.

the diaphragm at the bottom of the draw-tube, and it has since been found that in many students' stands the opening of this diaphragm is as small as $\frac{3}{8}$ inch. This is altogether insufficient, and causes restriction to the passage of rays from the objective. It would be well to see that this diaphragm has an opening of at least $\frac{3}{4}$ inch.

Some cheaper students' microscopes, instead of being provided with a rack and pinion for the coarse adjustment of the object-glass, are made with the body to slide in a fixed tube. This is a very rough-and-ready arrangement, and accuracy of centring cannot be maintained as with a rack and pinion. These instruments are, however, largely used in hospitals and medical schools, and possessing one element of advantage—namely, cheapness—other considerations are often made subsidiary. Instruments of this kind are generally cast aside or disposed of in favour of an instrument having rack and pinion after a very short time, and anyone purchasing a microscope with a view of adding apparatus to it would be well advised in having one with a little less apparatus, but with a rack and pinion instead of a sliding body.

TAILPIECE AND MIRRORS.

The mirror should be hung on a tailpiece which can be swung aside. This arrangement is of great utility, as it permits of light from the lamp being directed without obstruction, through the sub-stage condenser, for critical work. A few years since it was a rule in manipulation to obtain oblique light by swinging the mirror to an acute angle with the stage for the exhibition of striæ on finely-marked diatoms, etc.; also the sub-stage itself could be swung on an arc, having its centre on the upper surface of the stage in the optical axis. In the sub-stage, various condensers were used at the point giving the most oblique ray that the objective could receive. The instrument on page 41 illustrates the arrangement. This method is not now adopted in England, the advent of the achromatic condenser having rendered it superfluous; but our American

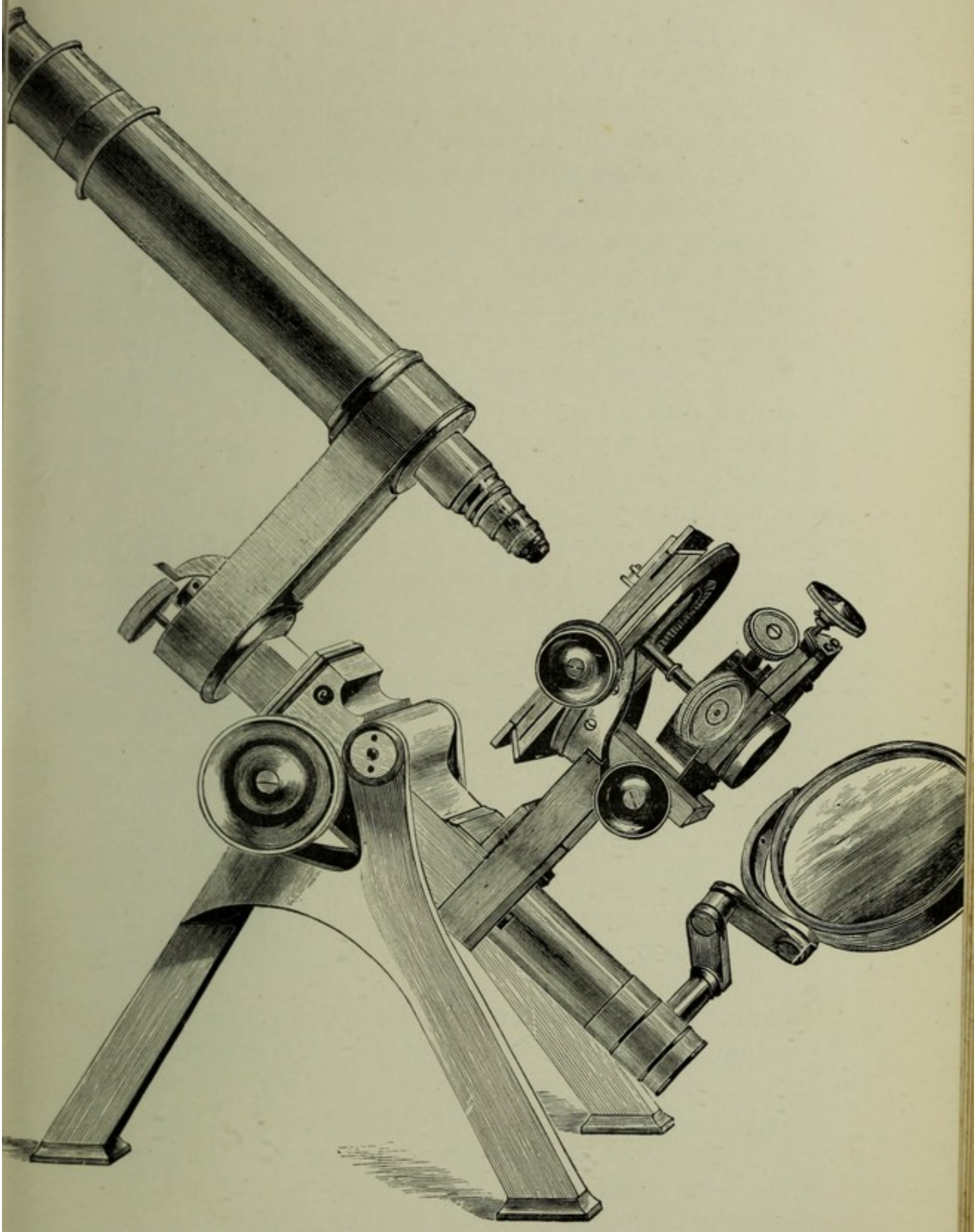

FIG. 5.—Powell and Lealand's No. 1 Microscope. A typical high-class microscope, used by nearly all the most renowned workers.

friends still cling tenaciously to it, and advocate its use. The mirror, in this form, can be brought above the stage for opaque illumination, and this element of advantage largely influences in its favour. If oblique light be desired it can be obtained to the nicest degree with a condenser in the sub-stage in the optical axis, by means of stops, with slots cut in them, placed beneath the optical combination.

The Mirrors should be plane and concave, hung in a gymbal, giving universal movements, and have a means of adjustment to focus in a vertical direction. The plane mirror is always used with the condenser, spot lens, etc., and with very low-power objectives, but the concave, when the condenser is not employed and the maximum amount of light is desired.

A constant source of trouble and annoyance is an imperfectly worked plane mirror, which will give several reflections of the image of the lamp flame. The plane mirrors usually fitted invariably do this. It should be insisted, in a microscope with which high-class work is to be done, that a parallel-worked mirror, which will give only one reflection, should be supplied. It is a trifle more costly than the ordinary kind, but the additional efficiency is very appreciable. The parallelism of a mirror may be tested by holding it just below the level of the eye in the direction of a row of objects, such, for instance, as chimney-pots; and on observing the reflections, each subject should stand out singly and clearly. If the mirror is not parallel-worked several reflections of the same object will appear superimposed in the mirror.

Care is needful in the use of the concave mirror, if the best result is to be obtained with it. It should be so arranged that the apex of the cone of rays that it transmits may be exactly in focus on the object. Many microscopes are provided with mirrors that are unsuited to the instrument, being either too long or too short in focus, and consequently do not produce good effects. To test the mirror, a piece of white paper should be placed upon the stage of the instrument, which must be set horizontally, and light

from a lamp reflected by the concave mirror on this; then, by sliding the mirror up and down on the tailpiece, it can quickly be seen if the focal point can be obtained upon the paper.

TESTING A MICROSCOPE.

The following are some points to be specially examined when purchasing a microscope: The motions should be perfectly smooth, with no lumpy feeling, and there should be no backlash. This latter can be detected best by holding gently the part that is actuated by the pinion, and then attempting to rotate the pinion. If the pinion rotates at all, or a movement of it can be detected without a corresponding motion on the movable part, there is backlash. Then, there should be no shake in any of the fittings. In a badly-constructed microscope, even when the fittings are in their most advantageous position, by holding them and shaking them slightly, a movement in the slides can be detected. The body should be racked up a considerable distance to see whether any rock or shake beyond that of the tension on the bearings can be detected. An instrument sound in construction should exhibit none whatever. The stage should be treated in the same way. A good idea of the comparative quality and finish can often be obtained by examining some hidden or unnoticed part, and observing whether the same care in finishing has been exercised there as in parts that are seen. For instance, if some microscopes be examined underneath the foot, they will be found left in the rough as cast, and merely blackened over; while another instrument will be found carefully finished in that part. It would not necessarily follow that the former was a bad instrument, but it would often be found, if taken to pieces, that there was not a careful fitting in working parts that did not catch the eye, and there would be a probability of its not proving so durable as the better-finished instrument.

BINOCULAR MICROSCOPES.

We have hitherto been treating principally of the monocular microscope, and this, it must be understood, is

the only form that can be used for critical high-power work —in fact, the Continental firms as a rule do not make binocular microscopes at all, regarding them as unnecessary. Two or three of them, however, make a binocular eyepiece, which will be found described under the head of eyepieces. The advantage of a binocular microscope is, that both eyes can be employed simultaneously, saving the strain on the vision which is apt to ensue through the constant employment of the monocular microscope, and the endeavour to see in the best manner the detail in the specimens examined. We should recommend every user of the monocular microscope to train himself to work with either eye, keeping the one not in use open; this will be found of the very greatest service. The universally understood binocular microscope is provided with a prism, designed by Wenham, which admits of the light going up a direct tube, and reflects light also into a second tube. By this means objects can be seen more naturally than with the monocular microscope, for the reason that stereoscopic vision is obtained, and objects having a certain amount of depth may be seen completely with the binocular microscope, whereas with the monocular it would be necessary to focus in successive stages through the entire depth. Especially is this true regarding opaque objects, with low powers. The stereoscopic binocular conveys an impression of the objects viewed that is almost startling in its beautiful effect. Subjects stand out in relief, exhibiting their natural contour, and at once the worker is able to decide the shape and form of an object in a way that it is impossible to do by focussing through the several planes with a monocular instrument. The binocular microscope is *par excellence* the instrument for the amateur. To him the beautiful appeals in a manner that it perforce cannot do to the scientific man, who, being intent on the pursuit of knowledge on some obscure point, has no time to notice, or if to notice, cannot linger to reflect upon the æsthetic aspect. In the examination of rotifers and other inhabitants of

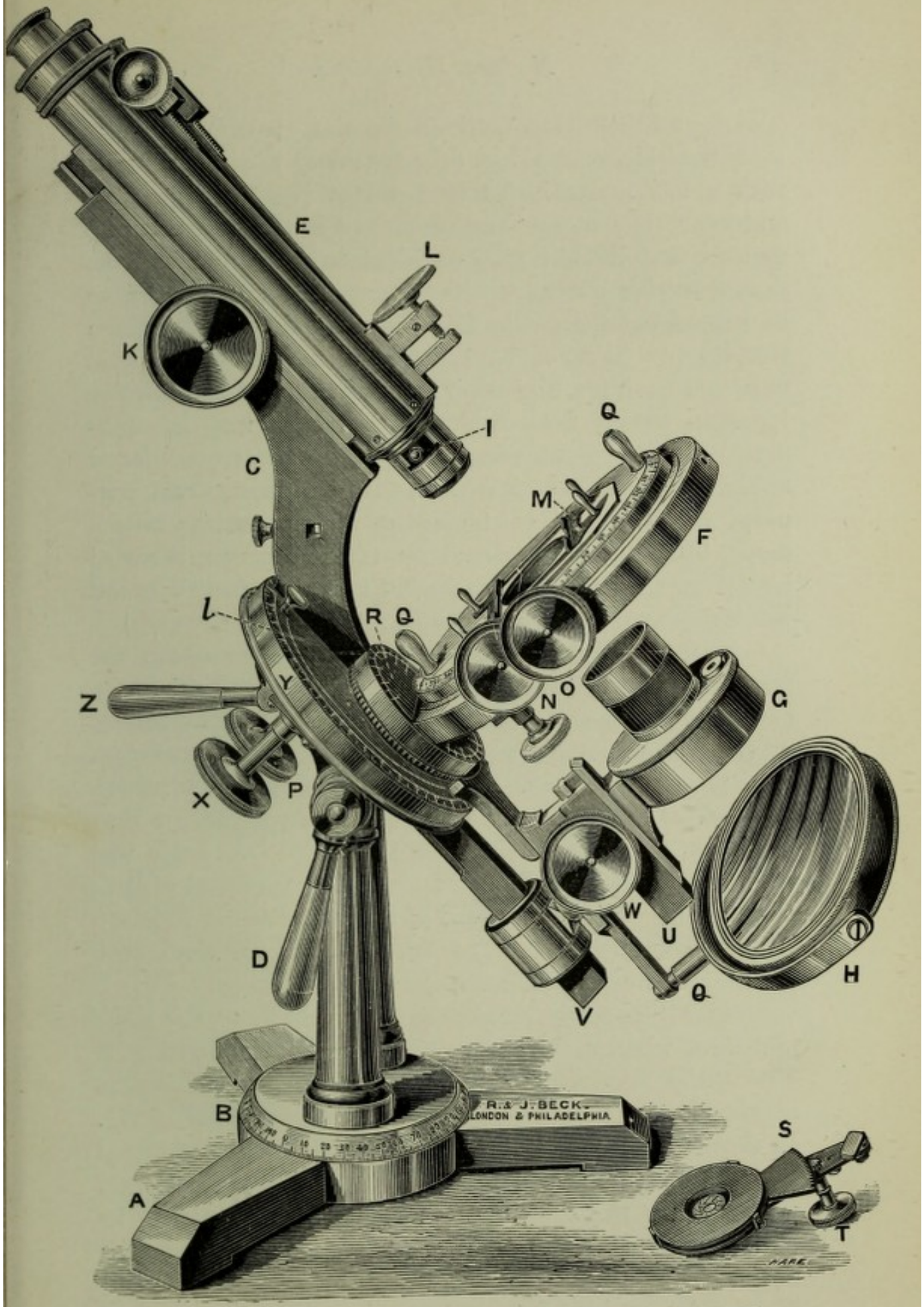

Fig. 6.—R. and J. Beck's International Microscope-stand with Swinging Sub-stage (un 'modèle de luxe').

'ponds and rock pools,' perhaps the most charming subjects that the microscope has ever revealed, the microscopist with a monocular instrument cannot possibly appreciate and interpret structure and movement in the same accurate manner that the binocular enables him to do. These facts should receive careful consideration when a microscope is to be chosen, but it must be borne in mind that the Wenham stereoscopic form of binocular cannot be used with an objective having a higher air angle than 40°. Provision is, however, always made whereby the prism may be withdrawn, and the light then only goes up the monocular or straight tube, and the instrument is to all intents and purposes as useful and convenient as the monocular microscope, while the unemployed eye of the observer is rested by looking into the blank binocular tube; the fact of its not being illuminated will scarcely be noticeable.

For use with the binocular microscope, the closer the posterior lens of the objective is brought to the prism the better. In fact, some makers have constructed objectives in very short mounts, working quite on to the prism, up to a power of $\frac{1}{8}$ inch. These, however, are not recommended. Dr. Carpenter some time since pointed out that when an objective having more than 40° air angle was employed with the Wenham binocular, spherical objects became distorted, and instead of appearing round in shape they became conical. The special apparent advantage gained is therefore useless.

Special high-power prisms are also made by two or three opticians, whereby higher powers may be employed with a binocular microscope; but we doubt whether the results obtainable with them are really worth the additional outlay. In those we have seen there has been such a very unequal illumination in the two tubes, that, personally, we should prefer using the instrument monocularly. It must be understood that all vision through the microscope in the ordinary way is inverted, that is, the object is seen upside down. A very good form of binocular microscope, devised

by Stephenson and made by Swift and Son, erects the image, or, in other words, enables it to be seen the right way up. It is well spoken of for dissecting purposes, and high powers can be employed with it; still, it cannot be described as an all-round microscope, and would have to be classed with instruments for special work. Our advice on the question of a monocular or binocular microscope is: If the instrument be required for strictly educational, scientific, or photographic work, the monocular must be chosen. The bulk of the general amateur's work is done with comparatively low powers, and in such cases the binocular is unquestionably of advantage, and to be preferred. If it is proposed to combine scientific with general work, a good plan is to have two separate bodies—monocular and binocular—interchangeable in the same bearings. The maximum facility is then at the disposal of the user. It should be noted that when the two bodies are chosen, it is well to have centring screws to the rotating stage, as described page 23, because the bodies rarely have identically the same centres, and the stage could not otherwise be made to rotate concentrically with both bodies.

DISSECTING MICROSCOPES.

These as a rule are of plain construction, sometimes fitted with simple unachromatized lenses, but in the best kinds with aplanatic lenses. The most important points in these instruments are, that they should afford convenient rests for the hands, should not be very high from the table, and be provided with a glass stage. The desirability of always using well-corrected lenses for all purposes cannot be too strongly advised. The difference in cost in this respect in a dissecting microscope need not exceed £1, and very little comparison is required for the difference to be appreciated. The Steinbeil Loupes of Zeiss are excellent lenses for dissecting purposes, or as pocket magnifiers, and can be had mounted on a plain stand, or can be adapted to most of the ordinary dissecting microscopes, particulars of which can be obtained from the various makers' catalogues.

CHAPTER II.

OPTICAL CONSTRUCTION.

Preliminary Note.

In the former part of this book we have dealt exclusively with the stand, or mechanical means of employing the optical system and accessories; and important as it is that those details shall be very efficient, it is, if anything, still more so that the eyepieces, objectives and illuminating apparatus shall be of the most perfect description, properly adapted and intelligently employed, as on the optical combinations depend the results that are to be obtained with the stand; and although care and trouble may enable a person to use a bad stand, no good stand can ever compensate in any way for bad objectives. It requires constant practice and a long apprenticeship to learn to use the microscope to the utmost advantage. Every special subject of examination calls for special manipulative treatment if it is to be correctly understood and appreciated. Experience alone can guide in obtaining the best result under varied circumstances, and that experience must be based on a knowledge and understanding of correct methods in working.

Definitions.

Some of the following terms will be made use of in this book, and are constantly met with in literature on microscopical subjects; a brief explanation of them may therefore prove of service:

Aplanatism.—A freedom from spherical aberration (see below).

Chromatic Aberration.—White light, in its passage through a lens, is decomposed into its component colours

in the same manner as with a prism in a spectroscope. These colours are refracted or bent from their original course; but red rays are not bent to the same extent as orange, and the orange rays are not so much bent as the green, and so on through the spectrum, the red rays being the least refrangible, or bending to the smallest extent. The result is that on emergence from the lens, the rays have different foci for the different colours of the spectrum, the violet coming to a focus nearest to the lens and the red at the greatest distance from the lens. The non-union of these rays in one focal-point is termed chromatic aberration.

Diaphragm.—This is generally understood in optical instruments to be a circular opening in a plate that is used to cut off the marginal portions of a beam of light, and in this sense is referred to in this book. The diaphragm is often improperly called a *stop*.

Diffraction Spectra.—If we look through a finely-ruled grating at a gas or candle flame, we shall see a large number of images of that flame having the colours of the spectrum. This effect is due to diffraction. In the microscope, according to the Abbe theory of microscopic vision, objects having fine detail diffract the light in the same manner, with the result that, unless the interstices are sufficiently wide apart for the objective to receive these spectra, the image that we see does not actually represent the real structure of the object under examination. A further note on this interesting subject by Dr. G. Johnstone Stoney, F.R.S., will be found in an appendix on page 107.

Female and Male Screws.—The former is a threaded fitting which receives a screw, and the latter a screw which goes into the female fitting. In the case of a bolt and nut, the former would have the male, and the latter the female, screws.

N.A. — Abbreviation for numerical aperture. See page 54.

Negative Eyepiece.—This is an eyepiece for examining an image formed at the diaphragm set between the two com-

ponent plano-convex lenses. The Huyghenian is the best known form of negative eyepiece.

O.I.—Abbreviation for optical index. See page 58.

Over correction is chromatic aberration in which the colours tending to the red end of the spectrum are predominant.

Positive Eyepiece.—This is an eyepiece for examining an image situated beyond the field lens. It can consequently be used as a magnifying-glass, etc.

Refractive Index.—When a ray of light passes obliquely from one medium into another of different density, the path of that ray is bent or altered in its course. The ray which enters the medium is the incident ray, and the ray that emerges from the medium is termed the refracted ray. The refractive index is obtained by dividing the sine of the angle of incidence by the sine of the angle of refraction. The angle of incidence is the angle between the incident ray and the perpendicular, and the angle of refraction is the angle between the refracted ray and the perpendicular.

Spectrum.—A band of colours produced by the splitting up of white light by means of a prism. The order of the colours is: red, orange, yellow, green, blue, indigo, violet.

Stop.—In an optical instrument this is a means of obstructing the passage of the central portion of a beam of light.

Spherical Aberration.—Rays of light passing through the marginal portion of a lens come to a focus nearer to the lens itself than those rays which pass through the centre of the lens, and the interval between the focal-points of rays which pass through the marginal and the central parts of that lens is the spherical aberration. In compound lenses this spherical aberration can be corrected for one or more special rays; and a lens so corrected is called aplanatic. It is only truly aplanatic for the particular rays for which it has been accurately corrected.

Under correction is chromatic aberration in which the colours tending to the violet end of the spectrum predominate.

MAGNIFYING POWER.

It is often supposed by the novice that magnification is dependent on the size of the instrument, and a large one is frequently described as a very powerful one. This is quite an error. Given similar eyepieces and objectives, the same magnification is obtained on a small microscope as on a large one. It is entirely dependent on the two optical parts, the objective, and the eyepiece or ocular. Under the head of 'Objectives' in the makers' catalogues it will be noticed that the powers are expressed as 1-inch, $\frac{1}{4}$-inch, $\frac{1}{8}$-inch, etc. The figures do not indicate the distance at which the lenses focus on the object, but are intended to approximately convey the actual magnifying power of the objective. To understand this description, imagine an objective to be placed so as to form an image of an object 10 inches from its back lens. Then, an objective which, when so placed, formed an image on the screen which was ten times (diameters) the real size of the object would be described as a 1-inch objective; one which formed an image twenty times the size of the object would be called a $\frac{1}{2}$-inch objective, and in general the result given, when the magnification of the image formed on the screen is divided by ten, is what is spoken of as the focal length of the objective; and conversely the focal length of an objective divided into ten gives its magnifying power—thus a 2-inch should magnify 5, a $\frac{1}{4}$-inch 40, and a $\frac{1}{8}$-inch 80, diameters.* This is termed the initial magnifying power of an objective.

The foci of German objectives are usually expressed in millimetres, 250 millimetres (about $9\frac{13}{16}$ inches) being taken as the normal vision distance, and the focal length of the objective divided into 250 gives the initial magnifying power.* Thus a 3-millimetre objective should have an initial power of $83\frac{1}{3}$, and a 4-millimetre of $62\frac{1}{2}$, diameters, and so on.

The image formed by the objective is again magnified by the eyepiece. Unfortunately, the latter is rarely marked

* A tube-length of ten inches must be used to obtain these results.

with its magnifying power, the general rule being to call the different powers by the letters A, B, C, D, etc., or 1, 2, 3, 4, etc. This is not very intelligible, and it would be far better either to express their focal power, as in the case of objectives, or to have the magnifying power in diameters marked on the cap. We will take it that the 'A' eyepiece yields a magnification of 5 diameters. When this, therefore, is used in conjunction with the 1-inch objective, which, as we have said, should have a power of 10 diameters with a ten-inch tube-length, the resultant combined power is 50; that is, the powers of the objective and eyepiece multiplied together. The method of estimating the power with short tube-lengths is referred to on page 68.

OBJECTIVES.

For our purpose we shall divide the subject of objectives into two classes—(1) the apochromatic, and (2) the achromatic. The immersion objective which may belong to either of these two classes is referred to separately. As in our remarks on objectives we shall constantly use the two terms, we will describe their reference.

Apochromatic Objectives.—The introduction of these objectives by the firm of Carl Zeiss, of Jena, Germany, placed the science of microscopic optics on a far higher level than had hitherto been attained, and as a result many of the traditional modes of working have been altered. Greater precision has been necessitated in the microscope-stand, and the provision of sub-stage condensers of corresponding optical quality to the objectives has been essential. Messrs. Zeiss were granted a subsidy by the German Government with a view to the promotion of optical research, and aided by this they were able to produce several varieties of new optical glass. The employment of these new glasses in conjunction with fluorite, based upon the careful and elaborate calculations of Professor Abbe, resulted in the production of apochromatic objectives. In these lenses, aberrations which were inherent

in the older systems were eliminated or minimized—that is to say, the secondary spectrum was practically removed, and spherical aberration was considerably reduced. The objective, therefore, produced, to all intents and purposes, a colourless image. Higher apertures were obtainable, and in consequence of the improved corrections, accompanied by greater brilliance of the field, the use of eyepieces of high power was rendered permissible and advantageous.

The new kinds of glass were placed at the disposal of opticians throughout the world, and the apochromatic objectives have been since manufactured by other firms, and notably by Messrs. Powell and Lealand, of London, whose productions compare favourably with the best of the originators' lenses.

Special eyepieces, termed 'compensating oculars,' are necessary when using the apochromatic objectives. They will be found described on page 67.

ACHROMATIC OBJECTIVES.—All objectives that are not actually comprised in the apochromatic category—that is, in which the secondary spectrum is not eliminated—are included under this heading. So far as the principal opticians are concerned, it comprehends a better class of objectives than it did at the period when apochromatic lenses were introduced. By the use of the new optical glasses previously referred to, and in consequence of keen competition amongst manufacturers, a new class of achromatic objectives, tending towards apochromatism, has been introduced. In many of these the secondary spectrum and the spherical aberration are diminished, but not altogether removed, and in one or two instances they almost vie with the apochromatics in performance.

ACHROMATIC *versus* APOCHROMATIC OBJECTIVES.—In view of the foregoing facts, it will be well to consider which series of objectives should be selected for specific work. In the first place, the apochromatic objectives are very expensive, and, generally speaking, are beyond the reach of the ordinary amateur, who, taking up micros-

copy without special scientific aims, endeavours to find his recreation in the minute, and excepting to a trained critical eye they would not be found to possess the extraordinary merit that is claimed for them. The question, therefore, naturally occurs, 'Is it worth while paying four times the price of a good high-class *achromatic* objective for an *apochromatic* objective, and in addition to this the incident cost of special eyepieces?' In reply, we would say, 'Yes' and 'No.' Yes, if the intending purchaser proposes to do his work on the most exact and highest scale, and to conduct original research; No, if his aim is only to examine into nature's small things without attempting to obtain the apparently impossible in results, or to detect structure not hitherto discovered. We would here quote from the words of Professor H. L. Tolman, President of the Illinois State Microscopical Society: 'Of the apochromatic objectives and their comparative advantages with the ordinary highest-class lenses, it must be remembered that the difference in degree between the performance of the best American and English achromatic lenses and the Zeiss apochromatics is often only detectable by the experienced eye; and, secondly, that the vast bulk of microscopical work is done with medium or low powers, where the diffracting beams play a much less important part proportionately, the image being chiefly dioptric. As to the first point, the writer may even go farther, and say that it is a contested point among many of the highest authorities in America whether the Zeiss apochromatics have *any* marked practical superiority. The student may rest contented, therefore, that he can work for an indefinite time with any good American or English lenses without losing his time, or running any serious risks of making mistakes in what he sees.'

This opinion is borne out to a considerable degree by many of the most careful and practical observers in the microscopical world. Still, as before remarked, if original research is to be conducted and the very finest results

are to be obtained, and the minutest structure revealed in the plainest manner, the apochromatics must be selected.

There is one other point which should influence in the selection of objectives. On account of the reduction of spherical aberration, accompanied by the greater brilliance of field of the apochromats, a higher power of eyepiece can be advantageously employed with these than with the achromatic objectives. Few, indeed, are the specimens of the latter that do not commence to break down with an eyepiece having an initial power of 20 diameters. This is not the case with the apochromats. A series of compensating eyepieces is specially designed to work with them, having powers varying from 2 to 27 diameters. Supposing, therefore, we were working with a $\frac{1}{4}$-inch apochromat having an initial power of 40 diameters, with a ten-inch tube-length, we could by means of the searcher eyepiece ($\times$ 2) obtain a magnification of 80 diameters, and by using intermediate powers of eyepieces up to the $\times$ 27 produce any magnification that might be desired from 80 to 1,080 diameters ($\frac{1}{4}$-inch initial power of 40×27 eyepiece power $= 1{,}080$).

Further, these special eyepieces are all designed to work in the same focal plane at the tube-length for which the eyepieces and objectives are designed, with the result that practically very little refocusing is necessary on the exchange of an eyepiece during an observation. By this means the magnification with a low power objective having a long working distance and a fairly high N.A. for its power, as possessed by all of the apochromats, of Messrs. Zeiss' manufacture, can be gradually increased, and the advantage gained is one for which many microscopists sighed before the days of apochromats—namely, a wide range of magnifying power and great working distance. For many classes of work this convenience is very great. The best of the achromatic objectives will stand a fairly high eyepiece power, say up to 16 or even 20 diameters, but usually not in the perfect manner that the apochromats

do. The Huyghenian eyepieces that are used with the achromatics are very rarely designed to work one after the other in the same focal plane, with the result that it is necessary to refocus every time the eyepieces are exchanged, and the higher the power of the eyepiece that is employed the closer will the objective work to the object. The special convenience derivable with the apochromats is, therefore, practically non-existent with the achromatic objectives.

Magnifying power, however, is not the only feature to be considered with regard to an objective; there must be a power of delineating detail. This latter quality is dependent on the numerical aperture of the objective, referred to on page 54.

Immersion Objectives.—In using these objectives a film of a specified fluid is interposed between the front lens of the objective and the cover-glass of the object under examination, so that continuity is established between them. There are two media that are in regular use, viz., water and cedar-wood oil. Others, including glycerine and mono-bromide of naphthalin, are, however, occasionally employed. It may be taken that when a lens is referred to as a 'homogeneous or immersion' objective, that cedar-wood oil, or a mixture, of which that oil is the principal ingredient, known as immersion oil, is the correct medium for using with that objective. The refractive index of cedar-wood oil is about 1·52, and practically the same as crown-glass, consequently, when it is used for immersion purposes it has the effect of rendering the cover-glass part of the objective.

The question naturally arises, What advantage is gained by the use of an immersion medium? In reply, it may be briefly stated that the resolving power of the objective, the brilliance of the image, and the working distance, are all increased.

It is a well-known law that rays passing from a rarer to a denser medium are refracted towards the perpendicular, and *vice versa*. If, therefore, an object be examined with a dry objective, it is obvious that certain rays of light emerg-

ing from the denser crown cover-glass into the rarer medium, air, are refracted from the perpendicular, and so fail to assist in forming the image. By placing a medium between the cover-glass and the objective, these rays are utilized, owing to the influence of the dense medium, oil. The refractive index of air is 1·0, that of water 1·33, while that of cedar-wood oil is 1·52. It will be seen from this, that the utility of the oil must be very appreciable, in fact, an oil immersion lens receiving light at 82° and a water immersion lens receiving light at 96° admit the same rays as a dry lens of 180°, and, therefore, divide as many lines to the inch as the maximum number possible with a dry lens. If immersion lenses have greater apertures than the above named they will divide finer markings than any dry lens, and they can be theoretically carried to oil and water angles respectively of 180°.

