A practical course of general physiology : for medical students / by D. Noël Paton, M.D., F.R.C.P.Ed., Professor of Physiology, University of Glasgow, and G. Herbert Clark, M.B., D.P.H., Muirhead Demonstrator of Physiology, University of Glasgow.

GUIDE

TO

THE PRACTICAL STUDY OF HISTOLOGY

FOR MEDICAL STUDENTS

BY

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GUIDE

TO

THE PRACTICAL STUDY

GUIDE TO THE PRACTICAL STUDY OF HISTOLOGY

INTRODUCTION.

THE practical Study of Histology may be divided into three parts:

1st—The methods of preparing specimens for microscopic examination.

2nd—The method of examining and reporting on specimens.

3rd—The structure of the normal tissues, organs and fluids of the body.

PART I.

METHODS OF PREPARING SPECIMENS.

A. **FLUIDS.**

1. **Opaque Fluids** (such as blood and milk).—These usually contain solid particles uniformly distributed. (*a*) *Examination in fresh condition.* A drop of the fluid is placed on a microscopic slide, covered with a clean cover-glass, and examined first, unstained; second, after staining or treatment with reagents if necessary.

(*b*) *Permanent preparations* may often be made by spreading a thin layer on a cover-glass, drying, fixing, staining, and mounting in Canada balsam. (See Blood Films, p. 13.)

2. **Transparent Fluids.**—These have usually few solid particles, but they may be set aside to allow of the precipitation of solid matter as a deposit. Such precipitation may be accelerated by centrifugalisation. Some of the deposit is taken in a pipette, placed on a slide, covered and examined.

Such deposits may be *preserved* either by drying, staining, and mounting in balsam, or, in the case of urinary crystals, by the addition of glycerine jelly.

B. SOLIDS.

Solid structures should be examined, (*A*) in the fresh condition; (*B*) after preparation.

A. To examine in the **fresh condition** the following methods may be employed:

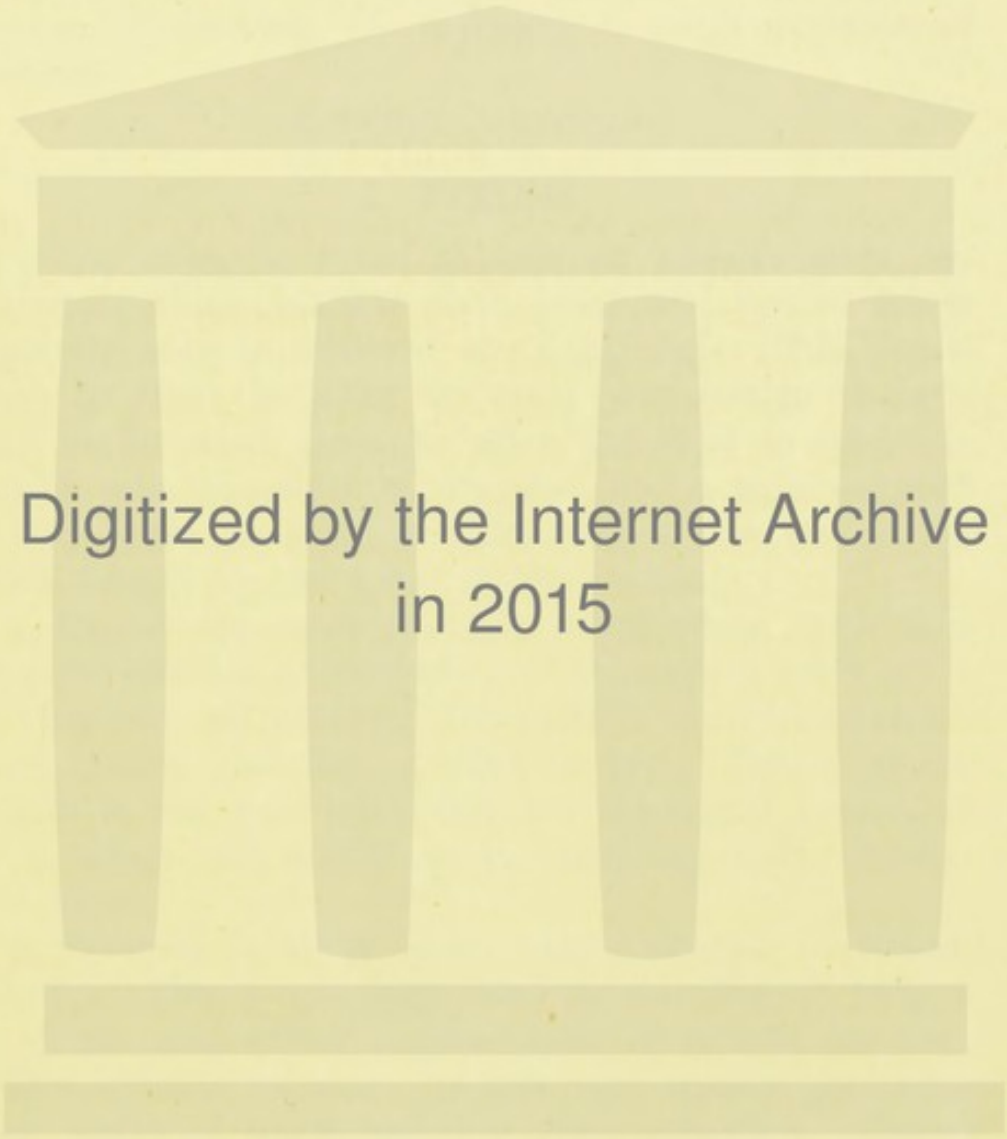
1. If the tissue is soft, the surface may be *scraped*, the scrapings placed on a clean cover-glass, and another cover-glass pressed upon it. The two cover-glasses are then slid along one another till they are separated, and the thin layer of tissue left upon one is mounted in a drop of some indifferent solution, such as a 0·75 per cent. solution of common salt.

