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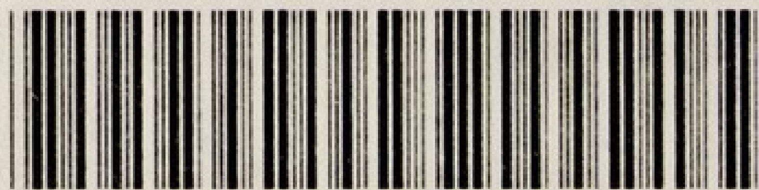

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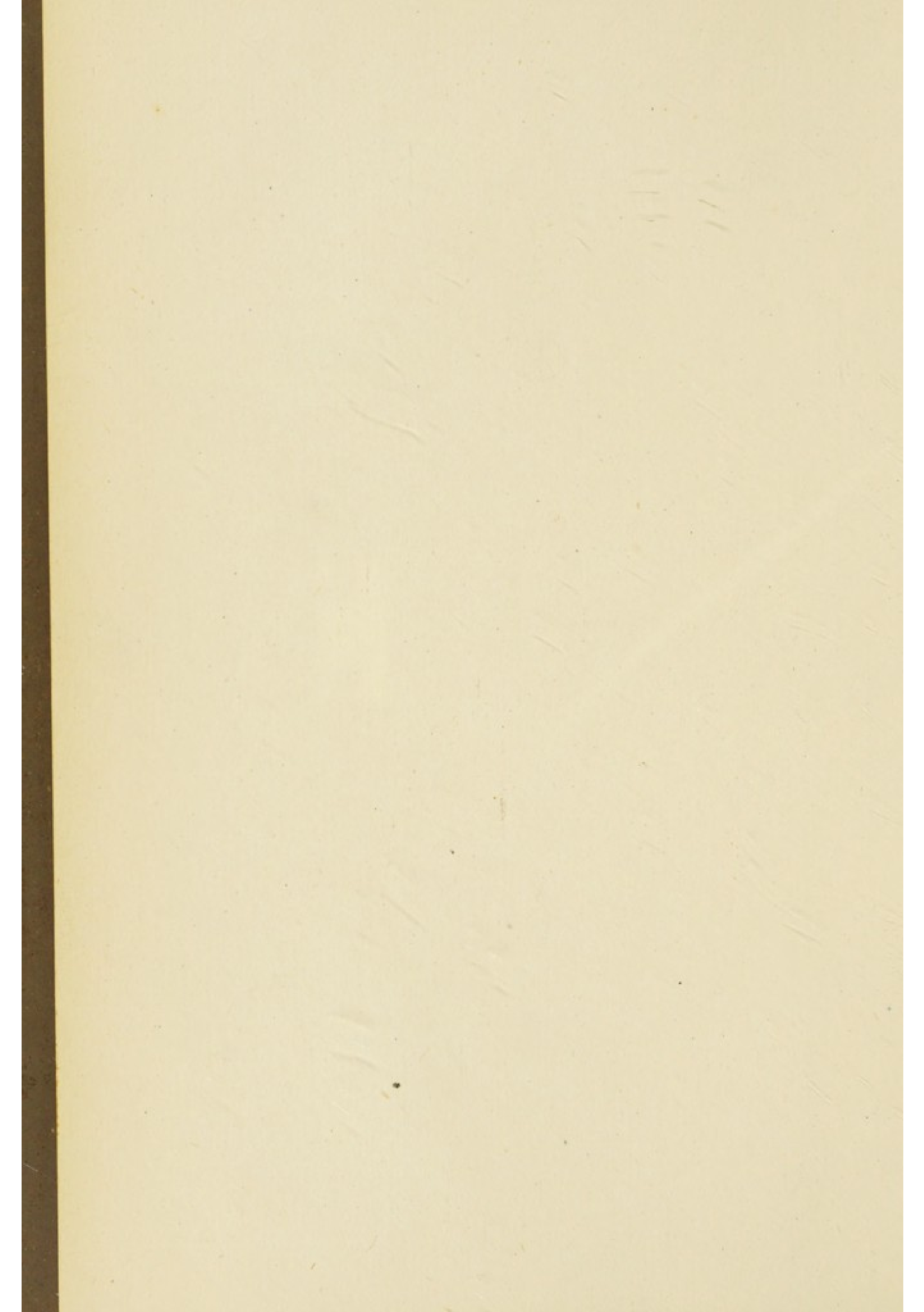

SECTION CUTTING AND STAINING

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SECTION CUTTING

AND

STAINING

A PRACTICAL GUIDE TO THE PREPARATION OF NORMAL AND MORBID HISTOLOGICAL SPECIMENS

BY

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PREFACE.

THIS little book is intended, not as an exhaustive account of different histological methods, but as a practical guide for those who are beginning to work at the subject. In it, methods which have been found most useful for general purposes are described fully, but in order to keep the book of as moderate size as possible, methods that are only required for special investigations have been described more briefly, or omitted altogether. No attempt has been made to give any description of the microscopical appearances of the sections, as these would require to be so condensed as to be of little value. The best and most lasting knowledge of histology will be attained by a careful study of one's own sections, assisted by the descriptions in such books as Klein's *Histology*, Quain's *Anatomy*, Woodhead's *Practical Pathology* and Crookshank's *Bacteriology*.

In addition to the books of reference given, I

have been much indebted to the courses of Practical Physiology and Practical Pathology in the University of Edinburgh. I have also much pleasure in expressing my thanks to Professor Greenfield, for leave to insert many methods learned while working as his Assistant; to Dr. Sims Woodhead for permission to copy several of the formulæ from his *Practical Pathology;* to Mr. A. Frazer, M.A., for other formulæ, and to Dr. Beevor and Dr. Tooth for information about the celloidin method, and the staining processes of Weigert and Pal.

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NATIONAL HOSPITAL,
QUEEN'S SQUARE, W.C.
October, 1888.

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SECTION CUTTING AND STAINING.

CHAPTER I.

APPARATUS, ETC., REQUIRED.

PERHAPS there is nothing more perplexing to a beginner than to decide what apparatus is required. If he consult a price list he has the greatest difficulty in deciding which articles will be necessary, and which will be either luxuries, or required only for special investigation. In the following account of requisites, those only will be described, which it is useful to have always by one. They will be found ample for ordinary work, but for special investigations more elaborate apparatus will often be required.

If economy is any object, all staining and other reagents should be made as far as possible by the worker himself according to the directions given in this book. This should at any rate be done at first, as the knowledge thus gained will prove

invaluable. It will also effect a great saving if articles that are used in any quantity, such as methylated spirit, distilled water, etc., are bought by the gallon, and not in small quantities.

Almost all the processes described here can be carried out without the use of a fully equipped laboratory, in fact, in an ordinary room. The only furniture required is a firm table, and a cupboard for storing reagents.

The following should also be procured:—

Jars or **bottles,** with well fitting stoppers or corks, to contain the tissues while being hardened. They should not hold less than two ounces. Empty drug bottles which can usually be obtained from druggists for a few pence, serve very well.

Smaller bottles should also be procured for keeping specimens in spirit after they have been hardened until one is ready to cut sections. After sections have been cut from a portion of the specimen, the rest should be preserved, in case it is wanted for further investigation. Each specimen must be labelled, with a name or a number corresponding to a reference in the note-book, and a large number of specimens may then be

kept in the same jar. The best way to label them is to write the name or number on a piece of vegetable parchment in ordinary "marking ink," and warm it until the writing is black. The little label should then be fixed to a corner of the piece of tissue with a stitch or by a fine pin, and it may be identified years afterwards. The importance of keeping tissues, sections, slides, etc., distinctly **labelled** cannot be too strongly impressed on the beginner. At first he will be inclined to neglect this as he will recognize his pieces of tissue and sections so readily merely by their shape and general appearance. But as time elapses and similar specimens accumulate, he will find it most difficult or even impossible to identify one from the other. The label should mention the date, the name of the organ, the hardening fluid etc. If a pathological specimen, the patient's name, the name of the disease, and of the organs that are in the jar, and the date of the post-mortem, should be mentioned.

A number of 1 oz. and 2 oz. **stoppered bottles** for staining reagents.

The stopper of these should be fitted with a rod,

This is done by simply heating the lower end of the stopper and the upper end of a piece of glass rod of suitable length in a blow-pipe, until they are plastic, and then pressing them together.

Watch glasses. At least a dozen watch glasses, in which to perform the operations of staining, clarifying, etc. Those with a flat bottom should be employed as they are less easily upset than the others.

Plenty of **filter papers.**

Both coarse ones, for use in the manufacture of reagents, and small fine white ones (2½ inch) for filtering the staining fluids immediately before using them, should be procured. Before using them a few drops of alcohol or distilled water should be placed in them to saturate the paper. This not only allows the fluid to pass through more rapidly, but prevents a portion of it being wasted through being absorbed by the pores of the paper.

Several **needles** mounted in handles.

They must be kept very bright and smooth, and care must be taken that the point does not get turned up.

A large and small **funnel.**

Two **pipettes.**

These may be easily made. Take a glass tube with a bore of about $\frac{1}{16}$ in. about eighteen inches long. Stop up one end of it. Hold a portion of the tube six inches from the closed end in the flame of a Bunsen's or other burner until it is red hot. Then blow gently down the tube so as to form a bulb at the heated spot. The tube should then be heated about two inches from the closed end, and when red hot should be drawn out to a fine point. The tube should then be filed and broken across about two inches beyond the bulb and the pipette is made.

Section lifter is used for transferring sections from one reagent to another, or from oil of cloves, etc., to the slide. It should be made of thin sheet copper, which allows the blade to be bent at any angle to the stem. The stem or handle should be about six inches long, and continuous with, and at an angle to it, a flat blade about $\frac{3}{4}$ in. square with the corners rounded off. The surface of the blade should be brightly polished, and kept scrupulously clean.

If the student intends to work much with strong acids, as in preparing specimens of the tubercle bacillus, he should use an aluminium lifter which, however, is more expensive than the copper one.

Ordinary dissecting **forceps.**

One or two **scalpels.**

A pair of fine **scissors.**

A **razor** or other instrument for cutting sections.

A smooth **oil stone** for keeping the razors and knives properly sharpened.

A **spirit lamp** for warming the staining fluids.

A few **test tubes.**

A **minim measure.**

Scales and small weights.

A gross of ground glass slides 3 × 1 in.

Half a gross of ground glass slides 3 × 1½ in.

Half an ounce thinnest coverslips, ⅞ in. diameter.

Quarter of an ounce of thinnest coverslips, 1¼ in. diameter.

Microscope.—This is not the place for a description of the microscope as an optical instrument, but some hints as to the selection of one may be found useful.

Showy microscopes with much brass work should

be avoided, simplicity of construction being a great recommendation. The microscope should have a large heavy base, either of the horse-shoe or tripod pattern large enough to afford a firm base when the microscope is tilted.

Mechanical stages should be avoided, as they add greatly to the expense, and very little to the utility of the instrument for histological work. Binocular arrangements also are of little use for this purpose.

The microscope should be provided with a coarse and fine adjustment. These should be most carefully tested before purchasing the instrument. They should move freely and smoothly, and the slightest turn in either direction should at once alter the focus.

There should be a **reversible mirror,** one side being concave and the other plane. The concave surface is the one usually employed, the plane surface being chiefly used in conjunction with the sub-stage condenser. There should be an **eye-piece** of moderate magnifying power. Very powerful eye-pieces do not reveal additional details, but merely enlarge the image, and with it

any defects that may be produced there by faults in the objective.

At least two **objectives** should be provided, 1 inch and ¼ inch are very suitable, or 3 and 7 of most Continental makers. For bacteriological work, a higher power immersion lens should be added. The objectives should be fixed on to a **double or triple nose-piece** which can now be obtained with any instrument. It should always be used, as it saves much time and allows a section to be thoroughly examined with much ease. Those whose microscopes are not already fitted with this appliance should have one fitted on, and the cost (about 17*s.*) will speedily be repaid by the saving of time and trouble.

For bacteriological work, an arrangement of lenses beneath the stage for focussing the light on the section is required. The most usual form is an **Abbe's illuminator.**

The cost of microscopes vary from about two guineas to a hundred. There are many excellent instruments in the market, and of these several may be mentioned which the writer has found to work satisfactorily.

For students' purposes the following cheaper forms are excellent.

Beck's star microscope. (Fig. 1.)—May be obtained with coarse and fine adjustment, nose-piece, and 1 inch and $\frac{1}{4}$ inch objectives for £5. The quality of the lenses is very high, and it is doubtful if the student can obtain better value for his money.

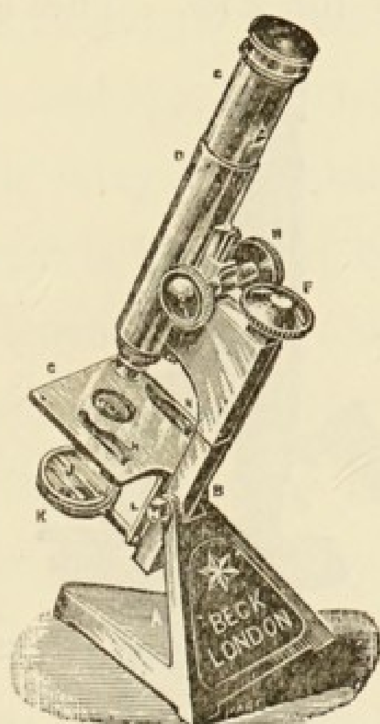

Fig. 1.—Beck's "Star" Microscope.

Leitz puts out two excellent instruments for £3 10*s.* and £5 5*s.* They are of simple construction and the lenses extremely good.

Parkes, of Birmingham, has some almost as good, the prices being £4 to £6.