There is another feature of advantage gained by the use of an oil immersion lens. The refraction caused by the influence of the cover-glass thickness referred to on page 59 does not take place, on account of the continuity established between the objective and the cover-glass by the immersion oil. There is, therefore, no necessity for such objectives to be provided with a correction collar for variations in thickness of cover-glass; a slight correction of the same kind has, however, sometimes to be made on account of the distance which the object may be beyond the cover-glass when the mounting medium has not the same refractive index as the cover-glass. This can be efficiently effected by either extending or shortening the body-length. Water immersion objectives do not yield so high an aperture as the oil immersions, and as the immersion medium is not of the same density as the cover-glass a correction collar is essential, but there are subjects with which oil could not be suitably used, and in such cases the water immersion lenses have to be chosen.

Mono-bromide of naphthalin is, at present, only used with one form of objective, a $\frac{1}{10}$ inch, by Carl Zeiss, of Jena,

having a numerical aperture of 1·63. The refractive index of this medium is 1·657, and special flint cover-glasses of the same density have to be employed with it. This restriction, together with its high price—£40—has prevented its being largely used. Those who have had an opportunity of working with one have spoken in high terms of the beautiful effects it yields.

It must be borne in mind that objectives that are intended to be used immersed are specially corrected for the specific medium to be employed. Ordinary lenses intended for use dry cannot be advantageously worked immersed.

APERTURES OF OBJECTIVES—ANGULAR AND NUMERICAL.

For reasons which will be stated hereafter, it will be seen that on the aperture possessed by an objective depends the fineness of detail that it is capable of delineating, that is, the number of lines per inch that it will separate.

Angular Aperture.—Before the introduction of immersion objectives the ability of an objective to resolve fine structure was known to be dependent on the angle formed by the extreme rays issuing from the object that could be received by the objective. This, which was called the angular aperture of a lens, is, in other words, the angle of the cone which envelopes the pencil of light that is received by the objective from a point on the object.

As we have stated in the description of immersion lenses, an oil immersion lens receiving light at 82°, and a water immersion, receiving light at 96°, would each divide as many lines to the inch as a dry lens having the limiting aperture of 180° (which in practice can never be quite reached), and as the immersion lenses can theoretically be carried to oil and water angles respectively of 180°, it is obvious that in order to express the efficiency of such objectives a notation must be employed which takes cognizance of the medium which surrounds the front of

the objective, and the result it has in the formation of the image. This is achieved by means of the system termed NUMERICAL APERTURE, which was introduced by Professor Abbe. This expresses the efficiency of an objective to allow pencils of light to pass so as to include them in the light forming the image. Numerical aperture is expressed in the formula $n \sin u$. n signifies the index of refraction of the medium by which the objective front is enveloped, and u equals half the angle of aperture. Therefore, by multiplying the sine of the semi-angle of aperture by the refractive index of the medium in which that angle has been measured the numerical aperture ($n \sin u$) is obtained.

It follows from this that the greatest value which the numerical aperture can have in the case of dry lenses is unity, corresponding to an angular aperture of 180°.

If we are aware of the numerical aperture of an objective we can readily ascertain the number of lines per inch or millimetre which it is capable of dividing, or, in other words, its extreme power of resolution. The formula is,—twice the numerical aperture, multiplied by the wave-frequency* of the light used, equals the extreme number of markings per inch or millimetre, according as the calculation may be made, that the lens will resolve.† Conversely, if the extreme limit of resolving power be known, the number of lines per inch or millimetre that it will separate, divided by the wave-frequency of light used, equals twice the numerical aperture.

These calculations are based on the assumption that annular or some other form of oblique illumination is used. With a solid cone of illumination equal to the numerical aperture of the objective no fine detail is visible; it

* The wave-frequency is the number of waves contained in an inch or millimetre, according to which measure is used.

† The mean wave-length of white light is ·5269 μ (=48,200 to an inch). Taking the numerical aperture of an objective as 1·0 N.A., and for this purpose doubling it, we find that with the aperture of 1·0 N.A. 96,400 lines (about) per inch can be resolved with white light (48,200 × double the numerical aperture = 2, produces 96,400).

becomes blurred, and, in practice, when using solid cones of illumination, it is usual to make them fill ¾ only of the back lens of the objective. This will be found treated on page 83, in connection with condensers.

Under these conditions the number of lines per inch that will be resolved by the objective will be ascertained by multiplying the wave-frequency of the light used by ½, and then multiplying the product by the numerical aperture.†

THE APERTOMETER.—To enable the numerical apertures of objectives to be taken without a calculation, Professor Abbe devised the apertometer. It consists of an almost semi-circular plate of crown-glass, having the diametrical

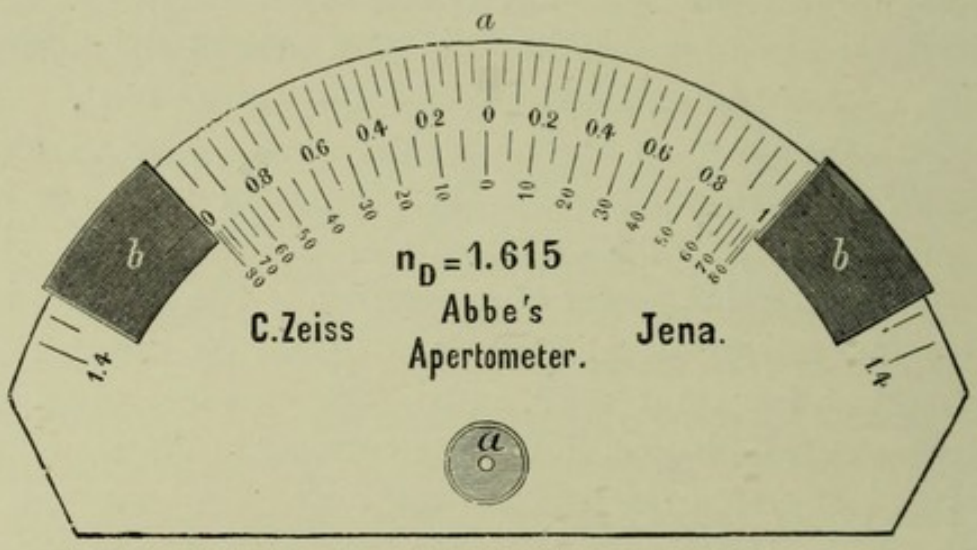

FIG. 7.—Abbe's Apertometer.

edge ground to an angle of 45°, while the circumference is a polished cylindrical surface. It is shown in Fig. 7.

The centre of the semi-circle is marked by a silver disc, *a*, having a very small central aperture, and on the upper surface on the periphery it is provided with a scale of divisions, indicating both angular and numerical apertures. The manner of using the apertometer is as follows: The microscope is placed in a vertical position, and the apertometer is laid on the stage, with the diametrical edge towards the limb of the instrument. The objective that it is desired to take the aperture of is then screwed

* E. M. Nelson, Journal of the Royal Microscopical Society, 1893, p. 15.

on, and the objective focused on the plain centre of the silvered disc. It is well now to fix the apertometer to the stage, either by springs or an elastic band, to prevent its moving. The two pointers, *b*, are then set on the edge of the circle to read zero. The draw-tube and eyepiece with which the silvered disc has been set are removed, and at the lower end of the draw-tube a special objective of low power, that is supplied with the apparatus, is screwed into the universal thread, which should be there fitted in all microscopes of high-class.

The cylindrical edge of the apertometer is then illuminated in front and at the sides by means of bull's-eye condensers and lamps, or, if daylight is available, it will be easier to get uniform brilliance all over the field by placing the microscope on a table in front of a window, and using bull's-eye condensers to increase the light.

The draw-tube carrying the special objective and the eyepiece is then replaced in the body of the microscope and the back lens of the objective that is being examined is brought sharply into focus by means of the draw-tube, when it will be found that the image of the pointers *b* in Fig. 7 are in the centre of the field, being reflected by means of the prismatic diametrical edge of the glass plate. While looking through the microscope these pointers are then each moved separately, in opposite directions, round the outer edge of the apertometer, until they are set exactly on the extreme margins of the back lens. The reading is then taken by the divisions on the face of the apertometer against which the pointers have now arrived. In the case of an oil or water immersion lens, the medium must, of course, be placed in front of the object-glass during the examination.

There are several further matters of importance that it would be well to consider.

Numerical Aperture and Power.—As we have before remarked, magnifying power is not the only quality necessary for the observation of minute structure. The power to delineate fine detail is still more dependent on the numerical

aperture of the objective. It has been explained by Dr. Dallinger in 'Carpenter on the Microscope,' and will be evident from a consideration of the preceding remarks concerning numerical aperture, that two objectives—one of much greater magnifying power than the other, but both having only the same numerical aperture—will only divide the same amount of detail, the higher power only exhibiting it on a larger scale. That is, supposing with a $\frac{1}{4}$-inch objective of 0·90 N.A. certain structure were presented, and then a $\frac{1}{8}$-inch objective with just double the magnification, but with the same N.A., were afterwards used, there would be no further power of resolution in the $\frac{1}{8}$ than in the $\frac{1}{4}$. It might be possible to make an objective of very low power of sufficiently high aperture to divide very minute details, but this would be useless unless the objective would bear a sufficiently deep eyepiece to enable the human eye to see it. It therefore becomes necessary that a ratio of aperture to power should be established. Mr. Nelson has suggested that a standard, termed the 'optical index' (O.I.), should be adopted for this purpose, to indicate the numerical aperture that should be given to an objective, if it be intended that the eye should see in the image as fine detail as it could divide in a real object of the same size. It is ascertained by multiplying the numerical aperture of an objective by 1,000, and dividing by the initial magnifying power of the objective.* If a microscope is required to show all that keen eyes are able to appreciate, then 0·26 N.A. must be given to it for every 100 diameters of magnification. If we limit the power of the eyepiece of such a microscope to 10, then the objective must have 0·26 N.A. for each 10 diameters of *initial* magnifying power. The optical index, therefore, of an objective which, with an eyepiece magnifying 10 diameters, will yield all that it is possible for a normal eye to appreciate, will be 26·0. In practice it is found possible to employ eyepieces giving higher magnifications than those men-

* E. M. Nelson, Journal of the Royal Microscopical Society, February, 1893.

tioned in Mr. Nelson's rule, and these would of course enlarge the image.

Although large apertures are the pride of those whose ultimate ambition in matters microscopical seems to be bounded by the endeavour to resolve the markings upon diatomaceous frustules, it is doubtful whether for the ordinary amateur there is a real necessity for the extremely large apertures. Lenses having such, require great skill and care in manufacturing and adjusting, and are consequently expensive, and if the ordinary work of an amateur is proposed to be conducted, and not original scientific research, objectives of medium aperture will usually be found to meet his requirements thoroughly.

THE INFLUENCE OF THE COVER-GLASS.

As a rule, objectives are corrected for a specified thickness of cover-glass, which is placed over the object to protect it. These cover-glasses, however, vary considerably in thickness, and consequently by refraction disturb the corrections of the objectives. An objective which gives crisp definition when an object that has no cover-glass to it is being viewed,

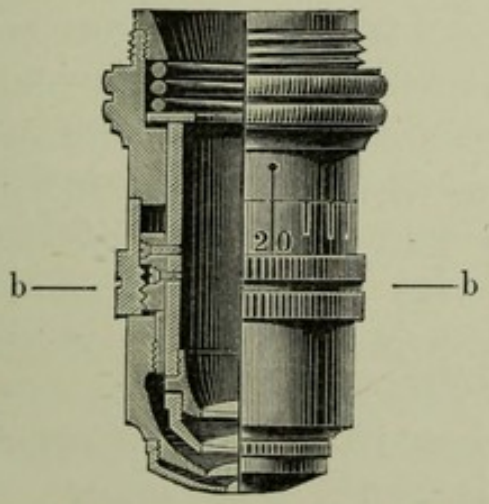

Fig. 8.—Correction Collar (Zeiss).

will not define so clearly if a thin one be applied, and the greater the thickness of the cover-glass the more will the image be deteriorated. Low powers are not so sensitive to this influence as high ones. There are two means of correcting this. Most dry objectives having a numerical aperture

of, or exceeding, N.A. 0·6, have what is termed 'a correction collar,' whereby the two back combinations of the objective are removed farther from, or brought closer to, the front lens or lenses. Fig. 8 shows the manner in which this is effected in one of Zeiss's lenses. bb is the correction ring, by turning which the distance between the upper lenses and the two lower lenses is varied. With such a correction collar a worker is undisturbed by thickness of cover-glass, because he has within certain limits the means at his disposal in the objective itself for correcting same. The use of this correction collar almost requires a personal demonstration, and to set it at the exact point that yields the best result is a matter of extreme delicacy, which can only be accurately done as the result of experience and with the aid of a critical eye. On this account it can hardly be recommended to students, because they will not usually afford the time and trouble necessary to get such perfect results; consequently there is a growing tendency, except in the apochromatic objectives, to have the lenses mounted in a rigid setting, corrected for a specific tube-length and thickness of cover-glass. With the fixed setting, if a different thickness of cover-glass be used than that for which the objective was designed, correction can be made by altering the tube-length of the microscope. This has the same effect as altering the distance between the lenses. Supposing we had an objective adjusted for a 6-inch tube, with a 'B' eyepiece, to a cover-glass 0·008 inch thick (this is about the average thickness adopted by opticians), and we wished to examine an object having another thickness of cover—say 0·005—we should at once notice that the performance was not so good, and in order to improve it we should have to make the body longer. This difference of cover-glass thickness, with a good $\frac{1}{6}$-inch objective, would necessitate a 10-inch body. On the other hand, if the cover were 0·01 inch thick, the length of the body would have to be diminished below that for which the objective had been designed, to obtain the best results—that is, for a thin cover the body-tube would have to be lengthened,

while for a thick one it would have to be shortened, and the finer the quality of the objective the more sensitive would it be to cover-glass thickness. This system of correcting by draw-tube, however, has one drawback, and that is, that the power is varied in correcting, and, of course, the focus is altered. From the considerations here named, it will be found advantageous if the microscope be provided with a means of lengthening the body by draw-tubes to 12 inches, and on the other hand, when the draw-tubes are closed, of having the body shorter than the Continental length (6 inches). In order that the best adjustment may be made, it is essential that one of the draw-tubes be actuated by rack and pinion, and the convenience of this arrangement cannot be too strongly urged upon microscopists. Messrs. Baker, and Watson and Sons have adopted it in their large models, and it has evidently met with considerable appreciation, for it is now made in a cheaper form by the latter firm and Messrs. Swift and Sons for their students' instruments, and anyone with a limited purse, but who wished to do his work in the most precise manner, could have it in an inexpensive form of instrument.

The difference between an objective designed for a 6-inch and that for a 10-inch tube, is, that in the latter case the back combinations of the objective are brought closer to the front lenses; this gives a slightly increased aperture. A large number of the commercial objects and the majority of cover-glasses that are purchased are more than 0·008 inch thick; 0·008 inch is a medium thickness of cover-glass, but the tendency is to use thicker ones. It will be found a great advantage to buy only such objectives as are corrected for the 10-inch tube, and having the rackwork before referred to fitted to the microscope tube, sufficient latitude would still be allowed if a thinner cover-glass were met with; but it would often be found necessary to close the draw-tubes down to 6 or 7 inches, in order to get the best correction for the thick cover-glasses that are commonly used.

We may here clear up another question that occasionally

arises. If a $\frac{1}{6}$-inch objective is corrected for a 6-inch tube-length, it does not give an initial magnification of 60 diameters at 6 inches. The powers of all objectives are calculated for a 10-inch tube-length, therefore the full total benefit is not obtained from an objective when used at 6 inches, but only six-tenths of it. Of course, with the lessened magnification at 6 inches a brighter field is produced, and a deeper power of eyepiece is found permissible. This is rather an important item in testing an objective, because an objective at 10 inches would be yielding about two-thirds more magnification than at 6 inches, and its powers would be much more severely tested than if employed at 6 inches.

It would be a great advantage to the microscopist if opticians would mark exactly the focal power and precise numerical aperture of their objectives upon them. In order that objectives might appear to have a large ratio of aperture to power, they are often put forward as possessing a considerably lower power than they actually have. For instance, a so-called 1-inch often turns out to be nearer $\frac{2}{3}$-inch, $\frac{1}{2}$-inch about $\frac{4}{10}$-inch, $\frac{1}{5}$-inch to have the power of $\frac{1}{6}$-inch, and $\frac{1}{12}$-inch in some instances to be $\frac{1}{14}$-inch. It has become such an acknowledged fact that the act of misrepresentation involved seems to be condoned. This is a state of things which should not be. Opticians must be aware of the misdescription and the immensity of trouble that is caused by it. We must, therefore, advise microscopists not to rely on the powers marked on their objectives, but to ascertain them for themselves, and the best way to do it is to project the image of a micrometer, without any eyepiece in the body-tube, on a screen 20 inches distant from the thread of the objective. Measure with a foot rule the distance apart of the lines so projected, and supposing that each hundredth of an inch measured on the screen 1 inch, that would represent a magnification of 100 diameters; reduce the result to the 10-inch tube-length by dividing by two, and the result would be approximately 50 diameters, or a $\frac{1}{5}$-inch objective.

TESTING OBJECTIVES.

It is a somewhat difficult matter for the novice to decide for himself as to the quality of object-glasses. Such work needs experience, judgment, and a critical eye. The writer has met with people who have not been able to distinguish the difference in performance between an uncorrected single French lens and a first-class achromatic. This, of course, was due entirely to a lack of that perception of microscopical detail which can only be acquired by intimacy with objectives and their qualities. Especially is this true in lenses of the highest grade. We propose, therefore, to give a few hints which, if not of so much use in the initial stage, may be of aid at a later period.

Flatness of Field.—This is an important feature in objectives of low power, and the best method of testing for this quality is either by examining the ruled lines of a stage micrometer or the surface of a piece of ground glass. With the higher powers this feature is sadly neglected, especially in lenses of Continental make, it being advocated by one or two leading workers that it is better to get the utmost perfection of definition in one central point rather than that definition should be sacrificed for flatness of field. It would certainly be a great advantage to the microscopist if the two points could be combined in a more satisfactory manner than at present. English manufacturers in the high powers generally provide a flatter field than their Continental contemporaries.

Colour.—Dr. Carpenter's old test for achromatism—the examination of the cells in a thin section of deal—will give a very good idea of the colour corrections of objectives. For high powers, the markings on a frustule of the diatom "Pleurosigma formosum" are an excellent test. With the apochromatic objectives these come out quite black and white, while with those of the achromatic series any outstanding colour is at once revealed. Another method is the mercury test adopted by opticians. A small globule of mercury is placed on a slip of ebonite, and a piece of whale-

bone or watch-spring is made to snap on it, causing the globule to split up into numerous particles of exceedingly minute size. These globules are then examined with the objective, and are usually illuminated by means of a bare gas-jet. The Abbe's test-plate made by Zeiss is designed specially for testing lenses for spherical and chromatic aberration, and also for ascertaining the thickness of cover-glass, for which the objective is designed and through which the objective works at its best. Full instructions are provided for working, with each test-plate, but it requires some considerable knowledge of microscopical technique to use it advantageously.

DEFINITION.—For objectives varying in power from 2 inches to $\frac{1}{2}$ inch, nothing is better as a test than the proboscis of a blowfly. The spines in the central portion of the tongue should each show a well-defined point. For high-power objectives the internal markings of Triceratum and Pleurosigma angulatum, also the markings on the scales of Podura (Lepidocyrtus curvicollis), are the most suitable. It is always well to test an objective with an eyepiece of high power. Generally speaking, its performance under such circumstances is an indication of the presence or absence of spherical aberration. The freer an objective is made from spherical aberration the higher will be the power of eyepiece that it will bear.

CHOICE OF OBJECTIVES.

The best objectives for a novice at starting would be 2-inch, 1-inch, and $\frac{1}{6}$-inch. The 2-inch will be found extremely useful for large specimens, while the 1-inch, which is considered the working-glass of the average microscopist, will with a higher power—namely, the $\frac{1}{6}$-inch—show him some of the minuter detail which sooner or later he will wish to make himself acquainted with. If more object-glasses than these be required, we should recommend the $\frac{1}{2}$-inch as an intermediate between the 1-inch and the $\frac{1}{6}$-inch, and for a higher power a $\frac{1}{12}$-inch oil immersion

objective should be added. It is well to buy only such low-power objectives as have double combinations. Some of the cheaper ones are composed of two or three lenses balsamed together only; with these there is an insufficiency of aperture, and good definition and flatness of field cannot be obtained. All the best low-power lenses are constructed with two pairs of lenses set a little distance apart, and can be readily recognised. Of the apochromatic series of Zeiss our choice would be the 24, 12, 6, and 3 (1·4 N.A.) mm. objectives. The 12 and 3 mm. are considered the finest of the series. All of them should be corrected for the 10-inch tube. Messrs. Powell and Lealand supply a $\frac{1}{12}$-inch apochromatic oil immersion objective of 1·4 N.A. which enjoys special favour, the price of which is only £10. Of achromatics that approach the apochromatics in performance the 8 mm. by Reichert and his 4 mm. (1·3 N.A.) homogeneous immersion are very fine, especially the latter. We have worked with both of these, and they have given most excellent results. The No. 6 and $\frac{1}{12}$-inch of the same maker are also extremely good. Leitz, of Wetzlar, is the maker of a very good series of achromatic objectives also. The productions of American opticians are but little known in England, yet they are said in many instances by competent judges to be of exceptional quality, Messrs. Bausch and Lomb and the Spencer and Smith Optical Company having high reputations for their lenses.

Of course, different requirements would necessitate the selection of special objectives, but a practical microscopist or any microscope manufacturer would be able to advise on the matter.

EYEPIECES.

The eyepiece commonly used with the microscope is what is termed the Huyghenian form, which consists of two plano-convex lenses placed at a distance apart equal to half the sum of their foci, with a stop in the principal focus of the eye-lens. This will be found to meet all ordinary requirements of microscopical work with achromatic objectives. Eyepieces

vary in power, and these powers are usually designated by the letters A, B, C, D, etc., A being the weakest power. On the Continent they are generally designated 1, 2, 3, 4, etc., while some firms express their power in focal units—for instance, an eyepiece having a power of 10 would be 1 inch. This last method, or that adopted by Zeiss for the compensating eyepieces, where the actual magnifying power is engraved on the cap of the eyepiece, is the only rational one. The letters A, B, C, etc., or Nos. 1, 2, 3, etc., convey no real idea of the magnifying power of the eyepieces, because each maker has his own formula for each eyepiece, and there is no correspondence in the powers of one eyepiece marked 'D' by one maker, and that supplied by another. It is often remarked that the Continental objectives stand a stronger power of eyepiece than the English, and on this account a superiority has been claimed for them; but it should be borne in mind that English manufacturers give in many instances as deep a power of eyepiece as 20 or 25, whereas Continental manufacturers rarely supply them of greater magnifying power than 10 or 12 diameters. In the English series the variation in power between two consecutive eyepieces is generally greater than in the Continental series. A comparison, therefore, between the merits of an English object-glass tested with, say, a 'D' English eyepiece and a Continental object-glass of the same power tested with a No. 4 eyepiece of Continental make would not be fair, as the former, having a deeper-power eyepiece on it, would be liable not to give such perfect results as the latter. And here we may mention that, although people very often buy deep-power eyepieces, it is advisable, with ordinary achromatic lenses, that no stronger power should be used than an eyepiece giving an initial power of 15 diameters. The best eyepiece for general purposes

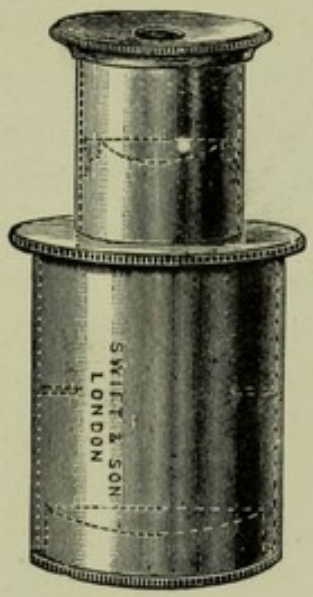

FIG. 9.—Huyghenian Eyepiece.

is the 'B.' This gives a convenient size of field, and is by far the most comfortable to work with of the whole series. Next, and in addition to this, we should recommend either the 'C' or the 'D.'

Microscopists having abnormal vision, and preferring to work without spectacles, should have an auxiliary cap made to fit over their eyepieces, carrying a lens of the power that corrects the error of vision. This is specially necessary where measuring has to be done, or where the microscope is arranged for a second person's inspection.

It is also convenient, especially in examining the back of the objective to observe diffraction phenomena, cones of illumination, etc., that a 'dummy' eyepiece be employed, that is, an ordinary eyepiece mount having no lenses in it. The aperture in the cap must, however, be a very small one.

At times it is desired to know whether an eyepiece can have its diaphragm enlarged so as to give a larger field. An easy method of ascertaining how much of the field lens is employed is to make a spot with ink near the margin, on the convex side of the field lens, and on placing the eyepiece in the microscope, if the diaphragm has a sufficiently large aperture, the ink will be visible; if not, it may be enlarged until it appears. The diaphragm should not be so large as to admit of more than the edge of the field lens being visible.

Compensating Eyepieces.

Under the description of 'Apochromatic Objectives' on page 51, reference is made to compensating eyepieces. These are specially designed to correct an outstanding colour defect (under correction) which is inherent in all high-power objectives, whether they be apochromatic or achromatic, on account of the peculiar construction of the front lens. For the sake of uniformity of eyepiece, Zeiss imparts the same colour effect to the lower-power lenses of the apochromatic series. The eyepieces, then, have an equal error of the opposite kind (over-correction), and when

the objective and eyepiece are combined, a perfect correction is obtained.

The apochromatic objectives are 'under-corrected,' while the achromatic objectives of low power are 'over-corrected.' The compensating eyepieces for the former are over-corrected, and the Huyghenian eyepieces for the latter under-corrected. With low powers of the achromatic type, the compensating eyepieces are disadvantageous, but with high powers, where the defects caused by the hemispherical front lens give rise to error identical with that in the apochromatic objectives, and which the compensating eyepieces are designed to overcome, these special eyepieces can be employed, but the result is not sufficiently beneficial to justify the purchase of them for use with high-power achromatic objectives.

A most advantageous feature is imparted to the Zeiss compensating eyepieces. They are all designed to work in the same focal plane, so that when two eyepieces of this series of different powers are interchanged in the body of the microscope no alteration in the focusing is necessary.

A number is engraved on each eyepiece, which, multiplied by the initial magnifying power of the objective, will, when used at the tube-length for which the eyepiece is designed, give the magnifying power that is being employed.

The compensating eyepieces designed for the 6-inch tube-length can be used on the 10-inch tube, and those for the 10-inch at the 6-inch tube-length, without detrimental effect; but in the former case about half must be added to the product of the multiplication of the power of the objective and eyepiece, and in the latter case about one-third must be deducted.

Kelner Eyepieces.

This is an achromatic form of eyepiece, giving an exceedingly large field, which is considerably used for the examination of animalculæ, pond life, etc. A certain amount of definition is, however, sacrificed in working with it, and although occasionally of use, we should recom-

mend the microscopist, before purchasing any, to judge for himself as to the desirability or otherwise of his having them; they are not by any means necessary adjuncts. We may mention that they give very good results photographically.

Projection Eyepieces.

These were designed specially for projecting objects on the screen and for photographic purposes. They give an exceedingly small field, but an exquisitely sharp one. In order to obtain good results with these, it is necessary to alter the position of the eye-lens, until the image of the diaphragm appears sharply projected upon the screen. For this purpose the eye-lens is mounted in a tube having in it a spiral slot, permitting of the eye-lens being moved to and fro with great precision. They are usually made in four powers, magnifying 2 and 4 diameters respectively for the short tube-length, and 3 and 6 diameters for the English tube-length. The most serviceable are the 4 for the short tube, and the 3 for the long tube. All photo-micrographers of note use these eyepieces, but as they have the same corrections as the compensating eyepieces, they cannot be employed with low-power ordinary achromatic objectives. With the higher powers, however, from, say, $\frac{1}{6}$-inch of N.A. ·80, they work well. For photographing with ordinary objectives of low power, the 'A' eyepiece gives good results.

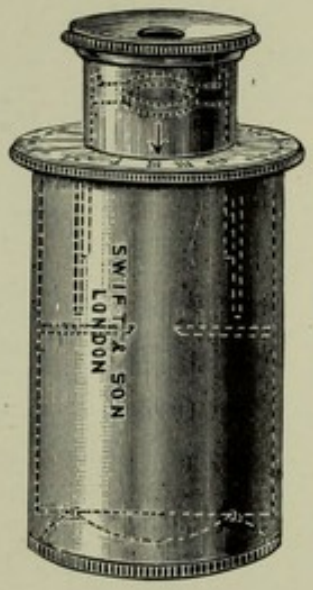

Fig. 10.—Projection Eyepiece.

Binocular Eyepieces

As we have mentioned under 'Binocular Microscopes,' most of the Continental firms do not make the binocular microscope. Therefore, for those who desire to be able to employ both eyes, they make a binocular eyepiece, but only for the Continental length of tube, and this should be particularly understood. The one with which we are

acquainted is that designed by Abbe, and manufactured by Zeiss, as shown in Fig. 11. It gives stereoscopic effects and most beautiful results with both high and low powers. If this were mounted in some lighter manner it

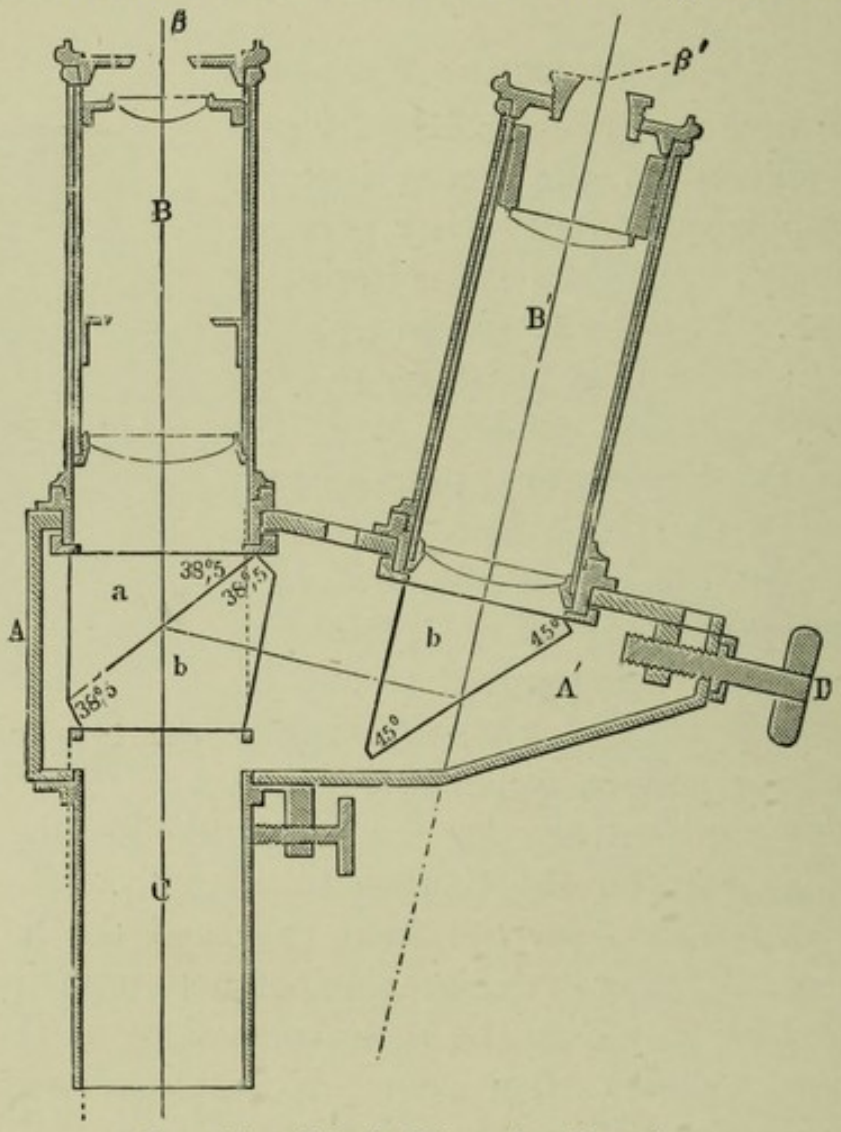

Fig. 11.—Zeiss's Binocular Eyepiece.

would perhaps become more generally used; its weight is very much against it when working with high powers; still, for the advantages it affords it is an adjunct which is by no means to be despised.

