4. *Swiss Retrospect.*

Histological Technique of the Central Nervous System. Methods of Staining. By A. MERCIER, M.D., Assistant Physician in Burghölzli Asylum, Zürich.*

In dealing with this subject I must mention the preliminary steps in the preparation of sections, and since the hardening of pieces of the central nervous system is perhaps the most important part of the whole process, it will be necessary to enter into many details which are not usually given. I shall also have to describe shortly the manipulation of sections, and, to ensure success, may have to describe things which may seem foreign to the subject, but which are really essential. The subject will be divided into sections.

SECTION I.

Portions of the central nervous system which are to be hardened must first be carefully removed. The best method after removal of the brain and spinal cord (the dura mater of the latter having been opened up) is to cut the tissue into smallish pieces, *e.g.*, not exceeding two or three c.m. across. The site whence each portion was obtained is to be recognized by placing each in a separate and labelled vessel. This should be done as soon as possible after death, care being taken that the pieces do not get soiled in any way. They must not be touched with the fingers, but the whole operation performed with scalpel and forceps only; these instruments must be handled most delicately. Pieces must not be washed before being put into the hardening fluid. If the pia-arachnoid is not easily separable the piece must be allowed to harden in the fluid till the membranes can be removed with safety.

As to hardening liquids, alcohol serves well for anatomical preparations, but not for the preparation of sections which are to be stained, since some reagents, *e.g.*, carmine, fail to stain sections which have been in alcohol, even for a short time.

Potassium bichromate is essential for hardening purposes when the myelin fibres are to be stained. The liquid most usually employed is known as Müller's liquid. Its formula is:—

Potassium Bichromate	20	grammes.
Sulphate of Potash	10	,,
Distilled water	1000	,,

Although this liquid enjoys a great reputation, its employment has appeared to me to have some disadvantages, *e.g.*, it hardens unequally. This is most observable when large pieces are taken; but even small pieces may not be hardened centrally when the outside is quite hard. This is the experience of others besides myself. Moreover, several methods of staining are not available when Müller's liquid has been used. The solution I prefer is a weak solution of bichromate of potash: such will harden the piece equally throughout.

A common fault in the hardening of tissues is the employment of too small a quantity of liquid. For five or six of the small pieces mentioned above the minimum quantity must be not less than 50 c.cm. For large pieces the quantity

* Having had an opportunity of seeing Dr. Mercier's beautiful sections of the cord in the laboratory of the Zürich Asylum last autumn, we requested him to favour us with his method of staining, etc.—EDS.

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to be employed must be proportionately large, *e.g.*, for the hemispheres four or five litres, for the cerebellum two or three litres, for the medulla oblongata, pons and crura approximately one litre.

The next fault, almost always committed, is to allow the pieces to remain too long in the same quantity of liquid. Fresh pieces decompose sooner or later even in solutions of bichromate of potash. The products of this decomposition foul the liquid, and after a short time such pieces become mouldy. It is no wonder then that pieces are so often badly hardened and cannot be properly stained. During the first eight days of hardening the liquid ought to be changed every 48 hours, after this time it should be changed weekly till the desired hardness is obtained. Bichromate of potash being an inexpensive substance fresh solutions should be prepared each time.

The position of small pieces need not be changed, since in the changing of the liquid this will take place to the needful extent. But when large pieces are taken it is necessary to change their position frequently. In the case of the hemispheres, cerebellum, etc., the parts should rest upon a layer of wadding, and wadding must also be inserted between the two hemispheres and between the cerebellum and the medulla in order to prevent the apposition of two brain surfaces. The position of these large pieces must, as has been said, be frequently changed. In each case the object in view is to have every part in contact with as much fluid as possible. I consider that vessels which contain pieces for hardening must always be kept in the dark, for daylight decomposes the bichromate. The vessels must also be well covered.

For the first days when small pieces are taken it is quite sufficient to employ a solution of $1\frac{1}{2}\%$. After this, and for large pieces earlier, the solutions should be stronger—$2\frac{1}{2}\%$ to $2\frac{1}{4}\%$. It is necessary to remember that pieces of the spinal cord harden more quickly than pieces of the brain and medulla oblongata, and that in the case of the lower animals pieces harden more quickly than in the case of the higher species. No general rule, however, can be laid down respecting this. The solutions should be made, especially at the commencement of the process, with distilled water. Solutions containing pieces keep better with the addition of small pieces of camphor.

The time which is necessary to obtain the desired degree of hardness is very variable; it depends on many circumstances, *e.g.*, the nature of the piece, its freshness, the care employed in changing the liquid, etc., and the temperature of the liquid (with regard to this last I do not hold that there is any advantage in raising the temperature, though, no doubt, a medium temperature is better than a low one).

