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I.—*A New Process of Preparing and Staining Fresh Brain for Microscopic Examination.* By BEVAN LEWIS, F.R.M.S., Pathologist and Assistant Medical Officer, West Riding Asylum.

(Taken as read before the ROYAL MICROSCOPICAL SOCIETY.)

PLATE CXLIX.

It has always appeared to me a matter of very essential importance that the microscopical examination of the brain should involve some process applicable to immediate use, and that we should not, as is so often the case, rest content with its microscopic appearances until the lapse of six or eight weeks has prepared the brain substance for examination by the ordinary processes of hardening and section cutting.

The earlier our examination is made after death the more probable is it that minute lesions will be detected and fallacies eliminated which are necessarily involved in processes more complicated and prolonged. In advocating a method which I now adopt for the examination of fresh brain I would wish my readers to fully understand that I do not for an instant assume that any such process can possibly supersede the now venerable method of hardening by chromic acid and its salts. Both methods should be employed in all cases, for each has its individual merits; thus the older plan preserves to us most faithfully the natural *relationships* of individual parts, which cannot be said for the new process; on the other hand, we gain by the fresh examination far greater *details* of structure and *differentiation* of the various constituents, besides other advantages naturally attendant upon so early an investigation. It will therefore be evident that whilst for researches in comparative histology the hardening reagents must be employed, for many purposes, especially *pathological*, the fresh method has its special advantages. In the pathological department of West Riding Asylum I have enjoyed especially good opportunities for thoroughly testing the question whether or not a process could be devised for examining in the fresh condition the structure of the cerebral and cerebellar cortex. I have no doubt that in this investigation I am working side by side with others equally anxious to solve the

question, and trust that the publication of my own results may, whilst it tends to prove that at least some advance has been made in the right direction, also serve as a stimulus and encouragement to the prosecution of what certainly is a praiseworthy object. Dr. Batty Tuke certainly has the merit of first applying the process of staining to fresh brain, but the method employed by him differs in many very essential points from the process which I have here to recommend. I cannot undertake to express any opinion on the merits of his process as I have not myself adopted it, but I have been told that it occasionally yields very good results. The aniline black which is so valuable an adjunct in staining the nerve-cells was not employed by Dr. Batty Tuke, attention having been directed to this reagent by Mr. Sankey last year.

Another more recent mode has been adopted by Mr. H. R. O. Sankey, and deserves special attention, as by its aid the most beautiful preparations may be obtained. His process, briefly described in the pages of the 'Lancet,' was afterwards made the subject of an able paper in West Riding Asylum Reports for 1875.

Some months ago I described in the 'Medical Times and Gazette' a rough and ready method for examining the brain during an ordinary post-mortem, by which method the varied histological constituents may be well differentiated and morbid textural changes at once detected. The difficulty of obtaining a fair film by this measure may be objected to by some, but as the process almost entirely depends upon manipulative dexterity, any practised microscopist will find no impediment in his way. The method, however, which I have now to describe is developed out of the sum total of my experiments, and as it invariably presents us with a beautifully delicate film independent of any special amount of manipulative skill, it offers peculiar advantages to the most inexperienced worker. For facility of description I will arrange the subject under three heads embracing three stages of the process, as follows:—1. The preparatory stage. 2. Staining and differentiation. 3. Permanent mounting.

First, or Preparatory Stage.—The convolution of which the structure is to be examined is stripped of its membranes and a portion excised of a bulk sufficient for convenient manipulation. It is held between the thumb and second finger of the left hand, the index finger being utilized as a support and guide for the blade. One of the larger razor section-knives 6 to 7 inches long by 1 to 1½ inch in breadth will be found most convenient for making these sections. The upper surface of the blade should be deeply concave and kept deluged with spirit. A clean sweeping cut should now be made perpendicularly to the cortical layers down through the white matter, passing from without inwards so as to expose a perfectly smooth even surface. Thin vertical sections of the cortex