There is no universally adopted size of fitting for eyepieces, consequently every maker has his own gauges, varying for nearly every instrument. This is much to be deplored, as it is a constant source of inconvenience to workers, especially when it is necessary to use eyepieces by different makers.

The Royal Microscopical Society in 1882 appointed a committee to consider the adoption of universal sizes for eyepiece fittings, and on page 595 of the society's journal for that year, the report of that committee is to be found. The sizes recommended were two that were already largely in use, viz., for microscopes of large size 1·35 inches diameter, and for small microscopes ·92 inch diameter. One optician confessedly makes no less than five different sizes of eyepiece fittings, and the writer has six adapters in regular use for eyepieces that he uses in his microscopes. Why cannot the recommended sizes be adopted by opticians? No loss of prestige could possibly ensue, and the additional facility for working would be of the greatest benefit.

CHAPTER III.

ILLUMINATION AND ILLUMINATING APPARATUS.

Monochromatic Light.

ABSOLUTELY monochromatic light is a light of one refrangibility—that is, a colour of one uniform wave-length. As used in microscopy, monochromatic light means light with a small range of refrangibility, and it is important that its function should be clearly understood.

If white light is divided into its component parts by means of a prism or a spectroscope, a regular band of colours is produced, termed the spectrum, commencing with red at one end, followed by orange, yellow, green, blue, indigo, and finishing with violet. In physical optics light is regarded as travelling in waves, the amplitude of each of which is very small, compared with the wave-length—not more than about 1 : 10,000. Now, the length of a light wave varies according to the portion of the spectrum that is used. At the extreme red end of the spectrum it measures ·76 μ*, and the wave-length decreases through the range of colours until at the extreme violet end it measures ·39 μ. From this it will be seen that nearly double the number of waves of light would be oscillating per millimetre with a violet light than with a red.

The numerical aperture of an objective is increased by the use of a dense medium enveloping the object and the front lens of the objective, as we have seen by the descrip-

* $\mu = \frac{1}{1000}$ of a millimetre, and is called a micron. There are 7½ to 8 microns in the diameter of a human blood-disc.

tion of immersion objectives. The oil or other medium employed shortens the wave-length of the light used, whatever its colour, and when we use a light of shorter wave-length than it would have when passing through air, we increase the effective aperture of the objective. Accordingly the resolving power of a lens is increased by shortening the wave-length of the light admitted to it, and this is accomplished in either of two ways—(1) By employing blue instead of white light, or (2) by converting the lens into an immersion lens, and interposing a layer of oil instead of air between it and the object. For instance, if a microscopic objective were used with white light, and its limit of power to resolve fine structure were 50,133 lines per inch with such illumination, its limit would be 54,342 lines per inch with monochromatic blue light (line F).

A natural conclusion from these statements would be that the farther towards the violet the monochromatic light were used, the more marked would be the results obtained ; but although this is correct theoretically, it is not true practically. Microscopic objectives are corrected for visual purposes for use with the brightest rays of white light, and if the extreme ends of the spectrum were employed—the objective not being calculated for these—rise would be given to spherical aberration, even in the best objectives, preventing the accomplishment of good work. If a lens were corrected for spherical aberration when used with light from the extreme blue end of the spectrum, under existing conditions of manufacture it would work at its best with the light for which it was designed, and if light lower down in the spectrum were employed, spherical aberration would be apparent. It must be borne in mind that light of extremely short wave-length is sensibly absorbed by glass, also the eyesight is not keen in extreme blue and violet lights, consequently the range of light that is practically available for monochromatic illumination is restricted.

Another advantage gained by the use of monochromatic light is, that as there is but one colour of the spectrum used, objectives of high-class make of the achromatic form are rendered practically equal to apochromatics by the removal of the secondary spectrum—that is, any chromatic aberration that may be present in the objective is annulled by the monochromatic light with which the illumination is effected. The more nearly the monochromatic light which is used approaches to that ray for which the spherical aberration in the objective is best corrected, the better will be the resulting definition. In some cases, so advantageous has this means of illumination proved, that, using two objectives of the same power alternately, one an expensive apochromatic, and the other an achromatic, it has been difficult to tell which was being employed.

True monochromatic light can be at the present time obtained by means of prisms only, and the best apparatus procurable is that designed by Mr. E. M. Nelson, and manufactured by Mr. C. Baker, of 244, High Holborn, London. It consists of prisms, slits, and condensing-lenses so arranged as to afford every facility for obtoining accurate effects.

Many experiments have been made with a view to producing by means of pigments and the combinations of coloured glasses, monochromatic light screens, and thereby obviating the necessity for prisms. So far these attempts have been only partially successful, all that have been made passing light of more than one colour, but it is to be hoped that the desired result may yet be achieved. Very material assistance is, however, afforded by these screens, not alone for actual monochromatic illumination, but also for general work. They are usually placed beneath the sub-stage condenser, and are employed in photo-micrography very considerably for neutralizing non-photographic colours in objects, and rendering the actinic and visual rays in an objective more nearly coincident, also in visual work for minimizing light-glare with large cones

of illumination. This latter applies equally to apochromatic and achromatic objectives, the screen often producing stronger contrast and a crisper image than could be obtained without one.

The most advantageous colour for all round visual purposes is the green-blue, and the best light screen at present available is Mr. J. W. Gifford's mixture of malachite green and picric acid, obtainable of most opticians. For photography discs of light and dark yellow and various shades of blue glass are constantly employed. The great desideratum in a light screen is that it shall pass a large quantity of light. No doubt mixtures could soon be made that would produce monochromatism, if only great transparency were not of importance. The prismatic apparatus before referred to gives most brilliant results, and when the condenser is adjusted with the image of the lamp-flame in focus, light of any degree of monochromatism can be obtained by narrowing the slit.

Mr. Nelson states* that monochromatic blue light 'makes a difference of about 14 per cent. in the case of low apertures, but beyond those of 0·9 N.A. its influence in increasing resolution is so small as to be hardly worth taking into account. What it does effect is the sharpening and clearing of detail already resolved.'

This is no doubt because only a small part of the first diffraction image, seen when looking at the back of the objective with the eyepiece removed, is utilized by the lens, and that this part consists of blue light whether it be blue or white light that is employed for illuminating, so that in both cases there are the same materials for resolving the detail of the object, and the only difference is that there is a haze of light of lower refrangibility (or greater wave-length) also present, which forms a luminous veil over the whole field. It is this veil which is removed by using monochromatic light, and therefore the effect is to sharpen and clear the detail that is already resolved.

* Journal of the Royal Microscopical Society, 1893, p. 15.

Sub-stage Condensers.

A few years since all that was considered necessary in order to illuminate in a proper manner an object under examination was the mirror, perhaps in conjunction with the bull's-eye stand condenser; and in many cases the mirror was hung on a tailpiece which could be moved in an arc round the centre of the stage, and by this means light at any angle could be reflected on the object. The day for this, however, has gone by, and anyone who requires to get even fair results must use a sub-stage condenser in some form or other. Especially does this apply to high-power objectives. Plenty of illumination can be obtained with the mirror only, for lenses up to, say, $\frac{1}{4}$-inch, but beyond this the object becomes ill-defined and the field dark. More especially since the study of bacteriology has taken so prominent a position has the condenser come to the front. Without its aid it would be almost impossible to distinguish between different species of these minute organisms. To the leading members of the Royal Microscopical Society, and especially to Dr. Dallinger and Mr. E. M. Nelson, is due the steady improvement that has taken place in the optical qualities of the sub-stage condenser. The two gentlemen named have been indefatigable in their appeals and demonstrations to microscopists, urging the pre-eminent position that it should occupy in manipulation, and the proper methods of using it.

One of the most largely used of condensers is a chromatic one, named the Abbe illuminator, originated by the firm of Carl Zeiss, and now supplied by nearly all opticians. It is made in two forms, one having a numerical aperture of 1·20, and the other of 1·40. The former is the more commonly employed, and is principally adopted by students. The optical portion is shown in Fig. 12. On account of its large aperture it gives a most brilliant illumination, with the highest power objective, while with the lower powers, by removing the top lens, good results can be obtained. Its price is low, but it has the great disadvantage of not

being achromatic, and having so large an amount of spherical aberration as to be almost useless for critical work. Nevertheless, it fills a distinct position in microscopical work, and is very easy to use.

The Abbe illuminator was followed some few years later by an achromatic condenser, having a numerical aperture of 1·0, which also originated in the firm of Carl Zeiss. This is also made by several London opticians, and works with low powers after the removal of the top lens. It is an immense improvement on the chromatic condenser, and we unhesitatingly advise its use in preference to the same. Should either of the above-mentioned condensers be adopted, it is most desirable that they should be of English make. Those of German origin are not usually intended for fitting into the English size of sub-stage, and require adapting. Even when they are supplied by foreign firms for the English microscope, they are invariably far too heavy and clumsy in the mounting, and are consequently apt to impart vibration, and are inconvenient to handle.

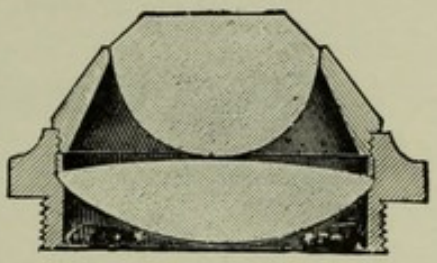

FIG. 12.—Abbe Illuminator, 1·20 N.A., optical part.

In Fig. 14 one of the achromatic condensers is shown, mounted on a carrier for the sub-stage. It is provided with an iris diaphragm similar to that illustrated in Fig. 13, by means of which any desired aperture may be quickly and exactly obtained. It will be found of utility to have the arc through which the lever controlling the iris diaphragm travels provided with a scale of divisions, so that results may be quickly reproduced or any special aperture may be obtained; but for this purpose it is necessary that the diaphragm shall respond immediately on the pressure of the lever handle; there must be no loss of time in the movement. The carrier is further provided with an arm, having a rotating cell, in which may be placed stops for producing dark ground illumination in the same manner

as with the spot lens, described on page 87; also stops for obtaining oblique illumination for the resolution of the markings on diatomaceæ, and for holding tinted glasses or

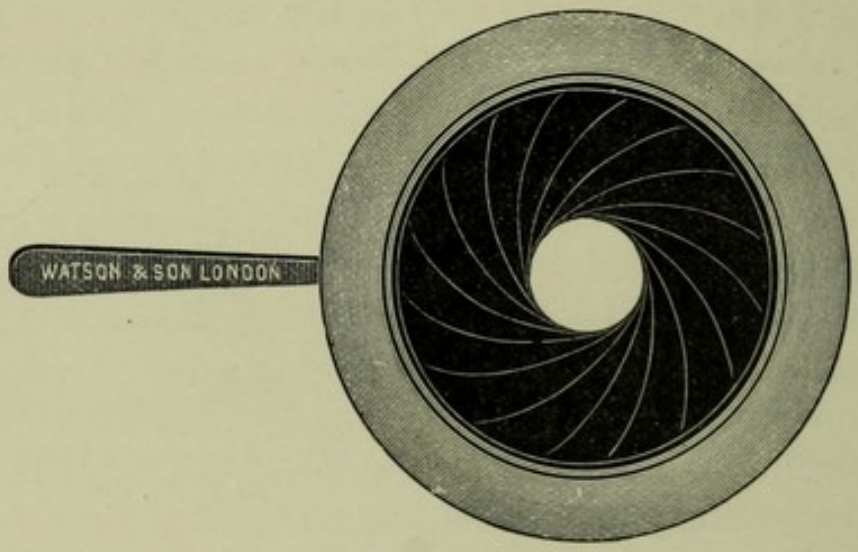

Fig. 13.—Iris Diaphragm as fitted to the Condenser Carrier.

light screens. This form of carrier is applicable also to the Abbe illuminator.

There is yet a higher class of condensers than either of those mentioned, made exclusively by Messrs. Powell and Lealand. This firm supplies two apochromatic condensers,

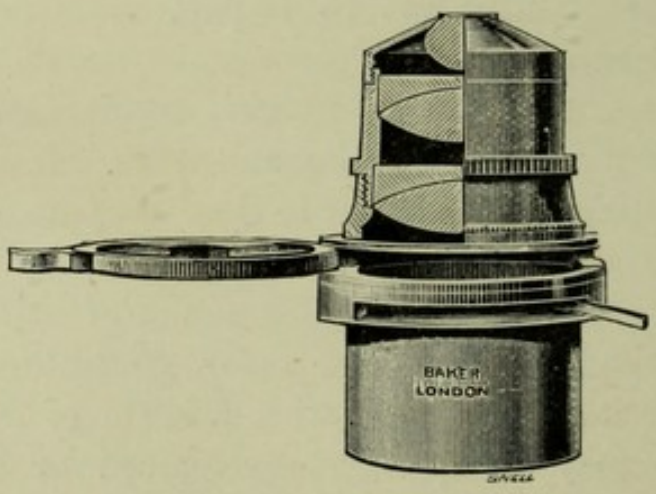

Fig. 14.—Achromatic Condenser 1·0 mounted for Sub-stage.

one having a N.A. of 1·0 for use dry, and the other an oil immersion having a N.A. of 1·40. In some of the finest modern critical work these condensers have been employed, and they have for several years been the very best that

could be obtained. They are especially appropriate for use with the apochromatic objectives.

A very efficient condenser can be oftentimes formed by fitting a low-power objective into a suitable carrier in the sub-stage. A small iris diaphragm, called the Davis's Shutter, having the universal male society screw at one end and the female screw at the other, together with a tube, fitting into the sub-stage and provided with the universal thread, into which the iris diaphragm may be fitted, is a very suitable carrier for the objective.

The value of a sub-stage condenser must not be reckoned by its numerical aperture, but by the aplanatic cone that it will transmit, or, in other words, by its perfection of correction, for it is the aplanatic cone alone that can be employed for critical work. The Abbe illuminator has a very small aplanatic cone, that of the 1·20 N.A. form being only about ·50 N.A.; the achromatic 1·0 form is considerably superior in this respect, and has an aplanatic cone of about ·70 N.A., while the Powell and Lealand apochromatic condensers of 1·0 and 1·4 have aplanatic apertures, as taken by Dr. Dallinger, of ·9 and 1·1 respectively. From this it will be seen how immensely superior for critical or high-class work a well corrected condenser is.

THE APLANATIC APERTURE of a sub-stage condenser is ascertained in the following manner:

The condenser is accurately centred, and both it and an objective are focused in the usual way on an object mounted in *Canada balsam*, the edge of the lamp-flame being employed. We will presume that the objective has a numerical aperture of ·5 N.A. The full aperture of the condenser is then used, and the object so placed that the balsam portion is still between the condenser and objective, but the object itself is not in the field. It would be well that a balsam-mounted object were always used for this experiment, as the result is slightly affected by different media. The eyepiece is now removed, and the back lens of the objective is examined. It may be found that it is com-

pletely filled with light, as in Fig. 15, under which circumstance the condenser has an aplanatic cone exceeding the N.A. of the objective. The aperture of the condenser can now be limited by means of a diaphragm, and an approximate value obtained for the size of diaphragm that is used. The edge of this diaphragm should be so set that its edge is just seen appearing at the margin of the objective as in Fig. 16. The aperture of the condenser when used with this size of diaphragm is therefore a shade less than N.A. ·5 —say ·45. An objective having a larger N.A., say ·95, is now employed, and it will be found that the back lens of the objective is no longer filled with light. Theoretically, this is the condition under which the aplanatic aperture should be estimated, but when a flat flame of a lamp is presented edgewise, its image has corresponding depth, and

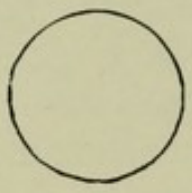

Fig. 15.

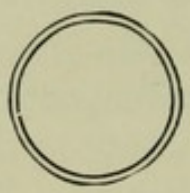

Fig. 16.

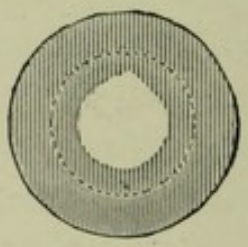

Fig. 17.

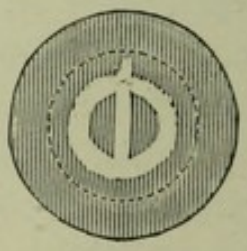

Fig. 18.

when one part is focused on the object, other parts of the image of the flame will necessarily be out of focus. There is therefore a certain range of adjustment of the condenser within which the effect (so far as it depends on focusing the light on the object) will be pretty much the same. But these different positions give different apertures to the condenser as judged by the light reaching the back lens of the objective. The condenser should then be gently racked upward until the disc of light is at its largest (Fig. 17); until on a further movement of the condenser two black spots appear, one on either side of the middle of the disc (Fig. 18), which increase as the condenser is further racked up. *The last point before the appearance of the black spots furnishes the position in which the condenser has the largest aperture consistent with its outstanding spherical aberra-*

tion not too much interfering with the highest results, and is the limit of the condenser for critical work. Any further advance of the condenser gives merely annular illumination, which, of course, is to be avoided, excepting when stops are used.*

How to use the Condenser.

The condenser requires as much care and skill in adjusting as the objective, for if it be improperly set up it will give rise to 'false images,' due to diffraction. For an objective to work at its best, it will be necessary to focus the image of the *edge* of the lamp-flame sharply upon the object on the stage, and the following will be the procedure:

A proper microscope lamp, as described on page 92, should be set in front of the instrument with the edge of the wick towards the microscope, and the light from the lamp may be allowed to fall directly upon the condenser, or a plane mirror may be used. The sub-stage condenser should now be centred, first having been placed in about the position that it will occupy when focused. The centring cannot, of course, be properly effected without a centring sub-stage; but where there is only a fixed under-fitting, it is well to set the condenser at the position where it is most central. It is understood that the under-fitting is centred with the optical axis of the microscope when sent out by the makers; but owing to the fitting-tubes being more or less elliptical, it often happens, if the condenser is rotated in the under-fitting, that it will be central in one position only, and at this position it should be placed for working when there are no centring screws. Some condensers have fitted on top of them a removable cap with a very small pin-hole. This pin-hole should be focused with, say, an inch or a ½-inch objective, and the condenser centred by it; the cap should then be removed from the condenser for

* E. M. Nelson, *English Mechanic*, November 16, 1888 (vol. xlviii., No. 1234), and 'The Microscope and its Revelations,' by Dr. Dallinger.

working. Condensers not provided with this cap can, as a rule, be centred by using a diaphragm having a very small aperture at the back of the lenses, and focusing the aerial image of it with a ½-inch objective; but the easiest way of centring is to make a very small spot in the middle of the top of the lens with a pen and ink; centre by this spot, and wipe it off. It will not make any difference to the performance of the condenser, and will ensure accuracy and save time. Having centred the condenser, it should be racked up until it touches the under-side of the slide, the objective being made to touch the cover-glass of the object on the upper side; see that the diaphragm of the condenser is open, reflect the light with the mirror, and thus illuminate the field; then rack the microscope body upwards until the object comes into view. If it is found that there is too bright a flood of light, the aperture of the condenser must be decreased a little by using a smaller diaphragm. Having focused the object on the upper side with the objective, it will be necessary to focus the condenser. Rack this downwards from the object very slightly until the image of the lamp-flame is seen in the centre of the field, the remainder being comparatively dark, as in Fig. 19. If now it be desired to have the whole field equally brilliant a bull's-eye stand condenser may be interposed between the lamp and the mirror, the plane side of the bull's-eye being towards the lamp; or the burner of the lamp may be turned round till the flat of the wick is towards the mirror. Where high powers are to be used, the object to be examined may with advantage be set upon the stage and focused with the ½-inch or other low-power objective, and the sub-stage condenser focused upon the object. The high power may then replace the low power, and the condenser will be in adjustment. If it be found that the image of the lamp-flame is not in the middle portion of the field on

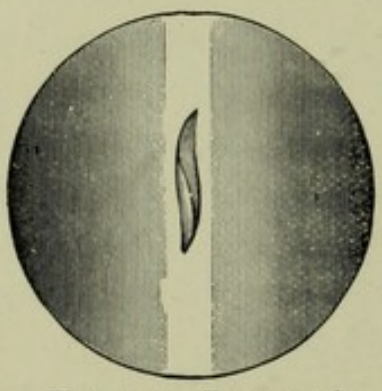

Fig. 19.—Image of Lamp-flame.

exchanging the objectives, it will show that the objectives have not exactly the same centres, and the image must be set central with the high power by altering the position of the condenser by means of the centring screws of the sub-stage.

The next question is, What amount of light should be admitted from the condenser in order to see the object at its best? Mr. Nelson has suggested that the aperture of the condenser should be about three-quarters that of the objective, and in order to arrange this it will be necessary to remove the eyepiece from the microscope, and look down the tube at the back of the object glass, opening the diaphragm of the condenser to its fullest extent. Bearing in mind the size of the circle of light seen, gradually diminish the opening of the diaphragm of the condenser until one quarter of the back lens of the objective is shut out; again put in the eyepiece, and the desired amount of illumination is arranged. The aperture employed should be varied slightly according to the transparency or opacity of the object under view.

When the condenser is centred and focused, and the back lens of the objective is three-quarters filled with light, a *critical image* is obtained; that is, the objective is understood then to produce the finest results it is capable of.

Mr. Nelson's $\frac{3}{4}$-cone method of illumination has been almost universally accepted as a most practical one; but the following plan, which was suggested to the writer by a microscopical friend, has given very satisfactory results. On examining the back lens of the objective with a striated object, such as *Pleurosigma angulatum*, resolved and focused upon the stage, there will be seen a central disc surrounded by six diffraction spectra similar to Fig. 20. With the $\frac{3}{4}$ cone of illumination, the surrounding spectra will, in some cases, appear to overlap the central disc, and in others will not appear to touch it. Our plan is to open the diaphragm of the condenser only to such an extent

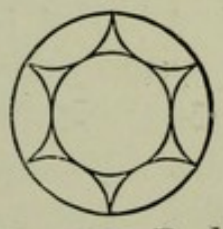

Fig. 20.—Back Lens of Objective.

that the spectra just touch, but do not overlap, the central disc. This would necessitate that in some instances we should employ rather less than a $\frac{3}{4}$ cone of illumination, and in others rather more than a $\frac{3}{4}$ cone. We have not been able to observe that any loss of resolution results from this practice; but, on the other hand, in our opinion detail is more clearly seen, and appears crisper under these circumstances of illumination than any other. This system is especially advantageous when monochromatic light is used.

It will be found of great advantage to become acquainted with the appearance of the back lens of the objective when working; many hints of importance may be gleaned from it, enabling manipulation to be effected with increased precision. For this purpose the 'dummy' eyepiece referred to on page 67 is a most useful adjunct.

When working with monochromatic light, the condenser must be focused so that the whole of the light which is visible on the back lens of the objective when the eyepiece is removed shall appear as nearly as possible of the same colour.

Condensers having a numerical aperture of 1·0 N.A. and over require to be immersed in order that they may work at their full aperture; that is, a drop of immersion oil or Canada balsam must be placed between the top lens of the condenser and the object. It will be found generally that the condenser is a little too long in focus for continuity between the top lens of the condenser and the underside of the object to be maintained. Under such circumstances an additional thin 3×1 inch slip, or a piece of cover-glass, should be placed under the object, which will enable the oil contact to be maintained. The distance between the condenser and the object will vary according to the thickness of the slip on which the object is mounted, and the intermediate contact glass will have to be selected accordingly. To use an oil immersion condenser effectively the object must be mounted in some medium, and not dry upon the cover-glass.

As before mentioned, with the majority of condensers, stops are supplied having the centres blocked out, as shown in Fig. 21, by means of which dark-ground and oblique illuminations are obtained. Dark-ground illumination gives a most beautiful effect to very transparent objects, such as infusoria, pond-life specimens, etc. In the form of carrier for condensers shown in Fig. 14, a cell is provided just above the iris diaphragm to carry the stops. One similar to *a* (Fig. 21) is placed in the cell, the iris diaphragm is opened completely, the condenser having been previously adjusted in the usual way, when it will be found that the object will be illuminated, but the ground on which it is seen will be black. Different objectives require stops of special sizes, which may be readily made of blackened cardboard, cut to the most suitable size for working with the objective.

FIG. 21.—Stops for Condensers.

These stops can be further used for strengthening the contrast in the image with large cones of illumination and objectives having high apertures. This method does not minimize in any way the effective working of the objective, for with objectives of large aperture rays may be present which only impart brightness to the field, but do not contribute to making visible the fine detail upon the object. If less than half of the lateral spectra, as shown in Fig. 20, are seen on looking down the tube at the back lens of the object glass, with a striated object in focus, then the central portion of the direct beam or central disc has no lateral image corresponding to it in the portions of the spectra that are visible. Under these circumstances that central portion of the central disc in no degree contributes in enabling the detail to be seen, but only produces a haze; by blocking it out the haze is removed, and there is a great improvement in the resulting definition. This produces oblique illumination in all azimuths.

FOR OBLIQUE ILLUMINATION IN ONE AZIMUTH one of the stops, *b* or *c* in Fig. 21, is fitted into the cell for dark-

ground stops, the iris diaphragm being completely opened. By this means striated objects, and especially the markings on diatomaceæ, are exhibited in a very striking manner, but this method gives rise to an image which very imperfectly represents the object and is not to be recommended.

THE CHOICE OF A CONDENSER.—That the condenser is an absolute necessity cannot be too strongly impressed. No good results can be obtained without it.

Condensers, like objectives, not only vary in aperture, but also in power, and the higher the power of the condenser the smaller will be the image of the lamp-flame that it transmits. Consequently, if a condenser of high power is used with a low-power objective, the illuminated portion of the field will be exceedingly small, while if a low-power condenser is used with a high-power objective, the image of the lamp-flame is so magnified that the whole field is bright, and it is not easy to tell when the condenser is exactly focused. Furthermore, under such circumstances as the latter, it is impossible to get the best effect with the objective. If work of the highest class be aimed at, it is desirable that two condensers shall form part of the equipment, a high and a low power. The Abbe illuminator and achromatic condenser 1·0 N.A. are low in power; the Powell and Lealand apochromatic condenser (1·0 N.A.) is of medium power ($\frac{4}{10}$ inch), and this firm's oil immersion condenser is of high power ($\frac{1}{6}$ inch). It has been cause for surprise that opticians have not hitherto produced a medium-power achromatic condenser, giving a large aplanatic cone (say ·90 N.A.); such a condenser ought to be made at a cost which would place it within the reach of the ordinary microscopist. We are happy to state that it is likely, at no very distant date, to be procurable. We have heard of several that are in the experimental stage, but it would be premature to give details of them at present. The high-power condensers have rather small lenses, and require greater skill in using than the low powers. In conclusion, we should advise that where high-class work is to be undertaken two condensers, such as the

Zeiss pattern achromatic (1·0 N.A.) and another of medium power, say ¼ inch, or Powell and Lealand's oil immersion (1·4 N.A.), should be taken.

For amateurs' or students' purposes the Zeiss pattern achromatic condenser (1·0 N.A.) will usually be found quite efficient.

The Abbe illuminator has its special points of advantage; it is easy to use, gives most excellent dark-ground illumination, and for oblique illumination with high powers is quite satisfactory, but on account of its want of chromatic correction it is not so desirable for general work as the condensers mentioned above. It is, however, very popular and largely used.

The Spot Lens.

Before the sub-stage condenser came into general use, the spot lens and paraboloid were largely used for obtaining dark-ground illumination. They have, however, been to a considerable extent superseded, owing to the perfection in which the same effect can now be obtained with the condenser. They are, nevertheless, often used, and preferred by some to the condenser. It must be understood that they cannot take the place of the condenser for ordinary direct illumination. The method of using is simple, the spot lens being intended for low powers up to ½ inch, and the paraboloid for higher ones. With both of them a plane mirror and the flat of the wick of the lamp should be used. If the spot lens be employed, the sub-stage that carries it should be moved up and down until a perfectly black ground is obtained; if additional brilliancy is required on the object, a stand condenser interposed between the lamp and the mirror, with the convex side of the condenser towards the mirror, will give a brighter effect. The paraboloid is adjusted in a similar manner, but instead of having a fixed black spot on the top of the lens it has an adjustable one, and the pin carrying this black spot should be moved up and down until the best effect is obtained. This latter is far more expensive than the spot lens, and the advantage gained is so slight that we can hardly recommend it.

For one class of work the spot lens is specially advantageous. Most sub-stage condensers have a very short focus, and if organisms in water in a trough are being examined, it is impossible to focus accurately the condenser through the trough and its contents. A spot lens has a longer focus, and gives under these circumstances the best results.

The Polariscope.

This consists of two parts, each composed of a Nicol prism of Iceland spar in a suitable mounting—one called the polarizer, which fits into the sub-stage, and the other the analyzer, which is inserted between the nosepiece of the microscope and the objective. By its means, light is split up into its component parts, and most beautiful colour

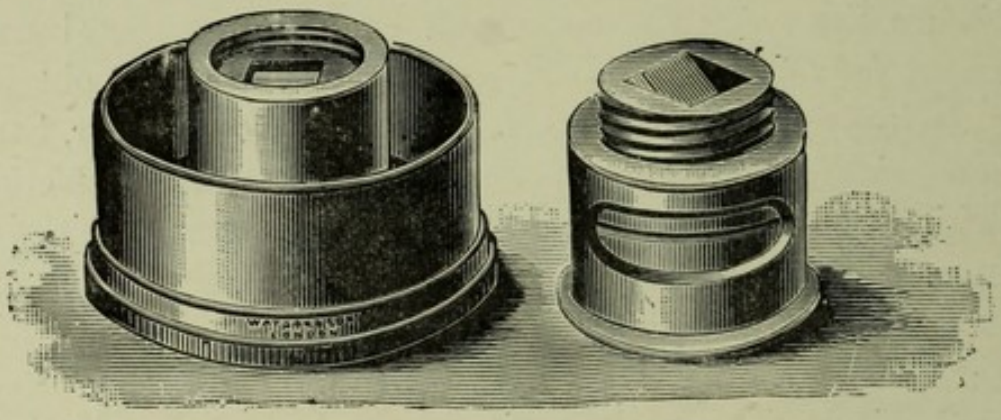

FIG. 22.—Polarizer. FIG. 23.—Analyzer.

effects are obtained. The polarizer has a flange beneath, by means of which it can be rotated, and in this way the colours are varied. In examining certain chemical crystals, geological slides, etc., it brings into view structure which without it would hardly be detected, and for this it is largely used in analytical work. In some instruments the analyzer prism is fitted in the body. This is rather an inconvenience unless the instrument be designed especially for petrology. For a binocular microscope, however, it is better not to have it between the nosepiece and the objective, as it creates a distance between these two, which interferes with the performance of the binocular prism; in fact, the closer the back lens of the objective can be brought to the binocular prism, the more perfect will the vision be.