If necessary, such preparations may be stained with aqueous solutions of aniline dyes, such as methylene blue, or with picrocarmine. Sometimes they may be preserved by drying, fixing, staining and mounting in balsam, in the same manner as blood films.

2. If the tissue is fibrous, a small piece may be *teased* out with needles, and examined in a drop of salt solution, either unstained or stained.

3. By *freezing* the tissue, thin slices may be cut with a sharp razor, floated in a 5 per cent. formalin solution, spread upon a microscope slide, and examined unstained or stained. Such preparations may afterwards be preserved.

Such methods of examination often give much information as to the nature of the structures examined, and are sometimes sufficient. In every case they afford indications of



the best mode of preparing and carrying out the further examination.

B. In order to make **permanent preparations,** it is in most cases necessary to carry out the following processes in order: (1) Fixing, (2) hardening, (3) embedding in some material which facilitates section-cutting, then (4) cutting, (5) staining, (6) mounting in some preservative medium.

GENERAL METHODS.

1. FIXING.

Tissues must be taken as soon as possible after the animal is killed, cut into small pieces, and thrown into a relatively large quantity of the fixing fluid. The tissues must be fresh in order to avoid post-mortem changes, must be in small pieces to allow the fluid to penetrate rapidly into the centre of the piece, and a large quantity of fluid must be taken in order that it may not be too much diluted by the water of the tissues.

1. One of the most generally useful fixing fluids is a *saturated solution of perchloride of mercury in water or in* 0·75 *per cent. salt solution.* The solution must be saturated while boiling, and then allowed to cool. Tissues should remain in this fluid until they are thoroughly penetrated by it, which will usually be in something under twenty-four hours.

2. *Formalin* (a 40 per cent. watery solution of formaldehyde). This is generally used in solutions of from 5 to 10 per cent. either in pure water or in 0·75 per cent. sodium chloride solution. It acts very rapidly, penetrates large pieces of tissue, and does not overharden. Tissues after lying in formalin solution till they are penetrated—a few minutes—may be frozen, and at once cut and stained.

3. *Absolute alcohol* is a very valuable fixative because of its extreme rapidity of action and great penetrating power. It allows the use of almost any stain. Absolute alcohol, or alcohol of some lower grade—*e.g.* 75 per cent.,

is often used instead of water to dissolve other fixing agents—*e.g.* perchloride of mercury, so as to render their action more rapid and energetic.

4. *Osmic acid* (1 or 2 per cent. solution in distilled water) is a rapid fixative, but does not penetrate far. It has the special property of blackening fat by the reduction of osmium, and the disadvantage that it is not easy to stain tissues after using it. Osmic acid is used in combination with chromic acid in Flemming's solution, which fixes mitotic figures well. (Appendix.)

