PRESERVATION OF THE FINE STRUCTURE OF ISOLATED LIVER CELL PARTICULATES WITH POLYVINYLPYRROLLIDONE-SUCROSE*

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PLATES 18 AND 19

The purpose of this brief communication is to illustrate the effectiveness of polyvinylpyrrollidone-sucrose in preserving the fine structure of cell particulates of rat liver during their isolation by homogenization and differential centrifugation.

As reported elsewhere (1, 2), the medium we have evolved is a modification of those of Woods (3) and Greenfield and Price (4). It is a mixture of 7.3 per cent polyvinylpyrrollidone (PVP) and 0.25 M sucrose, adjusted with alkali to pH 7.6–7.8. This yields a homogenate of pH 6.9–7.1.

Phase-contrast microscopy shows that the mitochondria retain, for long periods of time, the typical rod shapes seen in the intact cell. Their biochemical integrity is well preserved, as indicated by the latency of their ATP-ase and their ability to carry on oxidative phosphorylation in the absence of fluoride (1, 2).

For fixation, we have found a mixture of 7.3 per cent PVP, 0.25 M sucrose, and 2 per cent osmium tetroxide to be effective when adjusted to pH 7.0 (2).

An unpurified fraction isolated from PVP-sucrose homogenate is shown in Fig. 1. This section is chosen because it includes different particulate types. The mitochondria (*mt*) show the typical outer and inner membranes, and small inner granules (5–8). Most noteworthy is the uniform density of such mitochondria. This indicates that little, if any, loss of material has occurred during isolation.

The microsomal material (*mc*, Fig. 1; also Fig. 2) shows a striking resemblance to the basophilic material seen within liver cells in sections (9, 7, 10, 11). Except for length, these microsomes appear identical with the granule-coated membranes of the ergastoplasm or endoplasmic reticulum (12–14, 10).

Other cytoplasmic structures which are apparently well preserved when isolated from PVP-sucrose are Golgi membranes (*g*, Fig. 1) and the so-called "dense bodies" (*db*, Fig. 2; see also Novikoff *et al.* (15)). What appear to be microvilli of the bile canaliculi (*m*, Fig. 3) (9, 16, 11) and other membranes (*me*, Fig. 1) whose cytologic identity is not known are also present.

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Fig. 3 is a section of washed "nuclear fraction." The nucleus shows a dark granular nucleolus and a clearly double nuclear membrane. The nuclear membrane is discontinuous, interrupted by numerous openings. These seem to be enlarged "nuclear pores" (17). Through the openings a finely granular material appears to be leaving the nucleus. This probably represents a gel-like material which exudes from the nuclei when they are washed in aqueous media; it may contain the protein which Pollister and Leuchtenberger (18) showed to be lost from nuclei during their isolation from such media.

These illustrations suggest the value of PVP-sucrose for the isolation of intracellular structures from rat liver. They also point to the importance of electron microscopy of thin sections for the cytologic characterization of cell fractions used in biochemistry.