Under these circumstances the analyzer prism is best mounted over the top of the eyepiece and the monocular tube alone used. For use with the polariscope, varieties of tints and a background of colour can be obtained by the employment of selenite films. These, in the cheapest form, are mounted in the same way as ordinary microscopic objects; but a still greater variety of effect can be obtained by having selenites fitting into a carrier to come between the polarizer and the stage in a sub-stage microscope. We illustrate one (Fig. 24) by R. and J. Beck. In this form each of the selenites is provided with a ring which rotates. The three being one over the other, either two or all three can be rotated together or in opposite directions to one

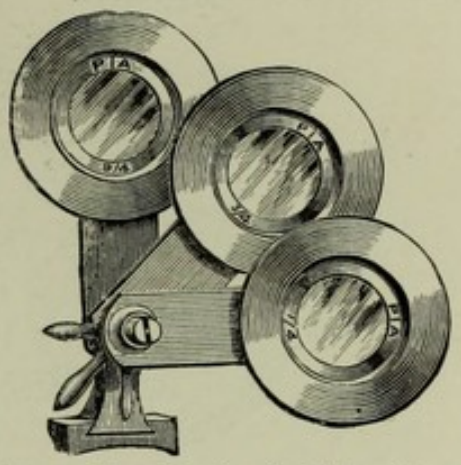

Fig. 24.—Darker's Selenites.

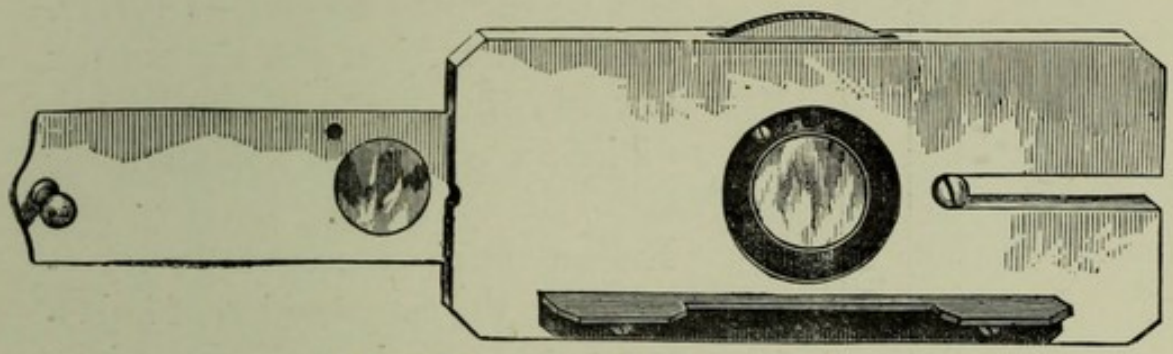

Fig. 25.—Mica-selenite Stage.

another, and the effect is most striking. This is an expensive item, but a modification of it is made by Swift and Sons, called the mica-selenite stage, as shown in Fig. 25. This consists of a film of mica made to rotate in a brass plate, upon which the object is laid, and beneath it is a carrier with three separate selenites. These can each be pushed separately beneath the mica and the latter rotated. By this means all the different tints obtainable with any number of selenite films can be produced. It can be employed on any microscope. To get greater brilliancy the polarizer can be made to fit into the sub-stage condenser

on the under side, and the Abbe chromatic and achromatic condensers referred to previously are particularly suitable for this arrangement.

The Bull's-eye Condenser.

Many objects, being opaque, cannot be viewed by light from beneath, and consequently have to be illuminated from above. In order to do this a bull's-eye condenser is necessary. These are generally mounted on a stand, and may be had in varying sizes. They consist usually of a plano-convex lens mounted, as shown in the figure. This has a ball and socket joint and a sliding bar, by means of which the lens can be placed in any desired position. The plane side of the lens should be turned towards the object and the convex towards the source of the illumination, whether it be daylight or artificial by lamp. Latterly Mr. Nelson has suggested improvements in the construction of bull's-eye condensers in order to reduce the large amount of spherical aberration which is a necessary accompaniment of the single lens. His improved form consists of either two or three lenses in combination, and the advantage obtained is well worth the additional outlay. To enhance the effects obtained with opaque objects with these stand condensers the side silver reflector will be found very convenient. The arm of this is attached to either the stage or limb of the microscope, or fitted between the nosepiece and the objective. It consists

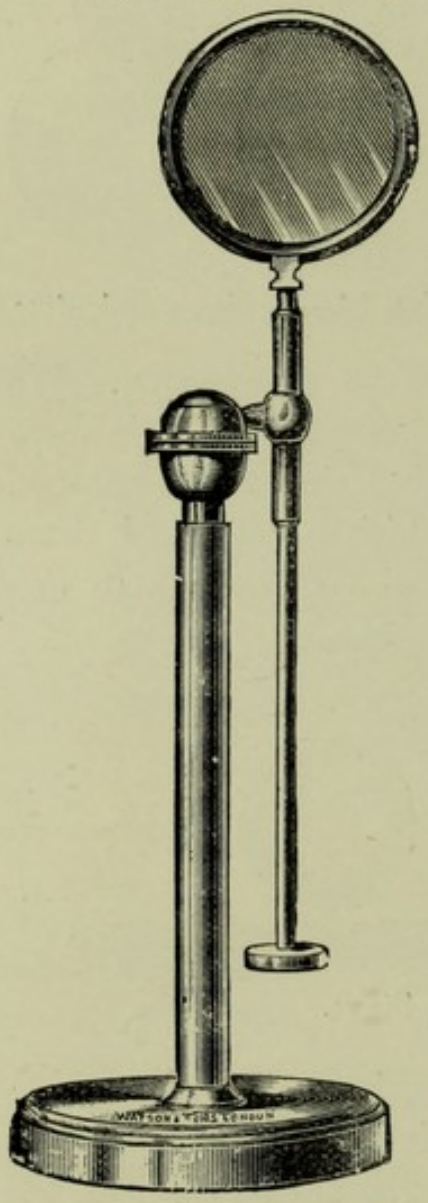

FIG. 26.—Stand Condenser.

of a highly-polished silver parabolic speculum. This reflector is placed by the side of the object, and light is thrown from the lamp through the bull's-eye on to its centre, and then thrown by the reflector on to the object. Most brilliant opaque illumination may be obtained by this means.

The Vertical or Disc Illuminator.

The merits of this piece of apparatus are comparatively little known. It is employed for examining the surfaces of opaque objects, such as metals, minerals, etc., under high powers, and by its aid it can be ascertained if a specimen is adherent to the cover-glass. This latter is a most important condition when an oil immersion objective is being employed on an object mounted dry. If the specimen is not on the cover-glass, it cannot be seen at all with this illuminator.

It is also used for the resolution of the markings on diatoms that are mounted dry on the cover-glass. It is, however, only useful with oil immersion objectives.

The simplest form of this piece of apparatus is that made by Messrs. R. and J. Beck, as per figure. The fitting *b* screws to the nosepiece of the microscope, above the objective, and has mounted inside it a disc of cover-glass fitted on a little clip having a small milled head, as shown at *a*. This disc of glass must be set at an angle of 45° to the optic axis. Light is received on this disc of glass through the small opening in the body of the fitting *b*, and it is totally reflected through the object-glass on the object, the objective acting as its own achromatic condenser. In order that the light may be focused on the object, the lamp wick from which the light is being obtained must be the same distance from the reflector as the latter is from the diaphragm of the eyepiece, if a positive eyepiece is being used, or to the eye lens, if a Huyghenian or negative eyepiece is employed.*

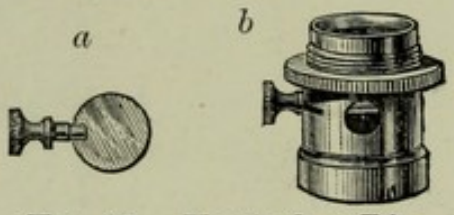

Fig. 27.—Vertical or Disc Illuminator.

* Dr. Dallinger, 'Microscope and its Revelations,' p. 285.

CHAPTER IV.

ACCESSORY APPARATUS.

The Lamp.

It is most important that the microscopist should possess a good and suitable lamp, otherwise he cannot work to the greatest advantage. The amateur will often be found working with a reading-lamp or an ordinary oil-lamp, but good work can never be done conveniently by this means. There are two or three important points which must be borne in mind. In the first place, if light is proceeding from the one illuminating point only, and the remainder of the room is dark, while using the microscope, a great deal better effect can be produced than if the whole room be illuminated. In the next place, a small brilliant source of light is far better than a large one. In recent years special attention has been paid to this matter, with the result that lamps have been constructed with which the best work may be accomplished. The following are desirable features which should be embraced by a good microscope lamp: The reservoir for oil should be large in diameter and flat, so that the light may be brought down very close to the table. For this reservoir, glass is usually preferable to metal, it being much cleaner, and the worker is able to tell when his oil is getting exhausted; whereas with a metal reservoir, unless careful reckoning is kept, in the middle of some important observation the light may go out from want of oil. A half-inch wick is generally found to be sufficient. We strongly deprecate the use of glass chimneys. They are always liable to get broken very

easily, and become a source of expense, in addition to which, if away from town, there is a possibility of not being able to get the right kind, and so work may be delayed. Far better will be found the metal chimneys now made by nearly all opticians, with a carrier for a 3×1 slip. It is obvious that if the slip be broken it can be immediately re-

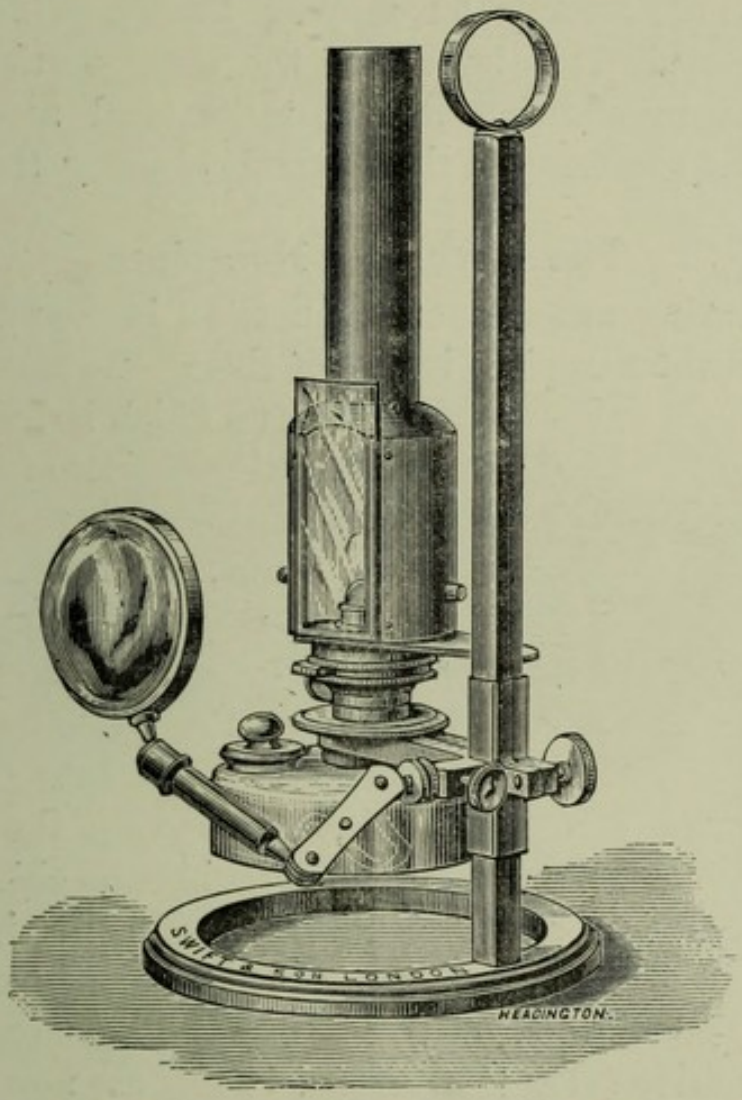

Fig. 28.—Microscope Lamp with Metal Chimney.

placed, it being part of the microscopist's average stock. It is also desirable that the bar on which the lamp is raised and lowered on the stand should be a square one. If round in shape, the lamp is apt to swing round on the stand and the whole to topple over. This is an impossibility with the square bar. Such a lamp is shown in Fig. 28, by Swift and Son, and modifications of it can be obtained

of most dealers. Messrs. Swift make a somewhat similar lamp to the above, called the 'Nelson-Dallinger' model, which has a short rackwork for vertical adjustment, and a quick acting screw for lateral adjustment. These mechanical arrangements enable the light from the lamp to be shifted just the slight amount that is often needed, and obviate the necessity for moving the lamp itself, as is usually done. This lamp has the usual vertical sliding movement in addition to the mechanical screws. We have already referred to the methods of using the lamp on page 81.

The Revolving Nosepiece.

Time-saving arrangements will often be found useful in work, and the nosepiece is one of these. This is a piece of apparatus which is screwed into the fixed nosepiece of the microscope, and is made to carry either two, three, or four objectives, termed respectively the double, triple, or quadruple nosepiece. Each of the objectives can in turn be rotated into the optical axis, thus saving the necessity of unscrewing an objective and screwing another on in order to get a variation of power. In hospitals, laboratories, etc., it is usual to have one of these fitted to nearly every instrument. They are now to be had made of an aluminium alloy which is extremely light, reducing the strain on the body tube. Any microscope having the universal size of thread for objectives will carry a revolving nosepiece; no special adaptation is required. When the revolving nosepiece is screwed home, the objectives not in use must point towards the middle of the front of the stage, otherwise, in rotating the objectives they are, with low powers, apt to foul the rackwork bar of the microscope.

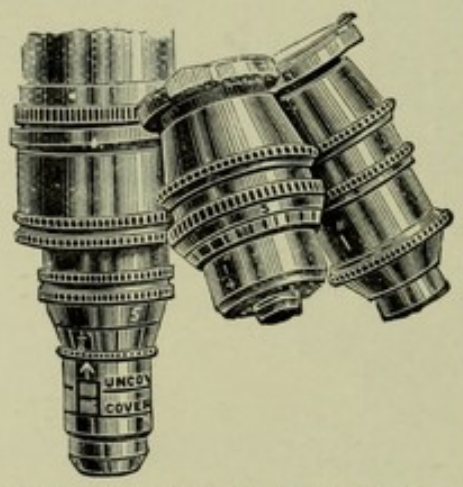

Fig. 29.—Triple Nosepiece.

The Nosepiece Iris Diaphragm, or Davis's Shutter.

This is a very compactly made iris diaphragm, which is placed between the nosepiece of the microscope and the objective. Its special function is to enable the aperture of an objective to be decreased, so that it may be used with dark-ground illumination, or to increase penetration when examining objects having several planes. For photographing opaque objects with low powers it enables the appearance of a small round object, such as a moth's egg, to be taken quite sharply. We have been shown photographs that were taken by a gentleman in America, with a low-power objective, having an iris diaphragm fitted between the combinations of lenses, by the limitation of the aperture of which considerable depth of focus was obtained. The writer has not heard of this scheme having been tried in England.

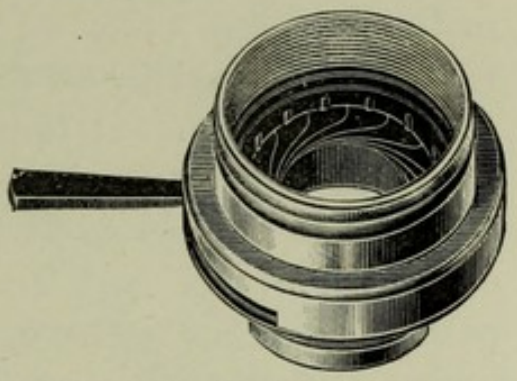

FIG. 30.—Davis's Shutter.

The Davis's shutter is furthermore very useful for examining and experimenting with the diffraction spectra seen on looking down the microscope tube, at the back of the objective, when the eyepiece is removed, and a striated object is being examined.

This iris diaphragm must have its aperture perfectly central, and the threads quite true. The aperture of the iris, when completely opened, should be as great as it is possible for the inside of the mount of an objective to be, but the box of the iris diaphragm must not be so large as to touch the bearings in which the tube of the microscope is raised and lowered. It is well to have the lever of the iris diaphragm working in front of the body, and so that this may be easily arranged, the male thread should be so mounted that it may be rotated, but should be very stiff in its rotary movement.

Camera Lucida.

This is designed to assist in drawing objects seen in the microscope. Photo-micrography has to a large extent superseded it; still, there are a great many who prefer this method to any other. Dr. Beale's neutral-tint reflector, which is supplied by all the opticians, is the cheapest and a very good form. It consists essentially of a neutral-tint glass—in which the image of the object is reflected—mounted in a frame to fit over the eyepiece. The method of using it is as follows: The microscope is set in a horizontal position, with the centre of the eyepiece 10 inches from the table. Illumination is arranged in the ordinary way. The cap of the eyepiece is removed, and the neutral-tint reflector is fitted in place of it, and is so arranged that the centres of the neutral-tint glass, and the eye-lens of the eyepiece are in alignment, the former being set at an angle of 45°. On looking on this neutral-tint glass from the upper side, a disc of bright light will be seen on it, and if a piece of white paper be spread below on the table, on further examination the outlines of the object will appear to be upon the paper. If a pencil be now taken, the specimen can be sketched in its magnified form. This will be found somewhat difficult at first, nearly every worker seeming to find it necessary to work in some special manner of his own, but the secret of success is to arrange the balance of illumination by turning the lamp-wick up and down until a degree of light is found at which the pencil-point and image can be distinctly seen. When using students' microscopes, the eyepieces of which have no caps, it is usual to remove the eyepiece, fit the tube of the reflector to the outside of the top of the draw-tube, then reinsert the eyepiece and set the neutral-tint glass in position. The tinted glass is usually mounted on an arm which has a joint, so that it may be turned out of the way when not required without detaching the piece of apparatus from the microscope. The distance of 10 inches between the eyepiece and the table is maintained,

whether the microscope has a 6 or a 10 inch tube-length. Of more expensive description, but considered the best at present made, is the Abbe camera lucida (Fig. 31). The microscope may be used in a vertical or any inclined position with this apparatus. Its construction and the manner of using is as follows: Mounted in a cap, which is fitted immediately above the eyepiece, are two right-angle prisms; these are cemented together and form a cube. One of the cemented surfaces is silvered, but a small central disc is left clear, through which the object is viewed in the ordinary

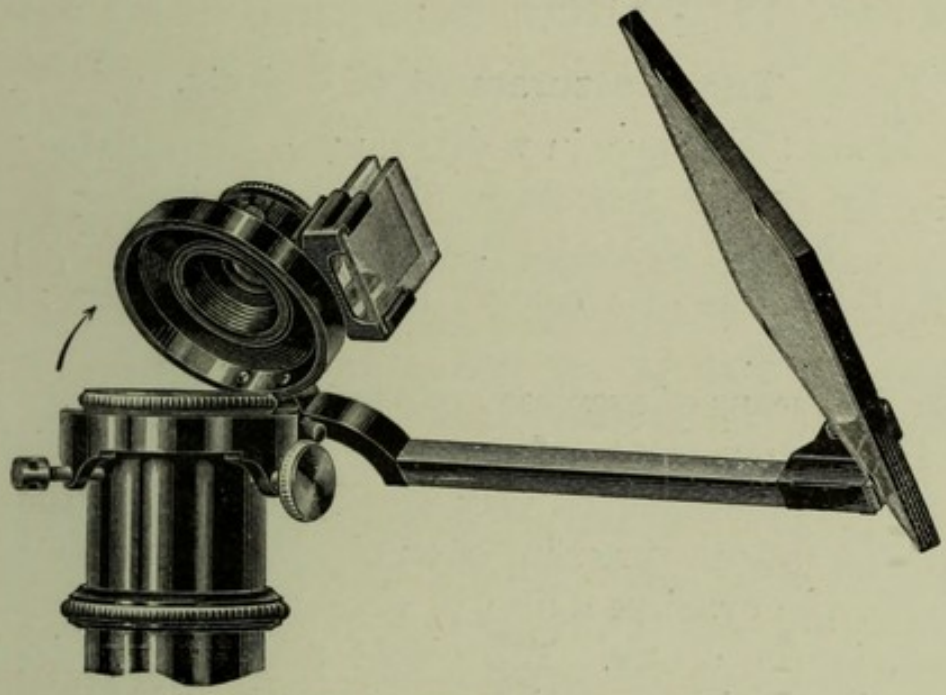

FIG. 31.—Abbe Camera Lucida.

way. The prisms are so set that the image of the paper on which the drawing is to be made, and which is reflected by a mirror to the prisms, is by them conveyed to the eye. Thus the pencils of light reach the eye coincidently from both the microscope and the paper, and when drawing the object, the pencil-point appears in the field of view very distinctly, and the minutest details can be exactly traced. Low-power eyepieces should be used with this camera lucida. There is another very commonly-used form of camera lucida, invented by Wollaston. In employing this it is necessary to put the microscope in a horizontal position,

but it is somewhat difficult to see the pencil-point with it when working. To do this it is necessary that one part of the pupil of the eye shall be over the prism and receiving the image, while the other part is looking down on the paper below. A little practice soon enables one to do this. Some workers prefer this to Beale's neutral-tint reflector, but in our hands no superiority has been apparent in it. There are many other forms peculiar to individual makers, possessing more or less merit, some of which may be used with the tube in any position, but particulars of these can easily be gained from catalogues.

The Measurement of Objects.

There are three ways in which this may be effected :

1. By having the stage divided—applicable to mechanical stages only.
2. By means of a camera lucida and a stage micrometer.
3. By means of eyepiece and stage micrometers.

1. If the movements of a mechanical stage are divided and read by verniers to very small parts of an inch or millimetre, the measurement of an object can be effected by having in the eyepiece a disc of glass with a diamond-cut line across the centre. The object that it is desired to measure is set with one point exactly against the diamond-cut line, which, of course, will appear in the field, and the reading of the stage divisions taken. The stage is then slowly moved along by means of the milled head until the other edge of the specimen to be measured is exactly touching the line. The stage divisions are again taken, and by subtracting one from the other the measurement will be ascertained. For quick work, and without extraneous appliances, this is fairly accurate, and largely used.

2. *Camera Lucida and Stage Micrometer.* — A stage micrometer usually consists of a number of lines photographed, or ruled with a diamond on a slip of glass to the scale of $\frac{1}{100}$th or $\frac{1}{1000}$th part of an inch, or the $\frac{1}{10}$th and

$\frac{1}{100}$th of a millimetre. This is put on the stage and focused like an ordinary object. The camera lucida is then fixed to the eyepiece, and the micrometer lines are projected on to a piece of paper in the same way as when drawing an object explained on page 96. The lines so projected are then measured, and supposing the lines of the micrometer, which are $\frac{1}{100}$th of an inch apart, appear when drawn on the paper 1 inch apart, it is at once known that the magnifying power in use is 100 diameters. The object may be measured in the same manner. Measurements should be taken about the centre of the field, and not towards the edge, especially with high powers, as, owing to curvature of the field, the outer edges appear more highly magnified than the centre.

3. *The Eyepiece Micrometer and Stage Micrometer.*—The stage micrometer, as previously described, is placed on the stage, and a somewhat similar micrometer is put into the eyepiece. This latter is generally divided into hundredths of an inch, but no exact value is needful so long as the lines are equi-distant. On focusing the stage micrometer the two sets of lines will appear in the field at once. It is now desirable to ascertain how many divisions of the eyepiece micrometer are included between one of the spaces—that is, $\frac{1}{100}$ of an inch—of the stage micrometer. Perhaps it will be found that there will be several lines of the eyepiece micrometer and a fraction in that space, and in order that this fraction may be obviated the drawtube should be slightly pulled out, which will give, of course, an increased amplification, until a certain number of the lines on the eyepiece micrometer are exactly equal to a division or divisions on the stage micrometer. We will imagine that the number of eyepiece micrometer lines that fill $\frac{1}{100}$ of an inch of the stage micrometer is five. The stage micrometer is now removed, and the object to be measured replaces it. The lines of the eyepiece micrometer will still be seen in the field, and bearing in mind that five of these lines equal $\frac{1}{100}$ of an inch, any part of the object can at once be

measured. It must be remembered, however, that with every objective an estimation of the value of the eyepiece micrometer is necessary.

To give greater facility and accuracy, a form of eyepiece micrometer is used, devised by Jackson, which is fitted in a frame, and by means of a micrometer screw traverses the object. If there be no mechanical stage to the instrument it is very difficult to set a special part of the object against the micrometer for measurement, especially with high powers. This form of micrometer surmounts this difficulty. The ordinary eyepiece micrometers necessitate no alteration to ordinary eyepieces, but the Jackson form requires that the outer tube of the eyepiece shall be cut to receive the carrier for the micrometer. Fig. 32 shows an eyepiece with the Jackson micrometer, *m*, in position.

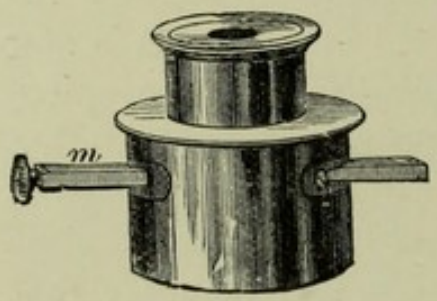

FIG. 32.—Jackson Micrometer fitted to Eyepiece.

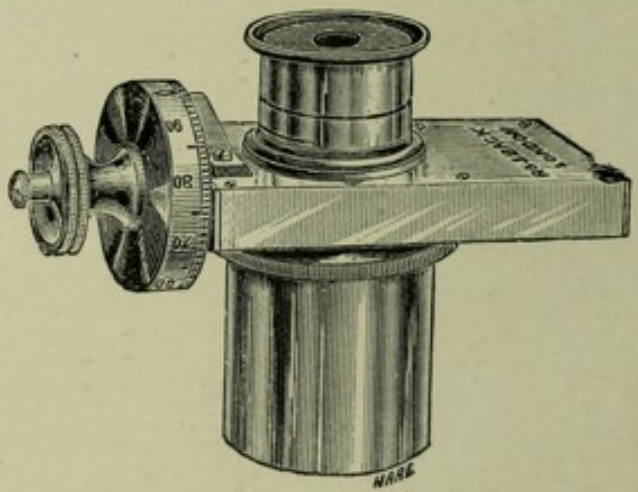

FIG. 33.—Ramsden Screw Micrometer.

There is yet another form of eyepiece micrometer, called the Ramsden screw micrometer, which consists of an eyepiece containing two wires, one fixed, the other travelling by means of a screw having 100 threads to the inch. The milled head of this screw is divided into 100 parts. Across the field are very small equi-distant V-shaped teeth, the interval between each of which corresponds to one complete revolution of the milled head. The value of these teeth is taken against the stage micrometer, and the object placed

on the stage. One edge of the object is then brought against the fixed wire, and the travelling wire moved to the other part that it is desired to gauge. By then counting the number of intervening teeth, and reading the fraction on the milled head, it can at once be ascertained what magnifying power is used. This is considered the most accurate and precise method of working, but it is an expensive piece of apparatus, and with care one of the previous methods named will be, as a rule, sufficient.

Persons having abnormal vision are likely to make errors in measuring. To obviate this, a cap carrying a lens that will correct the abnormality should be placed over the top of the eyepiece, as described on page 67, while measurements are being taken.

Troughs, Live-cages, Stage Forceps, etc.

Troughs.—These are made of various materials, including glass, vulcanite, brass, etc., and are used in the examination of infusoria and animalculæ alive under the microscope. The essentials of a trough are that a medium power, say ½ inch at least, can be used, that it may be easily cleaned, and that if broken it can be repaired. The ordinary

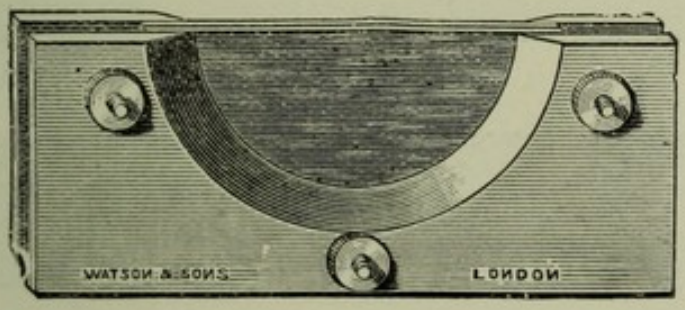

Fig. 34.—Botterill's Trough.

commercial glass troughs unfortunately do not meet these requirements. They are difficult to clean, they are invariably hard to mend when broken, and they very often leak when water is put in. The one that we have found most serviceable is the Botterill's trough, as shown in Fig. 34, which consists of two vulcanite plates between which

are placed slips of glass, which are separated by an india-rubber band, small bolts and screws passing through the whole to hold them together. This is not an ideal trough, but it certainly answers its purpose as well as any at present made.

Live-cages.—These are not used so largely for water objects as for insects, etc. They consist of a brass plate having a glass base-plate, over which a cap slides, having a very thin cover-glass. The subject to be viewed is placed between these two glasses and held firmly by compression. The best form is that designed by Mr. Rousselet, shown in

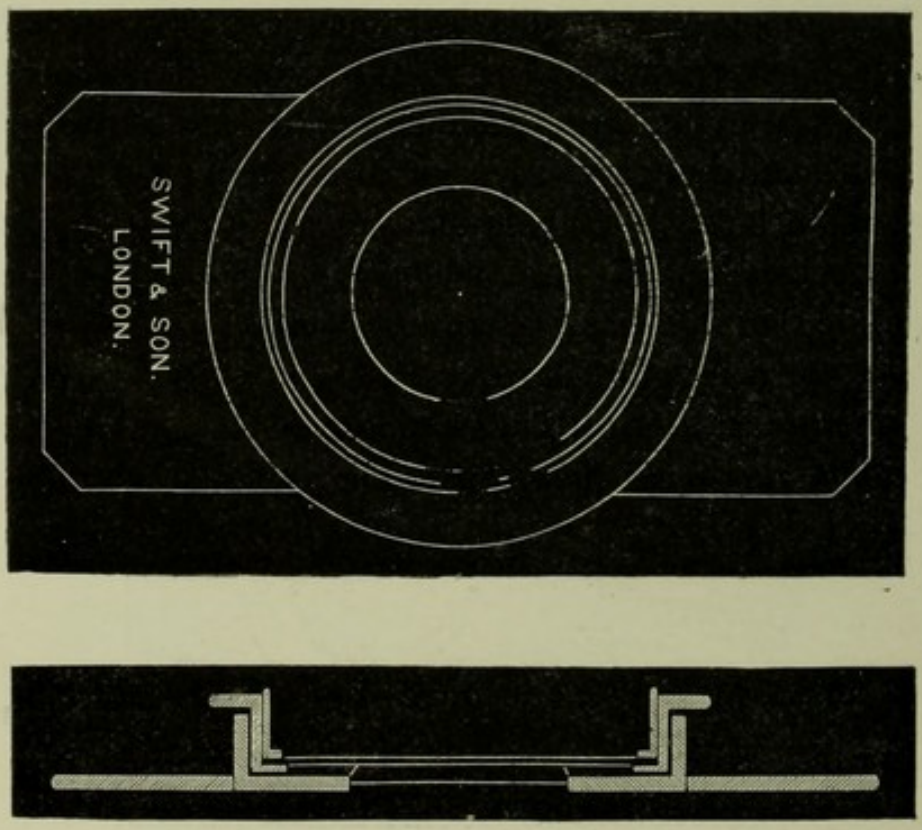

Fig. 35.—Rousselet's Live-cage.