The vapour of osmic acid may be used for fixation.

5. *Nitric acid* (3 per cent. to 50 per cent. in water) is mainly used in embryological work, and is then often combined with chromic acid (Perenyi). After tissues have been fixed with some other agent, or without this having been done, it is often used to decalcify them, either as Perenyi's solution, or in watery or alcoholic solutions, or with the addition of phloroglucin to protect the soft parts from its action. (Appendix.)

6. *Bichromate of potash* (2 to 5 per cent. in water) is a very weak fixative, but an excellent hardening reagent, especially for the central nervous system. It is generally used with sulphate of soda, as Müller's fluid. (Appendix.)

In most cases it is necessary to *wash out the excess of the fixing agent*, as the presence of many of these substances in the tissue interferes with staining. Twenty-four hours in running water is usually sufficient for this purpose, and that time should rarely be exceeded, as tissues are apt to become soft if kept longer in water. With weak fixatives, like picric acid, it is better, indeed, to transfer the object directly to alcohol.

2. HARDENING.

In most cases the process of hardening is completed by alcohol, no matter by what fixing agent it may have been commenced. The reason for this is twofold. Alcohol hardens *directly* by coagulating the albuminous parts of

the tissues, or by keeping them coagulated when that has already been done by the fixing agent, and also *indirectly* by withdrawing water from the tissues and so *dehydrating* them. This dehydration is a necessary step in the embedding processes most commonly used—paraffin and celloidin. It is important that the replacing of water by alcohol should take place gradually, in order that the tissues may shrink as little as possible, and that the small amount of shrinking which is probably inevitable in all hardened specimens may be uniform. This end is attained by passing the object through alcohols of gradually increasing strength, first for a few hours in 30 per cent. alcohol, then for some hours in 50 per cent. alcohol, next for the same time in methylated spirits, and last in absolute alcohol.

Where corrosive sublimate has been used as the fixing agent, the traces of it which have not been removed by washing the tissues may be cleared away by adding a little tincture of *iodine* to the alcohols used for hardening.

3. EMBEDDING AND SECTION-CUTTING.

There are comparatively few objects which, even when hardened, are sufficiently homogeneous in texture to allow of good sections being cut with the free hand, or unembedded with a microtome. Well-hardened liver is an example of the kind of object which does allow it. In most cases the objects are either too delicate or have some parts of greater density than others, so that sections cut with the free hand are too unequal. It becomes necessary, therefore, to infiltrate the tissue thoroughly with some substance which will allow it to be treated as if it were a solid block, which will fill even the minutest cavities, and which will accurately preserve the relations of parts. This may be done in several ways, either by means of a substance which is fluid at ordinary temperatures and becomes solid by freezing, such as a mixture of gum and syrup; by a substance which is fluid when heated and

becomes solid on cooling, such as paraffin; or by a substance which can be dissolved by solvents whose evaporation leaves it solid, such as celloidin or photoxylin.

(*a*) *The Gum and Syrup Method.*—This is mainly employed for tissues which are too brittle to be cut in paraffin. The tissues must be thoroughly hardened. Every trace of alcohol must be washed out of them by twenty-four hours' soaking in running water, as alcohol interferes with the freezing. They are then soaked in a thick solution of gum and syrup for twelve hours or more, according to the size of the piece taken. The tissue is then placed on the pedestal of a freezing microtome, the gum and syrup mixture is painted round it, and the whole is frozen by the use of ice and salt, or by the evaporation of ether or chloride of ethyl. The mass becomes solid, and should cut like cheese. The sections when cut are soaked in water to remove the gum and syrup, and may thereafter be preserved in spirit.

(*b*) *The Paraffin Method.*—(1) The tissue must be thoroughly dehydrated in absolute alcohol, which may require to be changed once or twice if the object is large. (2) It is then *cleared* in a solvent of paraffin, such as cedar oil, chloroform, or xylol, which removes the alcohol and renders it possible for the paraffin to pentrate the object. (3) The tissue is then placed in paraffin, melted in an oven with a water-jacket, which is kept at a constant temperature, just above the melting-point of the paraffin used. If this be 50° C., the oven should be regulated to 52° C. Tissues are spoilt if they are heated above 60° C. The object is kept in the melted paraffin until the solvent used has thoroughly diffused out into the paraffin, and has either been allowed to evaporate from this or has been removed by several changes of paraffin. Twenty-four to forty-eight hours is usually a sufficient time for all but the densest tissues. (4) The object is then placed in a paper boat and arranged in the paraffin, which is still fluid. The boat is then floated on cold water, so that the paraffin may cool rapidly and set homogeneously. (5) The block

when cool is trimmed to the desired shape and size, and sections are cut with such an instrument as the rocking microtome.