and a small portion of the medulla are now to be made by the same sweeping cuts, the precaution being observed of keeping the surface operated upon as well as the blade thoroughly and constantly moistened with spirit. Extremely delicate sections may thus be made after a little practice by rapid steady strokes of the knives, and all tearing avoided by the floating up of the section upon the moistened blade. I usually operate upon half-a-dozen sections simultaneously, as no further trouble is involved in preparing a large than a small number of such specimens. Having therefore the slides well polished placed before us, the finest and more delicate sections are selected and placed on the glass slips and a few large drops of Müller's fluid dropped over each section from a glass pipette or camel-hair brush. This fluid is allowed to thoroughly deluge the section above and below for some seconds, and a circular cover-glass dropped on in such a position that the section occupies about one-third to one-half the diameter of the cover. The covering glasses most serviceable for slides measuring 3 by 1 should be quite 2 centimeters in diameter. The blade of a knife or point of a strong mounted needle may next be placed on the centre of the cover and by steady gentle pressure the grey matter is flattened out into a thin almost transparent film. Practice soon enables the operator to so adjust the position of the cover that the extreme edge of this film still occupies the space covered by the circle. It will, however, be found of very slight disadvantage should the film be partly pressed outside the cover-glass, although I generally avoid such an occurrence, as it is not so likely to lead to the production of a film of uniform thickness. The superfluous fluid is removed by rapid rinsing in water, and the slide next transferred to methylated spirits. For this purpose I use a flat porcelain bath such as is employed by photographers, and which should contain sufficient spirit to cover the slides. In from thirty to forty seconds the film will be found in the best condition for removal without tearing. For this purpose the slide is removed, one edge of the cover-glass steadied with the finger, and the blade of a penknife gradually inserted beneath the opposite edge, and it will be found most advantageous to elevate that edge which impinges on the lower cortical layers or on the medullary substance. The cover is in this way most readily removed, and the most perfect films remain either loosely floating on the glass slip or closely and smoothly adherent to the cover. For rapid staining it is now advisable to remove all traces of spirit, and this is most readily accomplished by slightly inclining the slide or the cover to which the film is attached and allowing a gentle stream of water to flow from a large camel-hair brush over it. Having passed through this preparatory stage the film is ready for the second stage, that of staining.

Second Stage.—The film is now to be subjected to the agency

of some of the more suitable staining reagents, and it is necessary to remark that it is at present in the condition most suitable for histo-chemical research; the slightest tinting with a weak carmine solution just sufficient to render the cell elements visible enables the microscopist to apply his reagents to great advantage; at the same time metallic impregnation may be employed, as by the salts of gold, silver, platinum, or the hyperosmic acid. The dyes which I usually employ are the aniline and carmine solutions. The former as a 1 per cent. solution of aniline black is pre-eminently useful for staining the cells of the cortex and their processes, whilst carmine deserves the preference for the best differentiation of the delicate neuroglia basis or framework of the nervous system. The staining with aniline is thus accomplished: a large drop of the solution is placed on the film, which in a short time begins to assume a general and decided coloration. When the requisite colour has been acquired (a matter learned by experience only) the slides are transferred to a vessel containing water, and *very gently* lowered and allowed to rest on the flat bottom of the vessel. This immersion favours the staining and differentiation, for it will be observed that, as the superfluous dye floats away, aided by the gentle swaying to and fro of a brush in the fluid above, the film becomes deeper in hue and the staining perfectly uniform. The slide is now as carefully removed, all fluid drained off, and the preparation transferred to a bell-glass which protects it from injury and dust during the process of drying which it has next to undergo prior to mounting.

Third Stage.—Before the specimen can be permanently mounted in balsam it must necessarily undergo complete dehydration. For this purpose three methods present themselves for our choice, viz. 1. Spirit. 2. Artificial heat. 3. Spontaneous drying beneath the bell-glass, with or without the agency of concentrated sulphuric acid. The first method I have now completely discarded, as presenting numerous objections. The employment of artificial heat is also as far as possible to be avoided except where time is a matter of great import. The latter method is according to my own experience decidedly the best, and the addition of a small vessel of concentrated sulphuric acid beneath the bell-glass covering the slides very materially hastens the drying without in any way interfering with the delicate structures exposed in the slides. When perfectly dry a few drops of chloroform are placed over the film, which if examined in this moistened condition displays exquisitely the minute structure of the cortex. The chloroform must not be allowed to remain any length of time, a few seconds sufficing, and ere evaporation occurs a drop of a benzole or chloroform solution of Canada balsam is allowed to fall on the preparation, the cover-glass adjusted and gently pressed down. Oil of cloves may precede the

balsam in lieu of chloroform, but judging from the results obtained I find the plan advised preferable.