Fig 35, with which a condenser may be used conveniently. It is also so arranged that even if a specimen be fixed at the extreme edge of the glass plate, there is room for an objective to work on it. The ordinary live-cages are usually provided with a coverglass too small in diameter for this to be done. A very good plan is often adopted by amateurs

for viewing live objects as follows: A square, flat piece of glass is obtained, and on this an indiarubber ring is laid, into which the animalculæ can be placed; a thin piece of glass is now put over the top of the indiarubber ring, and this really makes a very serviceable trough.

Rousselet's Compressor.— Mr. Rousselet, the designer of the live-cage previously mentioned, is also the originator of the most efficient compressor at present obtainable. It is shown in Fig. 36, and is made by Mr. C. Baker. The upper arm has cemented to its under side a portion of a circle of thin cover-glass, which enables high-power objectives to be employed, and the disc of glass in the base-plate is not too thick to prevent the employment of condensers of large aperture. The compression is quite parallel in action, being effected by turning a milled head at the top of a drum containing a spring, which causes the upper plate to rise when the milled head is released. The cut off top of the cover-glass permits of different media being inserted while the specimen is under examination, and the arm can be turned aside when desired for cleaning, etc.

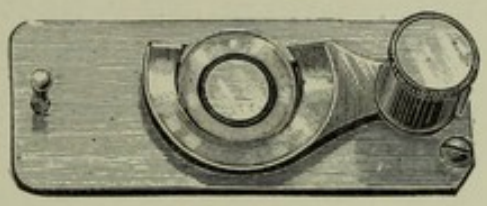

Fig. 36.
Rousselet's Compressorium.

Forceps.—Stage forceps are used to hold unmounted specimens in the field of view while they are examined, there being a fitting on the forceps to go into a hole provided in the limb or the stage of the instrument.

There are in existence many modifications of the apparatus described in the foregoing pages, the adoption or rejection of which must be left to the suggestions which will be naturally derived from practical experience. But the forms of apparatus most commonly worked with, and those whose merits particularly commend them to the writer's judgment, have been described.

For a more exhaustive treatment of the subject the following textbooks are recommended:

Carpenter's 'The Microscope and its Revelations,' edited by Dr. Dallinger.

'The Microscope,' by Dr. Henri van Heurck.

'Photo-micrography,' by Dr. Bousfield.

'Photo-micrography,' by Mr. Andrew Pringle.

And as periodicals the best are: *International Journal of Microscopy and Natural Science*, edited by A. Allen, Bath; and the *American Monthly Microscopical Journal*, edited by C. W. Smiley, Washington, D.C.

These journals both contain latest information with respect to the microscope.

HINTS REGARDING THE CARE OF THE MICROSCOPE.

Cleanliness is a most important feature in microscopical work. Never allow dust to accumulate upon the microscope, for it soon finds its way between the fittings, and causes mechanical screws to work with backlash. When not in use, the instrument should always be placed in its case or under a glass shade.

Dusting.—For dusting the microscope, a camel-hair brush should be used in the first place; by the aid of this dust can be removed from niches and crevices with great ease. For wiping over the stand, also for cleaning eyepiece lenses, the fronts of objectives, and other optical work, the writer has always employed handkerchiefs that are made of a mixture of silk and cotton. These should be washed out two or three times until they are soft and free from dust.

Adjusting a Microscope.—It is not to be recommended that other than microscope makers should take the instruments to pieces; but it is often necessary, where a person resides abroad, that he should be able to adjust his own microscope. It is difficult to give definite advice, because the fittings vary considerably in every make of microscope. If the rackwork of the coarse adjustment or sub-stage develop loss of time, it is more often than not due to the

bearings clutching on account of the presence of dust, or to their becoming dry. The way to adjust them is as follows: Rack the body up as far as it will go, and mark lines with a pen and ink on the pinion stem and the body of the microscope to correspond with one another. The object of this is to ensure the replacement of the body so that the rack engages the correct leaf of the pinion, and it is here presumed that a Jackson model microscope is used, and that it has a stop-pin to the rack, which prevents the body being removed from its fitting. Now remove the cock-piece, which holds the pinion in position, and take away the pinion itself, holding the body meantime, or it will run down on to the stage.

Remove the body from its fitting, wipe both bearings and the rack thoroughly with paraffin-oil and a clean rag, then dry them with another cloth. Now drop on, at most, two drops of watchmakers' oil on each side of the bearing fittings attached to the body, and replace the tube in its fittings. It should then be moved up and down until the motion is quite free, and if there are adjusting screws, they should be so set that there shall not be any shake in the fitting of the body, but that it may just, and only just, move in the bearings with its own weight when the instrument is set vertically. Carefully wipe the pinion leaves out, and then, after setting the ink-marks in correspondence again, the pinion may be attached, and this usually has adjusting screws to the cockpiece, which push it closer to, or allow it to remain farther from, the rack. These should then be so set as to give a soft movement. It is useless to attempt this procedure with a microscope that never has worked well, but where an instrument has, after use, become unsatisfactory in the mechanical parts, it generally is found to answer. Practically the same treatment is applicable to the mechanical movements of the stage, but very great care is essential, lest either of the plates become bent, an accident that is more easy of occurrence than would be deemed possible.

Objectives.—It is unwise for unskilled persons to unscrew microscopic objectives; they are frequently deranged by this means. If at any time it should, from some cause or another, be necessary to unscrew them, an ink-mark or small scratch should be made on each combination, so that when put together again they can be screwed up in the same positions as before. In many objectives it will be noted that three or four little spots are engraved in a line down the mount for the above purpose.

After using an oil immersion objective, the oil must be carefully removed from the front lens by wiping with the handkerchief. Undue pressure must not be used, but it must be thoroughly cleaned. If oil should become dried on the front lens at any time, it will be best to place some fresh immersion-oil over it; it should then be allowed to stand in a place free from dust for about an hour, when the whole may be cleaned off together.

Travelling.—When travelling with a microscope it is always well to pack the instrument round with tissue-paper, so that it cannot shake in its case. Screws frequently become loosened, and in some instances broken, and movements disordered, by severe shaking while in transit.

APPENDIX.

A short note concerning the influence of diffraction on the resolving power of microscopical objectives, and on the apparent colour of microscopical objects.

By Dr. G. Johnstone Stoney, F.R.S.

If we look from a distance at a flame through a thin feather or other uniformly ruled grating we see the flame, and around or on either side of it a number of lateral coloured images, which are wider and usually fainter the farther out that they lie. We thus learn that the light which passes through the grating becomes both a direct beam and a number of lateral, or diffracted beams as they are called. The proportions in which the light which passes the grating is distributed between the central beam and the several diffracted beams depends upon the ratio of the widths of the openings to the widths of the bars of the grating, as well as upon such particulars as whether each opening is a mere hole and each bar a mere obstruction, or whether they are occupied by material which acts on the light, especially if it act like a prism. It rarely happens that this distribution does not perceptibly differ for light of different wave-lengths. The direct beam consists of light in very nearly the same state as if it had passed through a simple opening of the size of the grating, except that it is fainter—usually fainter in some colours than in others.

Accordingly, if the eye when looking at the grating, or if the object-lens of a telescope, were to receive only this central beam wherewith to form an image of the grating,

the image would be almost identical with that which would be furnished by light coming through an opening covered by tinted glass, and no trace of the ruling would be seen in it. In order to see the ruling, the telescope lens must be able to catch and forward to its focus other rays which have passed through the grating than those of the central beam. The more of the lateral beams which it can transmit and combine at its focus with the light of the central beam (where they will by interference strengthen some parts of the image formed by the central beam, and enfeeble others, thus *introducing* detail), the more nearly will the image it forms resemble the actual grating in detail, and in freedom from false colour. If it succeeds in catching, along with the central beam, even some small portion of the nearest of the diffraction beams, the image will exhibit lines, and the proper number of lines, though it will not present correctly such minuter detail as the widths of the lines and of the spaces between them.

Cases exactly analogous to this occur with the microscope. When an object covered with dots, such as *Pleurosigma angulatum*, has been focused upon the stage, and is resolved, the diffraction beams may be clearly seen upon the back lens of the objective by removing the eyepiece and looking down the tube. With this diatom there will then be seen the central beam, and portions of the nearest of the lateral beams, six in number. A rather small cone of illumination is best to show them conspicuously if white light be used, and they can be seen with larger cones of illumination and very sharply defined if monochromatic green light, produced by prisms, be employed.

The markings on the *Pleurosigma angulatum* are spaced in each row at intervals which have been measured, and found to be equal to wave-lengths of red light. With so close a ruling the lateral beams are much diffracted or bent aside, and dry objectives can only take in the central beam and a portion of each of the nearest diffracted beams. This enables us to see with such an objective the markings

correctly so far as concerns their number and positions, but any further detail is not correctly presented. Immersion objectives can transmit nearly the *whole* of the six nearest lateral beams, which are those that would produce spectra of the first order. We now see some detail: the dots appear hexagonal, and are separated from one another by walls which are thin, and which look like a honeycomb. This is the first and the only step we can take towards learning what the actual detail upon this diatom is, since no objective is competent to supply to the image the second or subsequent diffraction beams; inasmuch as no immersion fluid can shorten the waves of visible light so much as would enable the object to emit and the lens to receive these further diffraction beams.

The unequal distribution of *colour* between the several beams is strikingly exhibited by the diatom known as *Actinocyclus Ralfsii*. The phenomenon may be conveniently examined through a half-inch apochromatic, over which is mounted an iris diaphragm—an adjunct which is useful for many purposes. Select a frustule which is blue when this upper diaphragm is partly closed. Remove the eye-piece, close the lower, and open the upper diaphragm. Then, on looking down the tube, it will be seen that most of the red is located in a ring of first lateral beams, with, of course, an equal defect of red in the central beam. Hence the blue colour seen when the image is formed by the central beam only. Now place a small central stop (which may be cut out of card) over the back lens of the objective, open the upper, and partially close the lower diaphragm. The image is then formed by the ring of lateral beams only, and will be found to be preponderatingly red.

PART II.

INTRODUCTION.

In publishing methods of preparing, staining, hardening, and mounting microscopic objects, I have adopted the system employed in my classes for some years past; that is, each separate stage of procedure is arranged in successive lessons or chapters. Of course, a subject such as this cannot be so lucidly described in writing as by demonstration, but it has been my aim to make it as clear as possible, so that if the instructions are carefully followed and practised, successful permanent work can be performed; but it is only by most scrupulous care and constant practice that any degree of success in this work can be attained.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING AND MOUNTING REAGENTS.

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Blood, human.	Dry on slide and cover.			
Do., amphibia.	Dry on slide.	Eosin and Methyl green.	C. Balsam.	Frog.
Epithelium.	2 % Bichromate potash.	Picrocarmine.	Farrant's.	Do.
Endothelium.	Nil.	Silver nitrate.	Farrant's or C. Balsam.	Rabbit.
White fibrous tissue.	Alcohol.	Nil.	Farrant's or glycerine jelly.	Tail of rat.
Yellow elastic tissue.	Chromic acid and spirit.	Nil.	Do., do.	Lig. nuchæ of ox.
Adipose tissue.	Methylated spirit.	Hæmatoxylin.	Do., do.	Any animal.
Tendon.	Do., do.	Do.	Do., do.	Sheep.
Adenoid tissue.	Müller's fluid.	Do., and eosin.	C. Balsam.	Cat.
Cartilage.	Chromic acid and spirit.	Do., do.	Do.	Do.
Bone.	Chromic and nitric acid.	Picrocarmine.	Farrant's.	Do.
Do., developing.	Do., do.	Hæmatoxylin and eosin.	C. Balsam.	Kitten.
Marrow.	Methylated spirit.	Hæmatoxylin.	Do.	Guinea-pig or cat.
Muscle, striated.	2 % Bichromate potash.	Do., and eosin.	Do.	Cat.
Do., non-striated.	Chromic acid and spirit.	Hæmatoxylin.	Balsam or Farrant's.	Colon of rabbit.
Nerve-fibres.	Osmic acid.	Osmic acid.	Farrant's.	Sciatic of frog.
Do., trunk.	Chromic acid and spirit.	Hæmatoxylin and eosin.	C. Balsam.	Do. of cat.
Bloodvessels.	Do., do.	Do., do.	Do.	Cat.
Lymphatic gland.	Müller's fluid.	Do., do.	Do.	Do.
Tonsil.	Methylated spirit.	Do., do.	Do.	Do.
Thymus gland.	Müller's fluid.	Do., do.	Do.	Human fœtus or calf.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING AND MOUNTING REAGENTS—*continued.*

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Skin.	Methylated spirit.	Hæmatoxylin and eosin.	C. Balsam.	Human palm of hand.
Nail.	Do., do.	Do., do.	Do.	Human fœtus.
Scalp.	Do., do.	Do., do.	Do.	Do.
Heart muscle.	Chromic acid and spirit.	Do., do.	Do.	Cat.
Trachea.	Do., do.	Do., do.	Do.	Do.
Lung.	Do., do.	Do., do.	Do.	Do.
Tooth.	Chromic and nitric acid.	Picrocarmine.	Farrant's.	Do.
Do., developing.	Do., do.	Do.	Do.	Kitten about two months old.
Tongue.	Chromic acid and spirit.	Hæmatoxylin and eosin.	C. Balsam.	Cat.
Œsophagus.	Do., do.	Do., do.	Do.	Do.
Stomach, cardiac end.	Absolute alcohol.	Soluble aniline blue.	Do.	Do.
Do., pyloric end.	Methylated spirit.	Hæmatoxylin and eosin.	Do.	Do.
Small intestine.	Chromic acid and spirit.	Do., do.	Do.	Do.
Large intestine.	Do., do.	Do., do.	Do.	Do.
Liver.	2 % Bichromate potash.	Do., do.	Do.	Do.
Pancreas.	Absolute Alcohol.	Do., do.	Do.	Do.
Salivary glands.	Do., do.	Do., do.	Do.	Do.
Spleen.	2 % Bichromate potash.	Do., do.	Do.	Do.
Supra-renal glands.	Methylated spirit.	Do., do.	Do.	Guinea-pig or cat.
Thyroid glands.	Do., do.	Do., do.	Do.	Cat.
Kidney.	2 % Bichromate potash.	Do., do.	Do.	Do.
Ureter.	Chromic acid and spirit.	Do., do.	Do.	Do.
Testicle.	Methylated spirit.	Do., do.	Do.	Do.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING AND MOUNTING REAGENTS—*continued.*

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Vas deferens.	Methylated spirit.	Hæmatoxylin and eosin.	C. Balsam.	Cat.
Epididymis.	Do., do.	Do., do.	Do.	Do.
Prostate.	Do., do.	Do., do.	Do.	Do.
Ovary.	Do., do.	Do., do.	Do.	Do.
Fallopian-tube.	Do., do.	Do., do.	Do.	Do.
Uterus.	Do., do.	Do., do.	Do.	Do.
Mammary gland.	Do., do.	Picrocarmine.	Farrant's or C. Balsam.	Do.
Spinal cord.	2 % Bichromate ammonium.	Aniline blue black.	C. Balsam.	Do.
Medulla oblongata.	Do., do.	Do., do.	Do.	Do.
Pons Varolii.	Do., do.	Do., do.	Do.	Do.
Cerebellum.	Do., do.	Do., do.	Do.	Do.
Cerebrum.	Do., do.	Do., do.	Do.	Do.
Eyelid.	Methylated spirit.	Hæmatoxylin and eosin.	Do.	Do. or human fœtus.
Cornea.	Müller's Fluid.	Do., do.	Do.	Do. or sheep.
Choroid.	Do., do.	Do., do.	Do.	Sheep.
Crystalline lens.	2 % Bichromate potash.	Picrocarmine.	Farrant's.	Cat.
Retina.	Müller's fluid.	Hæmatoxylin and eosin.	C. Balsam.	Ox.
Sclerotic.	Do., do.	Do., do.	Do.	Do.
Optic nerve.	Do., do.	Do., do.	Do.	Do.
Olfactory mucous membrane.	Do., do.	Do., do.	Do.	Rat.
Internal ear. Cochlea.	Do., do., and decalcify.	Carmine in bulk.	Do.	Guinea-pig.

LIST OF BOTANICAL SPECIMENS, AND THE MOST SUITABLE PRESERVING, STAINING AND MOUNTING MEDIA.

SPECIMEN.	PRESERVING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.
Stems, young.	Methylated spirit.	Hæmatoxylin.	C. Balsam.
Do., older.	Do., do.	Carmine and acid green.	Do.
Leaves.	Do., do.	Hæmatoxylin.	Do.
Ovaries.	Do., do.	Do.	Do.
Anthers.	Do., do.	Borax carmine.	Do.
Epidermis for stomata.	Macerate in water.	Methyl aniline.	Glycerine jelly.
Fibro-vascular tissues.	Do., do.	Acid aniline green.	Do.
Yeast.	Camphor water.	Unstained.	Camphor-water.
Green algæ.	Acetate of copper solution.	Do.	Acetate of copper solution.
Red algæ.	Dilute methylated spirit.	Do.	Glycerine jelly.
Protococcus.	Acetate of copper solution.	Do.	Acetate of copper solution.
Volvox.	Do., do.	Do.	Do., do.
Desmids.	Do., do.	Do.	Do., do.
Raphides.	Macerate in water.	Do.	C. Balsam.
Starches.	Methylated spirit.	Do.	Glycerine jelly.
Fertile branch of chara.	Do., do.	Do.	Camphor-water.
Antheridia and archegonia of mosses.	Do., do.	Do.	C. Balsam.

LESSON 1.

HARDENING AND PRESERVING ANIMAL TISSUES AND ORGANS FOR MICROSCOPICAL EXAMINATIONS.

Fresh untreated tissues are unsuited for microscopical purposes, but it is sometimes advisable to observe the appearance of some specimens, such as muscle-fibres, tendon, connective tissues, and nerve-fibres, while fresh. When this is desired, the tissue must be examined in certain fluids called normal fluids that will alter its character as little as possible. Those generally used are: (1) blood serum; (2) the aqueous humour from a fresh eye; and (3) normal or $\frac{3}{4}$ per cent. salt solution. The two former are difficult to obtain, but the latter can be made at any time, and it will answer for most purposes. Place a small piece of the tissue on a slide, add a drop or two of salt solution, take two needles fixed in holders and carefully separate the fibres from each other; this process is called teasing. When sufficiently teased, apply a cover-glass and examine. You may now wish to irrigate with some staining reagent; if so, place a few drops of the stain at one edge of the cover-glass, and apply a piece of blotting-paper to the other side; this will absorb the salt solution, and the staining fluid will follow and take its place around the tissue; the slide may then be placed under the microscope, and the action of the reagent observed.

These specimens cannot, as a rule, be kept. For permanent preparations the tissues or organs must be hardened This is accomplished by subjecting them to the action of certain hardening or fixing solutions. The following are most commonly used:

Absolute Alcohol.—Suitable for stomach, pancreas, and salivary glands. These organs must be perfectly fresh, and

they should be cut into small pieces, so that the alcohol may penetrate as quickly as possible.

Change the alcohol every day for the first three days. The hardening is usually complete in a week.

Chromic Acid and Spirit.—Chromic acid $\frac{1}{6}$ per cent., watery solution 2 parts, and methylated spirit 1 part. This reagent hardens in about ten days. Then transfer to methylated spirit, which should be changed every day until no colour comes away from the tissues. It is suitable for cartilage, nerve-trunks, heart, lips, bloodvessels, trachea, lungs, tongue, bladder, ureter, intestines, and œsophagus.

Potassium Bichromate.—Make a 2 per cent. watery solution. This will harden in about three weeks. Then transfer to methylated spirit, and change the spirit every day until no colour comes away from the tissues. It is suitable for muscle, spleen, liver, and kidney.

Ammonium Bichromate.—Make a 2 per cent. watery solution. It hardens in from three to four weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. It is suitable for spinal cords, medulla, pons Varolii, cerebellum, and cerebrum.

Müller's Fluid. — Bichromate of potash 30 grains, sulphate of soda 15 grains, distilled water $3\frac{1}{2}$ ounces. It hardens in from three to five weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. Suitable for lymphatic glands, eyeballs, retina, and thymus gland.

Methylated Spirit.—May be used universally, if preferred, but it has a tendency to shrink some tissues too much. It hardens in about ten days. Change the spirit every twenty-four hours for the first three days. Suitable for skin, scalp, testicle, penis, prostate gland, vas deferens, epididymis, ovary, uterus, Fallopian tubes, placenta, mammary gland, supra-renal glands, tonsils, and all injected organs.

Decalcifying Solution. — For bones. Make a $\frac{1}{6}$ per cent. watery solution of chromic acid, and for every ounce

add 5 drops of nitric acid. This fluid will soften the femur of a dog in about three weeks; larger bones will take longer. Change the fluid several times, and test its action by running a needle through the thickest part of the bone. If it goes through easily, the bone is soft enough; if not, continue the softening process a little longer. When soft enough transfer to water, and soak for an hour or two; then pour off the water and add a 10 per cent. solution of bicarbonate of soda, and soak for twelve hours to remove all trace of acid. Wash again in water, and place in methylated spirit until required. Bones and teeth should always be softened in a large quantity of the decalcifying solution.

Mr. A. Hopewell Smith's process of preparing teeth:

(1) Immerse a newly-extracted tooth in Müller's fluid for three to four weeks, then place in spirit for ten to twenty days.

(2) Remove from spirit and seal up apical foramen and soft parts with collodion.

(3) Place tooth in 15 c.c. of the following solution:

Hydrochloric acid	...	...	12 parts
Nitric acid ...	...	...	30 ,,
Distilled water	...	...	108 ,,

(4) After soaking fifteen hours, add 1·5 parts of nitric acid, and after forty-eight hours add 1·5 parts more nitric acid. After seventy-five to eighty hours, remove, wash for half an hour in a solution of 5 grammes of lithium carbonate in an ounce of distilled water.

(5) Divide tooth with a razor into several pieces, and wash again in water, and soak in gum mucilage for twelve to fifteen hours.

(6) Place on stage of freezing microtome, cut, and wash sections in water, and stain with orange-rubine, gold chloride, borax carmine, or Weigert's hæmatoxylin.

(7) Dehydrate in absolute alcohol, clear in cedar oil, and mount in Canada balsam.

Olfactory Region.—Divide with a saw the head of a freshly killed rabbit or guinea-pig longitudinally, and parallel with the nasal septum. Cut out the septum so as to expose the olfactory region, which is recognised by its brown colour. Dissect out a portion including some of the turbinated bones. Harden this in Müller's fluid for three or four days. Then transfer to chromic and nitric acid decalcifying solution, and soak until the bones are quite soft. Wash well in water to remove all trace of acid, and complete the hardening in methylated spirit.

Cochlea.—Dissect out the internal ear of a freshly-killed young guinea-pig, open bulla with bone-forceps, when a conical elevation, the cochlea, will be seen. Remove as much of the surrounding bone as possible, and place the cochlea in Müller's fluid for two weeks to harden the delicate nervous tissues. Then transfer to chromic and nitric acid decalcifying solution, and soak until the bone is soft. Place in weak spirit for a day or two, and then transfer to strong methylated spirit.

General Directions for Hardening Tissues.

1. Always use fresh tissues.
2. Cut the organs into small pieces with a sharp knife.
3. Never wash a specimen in water; when it is necessary to remove any matter, allow some normal salt solution to flow over the surface of the tissue, or wash in some of the hardening reagent you are going to use.
4. All specimens should be hardened in a large quantity of the reagent; too many pieces should not be put into the bottle, and they should be kept in a cool place.
5. In all cases the hardening process must be completed in spirit.
6. Label the bottles, stating the contents, the hardening fluid used, and when changed. Strict attention to these details is necessary for successful histological preparations, for if the hardening is neglected good sections cannot be made.

LESSON 2.

EMBEDDING TISSUES AND SECTION-CUTTING.

To Cut Sections with a Razor by Hand.—Take the tissue between the thumb and forefinger of the left hand. Hold the finger horizontally, so that its upper surface may form a rest for the razor to slide on. Take the razor, hold it firmly in the hand, keep the handle in a line with the blade, and draw it through the tissue from heel to tip towards yourself. While cutting, keep the razor well wetted with dilute methylated spirit, and as the sections are cut place them in a saucer of dilute methylated spirit.

Embedding in Paraffin Wax and Lard.—Melt together by the aid of gentle heat four parts of solid paraffin and one part of lard. A quantity of this may be made and kept ready for use at any time. Melt the paraffin mass over a water-bath. Take the specimen and dry it between the folds of a cloth to remove the spirit, so that the paraffin may adhere to its surfaces, place it in a pill-box in the desired position, and pour in enough melted paraffin to cover it, then set aside to cool. When quite cold, break away the pill-box and cut sections from the embedded mass with a sharp razor. When a number of specimens are embedded, and it is desired to keep them for some time, they should be preserved in a jar of methylated spirit.

To Infiltrate a Tissue with Paraffin.—Dehydrate the specimen in absolute alcohol for several hours, then transfer to chloroform or xylol, in which it must remain until perfectly saturated. When clear, place in a bath of melted paraffin of 45° C. (melting-point), and keep it at this temperature for several hours, so that the paraffin may penetrate to the middle of the tissue. Then remove it from the paraffin and put it into a small pill-box, pour in enough

paraffin to fill the box, and as the paraffin cools, add a little more to make up the shrinkage and set aside to cool. When cold, place in water for a few minutes; this will soften the paper, and facilitate the removal of the pill-box. You will now have a cylinder of paraffin with the specimen firmly fixed in its centre, and, if desired, the paraffin may be pared away from the sides until a square block is obtained. The sections may now be made by hand with a razor, or the block can be fixed to a microtome with a little melted paraffin. The sections must be placed in turpentine to remove the paraffin, then in absolute alcohol to remove the turpentine, and, finally, in distilled water to remove the alcohol; they may then be stained. Sometimes it is desirable to stain the tissue in bulk before it is embedded. In this case the sections need only go into turpentine or benzole to wash away the paraffin; they may then be mounted in Canada balsam.

The above process requires an embedding bath. This is usually an expensive affair, but one that will answer all ordinary purposes can easily be made.

Get a small potato steamer, and cut a hole in the lower vessel to admit a spirit or small paraffin lamp. Get a tinsmith to cut out a circular plate of tin to fit into the upper vessel, in which some holes must be cut to take the test-tubes, and to the sides of the vessel four small pieces of tin, bent at right angles, must be soldered to support the tin plate. A piece of tin must also be soldered over the perforated bottom of the vessel, so that it will hold water. When the alterations are complete, place a layer of cotton-wool or a piece of felt on the bottom of the steamer, to protect the test-tubes from breakage; half fill with water, add a thermometer, light the lamp, and on the desired temperature being attained, put some paraffin in the test-tubes, place them in the steamer, and when the paraffin has melted add the specimens.

After use dry the apparatus so that rust may not set in. If this is attended to it will last for years.

When a proper embedding bath cannot be obtained, tissues may be infiltrated with paraffin in the following way: Dehydrate the specimen in absolute alcohol; then place in a quantity of chloroform or benzole, ten or twelve times

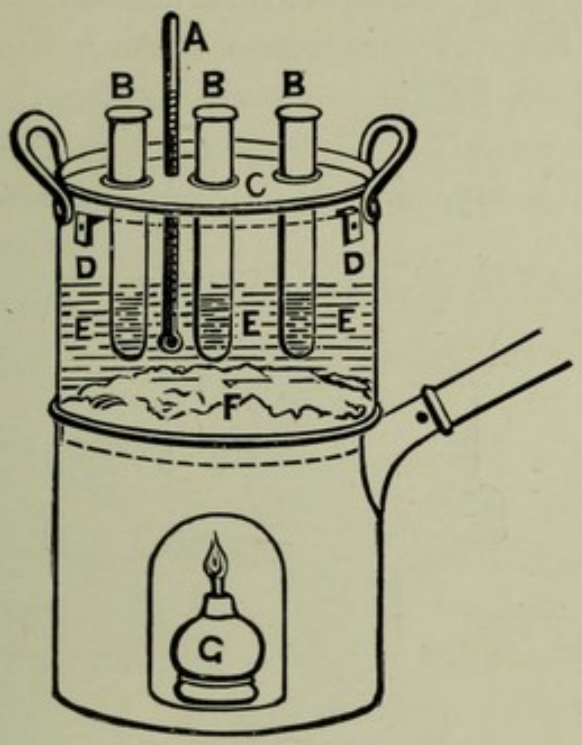

Fig. 37.—Potato-Steamer converted into an Embedding Bath.

(A) Thermometer. (B) Test-Tubes. (C) Disc of Tin. (D) Tin Supports. (E) Water. (F) Cotton-Wool. (G) Spirit or Small Paraffin Lamp.

the bulk of the tissue, until saturated; add small pieces of paraffin until no more will dissolve, and set aside for several hours. Apply gentle heat to drive off the solvent and melt the paraffin, after which, the tissue can be removed, and embedded in a pill-box of paraffin of the desired melting-point.

Cole's Microtome and Embedding in Carrot.—When a number of sections are wanted, or when a complete section of an organ is desired, a microtome should be used. A very good and simple instrument can be obtained from Messrs. Watson and Sons, 313, High Holborn. Screw the microtome firmly to the table, and with the brass tube supplied with the microtome punch out a cylinder of carrot to fit into the well of the microtome. Cut this in half

longitudinally, and scoop out enough space in one half of the carrot to take the specimen; then place the other half of carrot in position, and make sure that the specimen is held firmly between them, but it must not be crushed. Now put the cylinder of carrot and specimen into the well of the microtome and commence cutting the sections. A good razor will do, but it is better to use the knife which Messrs. Watson supply with the microtome. While cutting, keep the knife and plate of the microtome well wetted with

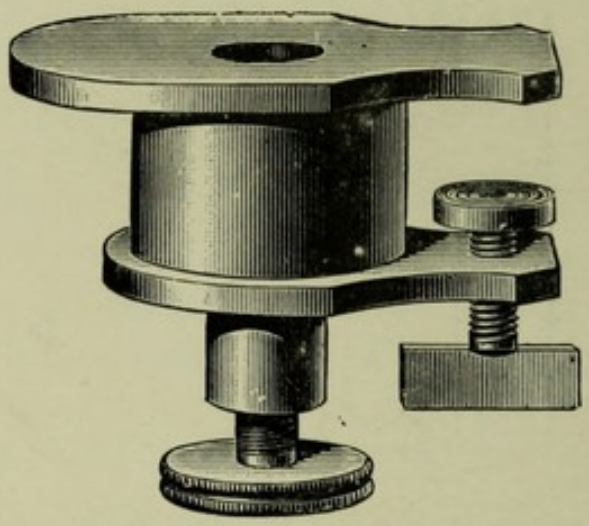

Fig. 38.—Cole's Pattern Microtome.

dilute methylated spirit, and as the sections are cut place them in a saucer of dilute spirit. A number of sections may be cut and preserved in methylated spirit until required.

