A NEW EMBEDDING TECHNIQUE FOR ELECTRON MICROSCOPY, COMBINING A WATER-SOLUBLE EPOXY RESIN (DURCUPAN) WITH WATER-INSOLUBLE ARALDITE

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A new water-soluble embedding agent, trial product CIBA X 133/2097[1] has recently been introduced for selected electron microscopic investigations (1). Durcupan subsequently proved to be miscible with the water-insoluble epoxy resin Araldite[2] which is commonly used for tissue embedding (2). In this way, animal tissues can be embedded in Araldite without being brought into contact with ethanol or acetone. By replacing Durcupan with Araldite as final embedding medium, ultrathin sections are more readily obtained than by employing Durcupan alone. The method described below displays some points of resemblance to the one devised by Craig *et al.* (3) for Epon 812.

MATERIALS AND METHODS

Liver, kidneys, and pancreas of white rats were fixed in 2 per cent osmium tetroxide for 1 hour at a temperature of 4°C. The tissue was then dehydrated at a temperature of 4°C in accordance with the following schedule:—

Time	Water	Durcupan
	per cent	*per cent*
15–30 min.	50	50
15–30 min.	30	70
15–30 min.	10	90
30–60 min	—	100
30–60 min.	—	100

The tissue, now free of water and impregnated with Durcupan at room temperature was embedded in Araldite by passing it through the following sequence of baths, Araldite II being kept at 50°C:—

Time	Durcupan	Araldite I	Araldite II
	per cent	*per cent*	*per cent*
1 hr.	70	30	—
1 hr.	50	50	—
Several hrs. or overnight	30	70	—
1 hr.	—	100	—
3 × 30 min.	—	—	100

Araldite I consists of Araldite M (10 ml) and hardener 964 B (10 ml); Araldite II contains in addition the accelerator 964 C (0.4 ml). The tissue was allowed to harden for 24 to 48 hours at a temperature of 50°C.

The blocks thus obtained were cut without difficulty. Ultrathin sections were produced by cutting with a diamond knife on a Porter-Blum or LKB microtome.

For the material shown in Figs. 1, 2, and 5 the staining agent used was lead hydroxide (4); in Figs. 3 and 6 it was a mixture of saturated uranyl acetate and 1 per cent potassium permanganate (5), without washing with citric acid, and in Fig. 4 it was saturated uranyl acetate.

The microscope employed was an Elmiskop I, operated with a double condenser and a 50 μ objective aperture.

OBSERVATIONS

Fig. 1 shows a segment from the cytoplasm of a rat liver cell. Besides the mitochondria with their characteristic internal structure, one can also see parts of the Golgi apparatus and of the endoplasmic reticulum, the structure of which is more

[1] Commercially available under the registered trade mark Durcupan (Fluka AG., Buchs, Switzerland).
[2] Registered trade mark of CIBA Ltd.

clearly visible in Fig. 2. A cut microbody (6) can be observed in the center of Fig. 1. If the section has been suitably cut, the dense core of the microbodies displays parallel striations, the period of which, measured with a microdensitometer, is approximately 48 A (Fig. 3).

Fig. 4 shows a section through three exocrine pancreatic cells bordering on a gland duct. Dense zymogen granules and a few mitochondria can be seen in the cytoplasm which is rich in endoplasmic reticulum. The nuclei of the pancreatic cells sometimes contain dense granules measuring 100 to 250 mμ in diameter (Fig. 5). It is not possible to decide whether there is a connection between these granules and the "perichromatin granules" described by Watson (7).

Fig. 6 represents a segment from an epithelial cell of the proximal tubule of the kidney. The invaginations of the cell membrane display a distinct double structure (insert, Fig. 6).

SUMMARY

A new method for embedding animal tissues without using either ethanol or acetone as dehydrating agent has been described. Ultrathin sections are readily obtained.

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Abbreviations

bm, basement membrane
cm, cell membrane
d, pancreatic duct
er, endoplasmic reticulum
G, Golgi apparatus
m, mitochondrion
mb, microbody
N, nucleolus
nm, nuclear membrane
z, zymogen granule

