TECHNICAL MODIFICATIONS IN MARAGLAS EMBEDDING

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Marglas 655 is proving to be a versatile embedding medium for biological electron microscopy (1–5) in that it provides favorable cutting qualities and excellent cellular preservation (Figs. 1, 2, 4). Since the publication of the original technic (1), we have found several useful technical modifications and developed methods for staining sections for light microscopy.

Two technical difficulties were encountered in the routine use of Maraglas-embedding medium. The first and most important, noted in an occasional block, was soft tissue surrounded by a hard matrix. Originally, we suggested double embedding to rectify this problem (1). It was apparent that soft tissue in a hard matrix was due to incomplete impregnation of the tissue by the Maraglas mixture (Maraglas, plasticizer, flexibilizer, and hardener). The embedding schedule has been modified to allow the tissue a longer period of impregnation in the Maraglas mixture prior to the final embedding and hardening. Complete dehydration has been achieved consistently by removing the tissue from absolute alcohol to pure propylene oxide and then to a 1:1 propylene oxide—Maraglas mixture. The tissue is then placed in pure Maraglas mixture in a stoppered vial and kept at 10°C in a refrigerator for 8 to 12 hours. The vial containing the Maraglas mixture and tissue is then warmed to room temperature before removing the stopper. Other than warming before exposure to room air, no precautions need be taken to avoid water condensation. In our experience, water condensation has not been apparent to the naked eye. Desiccated gelatin capsules are filled with fresh Maraglas mixture, the tissue is placed in the capsule, and the specimen is cured by dry heat in an oven at 60°C for 24 to 48 hours.

The second technical difficulty was bubble formation between the gelatin capsule and plastic. This probably would not be considered serious for the investigator using specimens sufficiently large to trim away the distorted area; however, for an investigator dealing with very small blocks or single cells, this distortion could be a serious deterrent. The formation of bubbles occurs after the capsules are removed from the oven and allowed to remain at room temperature for prolonged periods of time; therefore, it is desirable to remove the gelatin capsule from the plastic shortly after its removal from the oven. This is best accomplished by cooling to room temperature for 15 minutes, and then removing the gelatin capsule by soaking in cold tap water.

We have found a slight modification of Maraglas mixture E as originally published (1) to be the most reliable for biological work. The modified Maraglas mixture and the complete tissue processing schedule are listed below.

Maraglas Mixture[1]

Maraglas 655	68 ml
Cardolite NC-513	20 ml
Dibutyl phthalate	10 ml
Benzyldimethylamine (curing agent)	2 ml

The routine procedure for fixation, dehydration, and infiltration of tissue in this laboratory is:

Fixation (by desired technic)	
10 per cent buffered formalin (only used following osmium tetroxide fixation)	½ hr.
50 per cent alcohol	15 min.
70 per cent alcohol	15 min.
95 per cent alcohol	15 min.
Absolute alcohol (2 changes)	15 min.
Propylene oxide (2 changes)	15 min.
Propylene oxide—Maraglas mixture 1:1	30 min.
Maraglas mixture (at 10°C)	12 hrs.
Embedding in desiccated gelatin capsules and hardening in 60°C oven	24 to 48 hrs.

[1] Maraglas Epoxy Embedding Media obtained in kit form from Polysciences, Inc., Rydal, Pennsylvania.

Thick sections (1.0 to 2.0 μ) of Maraglas-embedded tissues may be stained for light microscopy with the resin *in situ* (Fig. 3). A modification of the method of Trump, Smuckler, and Benditt (7) can be used for staining with toluidine blue (Fig. 3). The method is as follows:

1) Flood slide with a *freshly* prepared solution of 0.5 per cent toluidine blue in 2.5 per cent Na_2CO_3 and place in a 60°C oven for 2 hours

2) Wash with water and follow differentiation in the light microscope

3) Dehydrate with graded alcohols (*prolonged immersion in the alcohol will fade the stain*)

4) Clear with xylol and mount with Permount.

