SCIENTIFIC APPARATUS AND LABORATORY METHODS

IMPROVING THE STAINING ACTION OF IRON HAEMATOXYLIN

IRON haematoxylin, the most useful stain available to the cytologist, has during the past few years caused a great deal of trouble for a number of microscopists through its erratic behavior. Sometimes the desirable crisp black and white contrasting stain would be obtained, but more often it was not. The cytoplasm would retain the stain with the resulting muddy appearance seen all too often of late years. Stock solutions became turbid and lost their staining action in a few weeks.

Three years ago it occurred to the writer that if neutral solutions were essential to blood stains, neutrality might be a factor in the successful use of iron haematoxylin. Our distilled water was known to be slightly acid (about pH 6.6). A trace of sodium bicarbonate was added to a fresh 0.5 per cent. solution of haematoxylin. The straw-colored solution changed at once to the rich dark red wine color recognized as typical for an aged sample of this stain. Sections stained in this solution differentiated perfectly.

This 0.5 per cent. solution remains clear for about six months or occasionally longer and stains well as long as it is clear. These results have been obtained equally with haematoxylin crystals of pre-war Grueb-ler make or with the recent C. P. product of McAndrew and Forbes of America. After a solution of 0.5 per cent. haematoxylin becomes turbid the staining action is uncertain and the solution should be discarded. Even though six months should mark the functional life of the solution this does not seem serious in view of the successful behavior of the stain during this period.

Inasmuch as there appears to be a rather wide latitude in the quantity of bicarbonate that may or should be added to the solution of stain, it has not seemed worth while to determine the exact quantity added nor the pH obtained. In practise, to a liter of solution a very small quantity of sodium bicarbonate is lifted on the point of a scalpel and dropped into the solution. This rule of thumb procedure has never failed to work, although the quantities of alkali added must have varied considerably.

The making of a 0.5 per cent. solution is greatly facilitated by preparing (according to the directions given in Kingsbury and Johannsen's "Histological Technique") a 10 per cent. stock solution of haematoxylin dissolved in 95 per cent. alcohol. This solution keeps indefinitely and 5 cc of it in 100 cc of water gives a staining solution of the proper strength. To this solution the sodium bicarbonate is added.

This procedure has been in use for three years in this and neighboring laboratories and has given uniform results.

ROBERT T. HANCE

UNIVERSITY OF PITTSBURGH

A DEVICE FOR MOUNTING ANATOMICAL PREPARATIONS

A SIMPLE and convenient method for mounting anatomical preparations is as follows: A plate is made by melting hard paraffin and pouring it into a form (as a box top, etc.) where it hardens to form a plate from one fourth to one half inches in thickness. During the process of melting, enough lampblack is added to give the mixture a deep black color, which is, of course, an advantage where a dark background is desired. To prevent curling of plates after immersion in preservative, two glass rods are embedded parallel to each other, one on each side of the plate, just before the process of hardening is begun. These rods may be of various diameters, but we have found that those from 5 to 8 mm in diameter are quite satisfactory. The length of the rods will depend upon the length of the plate as they are placed lengthwise within the plate (Fig. 1 a a). Before the rods

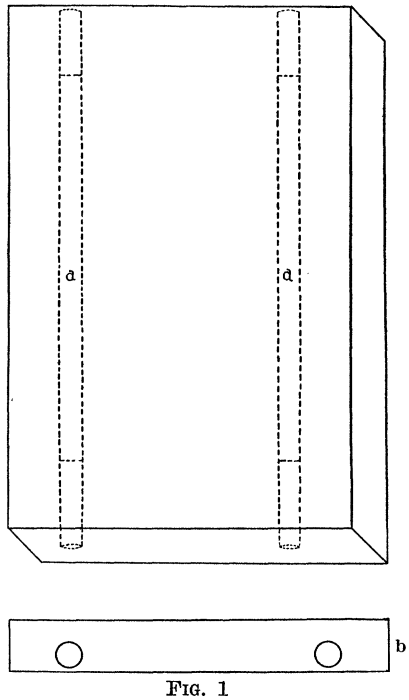

FIG. 1

are dropped in the melted paraffin a loop or two of heavy twine is wrapped around each end of each rod so that the rods will be suspended near the middle of the plate after it hardens (Fig. 1 b). Care must be taken to smear the inside of the box form with glycerine so that the hardened plate can be removed easily from the form.

The addition of some beeswax and resin to the paraffin-lampblack mixture will increase to a considerable extent the rigidity of the plate, although these constituents are not absolutely necessary.

