

III.—*On Cutting Sections of Animal Tissues for Microscopical Examination.* By JOSEPH NEEDHAM, F.R.M.S., &c., Demonstrator of Histology at the London Hospital Medical College.

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KNOWING the primary object of this Society to be the diffusion of practical knowledge amongst its members, I shall endeavour to further that object, this evening, by taking into consideration the various methods of cutting sections for microscopical examination.

It is not my intention to enter into the history of the subject, for time will not permit; we will, therefore, proceed at once to the more interesting part, which will be practically demonstrated.

We have three classes of tissues to deal with, each differing in consistence. Bone may be taken as the type of the first or hard; cartilage of the second or intermediate, and kidney of the third or soft.

I. Sections of *hard structures*, as bone, teeth, &c., are to be made by a gradual wearing away of the tissues on two opposite sides, corresponding in position, the planes of these sides being kept parallel to each other till the required thinness is attained; this may be accomplished in two ways, as follows:—

1st Method.—Deprive a bone of the ligaments, muscles, and tendons attached to it—in a way that will be presently described—and dry it; then firmly fix it in a vice, and divide it into thin plates by means of a very fine bow-saw, the blade of which should be made of watch-spring and held by screws: place a portion of bone so obtained on a flat surface, and remove the first excess by means of a file; several files may be used for this, commencing with a coarser and finishing with a finer. The section is to be now placed on a good flat hone, and rubbed down on both sides to the required thinness, being kept in contact with the stone by the pressure of the finger or thumb, or fixed on a piece of cork. Although the section is now thin enough, and sufficiently smooth for mounting in Canada balsam, yet when viewed as a dry object, will be found to exhibit

EXPLANATION OF FIGURES.

FIG. 1.—Section of plano-concave razor.

