## A Staining Method for Sections of Osmium-Fixed, Methacrylate-Embedded Tissue.*

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The increasingly broad application of electron microscopy to the study of cytological detail in both normal and pathological tissues has created a need for an improved staining method for light microscopy of material processed primarily for electron optical investigation. A staining technic that would produce results comparable to those obtained with hematoxylin and eosin should prove to be of value in correlating existing histological information with the findings of electron microscopy. Also, many problems of interpretation can best be approached by a comparison in the light and electron microscopes of adjacent sections through individual structures (3). Such a comparison is made difficult by the fact that the fixative of choice for electron microscopy (osmium tetroxide) alters or destroys the affinities of tissues for ordinary stains. Furthermore, it seems likely that additional loss of stainability may occur as a result of oxidative processes that accompany methacrylate embedding (1).

Houck and Dempsey (2) and others have utilized certain elastin and collagen staining methods for light microscopy of osmium-fixed, methacrylate-embedded tissues. In our laboratory the application of such stains as the periodic acid–Schiff reaction, ammoniacal silver, aldehyde fuchsin, various hematoxylin solutions, azocarmine, and a variety of "counterstains" have not consistently yielded highly satisfactory results. None of these stains alone or in combination approach the quality of microscopic differentiation achieved with classical histologic fixation and embedding techniques. Adequate demonstration of stained tissue by black and white photography is difficult and is achieved only with special photographic methods.

The purpose of this communication is to outline a staining method, currently in use for special problems in light microscopy, which we have adopted for demonstration of thin sections of osmium-fixed methacrylate-embedded tissue.

1. Sections were cut at 0.5 to 1.5 $\mu$ from tissue fixed in buffered osmium tetroxide (4), and embedded in methacrylate (5). Sections were mounted on slides or on narrow pieces of coverglass.

2. After the affixed sections had been dried by moderate heat, the methacrylate was dissolved by immersion of sections in 2 changes of toluene for 2 to 5 minutes each, depending on section thickness.

3. The sections were hydrated by passing them through graded alcohols (100 per cent, 95 per cent, 80 per cent, and 70 per cent) to water. One minute in each alcohol was usually sufficient.

4. The sections were transferred from water to a gallocyanin-chromium solution (*cf.* formula below) for 24 to 48 hours, depending on type of tissue and thickness of sections.

5. Washed in running tap water for 30 minutes to 1 hour.

6. Stained for 5 minutes in a 0.1 per cent aqueous solution of phloxin B (National Aniline Co.), to which has been added 0.1 gm. calcium chloride per 100 ml. of stain.

7. Differentiated by dipping very quickly in 3 changes of 95 per cent alcohol.

8. Dehydrated in 3 changes of absolute alcohol.

9. Cleared in 3 changes of xylene and mounted with clarite or equivalent. Typical results are illustrated in Figs. 1 to 4.

*Preparation of Gallocyanin-Chromium Solution:* (6, 7, 8)

Boil 0.15 gm. of gallocyanin (Schuchardt, Munchen, or National Aniline Co.) for 3 minutes in 100 ml. of 5 per cent aqueous solution of chromium potassium sulfate with continuous stirring. Cool and filter, then adjust the pH to 2.09 with 0.1 N HCl or 0.1 N NaOH. The solution will keep for approximately 1 month.

*Comment:*

The foregoing method has thus far been applied to human and animal tissues including kidney, liver, brain, and pancreas. Only steps 6 and 7 need to be adjusted to the staining characteristics of a particular tissue. Cytological detail

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has been remarkably well demonstrated in all tissues tested by working within the time limits of the various steps as outlined above.

The chromium lake of gallocyanin is insensitive to light and does not undergo reduction. Minute blue-stained granules are readily visible in the nuclei of all cells examined. The phloxin counterstain renders membranous structures in tones from transparent pink to bright red. In tissues such as liver, the mitochondria appear as brilliant pink structures that contrast well with the pale pink cytoplasmic background. Strongly osmiophilic structures, such as erythrocytes, remain brown to black, thus providing additional contrast.