The hardening process takes place generally in from two to four months when the pieces are of moderate size; the whole brain will not take less than five or six months, while on the other hand small pieces will harden in from four to six weeks.

The right degree of hardness is recognized by experience; a well hardened piece is capable of being cut easily in thin slices with a well whetted razor. The hardness should be combined with a slight elasticity, the colour of the grey and and white substance should be nearly the same. Over-hardening is a danger to be avoided, since pieces cannot then be well cut, as they are too brittle. Further, they stain badly afterwards. A well hardened piece, if for some reason it cannot be immediately cut, may be kept for some time longer in a weak solution of bichromate, $\frac{1}{4}$ to $\frac{1}{2}$ or 1%, to which a small piece of camphor has been added.

The next point to determine is the nature of the histological elements which we specially desire to study. On this question depends the further treatment of the hardened piece, the choice, *e.g.*, of a colouring agent.

Suppose that we wish to study the cells and axis cylinders of a continuous series of sections. We must then soak the piece in water to get rid of the excess of bichromate which it contains; clean water which has been boiled and cooled may serve for this purpose in lieu of distilled water. The water must be changed every day so long as it continues to be coloured by the bichromate. So soon as

this ceases the piece is imbedded in the microtom of Gudden and cut under water and stained according to appropriate methods.

The removal of the bichromate from the hardened piece is accelerated by using the water slightly warmed, and to this end the jar or vessel containing the piece may be placed in a warm place, *e.g.*, near a stove.

Should we desire to stain the myeline fibres as well as the cells and axis cylinders we shall have need to employ a combined or mixed method. The hardened piece is now embedded directly in the microtome of Gudden and cut into sections. Of the sections thus obtained we put aside a certain number for treatment by the method to be described later—for staining myeline fibres. The others, in which the cells and axis cylinders are to be coloured, are placed in distilled water, where they get rid of their excess of chrome salt. They are then stained with carmine or aniline, etc. The reason for this procedure is that the chrome salt is necessary to fix the colouring matter employed to stain the myeline fibres, whilst on the other hand cells and axis cylinders, which are strongly impregnated with chrome salts, will not take carmine and aniline dyes. We have therefore to keep the chrome salt in the one instance and to get rid of it in the other.

The preliminary preparation of *pieces* whose sections are to be stained for myeline fibres only is as follows:—The piece is placed in alcohol 70% and left there for a certain time; it is then imbedded in celloidin, and subsequently undergoes a special treatment to be described further on. Special microtomes, not that of Gudden, are employed to cut these sections.

Accordingly we may proceed according to three methods:—

a. We may cut with the microtome of Gudden and stain the sections for cells and axis cylinders only.

b. We may cut with the microtome of Gudden and treat the sections obtained in two ways—1st, for axis cylinders and cells as above, 2nd, for myeline fibres by a modified Weigert process.

c. We may adopt from the first a special method of cutting sections and of staining in order to demonstrate the myeline fibres—method of Weigert and of Pal.

(To be continued.)

PART IV.—NOTES AND NEWS.

MEDICO-PSYCHOLOGICAL ASSOCIATION.

The quarterly meeting of the Association was held at Brislington House, Bristol, on Friday, May 1st, at 3 p.m. The chair was taken by Dr. Yellowlees (the President), and among those present were:—Dr. Hack Tuke, Dr. Fletcher Beach, Dr. Thos. Webster, Dr. E. Markham Skerritt, Dr. Geo. H. Savage, Dr. David Nicolson, Dr. Charles S. Wigan, Dr. J. H. Paul, Dr. E. B. Whitcombe, Dr. R. Percy Smith, Dr. Samuel Craddock, Dr. T. Outterson Wood, Dr. T. Seymour Tuke, Dr. H. T. Pringle, Dr. Ernest W. White, Dr. A. Law Wade, Dr. A. C. Suffern, Dr. L. A. Weatherly, Dr. John Ewens, Dr. Augustin Prichard, Dr. J. Michell Clarke, Dr. Vincent Milner, Dr. Samuel Smith, Dr. Harry A. Benham, Dr. R. Shingleton Smith, Dr. F. G. Heyman, Dr. Wm. A. Moynan, Dr. H. Rayner, Dr. M. J. Nolan, Dr. Charles H. Fox, Dr. Bonville B. Fox, Dr. W. J. Fyffe, Dr. J. Hannocke Wathen.

The PRESIDENT—I think we must proceed to business, and happily for ourselves and our visitors the business shall be very brief. The first part is pleasant; it relates to the next annual meeting, and the Council have fixed the date of that meeting for the 23rd July, and the place of meeting will be