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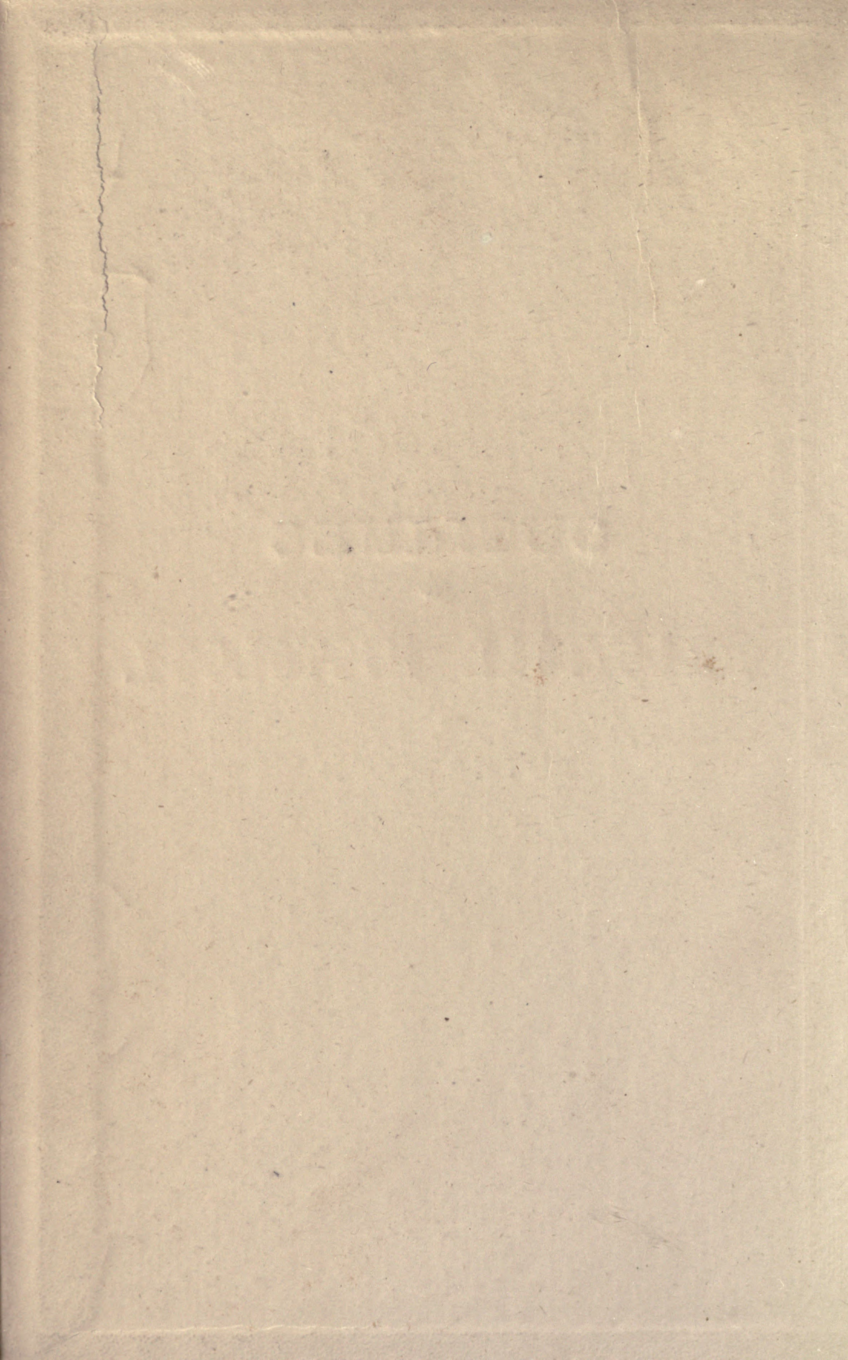
OUTLINES OF PRACTICAL HISTOLOGY.

RUTHERFORD.



OUTLINES

OF

PRACTICAL HISTOLOGY,

BEING THE NOTES OF THE HISTOLOGICAL SECTION OF THE CLASS OF PRACTICAL PHYSIOLOGY

HELD IN THE UNIVERSITY OF EDINBURGH.

BY

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PREFACE.

THE first edition of these Notes was published in the *Quarterly Microscopical Journal* for January 1872. Various circumstances having delayed the appearance of my "Text-Book of Practical Physiology," I have been induced to issue a new edition of the Notes, for the purpose of assisting those who are attending a course of Practical Histology for the first time. The present edition is considerably altered from the first, various new methods having been introduced. All figures of tissues, and most of those of apparatus, are reserved for my larger work. Pathological histology is almost entirely omitted, as this subject is taught practically by my colleague the Professor of Pathology. As the time at the disposal of most students of medicine is very limited, a course has been adopted, whereby students in classes, numbering from twenty to thirty, can be conducted through the leading points of practical histology in about thirty lessons, each lesson extending from an hour to an hour and a half. As

it is impossible in this short time to prepare all the tissues, special demonstrations previously made by the teacher are resorted to, in order that the *introduction* to the subject may be as complete as possible. The special working out of many complicated processes cannot be attempted in a general course of this sort, but must be done by the advanced student working in the laboratory for several hours daily. These Notes especially concern the course which is introductory to an advanced study of the subject, and the reader is desired to bear in mind that they are primarily intended for the students of the University of Edinburgh.

It is not professed that these Notes are more than an outline of the subject of Practical Histology. They merely give the points to which every student of medicine ought to attend. Care has been taken to render all instructions as definite as possible. Descriptions of structure are generally left to the ordinary text-books of descriptive histology. The structure of the microscope is also omitted. When the student desires a full account of the various methods proposed for the examination of the various tissues and organs, let him consult Frey on "The Microscope," and Beale "How to work with the Microsope." Such works should, however, only be used for reference; for although admirable encyclopædias, they can scarcely be regarded as guides for the beginner. Carpenter's book on "The Microscope" will be found an excellent treatise

on the structure of the microscope and its accessories, as well as on botanical and zoological histology.

When the student finds in the following Notes no statement about the preservation of a specimen, it is to be inferred that it is needless to preserve it.

In the course of the demonstrations the student is desired to read the general considerations which follow them (§ 75).

ABBREVIATIONS.

H. Means high power. A combination of lenses, such as Hartnack's ocular No. 3, and objective No. 7, with the tube of the microscope drawn out, magnifying about 300 diam., is the high power most useful for the student.

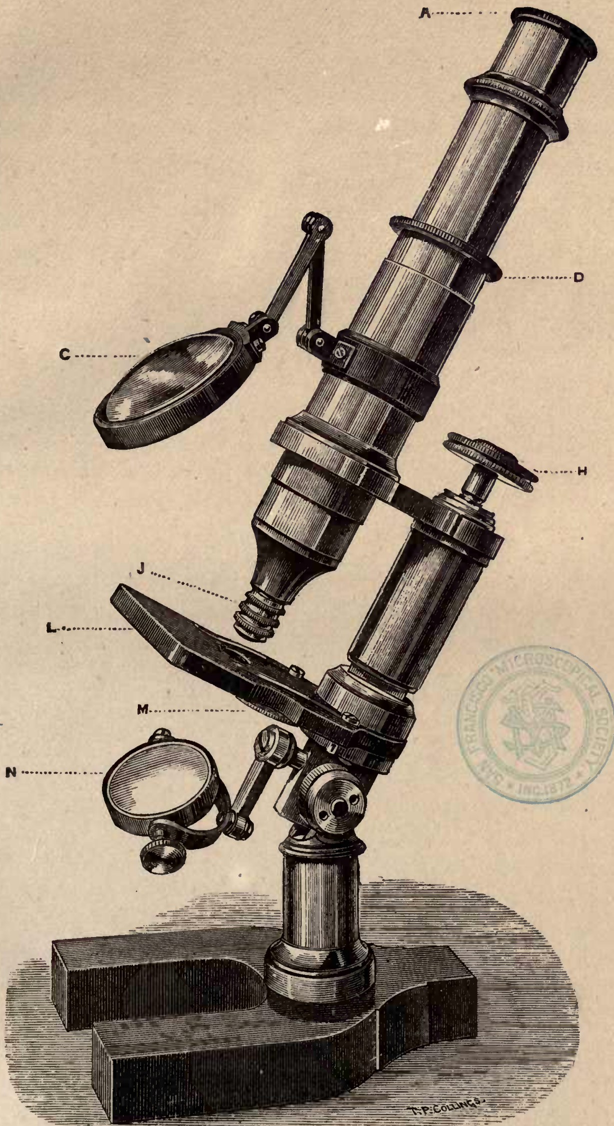
L. stands for low power. Hartnack's ocular No. 3, objective No. 3, tube drawn out, magnifying about 50 diam.

D. stands for demonstration, and indicates that the student is to prepare a microscopical demonstration.

V. S., L. S., and T. S., respectively mean vertical, longitudinal, and transverse section.

ERRATA.

P. 10, line 11 from top, for *p read p'*.
P. 49, last line of large type, *delete* (E.)



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Hartnack's No. IIIA Microscope. *A*, Eyepiece. *D*, Coarse adjustment. *C*, Condenser. *H*, Fine adjustment. *J*, Objective. *L*, Stage. *M*, Diaphragm. *N*, Mirror.

OUTLINES

OF

PRACTICAL HISTOLOGY.

INTRODUCTION.

1. In the histological section of the class of Practical Physiology in the University of Edinburgh, the student is supplied with a Hartnack's No. IIIA microscope, having a No. 3 and a No. 7 objective, and a No. 3 eyepiece—the No. 4 eyepiece commonly supplied by Hartnack being intentionally omitted as of little value. The highest magnifying power attainable with these lenses is 300 diameters; a power sufficiently high for most purposes for the beginner. He is also supplied with the following fluids for the preparation and preservation of the various tissues:—1. Distilled water. 2. Salt Solution. 3. Magenta Solution. 4. Carmine Solution. 5. Logwood Solution. 6. Absolute alcohol. 7. Clove oil. 8. Acetic acid. 9. Glycerine. 10. Weak spirit. 11. Potassium acetate solution. 12. Canada balsam, or Dammar mounting fluid. 13. Gold size varnish. 14. Dammar varnish. Other re-agents are only occasionally employed, and are provided when required.

2. *The student is desired to bring to the class* a razor ground flat on the side next the right hand when the face of the razor is held downwards (if the student is left handed, the left and not

the right side of the razor should be flattened); a pair of strong needles in handles; scalpels, forceps, scissors (such as are found in the ordinary dissecting case); three or four mounting clips; three or four camel-hair brushes of small size; two or three dozen ground class slides, 3 by 1 inch; ¼ oz. extra thin circular cover-glasses, ¾-inch broad; and a box to hold say fifty preparations. The student is thus enabled to preserve for himself the principal specimens made during the course.

3. At a future time, when the student undertakes histological work in private, he would do well to procure a Hartnack's microscope. This microscope is recommended because its defining power is excellent, its stage is not too high, and the whole instrument is small and easily used. Before purchasing a microscope it should invariably be tested by a competent observer. A drop of fresh human blood is perhaps the best test object. Unless the margins of the coloured corpuscles appear perfectly sharp *the definition is imperfect.* The only colour exhibited by a *single* coloured corpuscle is pale yellow: if therefore an isolated corpuscle when exactly in focus shows a red or green halo *the lenses are not achromatic.* If when the tube of the microscope is fully elongated the corpuscles in the centre of the field are sharp, while those near the periphery are misty, *the field is not flat.* The stand of the microscope must be perfectly steady, and the objective *must on no account move from side to side* when the fine adjustment is used. *A microscope should be rejected without hesitation for any one of these faults.*[1]

The advanced student should, in addition to the above lenses, procure Hartnack's No. 9 immersion objective, and, for a still higher power, Gundlach's No 8 immersion objective. Both are good lenses, and the latter is remarkably cheap. Gundlach's lenses, however, vary so much in quality that great care must be exercised in making a selection.

[1] Hartnack's microscopes may be obtained from Bryson, Optician, 60 Princes Street, Edinburgh; Tisley and Spiller, 172 Brompton Road, London, S.W.; Baker, 244 High Holborn, London, W.C.

Admirable lenses are made in this country by Ross, Powell and Lealand, Browning, and others, but their great expense places them within the reach of only a few persons.

The advanced student should also obtain a stage and a Hartnack's eyepiece micrometer, a freezing microtome, Valentin's knife, Chevalier's camera lucida, Stricker's hot stage, and, if need be, a colza oil or paraffin lamp, with a chimney of pale blue glass for night-work. He may also procure Hartnack's polarising apparatus, turn-table, and a small syringe for injections.

An achromatic condenser is not required, unless for very exceptional work. Other accessories are, speaking generally, of very little use.

Preparation of Tissues previous to their Examination.[1]

4. The following fluids require to be prepared :—I. ¾ *per cent. Salt Solution.* Dissolve 7.5 grammes of dried ordinary NaCl in 1000CC distilled water. II. 1 *per cent. Chromic Acid Solution.* Dissolve 10 grammes chromic acid in 1000CC water. This can be readily diluted when required. III. *Chromic Acid and Spirit Solution.* Dissolve 1 gramme chromic acid in 20CC water, and *slowly add it* to 180CC methylated spirit. IV. *Chromic and Bichromate Solution.* Dissolve 1 gramme chromic acid, 2 grammes potass. bichrom. in 1200CC water. V. *Chromic and Nitric Fluid.* Chromic acid 1 gramme, water 200CC, then add 2CC nitric acid. VI. *Müller's Fluid.* Dissolve 25 grammes potass. bichrom. and 10 grammes sodium sulphate in 1000CC water.

5. Most of the requisite tissues and organs may be obtained from the cat and guinea-pig. Feed the cat, and an hour or so afterwards place it in a bag ; drop chloroform over its nose until insensibility is produced. Open the chest by a linear incision

[1] This section is designed to assist the student when he undertakes histological work in private.

through the sternum ; speedily open the right ventricle, and allow the animal to bleed to death.

6. Divide the trachea immediately below the cricoid cartilage, and inject it with ¼ per cent. chromic acid fluid ; tie it to prevent the escape of the fluid, and place the distended lungs in the same fluid, and cover them with cotton wool. Change the fluid at the end of 18 hours. Allow them to remain in this fluid for a month, then transfer to meth. sp. until required.

7. Open by a linear incision the œsophagus, stomach, small and large intestine, and wash them with salt solution. Place a portion of the small intestine in chromic and bichromate fluid for a fortnight (change the fluid at the end of 18 hours), and then in meth. sp. until required. Place a portion of œsophagus, large intestine, and stomach in ¼ per cent. chromic acid for three weeks or a month (change the fluid at the end of 18 hours), and then in meth. sp. until required. Place a portion of the stomach in Müller's fluid until required for the preparation of non-striped muscle, and preparations of the gastric follicles. Open the bladder, wash it with salt solution, and prepare it in the same manner as the small intestine.

8. Remove the kidneys; divide one transversely and the other longitudinally, and place them in Müller's fluid. Change the fluid at the end of 18 hours. At the end of four weeks transfer them to meth. sp. until required. They will be ready after having been a fortnight in the spirit. It is well to prepare the kidney of the dog in the same way.

9. Cut half of the liver into small pieces, and prepare it in the same way as the kidneys. Place the tongue, divided transversely into five or six pieces, spleen, uterus, some thin muscles from the limbs or abdomen, in ¼ per cent. chromic acid. Change the fluid at the end of 18 hours. At the end of a month place in meth. sp.

10. Place testis of dog freely incised, and ovaries of cat or dog, in Müller's fluid. Change at the end of 18 hours, and at the end of three weeks transfer to meth. sp.

11. Remove both eyes. Divide them transversely behind the crystalline lens. Remove the vitreous. Place the posterior halves in the chromic and spirit solution III. Change at the end of 18 hours. Transfer to meth. sp. at the end of ten days. (Pritchard). Place the crystalline lens in Müller's fluid for five weeks, and then in meth sp. Place the cornea in $\frac{1}{4}$ per cent. chromic acid for a month, and then in meth. sp.

12. Cautiously open the cranial and spinal cavities. Remove brain and spinal cord, and strip off arachnoid. Partially divide spinal cord into pieces half an inch long. Partially divide the brain transversely into a number of pieces. Place them in a *cool* place in meth. sp. for 18 hours. Transfer the spinal cord to $\frac{1}{4}$ per cent. chromic acid for six or seven weeks. Change at the end of 18 hours. Prepare the sciatic nerve in the same manner. Place the brain in chromic and bichromate fluid. Change at the end of 18 hours, and then once a week, until the brain is hard and tough. If it be not tough and leathery at the end of six weeks, place it in $\frac{1}{6}$ per cent. chromic acid for a fortnight, and then in meth. sp. If the brain be placed in a too strong chromic acid solution, or if it be too long exposed to the action of even a moderately strong solution, it becomes friable and useless. Procure a portion of human cerebrum (vertex), as fresh as possible, and treat it in the same manner as the cat's brain. At the end of six or seven weeks transfer all the tissues to meth. sp. to complete the hardening. The brain and spinal cord should always be placed on cotton wool in their hardening fluid, in order that the fluid may reach every part equally.

13. Remove the muscles, but *not the periosteum*, from the bones of the upper or lower limb, and remove both from the lower jaw. Divide the jaw and the long bones transversely in two or three places, and put them into the chromic and nitric fluid. Change the fluid repeatedly until the bone is just sufficiently softened, and then transfer it to meth. sp. If the softening is not completed in a month, double the quantity of nitric acid in the fluid. The bone must not, however, remain

in the softening fluid after all calcareous matter has been removed.

14. Place a piece of human scalp, skin from palmar surface of finger, and skin of dog (for muscles of hair follicles), in chromic and spirit fluid. Change at the end of 18 hours. At the end of a month transfer to meth. sp.

15. Remove the petrous portion of the temporal bone, open the tympanum, pull the stapes from the oval fenestra, and place the cochlea in chromic and spirit fluid. Change it at the end of 18 hours, and again on the seventh day. But if a copious brown precipitate fall, change the fluid every third day. On the tenth or twelfth day transfer to chromic and nitric fluid. Change the fluid frequently until the bone is soft. Then place it in meth. sp. In the guinea-pig the cochlea forms a very evident projection into the tympanum, and is therefore very convenient for enabling the student to see how the cone is to be sliced when sections are to be made. Kill a young guinea-pig, sever the head from the body, disarticulate the lower jaw, open the tympanic bulla, remove the stapes, and prepare the cochlea as above. In a *young* animal the chromic and spirit fluid alone may suffice to soften the bone, and the chromic and nitric fluid may therefore not be required, or at any rate only for a short time.

16. Chromic acid requires from one to six weeks to harden tissues. It is important not to leave them too long exposed to its influence, otherwise they are very difficult to stain with carmine, and they may become friable and useless for the purpose of section. Therefore they are transferred to meth. sp. when the hardening is quite or nearly complete.

17. It is very important to place all the above tissues in a *cool* place. A cellar is best in summer. Protoplasm rapidly undergoes change in even a medium temperature.

The Mode of Working with the Microscope.

18. *Prepare the Microscope.* Before proceeding to prepare

the object for examination: *a.* See that the proper lenses are upon the microscope. *b.* Clean the tube, examine the lower surface of the objective, and the upper surface of the ocular, and see that both are clean. *c.* If the objective be Hartnack's No. 7 (one-sixth of an inch focus), place it *half an inch* above the level of the stage; if it be Hartnack's No. 3 (one-inch focus), place it *two inches* above the stage. As objective No. 7 will presently be required, fix that on the microscope. *d.* Arrange the mirror and illuminate the field of vision; use a small aperture of the diaphragm in the case of a high power. If any specks be visible on looking through the microscope, they are upon either the upper or lower glass of the eyepiece (probably on the upper surface of the latter), and must be removed. Muscæ volitantes must not be mistaken for such specks. The *latter* move as the eye changes its position. Dirt upon the *objective* is indicated by *no definite forms* on looking through the microscope, but *by a dimness of the object* which may be under examination. *e.* Clean one or more slides and cover-glasses. If it be found dificult to clean new cover-glasses, place them in a beaker; cover them with ordinary commercial sulphuric acid; heat to the boiling point in a water bath; *allow the acid to cool*, and then wash the glasses with water. *The latter should never be laid flat on the table, but should rest on their edges until required.*

19. *Prepare a Simple Object.* Place a *small* drop of milk upon a slide, and let a cover-glass fall *obliquely* upon the drop. The cover-glass may be applied most carefully by holding it near the margin with a pair of forceps. The small brass forceps supplied with Hartnack's microscope is well adapted for this purpose. If too large a drop be taken, remove the superfluous fluid from the edge of the cover-glass by means of blotting paper.

20. *Find the Focus.* The No. 7 objective is on the microscope, and it has been placed half an inch above the stage—that is, far outside its focal distance (one-sixth of an inch). Place

the object on the stage, and bring it into focus; in so doing, keep the slide moving gently by means of the left thumb and forefinger, seize the coarse adjustment with the right hand, and while the eye looks through the microscope, lower the body of the microscope very carefully in a *spiral manner* until the object is visible. Take care not to go *within* the focal distance, otherwise the *object* or the *objective may be damaged. When the object can be seen*, however dimly, *use the fine adjustment* and bring it accurately into focus. *But never use the fine adjustment until the object is visible.*

21. *Describe the Object.* It is very important that the student should be educated in the method of describing microscopic objects. To facilitate description, he should attend to the following: 1. Shape. 2. Size. 3. Border. 4. Upper and under surface. 5. Colour. 6. Transparency. 7. Contents. 8. Effects of re-agents.