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## EXPLANATION OF PLATE 186

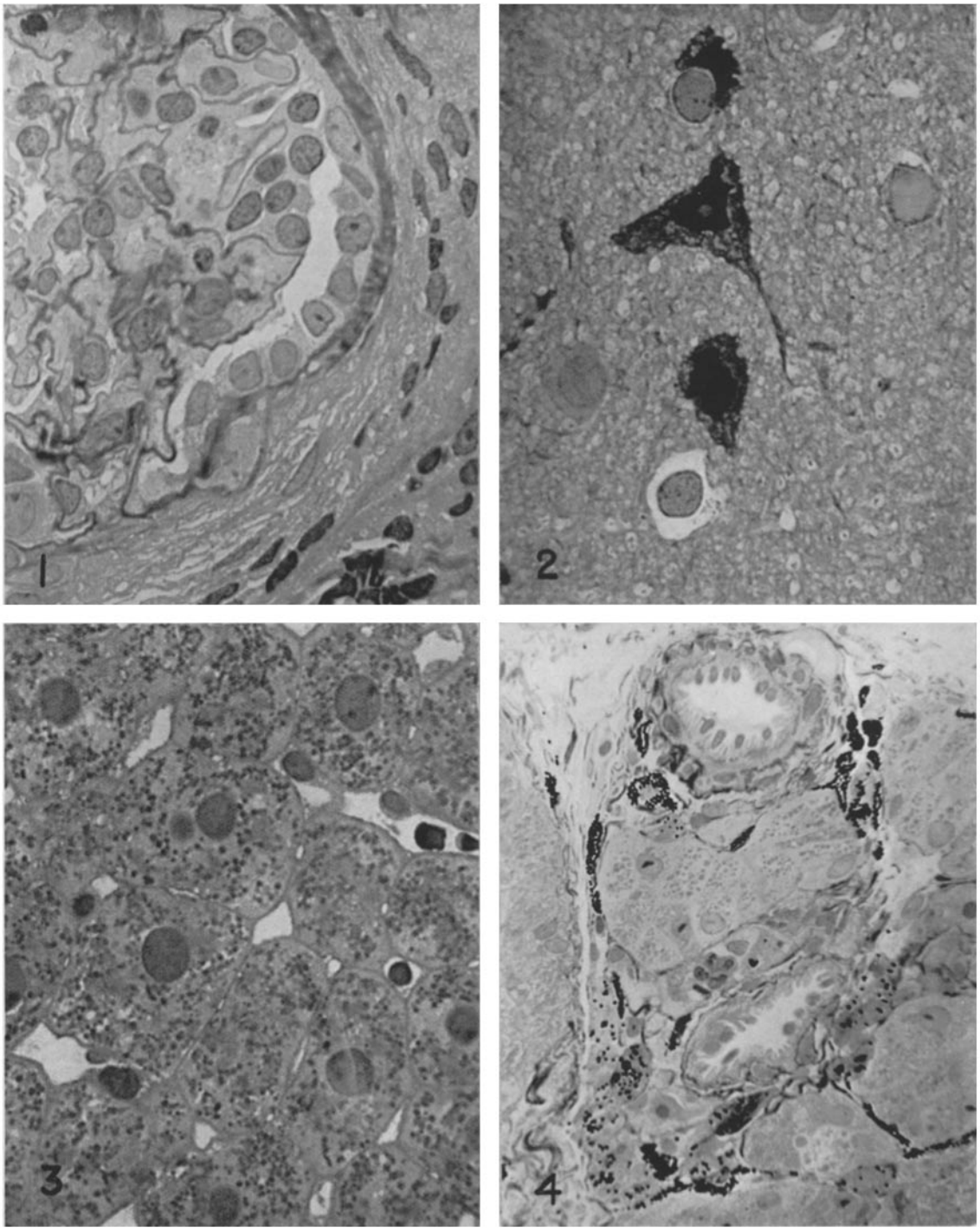
Tissues illustrated were cut at 1 micron using a Porter-Blum ultra microtome.

FIG. 1. Human kidney. Proliferative glomerulitis. Fine details of Bowman's capsular epithelium; glomerular endothelial and epithelial cells, and glomerular basement membrane are visible. In the original slide, the foot processes of the epithelial cells can be resolved by fine focusing. × 1250 (oil immersion).

FIG. 2. Human cerebral cortex. Portions of a triangular nerve cell and its processes appear near the center of the photograph. Oligodendroglial cells flank the nerve cell. A protoplasmic astrocyte with a large nucleus and nearly clear cytoplasm is seen at the bottom of the field. × 1250 (oil immersion).

FIG. 3. Rat liver. Nuclei, nucleoli, and mitochondria within a number of hepatic cells are clearly shown. Red blood cells in hepatic sinuses contrast sharply with the surrounding structures. × 1250 (oil immersion).

FIG. 4. Toadfish pancreas. Various structural details of acini and ducts are shown. The dark granules in clusters and rows are believed to be pigment (melanin granules). × 750 (oil immersion).




(Runge *et al.*: Staining method for osmium-fixed tissue)