First class microscopes by Swift, Pillischer, Reichert and Zeiss can be obtained for prices ranging from £8 to £10. They are of uniform excellence.

Among more expensive instruments may be mentioned—

R. and J. Beck's **"Pathological" microscope.** (Fig. 2.)—It is to be obtained fitted up with accessaries for £16. The lenses are excel-

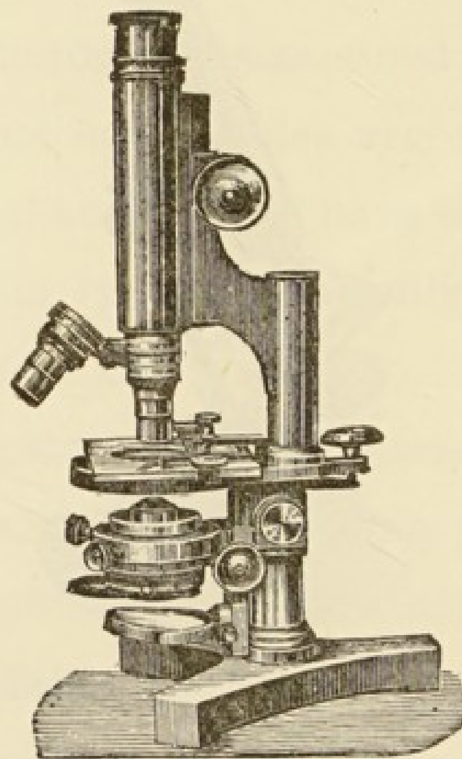

FIG. 2.—Beck's "Pathological" Microscope.

lent, and the sub-stage condenser is extremely good, and its large glass stage with the central "well" will be found most useful.

Zeiss's microscopes may be obtained fitted with high power lenses and accessaries, for from £15 to £25. The quality of these is absolutely reliable.

His agent in this country is C. Baker, of Holborn.

Reichert's microscopes.—Made after the pattern of Zeiss's, and appear to be almost equal to them in quality but are decidedly cheaper. A very useful one is Stand 3B fitted with ocular III. and objectives 3 and 7*a*, and triple nose-piece. This combination may be obtained for about £12 12*s*. His agent is Frazer of Edinburgh.

Oil immersion lenses may be obtained of any of the above makers, prices varying from £3 to £10 and upwards. Before buying them one should always have them most carefully tested by some competent person, as occasionally counterfeits of well-known makes find their way into the market.

CHAPTER II.

Hardening Processes.

For the satisfactory examination of tissues it is necessary that they should be "hardened" in certain fluids. The object of this is not only to give the specimens greater consistence, so that thin sections may be more readily obtained and more safely manipulated, but also to "fix" the tissue element as far as possible in the same relative position as in the living body. The hardening process also acts on the protoplasm of the cells, and prevents their swelling up when placed in water, and in the various staining fluids.

The fluid used must be one which will not itself injure the specimen, and which can be thoroughly removed by washing in water in order that it may not interfere with staining operations. The specimens should be kept while hardening in wide mouthed bottles, on the bottom of which a little cotton wool or tow has been laid. This allows

the hardening fluid to come freely in contact with the under surface of the piece of tissue, and prevents its being flattened against the hard glass bottom.

The hardening fluid requires changing occasionally. This should always be done at the end of twenty-four hours, in order to get rid of any deposit of blood, etc., that may have accumulated. Besides the tissue when placed in the fluid contained a good deal of water which will have diluted it and consequently an early change is desirable. Afterwards the fluid requires to be changed only as often as it becomes turbid, or any deposit occurs, usually about once a week.

While hardening, specimens should be kept in a cool place, as warmth favours changes in the cells, etc.

In manipulating the portions of organs, forceps should always be used and these with great gentleness. One should never impale the specimens with needles, or unsightly holes, which may even be mistaken for pathological appearances, will appear when a section is examined under the microscope.

It requires some practice to know when the tissue is sufficiently hardened. The object aimed at is to make them not really **hard** but **tough.** It is almost unnecessary to add that in testing this with the fingers the utmost gentleness must be observed, or serious damage may be done to the tissue.

When the tissue is sufficiently hardened the hardening fluid must be thoroughly dissolved out. This is best done by placing the specimen all night in a basin of cold water into which a tap is constantly running. The tissue may then be removed, (forceps always being used and never the needle) and placed in an embedding medium as subsequently directed; or if one is not prepared to cut it at once into equal parts of methylated spirit and water, in which it may be kept indefinitely, the fluid being changed if it becomes at all cloudy.

It is unnecessary for ordinary work to have more than the following hardening fluids:—

Müller's fluid.—

Potassium Bichromate	2¼ grms.	...	3½ drachms.
Sodium Sulphate . .	1 grm.	...	1½ drachms.
Water to . . .	100 c.c.	...	1 pint.

Two drachms of carbolic acid are sometimes added to each pint of the fluid but as a rule it is not necessary.

Müller's fluid is the most generally useful of the various fluids employed.

1. It causes very little shrinking of the elements of the tissue, and hence may be employed for most delicate objects, *e.g.* the retina and embryoes.

2. In consequence also of its not making the tissues shrink, it does not squeeze the blood out of the vessels and where the organ has been congested before death, we may, by using Müller's fluid, preserve a natural injection of the capillaries.

3. There is comparatively little danger of over-hardening the tissue and rendering it brittle.

4. Sections of organs hardened in Müller's fluid are usually firm and easy to manipulate. They do not tend to curl up or adhere to one another as much as those hardened in spirit.

5. The fluid readily permeates the tissues, and hence large portions of organs, or even the entire organ may be satisfactorily hardened in it.

6. The fluid is very cheap. A gallon can be made up for about eightpence.

The fluid has however certain slight drawbacks:—

1. The hardening process is a slow one occupying four to eight weeks.

2. The fluid gives a permanent dingy colour to the tissue. This does not cause any inconvenience for microscopic purposes, but it is a disadvantage where one has any idea of putting up the rest of the specimen, *e.g.*, a brain, as a naked eye preparation. In such cases the organ should be hardened in spirit.

Müller's fluid can be used for almost any tissue. It is especially useful for those which contain a large quantity of fluid, or of blood.

To harden a specimen in it at least twenty times the bulk of fluid must be employed.

The fluid must be changed on the third day, and afterwards about every week as may be required.

Methylated spirit is a very useful hardening agent. It hardens in one to three weeks according to the size of the tissue and the quantity of spirit used. Its disadvantages are:—

1. It is more apt to overharden than Müller's fluid.

2. It causes a good deal of shrinking of the tissue and thus squeezes much of the blood out of the vessels.

It is most useful in hardening tissues containing much epithelium, *e.g.* kidney, epithelioma, etc.

Spirit is also frequently employed to complete the hardening by Müller's fluid and to preserve tissues after they have been hardened.

About ten or fifteen times the bulk of spirit should be used for one of the tissues. The fluid should be changed at the third day and afterwards as required.

Müller's fluid and spirit. This is a useful combination for many purposes. It is made thus :— Müller's fluid, three parts ; methylated spirit, one part.

The fluid must be allowed to cool after mixing before being used, and if necessary filtered. It will harden specimens satisfactorily in three weeks.

Picric acid. May be used as a saturated watery solution, especially for skin, etc., more frequently it is used in combination with sulphuric acid.

Kleinenberg's solution. (Fol's formula)—

Picric acid . 1 grm. ... 45 grains.

Water . 100 c.c. ... 1 pint.

Mix and add

Sulphuric acid 6 c.c. ... 4½ drachms.

Filter from the precipitate which forms.

It gives a yellow stain to the tissues which is not entirely removed by washing in water. It is specially useful for delicate structures or those which require rapid hardening, such as embryoes, soft sarcomata, etc.

A large quantity of fluid should be used.

It should be changed early. After hardening the tissues may either be transferred to alcohol which is changed till it no longer becomes yellow, or may be washed in water, and sections cut after soaking it in gum. They are best stained in picrocarmine, but if it is desired to stain them in carmine or hæmatoxyline they must first be treated with a saturated solution of lithium carbonate and then washed thoroughly in distilled water before staining.

A saturated aqueous solution is also sometimes used as a decalcifying agent, especially for the bones of young animals or fœtuses.

Osmic acid. For rapidity of action, and for the way in which it fixes all the tissue elements in their natural position osmic acid is one of the best hardening reagents we possess.

Its disadvantages as a hardening agent are:—

1. Its expense (a 1 per cent. solution costs 3s. per ounce).

2. Its irritating and corrosive vapour.

3. The fact that only small pieces of tissue can be hardened in it, since it rapidly hardens the external surface, and thus prevents itself from penetrating into the centre of the lump.

It is most frequently used as a hardening agent for very delicate structures, such as the retina, or embryoes. It is most conveniently bought as a 1 per cent. solution, or the acid itself may be procured in sealed tubes. These should be broken in a bottle under sufficient distilled water to make a 1 per cent. solution. The bottle containing it should be covered with brown paper to exclude the light. For hardening purposes small pieces of the tissue, not much larger than a pea, should be placed in the acid, the 1 per cent. solution being diluted with five to ten volumes of distilled

water. The tissues may be left in this for from three to five days. They must then be **thoroughly** washed in distilled water and may then be preserved in methylated spirit.

Both the hardening and the subsequent washing must be carried on **in the dark.**

Osmic acid is also a most valuable staining reagent (see later).

Absolute alcohol. Used as a hardening agent where the tissues are to be examined for micro-organisms.

Small pieces must be used. The depth of the block should not exceed $\frac{3}{8}$ inch. The fluid should be changed on the third day. Hardening will be completed in about ten days or even earlier.

Its disadvantage is its expense, as at present it cannot be obtained duty free for scientific purposes. In most cases the student will obtain equally satisfactory results with methylated spirits.

Decalcifying fluids.—Used in the preparation of bone, tooth, osseous tumours, etc. The two best fluids for general use are **Chromic and Nitric fluid.**

This is made as follows:—

Chromic acid	1 gramme	...	45 grains.
Nitric acid	2 grammes	...	$1\frac{1}{2}$ drachms.
Water .	200 c.c.	...	1 pint.

If the bone is not very compact the fluid may be used diluted with an equal quantity of water. A large quantity of fluid should be used, and like all decalcifying fluids, it should be frequently changed.

As soon as the specimen is sufficiently flexible, it should be thoroughly washed in water containing about a drachm of carbonate of lithium to the to the pint, then in pure water, and then transferred to spirit until it is ready to be cut.

Von Ebner's solution.—

Hydrochloric acid	1 gramme	...	$1\frac{1}{2}$ drachms.
Common salt .	10 grammes	...	2 ounces.
Water to . .	100 c.c.	...	1 pint.

It is a very useful decalcifying agent, but causes the fibrous elements to swell up rather more than does the chromic and nitric fluid. A large quantity of the fluid must be used, and it should be changed daily.

CHAPTER III.

Section Cutting.

Embedding of sections. Before sections are made the tissues require to be embedded in some fluid, which will permeate their interstices, and is capable of being rendered firm so as to support the most delicate parts when the knife passes through the tissue.

The four most generally useful substances are—(1) gum, (2) gum and sugar, (3) celloidin, (4) paraffin or wax.

Gum.—Picked colourless gum arabic 2 parts, cold water 3 parts.

Leave with frequent stirring until dissolved. Add ten drops of carbolic acid to each ounce of the mucilage.

Specimens are thoroughly freed from all trace of the hardening fluid by washing in water, and the tissue is then placed in the gum solution for at least twelve hours, or if enough carbolic acid be added, it may be left there for an indefinite time. Gum and syrup is, however, a better preservative medium.

Gum solution made as directed above is the most suitable medium to use with the ether spray microtome. When frozen it forms a firm non-crystalline mass something like cheese, which supports the tissue on all sides, but does not injure the razor as it passes through. It must not be frozen too deeply, or it becomes hard and rather brittle and is apt to injure the razor.

Gum and syrup* was introduced by Hamilton as an embedding and preservative medium. It appears to have a greater penetrating power than simple gum, and so affords a better support to delicate tissues. After thorough washing in water, specimens are placed in the gum and syrup for at least twelve hours, or they may be kept there for an indefinitely long time.

Gum and syrup should always be employed when one is using an ice freezing microtome. It requires to be exposed to a lower temperature than simple gum before it freezes, and in consequence cannot as a rule be used with an ether spray microtome, as except in a cold dry atmos-

* Gum solution . . 5 parts.
Simple syrup . . 3 parts.

phere this will rarely yield a low enough temperature.

For delicate structures Hamilton suggested the addition to the gum, or the gum and syrup, ot an equal quantity of gelatine which had been previously soaked in water for twenty-four hours. The gelatine must be dissolved in the gum, etc. by a gentle heat, such as that of a water bath. The tissue should be plunged in it while fluid and left until it is firmly set. Place the specimen with the medium surrounding it on the microtome plate, and paint some gum round it to fix it. Cut sections in the ordinary way and transfer them singly to glass slides, and wash with distilled water and stain on the slide. If it is desired to remove the gelatine, heat the distilled water gently before washing.

After cutting in gum, or in gum and syrup, the sections are gently removed from the knife into distilled water by a soft camel's hair brush, and left there for an hour or two, until the medium is entirely dissolved out. They may then be stained and mounted, or they may be put away till a more convenient time either in methylated spirit,

or in "preservative fluid," which consists of equal parts of glycerine and water with ten drops of carbolic acid to each ounce of the mixture. They may be kept in either of these fluids for an indefinite time, and then stained and mounted.