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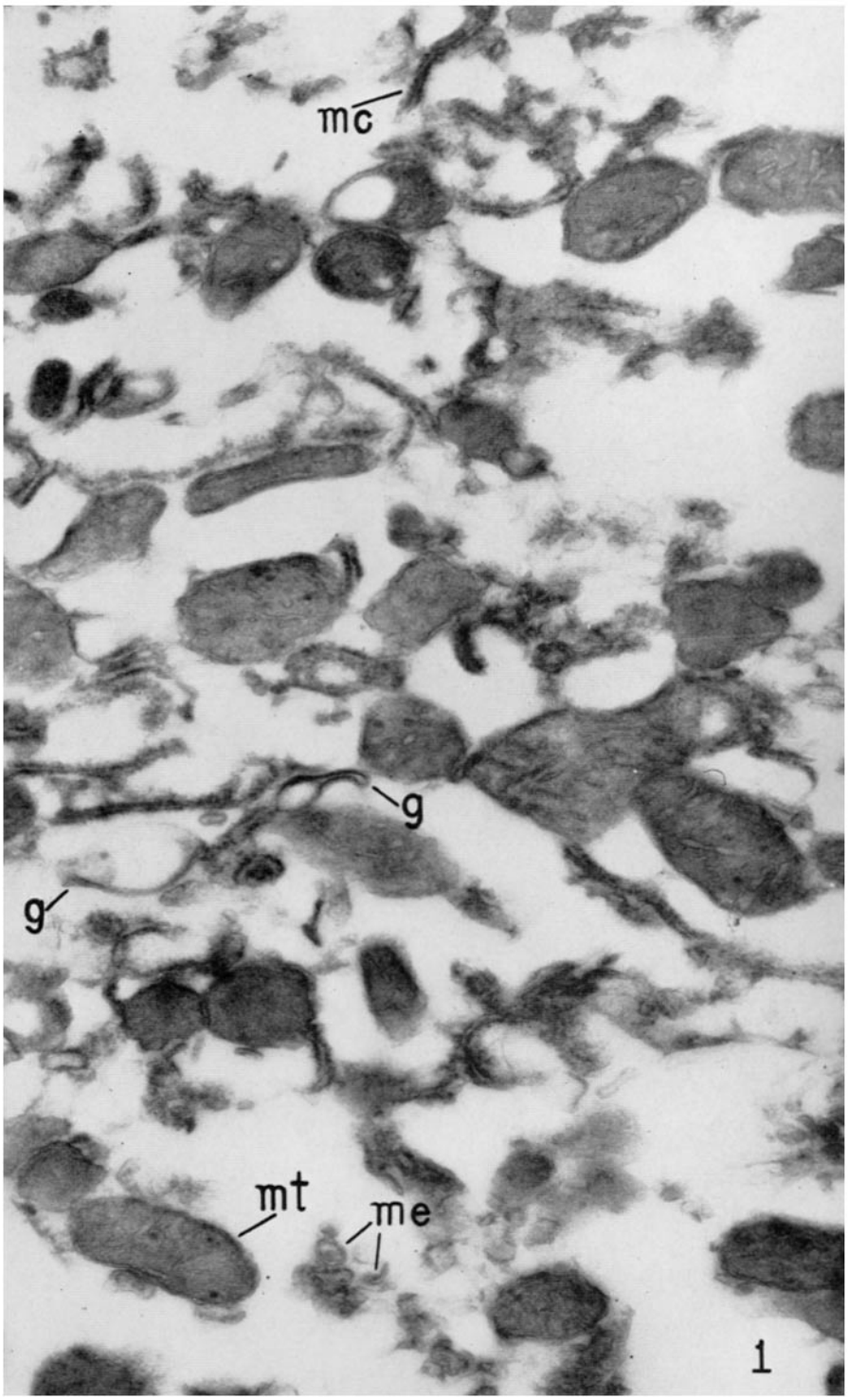
EXPLANATION OF PLATES

PLATE 18

FIG. 1. Unpurified fraction isolated from PVP-sucrose homogenate of rat liver. A very small pellet of the fraction was fixed in PVP-sucrose-OsO_4, pH 7.0, at 0°C. for 13 hours. *mt*—mitochondria, *mc*—microsomes, *g*—Golgi membranes, *me*—membranes of undetermined cytologic origin. See text for description. × 34,850.