FIGURE 1

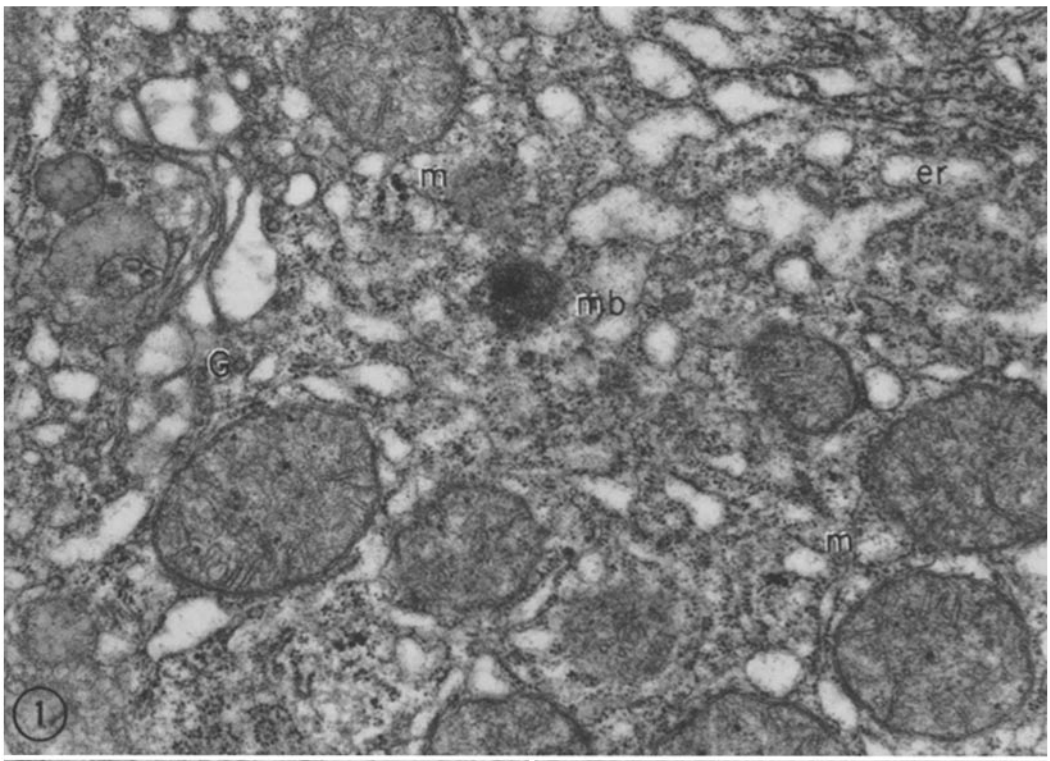
Part of a rat liver cell. × 27,000.

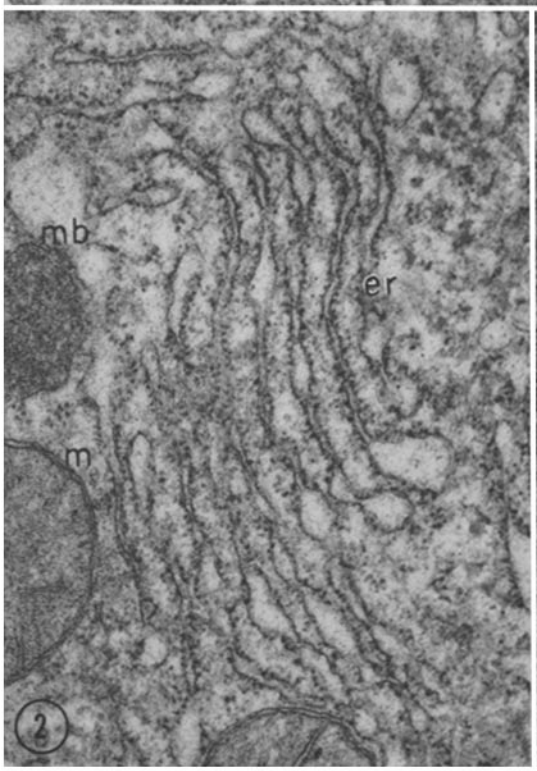
FIGURE 2

Ergastoplasmic profiles of a hepatic cell. × 28,000.

FIGURE 3

Microbody with dense core showing striations. × 128,000.





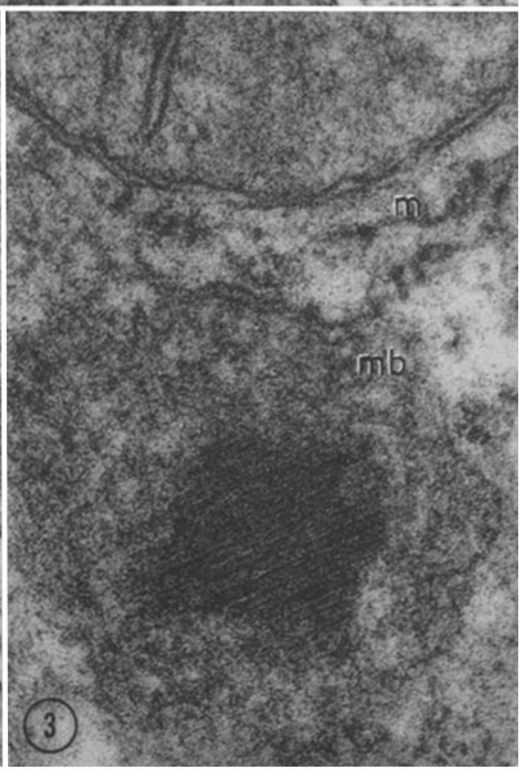


FIGURE 4

Exocrine pancreatic cells bordering on a pancreatic duct. × 5,000.

FIGURE 5

Nucleus of a pancreatic cell with intranuclear dense granules (arrows). × 17,000.

FIGURE 6

Part of an epithelial cell of the proximal tubule of rat kidney. × 36,000. Insert showing double structure of the cell membrane. × 90,000.