Celloidin.—Is in many respects almost an ideal embedding medium. (1) It has great penetrating power; (2) can be made of an admirable consistence for cutting purposes; (3) after sections are made it allows them to be very freely manipulated without fear of injuring them: (4) and being perfectly transparent and homogeneous in thin sections, it does not require to be removed from a section before mounting. It is made as follows:—

Take some pure celloidin ("Scherings" sold in boxes containing an ounce of shavings is very good) and pour on it about eight times its volume of a mixture of absolute alcohol and ether, equal parts. Allow this to stand all night until the celloidin is dissolved. The solution should be made about the consistence of ordinary mucilage.

To embed a specimen, dehydrate it for an hour or two in absolute alcohol; then place it for a

few minutes in ether, and then put it in the celloidin for at least twelve hours. Now take a cork, the end of which is about the same size as the piece of tissue, fix a piece of ordinary writing paper round the cork with a pin, making it project sufficiently beyond it to form a cell in which the specimen may be placed. Now paint in some celloidin to fill up the crevices in the cork, etc. Allow this to become firm. Then remove the tissue from the celloidin with forceps, and place it in position on the cork. Allow it to become fixed and then pour in celloidin until the tissue is covered. Let the cork stand exposed to the air until a film has formed on the surface of the celloidin, then fix the cork in the brass ferule supplied with the microtome and submerge it in methylated spirit, *not* in absolute alcohol which hardens it too much. If the brass ferule is not at hand, some lead or copper wire should be wrapped round the bottom of the cork to make it sink in spirit. It is left in spirit for twenty-four hours, by which time the celloidin will have become semi-opaque and opalescent, resembling white of egg in its appearance and consistence. The celloidin may be made

to set very much more rapidly, in fact almost instantaneously by immersing it in methylated chloroform.

The cork is fixed in the clamp of a microtome and sections are made. The knife used should have a longer blade than that for cutting in gum or paraffin, and should be drawn very obliquely through from heel to point. Without attending to these details it will be found extremely difficult to get thin sections. The celloidin mass must be kept moist from time to time by being brushed with methylated spirit, the knife being kept wetted, and sections brushed off into the same fluid. They may be stained in the same way as sections cut in other media. Aniline dyes, however, must not be used, as they stain the celloidin very deeply. Either Farrant's solution or Canada Balsam may be employed as a mounting medium. In the latter case the section must be transferred from the alcohol to the slide before clarifying, as the oil of cloves rapidly dissolves the celloidin. Some avoid this by using origanum oil or bergamot oil as the clarifying agent.

Many employ alcohol of 95 per cent. strength

instead of absolute alcohol, as the latter dissolves celloidin but not very rapidly.

Celloidin is only slightly stained by alum carmine, picrocarmine, and hæmatoxyline, but is very deeply stained by the aniline colours, which must not be used for sections cut in it.

Celloidin should always be used in Weigert's and Pal's hæmatoxylin process for staining the nervous centres, as it protects the section from being injured in the frequent transference from one fluid to another which is required. The stain is discharged from the celloidin by both the ferricyanide solution and by "Pal's solution."

Wax and Paraffin used to be very much used as embedding agents. For ordinary work, however, gum and celloidin will be found more satisfactory. The advantages they have over these latter are, that much less preparation of the tissue is required. Gum has the great advantage of being removed from the tissue by simple washing in water, and celloidin, while supporting the section after cutting (as wax and paraffin do, and as gum does not), does not require to be removed from the section before it is stained and mounted, like wax or paraffin.

The embedding masses are made as follows :—

1. White wax and olive oil equal parts.

2. Paraffin, five parts ; paraffin oil, one part ; lard, one part.

Dissolve at as low a temperature as possible. Allow to cool and keep the cakes in a cool place. If the weather or climate is very warm, a smaller proportion of the solvent should be used.

The specimen having been dehydrated in alcohol should be placed in a paper cell on the end of a cork (p. 26), and some of the embedding medium melted by as low a temperature as possible poured in. As soon as it is cool, sections may be made. The knife should be wetted with methylated spirit. The sections should be transferred from the knife, singly to a slide if very delicate, or collectively to a capsule containing oil of cloves or xylol to dissolve the paraffin or wax. They must then be transferred to alcohol, then to weak spirit, and then to distilled water, after which they may be stained in the usual way. If the section has been transferred to a slide, these reagents must be added one after the other by means of a pipette.

Microtomes.—After a large amount of prac-

tice, persons with a fair amount of manual dexterity may acquire sufficient skill to be able to cut very satisfactory sections of specimens embedded in wax, by hand. In fact in the Pathological Laboratory of a large German University, until quite recently the use of a microtome was prohibited by the Professor, himself a most distinguished histologist. The amount of time expended before one acquires the necessary skill, and the cheapness and great convenience of the modern microtome have combined to throw hand cutting into the background, and some form of microtome is now almost universally adopted.

Of these there are a very large number in the market, each having special advantages, and often special drawbacks. A few of the more generally useful only will be described. We have microtomes for cutting in gum frozen by ether spray or ice, and those intended for cutting in paraffin or celloidin.

Cathcart's **ether spray microtome.** (Fig. 3). —This, or its more recent modifications (*see* later), is perhaps the most useful and economical microtome for the purposes of the student. Its prime

cost is low, it is small and portable as well as being clean and inexpensive to work with.

It consists of an oak frame which can be firmly clamped on to a table. On this are two narrow parallel supports about two inches high, which are

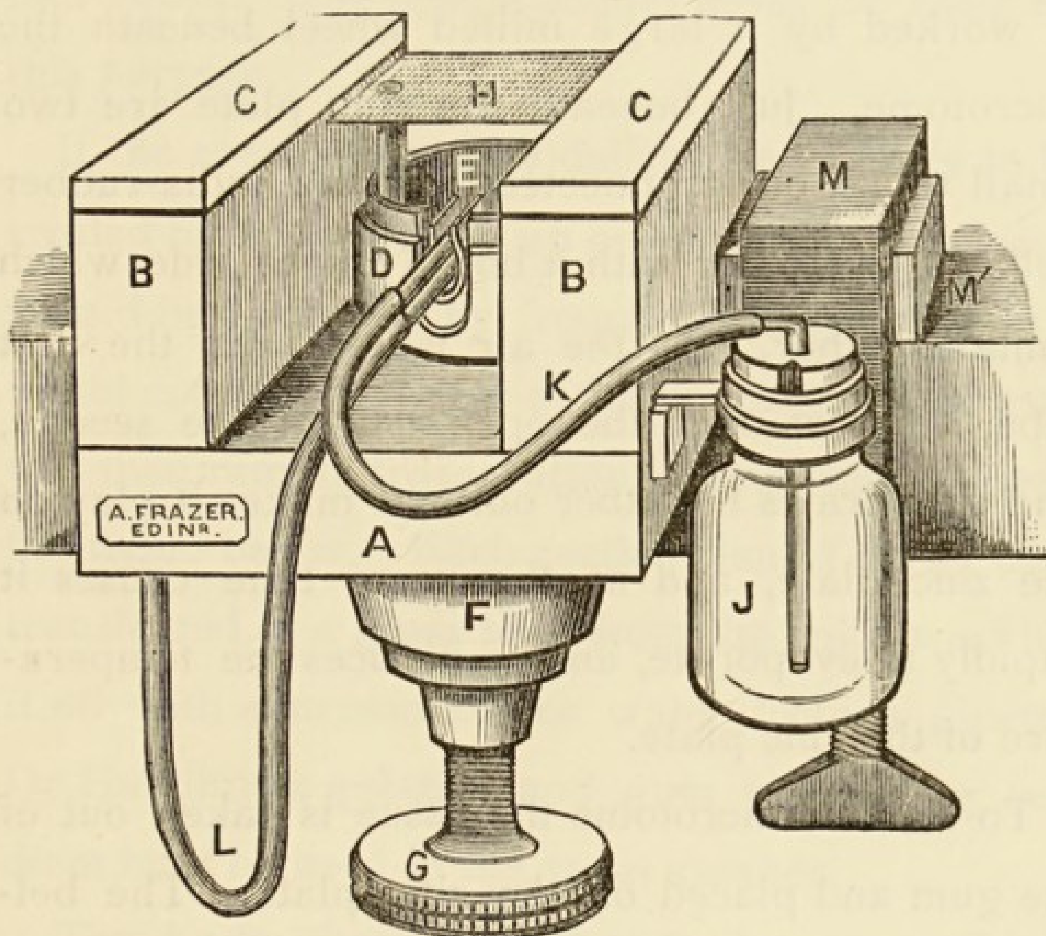

FIG. 3.—Cathcart's Ether Spray Microtome.

A, B. Wooden frame and supports. C. Glass runners. G. Screw for raising the zinc plate H. J. Ether bottle. L. Tube from air bellows.

covered by strips of plate glass, and serve as smooth rests along which the razor may glide in making sections. Between them is a brass well

and in this a zinc plate firmly fixed in the horizontal position, which is almost at the level of the glass runners. It is capable of being raised or lowered through about $\frac{3}{8}$ inch by means of a screw with a very fine and accurate thread. This screw is worked by a large milled wheel beneath the microtome. Just beneath the zinc plate are two small tubes, one connected with an india-rubber bellows, the other with a bottle at the side which contains ether. As the air issues from the first tube, it passes over the open end of the second, and thus draws the ether out and makes it play on the zinc plate, and at the same time causes it rapidly to evaporate, and so reduces the temperature of the zinc plate.

To use the microtome the tissue is taken out of the gum and placed on the zinc plate. The bellows are then worked until the gum on the zinc plate is completely frozen. The plate should be lowered by means of the screw until the surface of the piece of tissue is on a level with the glass runners. These and the razor should then be wetted with water. The razor being held firmly in the hand is pushed along the glass runners in a

rather oblique direction. The plate should then be raised by turning the screw below through a very small arc, another section taken off and so on. Sections are carefully removed from the razor to a vessel of water by means of a soft wet camel's hair brush. The needle should never be used for this purpose.

If the specimen is very delicate, and likely to be spoiled by being curled up on the knife, the latter should be kept cold by frequently dipping it in a vessel containing lumps of ice in water. The gum will then remain frozen after cutting, and support the tissue better. Each section should be at once transferred to a glass slide from the knife, washing it off with a stream of ice water from a pipette. Or Hamilton's gelatine and gum embedding medium may be used in the same manner.

The knife that is used may be an ordinary razor, preferably with the edge ground flat. It requires to be held steadily with both hands. As this is rather inconvenient, Dr. Sheridan Délépine suggested the employment of an ordinary plane iron such as is used in a carpenter's plane. This only requires one hand, and the other can be kept

on the head of the screw beneath to raise the plate at once after each stroke of the knife. Its disadvantages are that it is rather heavy for prolonged working, and that it is so narrow that it cannot be passed very obliquely through the tissue.

There is a very useful modification of this instrument where the glass runners are replaced by a glass plate with a central hole for the zinc plate. The glass plate is large enough to allow the "Swift's plough" to be used (*see* p. 38). This form is more expensive, and there is in addition the cost of the plough.

A. Frazer has recently introduced a valuable improvement in the Cathcart microtome (Fig. 4).

In this the brass frame carrying the zinc plate and ether spray tubes is surrounded by a brass cylinder, in which it fits accurately, and is pushed up as desired by turning the screw beneath the instrument. This brass frame and with it the zinc plate, etc., can be easily drawn altogether out of the outer tube, and replaced by a second brass well, which exactly fits its place and can be raised by the screw as desired. In this is a small toothed clamp which can be screwed up so as to hold

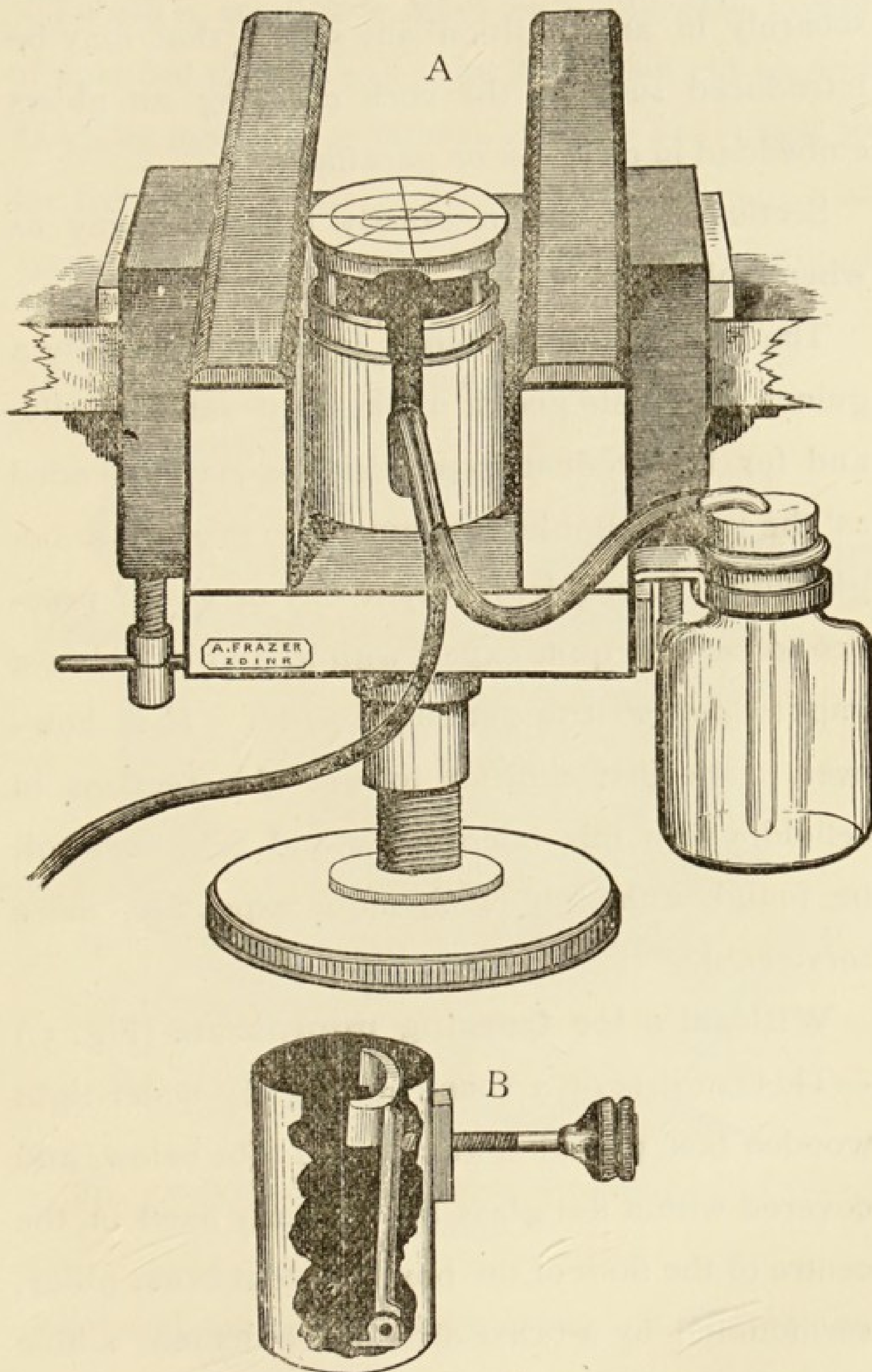

FIG. 4.—Frazer's Modification of Cathcart's Microtome.
A. Microtome arranged for ether spray. B. Cylinder with clamp for holding object embedded in celloidin, &c. to replace ether spray apparatus.

securely in any position any object that may be introduced, such as the cork carrying an object embedded in celloidin or paraffin.