The advantages of this method are that a complete series of sections of any object can be cut; that these sections are all of equal thickness, and can be cut very thin indeed; and that the relation of different parts is perfectly preserved in the sections.

After the sections have been cut, they must be *fixed to the slide* or to the cover-glass. This is best done by (1) floating the sections on warm water, at a temperature a good deal below the melting-point of the paraffin. They then flatten out, and can easily be disposed on the surface of the glass. (2) The water between section and glass is driven off by gentle heat, and the section adheres firmly to the glass. (3) The paraffin is removed by xylol, turpentine, or naphtha, and the section is passed through spirit into water, and thereafter stained. Sections fixed in formalin do not adhere readily, and the slide should be first coated with a 10 per cent. solution of egg white in water.

(*c*) *The Celloidin Method.*—(1) The tissue is dehydrated in absolute alcohol; (2) soaked in a mixture of absolute alcohol and ether; (3) then transferred to a thin solution of celloidin or photoxylin in absolute alcohol and ether; and (4) after some time to successively thicker solutions; or the initial solution may be allowed to concentrate itself by very slow evaporation, lasting over days or weeks. As soon as the mass of celloidin surrounding the object has acquired a certain consistence, the object is arranged in some receptacle, and the whole is hardened either in chloroform or in 85 per cent. alcohol. The block is then fixed to a microtome and cut by an obliquely-set knife sliding in a groove and kept wet with alcohol.

This method is not much used for general purposes in this country, but is employed for the central nervous system, for very large objects which are difficult to infiltrate with paraffin, and for objects, such as bone and

skin, which are so brittle as to make it difficult to cut them in paraffin.

Objects embedded in celloidin may be re-embedded in paraffin and cut dry.

4. STAINING.

Stains are generally used in order to differentiate the parts of a tissue. With this object it is usual to stain the nuclei of one colour, and the cell-bodies and formed material in the section, of another colour, which sharply contrasts with the nuclear stain. That this can be done depends on the fact that nuclear chromatin has an affinity for basic stains, while cell protoplasm and formed material generally take up acid stains.

The difference between these two sets of stains is best seen in the aniline dyes, where **basic stains** are those salts formed of a colourless acid and a coloured base, while **acid stains** are salts formed of a coloured acid and a colourless base. Some of the more important basic aniline stains are: Methylene blue, methylgreen, toluidin blue, fuchsin, gentian-violet. Carmine and hæmatoxylin, or hæmatein, act in many respects as basic stains. Some **acid stains** are eosin, orange, acid fuchsin and picric acid.

The stains most in use for general histological work are the aniline stains and picrocarmine and the combination of hæmatoxylin, hæmatein, or hæmalum and eosin.

(1) *Hæmatoxylin or Hæmalum and Eosin.*—Hæmatoxylin, or its active part hæmatein, is usually dissolved in alcohol or glycerine, and made up with a watery solution of alum. (Appendix.)

Staining takes place rapidly, in a minute or two, and as over-staining is apt to occur, it is usual to wash the section in water slightly acidulated with hydrochloric acid, in order to remove the stain from everything but the nuclei, and then to treat it with water rendered slightly alkaline with ammonia, or an alkaline carbonate, which renders the stain

blue and permanent. The section should then be washed in pure water and stained in a watery solution of eosin. Generally the washing with acid and alkaline waters may be dispensed with.

(2) *Aniline Stains.*—While this method of staining is sufficient for ordinary work, for finer work the aniline stains must be used. This may be done in different ways.

(*a*) Overstrain the tissue with a strong solution of a basic stain in alcohol, or water, or aniline water, and then wash out the excess with alcohol plain or acidulated, or with a solution of one of the acid stains, which substitutes itself for the basic stain in everything but the nuclei. It is of course important to stop the process of substitution at exactly the point required, and this is usually done by rapid dehydration and mounting in balsam. *Example*—

Nissl's Method.

