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Rapid Nuclear Staining Method for Frozen Sections

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To be of value, the frozen section technique must be simple, fast and must yield slides showing good definition. Most of the methods currently in use are rapid enough but fail to provide clear details; other methods give good definition but are too time-consuming.

The method described here is fast, shows good details and results in permanent slides. It makes use of the stain coelestine blue B, employed earlier by Proescher and others (1946) as a nuclear stain in combination with azophloxine as a cytoplasmic stain. The mixture of the two dyes is unstable and has to be made up immediately before use.

Gray et al. (1956), state that the staining properties of coelestine blue B would be improved by adjusting its pH to 0.8. At this hydrogen ion concentration the stain exists in the form of a colloid dispersion mixed with a true solution of the unchanged dye. The colloid particles are deposited on the nucleus while the solution of the dye stains the cytoplasm to some extent.

Methods

The staining solution is prepared by placing 1 g. of coelestine blue B into a 250 cc beaker, tilting it sideways, so that the dye accumulates in a small area. Then 0.5 cc of concentrated sulphuric acid are added and the mixture is rubbed to yield a paste which swells and effervesces, but sets after a few minutes to a friable mass. This can be reduced to coarse granules by means of a glass rod. These granules are then covered with a solution of 2.5 g. ferric alum in 100 cc of water, to which 14 cc of glycerol have been added. The mixture is kept at about 50 degrees C and constantly stirred, then cooled to room temperature. The pH is adjusted to 0.8 with concentrated sulphuric acid. This solution remains stable.

We have found that the practice of fixing the tissue with hot formaldehyde injures it; satisfactory results have been obtained by merely placing the fresh tissue in cold 10 per cent formaldehyde. Krajian (1940) suggests that the neutrality of the formaldehyde is important to the preservation of the staining properties of the nucleus. To obtain neutral formaldehyde, he adds 5 gm. of calcium carbonate or sodium bicarbonate to a gallon of commercial formaldehyde. We have cut and stained fresh tissue without fixation;

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these preparations were equal in quality to those fixed in formaldehyde.

Frozen sections are cut to 15 microns in the customary manner. The sections are then removed from the knife with a fine camels hair brush, and floated on tap water. After picking up, the sections are allowed to partially dry in the air.

The slides are dipped for one minute into the staining solution, put into tap water to remove excess stain, then carried through the graded series of alcohols, cleared in xylol, and mounted.

A more rapid technique is to flood the wet slide with stain from a dropper, rinse under the tap, flood with 95 per cent alcohol, xylol-alcohol, then with xylol; mount.

If the few minutes necessary for dehydration and clearing appear wasteful, the slide can be observed wet, then the technician may make up a permanent slide using another piece of tissue from the block.

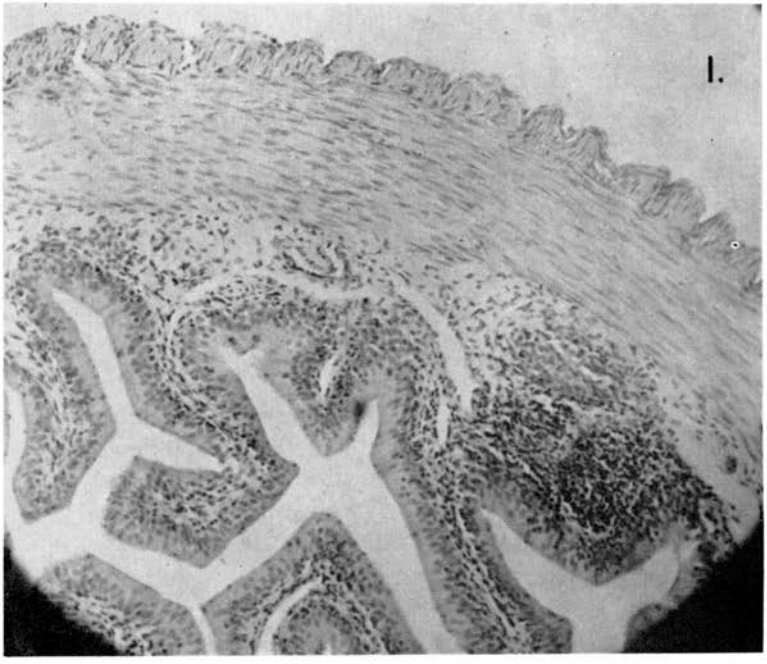
Results

The resulting slides show excellent nuclear staining of deep purple or black, and are clearly differentiated; the cytoplasm stains a light blue. The photomicrographs contrast coelestine blue B with thionin, a nuclear stain frequently used for frozen sections (Kohlmer and others, 1951). With thionin, the nuclei are visible, but the cytoplasm stains densely. The section of intestine stained with coelestine blue B (Fig. 1) shows clear differentiation of the nuclei; the mucosal cells are distinct. The thionin-stained intestine stains fairly well, but the nuclei are not distinct, particularly in the mucosa. (Fig. 4.) The photomicrographs were made under identical conditions. Thionin sections are generally observed wet and discarded. For purposes of comparison in this paper, the thionin sections were dehydrated and mounted; this procedure removed much of the stain. A wet thionin section has quite darkly staining cytoplasm, and fairly distinct nuclei.

The heart muscle stained with coelestine blue B (Fig. 2) also shows excellent cellular detail much better than the thionin section. (Fig. 3.) Frozen sections of a large variety of animal tissues were prepared, and in all instances, coelestine blue B produced slides which were equal or superior in quality to thionin or rapid hematoxylin slides. The simplicity of the process and the excellent results recommend its use as a method of staining frozen sections.