Nuclei stain dark blue and the cytoplasm stains light blue to blue-red. Kattine's modification of Lillie's allochrome technique (6) can be used for selective demonstration of mucopolysaccharide of connective tissue and cellular components. The intensity of the stain is proportional to the length of time stained (minimum time in the periodic acid and Schiff reagent is 90 minutes each).

The reproducibility, excellent specimen preservation, and methods for correlation of light and electron microscopic observations make Maraglas a desirable and useful embedding medium for biological electron microscopy.

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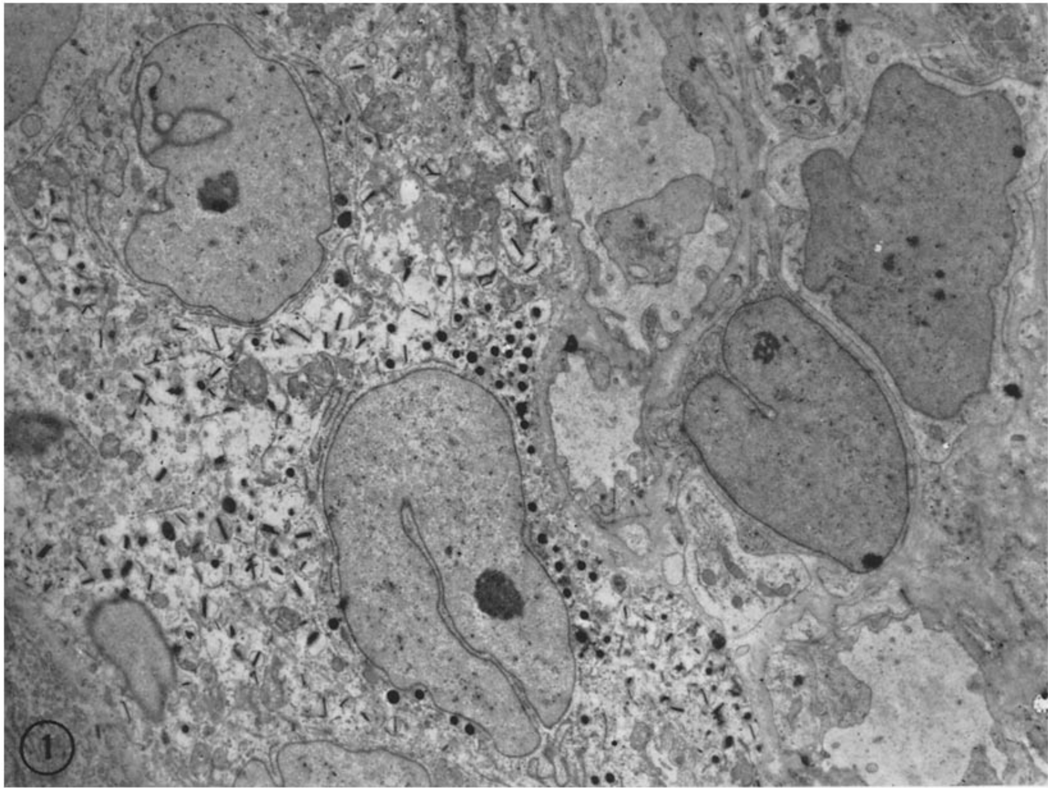
FIGURE 1

Electron micrograph of pancreas of dog. A portion of an islet of Langerhans. Mounted on 400 open mesh grid without membrane. Unstained. × 6,500.

