A ROUTINE METHOD FOR OBTAINING HIGH CONTRAST WITHOUT STAINING SECTIONS

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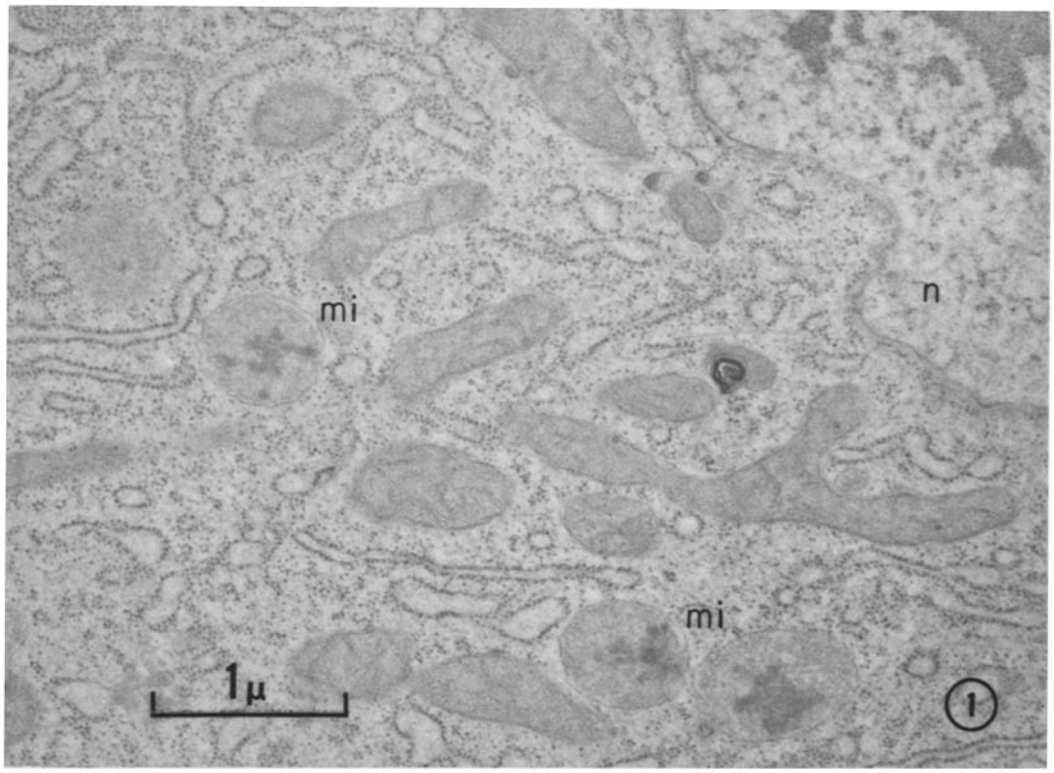
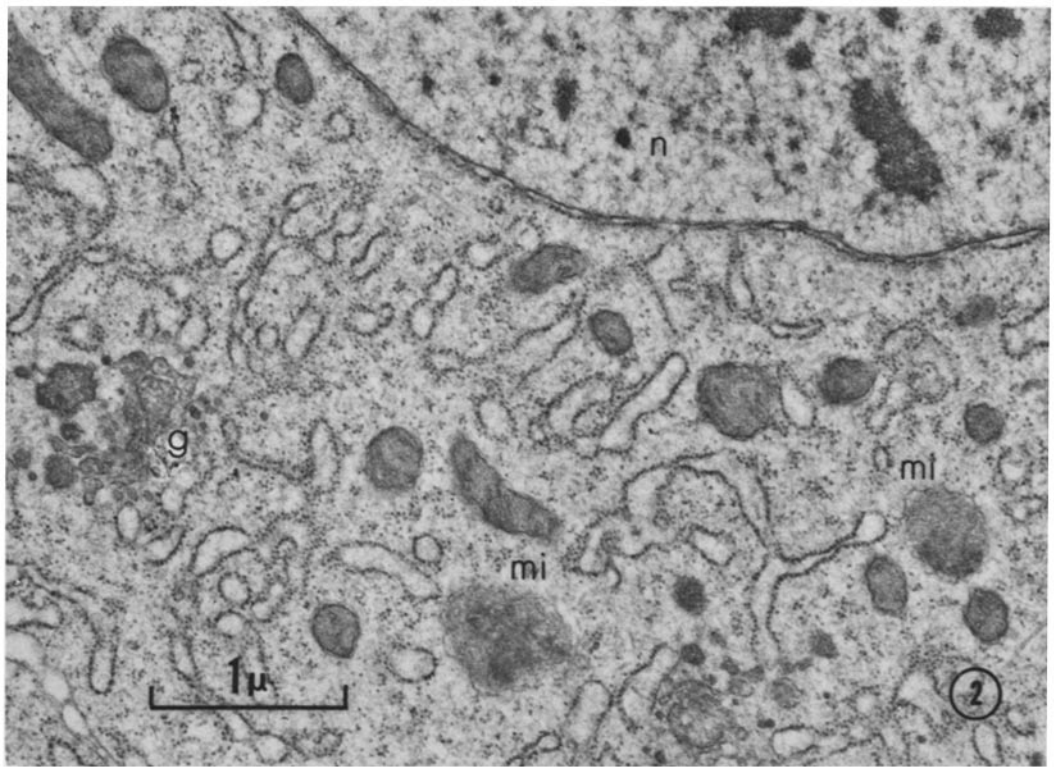
Immersion of tissue in aqueous uranyl acetate before embedding is commonly practiced to increase the contrast on sections which are subsequently stained (2, 3, 5). The treatment is only satisfactory for giving some degree of contrast to components such as membranes when they extend through the thickness of the section parallel to the electron beam. A simple modification of current practice