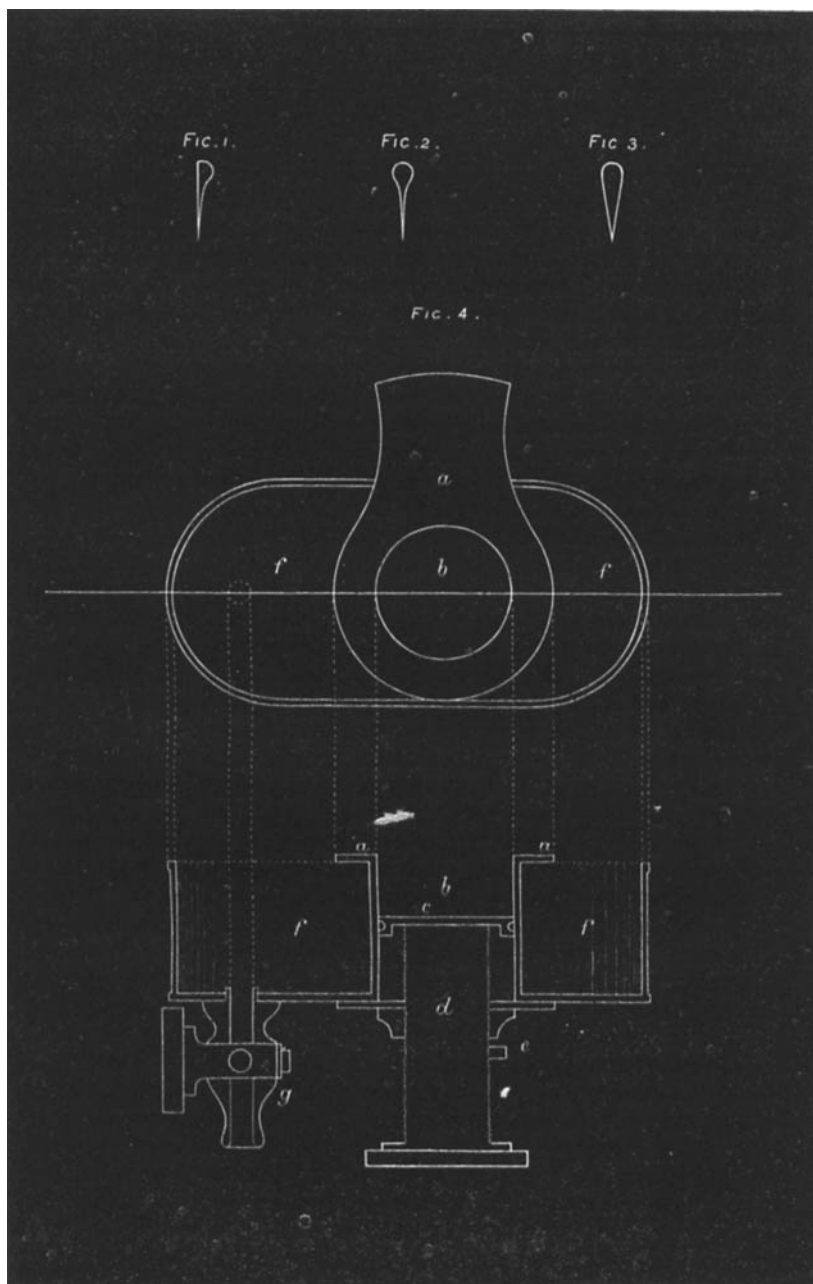
„ 2.—Section of bi-concave „

„ 3.—Section of flat „

„ 4.—Upper surface and vertical section of Refrigerating Microtome. *a*, brass

plate, with hole in centre; *b*, tube fixed to ditto; *c*, plug; *d*, graduated screw; *e*, indicator; *f*, oblong box to contain the freezing mixture; *g*, tap to carry off water.

Drawn to a scale of one-half.



numberless scratches, giving it a confused appearance; but this may be easily removed by polishing the section on a glass plate, with a little Tripoli or fine emery powder; a piece of leather firmly spread on a flat piece of wood, with a little powder sifted over it, will also answer the same purpose.

2nd Method.—The bone is sawn into lamellæ, as in the first method, or by a thin circular rotating saw, then ground down to a moderate thinness on a small grindstone, made to rotate in an ordinary lathe, the stone being kept moistened with water. It should then be further ground on both sides on a fine flat whetstone, also rotating, and finally polished as in the previous instance. The sections obtained by both processes should be cleaned in water, either with a camel's-hair brush, or—which is far preferable—a soft toothbrush, and dried; they are then ready to be put up in Canada balsam or dry. The last method was adopted and used by the late Mr. Carter for many years. A sufficient guarantee of its success is the splendid collection of sections of bones and teeth, made by him, in the possession of the Royal Microscopical Society.

Bones may be prepared either by removing the surrounding tissues with a scalpel, and drying, or, after cleaning in this manner, steeping them for some months in a large quantity of water, which should be changed occasionally to prevent putrefaction. During the maceration they should be scrubbed from time to time with a hard brush; and when perfectly clean, they should receive a final scrubbing in clean water, and dried by exposure to the atmosphere. By simply drying, a bone, saturated with fat, is generally the result, from which a dry, white specimen cannot readily be obtained; by the latter process, however, a perfectly white bone—especially if it be from a dropsical subject—will be the reward for time and patience expended on it.

II. Tissues of Intermediate Density.—Under this head may be classed decalcified bone, cartilage, tendon, and many tissues hardened by chromic acid, and other agents.

Sections of bone prepared by the methods already described, although very instructive and beautiful, do not show the soft organic structure, but only the bony framework; if we desire to exhibit the relations existing between the periosteum, blood-vessels, and nerves, it will be necessary to soften the bone; to effect this, after being cleaned from surrounding tissue, it is to be placed in a large quantity of one of the following solutions:—Chromic acid, 3 or 4 per cent.; or a mixture recommended by Professor Rutherford,* consisting of nitric acid, 2 per cent.; chromic acid, 1 per cent. When the softening is carried on in this solution, the tissues assume a bright green colour, due to the decomposition or reduction of the chromic acid into sesquioxide of chromium, Cr_2O_3 . Nitric

* Rutherford, No. 45, 'Quarterly Journal of Microscopical Science.'