Shape. Are the milk corpuscles globules, or are they circular discs like coins? To determine this, touch the edge of the cover-glass with a needle, and cause the corpuscles to roll over and over, or effect this by inclining the stage. Farther, attend to this; when the focus is altered a globule comes into and goes out of focus *gradually*, while a flat corpuscle passes into and out of focus *suddenly*. *Size.* Attend in this case to the relative, but omit the *absolute* size. *Border.* Is it smooth and regular, or notched? *Upper and under surface.* Elevate the objective, then slowly lower it, and carefully watch the upper surface as it comes into view. Is it smooth or rough? The character of the lower surface is unimportant in this case, but when a membrane such as the peritoneum is under observation, it is important. *Colour.* When exactly in focus, are the corpuscles coloured or colourless? *Transparency.* Bodies may be opaque, transparent or semi-transparent. The first transmits no light, through the second the outlines of subjacent objects are visible, the third transmits light, but the outlines of subjacent objects are not distinctly visible. *Contents.* Does the corpuscle appear to be

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homogeneous, or can included particles be seen on carefully focussing through the whole depth of the corpuscle?

22. *Draw the Object.* It is very important that the student should embrace every opportunity of making drawings of microscopical objects, for drawing necessitates a thorough inspection of an object, and it impresses its features deeply upon the memory. Excellent paper for drawing is the smooth but not too highly glazed "antique note paper" made by Pirie and Son, Aberdeen. The pencils used are H.B. and H.H.H.H. The shading should not be produced by lines, as in ordinary drawing and wood engraving, but it should be a continuous shade produced by a rapid to and fro movement of the pencil. The student should draw the object just as he sees it without altering its apparent size. In making drawings of complicated subjects, for the purpose of publication, it is often necessary to reduce the size, and to fill in the details of one drawing from many specimens, but with this the beginner has nothing to do. *The only*

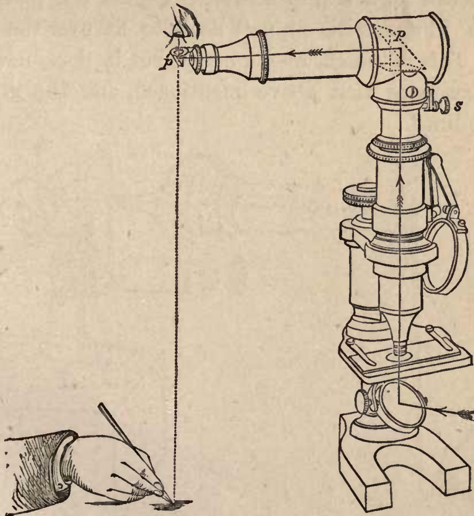

Fig. 1. Chevalier's camera lucida, with Hartnack's microscope.

practice advisible in his case is to draw things just as he sees them. It is important to practise even the drawing of *outlines* without the aid of a camera. The details of objects must indeed always be filled in without such aid, nevertheless it is difficult to get the exact size and the general form without a camera. Chevalier's camera lucida (Fig. 1.) is the best.

The eyepiece of the microscope is removed, and the camera inserted in its place, and fixed in any position with a screw (*s*). The rays penetrate a prism (*p*), undergo total internal reflection, emerge, are collected by a special eyepiece, and penetrate a second prism (*p*), where they are reflected to the eye of the observer, to whom the object appears to lie upon the table. The outlines of the object may be traced with a pencil as shown in the Fig. With Chevalier's camera the stage of the microscope is kept horizontal, so that it is perfectly serviceable in the case of fluids.

Beale's reflector is also a good arrangement for drawing (Fig. 2). It consists of a slip of neutral tint-glass (*r*), placed at an angle of 45° in a metallic support made to fit over the ordinary eyepiece. This is the cheapest camera, but it does not give so sharp an image as that above mentioned, and the microscope must be inclined.

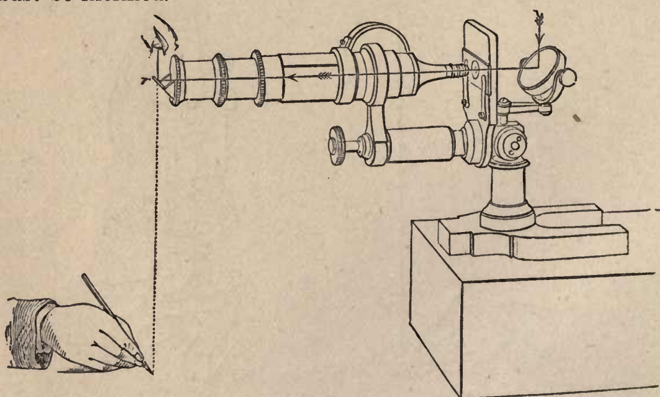

Fig 2. Drawing with Beale's Reflector.

In using either apparatus the *object must not be too brightly illuminated ; a black pasteboard shade should be placed between the window and the eye to cut off extraneous light, and the paper on which the image appears to lie should not be too brightly illuminated, otherwise the point of the pencil will not be clearly seen.* It is generally advantageous to close one eye. To all drawings a statement of the magnifying power should be added, and also a statement of the lenses used, *e.g.* Milk corpuscles × 300. Hartnack obj. 7, oc. 3. Tube drawn out.

Add acetic acid, observe its effects on the milk corpuscles. For this purpose place a drop of the acid on the slide close to the edge of the cover-glass. If it fail to pass under the cover, dip a strip of bibulous paper into the milk at the margin of the cover-glass opposite the acid. The acid will then speedily penetrate and *irrigate* the corpuscles. After it has affected them, those that lie near each other no longer remain distinct, but coalesce and form irregular masses ; from which it is inferred that each milk globule has an envelope that is soluble in acetic acid. From other chemical considerations this envelope is believed to consist of casein, a substance soluble in an excess of acetic acid.

23. *Estimate the magnifying power of the microscope.* Let the No. 7 objective and the No. 3 ocular be on Hartnack's microscope, with the tube fully elongated ; what is the magnifying power? Place a stage micrometer on the stage, and focalise a space that has in the process of manufacture been determined to be one-thousandth of an inch (0·001 in.) Hold the points of a pair of compasses *on a level* with the stage, keep both eyes open, look at the micrometer lines with one, and at the points of the compasses with the other eye, open or close the points until they include exactly one space of the micrometer ; then ascertain with the aid of an inch scale, to what extent the 0·001 inch has been enlarged. Suppose it to be magnified to 0·3 inch ; then 0·3 ÷ 0·001 = a magnifying power of 300 diameters linear. The tube may be shortened and the magnifying power again estimated,

and it will be found that the shorter the tube the lower the power.

24. *Estimate the size of an object.* This may be most accurately done with the aid of an eyepiece micrometer. This consists of a glass slip, with a fine scale engraved upon it. Hartnack's is the best. Measure the breadth of the human coloured blood corpuscle; use the No. 7 objective, elongate the tube, and substitute the micrometer ocular for the ordinary one. Bring a single coloured corpuscle into focus. It is seen through the micrometer lines. Suppose its broad diameter covers three of the smallest divisions. *Push in the tube; the corpuscle does not now cover so much of the micrometer; hence the value of the divisions of the ocular micrometer varies with the magnifying power*, and it is necessary to determine the value of the ocular micrometer divisions for all the powers employed. Therefore substitute a stage micrometer for the blood, elongate the tube, and focalise a 1/1000th inch division. Suppose it to cover ten divisions of the ocular micrometer, then evidently each division must, with this magnifying power, be equivalent to 1/10,000th inch. This ascertained, substitute the blood for the stage micrometer. Suppose that the broad diameter of a coloured corpuscle covers three divisions of the ocular micrometer, then 3/10,000ths=1/3333 inch. The corpuscles vary slightly in size; the average size being 1/3200th inch. This figure must be remembered, for the breadth of the human coloured blood corpuscle is commonly taken as the histological unit of size.

Histological Demonstrations.

25. *Granules aad Brownian movement.* (H.) Rub a piece of gamboge in a drop of water on a slide until the fluid is *faintly* tinged; cover and examine. The term *fine granule* is applied to a minute particle of any shape which has no light centre. The term *coarse granule* is applied to a particle of irregular shape having a light centre surrounded by a dark border. The dif-

ference between the optical characters of the former and those of the latter is entirely owing to size. Brownian motion is exhibited by all minute particles floating in a fluid. The motion is probably owing to thermal currents in the fluid; the solid particles merely indicating the directions of these currents.

26. *Hairs from stamens of Tradescantia virginica.* (H.) *a.* Examine in water; observe the cells, wall, nucleus, protoplasm, vacuoles, and movements of the protoplasm. Notice that the protoplasm is a colourless finely granular jelly.

b. Heat, and observe that a gentle heat accelerates the protoplasmic movement. A hot stage suitable for roughly heating a microscopic object may be made of a plate of tin, $9 \times 2\frac{1}{2}$ inches, with a small hole in the centre. Stricker's hot stage is employed when it is desirable to carefully graduate the temperature and keep it constant.

27. *Germinating yeast.*[1] (H.)

a. Examine different forms of the torulæ. The vacuole must not be mistaken for a nucleus.

b. Add magenta, and observe that as the pigment flows under the cover-glass the *buds* stain most rapidly. The protoplasm in many of the torulæ becomes stained, while the envelope *through which the staining agent must necessarily pass to reach the protoplast* remains colourless. A nucleus may be seen in some. The nucleus is generally the part of the cell which is most deeply stained by such agents as carmine, logwood, and magenta. Some torulæ never become stained. These are probably the oldest ones. Observe that the envelope is not equally thick in all the torulæ. In the youngest

[1] If beer yeast (torula cerevisiæ) cannot be obtained, procure German yeast, and sow it in sugar and water, or in the following modification of Pasteur's fluid:—

Water . .	8000	Potassic phosphate .	20
Cane sugar .	1300	Calcic phosphate .	2
Ammonium tartrate	100	Magnesium sulphate .	2

Keep the yeasty fluid gently warmed before the fire for a few hours.

31. *Linen fibres.* (H.) Examine in water. These are not twisted like cotton fibres.

32. *Disc-bearing tissue.* (H.) Examine a very thin vertical section of an ordinary cedar pencil, or of a piece of common fir in water, and observe the disc-like depressions on the fibres.

33. *Bacteria.* (H.) From a putrefying infusion of some albuminoid matter, *e.g.* muscle. Mince some muscle, allow it to soak in cold water for an hour, squeeze it in the water with the hand; filter through calico, and then through paper. Place the filtrate in a hot chamber, or near the fire, to keep it at summer heat, for 36 hours or so. Examine a particle of the pellicle that forms upon the fluid with the microscope. Observe the *definite* direction of the movements of the elongated bacteria *as long as they are alive.* Add magenta. The fluid stains and kills them. When dead, they exhibit only Brownian movement. The direction of this movement is *indefinite.* When living, the elongated bacteria wriggle like snakes, and thereby move in *definite* directions. The latter motion is vital, and results from contractility.

30, 31, 32, and 33 are examined because of the frequency of their occurrence in the urine, and other fluids examined by the medical microscopist.

34. *Blood of newt.*

Dry the tail, snip a piece from its extremity, and then express a small drop of blood upon a slide. Place the cover-glass upon it immediately.

D 1 (H.) *a.* Examine the coloured corpuscles. With the more obvious characters notice this, that the corpuscle at first appears to be homogeneous, but after a time a light oval spot appears in the centre of many; this is the position of the nucleus.

b. Examine the colourless corpuscles. There are several varieties of these. Some are very finely granular nucleated masses of protoplasm, as large, or even twice as

large, as the coloured corpuscles. These are the *common* white corpuscles. Others are coarsely granular. These are not nearly so numerous as the first. A third set consists of nucleated protoplasts, much smaller and finely granular than the others. Study the amœbiform movements of some of the large common finely granular corpuscles. If they move sluggishly, apply heat with the ordinary hot stage. Examine Stricker's hot stage and the mode of using it.

c. Add acetic acid at edge of cover-glass, and gently raise the cover to break the coagulum, and so allow the acid to penetrate. Notice that the coloured corpuscles suddenly become enlarged; they lose colour, and the nucleus becomes evident. The general mass of each white corpuscle becomes very transparent, while the larger ones usually exhibit three nuclei. The amœboid motion of the white corpuscle ceases.

D 2 (H.) The same blood. Cover, and add water at margin of cover-glass. The coloured corpuscles lose their colour, and often become more or less globular owing to endosmose.

D 3 (H.) The same blood. Add strong syrup. Mix the blood and syrup with a needle before covering. The coloured corpuscles shrivel, owing to exosmose.

D 4 (H.) The same blood. Before covering add the special magenta fluid used for staining corpuscles (see p. 63). It may be necessary to add afterwards a little of the ordinary magenta fluid. The nuclei of coloured and colourless corpuscles are stained.

D 5 (H.) The same blood. Add at margin of cover-glass fresh solution of tannic acid (2 grains in 1 oz. warm water. Allow it to cool). The coloured corpuscles often become globular, and after a time the hæmoglobin becomes slowly or suddenly extravasated from the corpuscle in the form of a bud adherent to the wall, or in

the form of fine granules scattered irregularly around the wall. With a [illegible] objective it may be seen, after the action of tannic acid, that the coloured corpuscle has a delicate envelope, and this envelope may sometimes be found ruptured when the ball has formed.

For D. 2, 3, and 4, frog's blood does perfectly.

D. 5 can also be done, though not so well, with the frog's corpuscles.

35. *Blood of bird.* (H.) Examine, and then add acetic acid.

36. *Blood of fish.* (H.) This must be quite fresh.

37. *Human blood.* (H.)

D. 1. Examine *a.* Coloured corpuscles. *b.* White corpuscles. *c.* Fibrin. *d.* Add acetic acid at side of cover glass, and observe that the coloured corpuscles become globular and lose their colour, while the white corpuscles become very transparent, and show in the larger ones usually three nuclei.

D. 2. The same blood. Allow the drop to partially dry before putting on the cover-glass, in order to get crenation of the coloured corpuscles.

D. 3. The same blood. Add to the blood, before applying the cover-glass, the special magenta fluid for staining blood corpuscles. No nucleus is revealed in the coloured corpuscle, but in the white corpuscle three nuclei usually appear.

D. 4. The same blood. Add tannic acid. Mix the drop of blood with a drop of the tannin solution (same as for newt's blood) before applying the cover-glass, and watch the change in the coloured corpuscles. The appearances are similar to those noticed in the newt's coloured corpuscles. Frequently, however, a delicate envelope may be seen outside the ball of extravasated hæmoglobin.

D. 5. Human blood, through which induced electricity has been passed, by the following arrangement. Take

the form of fine granules scattered irregularly around the wall. With 1-25th inch objective it may be seen, after the action of tannic acid, that the coloured corpuscle has a definite envelope, and this envelope may sometimes be found ruptured where the bud has formed.

For D 2, 3, and 4, frog's blood does perfectly.

D 5 can also be done, though not so well, with the frog's corpuscles.

35. *Blood of bird.* (H.) Examine, and then add acetic acid.

36. *Blood of fish.* (H.) This must be quite fresh.

37. *Human blood.* (H.)

D 1. Examine *a.* Coloured corpuscles. *b.* White corpuscles. *c.* Fibrin. *d.* Add acetic acid at side of cover-glass, and observe that the coloured corpuscles become globular and lose their colour, while the white corpuscles become very transparent, and show in the larger ones usually three nuclei.

D 2. The same blood. Allow the drop to partially dry before putting on the cover-glass, in order to get *crenation* of the coloured corpuscles.

D 3. The same blood. Add to the blood, before applying the cover-glass, the special magenta fluid for staining blood corpuscles. No nucleus is revealed in the coloured corpuscle, but in the white corpuscle three nuclei usually appear.

D 4. The same blood. Add tannic acid. Mix the drop of blood with a drop of the tannin solution (same as for newt's blood) before applying the cover-glass, and watch the change in the coloured corpuscles. The appearances are similar to those noticed in the newt's coloured corpuscles. Frequently, however, a delicate envelope may be seen outside the bud of extravasated hæmoglobin.

Examine human blood, through which induced electricity has been passed, by the following arrangements. Take

an ordinary slide, wet one surface with gum, press it firmly down on gold leaf, allow it to dry, scrape away the gold leaf so as to leave two triangles with their apices pointing towards each other at the centre of the slide, and reaching to an eighth of an inch from each other. Attach wires to two mounting clips. Place the slide on the stage and clamp each gold leaf triangle with a clip. Attach the wires to the secondary coil of a faradic machine. Place a drop of blood between the points of the gold leaf electrodes and apply a cover-glass. Transmit faradic shocks and watch effect. The coloured corpuscles become globular and lose their colour. The cause of this is probably electrolysis.

38. *Pus.* (H.) Examine and then add acetic acid. After the action of the acid the larger corpuscles usually show three nuclei, as in the case of the larger white blood corpuscles of human blood.

39. *Saliva.* (H.) Examine squamous epithelium and salivary corpuscles. Notice the Brownian motion of the granules in the latter. The salivary corpuscle appears to be a mucus corpuscle modified by the imbibition of fluid. Add magenta, and observe its effect on the cells. Observe the delicate fibrils of coagulated mucin.

40. *Mucus.* (H.) Examine mucus corpuscles obtained from urine, and compare with salivary and pus corpuscles. A mucus corpuscle has *usually* a single globular nucleus, sometimes, however, there is more than one.

41. *Squamous epithelium.* (H.) Omentum of guinea-pig or cat previously stained with silver nitrate. Mount in glycerine. (*This will be wanted for areolar tissue, fat-cells, and perhaps vessels, on a future day.*) Study the method of staining (§ 111).

42. *Ciliated epithelium.*

D 1 (L. & H.) Cilia from gill of salt-water mussel. Exa-

mals in sea water. Apply heat by means of the ordinary hot stage, and observe the accelerating effect of a moderate temperature on the ciliary motion.

2. (H.) Study the action of chloroform on the ciliary motion (Lister's experiment). For this purpose take a slide with a glass cell, or make a cell from a ring of putty, and cement it upon a slide. Place a small piece of the mussel's gill in a drop of sea water on the under surface of the cover-glass. Place this over the putty cell. Examine the cilia, then place a drop of chloroform on the floor of the cell, and watch the effects of the vapour. The motion gradually comes to a standstill. Study the mode of applying vapours to microscopic objects ([illegible] stage).

3. (H.) Scrape mucous membrane of frog's pharynx, and examine ciliated cells in ½ per cent. salt solution. (Or kill a cat by a blow on the head; chloroform must not be given, and scrape the mucous membrane of the trachea.) Notice the clear band at the broad end of the cell where the cilia are seen. If ciliated epithelium is stained with carmine or magenta, the nucleus and the finely granular protoplasm around it become stained, but the cilia and the clear band remain unstained; in this the latter resemble the contractile matter of muscle.

The study of preparations of ciliated epithelium for permanent preservation is postponed to the transverse section of trachea (see § 51).

43. Columnar epithelium. (H.) Scrape the mucous membrane of the small intestine of cat, prepared as directed in § 7, [illegible] teasing in water with the point of a knife or scalpel, and staining. Notice the finely striated band at the broad extremity of the columnar cell. This is more clearly seen with [illegible] lens. This preparation need not be preserved, for a section of the intestine (§ 59) gives all that it shows.

44. Transitional epithelium. (H.) Examine a preparation

or secondary slide and [illegible] with gum, press it firmly down on gold leaf, allow it to dry, scrape away the gold leaf so as to leave two triangles with their apices pointing towards each other at the centre of the slide and reaching to an eighth of an inch from each other. Attach wires to two mounting clips. Place the slide on the stage and clamp each gold leaf triangle with a clip. Attach the wires to the secondary end of a faradic machine. Place a drop of blood between the points of the gold leaf electrodes and apply a cover-glass. Then [illegible] shocks and watch effect. The coloured corpuscles become globular and lose their colour. The cause of this is probably electrolysis.

38. *Pus.* (H.) Examine and then add acetic acid. After the action of the acid the leucocytes generally show three nuclei, as in the case of the larger white blood corpuscles of human blood.

39. *Saliva.* (H.) Examine squamous epithelium and salivary corpuscles. Notice the Brownian motion of the granules in the latter. The salivary corpuscle appears to be a mucous corpuscle swollen by the imbibition of fluid. Add [illegible], and observe its effect on the latter. Observe the delicate threads of coagulated mucin.

40. *Mucus.* (H.) Examine mucus. Recognize [illegible] and compare with salivary and pus corpuscles. A [illegible] corpuscle has usually a single globular nucleus; sometimes, however, there is more than one.

41. *Squamous epithelium.* (H.) Omentum of [illegible] not previously stained with silver nitrate. Mount in glycerine. (This will be needed for another lesson, [illegible] and [illegible] mount on a [illegible].) Study the method of staining (§ 11.)

IV. *Ciliated epithelium.*

42. (L. & H.) Cilia from gill of [illegible] mussel. The—

mine in sea water. Apply heat by means of the ordinary hot stage, and observe the accelerating effect of a moderate temperature on the ciliary motion.

D 2 (H.) Study the action of chloroform on the ciliary motion (Lister's experiment). For this purpose take a slide with a glass cell, or make a cell from a ring of putty, and place it upon a slide. Place a small piece of the mussel's gill in a drop of sea water on the under surface of the cover-glass. Place this over the putty cell. Examine the cilia, then place a drop of chloroform on the floor of the cell, and watch the effects of the vapour. The motion gradually comes to a standstill. Study the mode of applying vapours to microscopic objects with Stricker's stage.

D 3 (H.) Scrape mucous membrane of frog's pharynx, and examine ciliated cells in $\frac{3}{4}$ per cent. salt solution. (Or, kill a cat by a blow on the head: *chloroform must not be given*,—and scrape the mucous membrane of the trachea.) Notice the clear band at the broad end of the cell where the cilia are seen. If ciliated epithelium be stained with carmine or magenta, the nucleus and the finely granular protoplasm around it become stained, but the cilia and the clear band remain unstained. In this the latter resemble the contractile matter of muscle.