In reviewing briefly the process now described it will be seen that the preparation of the film is a feature of most essential importance, and it will be readily understood that the employment of Müller's fluid can prove in no way detrimental to the delicate cellular apparatus with which we are dealing. This fluid medium has been, I believe, very universally acknowledged by most histologists as the best for the preservation of the most delicate nervous textures, such as the retina, &c., with the least possible alteration of textural relationships. By its means, also, we avoid the extreme friability which a film produced in water alone acquires, and at the same time we reduce to a minimum that adhesiveness natural to a thin lamella obtained from the brain by pressure, and hence render the removal of the cover-glass a matter of the greatest simplicity. The subsequent short treatment by spirit still further aids the last-mentioned object, and serves to free the film rapidly from all traces of the bichromate. Thus, then, is our primary object attained in the formation of a delicate lamella of the cortex, and the safe removal of the covering glass. Once this is obtained, we need no longer fear the friability induced by endosmose of water, and which would have been fatal to our object if induced prior to removal of the cover. The subsequent removal of spirit is found essential to a perfect and rapid staining by the aqueous solutions of aniline, and the further steps of the process explain themselves, I believe, too readily to our minds to need any further comment upon.

Those who have attempted the staining of fresh as well as hardened brain will readily allow that the dye acts far more energetically and with more favourable results in the former than in the latter case, and differentiation is ensured by the absence of that diffuse staining which so often complicates matters in the preparation of sections obtained from brain hardened by chromic acid. The idea therefore occurred to my mind that if hardening by chromic acid and its salts acts thus detrimentally in the subsequent staining process, could we not devise a method whereby the staining might be completed ere this unfavourable effect upon the germinal centres took place? I believe I have solved this question satisfactorily, for I find that staining of a most perfect character may be induced during the subjection of fresh brain to certain hardening reagents. Some of the best carmine preparations of the cortex which I possess were obtained by this method, and I do not hesitate to affirm that I believe it to be a decided improvement upon the older method. Time and space preclude me from giving even a brief outline of this process, but I can assure my readers they will find an adjunct of extreme utility in a mixture of Müller's fluid and carmine dye of the original strength given by Beale's formulæ. By the time that

hardening has proceeded sufficiently far for section cutting, it will be found that a very perfect and beautiful staining of all the layers of the cortex has been acquired, and the sections have simply to be washed, dehydrated, and mounted by Clarke's method. It will probably occur to the reader that if Müller's fluid does not interfere with the staining by carmine or aniline, a further simplification of my method of fresh-brain examination would be possible by carrying on the staining simultaneously with the preparation of the film, and so reducing the two stages to one. That this is possible I allow, but since the method I have described yields the best results, I have no hesitation in recommending it above all others for trial. The process of fresh-brain preparation can scarcely be called a crushing of the cortex, as I have heard it designated—this term decidedly originates a wrong impression. The neuroglia basis of the brain is of a very resilient character, and it is really surprising to what an extent its structure may be subjected to pressure without rupture and tearing of the nerve-cells and processes. If we assume with Gerlach that one of its essential constituents is purely elastic fibre, as seen in the neuroglia of the cord, this explains its peculiar immunity from injury under such rough usage, and the result is seen to be equivalent to the most delicate teasing of tissue, the processes being gradually unravelled from their dense networks, and the structural elements universally displayed to the best advantage. The accompanying Plate may serve to illustrate the appearance of the larger and smaller pyramidal cells of the cortex as obtained by this process. The sections (Plate CXLIX.) are from different portions of the ascending frontal gyrus, and are magnified 55 and 210 diameters respectively by an inch and a quarter-inch objective of Smith and Beck with No. 1 eye-piece.

Fungus in the orange & olive.