A Rapid Method for Localization of Tissue Structures or Lesions for Electron Microscopy.*

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Small structures or lesions are frequently difficult to find and identify for electron microscopic study. Pathologists in particular need an orderly procedure to reduce the time spent in random searching. In the method described here a relatively large block of tissue, which is expected to contain the desired focus, is initially fixed and embedded. From the methacrylate-embedded tissue a thick section is then cut and stained for a few seconds with toluidine blue. When the lesion or structure is located with the light microscope, the block is trimmed to a small area including the lesion and then sections are cut for electron microscopy.

This method offers several advantages. It enables rapid localization of desired lesions while sectioning. It ensures that the lesion is in that portion of the block trimmed for thin sections. The small area of the sections ultimately used reduces the time spent with the electron microscope hunting for the lesion and it also facilitates cutting the thinnest sections. In addition, the method allows determination of the depth and adequacy of the zone of optimal fixation in the original block. Examination of such sections by light microscopy often reveals details not seen in usual paraffin sections. Enhancement of detail may be obtained with phase microscopy, as others have observed.

Various staining procedures have been adapted by others for osmium-fixed tissues embedded in methacrylate (1–4). These do not seem to have both the rapidity and clarity of the method described here. Some more time-consuming special stains may be necessary to identify specific substances or lesions. The facility with which toluidine blue can be removed from the section allows the same slide, if necessary, to be used for other more complex stains.

Method

After fixation with buffered osmium tetroxide and embedding in butyl-methacrylate, the entire tissue block, 1 to 2 mm. on a side, is oriented in a microtome and sections 0.5 to 1 micron thick are cut from the entire face of the block. Sections in the water trough are flattened by holding over them a wooden applicator stick soaked in toluene (5). Two or three sections are picked up from the trough with a wire loop about 4 mm. in diameter. When the loop is momentarily touched to the surface of the acetone solution around the sections, the sections float on the droplet picked up. The sections are deposited onto a clean glass slide by touching it with the bottom of the droplet. They may be immediately blotted dry with bibulous paper. No special precautions are necessary to keep sections attached to the glass. The methacrylate is removed by placing slides in acetone for a few seconds. The sections are then rehydrated with water and placed in a 2 per cent aqueous toluidine blue solution for 2 or 3 seconds and then rinsed off with running tap water. The sections may be examined microscopically at this time by placing a coverslip over them. The metachromasia of some tissues enhances the staining of these aqueous mounts. If a permanent mount is desired, the coverslip is removed with water. The section is blotted with bibulous paper, rinsed with acetone, cleared with toluene, and mounted with permount (Fisher Scientific Co., New York). A permanent section may be obtained directly in less than 1 minute (Fig. 1).

When the desired lesion or structure is found in the light microscope, the block is trimmed to a small area that includes the lesion. Serial thin sections are cut for electron microscopy followed immediately by a thick section for toluidine blue staining and light microscopy. This gives close correspondence between thick and thin sections, a desirable feature if corresponding areas are to be studied or photographed.

An example of the usefulness of this method is the electron microscopic identification of the hyaline droplets seen by light microscopy in the glomeruli of rats following the injection of uranyl nitrate. The same droplet, about 0.6 micron in diameter, is shown in an epithelial cell by light microscopy (Fig. 2) and electron microscopy (Fig. 3).