An excellent preparation of ciliated epithelium for preservation will be obtained in the transverse section of trachea (see § 51).

43. *Columnar epithelium.* (H.) Scrape the mucous membrane of the small intestine of cat, prepared as directed in § 7. Diffuse the scraping in water with the point of a knife or needle; cover and examine. Notice the finely striated band at the broad extremity of the columnar cell. This is more clearly seen with a 1-25th lens. This preparation need not be preserved, for a section of the intestine (§ 59) gives all that it shows.

44. *Transitional epithelium.* (H.) Examine a preparation

made by scraping the mucous membrane of cat's bladder, prepared as directed in § 7, and preserved in glycerine. In this variety of epithelium the cells may be squamous, columnar, or quite indefinite in shape.

The various forms of *glandular epithelium* will be examined in the different glands.

45. *Elastic tissue.* (H.) Lig. nuchæ of calf. Snip off a *small* piece with scissors, place it on a slide, add a drop of water, and tease it thoroughly with needles, cover and examine. Remove the cover-glass, and add acetic acid. It does not affect elastic fibres. This tissue may be preserved in weak spirit or glycerine, but it may also be preserved by the acetic acid already added; therefore, take care that the space under the cover-glass is completely filled with the acid. All air bubbles must be removed. With bibulous paper thoroughly dry the slide outside the cover-glass, and then, paint a ring of gold size around and over the edge of the cover. When this has dried, put on a second coat, with or without the aid of a turntable. When this has dried, put on a final coat of dammar varnish. (See § 127.)

46. *Areolar tissue.*

D 1 (H.) *a.* Rapidly spread out with needles on a *dry* slide a small piece of subcutaneous tissue from a cat or rabbit. A dry slide is taken in order that the tissue may adhere to it, and so be spread out readily. The tissue must not however be allowed to dry. Add salt solution and examine. Notice that the white fibres consist of bundles of fibrils. The borders of the white fibres are faint and ill defined. An elastic fibre may be found here and there in the field; unlike the white fibre, its margin is sharply defined, owing to the substance being strongly refractile.

b. Take off cover-glass and add acetic acid. It causes no change in the elastic fibres, but the white fibres swell up, the fibrils disappear, and nothing marks their position

occupying a clear [illegible]. The nuclei of the connective tissue protoplasts are brought into view. Areolar tissue may be preserved in potassium acetate or weak spirit. Glycerine causes the white fibres to swell up and become transparent or hyaline. This preparation need not now be preserved, as specimens will be obtained in sections of the alimentary canal.

The splitting of the white fibres into fibrils is easily effected after maceration for a fortnight or so in a 10 per cent. solution of salt, often changed, or in lime or baryta water. (Rollett.) These agents appear to soften or dissolve a cement which unites the fibrils.

D. 2 (H.) Examine in a drop of aqueous humour (see § 7) a preparation of intermuscular fascia from the leg, or of subperitoneal tissue from the lumbar region of a frog recently killed. Remove a small portion of the tissue with scissors and gently spread it out on a slide in the aqueous humour. Observe the stationary and amoeboid connective tissue corpuscles. The latter, often termed the wandering cells of connective tissue, appear to be identical with white blood corpuscles. The former are the true connective tissue corpuscles. Endeavour to see the locomotion of the wandering cells. The changes of shape, but without locomotion of the connective tissue protoplasts proper may perhaps also be seen.

D. 3 (H.) Examine the preparation of omentum already made, and observe the connective tissue corpuscles amidst the fibres, and the epithelium here and there clothing the bundles of fibres. Wandering cells (no longer moving of course) may sometimes be seen singly or in groups here and there on the surface of the bundles.

4. Tendon. (H.) T.S. dried tendon of sheep. The tendon should be dried until it is just dense enough to be readily sliced. Make an extremely thin section with a scalpel wetted with

made by scraping the mucous membrane of [illegible] bladder prepared as directed in § 7 and [illegible] in [illegible]. In this variety of epithelium the cells may be squamous, columnar, or quite [illegible] in shape.

The various forms of *glandular epithelium* will be examined in the different glands.

35. *Elastic tissue.* (H.) Ligamentum nuchae of calf. Snip off a small piece with scissors, place it on a slide, add a drop of water, and tease it thoroughly with needles, cover and examine. Remove the cover-glass and add acetic acid. It does not affect elastic fibres. This tissue may be preserved in weak spirit or glycerine, but it may also be preserved by the acetic acid already added; therefore, take care that the space under the cover-glass is completely filled with the acid. All air bubbles must be removed. With bibulous paper thoroughly dry the slide outside the cover-glass, and then paint a ring of gold-size around and over the edge of the cover. When this has dried, put on a second coat, with or without the aid of a turntable. When this has dried, put on a final coat of dammar varnish. (See § 127.)

36. *Areolar tissue.*

D 1 (H.) *a.* Rapidly spread out with needles on a dry slide a small piece of subcutaneous tissue from a rat or rabbit. A dry slide is better because the tissue then adheres to it and so is spread out readily. The tissue must not however be allowed to dry. Add salt solution and examine. Notice that the white fibres consist of bundles of fibrils. The borders of the white fibres are faint and ill defined. An elastic fibre may be found here and there in the field; unlike the white fibre, its margin is sharply defined, owing to the substance being strongly refractile.

b. Take off cover-glass and add acetic acid. It causes no change in the elastic fibres, but the white fibres swell up, the fibrils disappear, and nothing marks their position

excepting a clear jelly. The nuclei of the connective tissue protoplasts are brought into view. Areolar tissue may be preserved in potassium acetate or weak spirit. Glycerine causes the white fibres to swell up and become transparent or hyaline. This preparation need not however be preserved, as specimens will be obtained in sections of the alimentary canal.

The splitting of the white fibres into fibrils is easily effected after maceration for a fortnight or so in a 10 per cent. solution of salt, often changed, or in lime or baryta water. (Rollett.) These agents appear to soften or dissolve a cement which unites the fibrils.

D 2 (H.) Examine in a drop of aqueous humour (see § 77) a preparation of intermuscular fascia from the leg, or of subperitoneal tissue from the lumbar region of a frog recently killed. Remove a small portion of the tissue with scissors and gently spread it out on a slide *in* the aqueous humour. Observe the stationary and amœboid connective tissue corpuscles. The latter, often termed the wandering cells of connective tissue, appear to be identical with white blood corpuscles. The former are the true connective tissue corpuscles. Endeavour to see the *locomotion* of the wandering cells. The changes of shape, but *without locomotion* of the connective tissue protoplasts proper may perhaps also be seen.

D 3 (H.) Examine the preparation of omentum already made, and observe the connective tissue corpuscles *amidst* the fibres, and the epithelium here and there clasping the bundles of fibres. Wandering cells (no longer moving of course) may sometimes be seen singly or in groups here and there on the surface of the bundles.

47. *Tendon.* (H.) T. S. dried tendon of sheep. The tendon should be dried until it is just dense enough to be readily sliced. Make an excessively thin section with a scalpel wetted with

methylated spirit. Water does not adhere to the knife. Examine the section in water. Observe the compressed branching spaces between the transverse sections of the longitudinal fibres of the tendon. Some of the spaces contain nucleated protoplasts. Bundles of fibres are seen running transversely around and between the longitudinal bundles. (This need not be preserved.)

Examine T. S. tendon hardened in chromic acid, and preserved in glycerine. In this, the spaces between the bundles are not so compressed as in the dried tendon.

Examine L. S. tendon hardened in chromic acid.

48. *Adipose tissue.* (H.) Examine the preparation of omentum of guinea-pig or cat already made. The nucleus of the fat cell may have been stained by the silver, and if so, it will be readily seen.

Examine a preparation of adipose tissue previously carminised or stained with logwood and preserved in glycerine. Magenta does not stain the nuclei of the fat cells readily. Such a preparation may be made by freezing a portion of fresh skin, slicing it, and then staining it with carmine.

49. *Adenoid tissue.* (H.) *a.* Examine an extremely thin section made with the freezing microtome of a fresh mesenteric gland of cat or dog. Brush away lymph corpuscles with a small camel-hair pencil, or, what is better, shake the section gently in a test-tube containing $\frac{3}{4}$ per cent. salt solution, in order to see the branched connective tissue corpuscles of which adenoid tissue consists—the branches of the corpuscles, however, having been transformed into fibres. Sections of lymph glands may be advantageously stained with logwood (see § 110). Preserve in glycerine. (*This preparation will be wanted on a future day.*)

Study the mode of using the freezing microtome (see § 85).

50. *Mucous tissue.* (H.) Examine a preparation of vitreous humour. (Such a preparation may be made by slicing a frozen eye and preserving the section of the vitreous in potass. acet.)

In the peripheral part of the vitreous the tissue consists of a jelly-like matrix, containing branched connective tissue corpuscles. Towards the centre of the vitreous no protoplasts can be found in the jelly.

51. *Cartilage.* (H.)

D 1. With scissors make a section of white fibrocartilage from intervertebral disc of sheep, tease it slightly in salt solution, and examine it in this fluid. The matrix contains numerous fibrils like those of white fibrous tissue. The protoplasts are rounded and enclosed in a capsule (cartilage capsule). The cells may be found in various stages of fission. (The capsule around the protoplast is most evident with a 1-25th lens.) Preserve in glycerine.

D 2. Section of yellow fibro-cartilage from epiglottis of sheep, hardened for a day in methylated spirit (nothing else hardens this so well). Examine and preserve in glycerine. When examined with a very high power it is evident that this tissue consists of cells, imbedded in a matrix formed of a spongy material. Here and there its processes resemble the fibres of elastic tissue, but it is erroneous to speak of the matrix as consisting of elastic fibres imbedded in a granular matter. The apparent granules are the cut ends of spicules of the spongy matter.

D 3. T. S. tracheal rings of cat, hardened in chromic acid (see § 6). Mount in glycerine. Examine hyaline cartilage and perichondrium. This is a valuable specimen if the tissue be cut with the aid of the freezing microtome, and its surfaces therefore not destroyed by imbedding in paraffin. It will show ciliated epithelium, glands in mucous membrane, hyaline cartilage and fibrous tissue.

D 4. T. S. costal cartilage of adult. Kept in methylated spirit for a day or so. Make the section through a point of calcification. Preserve in glycerine. Some of the

cells may have undergone fatty degeneration. Close to the point of calcification, the cells will be found in rows owing to proliferation. The matrix between the rows will probably be found to be fibrillated owing to its having been transformed into a calcified fibrous tissue.

D 5. V. S. articular cartilage and bone from head of softened bone of cat. (L.) Examine cartilage and cancelli. (H.) Examine bone corpuscles in lacunæ, canaliculi, osteoblasts in the cancelli, formation of bone corpuscles from cartilage corpuscles. Examine and mount in glycerine. Glycerine is apt to render softened bones too transparent; when such is the case, mount in meth. sp., and use *thick* dammar as the cement. This preparation ought to be one of much value. I have seen no preparations of softened bones so beautiful as those prepared in the manner indicated in § 13. Examine a preparation of osteoblasts under a 1-25th inch lens.

52. *Bone.* (H.) T. S. softened long bone of cat. (Treat as D 5, § 51.) Examine Haversian canals and the concentric arrangement of the lacunæ around these; the difference between the dense and the cancellated bone; the lamellæ and their arrangements; the periosteum.

Examine T. S. and V. S. of unsoftened long bone. Such sections are made with a fine saw, and are then ground sufficiently thin upon a hone. They are best mounted dry (in air).

53. *Tooth.* V. S. softened tooth and jaw of cat. Treat as D 5, § 51. (L.) General arrangement of tooth in alveolus, pulp cavity, dentine. (H.) Dentine and crusta petrosa. The enamel disappears under the influence of the acid. The odontoblasts may possibly be found on the inner aspect of the dentine, while their external processes—the fibres of Tones—may be seen passing into the dentine.

Examine sections of unsoftened teeth made in the same way as the above. (I.) In V. S. notice arrangement of enamel fibres; coloured lines in enamel, and contour lines in dentine; and the curvatures of the dentinal tubules. (II.) In T. S. notice the transverse markings in the fibres of the enamel. Families of the fibres will probably be found to have been cut transversely. These will probably show that each fibre is of a more or less hexagonal shape. Observe the fine secondary branchings of the dentinal tubules, giving rise to a feathery appearance. This is usually most evident near the outer part of the dentine. In this situation look for interglobular spaces. T. S. of dentinal tubes will probably be found at some part of the section; if not, examine a T. S. of tooth where the section has been made immediately external to the pulp cavity.

21. Muscle. (M.)

D 1. Strip off a small piece of non-striped muscle from intestine or stomach macerated for two months or so in Müller's fluid. Separate the fibres by teasing. Preserve in glycerine. It is difficult to see the nuclei unless the fibres be stained. Examine frog's bladder carminised. Notice the spindle-shaped and the branched fibres.

D 2. Fresh striped muscle of frog. Dissect it with needles in a drop of water, in order to study the sarcolemma. Look for the sarcolemma raised from the muscular substance, by imbibition, in the form of a vesicle, or stretched between the ends of fibres broken across, or prolonged as a funnel-shaped process from the end of a ruptured fibre. Add acetic acid. It renders the muscular substance very transparent and reveals the nuclei of the sarcolemma. The sarcolemma itself, being of the nature of elastic tissue, is unaffected.

D 3. V. S. (with a razor) stained muscle of cat or rabbit,

cells they have undergone fatty degeneration. Close to the point of ossification, these cells will be found to have undergone proliferation. The matrix between the cells will probably be found to be fibrillated owing to its having been transformed into a calcified fibrous tissue.

D 6. V. S. articular cartilage and bone from head of femur, boiled in oil. (L.) Examine cartilage and canaliculi. (H.) Examine bone corpuscles in lacunæ; canaliculi; cartilage in the process of formation of bone corpuscles from cartilage corpuscles. Examine and mount in glycerine. Glycerine is apt to render softened bones too transparent. When such is the case, mount in [illegible] and [illegible] darker to the [illegible]. This preparation [illegible] to be one of much value. I have seen no preparations of softened bones so beautiful as those prepared in the manner indicated in § 15. Examine a preparation of canaliculi under a 1-25th-inch lens.

31. Bone. (L.) T. S. softened long bone of ox. (Same as D 6, § 31.) Examine Haversian canals and the concentric arrangement of the lacunæ around them; the difference between the dense and the cancellated bone; the lamellæ and their arrangement; the periosteum.

Examine T. S. and V. S. of unsoftened long bone. These such as are made with a fine saw, and are then ground sufficiently thin upon a hone. They are then mounted dry (in air).

32. Teeth. V. S. softened tooth and jaw of cat. (Same as D 6, § 31.) (L.) General arrangement of tooth in its socket; pulp cavity; cement. (H.) Dentine and dental pulp. The enamel disappears under the influence of the acid. The odontoblasts may possibly be found on the inner aspect of the dentine, while their external processes—the fibres of Tomes—may be seen passing into the dentine.

Examine sections of unsoftened tooth made in the same way as the above. (L.) In V. S. notice arrangement of enamel fibres; coloured lines in enamel, and contour lines in dentine, and the curvatures of the dentinal tubules. (H.) In V. S. notice the transverse markings in the fibres of the enamel. Bundles of the fibres will probably be found to have been cut transversely. These will probably show that each fibre is of a more or less hexagonal shape. Observe the fine secondary branchings of the dentinal tubules, giving rise to a feathery appearance. This is usually most evident near the outer part of the dentine. In this situation look for interglobular spaces. T. S. of dentinal tubes will probably be found at some part of the dentine, if not, examine a V. S. of tooth where the section has been made immediately external to the pulp cavity.

54. *Muscle.* (H.)

D 1. Strip off a small piece of non-striped muscle from intestine or stomach macerated for two months or so in Müller's fluid. Separate the fibres by teasing. Preserve in glycerine. It is difficult to see the nucleus unless the fibres be stained. Examine frog's bladder carminised. Notice the spindle-shaped and the tri-radiate fibres.

D 2. Fresh striped muscle of frog. Dissect it with needles in a drop of water, in order to study the sarcolemma. Look for the sarcolemma raised from the sarcous substance, by imbibition, in the form of a vesicle, or stretched between the ends of fibres broken across, or prolonged as a funnel-shaped process from the end of a ruptured fibre. Add acetic acid. It renders the sarcous substance very transparent and reveals the nuclei of the sarcolemma. The sarcolemma itself, being of the nature of elastic tissue, is unaffected.

D 3. V. S. (with a razor) striped muscle of cat or rabbit,

hardened in chromic acid. Place the section in a drop of glycerine, tease with needles and examine the transverse stripes of the fibres and the fibrils. Remove the slide from under the lens, and forcibly press down the cover-glass with the *handle* of a knife or needle, and examine again. The pressure often causes the fibres to cleave transversely. The transverse cleavage takes place through a faint dim stripe running across the clear stripe. (This faint stripe can rarely be seen in this muscle without the use of a very high power.) In this preparation sarcous elements may be seen. These are oblong particles resulting from a transverse cleavage of the *fibrils.* Preserve in glycerine. The fibrils of striped muscle may be readily obtained from the muscle of the lobster by hardening it in meth. sp. for 24 hours and teasing.

An excellent preparation of T. S. and V. S. striped muscle will be obtained from hardened tongue (see § 59).

Examine T. S. striped muscle, carminised striped muscle, and injected muscle.

D 4. Muscle from the leg or thorax of water beetle (Dytiscus Marginalis). Add a drop of salt solution, gently separate the fibres, and examine immediately. Notice the light and dim stripes, Schaefer's "muscle rods" with their heads in the light stripe. A power of 1000 diameters is required to see the "rods" clearly. Study contractions. Even a ¾ per cent. salt solution rapidly alters the fibres, and, therefore, for prolonged study they should be surrounded by their own juice only (Schaefer).

Examine striped muscle of Dytiscus under 1-25th lens to see "muscle rods."

55. *Nerve-tissue.*

D 1 (H.) Fresh nerve of frog. Fray out one end of the nerve with a needle in salt solution or aqueous humour,

and examine axis cylinder and white sheath ("white substance of Schwann"). The grey sheath ("sheath of Schwann") may sometimes be seen projected as an infundibuliform process from the end of a ruptured fibre.

D 3 (H.) Sciatic of frog hardened in osmic acid (one-fifth per cent.) for two days. Wash in distilled water for a day, tease, and preserve in potass. acet. The acid blackens the white sheath. Ranvier's nodes may be readily seen in the course of the fibres.

D 2 (H.) T. S. with scissors white matter from spinal cord of cat or sheep. (Add no fluid.) An extremely small piece must be taken. Gently press down the cover-glass and examine the fibres. As the white fibres of the brain and spinal cord are not surrounded by a grey sheath, they readily yield to the influence of pressure, becoming ampullated and even broken into pieces. The fragments assume a rounded form; each one being marked like the original fibre by a double contour. This serves to distinguish them from nerve cells, for which they are apt to be mistaken by the inexperienced observer.

Examine fine white nerve fibres in frog's bladder, carminised, and blood-vessels injected with Beale's glycerine and Prussian Blue, and preserved in glycerine and acid. (Glycerine 1 oz.; glacial acetic acid 5 drops.) The acid renders the white sheath granular, and by this enables one at once to distinguish delicate medullated nerve fibres from white fibrous tissue.

D 4 (H.) T. S. sciatic nerve of cat hardened in chromic acid. Stain with carmine in the manner recommended in § 71. After staining and washing remove the water by first immersing in methylated spirit in a test or watch-glass, and then in absolute alcohol for two or three minutes. Lay the section on a slide, allow it to partially dry (let it just become glazed and whitish), then introduce a drop of clove oil under the section

and examine axial cylinder and white sheath ("white substance of Schwann"). The grey sheath ("sheath of Schwann") may sometimes be seen prolonged as an infundibuliform process from the end of a ruptured fibre.

D 2 (H.) Nerve of frog hardened in osmic acid (one-fifth per cent.) for two days. Wash in distilled water for a day, tease, and preserve in potass. acet. The acid darkens the white sheath. Ranvier's nodes may be readily seen in the course of the fibres.

D 3 (H.) L. S. with scissors white matter fresh spinal cord of cat or sheep. (Add no fluid.) An extremely small piece must be taken. Gently press down the cover-glass and examine the fibres. As the white fibres in the brain and spinal cord are not surrounded by a grey sheath, they readily yield to the influence of pressure, becoming ampullated and even broken into pieces. The fragments assume a rounded form ; each one being marked like the original fibre *by a double contour.* This serves to distinguish them from nerve cells, for which they are apt to be mistaken by the inexperienced observer.

Examine fine white nerve fibres in frog's bladder, carminised, and blood-vessels injected with Beale's glycerine and Prussian blue, and preserved in glycerine and acid. (Glycerine 1 oz., glacial acetic acid 5 drops.) The acid renders the white sheath granular, and by this enables one at once to distinguish delicate medulated nerve fibres from white fibrous tissue.

D 4 (H.) T. S. sciatic nerve of cat hardened in chromic acid. Stain with carmine in the manner recommended in § 72. After staining and washing remove the water by immersing first in methylated spirit in a test or watch-glass, and then in absolute alcohol for two or three minutes. Lay the section on a slide, allow it to *partially* dry (let it just become glazed and sodden), then *insinuate* a drop of clove oil *under* the section

with a sable-hair brush (camel-hair is not stiff enough). Allow the oil to *rise* through the section, *and so drive away the spirit.* Watch with L. the clarifying effect of the oil. Finish the clarification by touching the upper surface of the section with the oil. Lastly, cover with a drop of dammar or Canada balsam solution, put on the cover-glass, and gently press it down. Water and spirit are not miscible with dammar. The spirit replaces the water, the clove oil replaces the spirit, increases the transparency of the tissue, and is miscible with dammar or Canada balsam dissolved in benzole or chloroform.

