TECHNICAL METHODS

A Simple Method for Preparing Serial Blocks of Tissue

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The following method has proved of value in saving both time and materials in the preparation of long narrow organs or pieces of tissue for histological study. By means of it a number of transverse sections from different levels of the specimen are mounted, in order, on the same slide.

The organ or tissue, e.g., extrinsic eye muscle (Fig. 1), a vermiform appendix (Fig. 2), or a length of blood vessel, is fixed in formalin and impregnated with paraffin wax in the ordinary way. It is removed from the molten wax, laid on a smooth surface, straightened by hand, and allowed to cool. As soon as the wax has become firm a series of transverse cuts are made with a very sharp knife so that the tissue is divided into a series of small segments each about 3 mm. thick. The usual embedding box of two metal "squares" is arranged on a flat metal plate. A very little melted paraffin wax is poured in and the base warmed over a flame until a thin film covers the floor of the box. Each segment in turn is then laid on end and transferred, in order, to the embedding box (Fig. 3). The box is now passed over

a flame until the thin layer of wax over the bottom just melts. This fixes the serial blocks in position. Molten paraffin wax is now rapidly poured into the embedding box to fill it. It is important that the fresh wax poured in should fuse with the thin layer already present, so a small flame is passed over the surface of the wax in the box as the molten paraffin is poured in. As soon as a film has formed on the surface of the filled box the latter is cooled in water in the usual way. The whole block is now mounted on the carrier of the microtome and sections are cut and mounted in the

usual manner. Each section taken from the paraffin block, i.e., each slide, thus contains a series of sections, in order, at different levels through the tissue. The appearance of the finished slide is seen in Figs. 1 and 2. A complete set of serial sections can be made.

Essentially the same method can be used for tissues embedded in celloidin or low viscosity nitrocellulose. The tissue is embedded in the normal way and is blocked out after hardening. The block is cut so that it forms a rectangular mass with the minimum of celloidin around the tissue. This long block is then divided by transverse cuts, as in the paraffin method, and each small block is turned on end in series with and touching its neighbour in a paper box. This is filled with thick celloidin or low viscosity nitrocellulose and placed in a closed desiccator for several hours to allow the small blocks to unite into a whole, when the resulting large block is hardened in the usual manner. As in the paraffin method, each section from such a block contains a number of tissue sections.

FIG. 1

FIG. 2

FIG. 1.—Photograph of a series of transverse sections of an extrinsic eye muscle, cut from a single block, and mounted on a slide.

FIG. 2.—Photograph of a series of transverse sections through a vermiform appendix, also cut from a single block.

FIG. 3.—Diagram of embedding box with the "serial blocks" of an appendix in position, before the box is filled with paraffin wax. On the right is shown an appendix after removal from molten wax, straightened out before being cut into serial blocks.

This "serial block" method of embedding has been used successfully in various investigations. Cooper and Daniel (1949) and Cooper, Daniel, and Whitteridge (1951, 1955) used it in a study of muscle spindles in extrinsic eye muscles. In this work many of the "serial blocks" had serial sections cut from them, so that the whole length of the muscle could be studied in a set of sections which had taken perhaps a tenth of the usual time to cut, mount, and stain. The method is also valuable for the study of the whole brain or brain-stem of small animals, and was used successfully by Bosanquet, Daniel, and Vollum (1953) in a study of the exudate produced by intrathecal tuberculin in the guinea-pig. The spinal cord, hypothalamus, pituitary stalk and pituitary, the vermiform appendix, lengths of peripheral nerve, and lengths of arteries have also been examined by this method.

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The Filtration of C.S.F. in the Bacteriological Diagnosis of Tuberculous Meningitis

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The early detection of tubercle bacilli in the C.S.F. is an urgent and time-consuming occupation, and the earlier removal to hospital of suspected cases of tuberculous meningitis only makes the bacteriological problem more acute. The present report deals with an attempt to improve the chances of an early positive laboratory diagnosis by ensuring that all bacilli in the fluid are available for examination. This is of importance where there are only a few bacilli per millilitre of fluid and when, as Silverstolpe (1949) noted, many of the organisms do not in fact deposit on centrifugation. The centrifugal filter described by Elek and Hilson (1951) appeared to offer a convenient unit for making the total bacterial content of the specimen readily available on the plane removable surface of a collodion membrane.

Experimental Methods

Each specimen of 5 to 15 ml. of C.S.F. from suspected cases was divided equally as soon as possible after withdrawal from the patient (to obviate clotting), half being treated in (a) the routine fashion, i.e., centrifuging at 3,000 r.p.m. for 30 minutes, removing the supernatant, and using the deposit for building up films and for culture, and half being submitted to (b) filtration. Two to 7 ml. of C.S.F. was filtered through a membrane of A.P.D. 0.6 μ , five to seven minutes at 1,500 to 2,000 r.p.m. sufficing in most cases to complete the operation. After filtration the membrane was treated in a variety of ways to obtain satisfactory films, and whole or part of it was placed face-upward on a Löwenstein-Jensen slope of the same batch as that used in (a) above. The routine film and culture (a) was thus a control on the experiments under consideration.

Results

Of 18 specimens of C.S.F. examined, 13 were bacteriologically proven tuberculous, four were bacteriologically and clinically non-tuberculous, and one was bacteriologically negative but strongly suspect on clinical grounds as tuberculous.

Direct Films

Table I compares the results of methods (a) routine and (b) filtration. Five of 13 undoubted positive cases were positive by method (a), whereas only one