This is used to bring out the Nissl's granules in neuron cells. The tissue, fixed in perchloride of mercury, is stained for one hour in a saturated solution of toluidin blue. It is then partially decolorised in alcohol, dehydrated, cleared and mounted in balsam.

(*b*) Employ a combination of several stains in a single solution. The section is stained in this, and the different dyes are taken up by the structures which have a special affinity for them. The best known of these mixtures is the *Triacid* or *Ehrlich-Biondi* stain, which contains methylgreen as the basic stain, and the two acid stains—acid fuchsin and orange.*

(3) *Picrocarmine* or picrocarminate of ammonia, a salt formed by the decomposition of an ammoniacal solution of carmine and a solution of picric acid, is a double stain. Nuclei become red, formed material either pink or yellow —*e.g.* white fibrous tissue becomes pink, elastic fibres yellow. It is used in watery solution, and should be allowed to act on the section as long as possible, or its action may be

*This is best purchased in powder from one of Grübler's agents.

hastened by warmth. Sections stained with it are usually mounted in a glycerine medium.

5. MOUNTING IN A PRESERVATIVE MEDIUM.

The object of mounting is to enclose and impregnate the preparation with some material which will render it of a proper degree of transparency, which will not cause fading of the stains which have been used, which will preserve the tissue elements unaltered, and which, though fluid when used, will set afterwards so as to ensure that the section and cover-glass will not be displaced.

Glycerine, though very useful as a temporary mount, fails in the second and last of these respects, and Farrant's medium, a mixture of gum and glycerine, with some arsenious acid added as an antiseptic, only partly removes the difficulty. Its advantages are the fact that sections can be brought into it directly from water or a watery stain, that they can be covered at once, and that after some time it sets fairly well. But most stains rapidly fade in it, and in practice only sections stained with picrocarmine are usually mounted in it. A mixture of sugar and dextrin with a little thymol may be used instead of Farrant's medium. (Appendix.)

Canada balsam dissolved in xylol is the medium most in use. It renders tissues more transparent than the glycerine media, but it preserves tissues and stains, and sets rapidly and firmly. Sections to be brought into it must—(1) be dehydrated with alcohol, (2) cleared with xylol, clove oil, cedar oil, or some other of the clearing agents, and (3) then brought into the balsam and covered.

SPECIAL METHODS.

1. EXAMINATION OF BLOOD.

(*A*) Blood Films.

Spread a drop of blood from the finger in a thin layer between two **clean** cover-glasses by drawing them gently apart. Dry them in air. Place them face downwards in a solution of formalin (10 per cent.) in absolute alcohol for four minutes and wash them in water.

(α) To stain the film immerse in a saturated watery solution of eosine for five minutes. Wash in water. Dip in a saturated solution of methylene blue for half a minute. Wash in water. Dry thoroughly by warming and mount in balsam.

(β) *Hæmalum and Eosin.*

Stain as above with watery eosin solution for five minutes. Wash in water. Stain in hæmalum for 10 minutes or so. Wash in water. Dry and mount as above.

(γ) *Jenner's Stain* (see Appendix).

Previous fixation is unnecessary with this stain.

Immerse the cover-glass with dried film in the stain for from 1 to 3 minutes. Pour off excess. Rinse in *distilled* water till the film has a pink colour.

Dry and mount as above.

(*B*) Weigert's Method for Staining Fibrin.

(1) Harden in alcohol.
(2) Stain for 5 to 15 minutes in a strong aniline-water solution of gentian-violet.
(3) Wash in 0·75 per cent. salt solution.
(4) Dry on a slide.
(5) Place for two to three minutes on a slide in a solution of iodine 1 part, potassium iodide 2 parts, water 100 parts.

(6) Dry with blotting paper.
(7) Decolorise in aniline oil 2 parts, xylol 1 part.
(8) Remove the aniline-xylol with xylol.
(9) Mount in Canada balsam.

(*C*) Enumeration of Cells.

Thoma-Zeiss Hæmocytometer.

Erythrocytes.

1. *Dilution of Blood to Definite Proportion.*—Puncture the finger or lobe of the ear with a sharp needle. Suck the blood thus obtained into the pipette up to the mark 0·5 on the stem. After drying the point, suck Hayem's solution (appendix) into the pipette up to the mark 101, mixing thoroughly by gently shaking the pipette. The dilution is now 1 to 200.