Sections are cut in exactly the same way as when embedded in gum.

The cost of the combined instrument is only a guinea complete and it works most satisfactorily, and for the student may safely be recommended as the most suitable instrument to procure to begin with. It requires only a day or two's practice to become quite expert with it, and it is almost impossible for it to get out of order. It is, however, somewhat difficult to get *thin* sections in celloidin with this microtome, and Schanze's will be found, although much more expensive, more serviceable.

William's ice freezing microtome (Fig. 5.) —This consists of a round mahogany water-tight wooden box, provided with an exit tube below, and covered with a flat glass lid. Firmly fixed in the centre of the floor of the box is a stout brass pillar, surmounted by a brass disc, which fits into a hole in the centre of the glass lid, and is almost on a level with its surface.

To use it, the box is filled with alternate layers of pounded ice and salt; the lid is then put on and fixed by means of a lateral screw. The tissue to be frozen is gently removed from the gum and syrup, and placed on the brass disc and plenty of

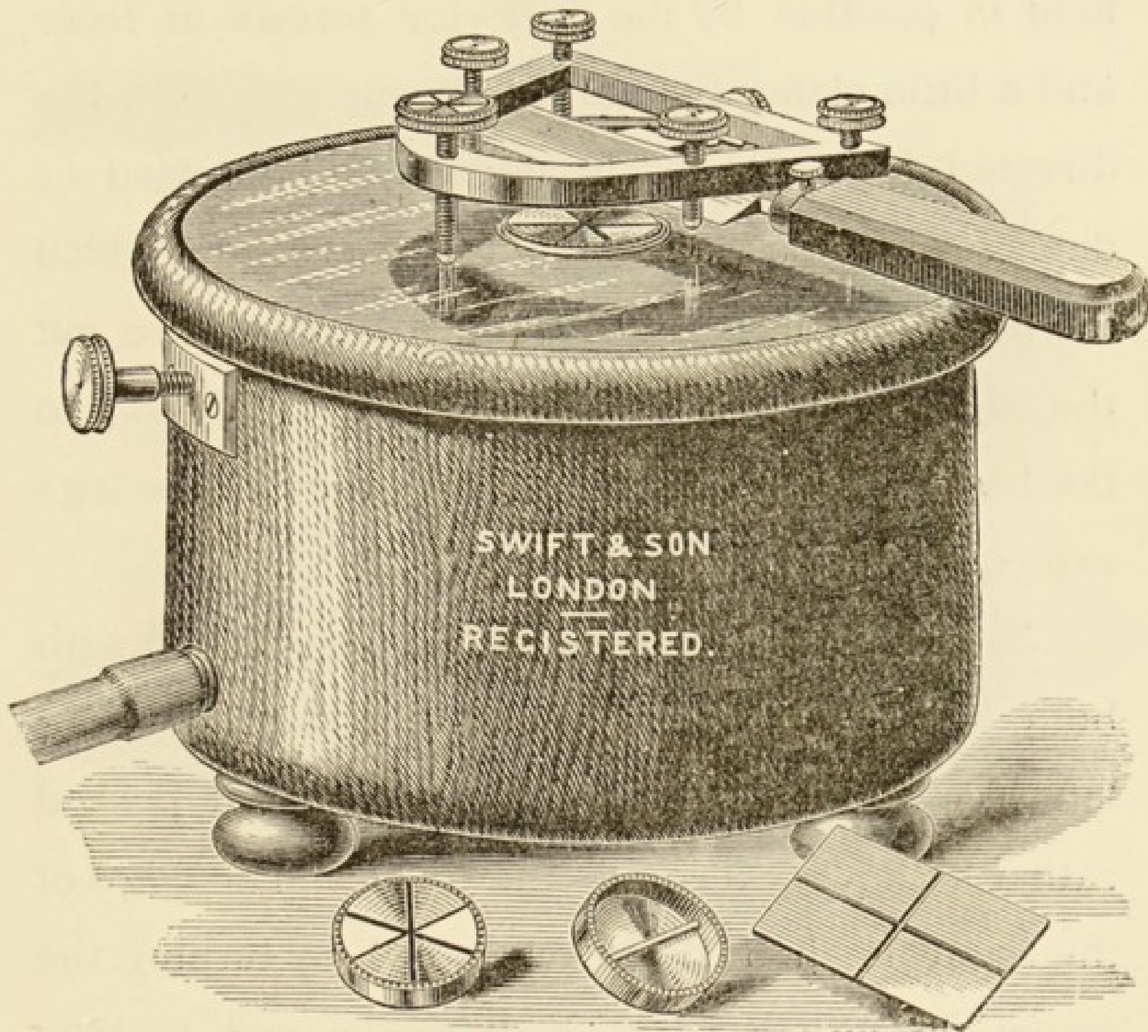

FIG. 5.—William's Ice Freezing Microtome with Swift's "Plough."

gum and syrup painted round it. It should then be covered with a tin cap for a few minutes until frozen.

Sections should be made with a **Swift's Plough.** This consists of a triangular brass frame, supported on three legs, each of which is a screw, tipped with ivory. There is one screw in front and two behind. Beneath the plate, and held in position by the posterior screws in front and a little clamp behind, is a razor with the edge directed forwards. The edge can be raised or depressed by turning the anterior screw, on which the frame is supported. Before sections are cut the edge of the razor should be brought down to the level of the tissue, taking care that all the legs are equal in length.

The plough should then be firmly grasped with both hands, (the index finger of one hand being left free to turn the anterior screw) and pushed rather obliquely through the tissue. The edge of the razor is then slightly lowered by turning the screw through a very small angle, and another section made and so on.

With a little practice very thin uniform sections may be made with great rapidity. Where a large number of sections have to be made for class purposes, etc., it is one of the best forms of freezing microtome to employ.

Schanze's microtome (Fig. 6) is the pattern used in the Leipsic laboratories. It consists of a heavy iron frame with a large base. The knife is carried in a clamp which slides along the full length

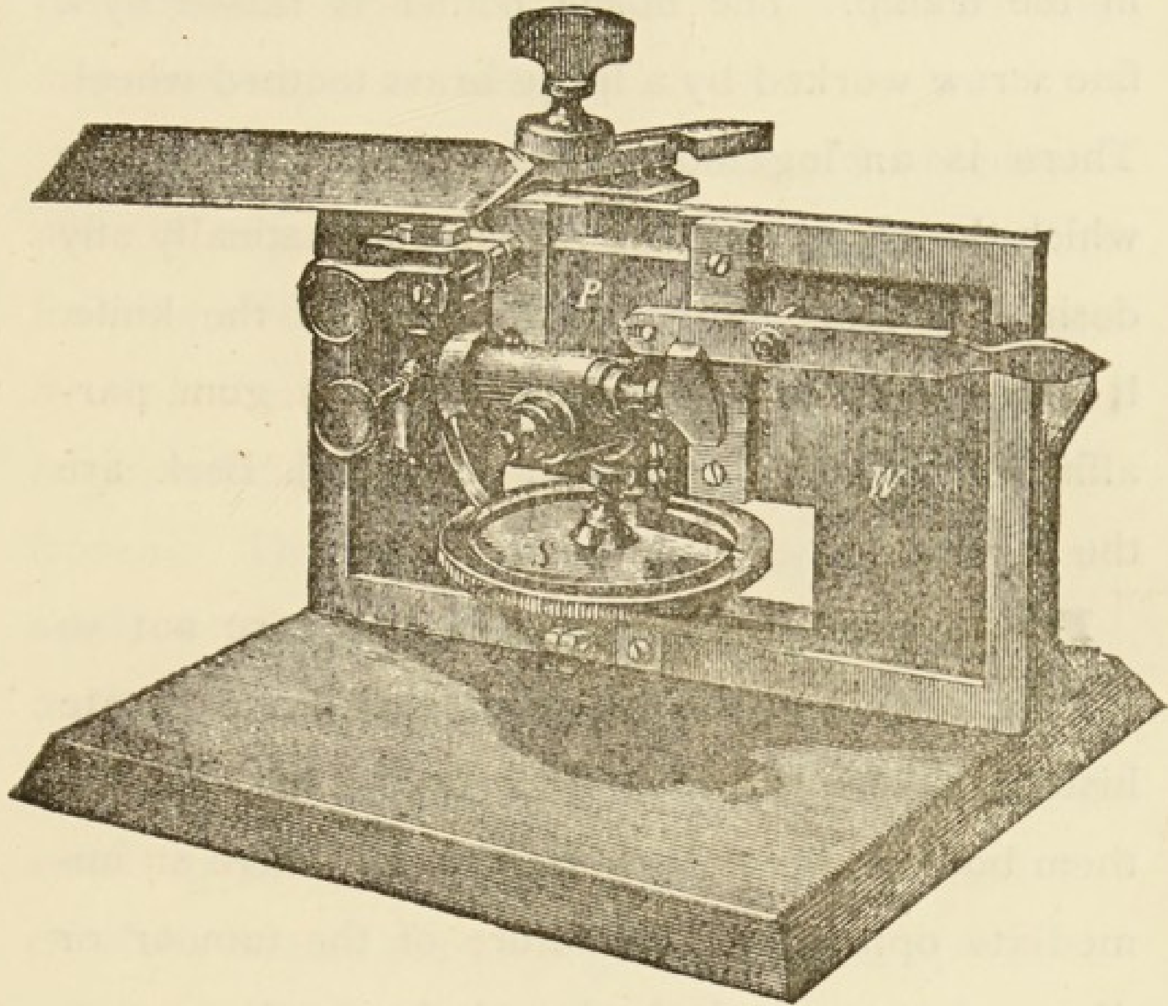

FIG. 6.—Schanze Microtome (see text).

of the instrument, gliding upon two smooth plates of iron which are arranged at an angle to one another. There are several object holders, which can be removed and interchanged, one connected with an ether spray apparatus, another suitable

for holding an object embedded in paraffin, and a third for grasping an object embedded in celloidin. When celloidin is employed, a specially long knife must be used, and it must be fixed very obliquely in the clamp. The object holder is raised by a fine screw worked by a large brass toothed wheel. There is an ingenious ratchet arrangement, by which the object may be raised automatically any desired distance, after each stroke of the knife. It gives most satisfactory results with gum, paraffin, or celloidin. (Messrs. R. and J. Beck are the agents).

Fresh sections.—Although these are not so satisfactory as hardened specimens for accurate histological work, it is often very useful to make them both in the post-mortem room where an immediate opinion of the nature of the tumour or diseased organ is desired, and also in the operating theatre. With a little practice sections may be cut, stained, and mounted, within ten minutes of the removal of the specimen from the body. In this way important information may be afforded to the operating surgeon, and in not a few cases it has caused the proposed treatment to be entirely

altered. Thus in one case a supposed chronic periostitis was shewn to be a sarcoma, and the limb was amputated. In another, a supposed sarcoma of the thigh was found to be a gumma of the muscles when a portion was removed and microscopically examined.

A portion of the specimen should be placed without any preparation on the zinc plate of the freezing microtome and some gum painted round it. It is then frozen. The serum in the tissues is not in sufficient mass to injure the knife when it is frozen. The knife should be wetted with, and sections transferred to, either pericardial serum or $\frac{3}{4}$ per cent. solution (70 grains to the pint) of common salt, neither of which causes the cells to swell up as plain water does. They should be carefully floated out on a glass slide, an operation which requires much more patience than with hardened sections, as fresh sections are less coherent and also more sticky, so that the edges tend to curl up on the knife, etc. They should then be examined, one unstained, simply mounted in salt solution; another stained with picrocarmine and examined in the saline solution; and a third

stained in picrocarmine, mounted in Farrant's solution, and preserved. The last usually gives the best results, the picrocarmine staining becoming quite brilliant after a week. The glycerine, however, is apt to make the sections shrink a good deal, and the weight of the cover-glass tends to break up the unhardened section.