and hydrochloric acids in a state of extreme dilution have been recommended by Dr. Frey* and Dr. Beale.†

Cartilage, whether hyaline or fibrous, needs no preparation. Glandular, nervous, and muscular tissues, and other soft structures, require hardening in methylated alcohol, solution of chromic acid and its salts, either singly or combined, or in union with sulphate of soda, varying in strength from 2 to $\frac{1}{8}$ per cent., or even less. Saturated aqueous solution of picric or carbazotic acid, strongly recommended by Ranvier;‡ solutions of osmic acid, $\frac{1}{4}$ per cent. (Schultze), bichloride of platinum (Merkel), bichloride of mercury, and chloride of palladium (Schultze). Of all these, chromic acid, its salts, and alcohol are preferable. It is not my province to enter into the subject of softening and hardening, suffice it to say that tissues may be made to assume—by subjecting them to the action of these solutions—a sufficient degree of softness in the first case, or of solidity in the last, to permit of sections being made with an ordinary razor or scalpel. For this purpose, a piece of cartilage, or of any tissue which has been softened or hardened to a density resembling it, may be held in the hand, or placed on a small, flat, plate of wax, being fixed in a convenient position by the middle finger and thumb of the left hand, whilst the thickness of the section is regulated by the nail of the forefinger of the same hand. The razor, which should be wetted with water, spirit, or glycerine, in this case must be held horizontally, with the cutting edge directed downwards; the section is made by drawing it from before, backwards. This method has been in use for some time at the London Hospital, and is universally liked.

For the purpose of hardening, the following agents may also be employed:—Aqueous solution of oxalic acid; drying, or boiling in a mixture composed of creosote, vinegar and water, and then drying; but they cannot be recommended; all have been deservedly superseded by those previously enumerated, and are now entirely relinquished.

III. We will now direct our attention to the preparation of sections from those tissues classed in the third division, which includes nearly all fresh material, from which good preparations cannot be obtained, without the assistance of some special arrangement, *e. g.* Valentine's knife, embedding, or freezing.

(A.) The double-bladed knife invented by Professor Valentine is especially recommended by Dr. Beale.§ It has been made to assume an endless variety of forms, every maker of the instrument modifying it in some way. Four forms only are worthy of notice. (1.) The original consists of two blades, differing in length; the longer is firmly secured in an ivory handle, the shorter is fixed to the longer

* Frey, 'Das Mikroskop und die Mikroskopische Technik,' American translation by Dr. Cutler.

† Beale, 'How to Work with the Microscope.'

‡ Frey, *loc. cit.*

§ Beale, *loc. cit.*

by means of a screw, the distance between the blades being regulated by a second screw situated nearer the cutting portion of the knife; the blades are sharp at the point and wide at the base, so that the cutting-edge slants downwards from the point. (2.) The second form is that made by Mr. Matthews: the whole of this knife is constructed of metal, the blades being continuous with the handle; they are short, and of equal breadth throughout; the cutting-edges are convex from point to base. An eye is attached to one blade, and on the other—in a corresponding position—is a longitudinal slot. In bringing the two portions of the knife together, the eye passes through the slot, and they are secured in position by sliding through the eye a spring-side thumb catch. The distance between the blades is regulated by one or two screws. (3.) The next form is that invented by Dr. Maddox, and manufactured by Mr. Baker, of High Holborn. It is a triple-bladed section knife. The circumstances that led to this invention are briefly described by him * as follows:—"I felt the want of some method by which a double section might be cut, so as to present, when removed, the opposite, but contiguous, surfaces of the part through which the section had passed, and which with the ordinary double-bladed knife is quite impossible." This knife overcomes the disadvantage referred to; at the same time by removal of one of the outer blades it is convertible into an ordinary double-bladed knife.

In using these knives a difficulty will be experienced in regulating the blades so that the interval between them may be equidistant throughout; to render this more easily accomplished, Mr. Hawksley, of Blenheim Street, has constructed an improvement on Matthews' form. (4.) A spring is fixed between the two blades, the distance being regulated by one screw, which has a graduated milled head. By this arrangement parallelism is obtained with facility; it also has the additional advantage of indicating and regulating the thickness of the section.