Study (H.) the section of nerve prepared as above. Notice the divided nerve fibres and the connective tissue (perineurium, neurilemma.)

Examine (H.) nerve fibrils in cornea stained with gold (see § 112). (H.) Grey fibres from sympathetic nerve prepared as follows. Tease and preserve in glycerine a portion of the cervical sympathetic of a rabbit recently killed.

D 5 (H.) Section of fresh Gasserian ganglion of sheep with scissors. Examine in salt solution. The nucleus, nucleolus, and general protoplasm of the cell can be readily seen. The processes are usually broken off.

The capsule that surrounds each cell may be demonstrated thus:—make sections of a frozen Gasserian ganglion (fresh), and stain with carmine or magenta.

Examine cells of spinal cord carminised, to see processes of nerve-cells.

The fibrillar structure of the processes of nerve-cells may be shown as follows. Cut the fresh spinal cord of a calf into pieces about a quarter of an inch in length. Place these for a month in a one per cent. potass. bichrom. solution. Remove a thin slice of the grey matter of the anterior horn with scissors, tease with needles, stain with carmine, and mount in glycerine.

Or from the fresh end of the cord make transverse sections. Place them in 1/10th per cent. acetic acid for twenty-four hours. Wash in water, and examine in potassium acetate, or place them for a day in iodised serum and then examine.

Examine a preparation of the pear-shaped cells of sympathetic ganglia. These may be prepared as follows (Beale): Remove the auricular septum of the frog's heart. Macerate for some days in glycerine (1 oz.) and glacial acetic acid ([illegible] drops), isolate the ganglia by teasing with needles under a dissecting microscope. Preserve in glycerine.

56. *Blood-vessels* (H.)

D. 1. Pia mater of sheep. With scissors remove a slice ⅛ inch or so in depth from the surface of a cerebral convolution. Lay it upon a slide, with the pia mater touching the glass. Fix one side of the slice with forceps, and with the back of a scalpel gently scrape away the brain matter. With a camel-hair brush and water, "pencil" away the remainder. Tease with needles and examine. Observe the nuclei in the capillaries. The elastic membrane, the muscular fibres, and the tunica adventitia in the larger vessels. Add acetic acid. The tunica adventitia swells up, becomes transparent (hyaline), and the nuclei of its connective tissue corpuscles appear. The muscular fibres become very transparent and show their nuclei. The elastic membrane is then more evident.

Examine elastic membrane of blood-vessels isolated. This may be readily prepared from the basilar or other larger artery at the base of the sheep's brain. Take a short piece of the artery, lay it open, add a drop of acetic acid, tease with needles, and mount in glycerine.

Examine blood-vessels with silvered epithelium (see p. 64), and T. S. blood-vessels. A preparation of T. S. blood-vessels may be readily obtained by making a T. S. of

Or freeze the fresh cord of the calf; make transverse sections. Place them in 1-5th per cent. osmic acid for twenty-four hours. Wash in water, and examine in potassium acetate, or place them for a day in iodised serum, and then examine.

Examine a preparation of the pear-shaped cells of sympathetic ganglia. These may be prepared as follows (Beale): Carminise the auricular septum of the frog's heart. Macerate for some days in glycerine (1 oz.), and glacial acetic acid (5 drops), isolate the ganglia by teasing with needles under a dissecting microscope. Preserve in glycerine.

56. *Blood-vessels.* (H.)

D 1. Pia mater of sheep. With scissors remove a slice $\frac{1}{4}$ inch or so in depth from the surface of a cerebral convolution. Lay it upon a slide, with the pia mater touching the glass. Fix one side of the slice with forceps, and with the *back* of a scalpel gently scrape away the brain matter. With a camel-hair brush and water, "pencil" away the remainder. Tease with needles and examine. Observe the nuclei in the capillaries. The elastic membrane, the muscular fibres, and the tunica adventitia in the larger vessels. Add acetic acid. The tunica adventitia swells up, becomes transparent (hyaline), and the nuclei of its connective tissue corpuscles appear. The muscular fibres become very transparent and show their nuclei. The elastic membrane is then more evident.

Examine elastic membrane of blood-vessels isolated. This may be readily prepared from the basilar or other larger artery at the base of the sheep's brain. Take a short piece of the artery, lay it open, add a drop of acetic acid, tease with needles, and mount in glycerine.

Examine blood-vessels with silvered epithelium (see p. 64), and T. S. blood-vessels. A preparation of T. S. blood-vessels may be readily obtained by making a T. S. of

hardened spinal cord with the pia mater intact (see § 72), or a V. S. of tongue (see § 59).

57. *Circulation in frog's web and mesentery.* *a.* Inject one, two, or three minims—according to size of frog—of a watery solution of curara, each minim of which contains 0·032 milligramme of the poison.

b. Tie a soft cotton thread round the longest toe, close to its tip, and another round the toe next to, and on one or the other side of this. Lay the frog on its back and stretch the web gently over a triangular window in a piece of cardboard (A. Fig. 3) by drawing the threads attached to the toes gently through the slits *a* and *á*. The web must not be permitted to dry.

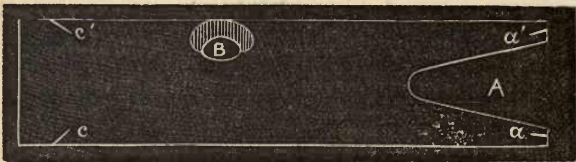

Fig. 3. Frog-plate of cardboard. The slits *c* and *c′* are only used when the frog is not curarised and requires to be secured in a bag.

c. (L.) Artery, vein, capillaries, and pigment cells. The velocity of the stream in the artery is greater than in the vein. This results from the calibre of the artery being less than that of the vein, and from the greater pressure of the blood in the former.

d. (H.) (Tube of microscope shortened.) Examine the manner in which the coloured and colourless corpuscles move along the capillaries—the former with comparative rapidity, owing to their polished surfaces, the latter tardily, owing to an apparent tendency to adhesion between the wall of the vessel and the corpuscle.

In the arteries and veins it may often be seen that the coloured corpuscles move principally in the centre of the stream, while the colourless corpuscles may often be found adhering to the

of the vessel, or moving lazily along it. The peripheral part of the stream is often devoid of coloured corpuscles (the "lymph space" of older observers). The smaller velocity of the peripheral, as compared with the central part of the stream, is owing to the friction between the vessel and the blood, and the adhesion [illegible] and those of the white corpuscles.

[illegible] Apply to the web a particle of strong mustard freshly made into a paste with water. Wash it off at the end of twenty minutes. While the mustard is acting, expose the mesentery by a vertical incision on the *left* side of abdomen, a vein immediately below the axilla must be avoided, and also the stomach and duodenum. With forceps gently draw out the intestines, and pin it to a cork mesentery close to a window in the corkboard (cf. Fig. 5).

Examine with I. and II. arteries, veins and capillaries. For continued observation it is necessary to place a slip of thin glass over the window in the corkboard, and also to cover the mesentery with a small cover-glass. It should be wetted with aqueous humour.

9. Wash off mustard and look at inflamed web with I. and II.

The diapedesis of the white corpuscles can be best seen in the tail of a tadpole, rendered unhealthy by keeping the animal for some days in a small quantity of water without changing it.

Study the mode of injecting vessels by a water pressure apparatus, and also with a syringe (see § 114).

B. *Lung.*

I. 1. (II.) Examine the section of trachea already prepared. Study ciliated epithelium and the layers of epithelial cells below those that are ciliated; the mucous glands and the cartilage.

II. 2. *a.* Make sections of chromic acid lung across a bronchus visible to the naked eye. Sections of lung, either fresh or hardened, are best made by freezing. Examine and

Transect spinal cord and pith the portion below [illegible] (Fig. [illegible])
[illegible] of strychnine [illegible].

III. Convulsions in frog's [illegible] and [illegible] [illegible] one, two or three minutes—according to the [illegible] of a watery solution of [illegible], each minim of which contains [illegible] of the poison.

5. Tie a soft cotton thread round the biggest toe, close to its tip, and another round the toe next to, and [illegible] on the other side of [illegible]. Lay the frog on its back and stretch the web gently over a triangular window in a piece of cardboard ([illegible] Fig. [illegible]), drawing the threads attached to the toes gently through the slits [illegible] and [illegible]. The web must not be permitted to dry.

Fig. [illegible]. [illegible] of [illegible]. The [illegible] and [illegible] are [illegible] when the frog is [illegible] between the [illegible] to be secured in [illegible].

6. (II.) Artery, vein, capillaries, and pigment cells. The velocity of the stream in the artery is greater than in the vein. This results from the calibre of the artery being less than that of the vein, and from the greater [illegible] of the [illegible] in the former.

7. (III.) [illegible] of microscopic [illegible]. [illegible] the manner in which the coloured and colourless [illegible] along the capillaries—the former with [illegible] rapidity, [illegible] their polished surfaces, the latter [illegible] an apparent tendency to adhesion between [illegible] of the vessel and the [illegible].

In the arteries and veins it may often be seen that the coloured corpuscles move principally in the centre of the stream, while the colourless corpuscles may often be found adhering to the

wall of the vessel, or moving tardily along it. The peripheral part of the stream is often devoid of coloured corpuscles (the "lymph space" of older observers). The smaller velocity of the peripheral, as compared with the central part of the stream, is owing to the friction between the vessel and the blood, and the adhesion here and there of the white corpuscles.

e. Apply to the web a particle of strong mustard freshly made into a paste with water. Wash it off at the end of twenty minutes. While the mustard is acting, expose the mesentery by a vertical incision on the *left* side of abdomen, a vein immediately below the axilla must be avoided, and also the stomach and duodenum. With forceps gently draw out the intestines, and pin it to a cork crescent close to a window in the cardboard (B. Fig. 3).

Examine with L. and H. arteries, veins, and capillaries. For prolonged observation it is necessary to place a slip of thin glass over the window in the cardboard, and also to cover the mesentery with a small cover-glass. It should be wetted with aqueous humour.

g. Wash off mustard and look at inflamed web with L. and H.

The diapedesis of the white corpuscles can be best seen in the tail of a tadpole, rendered unhealthy by keeping the animal for some days in a small quantity of water without changing it.

Study the mode of injecting vessels by a water pressure apparatus, and also with a syringe (see § 114).

58. *Lung.*

D 1. (H.) Examine the section of trachea already prepared. Study ciliated epithelium and the layers of epithelial cells below those that are ciliated; the mucous glands and the cartilage.

D 2. *a.* Make sections of chromic acid lung across a bronchus visible to the naked eye. Sections of lung, either fresh or hardened, are *best made by freezing.* Examine and

preserve in glycerine. (Sections may be stained with logwood and mounted in glycerine or dammar.)

b. (L.) Notice the general characters of T. S. bronchus and large vessels in order to recognize them readily with the high power. (H.) Examine epithelium of air vesicles and the fibrous tissue around these; T. S. bronchus showing epithelium, glands, muscle, cartilage; T. S. pulmonary artery and vein. The wall of the vein is thinner than that of the artery. Adenoid tissue may in some sections be seen around the larger blood vessels and in the bronchial mucous membrane. Examine a preparation of lung with blood vessels injected.

59. *Alimentary Canal.*

D 1. V. S. chromic acid tongue of cat. Examine and preserve in glycerine (L.) Papillæ, muscle, (H.) Epithelium, T. S. and V. S. striped muscle, T. S. and perhaps V. S. vessels, fibrous tissue. (Sections may be stained with carmine or logwood and mounted in dammar.)

Examine (H.) V. S. gustatory disc of rabbit's tongue for taste bulbs. This may be made from a tongue hardened in absolute alcohol. Stain with logwood, and mount in dammar.

Examine (L.) V. S. human tongue showing fungiform and filiform papillæ. (L.) V. S. injected tongue of cat or man.

D 2. V. S. chromic acid stomach of cat. This is best made by freezing. Preserve in glycerine. (L.) Relations of mucous, sub-mucous, and muscular coats. (H.) Follicles, muscularis mucosa, sub-mucous coat. Blood vessels, L. S. and T. S. non-striped muscle.

The various structures may also be beautifully seen in a stomach, macerated for six weeks or so in Müller's fluid (§ 7). This is well suited for the demonstration of the cells in the peptic follicles. In a very thin V. S., columnar

cells may be seen at the pyloric extremity, while two kinds of cells are seen in the cardiac portion of the tube, the so-called "peptic cells" placed externally, and the continuation of the columnar cells internal to these. In the [illegible] part of the glands the columnar cells are often [illegible] and irregular.

P. I. T. S. Small intestine of cat hardened in chromic acid and [illegible] fluid ([illegible]). Preserve in glycerine. (L.) Mucous, submucous, and muscular coats. (H.) Columnar epithelium of villi and Lieberkühn's follicles, muscularis mucosae.

P. I. T. S. Large intestine of cat hardened in chromic acid ([illegible]). Preserve in glycerine. (L.) Same as above. (H.) Lieberkühn's follicles.

Sections of stomach and intestine, if [illegible], may be mounted in dammar.

Examine (L.) V. S. injected stomach. T. S. injected small and large intestine. Inner aspect of small intestine injected. T. S. duodenum [illegible] showing Brunner's glands. T. S. small intestine [illegible] showing Peyer's follicles; and V. S. small intestine showing valvulae conniventes.

(H.) Contents of Peyer's follicle, seen in a T. S. or V. S. [illegible] small intestine with blood vessels injected.

Liver.

1 (H.) Cells of fresh cat or rabbit's liver. Examine in salt solution.

2 (H.) Cells of liver of cat. These are usually fatty.

3 [illegible] section of liver of cat hardened in Müller's fluid [illegible]. Make the section across some of the larger [illegible]. The sections are best made by freezing. Preserve in glycerine. (L.) Lobules. (H.) Appearance and [illegible] to another and to capillaries.

preserve in glycerine. [illegible] may be stained with logwood and mounted in [illegible].

8. (L.) Notice the [illegible] of the bronchi and large vessels in order to recognise them again under the high power. (H.) [illegible] epithelium of air vesicles and the fibrous tissue around them. T. S. bronchus showing epithelium, glands, muscle, cartilage. T. S. pulmonary artery and vein. The wall of the vein is thinner than that of the artery. Adenoid tissue may in some sections be seen around the large blood vessels and in the bronchial mucous membrane. Examine a prepara-tion of lung with blood vessels injected.

22. Alimentary Canal.

D. 1. V. S. circumvallate papilla and tongue of cat. Examine and pre-serve in glycerine (L.) Papillae, muscle, (H.) Epithelium, L. S. and V. S. striped muscle, T. S. and perhaps V. S. vessels, fibrous tissue. (Sections may be stained with carmine or logwood and mounted in dammar.)

Examine (H.) V. S. gustatory disc of rabbit's tongue for taste bulbs. This may be made from a tongue hardened in picrate alcohol. Stain with logwood and mount in dammar.

Examine (L.) V. S. [illegible] [illegible] papillae. (H.) V. S. [illegible] [illegible].

D. 2. V. S. fundus and stomach of cat. This is best made by freezing. Preserve in glycerine. (L.) Relations of mucosa, submucosa, and muscular coats. (H.) Folli-cles, muscularis mucosa, sub-mucosa. Blood vessels, L. S. and T. S. unstriped muscle.

The various structures may also be [illegible] seen in a stomach, macerated for [illegible] in Müller's fluid (§ 7). This is well suited for the demonstration of the cells in the peptic follicles. In a very thin V. S. [illegible]

cells may be seen at the patent extremity, while two kinds of cells are seen in the cæcal portion of the tube, the so-called "peptic cells" placed externally, and the continuation of the columnar cells internal to these. In the cæcal part of the follicle the columnar cells are often much shortened and irregular.

D 3. T. S. Small intestine of cat hardened in chromic and bichromate fluid (§ 7). Preserve in glycerine. (L.) Mucous, sub-mucous, and muscular coats. (H.) Columnar epithelium of villi, and Lieberkühn's follicles, muscularis mucosa.

D 4. T. S. Large intestine of cat hardened in chromic acid (§ 7). Preserve in glycerine. (L.) Same as above. (H.) Lieberkühn's follicles.

Sections of stomach and intestine, if carminised, may be mounted in dammar.

Examine (L.) V. S. injected stomach. T. S. injected small and large intestine. Inner aspect of small intestine injected. T. S. duodenum carminised showing Brunner's glands. T. S. small intestine carminised showing Peyer's follicles; and V. S. small intestine showing valvulæ conniventes.

(H.) Contents of Peyer's follicle, seen in a T. S. or V. S. carminised small intestine with blood vessels injected.

60. *Liver*.

D 1 (H.) Cells of fresh cat or rabbit's liver. Examine in salt solution.

D 2 (H.) Cells of liver of ox. These are usually fatty.

D 3. Thin section of liver of cat hardened in Müller's fluid and spirit. Make the section across some of the larger vessels. The sections are best made by freezing. Preserve in glycerine. (L.) Lobules. (H.) Appearances and relations of cells one to another and to capillaries

capsule of Glisson, T. S. portal vein, one of the larger bile ducts, and hepatic artery.

D 4 (L.) Section of liver of rabbit or cat injected from portal vein, and hardened in meth. sp. Preserve in glycerine or dammar.

Examine liver of rabbit (H.) with bile capillaries injected with soluble Prussian blue, and blood capillaries injected with carmine and gelatine, preserved in dammar. (L.) Liver with hepatic artery and venous system injected.

61. *Kidney.*

D 1. V. S. Malpighian pyramid of dog or pig's kidney, previously hardened in Müller's fluid and spirit. Examine and preserve in glycerine. The section is best made by freezing. It can also be tolerably well made with Valentin's knife. (L.) Arrangements of tubules in cortex and medulla, pyramids of Ferrein, Malpighian bodies. (H.) Epithelium of convoluted and straight tubules, Malpighian bodies and capsule, and larger blood-vessels.

D 2 (H.) Scrape the cut surface of cortex of fresh kidney of sheep to get isolated glomeruli, epithelium, and basement membranes. Examine in salt solution, and then add acetic acid, and notice effect on glomerulus.

D 3 (H.) T. S. Malpighian pyramid of Müller's fluid kidney near its apex. Examine and preserve in glycerine. (The section is best made from a frozen kidney, but may also be made with a Valentin's knife.) Study connective tissue matrix, and T. S. tubules.

D 4 (L. and H.) V. S. Malpighian pyramid of injected kidney. See afferent and efferent vessel of glomerulus and the vasa recta. Mount in glycerine or dammar.

62. *Urinary deposits.* (H.) The various forms of these are studied in the chemical division of the class of Practical Physiology. It is convenient however to study here the methods of

examining them, in order that the student may prepare specimens similar to those which he will afterwards be shown.

[illegible] and *Examination*.—Allow the urine to deposit in a conical glass and remove, with the aid of a long pipette, a portion of the deposit for microscopical examination, or remove the supernatant fluid with a syphon in order that the deposit may not be diffused through the supernatant fluid by the disturbing influence of the pipette. For the ordinary examination of the deposit it is generally convenient to place a drop of the urine on a slide with a shallow cell. The best cell for this purpose consists of a rounded shallow cavity ground in the centre of the surface of the slide. A cell made of a thin ring of Dammar varnish, allowed to dry, also answers the purpose. The cell prevents the diffusion of the deposit under the weight of the cover-glass. If Hartnack's No. 7 objective and No. 3 ocular be used, shorten the tube fully, as the power is a little too high with the tube elongated, that is to say, the lens requires, in the latter case, to come so near the cover-glass that the whole depth of the cell is not visible.

Mounting.—The deposit should be washed at least twice with the fluid in which it is to be mounted, or in rectified spirit in the case of such deposits as uric acid, etc., when they are to be mounted in dammar or Canada balsam. For this purpose allow the urine to deposit. Remove the supernatant fluid with a syphon or decant it; pour on the preservative fluid; allow deposit to form; remove fluid with a syphon or a pipette, again pour on preservative fluid, and keep the mixture until needed. When deposits are to be mounted in fluid media they should always be placed in a shallow dammar or balsam cell, made by painting a ring of fluid dammar, or Canada balsam, on a slide. With the aid of a pipette place a particle of the deposit in such a cell, add a drop of the preservative fluid, and seal up.

[illegible] *Crystals*.—Mount in weak spirit or glycerine solution (see [illegible]).

[illegible] *Casts*.—Mount in dilute acetic acid (ordinary pyroligneous acid 1 part, water 20 parts).

mounting them, in order that the student may prepare specimens similar to those which he will afterwards be shown.

Collection and Examination. Allow the urine to deposit in a conical glass, and remove, with the aid of a long pipette, a portion of the sediment for microscopical examination, or remove the supernatant fluid with a syphon, in order that the deposit may not be diffused through the supernatant fluid by the disturbing influence of the pipette. For the ordinary examination of the deposit it is generally convenient to place a drop of the urine on a slide with a shallow cell. The best cell for this purpose consists of a rounded shallow cavity ground in the centre of the surface of the slide. A cell made of a thin ring of dammar varnish, allowed to dry, also answers the purpose. The cell prevents the diffusion of the deposit under the weight of the cover-glass. If Hartnack's No. 7 objective and No. 3 ocular be used, shorten the tube fully, as the power is a little too high with the tube elongated, that is to say, the lens requires, in the latter case, to come so near the cover-glass that the whole depth of the cell is not visible.