When a specimen has a very irregular outline, it cannot be successfully embedded in carrot. Paraffin should then be used. Place the tissue in the well of the microtome in the desired position, pour in enough melted paraffin to cover it, and when cold cut the sections.

Freezing Microtome.—Cathcart's is the most simple and cheapest freezing microtome, and it can be obtained from any optician.

(1) Cut a slice of the specimen about $\frac{1}{8}$ inch thick, in the direction you wish to make the section.

(2) Place in water for an hour to remove the alcohol.

(3) Transfer to a mixture of gum-water 5 parts, saturated watery solution of loaf-sugar 3 parts, and allow it to soak in this for about twelve hours; or, if a few drops of carbolic acid are added to the mixture, tissues may remain in it for months without harm.

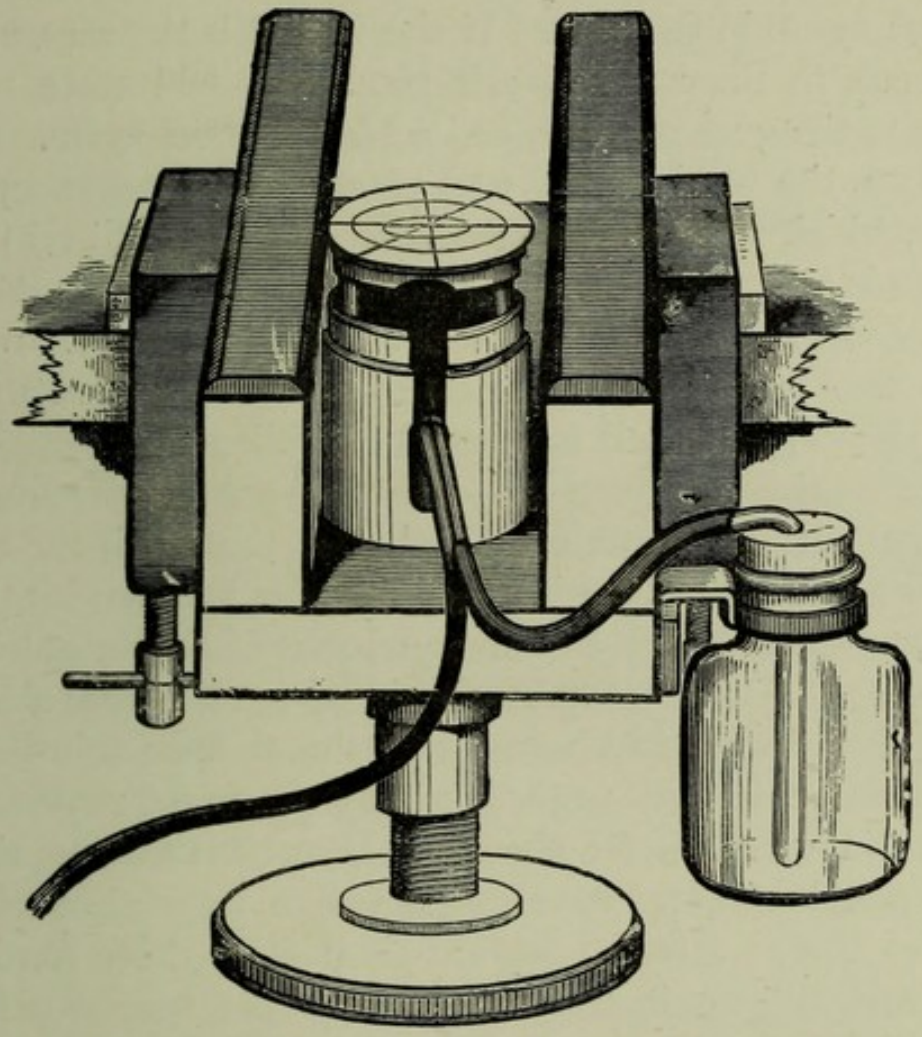

Fig. 39.—Cathcart Microtome.

(4) Clamp the microtome to a table, fix the ether spray in its place, and fill the bottle with ether. Methylated ether, Sp. gravity 720, will do.

(5) Put a little gum-water—not gum and syrup—on the zinc plate of the microtome, and place the tissue in it. Commence working the bellows, and as soon as all the gum has frozen add some more and freeze again, and so on until the tissue is completely covered and frozen into a solid mass.

(6) The best instrument for making the sections is the blade of a carpenter's plane. Hold it firmly in the right hand, and work the microtome screw under the machine with the left. Plane off the sections as quickly as possible. They should all collect on the plane iron. If they roll up or fly off, the tissue is frozen too hard, or there is not enough syrup in the gum. If the former is the case, allow the mass to thaw a little; if the latter, add some more syrup to the gum mixture, and soak the tissue again.

When the sections are cut, place them in a saucer of water, which must be changed several times until all trace of gum is removed. Water that has been boiled and allowed to cool will remove the gum sooner than cold water. When quite free from gum, the sections may be bottled up in methylated spirit until required for staining.

Embedding in Celloidin.—Dissolve Schering's celloidin in equal parts of absolute alcohol and ether until the solution is as thick as glycerine. Divide the solution into two parts, to one of which add an equal part of absolute alcohol and ether. Dehydrate the specimen in absolute alcohol for several hours, then transfer to the thinner solution of celloidin, and soak until perfectly saturated; place in the thick celloidin for about an hour, or until required. Take a cork and paint over one end a layer of celloidin, and let it dry; this will prevent air bubbles rising from the cork and lodging in the mass. Take the specimen from the celloidin and lay it on the cork, and let it stand for a minute or two, then add some more celloidin until the tissue is completely covered, and set aside, and when the mass has attained such a consistency that on touching it with the finger no impression will remain, place it in 50 per cent. alcohol for an hour or two to complete the hardening, or it may remain there until required. The embedded mass can now be placed between two pieces of carrot, and put into an ordinary microtome, and the sections made with a knife or razor, which must be well wetted with methylated spirit; or the embedded specimen can be

removed from the cork, and, after soaking in water, it can be transferred to gum and syrup, and the sections made with a Cathcart freezing microtome. If it is desired to remove the celloidin from the sections, soak them in equal parts of absolute alcohol and ether. When all the celloidin is removed, transfer to distilled water, then into the stain. After staining, wash in distilled water, dehydrate, clear in clove oil, and mount in Canada balsam.

When it is not desirable to remove the celloidin from the sections, they should be stained in borax carmine or hæmatoxylin. The former stains celloidin, but the colour is removed by washing in acidulated alcohol. Hæmatoxylin only stains it slightly. All the aniline dyes stain it deeply; they should not be used.

Tissues are usually stained in bulk before they are infiltrated with celloidin. When so, the sections must be dehydrated in methylated spirit, cleared in oil of bergamot, and mounted in Canada balsam.

When desirable, sections infiltrated with celloidin may be mounted in Farrant's medium or glycerine jelly. Wash away all trace of alcohol with water, and mount in either of the above media in the ordinary way.

When a number of celloidin masses are prepared for future use, they must be preserved in a vessel of methylated spirit.

The Rocking Microtome.—This machine is made by the Cambridge Scientific Instrument Company. It is only used for specimens infiltrated with paraffin, and it is automatic; that is to say, it can be set to cut sections of definite thickness, and every time the handle is pulled a section is cut, and the specimen is moved forward ready for another.

Infiltrate the tissue with paraffin in the ordinary way in a pill-box, and when the paraffin has set, remove the box and trim the paraffin into a rectangular block. Take care to keep the edges quite parallel, so that they may adhere together as the sections are cut and form a riband. The Cambridge Instrument Company makes an apparatus for

embedding, called imbedding L's. If these are used, perfectly rectangular blocks are formed ready for fixing to the brass cap at the end of the arm of the microtome, which

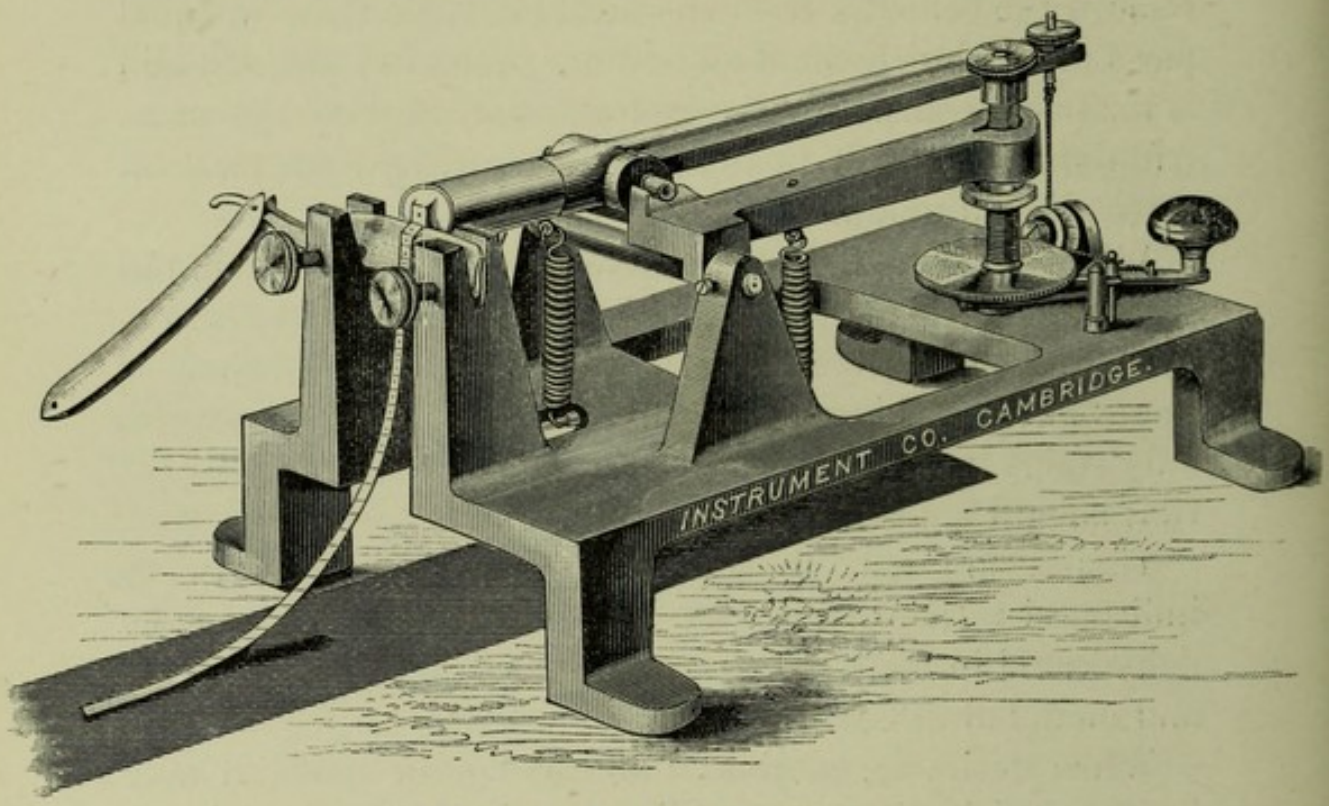

Fig. 40.—Rocking Microtome.

is filled with paraffin; this should be warmed over a spirit-lamp, and the block containing the specimen is to be pressed against the melted paraffin until it adheres firmly.

LESSON 3.

STAINING ANIMAL SECTIONS AND MOUNTING IN CANADA BALSAM.

All sections of organs and tissues should be stained with some colouring reagent, so that their structure may be made more apparent. Certain parts of the tissue have a special affinity for the dyes or stain; they therefore become more deeply tinted, and stand out clearly from the surrounding tissues.

The following staining reagents are the most useful:

Grenacher's Alcoholic Borax Carmine.—Carmine 3 grammes; borax 4 grammes; distilled water 100 c.c. Dissolve the borax in the water, add the carmine, and apply gentle heat until all is dissolved; then add 100 c.c. of 70 per cent. alcohol, filter, and keep in a stoppered bottle.

Staining Process.—(1) Place the section in distilled water to wash away the alcohol, then place a little of the carmine in a watch-glass, and immerse the section for from three to five minutes.

(2) Wash the section in methylated spirit.

(3) Take of methylated spirit 5 parts, and of hydrochloric acid 1 part, and mix them well together. A quantity of this acid solution may be made up and kept ready for use at any time.

Immerse the section in the above, and leave it to soak for about five to ten minutes, or, if overstained, until the desired tint is obtained. Sections of skin and scalp may be left until all colour is removed from the fibrous tissues; the glands, hair follicles, and Malpighian layer will then stand out clearly.

(4) Wash the section well in methylated spirit to remove all traces of the acid, then transfer to some perfectly clean and strong methylated spirit for from ten to fifteen minutes to dehydrate.

(5) Place some oil of cloves in a watch-glass, take the section from the spirit on a lifter, and carefully float it on to the surface of the oil, in which it must remain for about five minutes. This process is called clearing; the object of it is to remove the alcohol and to prepare the section for the balsam.

(6) Transfer the section to some filtered turpentine to wash away the oil of cloves, and mount it in Canada balsam. Sections may be mounted in Canada balsam direct from the oil of cloves, but it is better to wash in turpentine first, because if much oil is mixed with the balsam it will not dry; the oil also has a tendency to cause the balsam to turn a dark-yellow colour.

Logwood or Hæmatoxylin.—Hæmatoxylin 30 grains, absolute alcohol 3½ ounces, distilled water 3½ ounces, glycerine 3½ ounces, and ammonia alum 30 grains. Dissolve the hæmatoxylin in the alcohol and the alum in the water; mix the two solutions together, and add the glycerine and 3 drachms of glacial acetic acid. The mixture must now be left exposed to light for at least a month, then filter and keep in a stoppered bottle.

Staining Process.—(1) If the specimen has been hardened in any of the chromic solutions, place the section in a 1 per cent. watery solution of bicarbonate of soda for about five minutes, then wash well in distilled water. If it is a spirit preparation the soda will not be required, but all sections must be washed in distilled water before they go into logwood stain.

(2) To a watch-glassful of distilled water add from 10 to 20 drops of the logwood solution, and immerse the section for from ten to thirty minutes.

(3) Wash in distilled water, then in ordinary tap water; the latter will fix the dye and cause the colour to become blue.

When a section has been overstained with hæmatoxylin, the excess of colour may be removed by soaking it for a few minutes in a ½ per cent. solution of glacial acetic acid in distilled water, then wash again in tap water.

(4) Dehydrate in methylated spirit.

(5) Clear in clove oil, and mount in Canada balsam.

Double Staining with Hæmatoxylin and Eosin.—Stain the section in hæmatoxylin, as directed above, then place it in an alcoholic solution of eosin—about 1 grain of eosin to an ounce of methylated spirit is strong enough—and let it soak for about five minutes; wash well in methylated spirit, clear in clove oil, and mount in Canada balsam.

Aniline Blue-Black.—Dissolve 30 grains of nigrosine in 3½ ounces of distilled water, then add 1 ounce of rectified alcohol and filter. This stain is only used for sections of brain and spinal cord. Immerse the sections for from thirty

to sixty minutes, wash in water, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Aniline Blue.—Make a 1 per cent. solution of soluble aniline blue in distilled water and filter. Stain the section for five to ten minutes, wash in water, and place in methylated spirit, in which it must soak until the excess of colour is removed. Clear in clove oil and mount in Canada balsam.

This stain is useful for cardiac glands of the stomach, brain and spinal cord.

Golgi's Nitrate of Silver Methods.—These are chiefly employed for investigating the relations of cells and fibres in the central nervous system. Two methods are mostly used, as follows :

(*a*) Very small pieces of the tissue, which have been hardened for some weeks in bichromate solution or Müller's fluid, are placed for half an hour in the dark in 0·75 per cent. nitrate of silver solution, and are then transferred for twenty-four hours or more to a fresh quantity of the same solution (to which a drop or two of formic acid may be added.) They may then be hardened with 50 per cent. alcohol, and sections, which need not be thin, are cut either from celloidin with a microtome or with the free hand. The sections are mounted in Canada balsam, which is allowed to dry on the slide. They must not be covered with a cover-glass, but the balsam must remain exposed to the air.

(*b*) Instead of being slowly hardened in bichromate, the tissue is placed at once in very small pieces in a mixture of bichromate and osmic (3 parts of Müller's fluid to one of osmic acid). In this it remains from two to five days, after which the pieces are treated with silver nitrate, as in the other case. This method is not only more rapid than the other, but is more sure in its results.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of pure benzol, filter, and keep in an outside stoppered bottle. Clear the

section in clove oil, and place it in turpentine. Clean a cover-glass and a slide, place a few drops of balsam on the centre of the latter, take the section from the turpentine on a lifter, allow the excess of turpentine to drain away, and with a needle-point pull the section off the lifter into the balsam on the slide. Now take up the cover-glass with a pair of forceps, and bring its edge in contact with the balsam on the slide; ease it down carefully, so that no air-bubbles are enclosed, and with the points of the forceps press on the surface of the cover until the sections lie quite flat, and the excess of balsam is squeezed out. The slide must now be put aside for a day or two to allow the balsam to harden; the exuded medium may then be washed away with some benzol and a soft camel's-hair brush, after which dry the slide carefully with a cloth and apply a ring of cement. The above method answers well for mounting sections quickly, but when time will admit the following is a much better way. Clear the section and place it in turpentine; clean a cover-glass, and moisten the surface of a slide with your breath; apply the cover-glass to the slide, and make sure that it adheres. Place a few drops of balsam on the cover, into which put the section. Now put the slide away in a box, or in some place out of reach of dust, for twelve hours, so that the benzol may evaporate from the balsam. Clean a slide, warm it gently over the flame of a spirit-lamp; apply a drop of balsam to the surface of the hardened balsam on the cover-glass; take the cover up in a pair of forceps, and bring the drop of fresh balsam in contact with the centre of the warmed slide. Ease the cover down carefully, so that no air-bubbles may be enclosed, press on the surface of the cover-glass until the section lies quite flat; set the slide aside to cool. The exuded balsam may then be washed away with methylated spirit and a soft rag, and a ring of cement applied.

Staining in Bulk.—Place small pieces of the tissue in Grenacher's alcoholic carmine for from one to three days, then transfer to a ½ per cent. solution of hydrochloric acid

in methylated spirit for from one to twelve hours, according to the size of the tissue. Wash well in spirit, and soak for a day in 90 per cent. spirit. Dehydrate in absolute alcohol, clear in chloroform or xylol, and embed in paraffin.

Fixing and Staining Sections on the Slide.

Mayer's Albumen Method. — White of egg 50 c.c., glycerine 50 c.c., salicylate of soda 1 gramme; shake well together, and filter into a stoppered bottle. A thin layer of the cement is spread on a slide with a brush, and the section laid on it. Now warm gently on a water-bath. As the paraffin melts it is carried away from the section by the albumen. The section may now be washed with turpentine, benzole, and alcohol, and be treated with aqueous or other stains, without fear of it moving.

Shellac Method.—Make a solution of shellac in absolute alcohol—it should be about the thickness of oil—filter, and keep in a stoppered bottle. Warm some slides, and spread over them a layer of the cement with a brush, and put away to dry. When dry apply a very thin layer of creasote; this will form a sticky surface, on which the section must be carefully laid. Now heat the slide on a water-bath for about fifteen minutes at the melting-point of the paraffin; this will allow the section to come down on the shellac film, and at the same time evaporate the creasote. Allow the slide to cool, and wash away the paraffin with turpentine or benzole. If the section has been stained in bulk, a drop or two of Canada balsam is added, and a cover-glass applied.

To Stain a Section on the Slide.—Fix section on slide as directed above. Wash away the paraffin with rectified mineral naphtha, follow this quickly with a few drops of methylated spirit, and then with some distilled water. Now apply the stain, and place the slide under a bell-glass to prevent evaporation; or the slide may be plunged into a vessel containing the staining solution. When sufficiently

stained, wash with distilled water, dehydrate with methylated spirit, drain away the spirit, and apply a drop of clove-oil to clear the specimen. When clear, drain away as much of the oil as possible, add a drop of Canada balsam, and apply the cover-glass.

LESSON 4.

STAINING BLOOD AND EPITHELIUM, TEASING-OUT TISSUES, AND MOUNTING IN AQUEOUS MEDIA.

STAINING WITH PICROCARMINE, GOLD CHLORIDE, SILVER NITRATE, AND OSMIC ACID.

Double-staining Nucleated Blood Corpuscles.

Stain A.—Dissolve 5 grains of eosin in ½ ounce of distilled water, and add ½ ounce of rectified alcohol.

Stain B.—Dissolve 5 grains of methyl green in an ounce of distilled water.

Place a drop of frog's blood on a slide, and with the edge of another slide spread it evenly over the centre of the slip; now put it away out of reach of dust to dry. When quite dry, flood the slide with stain A for three minutes. Then wash with water, and flood the slide with stain B for five minutes. Wash again with water, and allow the slide to dry. Apply a drop or two of Canada balsam and a cover-glass.

Blood of Mammals, Non-nucleated Corpuscles.—Spread some blood on a slide and let it dry. Then put the slide in a turn-table, and run on a ring of any good cement; allow this to become perfectly dry, and then apply another coat, which must be allowed to *become nearly* dry; now clean a cover-glass and put it on the cement, and with a needle press it down until it adheres firmly to the cement.

Epithelium.—Kill a frog, cut off its head, and remove the lower jaw. Open the abdomen and take out the stomach, and slit it open. Place the head, lower jaw and stomach in a 2 per cent. solution of bichromate of potash for forty-eight hours. Then wash gently in water until no colour comes away from the specimens. Now place all three portions in picrocarmine for twenty-four hours. Remove the tissues from the carmine, and allow the stain to drain away from them. Take the lower jaw and scrape the tongue for squamous epithelium, and place the deposit obtained in a few drops of glycerine on a slide. Take the stomach, remove some columnar epithelium from its internal surface, and place it in some glycerine on another slide. Then take the head for ciliated epithelium, which will be found at the hinder part of the roof of the mouth; put some scrapings from this in glycerine on a slide as before. Clean a slide and place a drop or two of Farrant's medium on its centre; take up a little of the epithelium on the point of a needle, and put it into the medium. Now apply a cover-glass, and with the needle-point press it down until the epithelium cells are separated and spread evenly between the cover and the slide. Set the slide aside for a day or two, so that the medium may set. Then wash away the excess of medium with some water and a camel's hair brush, dry the slide with a soft rag, put it in a turn-table, and run on a ring of cement.

Portions of the tongue, trachea and intestine of a rabbit or cat may be treated in the same way.

Endothelium.—Take a piece of the omentum of any small animal, and rinse gently in distilled water to remove soluble matter. Place it in a $\frac{1}{4}$ per cent. solution of silver nitrate for ten minutes, or until it becomes a milky white. Wash well in ordinary water, and expose in a saucer of water to diffused sunlight, until it assumes a brownish colour. Cut out a small piece and mount it in Farrant's medium or glycerine jelly. In this specimen only the interstitial cement substance will be seen. To compare

with it, cut out a similar piece, wash it in distilled water, and stain it with hæmatoxylin for ten minutes; wash away all excess of stain with distilled water, and mount in Farrant's medium or glycerine jelly. In this specimen the nuclei will be seen stained blue. Specimens of mesentery showing endothelium may also be mounted in Canada balsam. When this is desired, stain the tissue as directed above, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Teasing-out Tissues.—Take a very small piece of the tissue, place it on a slide in a few drops of distilled water, and with a couple of needles mounted in holders carefully separate the fibres from each other. When the parts are sufficiently isolated, drain away the water, add a few drops of the mounting fluid, and apply the cover-glass. When teasing it is very important that a proper background should be used, so that the object may be easily seen. For a coloured specimen, a piece of white paper should be used, and a transparent white tissue will be seen better on a dark ground, such as a piece of black paper or American cloth; and as you proceed the slide should be examined from time to time under the low power of the microscope to ascertain when the tissue is teased out enough.

White Fibrous Tissue.—Harden some tendons from a rat's tail in methylated spirit for a week. Then soak a small piece in water to remove all trace of spirit, place it on a slide in a few drops of water, and tease it up until the fibres are separated from each other. Drain away the water, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

Yellow Elastic Tissue.—Place small pieces of the ligamentum nuchæ of an ox in chromic acid and spirit for ten days. Then proceed as above.

Striped or Voluntary Muscle.—Harden small pieces of muscle of a pig in a 2 per cent. solution of bichromate of potash for three weeks, then transfer to methylated spirit, in which it may remain until required. Soak a piece in

water to remove the spirit, place a very small fragment on a slide in a few drops of water, and with a couple of needles tease or tear the tissue up so as to separate the fibres. Drain away the excess of water, apply a drop or two of Farrant's medium and a cover-glass.

Non-striped Muscle.—Harden a piece of the intestine of a rabbit in chromic acid and spirit for ten days. Wash in water, strip off a thin layer of the muscular coats, and stain it in hæmatoxylin. Wash in distilled water, and then soak in ordinary tap-water until the colour becomes blue. Clean a slide, place a small fragment of the muscle on it in a few drops of water, and with needles separate the fibres. Drain off the excess of water, apply a few drops of Farrant's medium and a cover-glass.

Nerve Fibres.—Dissect out the sciatic nerve of a frog, and stretch it on a small piece of wood as follows: Take a match, make a slit in each end of it, into which put the ends of the nerve; now place it in a 1 per cent. solution of osmic acid for an hour or two. Wash in water, tease up a small fragment on a slide, and apply a few drops of Farrant's medium and a cover-glass.

When staining with gold chloride, solutions from $\frac{1}{2}$ per cent. to 5 per cent. in distilled water are employed. It is used for staining nerves and nerve-endings; it also brings out the cells of the cornea, fibrous connective tissues, and cartilage.

The tissue must be taken from the animal immediately after death, and be placed in the solution of gold for from half an hour to an hour; it is then removed to distilled water for twelve hours, and afterwards exposed to the action of diffuse sunlight in a saturated solution of tartaric acid or formic acid until it assumes a purple colour.

The future treatment will depend on the nature of the specimen.

If muscle has been stained for nerve-endings, place a small piece on a slide, tease it up, and examine with a low power until you find a nerve-fibre terminating in an end-

plate on a muscle-fibre, separate it from the surrounding fibres as much as possible, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

If cornea or cartilage, make vertical and horizontal sections with a freezing microtome, and mount in Farrant's medium or glycerine jelly. Sections of gold-stained tissues may also be mounted in Canada balsam, when this is desired, dehydrate in strong spirit, clear in clove oil, and mount in Canada balsam.

There are many ways of staining with gold, but the above is the most simple, and it gives very good results. For the other methods the student may refer to the larger works on practical histology.

Staining with Picrocarmine.—Rub up 1 gramme of carmine with 10 c.c. of water, and 3 c.c. of strong liquid ammonia; add this to 200 c.c. of a saturated solution of picric acid in distilled water. Leave the mixture exposed to the air until it evaporates to one-third of its bulk; filter, and keep in a stoppered bottle. Place some of the picrocarmine in a watch-glass, and immerse the section for from half an hour to an hour. Remove from the stain with a lifter, and place the section on a slide; drain away as much of the excess of stain as possible, and, if necessary, soak up what remains with a piece of filter-paper. Then add a few drops of Farrant's medium, and apply the cover-glass.

Picrocarmine stained tissues should never be washed; if they are, all the yellow colour will be removed, and the specimen will come out stained with carmine only. They improve by keeping, and the staining process goes on for several days after they are mounted; that is to say, some parts give up the stain, and others absorb it. Picrocarmine may be purchased in crystals, with which a 2 per cent. solution in distilled water should be made.

If it is desirable to mount a picrocarmine stained section in Canada balsam, proceed to stain as above; then make a saturated solution of picric acid in methylated spirit, filter, and dehydrate the section in it; then give it a final rinse

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

Farrant's Medium.—Take of glycerine and a saturated aqueous solution of arsenious acid equal parts, and mix them well together; then add as much powdered gum arabic as the mixture will take up, and let it stand for six weeks. Filter, and keep in an outside stoppered bottle.

The above is difficult to make; it is better to obtain it ready for use.

Glycerine Jelly.—Dissolve 1 ounce of French gelatine in 6 ounces of distilled water; then melt in a water-bath, and add 4 ounces of glycerine and a few drops of creasote or carbolic acid. Filter through paper while warm, and keep in a stoppered bottle. The above may be used instead of Farrant's medium. The jelly must, of course, be warmed before use. All tissues or sections must be well soaked in water before they are mounted in Farrant's medium or glycerine jelly, so that all trace of alcohol is removed.

Tissues containing much air should be soaked in water that has been boiled for about ten minutes and allowed to cool.

LESSON 5.

STAINING AND MOUNTING MICRO-ORGANISMS.

The investigation of bacteria may be carried out under various conditions.

(1) In fluids, such as milk, water, blood, pus, etc. (2) On solid media, bread, meat, potatoes, meat jelly, etc., or in the tissues and organs of animals. In the former case a drop of the fluid is placed on the centre of a cover-glass, and another cover-glass is placed on it; the two glasses are then to be rubbed together to spread the organisms evenly over their surfaces; they are then separated and allowed to dry. When bacteria are growing

on solid material, scrape off a small portion, put on a cover-glass, and treat as above; separate the covers, and allow to dry. When the cover is quite dry, take it up with a pair of forceps, organisms uppermost, and pass two or three times through the flame of a spirit-lamp; this will fix the albumen and fasten the bacteria to the glass.

To Stain Bacteria on Cover-Glasses.—They should be floated with the organisms downwards on a saturated watery solution of any of the following aniline dyes: Methyl blue, methyl violet,'gentian violet, fuchsin, vesuvin, or Bismarck brown. From ten to fifteen minutes is enough for the first four stains; vesuvin and Bismarck brown require about an hour. When the staining is complete wash the cover in distilled water. If the colour is too deep wash it in a ½ per cent. solution of acetic acid, and then again in water; put away to dry. When quite dry add a drop of Canada balsam, and mount on a slide in the usual way.

When bacteria are present in the organs of animals the tissues should be hardened in methylated spirit for about a week, and very thin sections with a freezing microtome cut from them. The sections may be stained in any of the above dyes, then wash in water, dehydrate in spirit, clear in oil of cedar or bergamot, and mount in balsam.

Staining Bacillus Tuberculosis.

Ehrlich's Method for Double-staining.—To 100 parts of a saturated watery solution of aniline oil add 11 parts of a saturated alcoholic solution of fuchsin, and filter. Place the covers or sections in the stain in a watch-glass, and warm slowly over a spirit-lamp until vapour rises. Wash in water, and then immerse for about a minute in dilute nitric acid, 1 part of acid to 2 parts water. Wash again in water, and stain again in a solution of methyl blue, 100 parts of distilled water to 20 parts of a saturated solution of methylated blue in alcohol for about twenty minutes. Wash in water, and in the case of sections dehydrate in

spirit, clear in oil of cedar or bergamot, and mount in balsam. The cover-glass preparations must be dried, then add a drop or two of balsam, and mount as above. The aniline oil solution is made by adding the oil to distilled water. Shake well, and let it stand for twenty-four hours before using. Water will only take up about 3 per cent of aniline oil.