Bubbles in fresh sections are almost inevitable. The only safe way to remove them, is to place the watch glass containing them under the receiver of an air pump. This is rarely available, and it is better to put up with them than to risk spoiling the section by placing it in alcohol, which makes the elements of the tissue shrink and often causes a fresh section to curl up into inextricable folds.

CHAPTER IV.

Staining Methods.

Much information may be obtained from unstained sections, and in most cases one section should be examined unstained, but in the majority of cases they are so transparent that it is difficult to study the details of the section. They are therefore usually prepared by treating them with some staining reagent, not merely to render them less transparent, but also to "differentiate" the elements of the section, by staining one part more deeply than another or of a different colour.

Thus hæmatoxylin stains the nuclei and rapidly growing parts of the tissue, leaving the formed material, as a rule, much more lightly tinted. Methyl violet again stains healthy tissues blue, and parts affected with waxy degeneration a red-violet colour. By combining stains also much differentiation of the tissue elements may be ob-

tained. The following are the most useful stains for general use:—

Sections should usually be stained with several as their effect on individual specimens varies a good deal.

Lithio-picrocarmine.

Prepared as follows:—

Carmine . . .	2.5 grms.	...	10 grains.
Saturated solution lithium carbonate	100 cc.	...	1 oz.

Dissolve, and add

Saturated solution picric acid	250 cc.	...	$2\frac{1}{2}$ oz.

Add a few drops of carbolic acid to each ounce.

It should stand a day or two in sunlight and then be filtered.

It should be kept in a stoppered bottle with a glass rod fused into the stopper.

When sections are to be stained with it, they are to be floated out on a clean glass slide as described later (p. 69). The slide should then be tilted to allow the water to drain off, and the slide should be wiped dry round the section. A drop or two of the stain should then be transferred to

the slide, which should be left lying quite flat for about ten minutes. Unless the room is very warm it is advisable to heat the slide very gently over a spirit lamp, as this causes the tissues to stain more brightly and more rapidly.

The excess of the picrocarmine should be allowed to run off the slide, and the latter wiped. Some of the stain should, however, be left on the section, as its effects go on increasing, and are often not fully seen until a few weeks have elapsed. They should be mounted in Farrant's medium. As a rule those mounted in Canada balsam do not give such good results. Should there be special reasons for using this medium, as in mounting spinal cord sections, etc., they should be dehydrated in an alcoholic solution of picric acid, (one part of a saturated alcoholic solution to five of alcohol), before clarifying in oil of cloves, as otherwise the alcohol will dissolve out the picric acid, and the differential staining effect will be lost.

The nuclei should be stained a bright crimson, the protoplasm of the cells yellow, or a dull pink, the fibrous elements a bright pink, red corpuscles

green, and all dead material, *e.g.*, caseous matter, bright yellow. It also stains nerve-cells, and the axis cylinders of nerve fibres very brightly.

Lithio-picrocarmine is more certain and satisfactory in its results than other forms, and is much more speedily made up. In the following pages when picrocarmine is mentioned, lithio-picrocarmine is always referred to.

Logwood.—This or its purified principle hæmatoxyline is almost as useful as a general stain as picrocarmine. The hæmatoxyline itself is preferable, giving more constant results, and less diffuse staining, but where large quantities are used it is much cheaper to employ the ordinary logwood extract.

For general staining purposes the two following will be found to give excellent results:—

Hæmatoxyline. **Schuchardt's formula**—

(*a*) Hæmatoxyline .	3 grms.	...	30 grains.
Absolute alcohol	16 c.c.	...	2½ drachms.
(*b*) Pure alum . .	3 grms.	...	30 grains.
Distilled water	100 c.c.	...	2 ounces.

Add (*a*) to (*b*) slowly and with constant agitation. Keep for some days exposed to diffuse daylight

until its colour is so deep that it will not transmit the light. It should then be filtered, and a few drops of an alcoholic solution of thymol added. It will not give very satisfactory staining reactions during the first week or two, and should be allowed to stand a month or six weeks before using. Whenever hæmatoxyline is made up with alum as in the above formula, an abundant reddish-brown precipitate forms after some time. This in no way interferes with the activity of the solution, but it must always be filtered before being used.

Barrett's formula.—Introduced by Dr. W. H. Barrett, of Edinburgh. It gives almost as good results as the above. It is made from ordinary English extract of logwood.

The extract should be dried, and finely powdered, and then extracted with absolute alcohol for several days.

Powdered extract of logwood 20 grms. ... 2 ounces.
Absolute alcohol . . . . 30 c.c. ... 3 ounces.

Filter and add slowly to

Alum . . . . 3 grms. ... 2½ drms.
Distilled water . . . . . 100 c.c. ... 10 ounces.

The strength of the solution will vary with

different samples of logwood and must be estimated by trial. This solution is comparatively cheap and is useful for class purposes.

In making up these solutions care must be taken that only distilled water is used, and that all the vessels employed have been previously rinsed out with it, otherwise precipitation of the hæmatoxyline will occur. In staining with it a watch glass is filled with distilled water and about half a dozen drops of the solution filtered into it. It is impossible to give exact directions as to quantity, as the strength of the reagent varies with the quality of the hæmatoxyline, the age of the solution, and so on. Sections may be left in it for twenty minutes or half an hour. This is usually much more satisfactory than more rapid staining with a stronger solution.

Should the section be overstained, this may be remedied by washing it in a half per cent. solution of acetic acid, until sufficient of the stain is discharged.

Hæmatoxyline stains the nuclei of the cells a beautiful violet colour, and also tints, more or less lightly the cell protoplasm and the fibrous ele-

ments. It also stains the axis cylinders of nerves, and is much used in special staining of the nerve centres as will be described later.

The stain is permanent. Sections may be mounted either in Farrant's solution, or in Canada balsam, the latter being preferable.

(For Weigert's and Pal's hæmatoxyline methods for investigating the nervous system see pp. 66, 67).

Eosine.—The writer has found much more satisfactory results from the commercial eosine (used in dyeing and in the manufacture of red ink), which occurs as an orange amorphous powder, than from the pure crystalline form.

It may be used as an aqueous solution ($\frac{1}{30}$ per cent.) or as solution in absolute alcohol ($\frac{1}{15}$ per cent.). Sections stained in the former should be passed through a one per cent. solution of acetic acid in order to "fix" the stain, and then washed in distilled water.

It is a very transparent stain, and the most delicate details of a section stained with it are perfectly visible.

It stains the nucleus but slightly, while it stains the cell protoplasm and fibrous tissues

and especially muscular tissues a beautiful rose colour.

It will be seen, therefore, that it stains those parts which are left unstained by hæmatoxyline, and vice versâ. This complementary action is applied in the following method.

Double staining with eosine and hæmatoxyline.—Sections having been stained in hæmatoxyline in the ordinary way, are washed in distilled water, and dehydrated in a solution (about 1 in 1500) of eosine in absolute alcohol. It should remain in this for about two minutes, and then be passed through oil of cloves and mounted in Canada balsam in the ordinary way.

This gives extremely useful and beautiful results with almost all tissues, and is equal if not superior to picrocarmine for differentiating the tissue elements. Thus, the nuclei are stained violet, the cell protoplasm a much paler and warmer violet, the fibrous tissues pink, and red blood corpuscles orange or brick red.

The alcoholic solution of eosine is also used as a contrast stain, after staining for micro-organisms with blue or violet dyes (*see* later).

Carmine.—Almost superseded now by picro-carmine and alum carmine.

It is made as follows :—

Carmine (best) .	2 . . .	1 drachm.
Strong ammonia	2 . . .	1 drachm.
Distilled water .	100 . . .	6 ounces.

Rub the carmine with a little water in a mortar, add the ammonia, when the liquid will turn black. Gradually add the rest of the water, rubbing it up all the time. It should be bottled, allowed to stand for a few days, and then filtered, and a piece of camphor put in the bottle.

Sections may be sufficiently stained in this fluid in from three to five minutes, but more satisfactory results are to be obtained by diluting it with twenty times its bulk of distilled water, and leaving sections in it for several hours.

After staining in carmine the sections must be passed through a half per cent. solution of acetic acid, in order to fix the carmine in the tissues, as otherwise the water will dissolve the stain out.

Borax carmine—

(*a*)	Borax . .	4 grms. . . .	3 drachms.
	Distilled water	100 c.c. . . .	5 ounces.
	Carmine . .	2 grms. . . .	$1\frac{1}{2}$ drachms.

Dissolve with the aid of heat and add slowly to (*b*).

(*b*) Alcohol . . 70 c.c. . . 3½ ounces.
Distilled water 30 c.c. . . 1½ ounces.

Allow to stand for a fortnight. Filter, and add a lump of camphor.

To use it, place sections, or the tissue in bulk, in it for twenty-four hours, according to size, and then transfer to alcohol (seventy per cent.) containing a drop to the ounce of hydrochloric acid, for twenty-four hours, and then wash thoroughly in water. It may then be placed in gum if it is to be frozen, dehydrated in alcohol if paraffin or celloidin is to be employed.

Its advantage is that it is very diffusible, and so can be used to stain large portions of tissue. It takes a considerable time to stain sufficiently deeply.

It stains nerve-cells and axis cylinders brightly, and also the connective tissue, bringing a sclerosed patch out very prominently.

Alum carmine—

Alum five per cent. solution in distilled water } 100 c.c.... 1 ounce.

Carmine pure 1 grm....4½ grains.

Boil for twenty minutes. Filter. Add a few drops of carbolic acid.

In using this re-agent it should be filtered into a watch glass, and the sections placed in it for at least an hour. There is no fear of overstaining, and they may be left all night. After they have been stained they must be thoroughly washed in water to remove the alum, as otherwise numerous crystals of it will be seen in the field when the section is mounted. Sections may be mounted in Farrant's solution or in Canada balsam. It improves very much after a few days.

If desired its staining action may be complemented by dehydrating it in an alcoholic solution, either of eosine (1 in 1500) or of picric acid (*see* p. 45), and then clearing up in oil of cloves, and mounting in Canada balsam.

By itself it gives a stain very like that of hæmatoxyline, only warmer. It picks out the nuclei and axis cylinders of nerves, stains cell protoplasm slightly, and the fibrous elements scarcely at all.

It may be used for the same purposes as hæmatoxyline. The colour is less attractive, and not so deep as that of the latter, but its property of not

overstaining sections, even when left in it for a couple of days, is a very useful one.

Osmic acid is invaluable for staining fatty particles in the cells. The one per cent. solution should be obtained and kept in the dark.

For ordinary use it should be diluted with ten times its bulk of distilled water, and sections stained in it all night in a dark cupboard, or the watch glass containing them may be placed inside a small box.

The sections must be washed **thoroughly** in plenty of water. If desired they may be stained in picrocarmine or methyl violet if waxy degeneration also be present. Sections should be mounted in Farrant's solution, as Canada balsam usually gives disappointing results.

It demonstrates the most minute fatty particles in degenerating cells, etc., staining them black. It may be employed to demonstrate the globules of fat blocking up the vessels in fat embolism.

It stains the myelin sheaths of nerves black, and will be again referred to when speaking of methods of staining the spinal cord.

Nitrate of silver is employed for staining the

intercellular cement of epithelial cells. It stains this substance a deep black, while the rest of the tissue takes on a brown colour. It is used as a half per cent. solution in **distilled** water, and kept in a stoppered bottle carefully covered up with brown paper. To use it take some epithelial tissue, *e.g.*, the omentum from a recently killed animal, or a section of some epithelial tumour, immediately after excision. Wash thoroughly in distilled water to remove all chlorides, and then place in a watch glass containing the silver solution. Keep this in the dark for half an hour and then wash thoroughly in plenty of water. The section should be mounted in glycerine or Farrant's medium and kept from the light or it will become too darkly stained.

Chloride of gold is employed to demonstrate the peripheral terminations of nerves. It can only be employed within the first half hour after the tissue has been removed from the living body. The pieces of tissue must be small and may be stained in bulk, sections being subsequently made.

A half per cent. solution in distilled water is employed. The tissue is transferred to this on its

removal from the body, until it becomes lemon coloured. It is then exposed in a one per cent. solution of acetic acid to a strong light until it assumes a purplish tinge, which takes from two hours to two days. Sections should be mounted in Farrant's medium. It stains the cells of the tissue, and nerve-cells reddish purple, and nerve fibrils, especially the terminal ones, rather more violet. This is very well seen in the cornea.

It is useful sometimes for clinical purposes to excise a portion of muscular tissue and examine the nerve endings by this method. Unfortunately the stain is somewhat uncertain in its action.

Methyl violet.—A very satisfactory solution may be obtained ready made in the "telegraphen tinte," prepared by Leonhardi, of Dresden, as recommended by Woodhead. It may also be used as a one per cent. solution in distilled water, a few drops of carbolic acid being added to prevent the growth of fungi. It is a very useful selective stain. It gives two reactions, red violet, and blue violet. Thus it stains the matrix of hyaline cartilage blue violet, but the cells red violet.

It has also a most important pathological application, as it picks out any parts which have undergone "waxy" or "lardaceous" degeneration, staining them red violet, but the rest of the section blue violet.

About ten drops of a one per cent. solution should be filtered into a watchglassful of water, and the sections stained for about five minutes. They must then be passed through a half per cent. solution of acetic acid and washed **thoroughly** for some time in a large quantity of water till no more colour comes away.

If these steps are not taken with care, the dye will diffuse out after the section has been mounted, blurring all details and spoiling the appearance of the section.

Sections must be mounted in Farrant's solution (to which a spot of formic acid may be added), not in Canada balsam, as the alcohol and oil of cloves both rapidly dissolve out the dye.

Magenta.

