SOME OBSERVATIONS ON THE BEVAN LEWIS METHOD OF PREPARING BRAIN TISSUE FOR THE MICROSCOPE.

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It would be entirely unnecessary for me to attempt to enumerate the many advantages of the Bevan Lewis method over that of the old one, chrome reagents. For there are but few of us who have not witnessed in the laboratory the deleterious effects on brain tissue of various hardening reagents, or carefully perused a description of their evil results from the distinguished author's writings on the subject. Nevertheless, I should like to call the reader's attention to some of the small but important points in the preparation of a good brain section. Especially to those who have but recently begun work by this method, I trust these few practical ideas will be of value.

In the beginning of this article, it is taken for granted that the freezing apparatus is in a position which will command both good light and plenty of room for work, and everything is in order to make brain sections.

I should first like to emphasize the importance of knowing the exact part of the brain from which the section is taken, so that a scientific record may be kept of the lesions found and their exact location in the cerebral cortex. To do this, a photograph of the brain should be made as it appears before section, or even before the lateral ventricles have been opened, so that the shape and contour may be preserved; such as the prominence or atrophy of certain lobes or convolutions. But if this can not be done through lack of knowledge of the camera, the next best thing is to make use of hectographs, and upon these mark the convolutions or areas 1, 2, 3, or a, b, c, etc., from which you have selected your specimen. The glass slides should also be marked in the same manner, corresponding to the number of letters on the hectographs. These hectographs can then be filed away with the autopsy report of the case. The glass slides can be marked by a glazier's pencil, so that they will retain their identity all through the process of washing and staining. Of course, the paper labels should be adjusted after the section has been stained and dried.

The brain should be kept free from water, otherwise ice crystals will hinder the cutting and staining. The pia, if allowed to remain intact, will in a short time be inspissated in such a manner that it will be impossible to remove it without considerable manipulation and laceration of brain tissue. Especially is this true in cases following inflammation of the membranes and outer cortical layers, as acute alcoholic mania, paresis, and chronic meningitis. Therefore the pia, while fresh, must be removed over those areas intended for examinations. I have been unable as yet to make good sections from brain with pia attached, as the firmness of the pia is in marked contrast to the pulsatious character of the gray matter.

In removing part of a convolution for section, carefully cut as nearly parallel as possible to the medullated nerve fiber running from the corona radiata to the ganglion cell in the cortex, so that the sections may show all the five or six layers of the cortex in the same plane.

For securing the specimen to the stand, while the process of freezing and cutting is going on, I have tried several different substances, but am unable to find anything better than a solution of gum arabic, although that has the disadvantage of allowing the specimen to slide on the stand before it has thawed, the gum melting a little more rapidly than brain tissue. This necessitates frequent freezing, especially when one is working alone and cares to cut only a few sections at a time.

The sections need to be very thin in order to take the stain well. If they are not so the stain will work at best but imperfectly and we get the peculiar foggy appearance of a poorly made section. The chief difficulty in trying to float thin sections in water is that they are apt to "dissipate like the morning mists" if taken from the knife while still frozen, and again, if they are held too long on the blade; it is almost impossible to remove them without spoiling the section, but with a little care and attention we can tell just how long to wait without getting these bad results. As for myself, I wait until the section resting on the blade begins to change from the white chalky appearance to a light gray color, frequently dipping the knife in ice-water, and quickly wiping it dry. If the knife is frequently dipped in ice-water and then hastily wiped dry it will materially aid in rendering the section less tenacious to the blade.

It is an important although difficult matter to tell how long the sections may remain in the water bath without material injury and yet be able to get them out whole, for upon this, in a great measure,

depends their success. It is only by long soaking of thin sections that the dendrons or cell processes stand out clearly with the cell. In some forms of insanity alterations in number and length of dendrons are among the most noticeable lesions; therefore, it will be seen that the above process plays no small part in the technique of a good slide.

We must exercise skill and deftness in removing sections from water, as the specimens, when soaked for ten or fifteen minutes, no longer cling to surrounding objects, and must be carefully dealt with lest the gray matter separates from the white, or the gray matter itself disintegrates and the relations of the sections be lost. A few minims of one-fourth of 1 per cent of the osmic acid solution in the baths renders the tissue stronger, but in doing this, one should bear in mind that if too much osmic acid is used in the bath or if the sections stay in too long, the tissue will be "burnt" and made worthless. My guide is to use four or five minims to the ounce of water and to remove the section as soon as it changes from the delicate pink appearance to a brownish hue. On withdrawal of the section, if osmic solution is not used in the bath, it should only be allowed to touch the section in its more concentrated form, then washed. If the osmic solution is used longer, the next dye of aniline blue black will stain unequally and imperfectly. Only too often I find a whole set of sections spoiled in this way by being over-stained by osmic acid. I can not impress the disadvantages of over-staining with osmic too strongly upon your attention. While some advantage is gained by applying the counterstain aniline blue black before the section is perfectly dry, yet it does not seem of sufficient moment to stop one from making thirty or forty slides first.

There are two preparations of aniline blue black in use, the English and German. The former is to be preferred, as it gives a clearer and sharper stain. Sometimes the specimens get doubled in staining; if so they can be easily readjusted by again floating them in the water bath, but this hinders re-staining in case the first application was insufficient. Therefore, the re-floating should be avoided until staining is complete.

In applying the dyes to the sections, I use common medicine droppers or pipettes exclusively and stain each section individually. I think whatever is lost in time is gained in good specimens.

My experience by this method teaches me that the older the brain, i. e., the longer time elapsing after autopsy, the more readily

the osmic acid acts, but the opposite is true with the blue black dye. In mounting, I make use of balsam in xylol since the xylol is of material advantage in clearing the section.

In conclusion I wish to state that in some cases of paresis and in other allied cerebral diseases with rapid destructive tissue change, it has been found, however perfect the stains may be, and however well they may be applied, yet the sections will stain only in spots, which, I think, rests upon some peculiar degenerate condition of the brain in these diseases, such as the fatty metamorphosis so often spoken of in connection with brain involution.

When all has been said that can be, of working on fresh brain tissue, there yet remain imperfections in the method which only a greater knowledge of the brain's normal structure and function can remove. For instance, all the dendrons are not certain to be stained and processes in which the disease may be most prominent will remain in the foreground hidden from the microscopist's eye. And the fact that the fresh method is desirable because brain tissue can be examined so soon after autopsy, is after all, not entirely to the method's advantage. The manipulation of soft and mobile brain, such as the removal of specimen from cortex, the cutting by microtome and handling in staining, slight as they may be, are yet sufficient to destroy the relation of one cell to another and disarrange their contiguity of dendrons. Again, great care is necessary to be able to say that an apparent diminution in or excess of a cortical layer is not due to the manner of cutting, instead of some brain abnormality.

Notwithstanding the foregoing remark, I am confident that by a much longer experience in examining fresh brain tissue we shall be able to say that with many so-called functional disorders of the mind there is an accompanying definite organic change, either in the brain's vascular structure or in its ganglionic network, which will be demonstrable under the microscope, and in that way throw more light on a nomenclature of mental diseases with a pathological basis; so that we shall be able to discard, in a measure, our present unsatisfactory and unscientific symptomatic classification.