The tissue to be operated on may be placed on cork, on the wax tablet before referred to, or on leather, and held steadily between the fingers and thumb of the left hand, or may be simply retained between the fingers and thumb. The method devised by Dr. Fenwick is remarkably good for membranous structures, *e.g.* a portion of stomach is drawn around the thumb and held in position by the fingers, the section being taken from that part covering the thumb nail. The knife must be drawn by one continuous stroke completely through the tissue, then the cutting-edge slightly turned, so that the section may be severed from the surrounding material. The section is now made, and is to be liberated by opening the blades and gently agitating the knife in water, when

* Maddox, No. 1, 'Monthly Microscopical Journal.'

it will float off; or it may be displaced from the blade with a camel's-hair brush. In removing thus, great care is necessary, otherwise the section will be lacerated. As regards wetting the blades, the same precaution obtains here as in the method described in Section II.

(B.) Finer sections can always be made when the tissue is firmly supported. For this purpose embedding is necessary. Various mixtures have been proposed by different authorities, of which the following is at best an incomplete list:—

Stricker* employs equal parts of white wax and olive oil. Drs. Urban Pritchard and Ferrier† recommend a mixture composed of solid paraffin, five parts; spermaceti, two parts; lard, one part. His‡ covers the object in pure paraffin, and a mixture of white wax and cocoa-butter is used by Mr. Moseley.§

Of the above mixtures, perhaps the best and cheapest is the wax and oil mass. In its preparation the finest white wax and purest olive oil must be used; the proportion of wax to oil will greatly depend upon the firmness of the tissue to be embedded. The greater the density, the larger will be the amount of wax required, and *vice versâ*; but the mass generally used is made as follows:—Equal parts of the ingredients are placed in a porcelain dish and heated till all the wax has melted, being continually stirred with a glass rod, that the mixture may be well incorporated; the mass is now ready for use. If the material from which sections are to be made has been hardened in aqueous solutions, it must be removed and steeped in ordinary methylated or absolute alcohol, so that the water may be replaced by spirit; this will occupy a longer or shorter time, according to the strength of the alcohol and size of the tissue. When perfectly saturated with spirit, an oblong piece is to be removed from it with a scalpel. A paper box must now be made according to the size of the piece, and about half as long again, the breadth and depth being in proportion; say, for example, the piece to be embedded is 1 in. long, and $\frac{1}{4}$ in. in breadth and depth. We must take a piece of stiff, well-glazed paper, $2\frac{1}{2}$ in. long and $1\frac{1}{2}$ in. broad, which is to be folded on itself for about $\frac{1}{2}$ in. on both sides in the long diameter, then the ends for a similar distance, so that it now appears to be only $\frac{1}{2}$ in. broad and $1\frac{1}{2}$ in. long. Now unfold the sides to half the distance, so that four walls are formed; the triangular pieces projecting from the corners are to be folded on the ends, so that they overlap each other, and, kept in position with a little gum or a pin, our box is now complete.

* Stricker, in Introduction of 'Manual of Human and Comparative Histology,' translated for New Sydenham Society, by H. Power, M.B., &c.

† Pritchard and Rutherford, p. 16, No. 45, 'Quarterly Journal of Microscopical Science,' and p. 382, No. 48, *ibid*.

‡ Frey, *loc. cit*.

§ Moseley, p. 337, No. 40, 'Quarterly Journal of Microscopical Science.'

