

ether mixture and then stored for various periods of time in a dry ice box before removal of the brains and analysis for lactic acid. There is a statistically significant though variable increase in lactic acid content in the stored normal brain while no significant change was noted in the stored poliomyelitic brain. This experiment requires confirmation before speculation on the mechanism would be advisable.

*Summary:* Lactic acid content of the brain is significantly decreased in mice infected with the virus

of poliomyelitis. This appears to be additional evidence for the view that the virus may interfere in a specific manner with cell metabolism.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A RAPID IRON HEMATOXYLIN TISSUE STAIN FOR ROUTINE LABORATORY USE

THE Heidenhain iron hematoxylin method for tissue staining is one of the most important and satisfactory cytological stains for the demonstration of fine nuclear and cellular detail. Its use as a routine stain would be highly desired. However, the length and relatively difficult technique of the iron hematoxylin stain restricts this method to the more or less special, non-routine procedures. Although it is possible to stain tissues with Heidenhain iron hematoxylin in as little as one to two hours, the most satisfactory results require from twelve hours to two days (Guyer,<sup>1</sup> Bensley and Bensley<sup>2</sup>). In view of the desirability of the use of this stain routinely, we have attempted to find a suitable modification whereby the technique might be shortened considerably and made more adaptable for common laboratory use. By incorporating a single change in the usual procedure, to be described below, the time necessary for staining the tissue is shortened to even less than that entailed for Delafield's or Harris's hematoxylin stain.

The modification simply consists in fixing and mordanting the tissue simultaneously in Bouin's fixative containing one and a half grams of ferric alum (Ferric ammonium sulphate) in 100 cc of fixative. The Bouin's acts as the fixative and the ferric alum functions as the mordant. The ferric alum does not impair the excellent fixative qualities of Bouin's, while Bouin's does not interfere with the mordanting of the tissue by ferric alum. The combined fixative and mordant has no deleterious effect upon the tissue.

After removal of the tissue from the combined fixative-mordant, it is prepared for mounting and staining by following the procedure usually employed

for hematoxylin stains. Either ethyl alcohol or dioxan may be used for the dehydrating agent and 0.5 per cent. hematoxylin to stain. Inasmuch as mordanting has already been accomplished during fixation, the sectioned tissue may be placed directly into the hematoxylin after passing the mounted sections from xylol to water, using either dioxan or alcohol in the intermediate steps. The tissue will overstain in from 3 to 5 minutes and destaining may be carried out as desired in 0.1 per cent. HCl (aqueous or 35 per cent. alcoholic solution). The acid destain is followed by an alkaline aqueous or alcoholic wash prepared by the addition of several cc of a saturated solution of lithium carbonate or 1 per cent. solution of sodium bicarbonate to 100 cc of water or 35 per cent. alcohol, respectively.

The modification of the accepted iron hematoxylin procedure has been used successfully on many different types of tissues and the resulting cytological picture is comparable to that observed after use of the lengthy Heidenhain iron hematoxylin method. There are, however, two cautions worthy of note: One is that although this technique is very adaptable for small pieces of tissues (5 to 6 mm cubed), larger pieces of tissue require longer fixation. This is to allow for sufficient penetration of the ferric alum to complete the mordanting process. For the smaller pieces of tissue, the combined fixative-mordanting process is completed by the time the tissue would be normally fixed when placed in the fixative alone. The second point is that in the process of staining the slides it is important that the transfer of tissue from dioxan or alcohol to the dye should be accomplished as rapidly as possible. If the tissue stays in the intermittent water wash for too long a period of time the ferric alum may be completely removed from the tissue and remordanting will be necessary.

*Summary:* A modification of the standard Heidenhain iron hematoxylin stain is described in which the process of fixation (in Bouin's) and mordanting (ferric alum) are executed simultaneously instead of independently. Consequently, mordanting just prior to

<sup>1</sup> M. F. Guyer, "Animal Micrology," University of Chicago Press, Chicago, 1936.

<sup>2</sup> R. R. Bensley and S. H. Bensley, "Handbook of Histological and Cytological Technique," University of Chicago Press, Chicago, 1938.

staining is unnecessary. Staining takes from 3 to 5 minutes. This modification of the usual procedure makes for a rapid and simple stain for routine laboratory use with the resulting stain comparable to that observed after use of the accepted and lengthy Heidenhain iron hematoxylin method.

