AN EMBEDDING TECHNIQUE FOR ELECTRON MICROSCOPY USING EPON 812

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Epoxy resins have been established as desirable embedding media for electron microscopy (1–5). Finck (6) described an epoxy resin, EPON 812 (Shell)[1], which in addition to its other desirable characteristics, is also appreciably miscible with water. He noted that prolonged alcohol dehydration was not necessary because of the water solubility of EPON 812. We, therefore, considered substitution of EPON 812 for alcohol. Such a procedure would be highly desirable for application of electron microscopy to histochemical investigation in which dehydration by organic solvents may result in considerable removal of lipid and lipid-soluble material from the tissue.

This report describes a technique for electron microscopy in which EPON 812 was employed to dehydrate and embed the tissue.

MATERIALS AND METHODS

Specimens of rat liver, spleen, kidney, and nerve were quickly removed, placed on a cold (0–4°C) dish, and cut into pieces 1 mm³ or less in size. These

[1] Received as a sample from the Shell Chemical Corp., Cleveland, Ohio.

pieces were transferred to a solution of 1 per cent OsO_4 containing 0.045 gram of sucrose per ml of solution (7), fixed for one hour at 0–4°C, washed for several minutes in an acetate-veronal buffer solution (8), pH 7.2, and then divided into two groups. The tissue in group I received a dehydration treatment similar to that employed preceding methacrylate embedding. Ascending concentrations of ethyl alcohol were used for dehydration followed by a solution of 50 per cent absolute alcohol and 50 per cent EPON 812 for 2 hours. The tissue was then transferred to 100 per cent EPON 812 solution for 2 hours, after which the tissue was embedded in the final EPON solution.

In group II the tissue was transferred from the buffered rinse solution into a solution of 25 per cent buffer and 75 per cent EPON 812 and allowed to remain for 1 hour. The tissue was placed in 100 per cent EPON 812 for 1 hour, then transferred to a mixture of 20 ml EPON 812 and 16 ml of hexahydrophthalic anhydride (HHPA). Because HHPA is a solid below 38°C, it was necessary to warm the HHPA and the EPON 812 to 38°C before mixing. After the tissue had remained in this mixture for 2 hours at room temperature, it was transferred to a fresh solution of EPON 812 and HHPA, containing 1.5 to 2.0 per cent benzyldimethylamine (BDMA), an accelerator, for 2 hours. This mixture was found

FIGURE 1

Micrograph of a portion of the cytoplasm of a hepatic cell from the liver of a normal rat. Note the endoplasmic reticulum and the smooth outer membranes of the mitochondria. Tissue from Group I. × 26,000.

FIGURE 2

Micrograph showing cell borders of adjacent cells near a bile canaliculus. A terminal bar can be seen at the lower left. Tissue from Group I. × 26,000.

FIGURE 3

Micrograph of the cytoplasm of a rat hepatic cell. Mitochondria are numerous. Note the excellent preservation of the membranes in the left lower corner. Tissue from Group II. × 26,000.