It should be placed on a flat piece of cork and filled with the melted mass, sufficiently to cover the piece of tissue; as soon as the wax mixture begins to solidify around the edges of the box, the tissue is to be introduced as follows: *—"A needle is stuck slightly into the end opposite to that from which sections are to be cut, and the bit is plunged into the mass with its long diameter horizontal, and in such a position that the end furthest from the needle is near, but not in contact with, the side of the box, and consequently the other end is at a considerable distance from the side. In this way, although the whole is surrounded with the wax mass, there is a greater thickness around the end into which the needle is stuck, so that the whole can be securely and conveniently held." By passing the needle directly through the tissue and into the cork upon which the box rests, "the operator is saved the trouble of holding the needle till the wax mixture solidifies. In finally withdrawing the needle, the greatest care must be taken to give it a twisting motion, as otherwise, especially if the object is thin, it is apt to be displaced." "If a thin membrane is to be embedded, of such tenuity that a needle could not be introduced without danger of destroying it, the following method may be used:—A box is half filled with the mass, and then, as soon as it begins to solidify, the membrane is applied to the half solid surface; the box is then filled with a thoroughly fused mass, care being taken that it is not too hot." In embedding any of the fatty masses, great care should be taken that the surfaces of the piece are dry previous to immersion in the mass, otherwise the medium will not adhere to it.

Besides the media already mentioned, others, as gum,† and a mixture of gelatine and glycerine,‡ have been used for some tissues with great success. The first is prepared by making a clear concentrated solution of the pulverized gum acacia. The gelatine mixture is prepared as follows:—Two parts of concentrated solution of isinglass and one part of pure glycerine. It is not necessary to place the tissues in alcohol previous to embedding in these. A paper box or cone having been prepared, it is filled with the mixture—the gum cold, the gelatine hot; the piece of tissue is then thrust into it. The gelatine mass when cold becomes solid. Both are then placed into common alcohol until a sufficient degree of hardness is attained; on releasing them from the paper they are ready for further treatment.

When cavernous structures, as lung-tissue, cochlea, &c., are embedded by the foregoing methods, good sections cannot always be obtained, in consequence of the tissue not being supported within, as well as without; but if it be placed in any one of the melted

* Klein, in 'Handbook for the Physiological Laboratory.'

† Stricker, *loc. cit.*

‡ Klebs in Frey, *loc. cit.*

embedding mixtures, or in the gum mass cold, under the receiver of an air pump, and exhausted till bubbles cease to come from the tissue, the mass will penetrate into the spaces, previously occupied by air; after solidification has taken place they may be dealt with as an ordinary embedding, and in this way it will be possible to obtain fine preparations.

Professor Quekett used to inject hot tallow through the bronchi into the air cells of an injected lung, which after cooling and drying yielded a splendid mass. All transparent lung injections put up by Mr. Topping are prepared in this way; although this plan answers the purpose very well, it is doubtful if it be even equal to the last.

For making sections of the tissues thus embedded, razors or section knives are used. I have employed for a considerable time the flexible-edged, concave-sided razors made by John Heiffor, and stamped "made for the army"; they are extremely thin for some distance from the edge, and the hollowed-out surface holds plenty of alcohol; this is absolutely necessary to prevent the section sticking to the blade. Mr. Moseley* also strongly recommends them for histological work. Dr. Klein's section knife has one side flat, and the other concave; the blade is eight inches in length. The razors previously referred to are equal and considerably cheaper, being obtainable from any cutler for the small sum of one shilling each. Previous to cutting the tissue embedded in any of the fatty masses, the instrument must be wetted with ordinary spirit, or, still better, absolute alcohol; in which liquid the sections must be placed as soon as made, to clean them from the surrounding material, after which they are ready for staining. If embedded in gum or gelatine, water or glycerine must be used to moisten the knife, and the sections cleaned in water. For lung injected with tallow, the razor is kept wet with turpentine, in which fluid the sections must be immersed; the tallow will readily dissolve, and the sections may be put up at once in Canada balsam, or solution of damma or balsam in benzole.†

(C.) It is often desirable to obtain sections of perfectly fresh tissue, for the purpose of immediate examination, or perhaps for impregnation with some metallic salt. It is obvious that no assistance can be looked for from hardening fluids or embedding, and very little indeed by means of the double-bladed knife. We must have recourse to one of the refrigerating methods. (1.) The process generally adopted is that described by Klein in the 'Handbook for the Physiological Laboratory,' from which the following is quoted:—"A freezing mixture is prepared by introducing alternately small quantities of broken ice, or snow (not so advantageous), and of