Mounting. The deposit should be washed at least twice with the fluid in which it is to be mounted, or in rectified spirit in the case of such deposits as uric acid, etc., when they are to be mounted in dammar or Canada balsam. For this purpose allow the urine to deposit. Remove the supernatant fluid with a syphon or decant it; pour on the preservative fluid; allow deposit to form; remove fluid with a syphon or a pipette, again pour on preservative fluid, and keep the mixture until wanted. When deposits are to be mounted in fluid media they should always be placed in a shallow dammar or balsam cell, made by painting a ring of fluid dammar, or Canada balsam, on a slide. With the aid of a pipette place a particle of the deposit in such a cell, add a drop of the preservative fluid, and seal up.

a. Urates. Mount in weak spirit or glycerine solution (see *d*).

b. Cystine. Mount in dilute acetic acid (ordinary pyroligneous acid 1 part, water 20 parts).

c. Triple phosphate. Mount in strong ammonia 1 part, water 6 parts.

d. Uric acid and oxalate of lime. Mount in *glycerine and camphor water*, equal parts (camphor water is thus prepared: wrap 3 grammes of camphor in a piece of muslin, place it in a bottle containing 1500CC of water; shake repeatedly. The fluid is ready for use in three or four days). Or they may be mounted in glycerine jelly as follows. Wash the deposit in weak spirit. Heat the glycerine jelly in a water bath until it becomes fluid. Place a drop on a warm slide, add the urinary deposit, and after a few seconds apply a warm cover-glass. When the jelly is cold, scrape it away from the edge of the slide, and seal up with dammar varnish, to prevent the hygroscopic jelly from attracting moisture from the air. As the jelly answers admirably, the use of Canada balsam or dammar is unnecessary; and this is fortunate, for the use of these media in this connection is attended with considerable difficulty.

e. Casts and epithelium. Pus and mucus may be preserved in naphtha and creosote fluid prepared as follows,—(Beale) A. Creosote 35CC. B. Wood naphtha, 28CC. C. Precipitated chalk 130 grammes. D. Water 600CC. Mix thoroughly A. and B. and C. in a mortar, very gradually add D., keeping the mixture in motion with the pestle all the while. Place the whole together with a few pieces of camphor in a lightly covered vessel for two or three weeks, occasionally shaking or stirring the mixture, then filter, and preserve in corked or stoppered bottles (Beale).

63. *Lymphatics.* (H.) Examine (*a*) a silvered preparation of the septum cysternæ lymphaticæ magnæ of the frog, preserved in glycerine, showing the stomata, or apertures of communication between the cavity of the peritoneum and that of the lymph sac. (*b*) A silvered preparation of the centrum tendineum of the rabbit, preserved in glycerine, showing lymphatic vessels with their epithelial lining. (*c*) A silvered preparation of the mesentery of the rabbit preserved in glycerine, showing lymphatics stained.

Study the method of staining (§ 111), and the puncture method of injecting lymphatics (§ 116).

64. *Lymphatic gland.* (H.) Examine the section previously prepared. Study the relations and appearances of the trabeculæ, lymph paths, and glandular tissue.

Excellent sections of lymphatic gland may be obtained by freezing a gland of the dog or cat, after hardening for a few days in 1-6th per cent. chromic acid.

65. *Spleen.*

Examine, without the microscope, the cut surface of fresh spleen of ox, to see pulp and splenic corpuscles; then scrape the pulp away, in order to see the trabeculæ.

Section of chromic acid spleen of cat; mount in glycerine. (L.) Trabeculæ, capsule, splenic corpuscles. (H.) Structure of trabeculæ, splenic corpuscles and pulp.

Sections of spleen tinged with carmine may be mounted in dammar.

66. *Thyroid gland.* (L. and H.) Examine sections of human thyroid gland injected and uninjected. The uninjected gland is best hardened in Müller's fluid for a month, and then in meth. sp. for a fortnight. Preserve in glycerine, or stain with carmine or logwood, and mount in dammar. The injected gland may be hardened in $\frac{1}{4}$ per cent. chromic acid for a fortnight, and then cut by the freezing method, stained with logwood or carmine, and mounted in dammar.

67. *Suprarenal Capsule* (L. and H.)

Examine V. S. suprarenal capsule prepared as follows:—Harden in Müller's fluid for a month, and then in methylated spirit for a fortnight. Mount the sections in glycerine or dammar.

68. *Skin and Epidermic Structures.*

D 1. V. S. skin of palmar surface of finger hardened in chromic acid. Preserve in glycerine, or stain with car-

mine or logwood, and mount in dammar. (L.) Horny layer and rete mucosum of epidermis, cutis vera, papillæ, possibly sweat glands. (H.) Epidermis, papillæ, touch (Wagner's) corpuscles, fibrous tissue, vessels, fat cells, possibly nerves and Pacinian corpuscles.

Excellent preparations of the skin may be made by the following method devised by my assistant, Dr. William Stirling:—Mix 1CC pure hydrochloric acid with 500CC water at 38° C. (100° F.), and add 1 gramme pepsine.[1] After keeping the mixture at 38° C. for three hours, shake it thoroughly. Stretch a piece of skin of man or dog, as fresh as possible, over the mouth of a glass dialysing jar, and tie it firmly round the jar to keep it stretched. Digest the skin in the above fluid at 38° C. for a period varying from two to eight hours, according to the size and age of the skin. Young skin digests more quickly than that which is old. It is advantageous to use only about 100CC of the digestive fluid at one time, and to change the fluid every second hour if the piece of skin be large, in order to remove the peptones, and thereby facilitate the digestive process. After partial digestion, place the skin in water for twenty-four hours. In this it becomes swollen and transparent. It can then be hardened in the ordinary fluids, and stained with logwood or carmine. By the above process the white fibrous tissue swells up and becomes extremely transparent, thus permitting of a clear view of the other tissues.

Examine (L.) V. S. skin of human heel for sudoriparous glands. (L.) V. S. skin from back of sheep's neck for sebaceous glands. (L.) V. S. skin of dog, showing the sudoriparous glands opening into the hair follicles (Wm. Stirling). (L. and H.) V. S. skin of dog for erector muscle of hair follicle. (H.) V. S. skin of finger for tactile (Wagner's) corpuscles. (L. and H.) Pacini's corpuscles.

[1] The *pepsina porci*, prepared by Bullock and Reynolds, Hanover Street, Hanover Square, London, should be employed, if the student does not desire to prepare it from the stomach himself.

Pacinian corpuscles may be obtained by making a V. S. of the pad of the cat's foot, hardened in chromic acid. Stain with carmine or logwood, and mount in dammar. They may, however, be found with far greater facilities in the mesentery of the cat, where they are evident to the unaided eye as small, clear, oval bodies. They are best studied in the fresh condition, being difficult to preserve. Glycerine is a tolerably good preservative medium.

D 3 (H.) Human hair.

D 4 (H.) Hair of sheep.

D 5 (H.) Hair of rabbit.

D 6 (H.) Scales of moth or butterfly.

Mount preparations 3, 4, and 5 in dammar.

Examine (H.) T. S. and V. S. human scalp, stained with carmine or logwood, for the contents of hair follicles.

92. *Eye.*

D 1. V. S. chromic acid cornea of cat. Preserve in glycerine. (H.) Anterior epithelium, and the dense fibrous band corresponding to Bowman's membrane in the human eye. Posterior epithelium and the elastic lamina (membrane of Descemet). Fibrous tissue and corneal corpuscles.

Examine cornea of guinea-pig, showing nerves and corneal corpuscles stained with gold. (See § 112.)

D 2. (H.) Fibres of crystalline lens of ox, cat, or dog, boiled for ten minutes in dilute sulphuric acid (1 per cent.). Tease and preserve in glycerine or potassium acetate.

Examine (H.) T. S. fibres of lens, hardened in Müller's fluid and spirit, and preserved as in D 2.

D 3. (H.) Hexagonal pigment cells from eye of cat or dog, hardened in Müller's fluid or chromic acid. Preserve as in D 2.

D 4 (H.) Retina hardened in chromic acid and spirit. Freeze and make V. S. of retina alone, or of all the tunics of the

Pacini's corpuscles may be obtained by making a V. S. of the pad of the cat's foot, hardened in chromic acid. Stain with carmine or logwood, and mount in dammar. They may, however, be found with far greater readiness in the mesentery of the cat, where they are evident to the unaided eye as small, clear, oval, bead-like bodies. They are best studied in the fresh condition, being difficult to preserve. Glycerine is a tolerably good preservative medium.

D 3 (H.) Human hair.

D 4 (H.) Hair of sheep.

D 5 (H.) Hair of rabbit.

D 6 (H.) Feathers of moth or butterfly.

Mount preparations 3, 4, and 5 in dammar.

Examine (H.) V. S. and T. S. human scalp, stained with carmine or logwood, for the contents of hair follicles.

69. *Eye.*

D 1. V. S. chromic acid cornea of cat. Preserve in glycerine. (H.) Anterior epithelium, and the dense fibrous band corresponding to Bowman's membrane in the human eye. Posterior epithelium and the elastic lamina (membrane of Descemet). Fibrous tissue and corneal corpuscles.

Examine cornea of guinea-pig, showing nerves and corneal corpuscles stained with gold. (See § 112.)

D 2 (H.) Fibres of crystalline lens of ox, cat, or dog, boiled for ten minutes in dilute sulphuric acid (1 per cent.). Tease and preserve in glycerine or potassium acetate.

Examine (H.) T. S. fibres of lens, hardened in Müller's fluid and spirit, and preserved as in D 2.

D 3 (H.) Hexagonal pigment cells from eye of cat or dog, hardened in Müller's fluid or chromic acid. Preserve as in D 2.

D 4 (H). Retina hardened in chromic acid and spirit. Freeze, and make V. S. of retina alone, or of all the tunics of the

eye. Preserve in glycerine, or carminise and mount in dammar. Study—1. Inner limiting membrane and fibres of Müller. 2. Fibrous layer (fibres of optic nerve). 3. Cellular layer (nerve cells). 4. Inner granular layer. 5. Inner nuclear layer. 6. Outer granular layer. 7. Outer nuclear layer. 8. Outer limiting membrane (it is difficult to see this). 9. Jacob's membrane: the rods are readily seen, the cones are seen with difficulty.

D 5 (H.) Retina of frog, hardened in $\frac{1}{4}$ per cent. osmic acid for 36 hours, washed in distilled water for 24 hours. Cut with scissors or by freezing. (This method of preparation is well adapted for showing outer and inner segments of rods, outer limiting membrane, etc.) Preserve in potass. acet.

Examine preparations showing (L.) V. S. optic nerve entering eyeball; V. S. ciliary muscle, iris, and sclerotic: inner aspect of injected ciliary processes. (H.) Branched pigment cells of choroid. (The vitreous has already been examined, § 50.)

70. *Cochlea.*

Examine V. S. cochlea. (L.) Observe the modiolus, lamina spiralis ossea, limbus laminæ spiralis, basilar membrane, ligamentum spirale, rods of Corti, membrane of Reissner, scala vestibuli, scala media, and scala tympani. (H.) Observe inner and outer rods of Corti, cells of Corti, nerves, membrana reticularis, membrana tectoria, together with the parts before mentioned. Examine also (H.) a superficial view of the heads of the rods of Corti and the membrana reticularis.

The following will be found an excellent method of preparing the cochlea, devised by my former assistant, Dr. Urban Pritchard, to whom I gave this subject for a graduation thesis (*Edinburgh Prize Thesis*, 1871):—

The structure of Corti's organ may be investigated in the fresh

condition, but, except in very young animals, little can be learned, by such a mode of examination, regarding the relations of the various soft tissues. For this purpose, Corti's organ must be hardened, its bony surroundings softened, and the cochlea sliced vertically. It is of the greatest importance that the organ be placed in preservative fluid as soon after death as possible. If the human cochlea be prepared, it should be taken not later than twelve hours after death; and in order to preserve it as thoroughly as possible, it is well, immediately after death, to inject methylated spirit into the tympanic cavity with an ordinary subcutaneous syringe, thrust through the membrana tympani.

Osmic acid and chromic acid are the best hardening agents. The latter is to be preferred when sections are to be made of the surrounding bone. The best chromic fluid is the mixture of chromic acid and spirit, devised by Dr. Pritchard (§ 4, Solution III.). This fluid is used in the manner indicated in § 15.

In the case of fœtal cochleæ, the bone may be softened by chromic acid alone, but for older cochleæ a stronger acid should be added. German authors recommend for this purpose hydrochloric acid diluted with water ten times, but far better results are obtained by using the bone-softening fluid already mentioned. (See § 4, Solution V.). A large quantity of this fluid is necessary, and, in the case of the larger cochleæ, they should be kept in motion by some such means as an ordinary meat-jack. With the aid of this device, even a large cochlea may be softened in a few days.

When sections are to be made of the softened cochlea, the lamina spiralis and organ of Corti require support to maintain them *in situ*. Melted wax and a hot solution of gelatine have both been used for this purpose with some success, but Stricker's method of imbedding in gum is decidedly preferable. Remove the cochlea from the softening fluid, and place it in a small cone of bibulous paper, containing a strong solution of gum arabic (§ 85), for four or five hours, then immerse the cone in meth.

sp. for forty-eight hours or so, until the gum has become hard and tough; then remove the superfluous gum, and imbed the whole in the ordinary paraffin mixture (§ 102) in a microtome, with the modiolus of the cochlea placed at right angles to the axis of the tube of the microtome, so that Corti's organ will be sliced vertically. Or transfer the cochlea from the softening fluid to a solution of gum placed in the well of the freezing microtome, and, after letting it soak in the gum for some hours, freeze and make sections. After either the one or other mode of section, the slices should be immediately placed in meth. sp.

The sections may be stained with magenta, logwood, gold, silver, and carmine. The last two yield the best results. Carmine may be successfully used in the following way. Mix equal parts of water, methylated spirit, and Beale's carmine fluid, with all the ammonia retained (see § 107). (The spirit is added to the mixture in order to prevent the rapid swelling up and solution of the supporting gum). Transfer the sections from meth. sp. to the above carmine fluid, and let them remain in this for about twelve hours, until a deep diffuse staining is obtained. Then pour away the carmine and wash the sections very gently with weak spirit (meth. sp. 1 part, distilled water 3 parts). Next cover them with an acid washing fluid, consisting of proof spirit (rectified spirit 5 parts, water 3 parts), containing 1 part hydrochloric acid in 100 parts proof spirit. After washing in this fluid for six or eight hours, until all diffuse staining is removed, cautiously pour off the fluid and preserve the sections in ordinary meth. sp. until required. Silver staining is useful for the fresh tissue. It reveals the outlines of the epithelium of the cochlea. The process of staining is the ordinary silver method (§ 111).

Great care is necessary in mounting the sections lest important parts be injured. If the sections have not been stained, the gum must be carefully removed or softened by weak spirit, but if glycerine jelly be used for mounting, this precaution is unnecessary. The best preservative media are glycerine, glycerine jelly, potassium acetate, and dammar or Canada balsam. The

it is generally resorted to when the sections have been stained. For unstained, and even for stained sections, glycerine jelly is a good preservative medium. In mounting vertical sections of the retina, the preparation should always be placed across the glass slip to facilitate subsequent microscopical study.

V. *Olfactory Membrane.*

Examine (H.) olfactory cells from nose of newt prepared as follows: Macerate the nose for a week in one per cent. potash bichromate. Tease a small portion of the mucous membrane and preserve in glycerine. V. S. Schneiderian membrane of cat or dog, prepared in the same way as the cochlea, and preserved in glycerine or glycerine jelly. Observe epithelium and glands.

VI. *Spinal cord and brain.*

(1.) T. S. spinal cord of cat hardened with chromic acid as directed in § 12. Wash the section in water, and stain by the following method, first adopted because of its rapidity. Place a large drop of ½ per cent. watery solution of palladium chloride on a glass slide, and on another slide a large drop of an ammoniacal solution of carmine (ammonia 1 c.c., carmine 1 gramme. Dissolve with the aid of a gentle heat, add 200 C.C. distilled water, and filter). Allow the section to remain in the palladium for two minutes. Wash it in water, and place it in the carmine fluid for three minutes. Wash in water, and mount in dammar, as described in D 4, § 86. Observe (L.) grey and white matter, nerve roots—if present, fissures. (H.) nerve cells, T. S. nerve fibres, neuroglia, [illegible] processes in the grey matter, and in the substance of the cord, the central canal and its epithelial lining.

With regard to the staining of nerve tissues hardened with chromic acid, it is to be remembered, that the longer the exposure of the tissue to the influence of the acid, the less readily is it stained with carmine; indeed, after a very prolonged action of

ast two are only resorted to when the sections have been stained. For unstained, and even for stained sections, glycerine jelly is a good preservative medium. In mounting vertical sections of the cochlea, the modiolus should always be placed across the glass slip to facilitate subsequent microscopical study.

71. *Schneiderian Membrane.*

Examine (H.) olfactory cells from nose of newt prepared as follows:—Harden the nose for a week in one per cent. potass. bichrom. Tease a small portion of the mucous membrane and preserve in glycerine. V. S. Schneiderian membrane of cat or dog, prepared in the same way as the cochlea, and preserved in glycerine or glycerine jelly. Observe epithelium and glands.

72. *Spinal cord and brain.*

D 1. T. S. spinal cord of cat hardened with chromic acid as directed in § 12. Wash the section in water, and carminise by the following method, here adopted because of its rapidity. Place a large drop of ¼ per cent. watery solution of palladium chloride on a glass slide, and on another slide a large drop of an ammoniacal solution of carmine (ammonia 1CC, carmine 1 gramme. Dissolve with the aid of a gentle heat, add 23CC distilled water, and filter). Allow the section to remain in the palladium for two minutes. Wash it in water, and place it in the carmine fluid for three minutes. Wash in water, and mount in dammar, as described in D 4, § 56. Observe (L.) grey and white matter, nerve roots—if present, fissures. (H.) nerve cells, T. S. nerve fibres, neuroglia, bloodvessels in the pia mater, and in the substance of the cord, the central canal and its epithelial lining.

With regard to the staining of nerve tissue, hardened with chromic acid, it is to be remembered, that the longer the exposure of the tissue to the influence of the acid, the less readily is it stained with carmine; indeed, after a very prolonged action of

chromic acid, carmine has scarcely any effect unless it be preceded by the action of palladium chloride (Merkel). In any case, the method above recommended yields the most rapid results. *The staining is most perfect, however, when the tissue has not been over-hardened, and when it is slowly tinged by a dilute solution of carmine alone.* A dilution of Beale's carmine fluid, with all the ammonia retained, answers the purpose admirably (see § 107).

Examine (L. and H.) T. S. spinal cord unstained. V. S. spinal cord carminised, showing a nerve root crossing a white column. (L.) T. S. injected spinal cord. (L. and H.) T. S. medulla oblongata.

D 2. V. S. cerebellum hardened in chromic acid, carminise and mount in dammar. Observe (L.) the relations of the various layers, and the general appearance of the convolutions, (H.) the flask-shaped cells at the inner part of the outer grey layer, the external processes of these cells, their branchings and the neuroglia with its nuclei amidst which the branches disappear; cells of the inner grey layer, nerve fibres and nuclei in the white matter.

D 3. V. S. convolutions from vertex of human cerebrum, hardened in chromic acid and carminised. Mount in dammar. (H.) Observe the pyramidal cells. The cells are seen to have a pyramidal shape only when the section is vertical as regards the cells.

Examine (L.) V. S. injected cerebral convolutions.

73. *Generative organs.*

D 1. (H.) With a scalpel or scissors remove a few tubuli from the testis of cat or dog, hardened in Müller's fluid. Tease and mount in glycerine. A complete section of the organ may be made by freezing.

Examine (H.) spermatozoids. (L. and H.) Section of ovary of cat showing Graafian follicles and their contents, prepared as recommended in § 10. The ovary should remain in meth. sp. for forty-eight hours after removal

in Müller's fluid. The hardening should then be completed by immersion in absolute alcohol for forty-eight hours or so. Imbed in paraffin and make sections. The sections may be mounted unstained in glycerine, or they may be stained with carmine or logwood, and mounted in balsam.

B. [illegible] T. S. [illegible] of rat hardened in chromic acid. Preserve in glycerine. Study the uterine glands.

Development.

Examine a series of preparations of the embryo chick, hardened with chromic acid, stained with carmine, and mounted in damar. A description of the mode of preparing the chick is quite beyond the limits of these notes. The student will find a full account of the subject in the *Elements of Embryology*, by Foster and Balfour. (London: Macmillan. 1874.)

GENERAL CONSIDERATIONS REGARDING HISTOLOGICAL METHODS.

RELATION OF THE TISSUES TO EXAMINATION MEDIA.

172. When it is not desirable to examine the textures in their living condition, they should be placed as fresh as possible in indifferent media. At death, the optical characters of many tissues undergo rapid changes. Generally speaking, transparency diminishes.

Some tissues, such as hair, teeth, and bone, may be examined in air. This, however, is generally objectionable; for, owing to the great disparity between the refractive powers of the air and the tissues, the outlines are too broad and too dark.

Some fluids alter the normal characters of the textures, e.g., the blood corpuscles swell in water, while in a concentrated solution of sugar or salt they shrivel. Acetic acid causes the

from Müller's fluid. The hardening should then be completed by immersion in absolute alcohol for forty-eight hours or so. Imbed in paraffin, and make sections. The sections may be mounted unstained in glycerine, or they may be stained with carmine or logwood, and mounted in dammar.

D 2. (L. and H.) T. S. uterus of cat hardened in chromic acid. Preserve in glycerine. Study the uterine glands.

74. *Development.*

Examine a series of preparations of the embryo chick, hardened with chromic acid, stained with carmine, and mounted in dammar. A description of the mode of preparing the chick is quite beyond the limits of these *notes.* The student will find a full account of this subject in the *Elements of Embryology,* by Foster and Balfour. (*London:* Macmillan. 1874.)