2. *Enumeration of Corpuscles in a Definite Volume of Diluted Blood.*—Blow a few drops of the mixture out of the capillary part of the pipette and then place one drop on the appropriate place on the counting slide. Cover with a cover glass. In putting on the cover glass, care must be taken to allow none of the mixture to pass into the trench which surrounds the platform on the counting slide.

Allow a minute to elapse before commencing to count.

Each ruled square has resting upon it when the cover glass is in position $\frac{1}{4000}$ c.mm. of the diluted blood. Hence the number of corpuscles in 1 c.mm. of diluted blood can be taken as the average number of corpuscles on one square multiplied by 4000.

The dilution of the blood was 1 to 200 so that by further multiplying by 200 the number of corpuscles per c.mm. of *undiluted* blood may be found.

The average number per square is arrived at by counting 3 sets of 16 squares.

Leucocytes.

Collect the blood as above in the appropriate pipette so that the dilution is 1 to 20 in a 0·3 per cent. glacial

acetic acid solution tinged with methyl violet to stain the leucocytes.

Count the white corpuscles in all the 256 small squares.

The average per square multiplied by 4000, as above, gives the number of leucocytes per c.mm. of diluted blood. As the dilution is 1 to 20, further multiplication by 20 will give the total number per c.mm. of *undiluted* blood.

2. DISSOCIATING TISSUES.

It is sometimes necessary to make a tissue easily disintegrated, so that the individual elements may be separated from one another. This may be done by macerating it in a solution of one-third alcohol (Ranvier's alcohol) or in a 2 per cent. solution of yellow chromate of potash.

3. STAINING WITH NITRATE OF SILVER.

This is usually used in order to demonstrate the outlines of epithelial or endothelial cells or connective tissue spaces, and its use can be best described as it is carried out with the omentum. This is taken *fresh*, and is placed in a 1 per cent. solution of nitrate of silver in the dark until the membrane turns grey. It is then washed in distilled water and exposed to sunlight in 50 per cent. alcohol until brown.

4. CAJAL'S METHOD FOR NEURON PROCESSES.

By this method the processes are rendered opaque by the deposition of silver.

Small pieces of tissue, not more than 3 mm. cube, are placed:

(1) In alcohol with 1 per cent. of ammonia and kept for 24 hours at a temperature of from 30° to 35° C.

(2) After washing in distilled water they are removed to a 1 to 2 per cent. solution of silver nitrate and again kept at the same temperature for 5 to 8 days.

(3) After thoroughly washing in distilled water the tissue is transferred to a reducing solution of pyrogallic acid (Appendix) for 24 hours. A yellowish brown colour on the cut surface shows that the process has been properly carried out.

(4) After washing in distilled water the tissue is dehydrated and cut in paraffin or celloidin.

The process must be carried out in the dark until after the reduction in the pyrogallic acid solution is completed.

5. GOLGI'S METHOD FOR NEURONS.

Cox's Modification.

With this method, not the substance between the cells, but the cells themselves are impregnated with chromate of mercury. It is used mainly for the nervous system. Small pieces of tissue are taken fresh, and are left for two or three months in a mixture of chrome salts and a mercuric salt. (Appendix.)

Thick sections are cut with the free hand and put into a saturated solution of lithium carbonate for 1 hour, washed in water, dehydrated, cleared rapidly, and mounted in balsam without a cover-glass.

The neuron cells and their processes are impregnated with the chromate of mercury, and appear black by transmitted light.

6. WEIGERT'S METHOD FOR NERVE FIBRES.

This method is used for staining the white sheath of nerve fibres. The tissue may be fixed in formalin and Müller's solution, then imbedded and cut in celloidin.

The sections are—(1) stained in a solution of 1 grm. of haematoxylin dissolved in a little alcohol in 100 c.c. of 2 per cent. acetic acid for 3 to 24 hrs. till black; (2) washed in water; (3) put into potassium permanganate 0·25 per cent. for 30 sec.; (4) transferred to 50 per cent. sulphurous

acid till decolorised; (5) washed in water; (6) put in a saturated solution of lithium carbonate till blue; (7) washed in water; (8) dehydrated, cleared and mounted in balsam.