FIGURE 4

Micrograph of a portion of the cytoplasm of a hepatic cell. Note the endoplasmic reticulum with associated RNP particles. The outer membranes of the mitochondria are very irregular. Tissue from Group II. × 26,000.
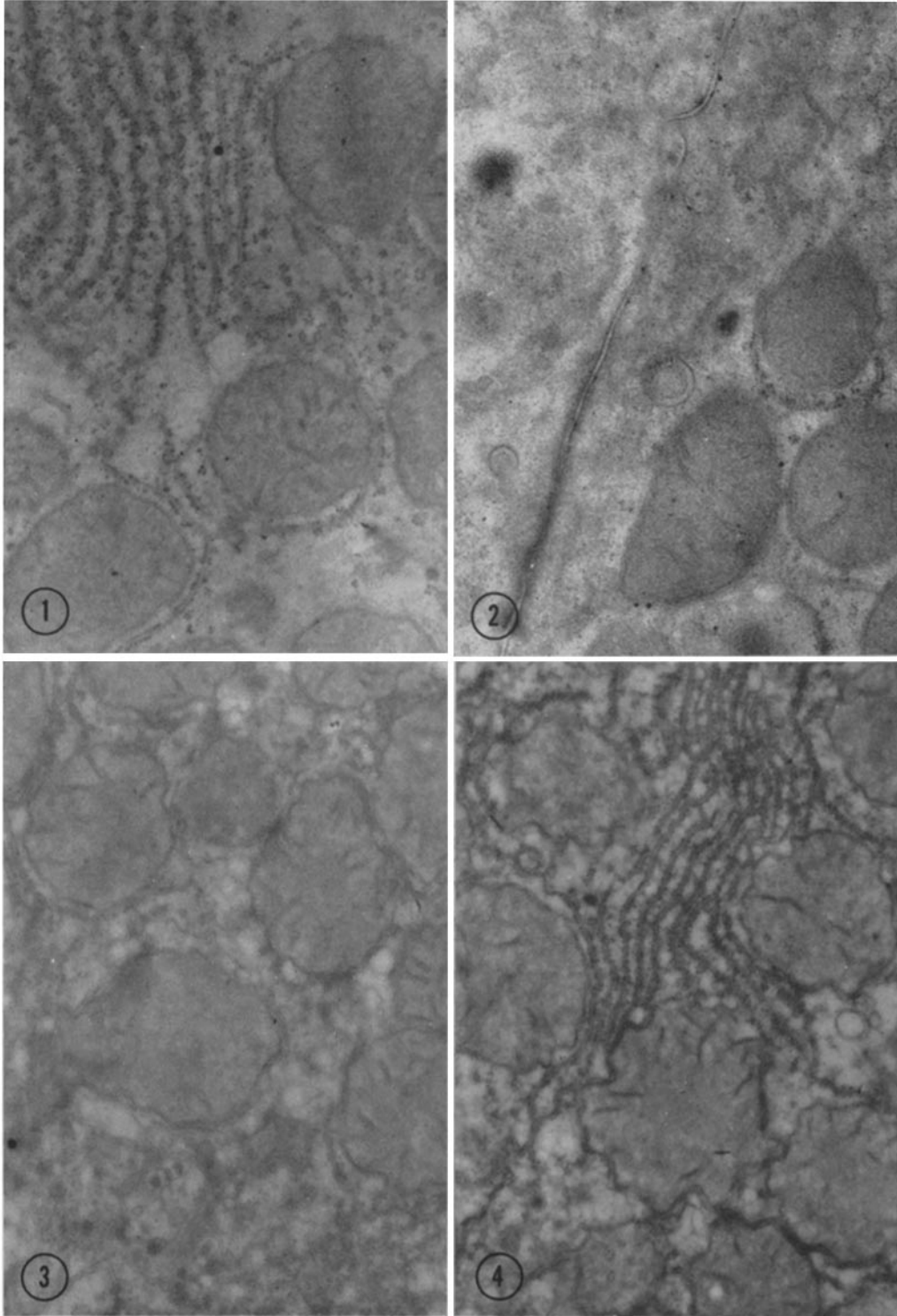
to produce blocks with the best cutting properties and was arrived at by several trials in which the amount of HHPA and BDMA were varied. The tissue was transferred to the final embedding solution, a fresh mixture of EPON 812, HHPA, and BDMA (in the proportions mentioned above) previously warmed in the oven.

Throughout the impregnation procedure, frequent changing of the solutions and constant agitation (on a circular-motion shaking device) were imperative to insure adequate removal of the water and penetration of the embedding solutions. The capsules were placed in a 55°C oven and allowed to polymerize overnight. The final product was a clear, straw-colored block which was hard, tough, showed minimal shrinkage, and seldom contained bubbles. These blocks were then prepared and sectioned by procedures routinely employed for methacrylate blocks.

OBSERVATIONS

Sections of hepatic parenchymal cells from specimens dehydrated in alcohol and embedded in EPON 812 were compared to those dehydrated and embedded in EPON 812. The homogeneous matrix of cells from tissue dehydrated with alcohol (Figs. 1 and 2) contained a complex of structures of variable size. The membranes of the endoplasmic reticulum were distinct and appeared associated with sharply defined ribonucleoprotein particles (RNP). The mitochondria were oval to round and possessed smooth limiting membranes. There were a few cristae which did not completely span the width of the mitochondria. The cell membranes of adjacent cells were in close apposition. In Fig. 2 a terminal bar is clearly discernible.

The cytoplasm of cells dehydrated in EPON 812 (Figs. 3 and 4) showed several minor differences when compared with cells from tissue dehydrated in alcohol. Most notable of these is the shape of the limiting membranes of the mitochondria. While retaining their characteristic double structure, the limiting membranes of mitochondria in cells dehydrated in EPON 812 were convoluted.