Before the plate has hardened, and is still plastic, the anatomical specimen is pressed down slightly into the soft plate to hold it in position and is fastened there with thread looped around the parts of the specimen, passed through holes, and tied at the back of the plate. Necessary labels are now attached.

When completed, the preparation is immersed in a preservative in a museum jar. Such preparations will last for an indefinite time.

H. W. SCHOENBORN
C. P. HICKMAN

DEPAUW UNIVERSITY

SPECIAL ARTICLES

THE PARTICLE SIZE OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS

A MOST interesting epidemic of acute encephalomyelitis occurred among horses and mules in various local districts of California during the summer and fall of 1930, 1931 and 1932. Bacteriological and pathological studies of the disease were conducted by Meyer, Haring and Howitt and these workers have reported elsewhere[1,2,3] the salient characteristics of the virus and its effects on animals. In this work they recorded the filterability of the virus through Berkefeld V and N candles. However, filtration experiments utilizing the usual forms of candles, furnish no adequate basis for estimating the particle size of the virus because many factors other than mechanical sieve action have been shown to condition the filterability of a microorganism through such filters.

In order to obtain some idea of the virus particle size filtration experiments were performed with the acetic collodion gel ultrafilter series described by Krueger and Ritter[4] which possess the advantage of uniformity and low adsorbing surface area. The pore sizes of these membranes depends upon the percentage of nitro-cellulose dissolved in the glacial acetic acid and have been estimated by testing the permeability both to colloidal sols of known particle size and to water under certain standard conditions. The two methods of estimation give figures of the same order of magnitude with a relatively small constant difference between them. Manegold and Veit[5] have shown that this difference may be materially reduced by basing the pore size calculations from water permeability data upon the assumption of a random pore distribution as contrasted to parallel capillary bundles such as we had assumed to exist in the membranes. The result is a practical coincidence of the two pore size curves.

During the past year and one-half we have used different materials in making the membranes and have made minor alternations in the technique of preparation. The pore sizes of the series necessarily

ULTRAFILTRATION OF SUSPENSIONS OF ENCEPHALOMYELITIS BRAINS

No.	Dilution of original supernatant from 20 per cent. brain suspension	Per cent. collodion (Ultrafilter)	Bacterial cultures from filtrate	Animal inoculation filtrate
1	Undiluted	3.0	0	Typical disease
2	Undiluted	3.0	0	Typical disease
3	1:2 with saline	3.0	B. subtilis	Typical disease
4	1:2 with saline	3.0	B. subtilis	Negative
5	Control undiluted	Unfiltered	0	Typical disease
6	Control 1:2 with saline	Unfiltered	0	Typical disease
7	1:2 with broth	3.0	0	Typical disease
8	1:2 with broth	3.0	0	Typical disease
9	Control undiluted	Unfiltered	0	Typical disease
10	Control 1:2 with broth	Unfiltered	0	Typical disease
11	Undiluted	4.0	0	Negative
12	Undiluted	4.0	0	Negative
13	Control, undiluted	Unfiltered	0	Typical disease
14	Control, undiluted	Unfiltered	0	Typical disease
15	Undiluted	3.5	0	Negative
16	Undiluted	3.5	0	Negative
17	Control, undiluted	Unfiltered	0	Typical disease
18	Control, undiluted	Unfiltered	0	Typical disease
19	1:2 with broth	3.5	0	Negative
20	1:2 with broth	3.5	0	Negative
21	Control, undiluted	Unfiltered	0	Typical disease
22	Control 1:2 with broth	Unfiltered	0	Typical disease

[1] K. F. Meyer, C. M. Haring and B. Howitt, "The Etiology of Epizootic Encephalomyelitis of Horses in the San Joaquin Valley, 1930," SCIENCE, 74: 227–228, 1931.

[2] C. M. Haring, J. A. Howarth and K. F. Meyer, "An Infectious Brain Disease of Horses and Mules." (Encephalomyelitis)," University of California Agricultural Experiment Station Circular 322, August, 1931.

[3] B. Howitt, "Cross Immunization Experiments with Poliomyelitis Virus and that of Encephalomyelitis in Horses," *Proc.* Soc. Exp. Biol. and Med., 29: 118–120, 1931.

[4] A. P. Krueger and Ritter, "The Preparation of a Graded Series of Ultrafilters and Measurement of their Pore Size," *Jour. Gen. Physiol.*, 13; 409, 1930.

[5] E. Manegold and K. Veit, "Über Kapillarsysteme, XI," Kol. Zeitschr., Bd. 56, H. 1, 1931.