Ziehl Neelsen's Method.—Fuchsin 1 part, 5 per cent. watery solution of carbolic acid 100 parts, absolute alcohol 10 parts. Remove the section from the alcohol, and immerse in the above stain for fifteen minutes.

Decolorize in a 5 per cent. watery solution of sulphuric acid, wash well in water to remove acid, and counter-stain in the following for five minutes: Saturated alcoholic solution of methyl blue 1 c.c.

Nitric and sulphuric acid is liable to injure some sections; when this is the case, Mr. Watson Cheyne recommends the following: After staining with fuchsin the sections are transferred to distilled water, rinsed in alcohol, and placed for two hours in the following contrast stain: Saturated alcoholic solution of methylene blue 20 c.c., distilled water 100 c.c., formic acid (sp. gr. 1·2) 1 c.c., caustic potash, 1 in 10,000 of water, 200 c.c. Wash in distilled water, dehydrate in absolute alcohol, clear in oil of cedar, and mount in Canada balsam.

Gibbe's Double Stain. — Rose aniline hydrochloride, 2 grammes; methyl blue, 1 gramme; rub well together in a mortar. Then dissolve aniline oil 3 c.c. in 15 c.c. of rectified spirit, and add the crystals to the mixture; shake well, and when all are dissolved, add 15 c.c. of distilled water. Place the cover-glass preparations or sections in the stain in a watch-glass, and warm gently over a spirit-lamp; then let them soak for four or five minutes. Wash in methylated spirit until no colour will come away, clear in oil of cedar, and mount in Canada balsam.

Cover-glass preparations will not require clearing; they are allowed to dry, then add a drop of balsam and mount on a slide.

Gram's Method.—*Solution A.*—Saturated alcoholic solution of gentian violet, 11 parts; saturated watery solution of aniline, 100 parts. Mix well together, and filter.

Solution B.—Iodine, 1 part; iodide of potassium, 3 parts; distilled water, 300 parts.

Solution C.—Saturated aqueous solution of vesuvin.

Take the section from alcohol, and place in Solution A for one to three minutes. Wash in alcohol, and transfer to Solution B for three minutes. Wash in alcohol, and place in Solution C for five minutes. Wash in distilled water, dehydrate, clear in oil of cedar or bergamot, and mount in Canada balsam.

Anthrax Bacillus.

Alkaline Blue Method.—To 100 parts of a solution of caustic potash (1 in 10,000) in distilled water, add 30 parts of a saturated alcoholic solution of methylene blue. Immerse the sections for an hour. Wash in distilled water, and then in a ½ per cent. solution of acetic acid in distilled water. Wash away the acid with water, dehydrate in alcohol, clear in oil of cedar or bergamot, and mount in Canada balsam.

Anthrax bacilli may also be stained by Gram's method.

Leprosy Bacillus.

Proceed as for tubercle bacillus by Ehrlich's method.

Bacillus of Diphtheria.

Employ Gram's method, as for tubercle bacillus.

Glanders Bacillus.

Kuhne's Method.—Methylene blue, 1 to 5 grammes; absolute alcohol, 10 c.c. When all the blue has dissolved, add 100 c.c. of a 5 per cent. watery solution of carbolic acid.

The sections are transferred from alcohol to the above stain for half an hour. Wash in water, and place in a weak solution of acetic acid in distilled water until they are of a pale-blue colour; watch carefully, or too much colour may

be removed; they are then rinsed in lithia water (1 in 70) 1 c.c., water 30 c.c., and transferred to water. The sections are now to be taken up one at a time on the point of a needle and dipped into absolute alcohol, in which some methylene blue has been dissolved. Dehydrate in methylene aniline oil, made as follows: Rub up about 10 grammes of methylene blue with 10 c.c. of aniline, and let the mixture settle. When dehydrated, rinse in aniline, and place for a few minutes in turpentine to clear, then mount in Canada balsam.

Schutz's Method.—Stain the sections or cover-glass films in methylene blue, 1 gramme; rectified spirit, 20 c.c.; distilled water, 80 c.c., for several hours. Wash in a $\frac{1}{2}$ per cent. solution of acetic acid, dehydrate in absolute alcohol, and clear in cedar oil, and mount in Canada balsam.

Actinomycosis.

Stain the sections in the following for ten minutes, warmed to about 45° C.: Magenta, 2 parts; aniline oil, 3 parts; rectified spirit, 20 parts; distilled water, 20 parts. Wash in water. Place in a concentrated alcoholic solution of picric acid for five to ten minutes. Wash in water. dehydrate in alcohol, clear in clove oil, and mount in Canada balsam.

Weigert's Method.—Glacial acetic acid, 5 c.c.; absolute alcohol, 20 c.c.; distilled water, 40 c.c.; add orseille until a dark-red fluid is obtained. Stain the sections in the above for an hour; rinse quickly in alcohol. Clear in cedar oil and mount in Canada balsam.

LESSON 6.

INJECTION OF BLOODVESSELS.

Carmine and Gelatine Injecting Mass.—

Pure carmine	60 grains.
Liq. ammonia fort.	2 drams.
Glacial acetic acid	86 minims.
Gelatine solution (1 ounce in 6 ounces of water)	2 ounces.
Water	2 ounces.

Dissolve the carmine in the ammonia and water in a test-tube, and mix it with one-half of the warm gelatine. Add the acid to the remaining half of gelatine, and drop it little by little into the carmine mixture, stirring well all the time with a stick or glass rod. Filter through flannel, and add a few drops of carbolic acid to make the mass keep. The principle to be remembered in making this mass is this: the carmine, if alkaline, would diffuse through the vessels and stain the tissues around them; if acid, the carmine would be deposited in fine granules, which would block up the capillaries; hence the necessity for a *neutral* fluid. The best guides are the colour and smell of the fluid. It should be a bright red, and all trace of smell of ammonia must be removed. The gelatine solution is made by putting 1 ounce of gelatine into 6 ounces of water; it must then be left until the gelatine becomes quite soft; then dissolve over a water-bath.

Prussian or Berlin Blue and Gelatine Mass.—Take 1½ ounces of gelatine, place it in a vessel and cover it with water; allow it to stand until all the water is absorbed and the gelatine is quite soft. Then dissolve in a hot-water bath. Dissolve 1 dram of Prussian or Berlin blue and 1 dram of oxalic acid in 6 ounces of water, and gradually mix it with the gelatine solution, stirring well all the time; then filter through flannel.

Watery Solution of Berlin Blue.—Dissolve 2½ drams of the blue in 18 ounces of distilled water and filter. This fluid is useful for injecting lymphatics.

Injecting Apparatus Required.—An injecting syringe fitted with a stopcock, and several cannulæ of various sizes.

Directions for Injecting.—The animal to be injected should be killed by chloroform, so that the vessels may be dilated, and injected while warm; if possible it should be placed in a bath of water at a temperature of 40° C. Expose the artery of the parts to be injected, clear a small portion of it from the surrounding tissues, and place a ligature of thin twine or silk round it. With sharp scissors make an oblique slit in the wall of the vessel, insert the cannula, and tie the ligature firmly over the artery behind the point of the cannula, into which put the stop-cock. Fill the syringe with injection fluid, which must not be too warm, and take care not to draw up any air-bubbles; now insert the nozzle of the syringe into the stopcock and force in a little fluid; remove the syringe, so that the air may escape, insert the syringe again, and repeat the process until no air-bubbles come out of the stopcock. You may then proceed slowly with the injection. Half an hour is not too long to take over the injection of an animal of the size of a cat. The completeness of an injection may be judged by looking at the vascular parts, such as the tongue, eyelids, and lips. When the injection is complete shut the stop-cock, remove the syringe and cannula, and tie the ligature round the artery. Now place the animal in cold water for an hour to set the injection-fluid. When quite cold dissect out the organs, cut them up into small pieces, and place them in methylated spirit to harden, and change the spirit every twenty-four hours for the first three days. The hardening will be complete in ten days.

Injection of Lymphatics (*Puncture Method*).—A small subcutaneous syringe is filled with a watery solution of Berlin or Prussian blue, and the nozzle is thrust into the pad of a cat's foot. The injection is to be forced into the

tissues. Then rub the limb from below upwards. This will cause the injection-fluid to flow along the lymphatics, and find its way into the glands of the groin.

To Inject Lymph-sinuses of Glands.—Force the nozzle of a subcutaneous syringe into the hilum of a lymphatic gland of an ox, and inject a watery solution of Prussian or Berlin blue until the blue appears on the surface of the gland. Then place it in methylated spirit to harden.

When *blue* injection-fluid is used, add a few drops of acetic acid to the spirit while hardening the tissues.

LESSON 7.

CUTTING, STAINING, AND MOUNTING VEGETABLE SECTIONS.

Stems, leaves, roots, ovaries, etc., should be hardened in methylated spirit for a week or ten days, and the spirit changed every twenty-four hours for the first three days. The stems must not be too old. One, too, and three years' growth will show all that is required.

Section-cutting, by hand and with a microtome, should be done in the same manner as described in Lesson 2.

Bleaching.—Vegetable sections generally require bleaching before they can be properly stained. Chlorinated soda is used for this purpose. Take of dry chloride of lime 2 ounces, of washing soda 4 ounces, and distilled water 2 pints. Mix the lime in 1 pint of the water and dissolve the soda in the other. Mix the two solutions together, shake well, and let the mixture stand for twenty-four hours. Pour off the clear fluid, filter, and keep in a stoppered bottle in a dark place, or cover the bottle with paper. Soak the sections in distilled water. Pour off the water and add a quantity of bleaching fluid. Allow this to act for from one to twelve hours. Wash well in water, which must be changed several times to remove all traces of soda. The

sections may now be stained, or they may be preserved in spirit until required.

Staining—Borax Carmine (suitable for ovaries, fruits, etc.).—Pure carmine 1 dram, liq. ammoniæ fort. 2 drams. Dissolve the carmine in the ammonia, and add 12 ounces of a saturated solution of borax in distilled water. Filter and keep in a stoppered bottle.

(1) Put some stain in a watch-glass, and immerse the section three to five minutes.

(2) Wash well in methylated spirit.

(3) Take of hydrochloric acid 1 part, and of methylated spirit 5 parts; mix well together, and soak the section until the colour changes to a bright scarlet, which takes about five minutes. The acidulated spirit may be kept ready for use at any time.

(4) Wash well in methylated spirit. Then place in some strong methylated spirit, and soak for at least ten minutes to dehydrate.

(5) Place the section on the surface of a small saucer of clove oil, and let it soak until clear.

(6) Remove from the clove oil and place in turpentine, and then mount in Canada balsam.

Hæmatoxylin. — Hæmatoxylin, 30 grains: absolute alcohol, 3½ ounces; distilled water, 3½ ounces; glycerine, 3 ounces; ammonia alum, 30 grains; glacial acetic acid 3 drams. Dissolve the hæmatoxylin in the alcohol and the alum in the water; then add the glycerine and acetic acid. Mix the two solutions together, and let the mixture stand for at least a month before use.

(1) Add about 30 drops of the above to an ounce of distilled water, and stain the section for fifteen to thirty minutes.

(2) Wash well in distilled water, and then in ordinary tap-water. This will fix the colour and make it deeper.

(3) Dehydrate in strong methylated spirit for at least ten minutes.

(4) Clear in clove oil and mount in Canada balsam.

Double-staining.—

Green Stain.—Acid aniline green	...	2 grains.
Distilled water	...	3 ounces.
Glycerine ...	...	1 ounce.

Mix the water and glycerine together, and dissolve the green in the mixture.

Carmine Stain A.—Borax	...	... 10 grains.
Distilled water	...	1 ounce.
Glycerine ...	...	½ ounce.
Alcohol rect.	...	½ ounce.

Dissolve the borax in the water, and add the glycerine and alcohol.

B.—Carmine ...	...	10 grains.
Liq. ammoniæ	...	20 min.
Distilled water	...	30 min.

Dissolve the carmine in the water and ammonia. Mix A and B together and filter.

(1) Place the section in green stain for five to ten minutes.

(2) Wash in water.

(3) Place in carmine for ten to fifteen minutes.

(4) Wash well in methylated spirit.

(5) Dehydrate and clear in clove oil. Wash in turpentine and mount in Canada balsam.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of benzole. Filter and keep in an outside stoppered bottle.

(1) Clean a cover-glass, moisten the surface of a slide with the breath, apply the cover-glass to it, and make sure that it adheres.

(2) Place a few drops of balsam on the cover-glass.

(3) Take the section out of the turpentine on a lifter, and put it into the balsam on the cover.

(4) Put away out of the reach of dust for twelve hours, to allow the benzine to evaporate from the balsam.

(5) Warm a slide over a spirit-lamp and apply a drop of balsam to that on the cover-glass; take it up with a pair of forceps, and bring the drop of fluid balsam in contact with the centre of the warmed slide. Ease the cover down carefully, so that no air-bubbles may be enclosed, and press it down with the point of the forceps until the section lies quite flat and the excess of balsam is squeezed out. Allow the slide to cool, and the excess of balsam may then be washed away with some methylated spirit and a soft rag.

Eosin.—Make a 1 per cent. solution of eosin in alcohol. This stain is useful for staining sieve-tubes and plates. Make longitudinal and transverse sections of the stem of vegetable marrow, immerse in the above for ten minutes, wash in methylated spirit, clear in clove oil, and mount in Canada balsam. Eosin may also be used as a counter-stain with hæmatoxylin. Stain the section with hæmatoxylin in the ordinary way, then transfer to dilute acetic acid in distilled water. Wash away all trace of acid with repeated changes of distilled water, and immerse the section in ordinary tap-water for ten to fifteen minutes; place in the eosin for five minutes, wash in methylated spirit, clear in clove oil, and mount in Canada balsam.

Sections of ovaries, young roots, and stems stain very well by the above method.

Pollens.—Place some mature anthers in a large pill-box, and allow them to become perfectly dry. Shake the box well until all the pollen is set free; then remove the anther sacs with a pair of forceps, and place the pollen in a bottle of turpentine; soak for several days to remove all trace of air, then pour off the turpentine; take up a little of the pollen on the point of a penknife, and place it in a few drops of Canada balsam on a cover-glass; stir up with a needle to spread the grains evenly over the cover, and put away to dry. When the balsam has dried, add a few more drops of balsam, take up the cover with a pair of forceps, and mount it on a warm slide. This method of mounting must always be employed for pollens, because, if they

are put up in any other way, the balsam only hardens at the edge of the cover, and remains in a more or less fluid state in the centre; with the result that, if the slide were placed on its edge, the specimens would run together in a heap at the lower side of the cover.

Pollens may also be mounted as opaque objects; see Lesson 13 on Dry Mounts.

Anthers.—Place some immature anthers in methylated spirit for several days, then transfer to equal parts of absolute alcohol and ether, and soak for two or three hours. Pour off this, and add a thin solution of celloidin, and soak for several days. Remove a specimen on the point of a needle, and hold it exposed to the air for a minute or two so that the celloidin may set around it. Now place in methylated spirit for several hours to complete the hardening. Embed between two pieces of carrot, and make transverse sections in an ordinary well microtome.

Stain the sections with borax carmine, dehydrate in methylated spirit, clear in oil of bergamot, and mount in Canada balsam.

Ovaries.—Gather some specimens before the bud has opened, and others immediately after, and place them in methylated spirit for a week. Make transverse sections, stain in hæmatoxylin, and then in alcoholic eosin. Wash in methylated spirit, clear in clove oil, and mount in Canada balsam.

LESSON 8.

THE PREPARATION OF VEGETABLE TISSUES FOR MOUNTING IN GLYCERINE JELLY, ACETATE OF COPPER SOLUTION, ETC.

Epidermal Tissues for Stomata.—Get some leaves, cut them into small pieces, and place them in a jar of water, in which they must remain for several weeks, or until the cellular tissue rots and the epidermis is set free. Wash

well in water, stain in a watery solution of methyl violet, and mount in glycerine jelly.

Annular Vessels.—Get some stem of maize, cut it into pieces about half an inch long, and then cut again into thin longitudinal slices; place these in water until rotten. Now put some of the broken-up material on a slide and examine with a microscope; pick out the annular vessels on the point of a needle, place them in some clean water, and wash well. Stain in a weak watery solution of acid green, and after washing in water, mount in glycerine jelly.

Scalariform Vessels.—Treat pieces of the rhizome of Pteris aquilina in exactly the same way as stem of maize.

Spiral Vessels.—Treat pieces of the stem of rhubarb in the same manner as annular vessels.

Raphides may be isolated, or they can be mounted *in situ* in the tissues in which they occur. For the former, take some leaves of cactus, stem of rhubarb, and root of Turkey rhubarb, cut them up into thin slices longitudinally, and place them in a jar of water, covered up to keep out dust, and put away until the tissue has become perfectly disintegrated. This will take several weeks, and the process is more easily carried out by keeping the jar in a warm place. When all the material has broken up, stir well with a glass rod, and strain through a piece of coarse muslin into a shallow vessel, such as a soup-plate; stir up again, and then allow to settle for a minute, so that the raphides may fall to the bottom of the plate; now pour away as much of the dirty water as possible, add more clean water, and repeat the process until you have got rid of all the disintegrated vegetable fibre. Now pour the raphides into a bottle, and if they are quite clean pour off the water, and add methylated spirit, in which they may be preserved until required for mounting.

To mount isolated raphides, clean a cover-glass, fasten it to a slide with the aid of your breath, take up some of the raphides in a dipping-tube, place them on the cover-glass, and spread them evenly over its surface with a needle.

Place the slide out of reach of dust until all the spirit has evaporated and the raphides are quite dry; add a few drops of Canada balsam, and put the slide away again for twelve hours; then add a few drops more balsam, take up the cover with a pair of forceps, and mount it on a warmed slip. When the raphides are very large, they must be mounted in balsam that is rather thicker than is usually used.

Raphides in situ in Tissues.—Harden the stems, roots, or leaves, in methylated spirit, and make sections in the ordinary way; dehydrate, clear in clove-oil, and mount in Canada balsam.

Raphides in Scale-Leaves of Bulbs, such as Onion, Garlic, Lily, Hyacinth.—Strip off a thin portion of the cuticle, place it in methylated spirit for a few hours, and when dehydrated clear in clove-oil and mount in Canada balsam.

Sometimes raphides are rendered too transparent when mounted in balsam. When this is the case they must be put up in glycerine jelly in the following way:

Isolated Specimens.—Pour off the methylated spirit, and add water; pour off the water, leaving the raphides at the bottom of the bottle. Clean a cover-glass and a slide. Place a few drops of warmed glycerine jelly on the centre of the slide; take up a few of the raphides on the point of a penknife, and place them in the glycerine jelly, but do not stir them up. Now apply the cover-glass, and press it down carefully with a needle, giving it at the same time a twisting motion, to spread the raphides evenly between the cover and slide. Put away for an hour or two, scrape off the excess of jelly with a penknife, wash in water, and then in methylated spirit, dry with a cloth, and apply a coat of black enamel or gold size. When raphides in the tissues are prepared in glycerine jelly, wash away all trace of spirit with water, and mount in glycerine jelly as above.

Starches.—*Isolated Specimens.*—If the tissue is fresh, scrape the cut surface with a knife, and place the scrapings in a bottle of water; shake well, and then strain through

fine muslin into a shallow vessel; let the starch settle, pour off the water, and wash again with some clean water until the starch is quite clean; then place it in a bottle, and when it has settled to the bottom, pour off the water, and add methylated spirit.

Dried Specimens.—Place in water until the tissue swells up, then, if the material is large enough, it may be scraped and treated as above. If too small—small seeds, for instance—place them in a mortar in some water, and carefully break them up; strain through muslin, wash with water until quite clean, and preserve in methylated spirit.

Starches may be mounted in Canada balsam or glycerine jelly. If the former is desired, spread a little starch evenly on a cover-glass, let it dry, apply some Canada balsam, and mount in the ordinary way. For glycerine jelly pour off the spirit and add water, then allow the starch to settle to the bottom of the bottle; pour away the water. Place a few drops of glycerine jelly on a slide, take up some starch on a penknife, and place it in a little heap in the jelly; now apply a cover-glass, and press down with a gentle twisting movement until the starch is evenly spread. Let the jelly set, scrape away the excess, wash in water, then in spirit, dry, and apply a coat of cement.

In mounting starch in glycerine jelly, care should be taken that the jelly is not too hot; if it be, the form of the starch will be altered.

Yeast.—Get some fresh baker's yeast, place a little of it in a bottle of sugar and water, and stand in a warm place for twenty-four hours. Pour off the sugar-water, and add camphor-water. Make a cell on a slide with black shellac cement, and let it dry; then apply a second coat of cement, and let this stand for a few minutes. Now take up some of the yeast in a glass tube and place a few drops in the cell; clean a cover-glass, and bring its edge in contact with the cement on one side of the cell; ease it down carefully, so that no air-bubbles may be enclosed; now press on the surface of the cover with a needle until it adheres firmly to

the cell all round, drain off the excess of fluid, dry the slide with a clean cloth, and apply a coat of cement.

Preserving Fluid for Green Algæ.—Acetate of copper, 15 grains; camphor water, 8 ounces; glacial acetic acid, 20 drops; glycerine, 8 ounces; corrosive sublimate, 1 grain. Mix well together, filter, and keep in a stoppered bottle. The above fluid preserves the colour of chlorophyll for a long time; it may also be used as a mounting fluid. For very delicate specimens leave out the glycerine.

The specimens should be well washed in water; then pour off the water, and add a quantity of the copper solution.

To Mount in the above.—For example, take spirogyra as a filamentous alga. Make a cell with some black cement, and let it dry; then apply a second coat of cement, and allow this to nearly dry. Place some spirogyra in the cell, and with needles separate the filaments; add a few drops of copper solution, and apply a cover-glass as directed for yeast.

Protococcus.—This can be obtained by scraping the bark of trees. Place it in a bottle of water, and let it stand for a few hours; now add a little copper solution—this will kill the specimens, and they will sink to the bottom of the bottle; pour off the water, and add more copper solution. Now make a cell as for spirogyra; take up some of the protococcus in a dipping-tube and place them in the cell; wait a minute for the forms to settle on the bottom of the cell, and then apply a cover-glass; drain off the excess of fluid, dry the slides with a cloth, and apply a coat of cement.

Desmids and Volvox.—Treat as above.

Red Algæ.—The copper solution does not suit red algæ; they must be preserved in dilute methylated spirit, or dried between folds of paper. To mount same, remove from the spirit, and soak in several changes of water until all trace of spirit is washed away, then mount in glycerine jelly. The dried specimens only require soaking in water until they

come off the paper. They are also to be mounted in glycerine jelly.

Antheridia and Archegonia of Mosses.—Place some male and female heads of mosses in methylated spirit for a few days, then transfer to equal parts of absolute alcohol and ether, in which they must be soaked for several hours. Pour off the alcohol and ether, and add a thin solution of celloidin, and soak for two or three days; then remove the stopper of the bottle, and let the celloidin evaporate to about half its original bulk. Now remove a specimen from the celloidin, and hold it in a pair of forceps until the celloidin sets, then place it in methylated spirit and soak for an hour or two to complete the hardening. The embedded specimen may now be fastened to a cork with a little celloidin, and longitudinal sections made in a Cathcart microtome, or it can be placed between two pieces of carrot, and the sections made with any ordinary well microtome. The sections must then be dehydrated in methylated spirit, cleared in oil of bergamot, and mounted in Canada balsam; or, if desired, they may be soaked in water to remove spirit, and be mounted in glycerine jelly.

Fertile Branch of Chara.—Chara is usually very dirty; to clean it, wash well in repeated changes of water, then in very dilute acetic acid for a few minutes only; again wash in water, and preserve in camphor water.

Make a cell with shellac cement as directed above, place a fertile branch of chara in it, and examine under a dissecting microscope or lens; with needles clear away the leaves from the archegonia and antheridia, fill the cell with camphor water, and apply a cover-glass.

When a deep cell is required for a specimen to be mounted in acetate of copper, never use one made of any metal. Vulcanite or glass cells must be used. To one side of a cell apply a coat of shellac cement and let it dry; now take a slide and warm it over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warmed slide, and press it down until it adheres firmly; then add another coat of

cement to the upper side of the cell, and let it nearly dry, put in the specimen, fill the cell with solution, and apply the cover-glass.

LESSON 9.

CUTTING, GRINDING, AND MOUNTING SECTIONS OF HARD TISSUES.

Bone.—Take the femur of a dog or cat, remove as much of the muscles as possible, and macerate in water until quite clean, then allow it to dry.

(1) With a fine saw make transverse and longitudinal sections.

(2) Take a hone (water of Ayr stone), moisten it with water, and rub one side of the section upon it until it is quite flat and smooth.

(3) Wash in water, and set aside until quite dry.

(4) Take some dried Canada balsam, place a piece on a square glass, and warm gently over a lamp until the balsam melts; allow it to cool a little, and then press the smooth side of the section into it, and set aside until cold.

(5) With a fine file rub the section down as thinly as possible.

(6) Take the hone again, and grind the section down until thin enough, using plenty of water.

(7) Place it, glass and all, in methylated spirit until the section comes away from the glass, then wash well in clean water.

(8) Make a cell with black shellac cement and allow it to dry; then apply another coat of cement, and after waiting a few minutes fill the cell with camphor water, and put the section into it; apply a cover-glass, and press it down until it adheres to the cement all round. Sections of teeth are made in the same way.

Rock Sections.—Small pieces or slices of rock are to be ground on a zinc plate with the aid of emery-powder and water until one side is quite flat and smooth. Then fasten the polished surface to a square of glass with some dried

Canada balsam, as directed for bone, and allow it to cool. Grind the other side on the zinc plate with coarse emery and plenty of water. When moderately thin, take a piece of plate-glass and some fine flour-emery, and rub the section down as thinly as possible. When thin enough, wash well in water and dry; then warm over a spirit-lamp, and with a needle push the section off the glass into a saucer of benzole or turpentine, and allow it to soak until all the balsam is dissolved. Wash again in some clean benzole, and mount in Canada balsam in the usual way. Sections of echinus spines, shells, and stones of fruit are prepared in the same way as bones and teeth; but when the grinding is finished, the sections are to be passed through alcohol into clove oil, then mount in Canada balsam in the usual way.

Sections of coal containing fossils, limestone, spines of echinus, and other friable specimens, should be cut with a very fine saw, and then soaked in benzole for several hours. When the benzole has saturated the tissue, transfer to ordinary solution of Canada balsam in benzole, and soak again until the balsam has penetrated to the centre. Take a 3×1 inch slide, place the section on its centre, and add sufficient balsam to cover it. Put away out of reach of dust until the benzole has evaporated from the balsam. Then place on a hot plate, apply gentle heat with a spirit-lamp, and bake until the balsam is quite hard. Grind down to the required thinness on a hone. Wash well with water, dry, add a few drops of fluid balsam in benzole, and apply a cover-glass.

LESSON 10.

PREPARING AND MOUNTING ENTOMOLOGICAL SPECIMENS FOR THE MICROSCOPE. MR. ROUSSELET'S METHOD OF PRESERVING ROTATORIA, ETC.

Insects should be killed with chloroform. They are then to be placed in methylated spirit, in which they may remain until required for mounting.

To Prepare a whole Insect for Mounting with Pressure in Canada Balsam.—(1) Transfer from methylated spirit to water, and let it soak for three or four hours to remove spirit.

(2) Place in liq. potassæ, 10 per cent. of caustic potash in distilled water until soft. Some specimens will only require a few hours in the potash, others want days, and some even weeks, to soften. In all cases they must be carefully watched and the action of the potash tested. This can be ascertained by pressing on the thorax or chest of the insect with some blunt instrument, such as the head of a pair of curved-pointed forceps.

(3) When soft enough, pour away the potash, and add water, which must be changed several times until all the potash is washed away.

(4) Pour away the water and add concentrated acetic acid, and soak for twelve hours, or until you are ready to go on with the work.

(5) Transfer from acetic acid to water, and soak for about half an hour; then place in a shallow saucer full of water, and with the aid of a needle and a camel's-hair brush spread out the wings, legs, etc. Now take a slide and place it in the water under the insect, lift the slide up carefully so that the insect may be stranded on the surface of the slide with all its parts expanded. Drain off the excess of water, and lay the slide down on a piece of white paper, and with the aid of needles or brushes carefully place all the limbs, wings, antennæ, etc., in their natural positions. Now put a narrow slip of paper on each slide of the insect, and carefully lay another slide over it, press it down until the insect is squeezed quite flat, tie the two slides together with a piece of twine, and place them in a jar of methylated spirit for at least twelve hours, or until required.

(6) Remove the glasses from the spirit, carefully separate them, and with a soft camel's hair brush push the insect off the glass into a saucer of spirit.

(7) Take the insect up on a lifter, and float it on to the surface of a small saucer of clove oil, and allow it to soak until perfectly clear.

(8) Remove from clove oil and place in turpentine for a few minutes.

(9) Mount in Canada balsam as directed for animal and botanical sections.

To Mount an Insect in Canada Balsam without Pressure.—Treat with potash as above, wash in water, and place in acetic acid. Wash away the acid with water, and transfer to a shallow saucer of methylated spirit. Take two needles and lay out the various parts as quickly as possible; if any parts are troublesome, hold them in position until the spirit has fixed them. Now let it soak for an hour, or until required. Remove from spirit, place in clove oil, and when clear place in turpentine.

Take a *tin* cell just deep enough for the specimen, and apply a coat of black shellac cement to one side of it. Allow this to nearly dry. Clean and warm a slide over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warmed slide; press on the upper side of the cell, until it adheres firmly to the slide, and put it away to dry. Fill the cell with Canada balsam, and see that it also flows over the upper edge of the cell, so that it may serve as a cement to fasten on the cover. Take the insect from the turpentine on a lifter, put it in the cell, and with needles re-arrange the parts if necessary. Put away out of reach of dust for twelve hours to harden the balsam. Place a drop of balsam on one side of the cell. Clean a cover-glass of the same size as the cell, take it up in a pair of forceps, and warm it gently over a spirit-lamp, and bring its edge in contact with the drop of fresh balsam; ease down carefully, so as to avoid air-bubbles, and press on surface of cover with a needle until it rests on the cell all round. Now take a soft brush and some benzole, and wash away the exuded balsam; dry with a clean rag, and apply a ring of cement.

To Mount an Insect in Glycerine without Pressure.—Many small, soft insects and their larvæ may be mounted in glycerine while fresh. The larger and harder kinds must be soaked in potash to render them transparent. Make a cell of the required size, and fasten it to a slide with black shellac cement as directed for balsam mounts. Apply a coat of cement to the upper side of the cell, and allow it to nearly dry. Fill the cell with glycerine, and put the insect into it; spread out the wings, legs, etc. Clean and warm a cover-glass, and apply its edge to the cell; press down, and be sure that it adheres to the cement all round. Wash away the excess of glycerine with some water, and dry the slide with a soft cloth. When quite dry, apply a ring of cement, and when this has dried, add another coat of black shellac cement.

The processes described only refer to the study of the external parts of insects; all the soft tissues and internal organs will, of course, have been destroyed by the potash. Soft internal organs must be dissected out of the specimen while under water.