GENERAL CONSIDERATIONS REGARDING HISTOLOGICAL METHODS.

RELATIONS OF THE TISSUES TO SURROUNDING MEDIA.

75. When it is not desirable to examine the textures in their living condition, they should be placed as fresh as possible in preservative media. At death, the optical characters of many tissues undergo rapid change. Generally speaking, transparency diminishes.

Some tissues, such as hair, tooth, and bone, may be examined in air. This, however, is generally objectionable; for, owing to the great disparity between the refractive powers of the air and the tissues, the outlines are too broad and too dark.

Some fluids alter the normal characters of the textures, *e.g.*, the blood corpuscles swell in water, while in a concentrated solution of sugar or salt they shrivel. Acetic acid causes the

fibrils of white fibrous tissue to assume the appearance of a transparent jelly; chloroform dissolves the medullary substance of a white nerve fibre, while water coagulates it. In selecting a medium in which to place a tissue, the physico-chemical nature of the tissue and the medium must therefore be taken into account. The points of greatest importance in this connection are:—*a.* The effect of the medium upon the vitality of the tissue. *b.* The hardness or softness of the tissue. *c.* The solubility of the tissue in the medium. *d.* The chemical relations between the tissue and the medium. *e.* The transparency of the tissue. *f.* The refractive index of the medium.

Nonpreservative media employed in the examination of the tissues.

76. To study the *normal* characters of *living* tissues, examine them in the fluids which bathe them during life, or in fluids which do not alter their vital properties. Blood, salivary, mucus, and pus corpuscles are examined in their own fluids.

77. *Serous fluids.* The fluid which bathes most tissues is lymph. *Blood serum*—seeing that it has a composition resembling that of lymph, is sometimes employed for moistening living tissues; but the blood corpuscles which it contains are an inconvenience. *Aqueous humour* is the most available serous fluid when only a small quantity is desired. It can always be readily obtained from the eye of a sheep or ox.

78. Serous fluids are also used for the examination of various delicate tissues, even when these are not living,—when they are required in considerable quantity, amniotic fluid, pericardial fluid, iodised serum, or dilute albumin, are employed. These may be preserved for a considerable time by placing them, together with a piece of camphor, in glass vessels, which have been thoroughly cleansed by *boiling* water, to destroy bacterial germs or other causes of putrefaction. *Iodised serum* is sometimes employed for macerating nerve cells and white nerve fibres, in order to show the "primitive nerve fibrils" (Schultze).

fluids of which there is neither the appearance of a transparent jelly; chloroform dissolves the medullary substance of a white nerve fibre, while water coagulates it. In selecting a medium in which to place a tissue, the physico-chemical nature of the tissue and the medium must therefore be taken into account. The points of greatest importance in this connection are—*a.* The effect of the medium upon the vitality of the tissue. *b.* The hardness or softness of the tissue. *c.* The solubility of the tissue in the medium. *d.* The chemical relations between the tissue and the medium. *e.* The transparency of the tissue. *f.* The refractive index of the medium.

Fluids and other media employed in the examination of the tissues.

76. To study the normal characters of living tissues, examine them in the fluids which bathe them during life or in fluids which do not alter their vital properties. Blood, salivary, mucus, and pus corpuscles are examined in their own fluids.

77. *Serous fluids.* The fluid which bathes most tissues is lymph. Blood serum—owing to the fact that it has a composition resembling that of lymph, is sometimes employed for moistening living tissues; but the blood corpuscles which it contains are an inconvenience. *Aqueous humour* is the most available serous fluid when only a small quantity is desired. It can also be readily obtained from the eye of a sheep or ox.

78. Serous fluids are also used for the examination of various delicate tissues, even when these are not living. When they are required in considerable quantity amniotic fluid, pericardial fluid, iodized serum, or dilute albumin, are employed. These may be preserved for a considerable time by placing them, together with a piece of camphor, in glass vessels, which have been thoroughly cleansed by boiling water, to destroy bacterial germs or other causes of putrefaction. *Iodized serum* is sometimes employed for macerating nerve cells and white fibrous tissue, in order to show the "primitive nerve fibrils" (fibrillae).

It is thus prepared : add 1 part tincture of iodine to 100 parts *fresh* amniotic fluid ; filter, and place a small piece of camphor in the fluid.

79. *Dilute albumin* may also be employed for the maceration of delicate tissues. It should, however, only be had recourse to when serous fluids cannot be obtained. It is thus prepared :— dissolve 2 grammes dried sodium chloride in 250CC water; add 28 grammes egg albumin, 2·3CC tincture of iodine, and filter. Place a small piece of camphor in the fluid (Frey).

80. *Salt solution.* This is a $\frac{3}{4}$ per cent. solution of common salt, prepared thus :—heat sodium chloride to redness, cool it over sulphuric acid, and dissolve 7·5 grammes in 1000CC distilled water.

The serous fluids, dilute albumin, and salt solution, are sometimes termed "neutral" or "indifferent" fluids, on the supposition that they do not alter the normal appearances of the tissues. *This, however, is only true of the serous fluids.*

81. *Water* is only employed for dense tissues, such as elastic tissue, hair, and epidermis. It destroys the normal characters of soft tissues.

The above-mentioned fluids are not adapted for the permanent preservation of the tissues.

The preservative fluids, those which clarify, and those which stain the tissues, are also used as media in which to examine them.

Methods of Hardening the Tissues.

Some tissues are too soft, others too hard, to permit of their being cut or dissected ; it is therefore necessary to alter their consistence.

82. *Drying and boiling* were formerly much resorted to, but are now rarely employed. The former may be used for tendon, when transverse sections are wanted merely to show the relations

of the various systems of fibres; moreover, it is sometimes advantageous to *partially* dry a tissue that has been hardened in alcohol before attempting to cut it. Excellent sections of the skin may be obtained in this way. If, however, protoplasm and other soft matters have not been previously hardened they are hopelessly spoilt by desiccation. Boiling is useful for hardening the fibres of the crystalline lens, and also those of muscle, when coarse dissections are to be made of the latter.

83. *Freezing* is a method of much value. With its aid, sections of any desirable thinness may be readily made of all the soft parts of the body in a perfectly fresh condition. Lung, kidney, spleen, lymphatic gland, vitreous humour, brain, &c., may all be removed from the living animal, frozen and sliced in a few minutes.

Freezing is suitable for hardening the brain and spinal cord, *only* when slices of the fresh tissue are desired for the isolation of nerve cells, &c. These organs are best hardened in chromic acid, potassium bichromate, &c., not because they can be more readily cut when hardened by these agents, but for the reason that, when the tissue has been previously hardened in these agents, the slices do not go to pieces when they are manipulated, as they are apt to do when the *unhardened* frozen tissue thaws after freezing. For the same reason, it is advantageous in the case of the kidney, lung, liver, spleen, and such like, to have the process of hardening carried to some extent by Müller's fluid, potassium bichromate, or chromic acid, before freezing them for the purpose of section. The special cases where freezing is of service have been indicated in the preceding demonstrations.

Freezing has been had but little recourse to, owing to the clumsy and inconvenient method hitherto adopted. This has simply consisted in placing the tissue on tinfoil, or in a platinum capsule, and setting it in a freezing mixture. It is very difficult and tedious to freeze any large piece of tissue in this way, and when it is removed from the freezing mixture, for the purpose of

[illegible] of acting to thaw, and thereby gives rise to so much in-
convenience, and is so little satisfactory, that the method of
freezing has been almost neglected. The method is rendered
simple and satisfactory with the aid of the freezing microtome.
When this apparatus becomes generally known, freezing will be
recognised as the best method for obtaining sections of fresh or
[illegible] hardened tissues.

Fig. 1. The Freezing Microtome.[illegible]

As the freezing microtome is a novel instrument, it is here
figured for the assistance of those who may not yet have seen it.
It (Fig. 1) consists of a plate of [illegible] metal ([illegible]), with a circular
opening in its centre ([illegible]). The opening leads into a well ([illegible]),

[illegible] The Microtome here figured is a modification of that originally [illegible]
[illegible] by me in the Journal of Anatomy and Physiology, May 1871. Other
modifications have been proposed by others, but I cannot regard them
[illegible] anything but the reverse of improvements. The apparatus is made
[illegible] Young, surgical instrument maker, North Bridge, Edinburgh,
[illegible] advisable.

section, it begins to thaw, and thereby gives rise to so much inconvenience, and to results so unsatisfactory, that the method of freezing has been almost neglected. The method is rendered simple and satisfactory with the aid of the freezing microtome. When this apparatus becomes generally known, freezing will be resorted to as the best method for obtaining sections of fresh or imperfectly hardened tissue.

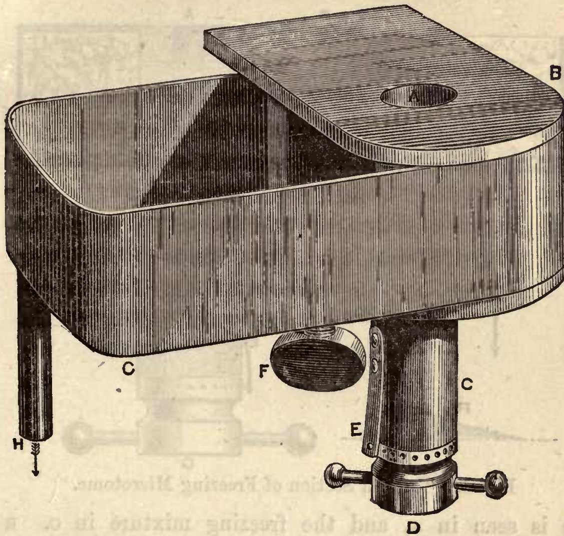

Fig. 4. The Freezing Microtome.[1]

As the freezing microtome is a novel instrument, it is here figured for the assistance of those who may not yet have seen it. It (Fig. 4) consists of a plate of gun metal (B), with a circular opening in its centre (A). The opening leads into a well (E),

[1] The Microtome here figured is a modification of that originally described by me in the *Journal of Anatomy and Physiology*, May 1871. One or two alterations have been proposed by others, but I cannot regard these as anything but the reverse of improvements. The apparatus as manufactured by Young, surgical instrument maker, North Bridge, Edinburgh, answers admirably.

closed inferiorly by a brass plug (K, Fig. 5), capable of being moved up or down by means of a screw (D). The tissue to be frozen is placed in the well, and sections are made by gliding a knife over the top of the well. The thickness of the sections is regulated by an indicator (E). A freezing mixture is placed in the box (C), the water from which flows away by the tube (H). The microtome is clamped to a table by the screw (F). The

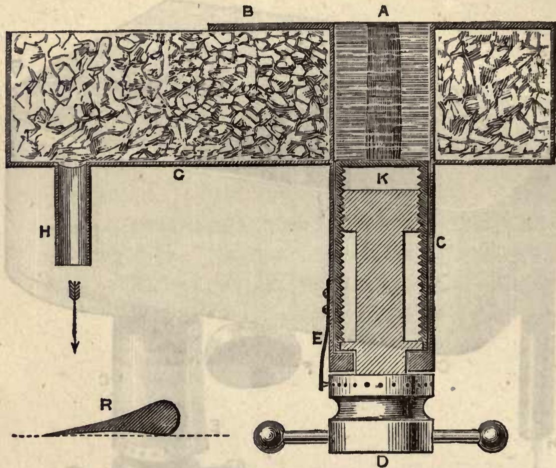

Fig. 5. Vertical Section of Freezing Microtome.

tissue is seen in A, and the freezing mixture in C. R is a section of the knife employed.

The freezing microtome is essentially the well-known microtome devised by Mr. Stirling, Sub-Curator of the Anatomical Museum of the University of Edinburgh, modified for freezing. It serves a double purpose. 1. *For cutting tissues hardened in the ordinary way by chromic acid or spirit*, &c. 2. *For cutting tissues hardened by freezing.* The second method of using the machine will be more readily comprehended after a description of the first.

84. *When the microtome is used in cutting an unfrozen tissue,*

[illegible] into the well a mixture of solid paraffin (five parts), and hog's lard (one part), melted by a gentle heat. The temperature employed should be just sufficient for this purpose and no more, in order that the tissue may be heated as slightly as possible, and also in order that the paraffin may, on cooling, shrink to the smallest [illegible] extent. With a pair of forceps dip the tissue—previously exposed to the air for a short time in order to dry its surface—into the melted paraffin, at once withdraw it, and, when the paraffin film has cooled, replace it permanently in the well of paraffin, close to the margin from which the face of the knife is to be moved in the process of section. The side nearest the operator is most convenient. A mixture of equal parts of bees-wax and sweet oil is much recommended for the same purpose as the paraffin in the above case. Certainly the wax and oil mixture is excellent for imbedding tissues, and it can be cut with ease. But it melts at a higher temperature than paraffin, and, owing to the great thermal expansion, it retracts from the side of the tube of the machine, and so the wax cylinder becomes loose. The only way in which this can be prevented is by heating the machine to a like temperature before introducing the wax. This is tedious; and, inasmuch as it is unnecessary in the case of paraffin, this is to be preferred. Even with this, however, the paraffin cylinder is apt to become a little loose, and to turn round in the machine; hence it is important that there be an eccentric hole in the brass plug (c, fig. 5) with a projecting pin fixed in it. The rotation of the paraffin cylinder is thus prevented.

8th. When the microtome is used *for freezing*, surround the freezing-box with a thick jacket of felt or flannel to prevent the entrance of heat. Unscrew the plug (a, fig. 6), and oil both its exterior and interior, so that it may not become fixed by the freezing. The tissue to be frozen, together with an imbedding fluid, are placed in the hole. If a watery fluid be employed, it becomes crystalline when frozen, and is therefore badly adapted

gauge, adjustable by a brass plug (a). Fig. 5) [illegible] moved up or down by means of a screw (b). The tissue to be frozen is placed in the well, and [illegible] the top of the well. The thickness of the section is regulated by an indicator (c). A freezing mixture is placed in the box (e), the water from which flows away by the tube (f). The microtome is clamped to a table by the screw [illegible].

Fig. 5.—Vertical Section of Freezing Microtome.

tissue is seen in [illegible], and the freezing surface in [illegible]. B is a section of the knife employed.

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54. When the microtome is used in cutting an imbedded tissue,

pour into the well a mixture of solid paraffin (five parts), and hog's lard (one part), melted by a *gentle* heat. The temperature employed should be just sufficient for the purpose and no more, in order that the tissue may be heated as slightly as possible, and also in order that the paraffin may, on cooling, shrink to the smallest possible extent. With a pair of forceps dip the tissue—previously exposed to the air for a short time in order to dry its surface—into the melted paraffin, at once withdraw it, and, when the paraffin crust has cooled, replace it permanently in the well of paraffin, close to the margin from which the face of the knife is to be moved in the process of section. The side nearest the operator is most convenient. A mixture of equal parts of bees-wax and sweet oil is much recommended for the same purpose as the paraffin in the above case. Certainly the wax and oil mixture is excellent for imbedding tissues, and it can be cut with ease. But it melts at a higher temperature than paraffin, and, owing to the great thermal expansion, it retracts from the side of the tube of the machine, and so the wax cylinder becomes loose. The only way in which this can be prevented is by heating the machine to a like temperature before introducing the wax. This is tedious; and, inasmuch as it is unnecessary in the case of paraffin, this is to be preferred. Even with this, however, the paraffin cylinder is apt to become a little loose, and to turn round in the machine : hence it is important that there be an *eccentric* hole in the brass plug (K, fig. 5) with a projecting wooden pin fixed in it. The rotation of the paraffin cylinder is thus prevented.

85. *When the microtome is used for freezing*, surround the freezing box with a thick jacket of felt or flannel to prevent the entrance of heat. Unscrew the plug (K, fig. 5), and oil both its exterior and interior, so that it may not become fixed by the freezing. The tissue to be frozen, together with an imbedding fluid, are placed in the hole. If a watery fluid be employed, it becomes crystalline when frozen, and is therefore badly adapted

to serve as the imbedding agent. This difficulty, however, is entirely overcome by using a solution of gum arabic. *Frozen mucilage can be sliced as readily as a piece of cheese.* The use of gum water for this purpose was suggested to me by my late assistant Dr. Urban Pritchard, and it is a suggestion of much value. The solution of gum is thus made:—Add to 10 oz. of water 120 minims of camphorated spirit, and 5 oz. of picked (*clean*) gum arabic; when the gum has dissolved, strain the fluid through calico or tow, and preserve it for use in a corked bottle. The gum solution should be placed in the microtome well first, and, when a film of ice has formed at the periphery, the tissue should be introduced and held against the advancing ice until it is fixed. In this way the tissue may be secured in any position for the process of section. Lay a piece of thin vulcanised indiarubber upon the microtome plate, so as to cover the well, and prevent the entrance of heat and of salt from the freezing mixture. Secure the indiarubber by a weight. Place in the freezing box small quantities of *finely powdered* ice, and salt alternately; constantly stir them with a glass rod around the well, and keep the tube (H) open to permit of the egress of water from the melting ice. This process is really very simple, and can be fully carried out in from ten to twenty minutes. Of course the period is shorter the colder the surrounding air. It is possible, especially in winter, to have the tissue frozen too hard to permit of its being readily cut. It splinters when it is too hard. This is prevented by discontinuing the further addition of the freezing mixture, or by dropping water or a $\frac{3}{4}$ per cent. salt solution at the ordinary temperature on the surface of the frozen tissue, or by heating the razor slightly.

A number of tissues may be frozen and cut at the same time. If they have been previously immersed in spirit, they must be steeped for a night in a large quantity of water ere they can be frozen. In all cases, where it is possible, it is well to steep the tissues for an hour or two in the solution of gum before freezing them, in order that the gum may thoroughly penetrate the tissue.

to serve as the imbedding agent. This difficulty, however, is entirely overcome by using a solution of gum-arabic. Fresh specimens can be [illegible] on a piece of [illegible]. The use of gum water for this purpose was suggested to me by my late assistant Dr. Urban Pritchard, and it is a suggestion of much value. The solution of gum is thus made:—Add to [illegible] water 120 minims of carbolised spirit, and 5 oz. of picked (clean) gum arabic; when the gum has dissolved, strain the fluid through calico or lint, and preserve it for use in a corked bottle. The gum solution should be placed in the microtome well first, and, when a film of ice has formed at the periphery, the tissue should be introduced and held against the [illegible] ice until it is fixed. In this way the tissue may be secured in any position for the process of section. Lay a piece of thin vulcanised indiarubber upon the microtome plate, so as to cover the well, and prevent the entrance of heat and of salt from the freezing mixture. Secure the indiarubber by a weight. Place in the freezing box small quantities of finely powdered ice and salt alternately; constantly stir them with a glass rod around the well, and keep the tube (a) open to permit of the escape of water from the melting ice. This process is really very simple, and can be fully carried out in from ten to twenty minutes. Of course the period is shorter the colder the surrounding air. It is possible, especially in winter, to have the tissue frozen too hard to permit of its being readily cut. It splinters when it is too hard. This is prevented by discontinuing the further addition of the freezing mixture, or by dropping water or a ½ per cent. salt solution at the ordinary temperature on the surface of the frozen tissue, or by heating the razor slightly.

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A great advantage of the freezing method for delicate organs, such as the retina, trachea, embryo, etc., is that the imbedding agent can be readily removed from the tissue by immersion in water.

86. A razor answers for all ordinary purposes. The blade should always be *hollow* on both surfaces (R, Fig. 5). It is a mistake to employ a flat knife, for it is scarcely possible to keep the surface of the brass table of the machine smooth enough to permit of the knife lying quite flat. The operator should cut *outwards* from himself by drawing the knife obliquely through the tissue, which should be cut at one sweep. This is not possible with a frozen tissue if the ice be too hard. For an unfrozen tissue, imbedded in paraffin, the knife should be wetted with methylated spirit. This should be dropped upon the knife from a funnel with an elastic tube depending from it, clamped by a Mohr's clip. The funnel should be suspended at a convenient height above the machine. The spirit keeps the blade perfectly clean, and it forms a pool upon it, *which enables the section to float over the blade.* In the case of freezing, it is not necessary to wet the knife, for the melting ice readily does so.

87. *Chromic acid* is a much used hardening agent. It is however not well adapted for kidney, liver, lymphatic gland, vitreous humour or crystalline lens, and it is apt to spoil the epithelium of the intestine. One-fifth per cent. watery solution is employed for the embryo, $\frac{1}{4}$ per cent. solution for the spinal cord, stomach, cornea, skin, lung, etc. A mixture of chromic acid and potassium bichromate (see IV., § 4) is used for the brain. Chromic acid solution in weak spirit is used for the retina, cochlea, etc. (see III., § 4).

Chromic acid hardens the tissues in from two days to two months. The tissue should be placed in a small quantity of fluid for the first eighteen hours. This should be changed at the end of this period, and a *large* quantity of fluid substituted. The process of hardening by means of chromic acid appears to be

analogous to the process of *tanning*. The tissue becomes tough, like leather. The chromic acid is removed from the fluid, and possibly forms some compound in the tissue, hence, a large quantity of the chromic solution is necessary. The same object cannot be attained by using a strong solution of the acid, for in such a case an impervious crust forms around the outer part of the tissue, while the centre remains soft and becomes rotten. When the tissues are sufficiently hard, they are removed from the acid and placed in methylated spirit, if it be necessary to preserve them until sections are made. Very delicate tissues that are apt to spoil in the chromic acid, *e.g.* the embryo, should be placed in methylated spirit ere they are sufficiently hard. The spirit completes the process of induration. Spirit removes much of the yellow colour given to the tissues by the chromic acid.

88. *Picric* or *Carbazotic acid,* 1 per cent. watery solution, hardens more rapidly than chromic acid, but the chromic acid is to be preferred, because it does not colour the tissues so much.