7. MARCHI'S METHOD FOR NERVE FIBRES.

This method is used for tracing the course of degenerated white nerve fibres, and it depends on the fact that the myelin substance while disintegrating, stains black when treated with a mixture of a chrome salt and osmic acid. The tissue is kept in Müller's solution for 8 days and then placed for 4 days in a mixture composed of 2 parts Müller's fluid and 1 part of a 1 per cent. solution of osmic acid. It is then carried through alcohols of increasing strengths and cut in celloidin.

Busch secures better penetration by using osmic acid 1 part, sodium iodate 3 parts, in 300 of water, instead of osmic acid and Müller's solution.

8. METHYLENE BLUE METHOD FOR NEURON FIBRES.

Spread out the tissue as thin as possible and wash in normal salt solution at 37·5° C. Place it in an open dish in an incubator at 37·5° C. and immerse in 1 in 1000 saline solution of Grübler's Bx methylene blue.

Keep it moist by adding a few drops from time to time.

Examine with a microscope every 15 to 30 minutes.

The stain takes from 2–6 hours and stains the nervous elements in the following order:

(1) Fine terminations.
(2) Nerve fibres.
(3) Nerve cells.

Specimens may be fixed by placing them in a saturated solution of ammonium picrate for an hour.

Mount in glycerine to which a drop of ammonium picrate has been added.

9. GOLD CHLORIDE METHOD FOR NEURON FIBRES.

Pieces of fresh tissue are placed in

(1) 25 per cent. formic acid for two minutes.

(2) 5 per cent. gold chloride solution for 20–30 minutes.

(3) 25 per cent. formic acid solution for 24 hours in the dark.

The specimens are mounted in glycerine acidulated with 1 per cent. formic acid.

10. STAINING FOR IRON.

The detection of the products of the disintegration of hæmoglobin may be effected by taking advantage of the fact that iron in simple compounds gives a colour reaction with certain reagents.

(1) A scraping of the tissue spread upon a slide—*e.g.* spleen—is treated with *ammonium sulphide*, covered and examined at once. The iron is dark green.

(2) Sections are washed, then placed for 10 minutes in a mixture of 4 per cent. *potassium ferrocyanide* and 2 per cent. *hydrochloric acid*. The iron is stained blue. The sections may then be washed, counter-stained for 10 minutes with carm-alum, dehydrated, cleared and mounted.

11. STAINING FOR GLYCOGEN.

Glycogen stains a dark brown with *iodine*.

(1) Scrapings of fresh liver may be treated with a solution of iodine in iodide of potassium and examined at once.

(2) Sections of tissues hardened in alcohol may be stained with iodine as above, dehydrated, cleared with alcohol and xylol containing iodine, and mounted in balsam.

12. STAINING FOR FAT.

Sections of tissues frozen directly or after fixing in formalin are generally used:

(1) *Osmic acid.* The sections are placed in 0·5 per cent. solution of osmic acid for about five minutes, then washed.

(2) *Sudan III.* The sections are placed in a saturated solution of sudan in 80 per cent. alcohol for ten minutes, then decolorised in 80 per cent. alcohol—usually for about three minutes, and mounted in Farrant's medium. They may be counter-stained with hæmatein.

13. STAINING ELASTIC FIBRES.

These may be stained by *magenta*, *picric acid*, or *orcein*—1 per cent. orcein in absolute alcohol with 1 per cent. hydrochloric acid. In this solution the sections are kept warm for some time, then decolorised with alcohol, dehydrated, and mounted in balsam.

PART II.

METHOD OF EXAMINING SPECIMENS.

(A) Examine fresh and unstained (p. 4). (B) Examine prepared and stained (p. 5 *et seq*).

1. First with the **naked eye**, and then with the low power, study the general arrangement, and distinguish the separate parts to be studied, making a sketch or plan and an inventory of the various parts to be studied in detail.

2. Study the separate parts in detail with the **low power**:

(i) *Colour.* (ii) *Shape.* (*a*) Length, (*b*) breadth, (*c*) depth (thickness). (iii) *Outline.* Sharply or not sharply defined —double or single contour. (iv) *Texture.* Homogeneous, granular, fibrous, etc. (v) *Size.* (*a*) Compare with Human Erythrocyte, which is 7·7 micros. in diameter, under the same power; (*b*) measure with the micrometer.