1. Woodhead's formula for ordinary purposes.

Magenta . . .	1 grm.	...	$1\frac{3}{4}$ grains.
Rectified spirit .	20 c.c.	...	3 drachms.
Distilled water to	200 c.c.	...	4 ounces.

Is specially useful for staining fresh tissues.

2. Rutherford's formula.

Magenta . .	25 grms.	...	1 grain.
Distilled water	37 c.c.	...	2½ drachms.

Dissolve and add

Rectified spirit	12·5 c.c.	...	1 drachm.
Glycerine . .	50 c.c.	...	3½ drachms.

A most useful stain for fresh specimens of blood, as the glycerine gives it almost the same specific, gravity, and hence it does not cause the red corpuscles to swell and rupture as simple aqueous solutions do.

Safranine—Employed as a freshly made saturated solution in aniline oil water warmed to 60° C. (140° F.). Filter into a watch glass. Stain for not more than a minute. Dehydrate in alcohol which will remove much of the stain, clarify in oil of cloves or origanum oil. Mount in balsam.

Another method is to stain for about ten minutes, and then leave for a minute in Gram's iodine solution. The sections are then washed in alcohol, dehydrated, clarified in oil of cloves, and mounted in balsam. By these methods the stain is

withdrawn except from certain elements, *e.g.*, those undergoing colloid or calcareous degeneration.

A quarter per cent. watery solution is sometimes employed as it stains nucleoli and actively dividing nuclei very brightly, while the rest of the cell is stained faintly. It may be employed to study karyokinesis in the cells of a rapidly growing cancer.

Although bacteriology is now in itself a special study, a short description of the general methods of staining micro-organisms in sections is given here. Those who require to do serious work in this direction are recommended to master Prof. Crookshank's *Bacteriology* from which the following account is mainly taken.

The following solid dyes should be procured:—

Gentian violet.

Methyl blue.

Methyl violet.

Bismarck brown.

Fuchsine.

For bacteriological work the following solutions of the above are necessary:—

1. Saturated alcoholic solutions of the above, which may be kept in stoppered bottles.

2. One per cent. watery solutions of the same, which must be freshly made just before using, as micro-organisms are very apt to develope in them.

An alkaline solution of methyl blue prepared thus:—

Saturated alcoholic solution	2 c.c.	...	4 minims.
Potash solution (10 per cent.)	1 c.c.	...	2 minims.
Distilled water	200 c.c.	...	1 ounce.

Should be freshly made before using.

Aniline oil water.—Made by shaking a little aniline thoroughly with distilled water for some time, and then filtering the emulsion.

Gram's solution.

Iodine	1 grm.	...	1½ grains.
Iodide of potassium	2 grms.	...	3 grains.
Distilled water .	300 c.c.	...	1 ounce.

Half saturated solution of carbonate of potash.

Nitric acid 10 per cent.

Sulphuric acid 1 in 8.

The following are the general methods of employing reagents for the purpose of staining organisms in sections. Special methods are required for special organisms, but one or two only can be given.

1. **Weigert's method.**—The sections must be placed in a freshly made one per cent. aqueous solution of methyl violet, gentian violet, fuchsine, etc. The solution may be kept at the temperature of the body in an incubator. The organisms will often stain more readily if the section be passed through a 1 in 2000 solution of corrosive sublimate before putting it into the staining fluid. After staining the section is washed in distilled water and then in methylated spirit until it appears almost decolorised. A contrast stain may then be used, such as picrocarmine, after which the section may be mounted in Farrant's medium: or a weak solution of another aniline colour, after which the section is clarified in clove oil, and mounted in balsam dissolved in xylol. The section must not be left too long in the oil of cloves which discharges the stain from the organisms after a time.

Some prefer to decolorise the tissues by washing in a half per cent. solution of acetic acid instead of methylated spirit.

Gram's method.—Place some aniline oil in a test tube and put in some distilled water. Close the end with the thumb and shake very thoroughly.

Filter into another clean test tube, and add ten drops of a saturated solution of gentian violet or some similar dye. Filter the mixture into a watch glass. Stain sections in it for from three minutes to half an hour. Wash in distilled water, and transfer to Gram's iodine solution until they become dark brown in colour, usually in a few minutes. They are then decolorised in absolute alcohol. This often takes some time. It may be hastened, as Crookshank suggests, by placing the section in clove oil, retnrning to alcohol, and so on. As soon as it is decolorised it may be treated with a contrast stain, the most suitable being alcoholic solutions of eosine or bismarck brown if a blue stain have been employed, or methyl blue if fuchsine has been the first stain used.

The following will be found the most useful stains and contrast stains:—

Stains.	Contrast Stains.
Gentian violet. Methyl violet. Methyl blue.	Picrocarmine. Eosine. Bismarck brown. Safranine.
Magenta. Fuchsine.	Methyl blue.

And *vice versâ.*

Much practice is required in using either of these methods before one can judge accurately how long to leave sections in the staining reagents or decolorising agents, and the beginner must not be discouraged if at first he is unable to obtain good results although he follows the book directions most minutely.

Ehrlich's method for tubercle bacilli.—Sections are stained for six to twenty-four hours in a one per cent. solution of gentian violet, methyl violet, methyl blue or fuchsine. They will stain more rapidly if the staining fluid be kept at the body temperature. They should be removed from the staining fluid, and washed in distilled water, and then transferred (preferably on an aluminium section lifter) to a ten per cent. solution of nitric acid in distilled water until they are nearly decolorised. They should then be passed through lithium carbonate solution, and thoroughly washed in distilled water. They should then be treated with some suitable contrast stain (p. 62) and mounted in Canada balsam.

Neelsen's stain for tubercle bacilli.—

Fuchsine . . . . 1 grm.... 4¼ grs.
Sol. carbolic acid (5 per cent.) 100 c.c. ... 1 ounce.

Dissolve and add

Alcohol . . . . 10 c.c. ... 45 minims.

The solution will keep only for about a fortnight. Sections are placed in this solution (which should be warmed) for ten minutes to half an hour. They are then decolorised by passing them into a solution of sulphuric acid. Twenty-five per cent. is the strength originally recommended, but a ten per cent. solution does equally well and injures the section less. They are then thoroughly washed in distilled water, and afterwards may be treated with a contrast stain.

Gibbes' double stain for tubercle bacilli.—

(1) Rosaniline hydrochlorate 2 grms ... 25 grs.
Methyl blue . . . . . 1 grm. ... 12·5 grs.

Triturate in a glass mortar,

(2) Aniline oil . . . . . 3 c.c. ... 37·5 grs.
Rectified spirit . . . . 15 c.c. ... 3½ drms.

Dissolve and add slowly to (1).

(3) Lastly add slowly to the mixture
Distilled water . . . . 15 c.c. ... 3½ drms.

Some of the solution is filtered into a watch glass and warmed. The sections are placed in it and left for some hours. They are then washed

in methylated spirit till they are sufficiently de-colourised, and then rapidly passed through absolute alcohol and oil of cloves and mounted in balsam and xylol. It is a very useful stain for examining the sputum for tubercle bacilli.

In order to stain fluids, such as blood, pus, or sputum, for organisms, a very thin layer should be obtained by placing a little of the fluid between two clean cover glasses and pressing them together. They are then separated and allowed to dry. The film is fixed by holding the cover glass in a pair of forceps, and passing it slowly through the flame of a spirit lamp two or three times. When it is cool it should be floated with the film downwards on the staining fluid in a watch glass. After staining it is decolorised, and "contrast stained" in exactly the same way as sections. It is sometimes difficult to tell which side of the glass is the one that has the film. This is best done by looking at the glass obliquely, so as to get a reflection from a window. The side which is coated will appear rough, while the other is bright and smooth. It will also help to avoid confusion if one blade of the forceps be marked, and

this blade always correspond to the coated slide of the cover glass.

Staining the nerve centres.—The following account of the special processes now most in vogue for staining the nerve centres may be found useful.

Weigert's hæmatoxyline method.—The tissue should be thoroughly hardened in Müller's fluid. If it have been hardened in spirit it must be kept in Müller's fluid for a week, previous to cutting sections. They should be transferred direct from the Müller's fluid to alcohol, and dehydrated, and then embedded in celloidin before the bichromate has been entirely removed. As soon as sections are cut, they are transferred to Weigert's hæmatoxyline solution.

Hæmatoxyline . .	1 . .	4 grains.
Alcohol	10 . .	45 minims.
Distilled water . .	100 . .	1 ounce.

It should not be more than a month old.

They are stained in this until they are quite black, which takes from six hours to two days, according to the temperature. The most suitable temperature is that of the body. After they

are sufficiently stained they are transferred to Weigert's differentiating solution.

Borax.	2 . .	8 grains.
Potass. ferricyanide .	2·5 . .	10 grains.
Distilled water . . .	100 . .	1 ounce.

They may be kept in this solution for half an hour to two hours, according to the depth of the stain. The solution discharges the blue colour from all the tissues except the medullated nerve fibres. These are left as blue-black streaks. The nerve cells are stained a dark brown, and the neuroglia a yellowish brown. Patches of sclerosis are also stained light brown.

Sections may then be stained with eosine or picrocarmine if desired.

Sections will often stain more satisfactorily if they are transferred, as soon as they are cut, to a half saturated solution of acetate of copper in distilled water. They should remain in this for twenty-four hours, and then be passed through distilled water and stained in hæmatoxyline as before.

Pal's method.—Weigert's method has been modified by Pal. In this method treatment with

acetate of copper is not used. The sections are treated with a solution of hæmatoxyline, prepared by adding an equal bulk of distilled water to Weigert's solution. Just before using it a few drops of a solution of lithium carbonate in distilled water are added, which turn the fluid from brown to purple. Sections are stained as before until quite black. They are then washed in distilled water, and placed for a few seconds in a three-fourth per cent. solution of permanganate of potash, then washed in distilled water, and then differentiated in Pal's solution.

Potassium sulphite .	1 grm. .	8 grains.
Oxalic acid . . .	1 grm. .	8 grains.
Distilled water . .	200 c.c. .	4 ounces.

They are kept in this for about five minutes, until the white and grey matter are clearly defined. They should then be washed thoroughly in distilled water, and then stained with eosine, alum carmine, or picrocarmine.

With this method the nerve fibres are stained as brightly as in Weigert's method. It is more speedy, and the colour is more thoroughly discharged from the neuroglia, so that contrast stains are much more effective.

CHAPTER V.

Section Mounting.

1. By **flotation.**

In this method the section whether stained or unstained is placed in a bowl of water, preferably distilled. A clean slide is then introduced into the water at an angle of about 60°, a little more than half of its length being submerged. The section is then brought up by the needle and floated as far as possible into position on the slide. One corner is then fixed by the needle, and on gently withdrawing the slide the section should lie flat. If any folds are left no attempt should be made to smooth them out with a needle, but the slide should be reimmersed until the folded part of the section is under water. It should then be gently withdrawn when the fold will disappear. This manœuvre must be repeated until the section lies quite smoothly on the slide. Stained and unstained sections are floated out in this way before

being mounted in Farrant's medium, and unstained sections previous to staining in picrocarmine.

2. By **transference** with a **section lifter.**

This method is usually employed in mounting in Canada balsam in order to transfer the section from the oil of cloves to the slide. The lifter is polished, oiled, and then insinuated under the section. The section being held in position by the needle is now raised from the oil of cloves, the blade being kept horizontal, so as to carry as much oil as possible. The edge of the lifter is then brought into contact with the slide, and the blade tilted, when the section glides off with the oil, and lies quite flat on the slide. The excess of oil is removed by holding the section in position with a mounted needle and tilting the slide, so as to allow the oil to drain off. Any that remains is then carefully wiped from around the section with a soft linen cloth. A drop of balsam is then run on, and a cover-glass applied.

Removal of air bubbles from sections.—When sections contain many air bubbles, the best plan is to leave them in methylated spirit for a time. The bubbles then coalesce and escape from the section.

For delicate structures and for fresh sections the transference to spirit, and the subsequent flying out of the section when returned to water are risky, and the best method of treating these is to put the vessel containing them under the receiver of an air pump if one is available.

The most frequent cause of air bubbles in mounted sections, however, is the employment of cover-glasses which have not been thoroughly cleansed. Proper cleansing is best effected by placing the covers when bought in a shallow wide mouthed stoppered bottle containing liquor potassæ, and leaving them in this fluid for twenty-four hours. The liquor potassæ should then be drained off and water run through the vessel from a tap, until the washings no longer give an alkaline reaction with turmeric paper. The water should then be drained off, and the glasses covered with alcohol. They can be removed one by one and rapidly dried as required. With cover-glasses properly cleansed in this manner, not only will air bubbles be avoided, but the covers will be dried much more easily with the cloth, and fewer will be broken in the process. Another very fre-

quent cause is the transference of air bubbles with the mounting medium on the glass rod. This occurs especially if the rod be fused to the stopper. The proper bottles to use, both for Farrant's medium and balsam are "balsam bottles" which have no stopper, but the mouth is closed by a glass cap which fits accurately. A short glass rod is kept loose in the bottle, and is used to transfer the medium to the slide.

Treatment of folded sections.—The folding may be due:—

(1) To the section having creased through being cut with a knife whose surface was not perfectly smooth. This is best remedied by placing the section in methylated spirit for a minute, and then transferring it to a bowl of clean water, when the section will rapidly rise to the top, and spread itself out flat on the surface of the water, in consequence of the alcohol rapidly diffusing out at the edges into the surrounding water.

(2) To the section containing a large amount of **fat,** as in those of the skin and subcutaneous tissue. The fat may be removed from the fat cells without materially altering the appearance of the

section. This is done by dehydrating the section in alcohol, and then transferring to a watch glass containing ether or chloroform to extract the fat. The tissue should be washed free from ether in the alcohol and then transferred to the bowl of water, and allowed to float out. This process does not interfere with subsequent staining operations.