Summary

A rapid method of staining various tissues with coelestine blue B is described. The method is superior to the use of thionin, and results in permanent preparations.



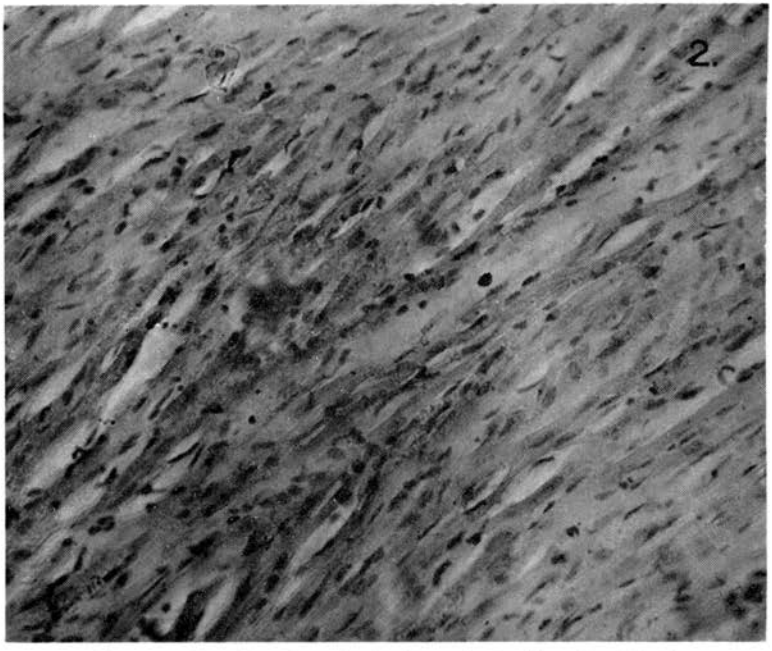
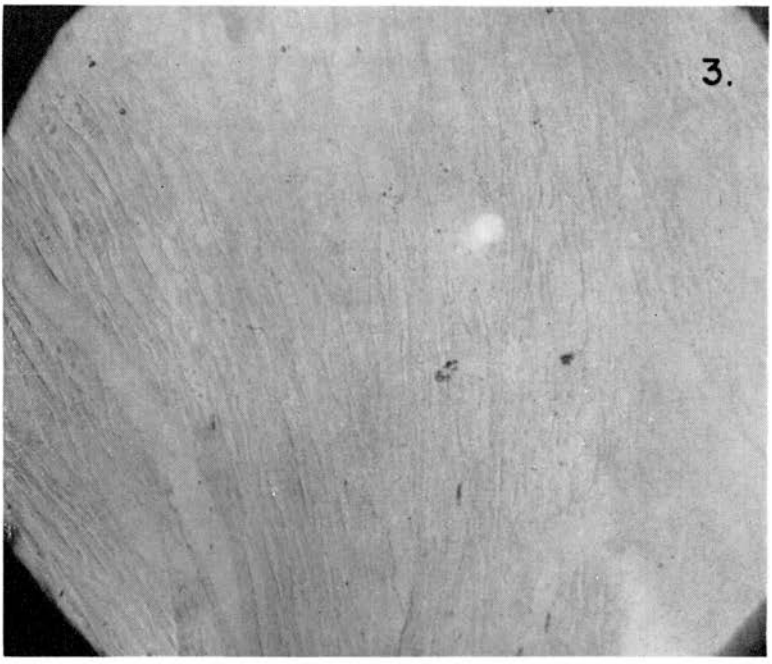


Figure 1. Frog Intestine stained with coelestine blue B. 180 X.
Figure 2. Rat heart muscle stained with coelestine blue B. 500 X.



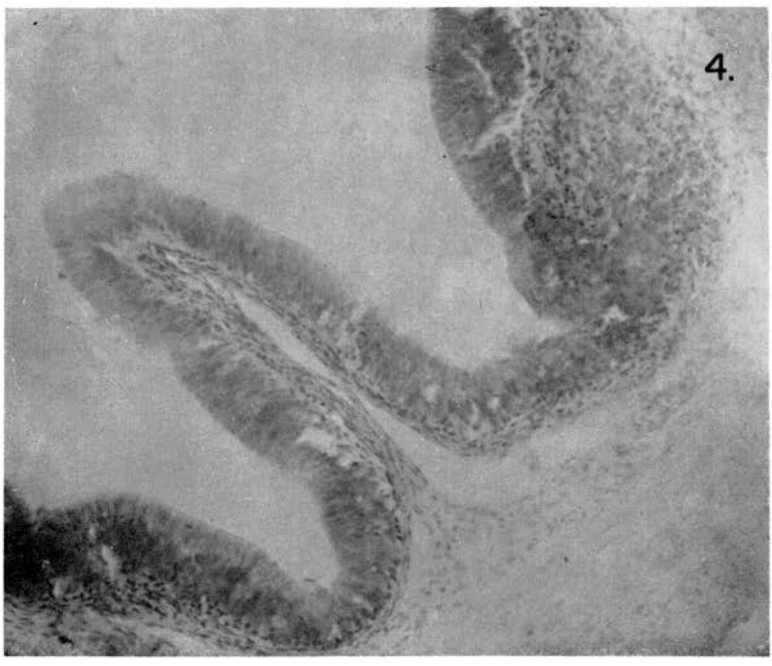


Figure 3. Rat heart muscle stained with thionin. 370 X.
Figure 4. Frog intestine stained with thionin. 370 X.

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