89. *Alcohol* has been recommended for gland tissues. They should be first placed in equal parts of methylated spirit and water for a day, then in methylated spirit for a similar period, and finally in absolute alcohol. It is most useful, however, for completing the hardening of tissues, *e.g.* the kidney, intestine, etc., that have been partially hardened in Müller's fluid or chromic acid.

Sections of tissues hardened in alcohol, chromic, or picric acids, generally require to be rendered transparent; for this purpose clove oil, turpentine, or glycerine, are commonly employed, and when clarified, the tissues are mounted in glycerine, dammar, or Canada balsam.

90. *Müller's Fluid* (potassium bichromate 2½ parts, sodium sulphate 1 part, distilled water 100 parts) is used for hardening the *liver, kidney, crystalline lens, stomach, ovary, testis,* etc. It requires from three to six weeks, and the tissues require to be

[illegible] to any pieces of tissue. The [illegible] be [illegible] the [illegible]. The chromic acid [illegible] and [illegible] forms some compound in the tissue, hence a [illegible] quantity of the chromic solution is necessary. [illegible] cannot be obtained by using a strong solution of chromic acid, for in such a case an impervious crust forms around the outer part of the tissue, while the centre remains soft and becomes rotten. When the tissues are sufficiently hard, they are removed from the acid and placed in methylated spirit, if it be necessary to preserve them until sections are made. Very delicate tissues that are apt to swell in the chromic acid, e.g. the embryo, should be placed in methylated spirit ere they are still [illegible] hard. The spirit completes the process of hardening. Spirit removes much of the yellow colour given to the tissues by the chromic acid.

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59. *Müller's Fluid* (potassium bichromate 2½ parts, sodium sulphate 1 part, distilled water 100 parts) is used for hardening the eye, kidney, spinal cord, brain, muscle, artery, heart, etc. It requires from three to six weeks, and the tissues require to be

afterwards frozen or placed in methylated spirit, or it may be, in absolute alcohol, to render them sufficiently hard. Mount the unstained tissues in glycerine, or stain them and mount in dammar.

91. *Osmic Acid* ($\frac{1}{10}$th to 1 per cent. in distilled water) for hardening the retina, epithelium, and white nerve fibres. It hardens tissues in from twelve to forty-eight hours. When hard, place them in distilled water for a day or two. Mount the tissues in a saturated solution of potassium acetate (Schultze).

Methods of Softening the Tissues.

92. *Removal of calcareous matter.* Chromic and nitric acid both harden albuminous tissues, but soften those which owe their hardness to the presence of calcareous salts. Bone and tooth may be softened in water containing a-half per cent. chromic, and one per cent. nitric acid. (See V., § 4, and § 13.) This fluid hardens the protoplasts of the tissue while it removes calcareous matter. The results of its use are extremely satisfactory.

93. *Softening of connective substances.* It is sometimes desirable to soften or dissolve various connective substances, such as white fibrous tissue, the cement which connects the fibres of the lens and the fibrils of striped muscle. White fibrous tissue may be softened: *a.* By boiling in water. *b.* By maceration, at a temperature of 116° Fah., in dilute sulphuric acid ($\frac{1}{10}$th per cent.); these methods are useful for coarse dissections of muscle. *c.* By maceration in glycerine (1 oz.), and glacial acetic acid (5 drops) (Beale). This is valuable for enabling us to trace fine nerves in many tissues. The white fibrous tissue is rendered very transparent and gelatinous, while medullated nerve fibres are rendered *granular*. In this way the nerve elements are readily recognised. This mode of tracing fine nerves has now been largely superseded by the method of staining them with perchloride of gold. *d.* By prolonged

maceration in a 1 per cent. solution of potassium bichromate or Müller's fluid. This permits of the isolation of the gland elements of kidney, intestine, stomach, etc., when specimens are to be prepared by teasing. The cement between the fibrils of the nerve axial cylinder may be dissolved: *e.* By maceration in the above acid glycerine. *f.* By maceration in iodised serum. The cement joining the fibrils in striated muscle may be dissolved by prolonged maceration in $\frac{1}{4}$ per cent. chromic acid. The cement connecting the fibres of the lens may be dissolved by boiling for five or ten minutes in dilute sulphuric acid (1 per cent.).

94. *Methods of separating tissue elements.* *a.* By dissection with needles. Delicate textures and organs should be dissected in some fluid, such as water, or $\frac{3}{4}$ per cent. salt solution. *b.* By gently pressing a small piece of tissue under a cover-glass, after placing the tissue on a slide in the usual way.

Methods of Cutting the Tissues.

95. Some tissues are too soft, while others are too hard, to permit of satisfactory section. When such is the case, harden or soften them by the methods above described.

96. *Saw.* Sections of unsoftened bone and tooth are made with a fine saw. The sections are then ground sufficiently thin upon a hone.

97. *Scissors* are often useful for removing a thin portion of unhardened tissue.

98. *Valentin's Knife* has two parallel blades, the distance between which can be adjusted by means of a screw. The thickness of the slice depends on the distance between the blades. This knife is useful for slicing soft organs, such as the liver, kidney, and lung, in their fresh condition. Sections of fresh organs made with this knife are, however, far inferior to those made with a single-bladed knife used with the freezing microtome.

99. *Single-bladed Knives.* A scalpel and a razor are the knives most commonly used for cutting any tissue which by softening or hardening has been brought to a suitable consistence. The razor should be ground flat on one side if it is not to be used with a microtome. For a right-handed person the flat side should be that facing the right when the cutting edge of the razor is held downwards. In giving this direction, it is presumed that the concave side of the knife will be held uppermost in the process of section, so that it may hold a pool of fluid over which the slice may readily float. When a microtome is not employed, the razor should be *pulled, not pushed,* obliquely through the texture, and the cutler should be instructed to bear this in mind in setting the blade.

100. *Cutting Instruments must always be wetted.* *a*, Water, *b*, salt solution, *c*, methylated spirit, *d*, absolute alcohol, are used for this purpose. Of these, *c* and *d* are the best, but they are only to be used in cases where spirit may be added to the tissue without altering it—*e.g.*, when the tissue has been hardened in spirit, Müller's fluid, or chromic acid. The sections must *float* over the surface of the knife, and *they must always be manipulated with sable or camel-hair brushes.*

101. *Imbedding in Carrot.* The piece of tissue is often so small that it is impossible to hold it in the hand, and it is sometimes so brittle that it is apt to go to pieces when cut. To overcome these difficulties, it is necessary to imbed the tissue, so as to support and hold it firm during the section. Carrot may be used for this purpose. For holding in the hand a piece of softened bone or hardened spinal cord, etc., it is extremely convenient. A hole rather smaller than the organ should be scooped in the carrot, near its centre. An incision should then be carried from the hole to the periphery, so that the hole may be enlarged for the admission of the organ, round which the carrot closes like a hollow spring. The carrot does not cling either to the knife or to the texture.

102. *Imbedding in Paraffin.* When a very firm support is required, paraffin is better than carrot. Ordinary solid paraffin is too hard, but if five parts solid paraffin be mixed with one part hog's lard, its consistence becomes suitable for the purpose of section, and when a section-machine is employed, it is much to be preferred to a mixture of equal parts of beeswax and sweet oil—for the latter having a higher melting point, contracts so much when it cools, that it separates from the wall of the box of the section-machine. Melt the paraffin mixture in a water bath, at as low a temperature as possible, pour it into a paper cone, or paper saucer, or into the well of a microtome. With forceps, dip the tissue into the paraffin, at once remove it, and, when the layer of paraffin has cooled, replace it finally in the paraffin. By this means, the overheating of the tissue will be avoided. Tissues to be imbedded in paraffin should be as dry as possible, otherwise the supporting substance will not cling to the tissue. The water may be removed from the surface of the tissue by immersing it for a short time in spirit, and then allowing it to dry.

103. *Imbedding in Gum.* Very delicate tissues may be imbedded in a strong solution of gum (Stricker). Remove all alcohol from the tissue to be imbedded, by immersing it in water for twenty-four hours. Place the tissue for four or five hours in a cone of blotting paper containing mucilage (see § 85). Then set the cone with its contents in methylated spirit, for forty-eight hours or so, to remove the water and thereby stiffen the gum. This may be more rapidly done by absolute alcohol. Lastly, imbed in paraffin and make sections.

104. *Microtome.* In making sections the student ought to practise as much as possible the ordinary method of holding the tissue in the one hand and the knife in the other. If the piece of tissue be small it is generally advantageous to imbed it in carrot or in the paraffin mixture. It is, however, always difficult to hold the tissue and the knife perfectly steady, and when a large section is wanted, say of kidney, spinal cord, or brain, a great

the sections have to be thrown away as imperfect. This difficulty is overcome by using a microtome. Various microtomes have long been in use. The freezing microtome,* already described, serves the purpose admirably. (See § 88.)

The knife used with the microtome may be an ordinary razor, [illegible] both sides, but with the face and back perfectly [illegible] from point to heel. For sections more than a third of an inch in diameter the blade of a razor is too short. For large sections, therefore, the blade of the knife should be a foot in length, and two and a half inches in breadth. The side of the blade (the left) that rests on the table of the microtome should be concave, while that which receives the slice should be flat, or so slightly concave that the movement of the slice of tissue over the blade will not be retarded by its edge coming in contact with the blade when it approaches the back. A pool of fluid over which the slice may float is essential. (See § 88.) The knife should be steadily drawn from heel to point through the tissue, but with the face turned from the operator, so that he may cut away from himself.

Methods of increasing the Transparency of the Tissues.

90. The transparency of the tissues may be increased—1. By impregnating them with fluids which strongly refract light, e.g. glycerine, turpentine, clove oil, Canada balsam, dammar. 2. By partially or completely dissolving certain elements of the tissues, so as to permit of others being seen. Acetic acid, caustic soda or potash, are used for this purpose. These partially or completely dissolve the soft albuminous parts of most tissues. Glycerine and glacial acetic acid may be used together. (G. 1 oz., acid 5 drops.) (Beale.) The acetic acid

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102. *Embedding in Gum.* Very delicate tissues [illegible] be [illegible] in a strong solution of gum [illegible]. [illegible] into the tissue to be imbedded [illegible] for twenty-four hours. Place the tissue for [illegible] in a [illegible] of blotting paper [illegible] (see § [illegible]). Then [illegible] the cone with its contents in methylated spirit, for forty-eight hours or so, to remove the water and thereby [illegible] the gum. This may be more rapidly done by absolute alcohol. Lastly, imbed in paraffin and make sections.

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The knife used with the microtome may be an ordinary razor, *concave* on both sides, but with the face and back perfectly straight from point to heel. For sections more than a third of an inch in diameter the blade of a razor is too short. For large sections, therefore, the blade of the knife should be a foot in length, and two and a half inches in breadth. The side of the blade (the left) that rests on the table of the microtome should be concave, while that which receives the slice should be flat, or so slightly concave, that the movement of the slice of tissue over the blade will not be retarded by its edge coming in contact with the blade when it approaches the back. A pool of fluid over which the slice may float is essential. (See § 86.) The knife should be steadily drawn from heel to point through the tissue, but with the face turned *from* the operator, so that he may cut *away* from himself.

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commonly used is ordinary pyroligneous acid. The solution of soda is, caustic soda 1 part, water 20 to 30 parts. These four fluids all mix with water, hence they are employed to clarify tissues from which water has not been removed.

Turpentine, clove, or other essential oil, creasote, and carbolic acid, render most tissues transparent. As these do not mix with water it must be previously removed by drying the tissue, or by immersion in alcohol, and then allowing the alcohol to escape.

Tissues hardened in alcohol or chromic acid are rendered opaque, and must in general be clarified. This is generally done by adding, *a*, clove oil; *b*, turpentine; *c*, glycerine. These fluids have different powers—*a* is stronger than *b*, and *b* is stronger than *c*. Glycerine is employed for tissues that must not be made too transparent, *e.g.* unstained spleen, alimentary canal, lung, livér. Generally speaking clove oil and turpentine are too powerful as clarifying agents for tissues not stained with such dyes as carmine and logwood. When glycerine is used, the tissue is preserved in it. When clove oil or turpentine is employed, the tissue is preserved in dammar, or Canada balsam.

Methods of Staining the Tissues.

106. In staining the tissues, there are two points to be especially borne in mind. 1. The staining fluid may affect some parts of the tissue more than others, or it may stain some parts, and others not at all, *e.g.*, the nucleus is more deeply tinged with carmine or magenta than the surrounding protoplasm, epithelial cement is deeply stained by silver nitrate, nerve fibrils by gold chloride. 2. The staining fluid may affect the tissue with tolerable uniformity, and prove serviceable by merely rendering very transparent, colourless parts more evident.

107. *Carmine.* The use of this valuable re-agent was first recommended by Gerlach. It tinges protoplasts, the axial cylinders of white nerve fibres, but not the *modified* protoplasm of which cilia and muscular fibre consist. It is useful to have

[illegible] Weak carmine fluid. — An exceedingly good carmine fluid, for general purposes, is the following modification of that proposed by Beale. Beale's fluid is thus prepared: Carmine 10 grains, strong ammonia 30 minims, glycerine 2 oz., distilled water 2 oz., rectified spirit ½ oz. Place the carmine in a test tube, add the ammonia, boil for a few seconds, let the mixture stand for an hour with the test tube open, add the water, filter, add the spirit and the glycerine, and allow the mixture to stand exposed to the air until the odour of ammonia is scarcely perceptible, then keep it in a stoppered bottle. This fluid is, owing to its density, slow in its action, and, although it may yield excellent results, it is uncertain in its operation. Density and uncertainty of action may be avoided by retaining all the ammonia in the fluid. Therefore, prepare the fluid thus: place the carmine in a test-tube, add the ammonia, bring to the boiling point, add the water and filter, then add the spirit and glycerine, and preserve in a stoppered bottle. When the fluid is to be used, dilute it [illegible] times with water (Atkinson), filter, and place the tissues in a large quantity of the fluid from 2 to 24 hours. This fluid is therefore not at all suitable for rapid staining, but carmine staining is generally most beautiful when it takes place slowly. Tissues which have been steeped in chromic acid, potassium bichromate, or Müller's fluid, must always be thoroughly washed in water ere they are placed in carmine. Remove superfluous pigment by washing the stained tissues in water, or proof spirit (rectified spirit 5 parts, water 3 parts), to which 1 per cent. of hydrochloric acid has been added (Pritchard), and mount in pure glycerine or dammar (see § 100). Beale's method is to stain the tissues with his carmine fluid undiluted, then to wash them for a day or two in diluted glycerine (glycerine 2 parts water 1 part), and lastly, to mount them in acid glycerine (glycerine 1 oz. glacial acetic acid 5 drops, or 5 drops hydrochloric acid in place of the acetic acid). The acid brightens the colour. 3. *Strong carmine fluid.* — It is useful to have a strong carmine fluid for rapidly staining nerve

commonly used is ordinary pyroligneous acid. The solution of soda is, caustic soda 1 part, water 20 to 30 parts. These four fluids all mix with water, hence they are employed to clarify tissues from which water has not been removed.

Turpentine, clove, or other essential oil, creosote, and carbolic acid, render most tissues transparent. As these do not mix with water it must be previously removed by drying the tissue, or by immersion in alcohol, and then allowing the alcohol to escape.

Tissues hardened in alcohol or chromic acid are rendered opaque, and must in general be clarified. This is generally done by either a, clove oil; b, turpentine; c, glycerine. These fluids have different powers—a is stronger than b, and b is stronger than c. Glycerine is employed for tissues that must not be made too transparent, e.g., [illegible] spinal cord, alimentary canal, lung, liver. Generally speaking clove oil and turpentine are too powerful as clarifying agents for tissues not stained with such dyes as carmine and logwood. When glycerine is used, the tissue is preserved in it. When clove oil or turpentine is employed, the tissue is preserved in dammar, or Canada balsam.

Methods of Differential Staining.

106. In staining the tissue, there are two points to be especially borne in mind. 1. The staining fluid may affect some parts of the tissue more than others, or it may stain some parts, and others not at all, e.g., the nucleus is more deeply tinged with carmine or magenta than the surrounding protoplasm, epithelial cement is deeply stained by silver nitrate, nerve fibrils by gold chloride. 2. The staining fluid may affect the tissue with tolerable uniformity, and prove serviceable by merely rendering very transparent, colourless parts more evident.

107. *Carmine.* The use of this [illegible] was first recommended by Gerlach. It stains protoplasm, the axial cylinders of white nerve fibres, but not the modified protoplasm of which cilia and muscular fibre consist. It is useful to have

two fluids. *a. Weak carmine fluid.* An exceedingly good carmine fluid, for general purposes, is the following modification of that proposed by Beale. Beale's fluid is thus prepared. Carmine 10 grains, strong ammonia 30 minims, glycerine 2 oz., distilled water 2 oz., rectified spirit ½ oz. Place the carmine in a test tube, add the ammonia, boil for a few seconds, let the solution stand for an hour with the test tube open, add the water, filter, add the spirit and the glycerine, and allow the mixture to stand exposed to the air until the odour of ammonia is scarcely perceptible, then keep it in a stoppered bottle. This fluid is, owing to its density, slow in its action, and, although it may yield excellent results, it is uncertain in its operation. Tardiness and uncertainty of action may be avoided by retaining all the ammonia in the fluid. Therefore, prepare the fluid thus:—place the carmine in a test tube, add the ammonia, bring to the boiling point, add the water and filter, then add the spirit and glycerine, and preserve in a stoppered bottle. When the fluid is to be used, dilute it *seven* times with water (Atkinson), filter, and place the tissues in a large quantity of the fluid from 2 to 24 hours. This fluid is therefore not at all suitable for rapid staining, but carmine staining is generally most beautiful when it takes place slowly. Tissues which have been steeped in chromic acid, potassium bichromate, or Müller's fluid, must always be thoroughly washed in water ere they are placed in carmine. Remove superfluous pigment by washing the stained tissues in water, or proof spirit (rectified spirit 5 parts, water 3 parts), to which 1 per cent. of hydrochloric acid has been added (Pritchard), and mount in pure glycerine or dammar (see § 119). Beale's method is to stain the tissues with his carmine fluid undiluted, then to wash them for a day or two in dilute glycerine (glycerine 2 parts, water 1 part), and lastly, to mount them in acid glycerine (glycerine 1 oz., glacial acetic acid 5 drops, or 2 drops hydrochloric acid in place of the acetic acid). The acid brightens the colour. *b. Strong carmine fluid.* It is useful to have a strong carmine fluid for rapidly staining nerve

tissues by the method recommended in § 72. This is prepared as follows. Dissolve 1 gramme carmine in 1CC strong ammonia in a test tube with the aid of a gentle heat, add 23CC distilled water, filter, and preserve in a stoppered bottle. This fluid is of the proper strength for staining by the method above referred to (§ 72). For ordinary purposes it tinges too rapidly, and for tissues unhardened in chromic acid or Müller's fluid it contains too much ammonia. Therefore should this fluid be used for staining in the ordinary way. Expose it in a beaker to the air for two days to let the excess of ammonia escape, then dilute it with five times its bulk of distilled water, filter, and preserve in a stoppered bottle.

108. *Picro-carmine.* A double staining, with picric acid and carmine, may be obtained by a fluid prepared as follows (Ranvier). Drop a strong ammoniacal solution of carmine into a saturated solution of picric acid until the fluid is neutralised. Then filter, and preserve in a stoppered bottle. The picric acid gives a yellow tinge to epithelium and muscle, while the carmine stains nuclei, etc. Sections of skin may be beautifully stained by this fluid. The epidermis, hair, and muscles of the hair follicles are rendered yellow, while the cutis vera is reddened.

109. *Magenta* (rosaniline nitrate) is useful for staining tissues rapidly in the ordinary process of examination. Like carmine, it stains protoplasts, the axial cylinders of white nerve fibres, etc. Unlike carmine, it fades, and is therefore not suitable for permanent preparations. Nevertheless, the colour can be retained for a long time by mounting the stained tissue in one-third per cent. watery solution of corrosive sublimate. Two fluids are necessary—*a.* Magenta crystals 1 decigramme, rectified (*not* methylated) spirit 9CC, distilled water 213CC. Dissolve the magenta in the water, and then add the spirit. Preserve in a stoppered or well-corked bottle. This fluid is used for the tissues generally. The following fluid, devised by my former

[illegible] Mr. Farrer, is used for staining blood corpuscles. [illegible] of a specific gravity similar to that of the liquor sanguinis, the coloured corpuscles [illegible] retain their shape while they become stained. To make, dissolve 1 decigramme crystallized magenta in 100·0 distilled water, and add 100·0 rectified spirit, and [illegible] glycerine.

110. *Logwood* is like carmine, suitable for staining tissues for permanent preparations. Speaking generally, it stains the same elements of the tissues as carmine and magenta. The fluid is [illegible]. The tissues may either be fresh, or they may have been hardened in chromic acid or alcohol. It stains the nuclei of epithelium, and the protoplasm of connective tissue especially well; the lung, skin, cornea, tongue, etc., can be beautifully tinged by this agent. Böhmer, who introduced logwood, [illegible] used hæmatoxyline; but a fluid prepared of logwood is cheaper and gives admirable results. Make an infusion of logwood thus,—pour [illegible] water on [illegible] logwood chips, and allow to stand at the side of the fire for six hours or so. The infusion must not reach the boiling point. Filter and add to the filtrate a sufficient quantity (about [illegible]) of a ½ per cent. watery solution of alum, to give a fine purple colour. Then add 30·0 rectified spirit. Before staining, filter the fluid. A fresh tissue is tinged by this in three or four minutes. It is better, however, to tinge a tissue more slowly than this, therefore place ten or a dozen drops of this fluid in a watch-glass, half filled with water. From half an hour to an hour will be required. Wash the tissue in water, or in ½ per cent. alum solution, to remove superfluous pigment, and mount in glycerine or dammar.