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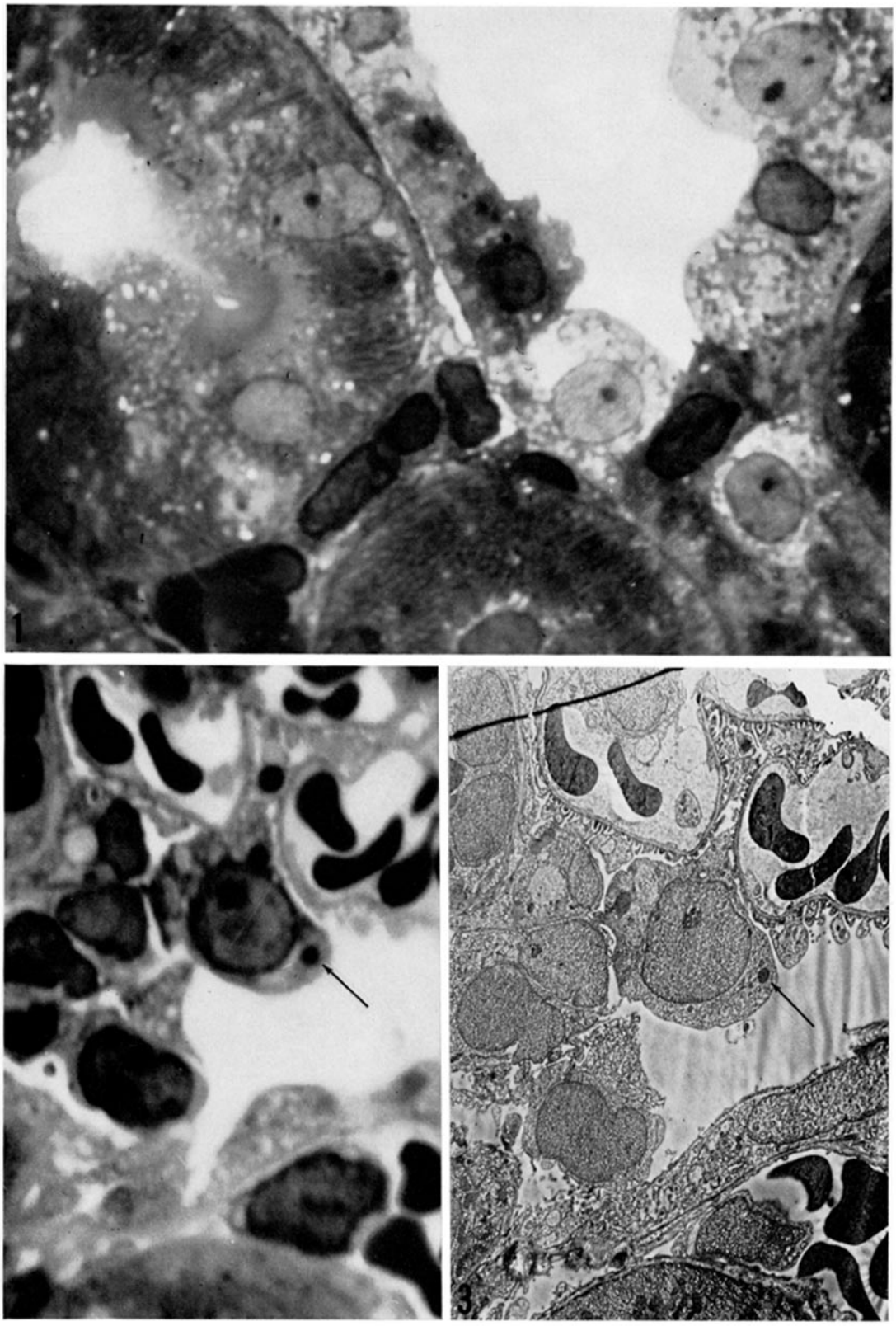
Explanation of Plate 219

Fig. 1. Toluidine blue stain of a normal rat kidney. Mitochondria and other cytoplasmic structures are well demonstrated by this procedure. On the left is a proximal convoluted tubule, and toward the right is a collecting tubule with both light and dark or intercalated cells. In the lower center is a portion of a distal convoluted tubule. × 2,500.

Fig. 2. Toluidine blue stain of a renal glomerulus of a rat given uranyl nitrate. In the center of the field is an epithelial cell containing a hyaline droplet (arrow) which is also seen in the electron micrograph in Fig. 3. Other droplets and less-dense cytoplasmic bodies as well as some foot processes of epithelial cells are visible. × 3,000.

Fig. 3. Electron micrograph of a section in the ribbon adjacent to that used for Fig. 2. The hyaline droplet seen in Fig. 2 is identified here as a dense, osmiophilic, sharply circumscribed round body (arrow). Some other details do not completely correspond with those in Fig. 2, because of the slightly different plane of sectioning. Because the section has been compressed during cutting, the magnification, as compared with Fig. 2, is less in the horizontal than in the vertical direction. The horizontal magnification is 3,000.



(Bencosme *et al.*: Method for localization of tissue structures)