Procure a gutta-percha dissecting-dish, lay the insect in it, and secure with pins in the desired position. If the abdominal or thoracic viscera are required, lay the insect on its back; if the nervous system, on its ventral surface. Fill the dish with water, and with a pair of sharp-pointed scissors cut through the chitinous skin on each side of the abdomen, taking care not to cut too deeply so as to injure the internal organs; then with a pair of forceps raise and remove the skin. The organs may now be removed with the aid of a pocket-lens, and washed in distilled water; then stain in borax carmine for several minutes, wash in methylated spirit; then immerse in acidulated alcohol for a few minutes, dehydrate, clear in clove oil, and mount in Canada balsam.

If desirable to mount the specimen in glycerine, stain as above, then wash away all trace of spirit with water, and mount in glycerine jelly; if the specimen requires a cell, it must be mounted in glycerine.

Salivary glands of cockroaches and crickets, gizzards of beetles, and stings of bees and wasps, may be easily removed in the following way: Place the specimen whole and while quite fresh in water, cover with a piece of paper or anything to keep out dust, and let them soak for several days until the smell becomes rather unpleasant; then wash in clean water, hold the insect between the fingers, and with a pair of forceps carefully pull off the head, which should bring with it the œsophagus, salivary glands, and stomach. For stings of wasps and bees proceed as follows: Gently squeeze the abdomen of the specimen between the fingers of the left hand until the sting protrudes, then catch hold of it with a pair of fine forceps, and gently pull it out. If properly done, the poison glands and duct should come away with it. Wash in water, and place it on a slide under a dissecting microscope, and with a fine needle-point draw the stings from their sheath; this is done by putting the needle under the stings at the base of the sheath and carefully drawing it towards the apex. Stain in borax carmine, wash in alcohol, then in acidulated alcohol, and place in water; now lay out on a slide, place another slide over it, tie with thread, and immerse in methylated spirit for several hours; remove from glass, clear in clove oil, and mount in Canada balsam.

Small insects, such as parasites, may be mounted whole in a cell in glycerine without treatment with potash, so that their internal organs may be seen *in situ*, but they usually require clearing. Take of Calvert's carbolic acid, solid at ordinary temperatures, 2 ounces, melt, and add about $\frac{1}{2}$ a drachm of glycerine to prevent it becoming solid again. Soak the insect in this until transparent; some specimens will only require an hour or two, others a week or more. When clear, make a cell as previously directed with any good shellac cement, and when dry, run on a coat of cement to its upper surface, let this become about half dry, then place in the cell, fill it up with glycerine, and apply a cover-glass, which must be carefully pressed

down with a needle-point until it adheres to the cement all round. The slide can then be washed with water to remove all trace of excess of glycerine; put away until all the water has evaporated, then apply a coat of shellac cement, and when this has dried, rub away any water-marks that may be left on the slide with a soft cloth, and add another coat of cement.

Wing-cases, legs, heads, and feet of diamond beetles should be mounted in opaque cells in Canada balsam. Take a slide, and with a turn-table run on a disc of black varnish of the required size; allow this to dry thoroughly. Take a piece of black gummed paper and punch out a disc of the same size as that on the slide to which it is to be fastened. Now take a *tin* cell of the required depth—on no account use brass or vulcanite cells; they are affected by the balsam, and the mount will be spoiled—lay the cell on a slide, and apply a coat of cement to its upper surface; allow this to become nearly dry, then take up the cell in a pair of forceps, and bring its cemented surface in contact with the paper disc on the slide, and with the point of the forceps press the cell down until the cement adheres to the paper. Now put away to dry in some place protected from dust. Take the specimen to be mounted, examine it under a microscope, and if dirty, wash in some benzole, and then let it dry again. Now place a small quantity of gum-water in the centre of the cell, and put the specimen into it in the desired position; make sure that it adheres securely to the gum, and put the slide away again until everything is quite dry. Put the slide in a turn-table, and run on a coat of shellac cement to its upper surface, and allow it to become nearly dry; then fill up the cell with Canada balsam, clean, and apply a cover, which must be well pressed into the cement until it adheres firmly; put away for an hour, and then wash away the exuded balsam with a soft brush and some turpentine; dry the slide with a soft rag, and apply a coat of black shellac cement.

Heads of flies having coloured compound eyes, such as

Tabanus, lace-wing flies, etc., should be mounted in opaque cells in glycerine. Make the cell in exactly the same way as directed for balsam mounts, but take care that the cell is only just deep enough to take the specimen, as the object has to be retained in the centre of the cell by slight pressure on the part of the cover-glass. When the cell is quite dry, apply a coat of shellac cement to its upper surface, and let it nearly dry ; then take a brush and some clean water and moisten the inside of the cell. This is done to prevent the formation of air-bubbles, for if glycerine is put into a dry cell, bubbles are sure to give a lot of trouble. Now fill the cell with glycerine and put in the specimen, which should be previously soaked in dilute glycerine for an hour or two, and with a needle place it in the desired position ; apply the cover-glass very carefully, so that no air-bubbles may be enclosed, and let it settle down by its own weight until it rests on the surface of the cell ; then press it down with a needle-point until securely embedded in the half-dried cement, and set aside for an hour or two to dry. The exuded glycerine may then be washed away by holding the slide under a water-tap. When all trace of glycerine is removed, dry the slide with a soft cloth, and apply a coat of black shellac enamel.

Heads of large insects may be secured in the centre of the cell in the following way : Take a fine needle, thread it with a hair, and run it through the specimen. Unthread the needle, take up each end of the hair with the object suspended and stretch it across the cell so that it may be embedded in the cement on each side. Now apply a cover-glass, press it down until securely fixed, and if the specimen is not in the middle of the cell, adjust it by pulling on the hair on one side. Put away to dry, cut off the ends of the hair close to the edge of the cell, wash away excess of glycerine, dry, and apply a coat of shellac enamel.

METHOD OF PRESERVING ROTATORIA.*

PUBLISHED BY KIND PERMISSION OF MR. C. F. ROUSSELET, F.R.M.S.

The principle of the process consists of narcotizing, killing, fixing, and preserving in a watery fluid, not appreciably denser than water.

For narcotizing the following mixture is used:

Two per cent. solution of cocaine ...	3 parts.
Methylated spirit, prepared with wood naphtha	1 part.
Water	6 parts.

If a small quantity of this solution be added to the pond-water in which the rotifers are, they will at first not be affected at all, but continue to swim about as usual. After some minutes (five to fifteen) their motion will become slower and slower, and, in the most successful cases, they will finally sink to the bottom of the trough fully extended, with the cilia vibrating but feebly. If an attempt be made to kill them at once in this state, they will most likely contract and be spoilt.

It is necessary to watch them under the microscope until the cilia have just ceased to vibrate, and then, at least in the majority of species, is the right moment to kill them, as explained below. The different species vary very much in the length of time they require for narcotization; some require to be treated very slowly, others very rapidly, in order to kill and fix them fully extended, and for this reason it is best to treat each species separately. The general rule to be followed is to add a few drops only at first of the narcotizing fluid to the water in which the rotifers have been placed, and then, if the animals continue to expand or swim about, more should be added at intervals of a few minutes, until their movements begin to slacken. Most free-swimming species can be killed when still swimming about slowly, but with some it is necessary to wait

* More extended details of this subject are given by Mr. Rousselet in the Journal of the Quekett Microscopical Club, vol. vi., No. 36, pp. 5-13, March, 1895.

until the cilia have just ceased beating. In order to ascertain the right moment for killing an animal not before prepared, it is best to separate one or two individuals, and if these can be killed fully extended with a drop of osmic acid, then the others are also ready.

Of course, it is important to kill and fix the animals while in the narcotized state and still living, as swelling and other post-mortem changes begin at once after death.

KILLING AND FIXING.—When the rotifers have been sufficiently long under the influence of the cocaine solution, they are killed and fixed with a drop of a $\frac{1}{4}$ per cent. solution of osmic acid for half a minute or less; then wash out immediately and thoroughly in water for a few minutes to half an hour, and finally preserve and mount in $2\frac{1}{2}$ per cent. formalin, or, in some cases, in a $\frac{1}{20}$ per cent. solution of bichloride of mercury, to which has been added a little common salt to prevent the formation of crystals.

This process is best carried out in watch-glasses, under the dissecting microscope, and the creatures are transferred from one to the other by means of a very fine pipette. It is essential that the rotifers be transferred to perfectly clean water before beginning the operation, in order to make satisfactory mounts.

MOUNTING.—Hollowed out glass slips, which can be obtained of all sizes from $\frac{1}{4}$ inch to $\frac{3}{4}$ inch in diameter, and proportionately deep, are the best for mounting. These are always ready, and have the great advantage that the often very minute animals cannot go to the edge, and be obscured from view as in a cement cell. Some difficulty may be experienced at first in closing the cell with the cover-glass without air-bubbles. This will, however, soon be overcome by proceeding as follows: Place a drop of the $2\frac{1}{2}$ per cent. formalin solution in the cell, just filling it, and transfer the prepared rotifers with a pipette into the cell; then place another drop of solution on the slip by the side of the cell, about half an inch to the left, lower your clean cover-glass on to this last drop, which will present no difficulty; then with a needle pass the cover-glass slowly, and

by little jerks, over the cell, stopping short for a moment if the rotifers show any tendency to move to the edge of the cell. But before covering the cell, examine it under the dissecting microscope, and remove every fibre and every particle of foreign matter, however small, with a mounted bristle.

The superabundant fluid is then removed with blotting-paper until none is left round the cover; the cover must not, however, stick too fast, and you must be able to move it with a needle, otherwise the cement will be forced in at the side by atmospheric pressure. When ready, the cover-glass is sealed down by tipping some Miller's caoutchouc cement all round the edge with a fine sable brush. The cement must not be liquid, but thickened by exposure to the consistency of a very soft jelly. The edge of the cover-glass must be carefully looked over under the lens to see that the cement covers it everywhere, and that no air-bubble has been left at the edge. Cover-glasses have frequently small cracks running inwards some little distance; these must be carefully covered with cement to their ends, otherwise the fluid will slowly evaporate through these cracks, and in time an air-bubble will appear in the mount. When the cement is dry, the slide can be finished with a ring of Clark's cement (obtainable from Mr. Bolton of Birmingham), which seals it firmly and permanently.

LESSON 11.

CRYSTALS AND POLARISCOPE OBJECTS.

Crystals.—*Method* 1.—Make a strong solution of the material in distilled water, with the aid of heat if necessary, and filter; take up a small quantity of the solution in a dipping-tube, and drop it on a cover-glass. Prepare several covers in this way, and allow some to dry slowly, and evaporate others over a spirit-lamp. When dry, add a drop or two of Canada balsam, and mount in the usual way.

Method 2.—Make a strong solution in distilled water, and add a few drops of gum water or a small piece of gelatine; mix well, and filter. Apply some of the solution

to a cover-glass, and allow it to dry slowly in a place protected from dust. Mount in Canada balsam.

Method 3.—Place a small piece of the dry crystal on a slide, and apply a cover-glass; warm over a spirit-lamp until fusion results, press the cover down with a needle, and allow the slide to cool. Clean off the exuded material, and finish off with some good cement.

Some crystals are soluble in Canada balsam; in which case, mount in castor oil.

Crystallize the specimen on the cover-glass; make a thin cell with some shellac cement on a slide, and allow it to become perfectly dry; then apply another coat of cement, and when this has nearly dried, fill the cell with castor oil. Take up the cover with a pair of forceps, and bring the crystallized surface in contact with the oil, being very careful that no air-bubbles form. Ease it down gently, and when it rests on the cell, give it a press with the point of the forceps; this will squeeze out the excess of oil and embed the edge of the cover in the cement. Put away to dry; wash off the exuded oil with some turpentine, and apply another coat of shellac cement.

The following salts, etc., are easily obtained, and they all give very good results:

Chloride of barium.*	Sulphate of iron.*	Asparagine.
Chlorate of potash.*	Tartrate of soda.*	Quinidine.
Sulphate of copper.*	Salicine.	Santonine.
Spermaceti (fuse).	Stearine (fuse).	Tartaric acid.

Those marked * are more effective when crystallized in gum or gelatine.

Crystals of Silver.—Clean a cover-glass and fasten it to a slide with the breath; make a 1 per cent. solution of nitrate of silver, and place a drop of it in the centre of the cover-glass. Now add a very small fragment of copper, and put the slide away out of reach of dust until the crystals have formed, and all moisture has evaporated. Then make a shallow opaque cell, and place a small drop of gum water in its centre. Take up the cover with a pair of forceps, crystals uppermost, of course, and drop it into the

cell; now take a needle-point, and carefully press on the cover-glass between the crystals, until it lies quite flat, and air-bubbles, if any, have exuded. Put the slide away again until the gum has dried. Now put the slide into a turn-table; run on a coat of shellac cement to the upper surface of the cell. Allow this to become half dry, and then apply a cover-glass.

The following specimens from the vegetable kingdom make fine polariscope objects: Starches, hairs, scales from leaves, cotton and silk fibres, cuticles of leaves, and longitudinal and transverse sections of stems.

Starches can be obtained from most vegetable substances by scraping the cut surface with a knife. Place the scrapings in a bottle of water and shake well; then strain through muslin of sufficiently fine texture to allow the starch to pass, but to retain the fibres. Now put the strained material into a bottle, shake it up, and then allow to settle; the starch will fall to the bottom of the bottle in a few minutes. Then pour off the water; add some more, and repeat the process until all trace of cellular tissue is removed. When the starch is quite clean, take up a little in a dipping-tube; apply a drop to a clean cover. See that it spreads evenly all over the surface of the cover, and put away, protected from dust, until quite dry; then add a drop of Canada balsam, and mount in the ordinary way.

Starches may also be mounted in glycerine jelly (see Lesson 8), but they do not polarize so well as the balsam preparations.

Sections of Starch-bearing Tissues.—The stems, roots, and bulbs must be hardened in methylated spirits for a week; then make transverse or longitudinal sections. Dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Cuticles containing Raphides.—The most common are taken from the following bulbs: garlic, onion, lily, hyacinth. Strip off the cuticle from the fresh specimen; dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Cuticles of Leaves.—Cut up the leaf into small pieces, and soak in water until rotten; the cuticles can then be separated, washed in water, dehydrated in methylated spirit, cleared in clove oil, and mounted in Canada balsam.

Cotton, Hemp, Wool, Silk, Flax, etc.—Place the fibres in methylated spirit to dehydrate; then clear in clove oil, and place a little on a slide. Separate the fibres from each other with needle-points; apply a few drops of Canada balsam and a cover-glass.

Scales of Leaves.—Scrape the leaf with a knife, and put the scrapings into a bottle of turpentine, and soak until all trace of air has disappeared from the scales; then pour off the turpentine. Take up a little of the scales on the point of a penknife, and mount them in Canada balsam in the ordinary way. Some leaf-scales are very difficult to deprive of air; in fact, it is impossible to get them quite free.

The following animal tissues make good polariscope objects: Fish scales, palates of molluscæ, sections of hairs and quills, horns and hoofs, whalebone, claws of dogs, cats, and fowls, decalcified bones, muscular tissues.

Fish Scales.—Scrape the fish from the head towards the tail; if scraped the other way, nearly all the scales will be injured. Place the scrapings in a bottle of water, shake well, pour off the water, and repeat the process until quite clean. Examine with a microscope, and if you find that the scales are not clean, pour off the water, add liq. potassæ, and soak for an hour or two; then wash away the potash with repeated changes of water, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Sometimes fish scales buckle up in spirit, and they will not lie flat. When this happens, put them into water again, and soak a little while; then place them on a slide, and put another slide over them, press down until quite flat, and tie the two glasses together with twine, and place them in a vessel of methylated spirit to dehydrate under pressure. This method will answer for all tissues that have a tendency to twist during the process of dehydration.

Palates.—Dissect out, and soak in liq. potassæ for a few

days. Wash well in water, spread out on a slide; put a piece of paper on each side of it to prevent crushing, and place another slide over all in the same way as directed for insects; tie the glasses together with string, and place in methylated spirit for an hour or two. Then remove the palate from the glasses, and place it in clove oil until clear. Mount in Canada balsam.

Sometimes it is very difficult to dissect out the palates from small snails. This process answers just as well:—Cut off the head of the animal, being careful that you remove the buccal mass with it, and place in liq. potassæ for a few days; this will destroy all the soft tissues, but not the palate or radula. Wash away the potash with repeated changes of water, and proceed as directed above.

Sections of hairs and quills may sometimes be cut after soaking for a few days in methylated spirit; but some of the larger kinds, such as the whisker of walrus, will require softening in potash. Place in liq. potassæ for a few hours or days, in accordance with the consistency of the tissue. When soft enough, wash away the potash with water, and place in methylated spirit, in which they may be preserved until required. Then make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Small Fine Hairs.—Cut off a number of hairs, tie them up into a bundle with some cotton, and soak for a few minutes in warm water. Make up a strong solution of gelatine in water, and transfer the bundle of hairs to it, and soak it for several hours in a hot-water bath until the gelatine has penetrated to the centre of the bundle. Remove from the gelatine on the point of a needle, and hold it exposed to the air until the gelatine has cooled; then push them from off the needle into a bottle of methylated spirit, and soak for an hour or two to complete the hardening. Embed in carrot, put in a microtome, and cut transverse sections, and as they are cut place them in methylated spirit to dehydrate; then clear in clove oil, and mount in Canada balsam.

Horns, hoofs, whalebone, and claws all require steeping in liq. potassæ until soft; they are then to be washed in water, and preserved in methylated spirit until required. Embed in carrot, place in a well microtome, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Decalcified Bones (see Lesson 1).—Embed in carrot, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Muscular Fibres.—Take the tongue of a cat, harden it in methylated spirit for a week or ten days; then embed in carrot, and make transverse or longitudinal sections, dehydrate, clear in clove oil, and mount in Canada balsam.

LESSON 12.

CLEANING AND MOUNTING DIATOMS, POLYCYSTINA, AND FORAMINIFERA.

To Clean Diatoms Growing upon Algæ or Shells.—Place the algæ or shells in a basin, cover them with water, add hydrochloric acid, and stir until effervescence results; add more acid little by little, until effervescence ceases, stirring from time to time. Now strain through net of sufficiently fine texture to allow the diatoms to pass, but to retain the débris. Allow the strained fluid to settle down, pour off the acid water, and place the deposit in a large test-tube. Add pure hydrochloric acid, and boil for twenty minutes; add some pure nitric acid, and boil again for twenty minutes, and, while boiling, add some crystals of chlorate of potash until complete bleaching results. Remove all trace of acid or alkali by washing in water, and examine the forms under the microscope. If clean, bottle them up in distilled water for future mounting. If, as is sometimes the case, there has been animal matter present which has not been removed, boil in pure sulphuric acid for a few minutes. Wash away all trace of acid before bottling the diatoms in distilled water.

To Clean Fossil Diatomaceous Deposits.—Break the deposit up into small pieces, and place them in a large test-tube in a moderately strong solution of bicarbonate of soda, and boil gently for two hours, the disintegrated portions being from time to time poured off into a beaker and the boiling in soda continued until all the deposit has broken up. The alkaline solution must then be washed away, and the diatoms boiled for a short time in nitric acid, and when sufficiently clean wash away the acid in repeated changes of water, and bottle up the diatoms in distilled water.

To Clean Living Diatoms.—Remove all dirt or salt by washing well in water; shake well, and allow the diatoms to settle before pouring off the water. In this way all soluble impurities can be removed. When the water remains clear, pour it off, leaving the diatoms as nearly dry as possible, and cover them with strong alcohol, which will extract the endochrome; change the alcohol daily until it ceases to be tinged with green; then wash away the alcohol with water, pour off the water, and place the diatoms in a platinum capsule and heat them to a dull red over a spirit-lamp. This will separate the frustules into single valves, and finish the cleaning of the diatoms, and they may then be bottled up in distilled water.

To Clean Polycystina.—The polycystinous earth should be broken into small pieces and boiled for several hours in a strong solution of common washing soda, the disintegrated matter being from time to time poured off into a vessel, and the boiling in soda continued until all the earth is broken up. Wash the disintegrated matter in water several times to remove the soda, allow the polycystina to settle down, and pour off the water and place the forms in a test-tube; add some nitric acid, and boil for twenty minutes. Remove all trace of acid with water, and bottle up in distilled water.

To Clean Foraminifera.—All mud must be got rid of by repeated washing in water. Then boil the forms 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 Diatoms in Canada Balsam (*Unselected Slides*).—The diatoms are to be taken out of the bottle with a dipping-tube, and should be allowed to fall upon a clean cover-glass. The fall of the drop causes the forms to spread evenly over the cover. It should then be dried slowly over a spirit-lamp. When dry, a small drop of Canada balsam is to be applied, and the slide put away out of reach of dust to dry for twelve hours. Now place on a hot plate, and apply gentle heat from a spirit-lamp for about ten minutes. Allow it to cool. Take the cover up with a pair of forceps, and bring its balsamed surface in contact with the centre of a warmed slide. The balsam should then run to a neat bevelled edge all round the cover; should it not do so, warm the slide a little more until it does.

Unselected Polycystina.—Take the forms from the bottle with a glass tube, and spread them on a slide; dry them over a spirit-lamp. Now clean a cover-glass, fasten it to a slip with your breath, and place a drop or two of balsam on it; take up some of the polycystina on the point of a knife and place them in the balsam; stir them well up with a needle and put away for twelve hours. Bake over a spirit-lamp for ten minutes, and while warm stir up again gently with a needle, and spread the forms evenly over the cover. Warm a glass slide, and proceed as directed for unselected diatoms.

Unselected Polycystina as Opaque Objects. — Dry some polycystina on a slide, then take a platinum capsule, put the dried material into it, and heat over a spirit-lamp to a dull red. Clean a cover-glass, fasten it to a slide with your breath, and apply a few drops of balsam. Take up some of the dried forms, put them into the balsam, and stir up with a needle until they are evenly spread over the cover; put away out of reach of dust for twelve hours, so that the air may escape from the forms. Now place on a

hot plate and apply gentle heat for ten to fifteen minutes to bake the balsam. Clean another cover-glass, add a drop or two of balsam to the hardened balsam, and apply the second cover-glass; warm again, and with a needle press gently on the upper cover until it lies perfectly flat; then allow to cool, apply a coat of black shellac cement all over one side of the upper cover, and put away to dry. In the meantime take a slip, put it in a turn-table, and run on a disc of black varnish of the same size as the cover; let this dry, then add a drop of strong gum or glue; take up the covers with a pair of forceps, and put the blackened side into the glue; press down with a needle until the glue spreads evenly under the cover, and put away to dry. When dry, finish off with a coat of black cement.

Selected Diatoms and Polycystina.—Take an ounce of distilled water, add 6 or 8 drops of ordinary gum water, and filter. Clean a cover-glass, and place a drop of the diluted gum upon it; put away to dry.

Spread the diatoms or polycystina on a slip, and dry them over a spirit-lamp. Select the desired forms with a fine brush or bristle, and breathe upon the gummed surface of the cover, and place the forms upon it, When dry, apply a drop of balsam, and put away out of reach of dust for twelve hours. Bake and finish as directed for unselected slides.

In mounting selected polycystina, they must be between two covers; if on a single cover, the forms would be upside down when the cover was reversed. If a transparent mount is desired, the two covers can be fastened to the slide with a drop of balsam. If opaque, the forms must be burnt, and one side must be blackened; in other respects proceed exactly as you would for unselected opaque mounts.

Polycystina may also be mounted in a dry opaque cell. Take a slide, run on a disc of black varnish, and when this has dried, fasten a disc of black gummed paper over it. Then take a shallow cell, apply a coat of cement to one side of it, and let it nearly dry; then fasten to the paper

disc, and put away to dry. Apply a little dilute gum water to the bottom of the cell, select the specimens, and put them into the gum; if they do not adhere, breathe on the surface of the gum. When all are arranged, put the slide away until everything is quite dry; then add a coat of cement to the upper side of the cell, let it nearly dry, and then apply the cover-glass.

Foraminifera — Unselected Transparent Mounts.— Dry the forms on a slide with the aid of gentle heat, then scrape them off into a bottle of turpentine, in which they must soak until all trace of air has disappeared. Then clean a cover-glass, fasten it to a slide with condensed breath, and apply a few drops of balsam. Pour off the turpentine from the foraminifera, take up some of the forms on the point of a penknife, and put them into the balsam on the cover; stir up with a needle until spread evenly, then put away for twelve hours. Bake gently for ten minutes on a hot plate, cool, apply a drop of fluid balsam, warm a slide over a spirit-lamp, take the cover up in a pair of forceps, and bring the drop of fluid balsam in contact with the centre of the slide, ease down carefully, and press on the upper surface of the cover with a needle-point until it lies quite flat; or if the forms are very delicate, warm the slide again gently until the cover settles down by its own weight. Allow the slide to cool, then clean away exuded balsam with methylated spirit, and apply a coat of cement.

Foraminifera—Opaque Mounts.—Proceed in exactly the same way as directed for mounting dry opaque polycystina; but if the specimens are unselected, gum the bottom of the cell, dry the forms on a slide, and spread a quantity of them all over the surface of the cell. Let the gum dry, then shake out all that have not adhered, apply a coat of cement to the upper side of the cell, and when this has nearly dried, apply a cover-glass.

Spicules of Gorgonia or Sea-fan.—Boil in liq. potassæ until all the material has broken up, then wash away the

potash with repeated changes of water, allowing the spicules to settle to the bottom of the tube between each washing. When cleaned, preserve in a bottle of dilute spirit. Proceed with the mounting in exactly the same way as directed for unselected polycystina.

Spicules of Alcionium.—Proceed as above.

Spicules of Sponge.—Cut the sponge up into small pieces and boil in liq. potassæ until the ground substance has broken up and the spicules are set free; then proceed as for gorgonia.

Sections of Sponges.—Harden in methylated spirit; then transfer to equal parts of ether and absolute alcohol for several hours. Then place in a thin solution of celloidin for a day or two, transfer to a thicker solution of celloidin, and soak again for a few hours. Remove from the celloidin on the point of a needle, and hold exposed to the air for a few minutes to allow the celloidin to set around the specimen; then push it off the needle into a bottle of methylated spirit, and soak for a few hours to complete the hardening. Embed in carrot, place in a well microtome, and make the sections. Dehydrate in methylated spirit, clear in oil of bergamot, and mount in Canada balsam.

Sometimes sponge sections are rendered too transparent when in balsam. When so, mount in glycerine jelly, but be careful to wash away all trace of alcohol before they go into the jelly.

LESSON 13.

DRY MOUNTS.

Opaque Cells.—Place a slide in a turn-table, and run a disc of black varnish on its centre; allow this to dry. Take a piece of black paper and punch out a disc of the same size as the one on the slide, and gum it on to the varnish spot. Take a cell, either metal or vulcanite, of the required depth and fasten it to the paper disc with gold size, or black shellac cement, and put the slide away

until quite dry. Now place a very small quantity of gum on the centre of the paper disc, and put the specimen into it; but take care that the gum does not extend beyond the object, or the appearance of the mount will be spoiled. When the gum has dried, put the slide into the turn-table again, and run a ring of any good cement on the upper surface of the cell, and when this has become about half dry apply a cover-glass, which must be pressed down with a needle-point until it adheres firmly to the cement all round the cell. Put the slide aside for an hour or two, and then run on a good coat of black shellac cement.

Feathers of humming-birds, eggs of butterflies and moths, small microscopic seeds, gills of many fishes, skins of fishes, skins of snakes, and transverse or longitudinal sections of stems of plants, are all mounted as opaque objects in the same manner as above. The former should be arranged in the cell in a group. The gills, skins, etc., should be well washed with distilled water and dried under pressure between two glass slips tied together with twine.

Transparent Cells.—Take a cell of the desired depth and apply a coat of cement to one side of it, and allow it to become very nearly dry. Take a slide and warm it gently over a spirit-lamp; take up the cell with a pair of forceps and place it on the centre of the slide, the warmth of which should cause the cement of the cell to melt; if not, warm a little more, and press the cell down gently with a needle-point until it adheres firmly to the slide all round. If the specimen is small it must be fastened in the cell with some gum, as for opaque mounts, then put it away until the gum has dried, apply a cover, and finish off as directed for opaque mounts. Leaves of plants and wings of butterflies should be mounted on a thin slide, so that both sides may be examined. No gum will be required for these specimens, but a piece of the leaf or wing should be cut or punched out as nearly the size of the cell as possible, and a thin cell should be used, so that the cover may rest on the object and keep it flat. In all dry mounts

great care must be taken that all the cements used to fasten the object in position are quite dry before the cover is put on; if not, any moisture remaining will condense on the under surface of the cover and spoil the preparation.

LESSON 14.

FINISHING OFF SLIDES.

Canada Balsam.—*Quick Method.*—Take a small saucer of chloroform and a soft brush, and carefully wash away the exuded balsam. Allow the slide to dry, then place it in a turn-table and apply a coat of black shellac cement. Let this dry, then wash the slide quite clean with turpentine and apply another coat of cement.

Canada Balsam.—*Exposure Method.*—Put the slide into a saucer of methylated spirit, and with a small piece of soft rag gently rub away the excess of balsam; dry the slide with a clean cloth, and apply a coat of any good cement.

Glycerine Jelly.—Put the slide into a saucer of cold water and allow it to soak for a few minutes, then take a penknife and carefully scrape away the jelly from the edge of the cover. Give the slide a good wash in water, and place it in some methylated spirit, which will remove the water. Dry with a clean soft cloth, and apply a coat of black shellac enamel, and when this has dried add another.

Farrant's Medium.—Allow the slide to dry for a few days, then put it into a saucer of water and wash away the excess of medium with a soft brush. Drain off as much water as possible, and, if the cover is firm enough, dry the slide carefully with a soft cloth; if not, allow all the moisture to evaporate by exposure to the air. When quite dry, put it in a turn-table and apply a coat of cement, and when this has dried add another.

Dry Mounts do not require any washing, but they should have one or two coats of any good cement.

Asphalte and white zinc cement may be used when desired for balsam or dry mounts, but they are both useless for any of the aqueous or fluid media.

The best cements for ALL slides are Watson's *Special* Black or Club Black enamel (Silico Enamel Company's patent). The latter can be obtained at any cycle shop. As sold it is a little too thin for microscopical work, but if the cork is left out of the bottle for a few days it will evaporate to the proper thickness, and when too thick, dilute with methylated spirit. The brush must also be washed in methylated spirit after use.

When a ring is being applied to a slide, the turn-table should not be run too fast, and the extreme point of the brush should only just touch the glass. A thin coat must be run on at first, then give it about ten minutes to dry. A sufficient quantity of cement may then be added to finish the mount, but if too much is applied at first it will overflow.

Cleaning off Failures.—During a course of microscopical work many slides will be not worth keeping, but the slips and covers are quite good, and they can be used again. When a batch of failures has accumulated, make a strong solution of Hudson's soap-powder in warm water, and place some of it in two jars. Warm the slide over a spirit-lamp, and with a needle-point push off the cover into one of the jars and put the slip into the other; let them soak for an hour or two, then wash away the soap solution with repeated changes of warm water, and finally pour away all the water and add methylated spirit; soak for a little while, and then dry with a soft clean rag.

Sometimes slips and covers have a dull, cloudy appearance, which defies all attempts to remove it. When this is the case, make up a solution of hydrochloric acid in methylated spirit (about one part of acid in six of spirit), and immerse the glasses for a few minutes. Wash away the acid with methylated spirit, and dry with a soft rag.

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THE END.

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