FIGURE 2

Electron micrograph of cardiac muscle of rat. Mounted on Sjöstrand-type grid with formvar membrane. Lead hydroxide stain. × 12,500.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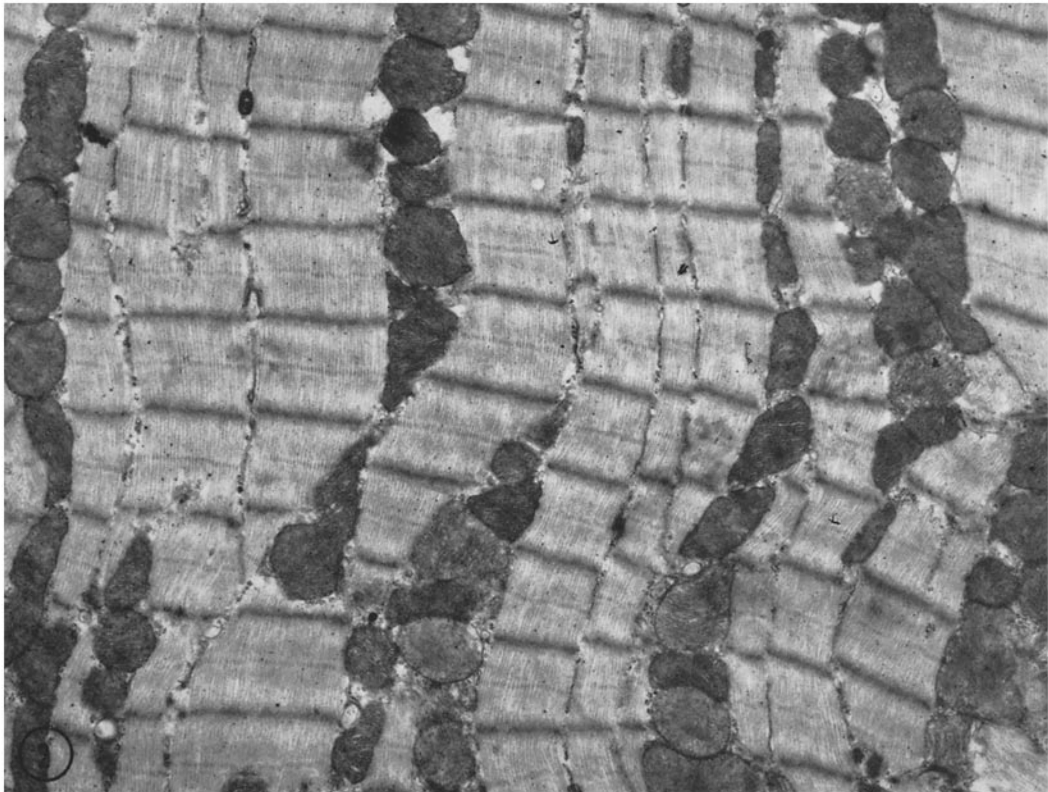
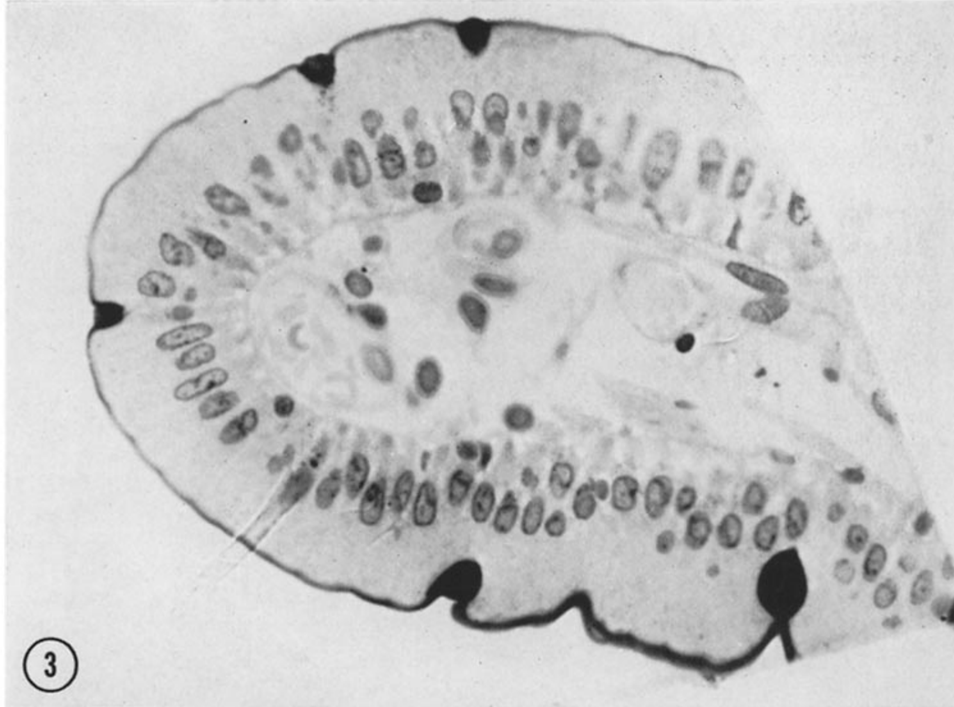