111. *Silver Nitrate.* Recklinghausen introduced this substance into histology. It is of great value in staining the stratified squamous epithelium of serous membranes, lymphatics and blood-vessels. Use ½ per cent. solution in distilled water. In staining a tissue e.g. the frog's mesentery, dip it two or three

tissues by the method recommended in § 73. This is prepared as follows:—Dissolve 1 gramme carmine in 10 C.C. strong ammonia in a test tube with the aid of a gentle heat, add 90 C.C. distilled water, filter, and preserve in a stoppered bottle. This fluid [illegible] the proper strength for staining by the method above referred to (§ 73). For ordinary purposes it stains too rapidly, and for tissues unhardened in chromic acid or Müller's fluid it contains too much ammonia. Therefore should this fluid be used for staining in the ordinary way. Expose it in a beaker to the air for two days to let the excess of ammonia escape, then dilute it with five times its bulk of distilled water, filter, and preserve in a stoppered bottle.

108. Picrocarmine.—A double staining, with picric acid and carmine, may be obtained by a fluid prepared as follows (Ranvier). Drop a strong ammoniacal solution of carmine into a saturated solution of picric acid until the fluid is neutralised. Then filter, and preserve in a stoppered bottle. The picric acid gives a yellow tinge to epithelium and muscle, while the carmine stains nuclei, etc. Sections of skin may be beautifully stained by this fluid. The epidermis, hair, and muscles of the hair follicles are rendered yellow, while the cutis [illegible] coloured.

109. Magenta (Roseaniline nitrate) is useful for staining tissues rapidly in the ordinary process of investigation. Like carmine, it stains protoplasm, the axial cylinders of nerves, nuclei, etc. Unlike carmine, it fades, and is therefore not suitable for permanent preparations. Nevertheless, the colour can be retained for a long time by mounting the stained tissue in one-third per cent. watery solution of corrosive sublimate. Two fluids are necessary—*a.* Magenta crystals 1 decigramme, rectified (not methylated) spirit 10 C.C., distilled water 290 C.C. Dissolve the magenta in the water, and then add the spirit. Preserve in a stoppered or well-corked bottle. This fluid is used for the tissues generally. The following fluid, devised by my former

assistant Dr. Ferrier, is used for staining blood corpuscles. Being of a specific gravity, similar to that of the liquor sanguinis, the coloured corpuscles of nonmammalian vertebrates alter but little in shape while they become stained. *b.* Dissolve 1 decigramme crystallised magenta in 15CC distilled water, and add 5CC rectified spirit, and 20CC glycerine.

110. *Logwood* is like carmine, suitable for staining tissues for permanent preparations. Speaking generally, it stains the same elements of the tissues as carmine and magenta. The tint is violet. The tissues may either be fresh, or they may have been hardened in chromic acid or alcohol. It stains the nuclei of epithelium, and the protoplasts of connective tissue remarkably well; the lung, skin, cornea, tongue, etc., can be beautifully tinged by this agent. Böhmer, who introduced logwood staining into histology, used hæmatoxyline; but a fluid prepared as follows is cheaper, and gives admirable results. Make an infusion of logwood thus,—pour 200CC cold water on 20 grammes logwood chips, and allow to stand at the side of the fire for six hours or so. The infusion must not reach the boiling point. Filter, and add to the filtrate a sufficient quantity (about 350CC) of a ½ per cent. watery solution of alum, to give a fine purple colour. Then add 30CC rectified spirit. Before staining, filter the fluid. A fresh tissue is tinged by this in three or four minutes. It is better, however, to tinge a tissue more slowly than this, therefore place ten or a dozen drops of this fluid in a watch-glass, half filled with water. From half an hour to an hour will be required. Wash the tissue in water, or in ½ per cent. alum solution, to remove superfluous pigment, and mount in glycerine or dammar.

111. *Silver Nitrate.* Recklinghausen introduced this substance into histology. It is of great value in staining nonstratified squamous epithelium of serous membranes, lymphatics and blood-vessels. Use ½ per cent. solution in distilled water. In staining a tissue, *e.g.* the frog's mesentery, dip it two or three

times into distilled water to remove the sodium chloride. Place it in the silver solution from one to three minutes. Remove the superfluous silver by washing in common water until the water ceases to become milky. Place the washed tissue in glycerine, and then expose to diffuse daylight until it becomes slightly brown. Mount in glycerine, or in glycerine jelly. In order to stain the epithelium of blood-vessels, wash out the blood-vessels of a newly-killed animal with distilled water, then inject ¼ per cent. solution of silver nitrate, allow it to remain in the vessels for five minutes or so, and then wash it out with distilled water. If the animal be warm-blooded, the water and the silver solution must be heated to 100° Fahr. Lastly, inject a warm solution, gelatine (gel. 1 part, water 6 parts), and expose to the light, and mount as above mentioned. This is a modification of Woodward's process by my former assistant Dr. Pritchard.

By exposure to light the silver salt is reduced, and the tissues are thereby blackened. If the immersion of the tissue in the silver solution be of very brief duration, only the outlines of the epithelial cells are blackened. The cells appear to be united by a colourless "cement," which readily reduces the silver salt when acted on by light. If the silver act for some time longer, the nucleus and general substance of the epithelial cells is blackened, also the nuclei of fat cells, connective tissue, corpuscles, etc. The silver process has been of great value in the investigation of the lympathic system and blood capillaries.

112. *Chloride of gold* (auric chloride) is of much service for staining *nerve fibrils*, connective tissue protoplasts, cartilage protoplasts, etc. The colour varies from bluish-grey to violet. Place the tissue, not later than fifteen minutes after its removal from the body, in one-half per cent. solution of auric chloride in distilled water, until the tissue becomes of a pale-yellow colour. From fifteen minutes to two hours will be required, according to the thickness of the tissue. Transfer to very dilute acetic acid

tissue into distilled water to remove the [illegible]. Place it in the silver solution from five to fifteen minutes. Remove the superfluous silver by washing in common water until the water ceases to become milky. Place the stained tissue in glycerine and then expose to diffuse daylight until it becomes slightly brown. Mount in glycerine or in glycerine jelly. In order to stain the epithelium of blood-vessels, wash out the blood-vessels of a newly killed animal with distilled water, then inject ½ per cent. solution of silver nitrate, allow it to remain in the vessels for five minutes or so, and then wash it out with distilled water. If the animal be warm-blooded, the water and the silver solution must be heated to 100° Fahr. Lastly inject a warm gelatine solution (gel. 1 part, water 6 parts) and expose to the light, and mount as above mentioned. This is a modification of Woodward's process by my former assistant, Dr. Pritchard.

By exposure to light the silver salt is reduced, and the tissues are thereby blackened. If the immersion of the tissue in the silver solution be of very brief duration, only the outlines of the epithelial cells are blackened. The cells appear to be united by a cement-like "cement," which readily reduces the silver salt when exposed to light. If the silver act for a longer time, the nuclei and general substance of the epithelial cells is blackened, also the nuclei of fat cells, connective tissue, corpuscles, etc. The silver process has been of great value in the investigation of the lymphatic system and blood capillaries.

112. *Chloride of gold* (auric chloride) is of much service for staining nerve fibrils, connective tissue [illegible], [illegible] etc. The colour varies from black-grey to violet. Place the tissue, not later than fifteen minutes after its removal from the body, in one-half per cent. solution of auric chloride in distilled water, until the tissue becomes of a pale yellow colour. From fifteen minutes to two hours will be required, according to the thickness of the tissue. Then lay in very dilute acetic acid

(1 or 2 per cent.) for five or ten minutes. Lastly, place the tissue in distilled water, and expose to the light, until a steel-grey or violet tint appear. This results from the reduction of the gold salt. The reduction is hastened by the acetic acid. The time of exposure varies with the intensity of the light; from one to two days usually suffice. Mount the stained tissue in glycerine, or in glycerine jelly.

The gold method was introduced by Cohnheim for staining the nerves of the cornea. The following method, devised by Klein (*Monthly Microscopical Journal*, vol. vii. p. 157), gives better results than Cohnheim's in this case. Remove the cornea from a rabbit or guinea-pig, within fifteen minutes after death, and place it in ½ per cent. gold solution, from one and a-half to two hours in the case of the rabbit, and rather more than an hour in the guinea-pig. Wash in distilled water, and expose to the light in the water for twenty-four or thirty-six hours, the water being changed twice or thrice. Then transfer the cornea to dilute glycerine (pure glycerine 1 part, distilled water 2 parts) for two or three days. Lastly, place the cornea in water, and gently brush away any precipitate that may have formed; make sections obliquely horizontal or vertical, and mount in glycerine. A cornea so treated is ash-grey or slightly violet.

113. *Osmic acid,* introduced by Max Schultze, is, as we have seen, a valuable hardening agent. Its property of blackening the white substance of Schwann and all fatty matter, as well as, though to a less extent, albuminoid matter, renders it an important staining agent. For the method of using it see § 91.

Methods of Injection.

114. *Injecting apparatus.* The fluid may be injected by means of a syringe. The piston of the syringe must be slowly screwed, not pushed, down, in order to prevent jerking. For most purposes the syringe has given place to a far more efficient apparatus, consisting of Wolff's bottles. The fluid is driven into

the vessels by water-pressure, which can be regulated with the greatest nicety. There is no jerking, no refilling of a syringe, no haphazard application of pressure, as is the case with the syringe. This valuable method we owe to Ludwig. Always begin with a low pressure and increase it gradually. In the case of the blood-vessels of a rabbit, the pressure may be raised to 110 millimetres (4½ inches) of a mercurial column.

115. *The injection of blood-vessels.* I. *Injection masses fluid at ordinary temperatures.* *a. Watery solution of soluble Prussian blue.* Soluble blue can now be procured in the market. The following, however, is the method in which it may be prepared (Brücke). A. Dissolve 217 grammes potassium ferrocyanide in 1 litre distilled water. B. Dissolve 100 grammes ferric chloride in 1 litre distilled water. C. Make a cold saturated solution of sodium sulphate. Mix 1 volume of A with two volumes of C, and mix 1 volume of B with 2 volumes of C. Pour B C very gradually into A C, stirring all the while, and let stand for a day. Pour off supernatant fluid, and filter off the remaining fluid from the precipitate. Wash the precipitate upon the filter, with repeated small quantities of distilled water, until it flows through distinctly blue. Press and dry the deposit. For the purpose of injection, a 2 per cent. solution of the blue in distilled water is employed. The fluid may be used cold, or it may be heated to the temperature of the body. After injection, prevent the blue from diffusing through the capillary walls by immersion in spirit (90 per cent.). If the colour fade, maceration in turpentine or in acid brings it back. Mount in dammar. *b. Beale's Prussian blue injection.* A. 1 oz. glycerine added to 4 ozs. water. B. 60 minims tincture of ferric chloride added to 1 oz. of solution A. C. 12 grains potassium ferrocyanide dissolved in 1 oz. of solution A. Pour B into C very gradually, with constant stirring and shaking of the mixture, then add the remainder of solution A, and also 1 oz. of spirits of wine. Mount in acid glycerine (glycerine 1 oz., H Cl

the arteries in water [illegible] should be performed with the greatest nicety. There is no [illegible] as backward application of pressure, as in the [illegible] syringe. This valuable method [illegible] begin with a low pressure and increase it gradually. [illegible] case of the blood-vessels of a rabbit, the pressure [illegible] to 110 millimeters (4 inches) of a mercurial column.

110. *The Injection of* [illegible] fluid of ordinary temperature, [illegible] watery solution of soluble Prussian blue. Soluble blue can now be procured in the market. The following, however, is the method in which it may be prepared (Brücke). A. Dissolve 217 grammes potassium ferrocyanide in 1 liter distilled water. B. Dissolve 100 grammes ferric chloride in 1 liter distilled water. C. Make a cold saturated solution of sodium sulphate. Mix 1 volume of A with two volumes of C, and mix 1 volume of B with 2 volumes of C. Pour B C very gradually [illegible] stirring all the while, and let stand for a day. [illegible] fluid, and filter off the remaining fluid [illegible] the precipitate upon the filter, with [illegible] distilled water, until it flows through [illegible] dry the deposit. For the purpose of injection a [illegible] solution of the blue in distilled water is employed. The fluid may be used cold, or it may be heated to the temperature of the body. After injection, prevent the blue from diffusing through the capillary walls by immersion in spirit (90 per cent.). If the [illegible] Mount in balsam. 2. *Beale's Prussian Blue* [illegible]. A. [illegible] glycerin added to 1 oz. water. [illegible] chloride added to 1 oz. of [illegible] B. [illegible] 12 grains potassium ferrocyanide [illegible] This is added very gradually, with constant stirring and shaking of the mixture, then add the remainder of solution A, and also 1 oz. of spirits of wine. [illegible]

2 drops). Prussian blue injections must not come in contact with any alkali. These injecting fluids are excellent when a perfect injection is not desired. II. *Injection masses solid at ordinary temperatures. a. Carter's carmine and gelatine injection.* This, the best of all the carmine injections, is prepared thus:— 60 grains carmine, 120 minims strong ammonia, 86 minims glacial acetic acid, 2 oz. solution of gelatine (1 of gelatine to 6 of water), 1½ oz. water. Dissolve the carmine in the ammonia and water with the aid of gentle heat, and filter; add to this 1½ oz. of hot gelatine solution, and mix thoroughly. Add the acid to the remaining half ounce of gelatine solution, and drop this into the heated carmine mixture with constant stirring. For the purpose of section, harden in weak and then in strong alcohol. Mount the injected tissues in dammar. *b. Prussian blue and gelatine injection.* A. Dissolve, with the aid of heat, 33 parts of gelatine in 200 parts distilled water. B. Dissolve 4 parts soluble Prussian blue in 300 parts distilled water. Gradually add B to A. For hardening the injected tissues, to permit of sections being made, use first weak and then strong alcohol, containing 1 per cent. H Cl, or use a ¼ per cent. solution of chromic acid for some weeks, and then alcohol. Preserve the injected tissues in dammar.

Inject the vessels immediately after death by chloroform. Previous to injection the vessels may be washed out with ¾ per cent. salt solution. This, however, is not essential. The injection and the salt solution, if it is employed, should be heated to the temperature of the blood (100° F.) in the case of a warm blooded animal. Tie the vessels when the injection is completed, and plunge the injected parts into *cold* meth. sp.

116. *The injection of lymphatics.* Use a solution of Alcannine in turpentine (Ludwig), or a watery solution of soluble Prussian blue. For the injection of lymphatics, Ludwig's method of puncture is adopted. Fill a subcutaneous syringe with the fluid, thrust the nozzle into the tissue, and slowly drive the fluid

wherever it will go. The lymphatics of the testis and spermatic cord of the dog can be readily injected by simply thrusting the nozzle of the syringe through the tunica albuginea.

117. *The injection of bile ducts.* Inject the portal vein with carmine and gelatine. Previous to this, open the common bile duct so that the distension of the blood vessels may press as much of the bile out of the ducts as possible. Then inject the bile capillaries from the common bile duct with an aqueous solution of soluble Prussian blue. It is not necessary, however, to previously inject the veins. The liver of the rabbit is well adapted for injecting the bile ducts. A pressure of from 20 to 25MM mercury is usually sufficient for the ducts, but it may require to be gradually increased to 40MM. The fluid being driven against the bile contained in the liver, an injection of the whole liver must not be attempted, but the injection should be discontinued as soon as a few lobules on the surface of the liver have become distinctly blue. After injection, place the liver in meth. sp. Mount the sections in dammar.

Methods of Preserving or Mounting the Tissues.

118. *The dry method.* Hair, and sections of dried bone and tooth, are sometimes mounted in air. The difference between the refractive powers of the tissues and the air is, however, so great, that the outlines are extremely dark. They show better when mounted in some more refractive medium than air, *e.g.* meth. sp. glycerine, glycerine jelly, or dammar. When a tissue is mounted in air, a ring of dammar rather smaller than the cover-glass is placed on the slide, and allowed to nearly dry. The tissue is then placed within the dammar *cell*, and the cover-glass placed over all, and pressed upon the dammar, to which it adheres. If the portion of tissue be thick, a glass cell is used.

119. *Dammar and Canada balsam.* All water must be

removed from the tissue by drying or by immersion, first in methylated spirit, and then in rectified spirit or absolute alcohol. The alcohol is driven away by floating the tissue upon oil of cloves, or oil of turpentine, and then the balsam or dammar is added, and the cover-glass put on (see § 56, D 4). If Canada balsam be employed, prepare it as follows. Place some pure Canada balsam in a saucer or other shallow vessel; cover the vessel with bibulous paper to exclude dust; dry it in an oven at a temperature not above 150° Fahr., until, when it cools, *it becomes as hard as ice;* dissolve this crystalline balsam in benzole. The old-fashioned method of mounting things in balsam was so troublesome, that one avoided it as much as possible. Undried balsam was taken and rendered quite liquid by heat. The slide, the cover-glass, and the tissue to be mounted, all require to be heated. It was difficult to get rid of air bubbles and to avoid overheating the tissue. But by using a limpid solution of *perfectly dried* balsam, all difficulty disappears. Dammar is now much employed as a substitute for Canada balsam. It renders the tissues more transparent than balsam. The following is an excellent method for preparing the dammar fluid :—dissolve $\frac{1}{2}$ oz. dammar resin and $\frac{1}{2}$ oz. gum mastic in 3 ozs. benzole, and filter. If rapid filtration be desired, use twice as much benzole, and then evaporate the fluid to half its bulk. Chloroform and turpentine are by some recommended as solvents of these resins, but the dammar fluid so made is turbid. If, however, it be prepared as above recommended, it is perfectly clear. Balsam or dammar is suitable for mounting unsoftened bone and tooth, hair, brain, and spinal cord, and indeed most tissues that have been hardened in alcohol or chromic acid, which require to have their transparency much increased. It renders softened bone and tooth too transparent. Speaking generally, it is more suitable for mounting stained than unstained tissues. The latter are often rendered too transparent. The tissues are very perfectly preserved in either of these substances, because all water is removed.

120. *Glycerine* is not adapted for white fibrous tissue and blood vessels, unless they have been hardened in chromic acid. Otherwise it causes the white fibres to swell up and lose their normal features, but it is well suited for tissues that are to be examined by very high powers. Liver, lung, alimentary canal, and skin, after having been hardened in chromic acid or alcohol, show better in glycerine than in balsam or dammar, unless they have been stained with carmine or logwood. It is suitable for mounting tissues that have been stained with silver nitrate or gold chloride. For tissues containing vessels injected with Prussian blue, use 2 drops H Cl in 1 oz. glycerine, to preserve the colour. It is somewhat difficult to mount things in glycerine. Air bubbles are difficult to eliminate, and if the glycerine pass outside the cover-glass, it is difficult to get rid of it. It can be removed most readily by sucking it up with a fine glass pipette. It is often necessary, however, to transfer the preparation to a new slide, on which a smaller drop of glycerine has been placed.

121. *Glycerine jelly* is a good preservative agent for many tissues, such as lung, blood vessels, tooth, bone, cochlea, etc. The tissues must be steeped in weak spirit previous to mounting. They cannot be transferred from glycerine to glycerine jelly directly. Fungi are very apt to grow in some kinds of glycerine jelly that are sold. Rimmington's glycerine jelly is the best. Its composition is unknown, but it appears to contain glycerine, gelatine or isinglass, and carbolic acid.

122. *Potassium acetate.* A saturated solution of this substance has long been used by botanists. It is the best medium for osmic acid preparations (Schultze). Glycerine renders these too transparent. The fluid may be prepared thus:—dissolve 56 grammes potassium acetate in 28CC hot water. When cold, add 2CC camphorated spirit, and filter.

123. *Weak spirit* (meth. sp. 1 part, distilled water 3 parts)

was formerly much employed. Muscle, connective tissue and blood vessels can be well preserved in it for a time, but very often they become granular and useless.

124. *Methylated spirit* may be used for things which have been hardened in spirit, if they do not require to be clarified. Softened bone shows best in spirit.

125. *Naptha and creosote fluid* is useful for preserving urinary casts (see § 62, *e.*)

126. *Cells for microscopic objects.* For dry objects use cells made of tinfoil, paper, cardboard, dammar varnish, or glass. For wet objects use glass cells, or cells made by painting a ring or square of dammar varnish upon a glass slide, and allowing it to dry. Delicate tissues that are apt to be spoilt by the pressure of the cover-glass should be placed in a dammar cell. The glass cells are cemented to the slide with marine glue. It is most convenient to buy them fixed to the slide.

127. *Cements.* When the object is mounted in Canada balsam or dammar, no cement is required; but if any other preservative substance be employed, even glycerine jelly, the margin of the cover-glass must be covered by cement. *First*, paint on a layer of *gold size, and allow it to dry*, then cover this with a layer of dammar varnish. The dammar mounting fluid, thickened by drying, is the best dammar varnish. If a tissue, *e.g.* a slice of softened bone, be mounted in meth. sp., use *thick* dammar varnish as the only cement, otherwise it will be apt to *run in* and spoil the preparation. The slide must be dried with bibulous paper ere the varnish is applied. Glycerine is apt to ooze through the varnish after a time; when this happens, run a stream of cold water over the preparation, then dry and paint on a fresh layer of dammar.

128. *Mode of keeping preparations.* The slides should lie upon the back, *not* upon the edge. All preparations should be carefully labelled. The things shown should be mentioned, the date and name of the mounting fluid ought also to be stated. *Never keep a bad preparation, and do not attempt to preserve everything.*

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