A. *Measurement of Objects.*

Prepare a micrometer scale by laying a sheet of white paper close to the right side of the base of the microscope and placing a stage micrometer ruled in divisions of $\frac{1}{10}$ and $\frac{1}{100}$ mm. under the objective. With the left eye look down the tube of the microscope, keeping the right eye open, and the lines on the micrometer will appear to be projected on to the paper. Mark these lines with a pencil. The spaces correspond to $\frac{1}{10}$s and $\frac{1}{100}$s of a mm. When an object has to be measured place it under the objective and treat it in the same way as the stage micrometer, and its image will appear projected on the spaces marked on the paper, and it may thus be measured.

B. *With the Eye-piece Micrometer.*

To set the eye-piece micrometer place the disc of glass on the ledge in the middle of the eye-piece so that the ruling is seen when the eye-piece is in position. Place a stage micrometer on the stage of the microscope and bring the ruling into focus. Now, by pulling out or pushing in the draw tube, bring one, two or more of the spaces of the eye-piece micrometer to correspond to a space of the stage micrometer. If the spaces on the stage micrometer are $\frac{1}{10}$ mm., then the length to which a space on the eye-piece micrometer corresponds may be calculated.

Points which cannot be made out with the low power are then to be examined with the **high power**.

Drawings of the whole specimens or of its various parts must be made under the low power, and if necessary under the high power.

PART III.

THE STRUCTURE OF THE NORMAL TISSUES AND ORGANS.

The structure of the tissues, organs and fluids is to be studied practically on a series of specimens prepared by the student or given out during the session. Drawings and descriptions of each of these must be made.

In revising this part of the work any of the standard text-books of Histology may be used.

APPENDIX.

Flemming's Solution.

1 per cent. Chromic acid,	15 parts.
2 ,, Osmic acid,	4 ,,
Glacial acetic acid,	1 part.

Perenyi's Fluid.

10 per cent. nitric acid,	4 parts.
Alcohol,	3 ,,
½ per cent. chromic acid,	3 ,,

Phloroglucin-Nitric Acid Solution for Decalcification of Bone, etc.

1 gram of phloroglucin.
10 c.c. of pure nitric acid (sp. gr. 1·4).

Mix and warm slowly and carefully. Dilute the resulting clear solution with 100 c.c. of distilled water and add 10 c.c. of pure nitric acid.

Tissues are decalcified very rapidly.

Excellent results may also be obtained by taking 1 gram of phloroglucin to 100 c.c. of from 5 to 40 per cent. pure hydrochloric acid.

Müller's Fluid.

Bichromate of potash,	2 parts.
Sulphate of soda,	1 part.
Water,	100 parts.

Mayer's Hæmalum.

Dissolve 1 gram of hæmatin in glycerine by rubbing up in a mortar, and make up to 1000 c.c. with ·5 per cent. potash alum solution.

Farrant's Medium.

Saturated solution of arsenious acid (filtered), }
Glycerine, - - - - - - - } equal parts.
Water, - - - - - - - - }

Add gum acacia until medium is of proper consistency, and then add 1 drop of carbolic acid.

Jenner's Stain.

Grübler's water-soluble eosine, - - 1·2 per cent. solution in water. }
Grübler's medicinal methylene-blue, 1 per cent. solution in water. } equal parts.

These are thoroughly mixed in an open dish and left for 24 hours. Filter and dry in an oven at 50° C.

For use ·5 gram of the powder is thoroughly shaken up in 100 c.c. of pure methyl-alcohol and filtered.

Hayem's Solution.

Corrosive sublimate, - - - - ·5 gms.
Sodium sulphate, - - - - 5·0 ,,
Sodium chloride, - - - - 1·0 ,,
Distilled water, - - - - - 200·0 c.c.

Sugar Mounting Fluid.

Candied sugar, }
Distilled water, equal parts, } - - 4 parts.
Dextrin, }
Distilled water, equal parts, } - - 1 part.

Mix and add thymol.

Fixing Solution for Golgi's Method (Cox's modification).

Potassium bichromate, 2 per cent.- - 50 parts.
Potassium chromate, 5 ,, - - 16 ,,
Corrosive sublimate, 5 ,, - - 20 ,,

Reducing Solution for Cajal's Method.

Pyrogallic acid, - - 1 gm.
Formol, - - - - 10 c.c.
Alcohol, - - - - 10 c.c. or so.
Distilled water, - - 100 c.c.

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