FIGURE 5

Micrograph showing granules of an eosinophile. The outer membrane of the granules appears intact. Tissue from Group II. X 26,000.

FIGURE 6

Micrograph of a portion of myelin sheath from the sciatic nerve of a rat. Parallel lamellae are apparent. Tissue from Group II. X 20,800.

FIGURE 7

Micrograph of a portion of a macrophage from the spleen of a rat. Large, dense clumps of disintegrating red cells can be seen. Note the distinct granules which are probably ferritin. Tissue from Group II. X 20,800.

FIGURE 8

Micrograph of cells from a rat kidney tubule. Note the nuclear pore (arrow). Tissue from Group I stained with lead hydroxide. X 13,500.

FIGURE 9

Micrograph of a large mitochondrion in a proximal convoluted tubule cell from tissue treated similarly to that shown in Fig. 8. At arrow 1 the crista appears continuous with the inner of the two limiting membranes, while at arrow 2 there seems to be a distinct discontinuity. X 28,200.

FIGURE 10

Micrograph of the basal portion of a proximal convoluted tubule cell. The prominent basal infoldings of the plasma membrane are clearly seen. Tissue from Group I stained with lead hydroxide. X 13,500.
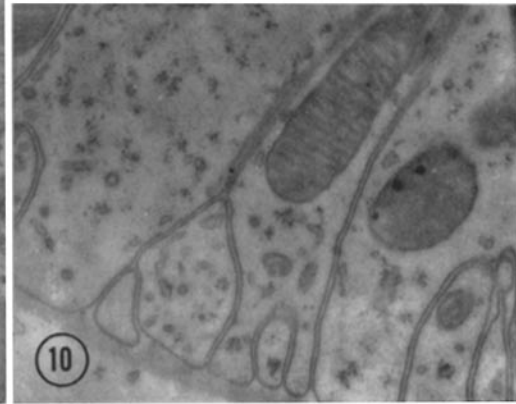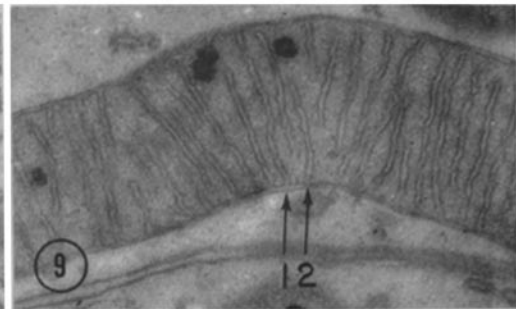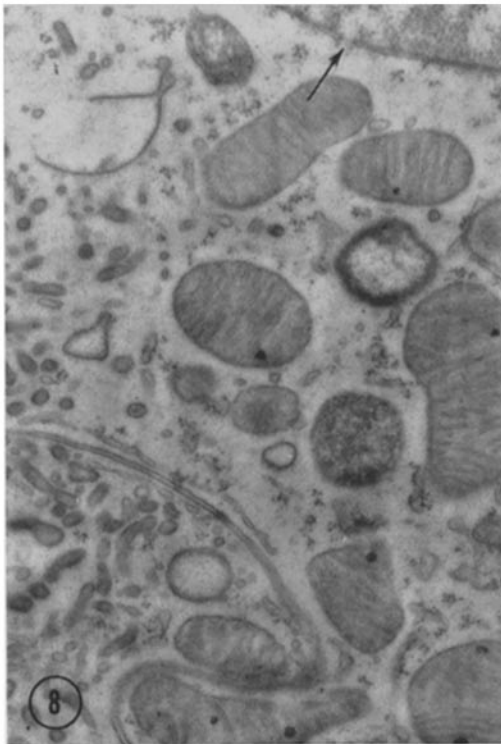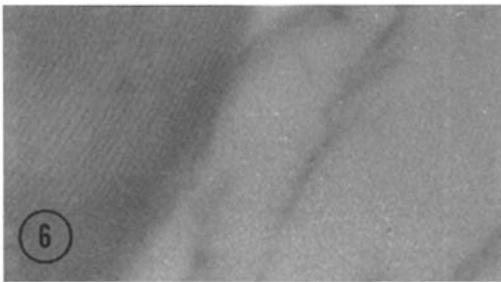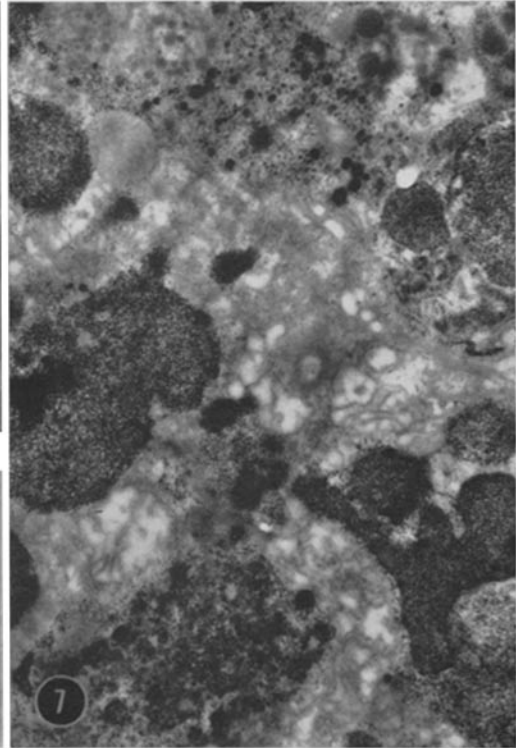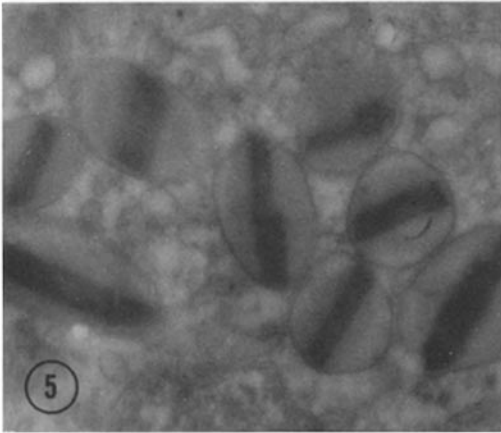
The cristae were similar in shape and distribution to those present in tissue dehydrated in alcohol. The endoplasmic reticulum was clearly defined and contained closely associated RNP particles. These particles were less distinct than those in cells dehydrated in alcohol. No appreciable differences were noted between the structureless cytoplasmic matrix of the cells compared.