Mounting Media.

Farrant's solution—

Gum arabic (picked, colourless) .	equal parts.
Glycerine	
Water	

In making this solution the best gum arabic must be used, and only the clearest pieces of this. "Powdered gum acacia" should be avoided, as though it looks white it often yields a brown mucilage, and besides is frequently adulterated with starch, etc.

The glycerine and water should be mixed and the gum arabic added. The mixture should be allowed to stand for some weeks, with frequent stirring until the whole of the gum is dissolved. Then allow it to stand for a week or two longer

in order that the dirt may subside, and the bubbles rise to the top. The scum should be removed and the clear fluid decanted from the sediment into a "Balsam bottle" (p. 72) containing a few drops of a saturated solution of arseniate of sodium solution and a small lump of camphor.

If properly made it is an extremely useful mounting reagent. It does not clarify the tissues too much, and in consequence of its containing gum it dries at the edges and cements the cover glass more or less firmly in a week or two. If this is not the case the medium contains too much glycerine and more gum must be added to compensate for this. This drying at the edge prevents any further evaporation and the glycerine keeps the section permanently moist.

The camphor and arseniate of sodium prevent the formation of fungi. Sections preserve their original appearance in this medium for many years. After a long time they are apt to become a little cloudy and granular.

Unstained sections should always be mounted in Farrant's medium. It is suitable for almost any tissue stained or unstained, but sections of the

nervous centres require to be mounted in Canada balsam.

Canada balsam.—Before being mounted in this medium, sections require to be dehydrated in alcohol, and clarified in some substance, in which it will dissolve, such as oil of cloves, turpentine or xylol. Consequently it must be used for stained sections, as the clarifying process renders unstained tissues so transparent that they can scarcely be seen under the microscope.

The medium is made thus :—

The ordinary Canada balsam is heated gently in a water bath for some hours. It is then allowed to cool to a yellow vitreous mass. Prepare as follows :—

Dried Canada balsam . .	equal parts.
Xylol	

Leave till dissolved, stirring occasionally.

Unless the solution be perfectly clear, it must be filtered through a very thin paper, previously wetted with xylol. If the medium be too thick more xylol should be added, if too thin, the xylol should be allowed to evaporate until the medium is of the consistence of a thin syrup.

If the medium is made too thin, much annoyance will be caused by its evaporating at the edge of the cover-glass, leaving an air-space, which will increase daily until the section is left quite dry. It should be remedied when it occurs by putting another drop of balsam at the edge of the coverslip and allowing it to run in and displace the air. A ring of cement should be put on as early as possible afterwards.

The bottle in which the balsam is preserved must be very carefully dried and then rinsed out with absolute alcohol, and afterwards with xylol. Turpentine or benzol are often used instead of xylol, and in the same proportion, but the latter appears to be less apt to dissolve out the aniline colours from the sections.

To mount sections in Canada balsam they must be transferred first to a watch glass containing absolute alcohol or an alcoholic solution of some staining reagent, and left in it, no attempt being made to spread it out, until it is perfectly dehydrated, *i.e.*, in about two minutes. It should then be transferred to oil of cloves on a mounted needle. This must be perfectly dry as any spot of moisture

that gets on to the section will resist the clarifying action of the oil of cloves, and will cause unsightly opaque areas when the section is mounted. Should this occur and white spots appear in the section while it is in the oil of cloves, it must be taken out with as little oil as possible, and again dehydrated in absolute alcohol.

As soon as the section is plunged into the oil, the alcohol rapidly diffuses out, so that the edges of the section fly out with it, and the section floats quite flat on the surface of the oil. As soon as it is clarified (in about half a minute), it should be transferred to the slide by the section lifter.

If the tissue is very delicate, and likely to be injured by changing from one vessel to another, or if it is larger than the section lifter will conveniently carry, it should be floated out on a glass slide, and, as much water as possible having been removed by a rag, should be dehydrated by adding a little alcohol with a pipette once or twice. Most of the alcohol should then be removed by tilting the slide, and before the remainder has evaporated, some oil of cloves should be added

from another pipette. The section will float on the oil at first, but the latter will gradually come through and appear on the top of the section. When this occurs the clarification is complete, and the oil may be run off and the section mounted in Canada balsam.

Cementing of cover-glasses.—The cover-glasses require to be cemented down to prevent their shifting and spoiling the specimen. If the cover-glass be circular, a Shadbolt's turntable should be used. It consists simply of a horizontal heavy brass disc, rotating easily on a pivot. There are a number of circles traced on the disc concentrically. The slide is then fixed on the disc by means of the clips, so that the circumference of the cover-glass corresponds to one of the circles. The disc is then rotated and the cement applied to the edge of the cover-glass with a brush.

Many materials are employed. The most suitable are:—(1) Canada balsam, which is almost colourless and transparent and looks very neat. (2) Gold size. (3) Marine glue.

When these are dry a finished appearance may

be given to the slide by laying on a ring of zinc white. This is made as follows:—

Oxide of zinc	½ drachm.
Benzole	half an ounce of each.
Gum dammar	

Preservation of sections.—They should be kept *flat*, and preserved from both light and dust. Very useful cardboard trays* are now sold in boxes made to contain twenty-four dozen slides for eight shillings, or suitable cabinets may be constructed by a carpenter.

* Can be obtained from F. Walters, Lambeth Palace Road, S.E., or from A. Frazer, Teviot Place, Edinburgh.

CHAPTER VI.

Directions for Preparing Individual Tissues.

Normal histology.—It cannot be too strongly impressed on the beginner that a thorough mastery of the normal appearances of tissues and organs is absolutely necessary before attempting to make an accurate study of morbid changes in them. He should not be satisfied with examining one specimen of an organ but as many as he conveniently can, in order to be fully acqainted with the many deviations from normal which may exist without actual disease. He should therefore obtain several animals, such as small dogs, cats, rabbits, frogs, etc., and remove their organs with all care and harden them in the various appropriate fluids. He should also obtain specimens of normal human organs from the post-mortem room. Many normal tissues (skin, muscle, tendon, bone, etc.), can also be prepared from a limb amputated for an accident to a healthy patient. By prepar-

ing specimens in this way one will not only become the possessor of a set of slides illustrating normal histology, but will find also that he has acquired that proficiency in hardening and staining the sections which practice alone can give.

The following account of the methods of preparing different tissues is merely intended to indicate the lines on which the beginner should proceed. After some practice he will be quite able to select the modes of hardening and staining which special circumstances or cases may seem to demand.

The first part of these directions will refer to the preparation of normal tissues, the second part to morbid histology.

Blood.—Should be examined fresh. It is obtained by pricking the finger or the lobe of the ear. The part pricked must not be squeezed. When sufficient has been obtained the cover-glass should be made to touch the drop. It is then transferred to the slide. Although one can best study the characters of fresh blood in this way, yet specimens will not keep.

In order to preserve the blood, it should be

spread out in a thin layer by pressing two cover-glasses together, with a small drop of blood between. The cover-glasses are then exposed to the fumes of osmic acid by placing them, blood downwards, on the unstoppered mouth of a one per cent. solution of osmic acid for three to five minutes. The layer is then allowed to dry and passed gently through the flame of a spirit lamp in order to fix it in position. The blood may then be stained with picrocarmine, magenta, eosine, hæmatoxyline, etc., and mounted in Farrant's medium or Canada balsam. Specimens thus prepared will last for several years. Fresh blood may also be stained by using Rutherford's magenta solution, which has the same specific gravity as blood, and so may be used with freshly drawn blood without making the red cells swell up and burst.

Both these methods are extremely valuable for the clinical examination of blood in cases of leucocythæmia, etc.

Blood crystals.—(1) Hæmoglobin crystals, obtained best from the blood of the rat, by adding a little water or a little ether to the blood, allow-

ing it to stand for a time, and then letting a drop slowly evaporate on a clean slide.

Hæmin crystals.—The student should make himself thoroughly familiar with these, as their presence affords positive proof of the existence of blood colouring matter in a stain.

To obtain them a drop of blood should be allowed to dry on a slide. The dried blood is then scraped into a little heap with a clean knife, and a drop of glacial acetic acid added. As it evaporates the minute reddish-brown acicular crystals will appear.

Hæmatoidin crystals.—Obtained from the site of a bruise, or an old hæmorrhage, *e.g.*, a cerebral apoplexy or a hæmatocele.

Simple squamous epithelium—(*Endothelium*). Carefully strip off the lining of the parietal pericardium or parietal pleura, of a recently killed animal or spread out its omentum on a piece of cork, and (1) stain the intercellular cement with nitrate of silver so as to reveal the outlines of the cells. (2) Stain other specimens with picrocarmine or alum carmine to reveal the nuclei.

Stratified squamous epithelium.—Best seen

in a section of any mammalian tongue. Specimens from skin of various parts should also be prepared. Harden in Müller's fluid. Stain in picrocarmine, and one section in nitrate of silver.

Transitional epithelium.—Occurs in the pelvis of the kidney, ureter and bladder. It is very readily detached, especially if not hardened immediately after death. Remove as early as possible. If the bladder is taken it should be cut open and pinned out as flat as possible. Harden in osmic acid, or Müller's fluid and spirit. Stain with picrocarmine or alum carmine.

Simple columnar epithelium.—Occurs in many parts. It may be studied in the salivary ducts, the intestine, kidney, etc., of any mammal. Harden in Müller's fluid or spirit. Stain one section in nitrate of silver. Others in picrocarmine or hæmatoxyline.

Goblet-cells.—Seen abundantly among the columnar cells of the intestinal glands, and in the mucous glands of the mouth and of the cervix uteri.

Stratified columnar epithelium.—Occurs only in the urethra. Harden the penis of a cat in

Müller's fluid, and cut transverse sections. Stain one section in nitrate of silver, others in picro-carmine or eosine and hæmatoxyline.

Ciliated epithelium.—Harden the trachea of a recently killed cat in osmic acid or Müller's fluid. Beautiful specimens may also be obtained from an ordinary nasal polypus, which should be put away into hardening fluid immediately after removal.

Ordinary areolar tissue.—Difficult to obtain free from fat. It may be studied in the subcut-aneous tissue of the section of the cat's penis al-ready made. A fragment of the tissue should also be removed and carefully teased in a drop of picrocarmine. Areolar tissue may also be studied in sections of skin, and in the capsules of the different internal organs.

Elastic tissue.—May also be studied in most sections of skin. If the ligamentum nuchæ of a large quadruped (horse, bullock), etc., is available it yields the best specimens, or the human stylo-hyoid ligament may be removed. Pin it out on a piece of wood or wax. Harden in Müller's fluid. Stain in picrocarmine. Both sections and teased specimens should be prepared.

Tendon.—Readily obtained from an amputated limb. Harden in Müller's fluid. Make transverse and longitudinal sections. Stain with eosine and hæmatoxyline.

A preparation should also be made by teasing a little of the fresh tendon in normal salt solution, and staining with picrocarmine or magenta.

Retiform or **lymphadenoid tissue.**—Seen in lymphatic glands and in the lymphoid follicles scattered along the sub-mucous coat of the alimentary canal.

Prepare sections in the ordinary way. Stain in eosine and hæmatoxyline or in picrocarmine.

Some sections should also be prepared by pencilling (*i.e.*, dabbing with a camel's hair brush) or by shaking sections up in a test tube with water or normal salt solution. By this means the leucocytes are removed, and the structure of the adenoid tissue itself becomes more evident.

Fat.—Best studied in sections of skin and subcutaneous tissue. One specimen should be stained with osmic acid and picrocarmine and mounted in Farrant's medium, and another in eosine and hæmotoxyline and mounted in Canada balsam.

Pigment cells.—Branched cells are best studied in the living foot of the frog, where amœboid movements may be seen in them when the light falling on the retina is made to vary in intensity. Permanent preparations are most conveniently made from the pallium of the common snail. The shell is removed, and the pallium snipped out with scissors. It is then pinned out flat, hardened for a day in methylated spirit, and mounted unstained in Farrant's medium.

Hyaline cartilage.—Specimens may be obtained from any joint, from the costal cartilages of young animals, or from the thyroid cartilage. It may be hardened in spirit. Stain with picrocarmine, eosine and hæmatoxyline, and with methyl violet.

Elastic cartilage.—Prepared from the epiglottis, or from the cartilages of the ear, *e.g.*, of a cat. Harden in spirit. Stain in picrocarmine or in magenta.

White fibro-cartilage.—Obtained from intervertebral disc. Prepare and stain as for hyaline cartilage.

Bone :—

Unsoftened bone.—Cut as thin a section as possible with a fine saw. Then rub it with the hand on a dry oil stone until it is as thin as possible. Then cement it by Canada balsam (liquefied by warming) to a piece of plate glass and continue the rubbing process with this, examining it now and then with the low power to see if it is thin enough. As soon as it is thin enough it is washed off the slide with methylated spirit, and washed. It should then be transferred to turpentine and may be mounted in balsam.

Softened bone.—Specimens may be obtained from an amputated limb or from the femur of a cat.

Specimens should be decalcified in chromic and nitric fluid, and the hardening completed in spirit. In studying the process of ossification, *e.g.*, in the head of the humerus of a kitten, it is best to embed the specimen in celloidin before cutting sections, as the trabeculæ of bone are very delicate, and easily detached.

Very beautiful double staining effects may be obtained with either picrocarmine, or eosine and hæmatoxyline.