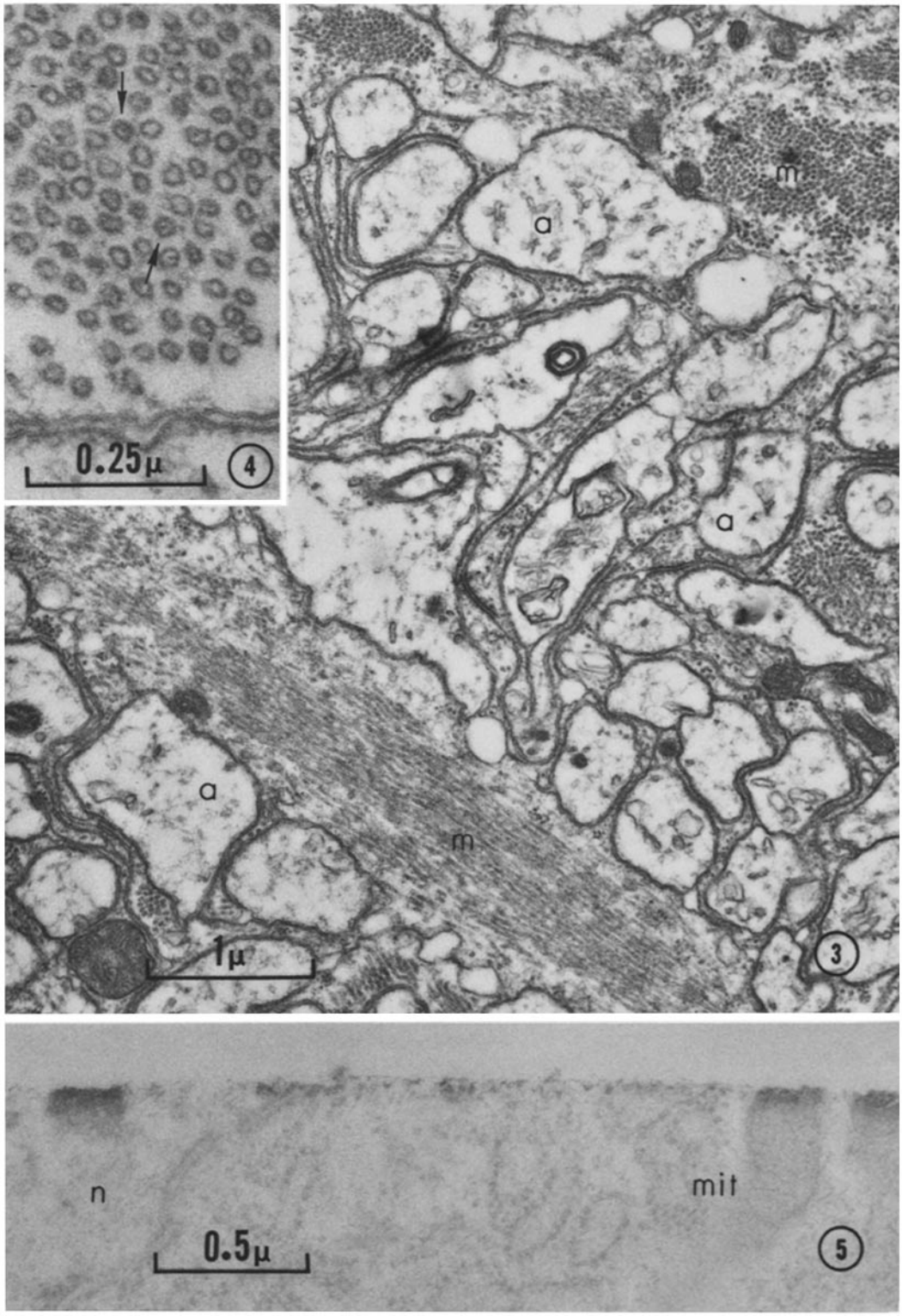




All figures are printed on Kodabromide F3 paper under standard conditions for comparing contrast. Figures 1–4 have only been stained in uranyl acetate before embedment.