FIGURE 3

Thick section (approximately 0.2 micron) of Maraglas-embedded rat intestinal villus. Aqueous toluidine blue stain. Light micrograph. × 600.

FIGURE 4

Electron micrograph of section of intestinal villus of rat. This is a portion of the villus depicted in Fig. 3. Uranyl acetate stain. × 5,000.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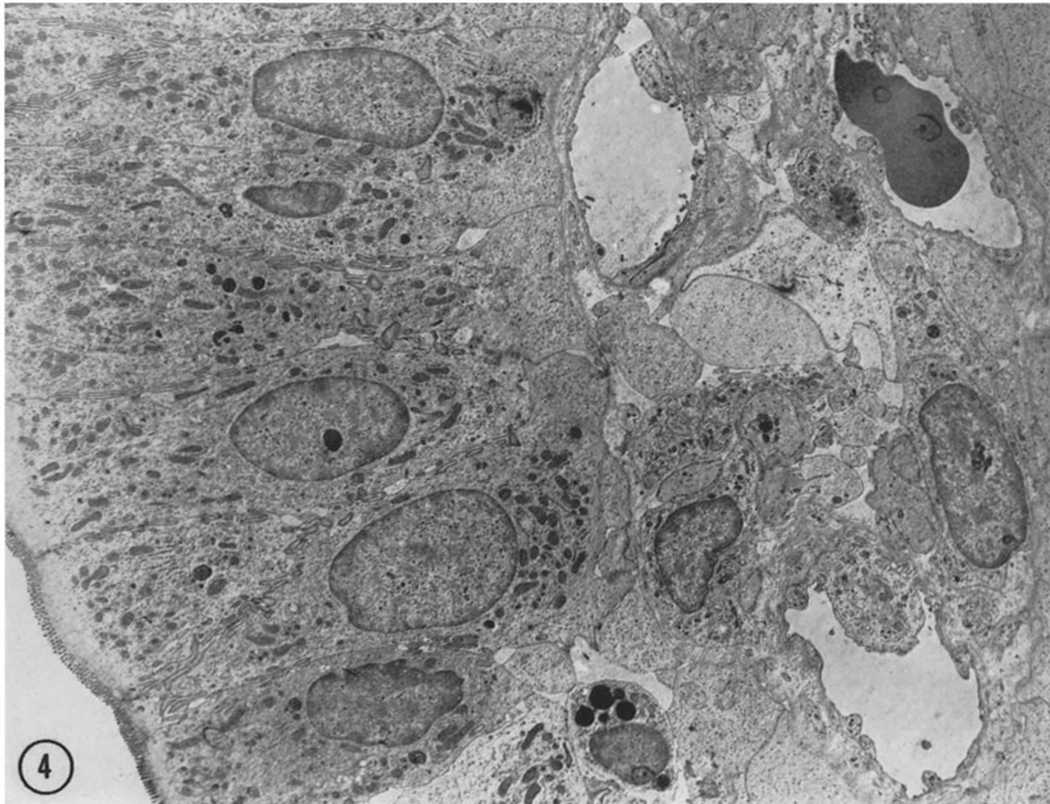
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