Bone marrow.—To obtain good specimens of bone marrow, take a piece of the clavicle or a rib, or of one of the carpal or tarsal bones. Decalcify in chromic and nitric fluid. Embed in celloidin. Stain with eosine and logwood, eosine and alum carmine, or alum carmine and picric acid. Mount in Canada balsam.

Tooth.—Best cut *in situ* from the jaw of a cat. Decalcify in chromic and nitric fluid, and cut both vertical and transverse sections. Stain in picro-carmine, or eosine and hæmatoxyline.

Developing tooth.—Extremely good specimens may be obtained from the jaw of a newly born kitten or puppy. One can readily get sections shewing a milk tooth and a developing permanent tooth by its side.

The enamel is dissolved by decalcifying fluids. To study it a specimen of unsoftened tooth should be made, according to the directions given for bone.

Striped muscle.—Should be studied in various animals.

The leg of an ordinary cockroach may be hardened in osmic acid. One leg should be hardened

in a straight position so as to fix the fibrils in the fully extended position, another should be bent up so as to get specimens of relaxed fibrils.

Portions of muscle should be removed, and teased on a glass slide in some staining fluid such as picrocarmine, a quarter per cent. solution of eosine or quarter per cent. of safranine.

Sections of amphibian and mammalian muscle should be prepared to show their differences in structure. The most convenient part to select is the tongue, as a view of the fibres is obtained both in longitudinal and transverse sections.

Fresh sections should be frozen and made to see "Colmheim's areas," etc. Others should be made after hardening in Müller's fluid, and stained in eosine and hæmatoxyline which gives a beautiful effect.

Heart muscle.—A portion should be teased fresh in picrocarmine or eosine, another portion hardened in Müller's fluid, and sections made and stained with eosine and hæmatoxyline.

Unstriped muscle.—May be obtained by teasing a fresh portion of the muscular coat of the small intestine of an animal, or by sections of the

hardened intestine, bladder or uterus. Stain in picrocarmine or preferably eosine and hæmatoxyline.

Nerves.—Take a nerve from a recently killed frog. Tease one portion in picrocarmine to study the axis cylinder, and another in osmic acid to show the myelin sheath. Part of a human nerve may also be removed from an amputated limb, and hardened in Müller's fluid.

Both longitudinal and transverse sections should be made, preferably in celloidin. Sections should be stained in osmic acid followed by picrocarmine; and mounted in Farrant's solution to study the myelin sheath and its nuclei, others in nitrate of silver to show the "nodes" and "crosses" of Ranvier, and others in eosine and hæmatoxyline, or by Weigert's method.

Nerve cells.—(1) Spinal nerve-cells of the various regions of the cord.

(2) Sympathetic nerve-cells from the various ganglia.

(3) "Antler" cells from the cortex of the cerebellum.

(4) Pyramidal nerve-cells.

A portion of the grey matter containing these cells is macerated for some weeks in a one-tenth per cent. solution of potassium bichromate, containing some carbolic acid, until it is quite soft. A little is then spread out on a slide in as thin a layer as possible, and stained with picrocarmine and mounted in Farrant's medium. The cells and their processes will in this way be obtained isolated along with a good deal of débris from the softened tissue.

Nerve terminations:—

Meissner's corpuscles.—Take the tip of an index finger immediately after amputation. Place part of it at once in chloride of gold solution, and the rest in Müller's fluid until it is hardened.

Sections stained with chloride of gold should be mounted in Farrant's medium. The other sections may be stained in picrocarmine or eosine and hæmatoxyline.

Pacini's corpuscles.—May be dissected out on the smaller branches of the digital nerves, or may be found in the mesentery of the cat. The latter should be spread out on wood, hardened in Müller's fluid, stained in hæmatoxyline, and mounted in balsam.

Other forms of tactile corpuscles may be studied in the tongues of frogs, ducks, or geese. A network of nervous fibrils should be studied in the cornea. Take the cornea of a newly killed frog or cat and stain with chloride of gold.

The end plates in which the nerves end in muscle may be studied by placing specimens of living muscle of some cold blooded animal into chloride of gold solution, and staining rather deeply.

Arteries.—Take a piece of the aorta, a piece of some medium artery as the renal or radial, and harden in Müller's fluid. Stain in picrocarmine and always in eosine and hæmatoxyline. Arterioles are best studied in sections of the various organs. Thus they are seen in each Malpighian body of the spleen, in the boundary zone of the kidney, and so on.

Veins.—Remove, harden, and stain in the same way.

Capillaries.—May be very well seen in the omentum of the frog. They should be studied both during life and after hardening.

Stun a frog by striking its head, or by chloro-

forming it. Open the abdomen. Draw out a loop of intestine and spread it out on a ring of cork and keep it moist by normal salt solution. The movement of blood in the capillaries, etc., can then be studied for an hour or two. As soon as the circulation ceases the mesentery should be spread out on a piece of wood, and hardened for a few days in Müller's fluid.

Stain with eosine and hæmatoxyline.

Lymphatics.—Commencement of lymphatics in serous membrane. Stain a piece of cat's omentum in nitrate of silver for some minutes. After washing keep in glycerine for about a week and then stain in hæmatoxyline and mount in Farrant's medium.

Lymphatic glands.—The lymphatic glands of the neck of the cat may be used. Harden in Müller's fluid. Stain in picrocarmine, eosine and hæmatoxyline.

Skin and sweat glands.—Sections should be made from pieces taken (*a*) from the sole, (*b*) from the skin of the body, (*c*) from the axilla of an adult to study the pigment. Harden in Müller's fluid. Stain in picrocarmine or eosine and hæmatoxyline.

Hairs and sebaceous glands.—Take a portion of the scalp, or of the skin of a puppy. Harden in Müller's fluid. Stain in eosine and hæmatoxyline, and mount others unstained.

Hairs from various parts of the body should also be soaked in liq. potassæ and mounted unstained in Farrant's medium.

Brain and spinal cord.—Must be removed from the body with extreme care, all stretching or squeezing being avoided. Harden slowly in Müller's fluid to which a fourth of its bulk of water may be added.

The best staining reagents to employ are eosine and hæmatoxyline, alum carmine or borax carmine, aniline blue-black, etc. For special work Weigert's or Pal's method may be required (see p. 67).

Eye.—Harden the eye of a recently killed bullock, cat, or other animal in Müller's fluid, puncturing the sclerotic in places to allow the hardening fluid to penetrate. In about six weeks, make a horizontal section through the eye. The anterior half (the lens having been removed) may be satisfactorily cut in gum. Sections of the

crystalline lens are not very satisfactory. The best way to get specimens of the fibres is to tease a piece of the fresh lens of a fish (*e.g.*, a cod) in a $\frac{1}{40}$ per cent. aqueous solution of eosine. Wash the eosine off the slide with $\frac{1}{2}$ per cent. acetic acid, and mount in Farrant's solution.

The posterior half should be embedded in celloidin, as otherwise it is extremely difficult to get sections of the retina in its proper relation to the other coats.

Mount some specimens unstained. Stain others with the ordinary stains.

Internal ear.—Decalcify the temporal bone of a cat, dog, guinea pig, etc., in chromic and nitric fluid. As soon as the bone is decalcified, complete the hardening of the soft parts in methylated spirit, embed in celloidin, and cut sections in the longitudinal axis of the cochlea.

The semi-circular canals will be most readily studied in the temporal bone of fishes, or of birds, *e.g.*, the common fowl. They also must be cut in celloidin, and stained in the ordinary way.

Nose and olfactory epithelium.—It is difficult to obtain specimens from the human subject,

but very satisfactory preparations may be made from the dog, or more conveniently in a new born puppy where the bones are still cartilaginous. Harden the latter in Müller's fluid, decalcify adult specimens in chromic and nitric fluid. Specimens of ciliated epithelium, etc., will be obtained from the lower part, and of the special olfactory epithelium from the upper part. Stain in eosine and hæmatoxyline.

Lungs.—Carefully remove the lungs of a cat without injuring the bronchi or trachea. Introduce a cannula into the trachea and gently inflate the trachea with air. Ligature the trachea and place the lung in Müller's fluid, a weight being attached to keep the organ submerged. Harden for about six weeks, and then make sections of the various parts.

To demonstrate the endothelium of the alveoli, inject instead of air, nitrate of silver. Allow it to remain in for half an hour, then remove it by washing, and harden in Müller's fluid.

Beautiful casts of the alveoli, etc. may be obtained by placing a cat's or human lung under the receiver of an air-pump, and when the air is com-

pletely exhausted, injecting fusible metal into the bronchus. The lung tissue is then removed by corrosion or by maceration. Portions of the casts should be removed, fixed in a glass cell with a spot of Canada balsam, and examined by reflected light.

Thyroid gland.—Best obtained from a young subject either human or an animal.

Harden in Müller's fluid. Stain in picrocarmine or eosine and hæmatoxyline. Also stain sections in safranine, which stains the colloid material, and also picks out any colloid degeneration that may be going on in the cells.

Thymus.—Remove from a fœtus or a very young animal, and prepare in the usual way.

Tongue.—That of the cat or rabbit serves very well.

Ordinary transverse sections should be made, and also sections through the circumvallate papillæ in order to study the "taste buds."

Salivary glands.—Those of a cat or dog do very well.

Sections should be made from each of the three glands.

Stomach.—That of the cat or dog should be studied. The organ must be removed immediately after death before any post-mortem digestion of the coats has occurred. The stomach should be opened, washed gently and pinned out as flat as possible without much stretching on a piece of wax or of wood, and may then be hardened in Müller's fluid with spirit.

Sections should be made (*a*) longitudinally through the cardiac end to show the transition from the œsophageal to the gastric mucous membrane, (*b*) from a portion of the greater curvature, (*c*) from the pyloric valve.

Eosine and hæmatoxyline form the best stain for the alimentary canal.

Intestine.—Prepare in the same way as the stomach. Make sections from (*a*) the upper part of the duodenum, (*b*) the ileum, (*c*) a Peyer's patch, (*d*) the vermiform appendix, (*e*) the colon.

Liver.—Make an injection of one specimen with carmine and gelatine. Harden in methylated spirit. Others should be hardened in Müller's fluid and stained in the usual way.

Kidney, supra renal, and pancreas.—Same preparation as for liver.

Spleen.—Harden in Müller's fluid.

Mount one section unstained. Shake another up with water in a test tube to shew the structure of the pulp. Stain others in eosine and hæmatoxyline.

Bladder.—Must be removed and pinned out immediately after death, as otherwise the epithelium will be macerated off. Consequently it must be taken from an animal, as a cat. Harden in osmic acid. Cut in celloidin as the coats are very apt to become detached.

Penis and testis.—Readily obtained from dog cat or rat.

Stain with eosine and hæmatoxyline.

Uterus, ovaries, and fallopian tubes.—May be obtained from the post-mortem room or from the lower animals. Harden in Müller's fluid, and make sections from the cervix, the body of the uterus, the Fallopian tube, and the ovary.

Embryological specimens.—For systematic work special manuals should be consulted.

Specimens should be hardened in osmic acid or in Müller's fluid, and cut in celloidin.

Cloudy swelling.—Specimens are obtained from organs of subjects who have died in the early stage of some fever. They should be always hardened in Müller's fluid, as the appearances alter if the tissue is kept in spirit for any length of time.

Fatty degeneration.—Prepare from patients who have died of exhausting diseases, phosphorus poisoning, etc.

Stain in osmic acid. Mount in Farrant's medium and keep in the dark.

Mucoid degeneration.—Study in goblet cells of normal intestine or of ovarian cysts.

Colloid degeneration.—Occurs in the thyroid gland, in the tubules of the kidney in many diseases, and the prostate of the old. Stain in safranine.

Waxy or lardaceous degeneration.—Best studied in liver, spleen or kidneys. It should be searched for in persons who have died from a long illness, accompanied by suppuration, *e.g.*, phthisis or bone disease. Mount one section unstained, stain another in methyl violet, a third in a weak solution of iodine, and examine the latter at once both by transmitted and reflected light. The stain is not

permanent. Another section should be stained in osmic acid, followed by methyl violet as waxy and fatty degeneration frequently co-exist.

Hyaline degeneration.—Seen in the arterioles of the spleen in some cases of typhoid and diphtheria. The ordinary staining methods must be used.

Calcareous degeneration.—Occurs after fatty degeneration in gummata and in atheromatous arteries. It also occurs in the matrix of the costal cartilages after middle life. Mount one section unstained and examine if possible with the polariscope. Stain others in safranine.

Pigmentary degeneration.—May be studied in brown atrophy of heart, nutmeg liver, etc. Harden in Müller's fluid and mount sections unstained.

It will be unnecessary to recapitulate the methods for hardening the various diseased organs as the directions for the normal organs hold good. If the presence of micro-organisms be suspected, harden in methylated spirit or preferably in absolute alcohol, but as a rule both for diseased organs and tumours Müller's fluid will be

found the most satisfactory reagent for general use.

It sometimes happens, however, that it is inconvenient to wait several weeks, until the Müller's fluid has hardened the specimen sufficiently, before making sections. In this case the best plan is to make fresh sections, or else to cut a slice about one-eighth of an inch thick, and harden for about three days in plenty of methylated spirit,

Tumours.—Müller's fluid should be employed, unless a more rapid agent is required.

Methylated spirit may be used in the case of epithelioma, adenoma, etc., but for sarcoma, myxoma, tumours containing cysts or much blood, Müller's fluid will yield by far the best results.

BOOKS OF REFERENCE.

Methods in Microscopical Anatomy—*Whitman.*

General account of Practical Histology—*Rutherford.*

Practical Pathology—*Woodhead.*

Manual of Bacteriology—*Crookshank.*

Manual for Physiological Laboratory—*Harris and Power.*

Practical Histology—*Schäfer.*

Practical Pathology and Histology—*Gibbes.*

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