Sections of cells from other tissues were examined to determine the reproducibility of this embedding procedure and its ability to preserve fine structure. In Fig. 5 the specific granules of an eosinophile display an inner dense component and an intact, single limiting membrane. In Fig. 6 the lamellae of a portion of the myelin sheath from the sciatic nerve show the characteristically parallel and evenly spaced lipoprotein sheaths. The lamellae have a periodicity of approximately 140 A. The cytoplasm of several macrophages containing large masses of ferritin granules, probably remnants of disintegrating red cells, was preserved with considerable structural detail (Fig. 7).

Fig. 8 illustrates the excellent morphologic preservation of the cytoplasm of kidney tubule cells which were dehydrated with alcohol and embedded in EPON. Fig. 9 is a micrograph of a portion of a mitochondrion from a proximal convoluted tubule, the cristae of which are well defined and display structurally variable, intimate relationships with the external membrane. The highly convoluted plasma membrane adjacent to and surrounding individual mitochondria is clearly defined in Fig. 10. Note the intimate contact between this membrane and the basement membrane.

DISCUSSIONS AND CONCLUSIONS

EPON 812 may be used for dehydration with little effect on the fine structure of a variety of cells. Endoplasmic reticulum (9) and other organelles appear undamaged. The exact mechanism of tissue dehydration by ascending concentrations of EPON 812 is not clear but it is probably similar to that of alcohol. Because EPON 812 is miscible with water (40 per cent by volume), the initial dehydration mixture should contain slightly less than 40 per cent water. The specimen may be transferred from the buffered rinse solution to 100 per cent EPON 812 without deleterious effect on the fine structure.

EPON 812 has limited lipid-solvent properties; however, the acid anhydrides and tertiary amine accelerators employed for its polymerization may add to this solvent effect. This effect appears to be much less drastic than that of the conventional solvents employed for routine dehydration, thus making EPON 812 a promising agent for the study of lipid-soluble, histochemical reaction products at the electron microscope level.

Sectioning of the embedded material presents several problems (6); however, with careful attention to technique sufficiently thin sections can be obtained. Prolonging the impregnation period and the polymerization time seems to improve the sectioning properties of the final block (12). Sections obtained demonstrate considerably less contrast than comparable sections from tissue embedded in methacrylate, presumably because the EPON does not sublime in the electron beam. This decrease in contrast can be overcome by subsequent staining with heavy metals (10, 11).

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