A Rapid Method for Staining thin Sections of Vestopal W-Embedded Tissue for Light Microscopy

Several methods for staining Vestopal W-embedded tissue for light microscopy have been advanced by SCHWALBACH, LICKFELD, and HOFFMEISTER[1]. However, as none of these methods is convenient for rapid making of survey-sections, I tested some of the stains recommended by RICHARDSON, JARETT and FINKE[2] for staining of thin Araldite-sections. The following method was thereby proved to give the best results: Staining solution; 0.1% methylene blue, 0.1% thionin and 0.1 *M* Na_2HPO_4 in distilled water.

Procedure. Sections of about 0.75 μ were cut with a LKB Ultrotome, transferred to a clean microscope slide, and thoroughly dried in an oven or with a microflame. The sections were subsequently covered with 3–5 drops of 95% alcohol and kept a few cm above a microflame. When the alcohol started boiling, the sections were flooded with the staining solution (3–10 drops), the slide being kept above the flame. After about 10 sec (the time may vary according to the type of tissue and thickness of section) the staining solution was rapidly washed off with distilled water and the slide was dried and mounted. As mounting medium only Eukitt (O. Kindler, Deckglaszuschneiderei, Freiburg/Br., West Germany) was used and has not as yet produced fading or diffusion of the stains (the oldest sections are now about 6 months old).

The Vestopal W around the tissue proper should be unstained, and differentiation is easily done in 70–95% alcohol. However, a weakly stained Vestopal is normally no disadvantage. Too low or too high temperature during the staining procedure proper may produce a less satisfactory result. However, the boiling alcohol gives the right starting temperature and the procedure is very easy to learn. The staining solution is stable at room temperature for at least 6 weeks, probably much longer.

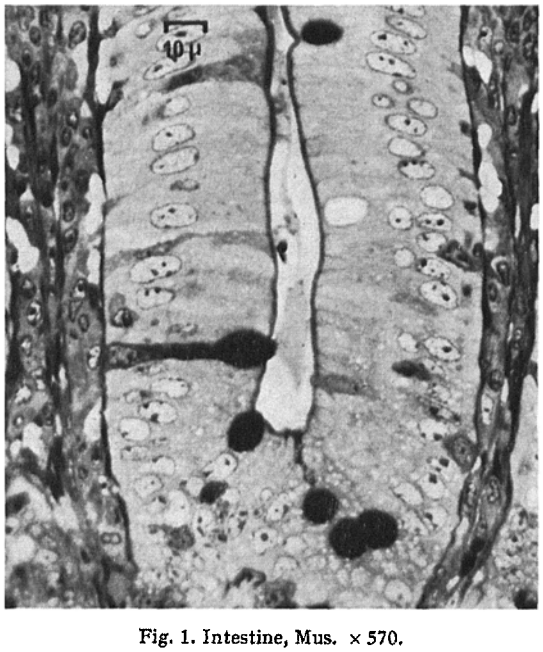

Fig. 1. Intestine, Mus. × 570.

Normally the cell nucleus is stained violet by thionin and the cytoplasm blue by methylene blue. Mucus cells are stained violet, fat drops – due to their high osmium content – greenish. The stain well demonstrates nucleoli, chromatin, many different types of cellular inclusions, membranes etc., and is mostly very good for survey sections.

The staining method has been tested on many different types of tissues, fixed in osmium tetroxide or glutaraldehyde and osmium tetroxide. As examples may be mentioned mammalian intestine (Figure 1), liver, and testis, cultures of avian retinal, nervous, and muscular tissue, amphibian pancreas (Figure 2), teleostian and elasmobranchian pineal tissue[3].

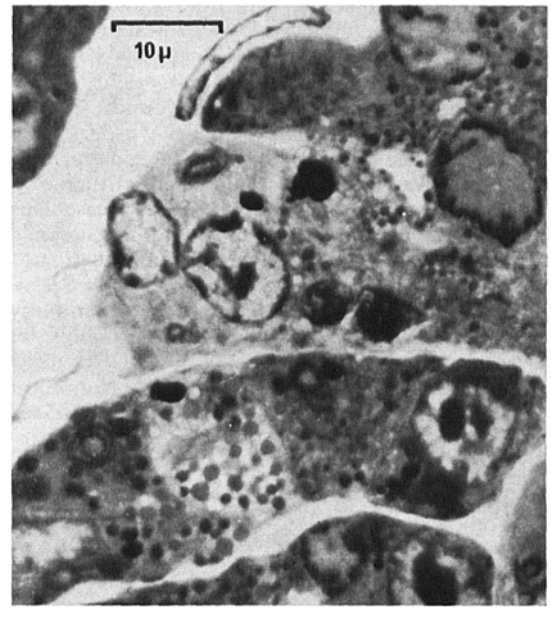

Fig. 2. Pancreas, Triturus, immersion lens. × 1440.

Riassunto. Viene descritto un metodo di colorazione con tionina e blu di metilene per un controllo, rapido e semplice, al microscopio ottico, di sezioni semifine di tessuti inclusi in Vestopal W.

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1 G. SCHWALBACH, G. H. LICKFELD and H. HOFFMEISTER, Stain Technol. *38*, 135 (1963).

2 K. C. RICHARDSON, L. JARETT and E. FINKE, Stain Technol. *35*, 313 (1960).

3 This work was supported by grants from the Royal Academy of Science, Stockholm and the Foundation of Helge Ax:son Johnsson Stockholm (Sweden).

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