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### A MYOTOME FOR BIOPSY OF MUSCLE<sup>1</sup>

IN a study of the chemistry and metabolism of muscle it is desirable to have a means by which small quantities of muscle may be removed simply and frequently from patients and experimental animals. Various tissue punches and methods of aspiration biopsy have been described.<sup>2-7</sup> These methods are well adapted for the removal of specimens of friable tissues. They are not useful, however, in obtaining specimens of less friable tissues such as muscle. An instrument has been devised for the removal of portions of muscle, 20 to 50 milligrams in weight, without disruption of the histological structure. This quantity of muscle is sufficient for various analyses by microchemical procedures and for histological studies.

The instrument or myotome consists of an outer stainless steel tube, the distal end of which is square and provided with cutting edges (Fig. 1). A second and shorter stainless steel tube fits within the outer tube and serves as a plunger which is kept in position by a steel spring. To its distal end is attached a flexible steel blade which moves through a double slot in the wall of the outer tube. This blade, like that in the large viscerotome of Soper, Rickard and Crawford,<sup>8</sup> acts as a knife to sever the base of the specimen of muscle entering the instrument. An obturator fits within the lumen of the inner tube.

The myotome is used in the following manner. After sterilization of the skin and its infiltration with novocain solution, a minute stab wound is made with a sharp-pointed knife. The myotome with the obturator inserted is passed through the skin incision and

through the subcutaneous tissue. Increased resistance is met when the myotome enters the muscle. At this time the obturator is withdrawn. The instrument is inserted further and directed at an angle of 60 degrees to the horizontal and in the longitudinal plane of the

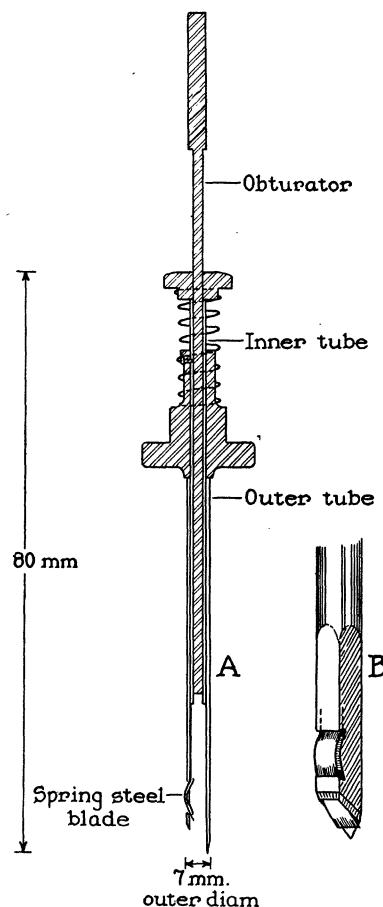


Fig. 1. A. Cross section of the myotome. B. Distal end showing the cutting edges.

muscle fibers. A portion of the muscle enters the outer tube. The inner tube or plunger is then pressed down to sever its attached end. Pressure is maintained on the plunger as the myotome is withdrawn. After withdrawal the plunger is released and the obturator inserted to expel the specimen. Several specimens may be taken through a single skin incision.

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<sup>1</sup> The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which are expressed in this paper.

<sup>2</sup> H. E. Martin and E. B. Ellis, *Annals of Surgery*, 92: 169, 1930.

<sup>3</sup> W. J. Hoffman, *Am. Jour. Cancer*, 15: 212, 1931.

<sup>4</sup> K. Lindblom, *Acta Radiologica*, 16: 295, 1935.

<sup>5</sup> C. C. Franseen, *New Eng. Jour. Med.*, 224: 1054, 1941.

<sup>6</sup> J. Tenopir and I. Silverman, *Radiology*, 36: 57, 1941.

<sup>7</sup> M. L. Weinstein, M. Schindler and E. L. Adams, *Ann. Surg.*, 115: 880, 1942.

<sup>8</sup> F. L. Soper, E. R. Rickard and P. J. Crawford, *Am. Jour. Hyg.*, 19: 549, 1934.

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