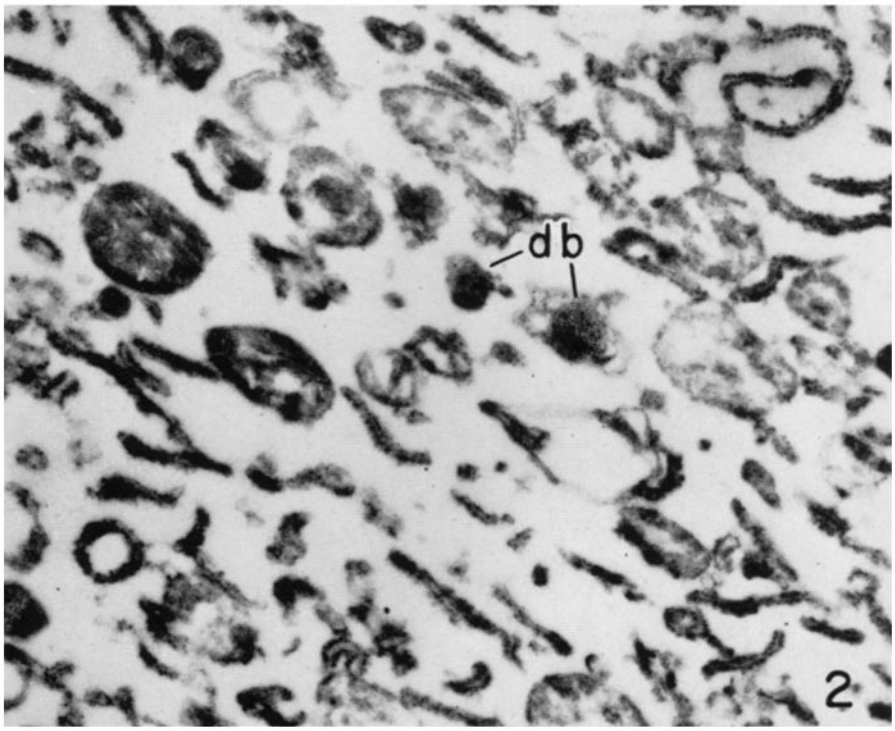


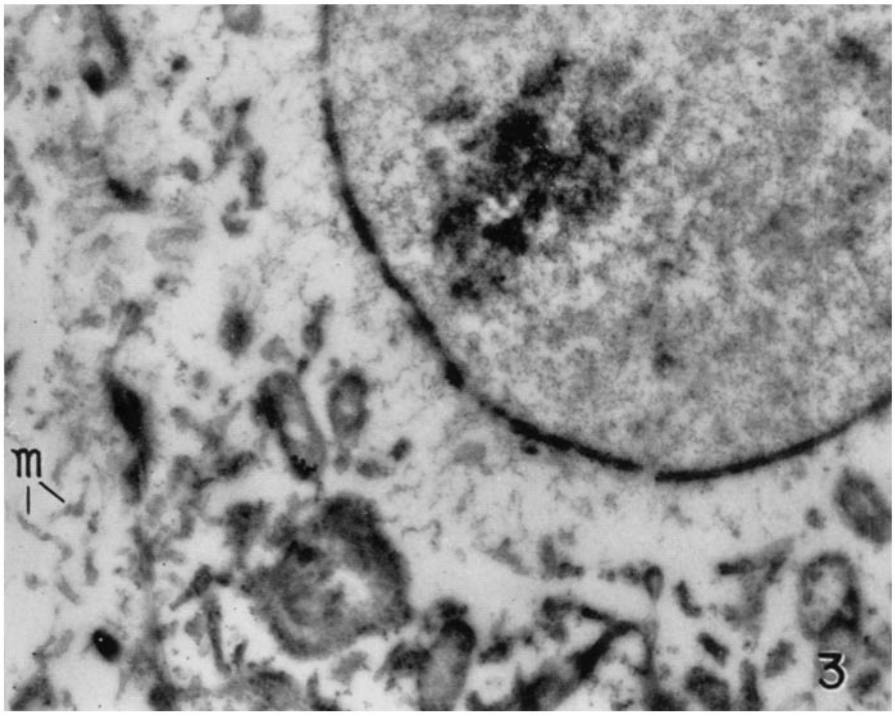
(Novikoff: Liver cell particulates)

PLATE 19

FIG. 2. Fraction isolated from PVP-sucrose homogenate of rat liver. A pellet of the fraction was fixed in PVP-sucrose-OsO_4, pH 7.0, at 0°C. for 13 hours. Note the abundant microsomal material (granule-studded membranes), some mitochondria (poorly preserved), and two "dense bodies" (*db*). × 31,500.

FIG. 3. Washed "nuclear fraction" isolated from PVP-sucrose homogenate of rat liver. A pellet of the fraction was fixed in PVP-sucrose-OsO_4, pH 7.0, at 0°C. for 15 hours. Note the nucleolus, double nuclear membrane, fine granular material leaving nucleus through enlarged "pores," and microvilli (*m*). × 22,300.





(Novikoff: Liver cell particulates)