FIGURE 1 The contrast obtained by treating with 2% uranyl acetate in absolute ethanol for 2 hr at room temperature. Organelles are discernible but the contrast is inadequate for viewing. × 26,000.

FIGURE 2 The contrast obtained by treating tissues with 2% uranyl acetate in absolute ethanol for 13 hr at 60°C. Contrast is now equal to that obtained by staining sections. Figs. 1 and 2: Fat body from *Calpodes* larvae. *n*, nucleus; *mi*, microbodies; *g*, Golgi complex. × 26,000.



FIGURES 3 and 4 The contrast obtained by treating tissues with 2% aqueous uranyl acetate for 20 hr at 60°C. Contrast is satisfactory for viewing and photography of sections suitable for both survey and high magnification. There is no deleterious tissue extraction or stain precipitation. T. S. nerve cord from *Calpodes* larva. *m*, microtubules in sheath cells; *a*, axons; arrows, microtubules with dense central cores. Figures 3, × 26,000. Figure 4, × 112,000.

FIGURE 5 The localization of stain in sections stained for 30 min in alcoholic uranyl acetate. Thick section of fat body stained, reembedded, and sectioned normal to the plane of the original section. The stain is localized at the surface with uneven penetration. *n*, nucleus; *mit*, mitochondria. × 48,000.

gives enough contrast for viewing even the thinnest sections without further staining.

In our staining of tissues with heavy metals, we were puzzled by the fact that alcoholic uranyl acetate (used typically as a modification [6] of the technique of Stempak and Ward [7] i.e., 1–10% uranyl acetate in 1:1 absolute methanol: 70% ethanol) caused more contrast when sections rather than unembedded tissues were immersed in it, in spite of the barrier to penetration caused by the resin. Tissues embedded in resin differ in two ways from those treated before embedment: they have been heated, and they are surrounded by resin. It seemed to us that the heat during the curing of the resin might denature tissue proteins to make them more reactive to stains, since proteins react more strongly with light microscope stains after heat denaturation (1). The following experiments showed that this is an unsatisfactory explanation but gave us a routine method for obtaining enough contrast by staining unembedded tissue to obviate the need for further tedious processing of sections.

Insect tissues were fixed in 2.5% glutaraldehyde with 0.05 M phosphate buffer at pH 7.2 for 3 hr and postfixed in 1% osmium tetroxide buffered with 0.1 M sodium cacodylate at pH 7.2 for 2 hr. Three samples were prepared: one treated with 2% uranyl acetate in absolute ethanol at room temperature for 2 hr in the dark; the second, similar but pretreated in alcohol at 60°C; and the third, similar to the first but stained at 60°C. The tissues were washed in absolute ethanol and embedded in Araldite. Sections were examined without further staining on an RCA 3F electron microscope operated at 100 kv. Photographs were taken under similar conditions on Kodak high contrast plates and printed on Kodabromide F3 paper for comparison of the staining treatments.

Heating the tissues in alcohol before treating them in uranyl acetate at room temperature had no effect on staining. Heat denaturation is therefore not the cause of the extra contrast obtained by staining sections. However, the tissues stained for 2 hr at 60°C showed greatly increased contrast over those exposed at room temperature (Fig. 1). This suggested that the in-block staining might be progressive and that longer treatment might achieve even greater contrast. Figs. 2 and 3 show the contrast after staining for 13 and 20 hr at 60°C. Hot aqueous uranyl acetate gives similar contrast to the stain in ethanol with no deleterious effects due to extraction. Stain is not removed during dehydration. These findings have led us to revise our staining procedure. We now routinely leave tissues in 2% uranyl acetate at 60°C overnight or longer before embedding. We then cut sections which need no further enhancement of contrast even for the thinnest sections at high magnification (Fig. 4). Lead citrate or phosphotungstic acid are useful counterstains for their specificity but are not needed for contrast at 100 kv.

These sections differ in appearance from all of our preparations up to this time since the organelles are stained uniformly through the thickness of the section and with much less graininess. We believe that these sections give a more faithful picture of the distribution of cellular components than sections stained by the conventional method of obtaining contrast. Fig. 5 shows a thick section stained in the same alcoholic uranyl acetate solution used to treat unembedded tissues, reembedded in Araldite, and sectioned normal to its original plane. Nearly all the contrast for such conventionally stained sections is caused by a grainy surface precipitate with penetration into the resin limited unevenly to about 500 A.

Up to the present time the high voltage electron microscope have been of limited value to biologists. Although the instrument can be used to view sections as thick as 1 μ, conventional staining techniques do not allow penetration of stain for more than about 1000 A (4). The advent of a staining technique which gives good and even contrast through sections of any thickness should increase the usefulness of high voltage microscopes to biologists.

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