* Moseley, *loc. cit.*

† Bastian, p. 96, No. 2, 'Monthly Microscopical Journal.'

finely-powdered salt, into a large vessel, mixing the two ingredients thoroughly after each addition. The object, which must be small, should be cut to an oblong form, and placed on a flat cork, much wider than itself. It must be pinned to this cork at the end opposite that from which the sections are to be cut. In the case of a membrane the object must be folded, and fixed in the same way. The whole is then placed in a platinum crucible, which has been previously plunged into the freezing mixture. The crucible must be at once covered, and a little of the freezing mixture placed on the top of it. The section knife, which must be sharp, is cooled by laying it on ice. As soon as it is ascertained by exploration with a needle that the preparation is firm enough, the knife is handed to an assistant, who wipes it, and holds it in readiness. The cork is then taken out with the forceps, and seized by the fingers of the left hand in such a way that they do not come into contact with the preparation. A succession of sections having been rapidly made, the number varying with the skill of the operator, the cork is replaced in the crucible." This method, perhaps giving very satisfactory results in dexterous hands, seems to be excessively tedious, awkward, and rather primitive, in comparison with that to be next described.

(2.) The best way to obtain sections of fresh, hardened, or softened tissues for immediate examination or further treatment, is, undoubtedly, by freezing in the refrigerating microtome. I refer to Mr. McCarthy's modification of Professor Rutherford's microtome, made by Khroné and Sesseman, of Whitechapel Road. It consists essentially of a brass plate having a hole in the centre; to the under surface a tube is fixed whose bore corresponds to, and is continuous with, the hole; a thin plug is accurately packed in the tube, capable of being moved up and down by means of a graduated screw: external to the tube is an oblong box, through the bottom of which the screw passes into the tube. A small tap communicates with the interior of the box to carry off water, if necessary, as produced by the ice. The plate rests on, and occupies about the middle fourth of two sides of the outer box. The whole is capable of being securely fastened to any table by a second screw. The machine is made of brass, and the sides are padded externally with leather.

The machine is first fastened to a table, a large square piece of flannel being interposed between them, the tap turned on, and the plug forced to the bottom of the tube, after displacing the graduated screw. The pieces of tissues—I say "pieces," because two or more different portions may be cut at the same time, if it be possible to distinguish the sections by the shape or colour from each other; *e.g.* lung and intestine may be readily distinguished by their shape, and easily separated after being cut into sections of extreme

tenuity—are placed in the desired position in the tube, which is then filled up with water, the aperture and contents being protected by placing a small piece of oil-skin over the plate. A thin layer of ice, divided into small pieces, is now placed in the box and on the oil-skin, over this is sprinkled a quantity of pulverized salt, another layer of ice is then added, and again more salt, and so on till the refrigerating mixture is piled over the plate; finally, the whole mass, machine included, is covered up with the flannel. After an interval of about twenty minutes from the completion of the operation, the tissues will be sufficiently frozen for cutting. All that is now necessary is to remove the ice mixture *above* the plate and sides of the machine, when the tissue embedded in *ice* will be exposed ready for cutting. By turning the graduated screw—the thickness of the section depending upon the number of turns or part of a turn given to this screw—the frozen mass will be elevated, a razor is placed at a slight angle on the brass plate and drawn obliquely through the mass projecting from the tube; the sections as they are cut can be easily floated off the razor into water. The razors used for this machine must be *flat and kept sharp* by frequent stropping.

Tissues hardened or preserved in spirit must be placed in water, or a very dilute solution of bichromate of potass to withdraw the spirit, otherwise the freezing of it will be retarded, if not entirely prevented.

By this method I have cut large and beautiful sections of *uniform* thickness of softened teeth and bone, of cartilage and tendon, and of hardened and fresh tissues of every description. Further, I may safely say that, if the given directions be rigidly followed, failure will be impossible; moreover, should you give this method a fair trial (a certain amount of experience in this, as in all things, being necessary), I am confident you will readily concur in Stricker's* assertion: "*The simplest and most elegant mode is that of refrigeration.*"

* Stricker, *loc. cit.*