ISOLATION OF A GOLGI APPARATUS-RICH FRACTION FROM RAT LIVER

I. Method and Morphology

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ABSTRACT

Golgi apparatus were released without fixatives from rat hepatocytes by gentle homogenization, concentrated by differential centrifugation, and purified by sucrose gradient centrifugation. Examination of sections of purified fractions by electron microscopy showed fields of morphologically intact units of Golgi apparatus consisting of stacks of parallel flattened cisternae, secretory vesicles, and small vesicular profiles. Negative staining of unfixed pellets revealed a complex network of anastomotic tubules continuous with platelike structures and secretory vesicles. These structures corresponded, respectively, to the small vesicular profiles and parallel flattened cisternae with attached secretory vesicles of sectioned material. Small fragments of granular endoplasmic reticulum were often closely associated with the peripheral tubules, suggesting sites of continuity in intact hepatocytes.

INTRODUCTION

Detailed studies on the chemistry and enzymology of Golgi apparatus have been hindered by lack of reproducible isolation and purification procedures that permit development of viable cell-free systems (12). Progress was reported with epididymis (7, 22) and later with plants (12, 17, 18), liver (4, 14), and kidney (14), but these preparations have not been subjected to extensive biochemical characterization. Our procedure for rat liver is simple, reproducible, excludes fixatives, and gives a concentrated Golgi apparatus fraction of useful quantity (20).

MATERIALS AND METHODS

Male rats, 200–250 g (50 days old, Holtzman Company, Madison, Wisconsin), fed Purina Laboratory chow, were anesthetized by intraperitoneal injection of 0.5–1 ml of pentobarbital (Nembutal) solution (20 mg/ml). Livers (approximately 10 g each) were drained of blood, minced thoroughly with scalpels, and transferred to 50 ml of chilled homogenization medium (37.5 mM Tris-maleate, pH 6.5; 0.5 M sucrose; 1% dextran and 5 mM $MgCl_2$ at 0–4°) for 5–10 min with occasional stirring. The liquid was decanted and replaced by 25 ml of fresh homogeniza-

tion medium followed by homogenization for 30–60 sec using a Polytron 20 ST (Kinematica, Lucerne, Switzerland) homogenizer operated at the slowest speed. The homogenate was squeezed through a single layer of Miracloth (Chicopee Mills, New York) and centrifuged for 20 min at 2,000 *g* (Spinco SW 39L).

Most of the supernatant from the 2,000 *g* centrifugation was removed by careful aspiration and cleared by centrifugation at 100,000 *g* for 30–120 min. The cleared supernatant was used for resuspension of Golgi apparatus and could be stored at 4° for several days or frozen for later use. The friable upper 1/3 of the 2,000 *g* pellet was resuspended in a small volume (1.0 ml) of cleared supernatant and then layered onto the following discontinuous sucrose gradient: 1.8, 1.6, 1.5, and 1.25 M sucrose in a v/v ratio to sample of 0.25:0.5:1:1. All gradient solutions were prepared in 37.5 mM Tris-maleate, pH 6.5, 1% dextran, and 5 mM $MgCl_2$. The gradient was centrifuged for 30–40 min at 100,000 *g* (SW 39L) to remove mitochondrial and endoplasmic reticulum contamination. The Golgi apparatus (density 1.12–1.14) collected in a cream-colored band at the 1.25 M sucrose-homogenate interface. Contaminating cell fractions occupied lower bands.

The band containing Golgi apparatus was carefully removed from the gradient with a Pasteur pipette, resuspended, and pelleted at 2,000 *g* for 30 min. So as to reduce contamination of the Golgi apparatus-rich fraction by plasma membrane, endoplasmic reticulum, and mitochondria, this step was normally followed by three to four centrifugation and washing cycles (2,000 *g* for 30 min in homogenation medium or distilled water). These procedures have been used or modified successfully in several different laboratories with comparable successes in each (8, 15, 16, 21, 23).

Samples of isolated fractions were prepared for electron microscopy by fixation for 1.5–2 hr in 2.5% glutaraldehyde (from a 50% stock solution, Fisher Biological Grade, treated with 0.16 g/ml of activated coconut charcoal for removing impurities) buffered with 0.1 M sodium phosphate, pH 7.2, followed by transfer to 1% osmium tetroxide in the same buffer at 4° for 16 hr. Specimens were dehydrated and embedded as described previously (2, 10). Negative staining of unfixed Golgi apparatus pellets was done on carbon-stabilized, collodion-coated grids by first resuspending a portion of the pellet in water and then mixing the suspension with an equal volume of 2% phosphotungstic acid (PTA) neutralized with potassium hydroxide to pH 6.8. Bovine serum albumin (0.05–0.1%) was sometimes used in the PTA for facilitating spreading of the sample (2). Specimens were observed and photographed in a Philips EM 200.

RESULTS AND DISCUSSION

Approximately 2–5 mg of dry Golgi apparatus (1.2–3.0 mg of protein) were obtained from each liver (0.4 mg of dry Golgi apparatus or 0.24 mg of Golgi apparatus protein per gram fresh weight of liver). Freeze-dried fractions were light yellow and the visible spectrum corresponded to that of (met) hemoglobin.

The yield and purity of the Golgi apparatus were critically dependent upon the homogenization procedure. With Polytron homogenization, large fragments of relatively intact Golgi apparatus were obtained which sedimented at low centrifugal force. Extensive homogenization yielded smaller membrane pieces which sedimented with the microsome fraction and were generally less suitable as a starting material for biochemical studies. Other details of the procedure appeared less critical and Mg^{++} could be eliminated without morphological alteration of the isolated Golgi apparatus.

The identification of isolated Golgi apparatus was based on their morphology which is so characteristic that it serves as a reliable marker. Thin sections of the pellets showed fields containing stacks or dictyosomes (11, 12, 14) comprised of parallel flattened cisternae surrounded by small vesicular profiles and secretory vesicles (Fig. 1). In low magnifications of negatively stained preparations (Fig. 2), the Golgi apparatus fraction appeared as a complex mixture of flattened platelike structures, tubules, and secretory vesicles. Appropriate planes of section showed continuity between flattened cisternae and the peripheral network of anastomotic tubules (Fig. 3). The latter usually appear in cross-section as small peripheral vesicles (Fig. 3). The tubular nature of this cell fraction was most striking in negatively stained preparations (Fig. 2) and was emphasized by the presence of 300–1000 A electron-translucent particles which caused distention of the tubule walls (Figs. 2 and 5). This extensive system of peripheral tubules was continuous with the platelike central regions of the cisternae in both thin-sectioned and negatively stained preparations (Figs. 3 and 4). These morphological data demonstrate that the platelike structures of negatively stained material correspond to the parallel flattened cisternae and that these are continuous with an extensive tubular network which appears as small vesicular profiles in thin sections.

Secretory vesicles of hepatocyte Golgi apparatus

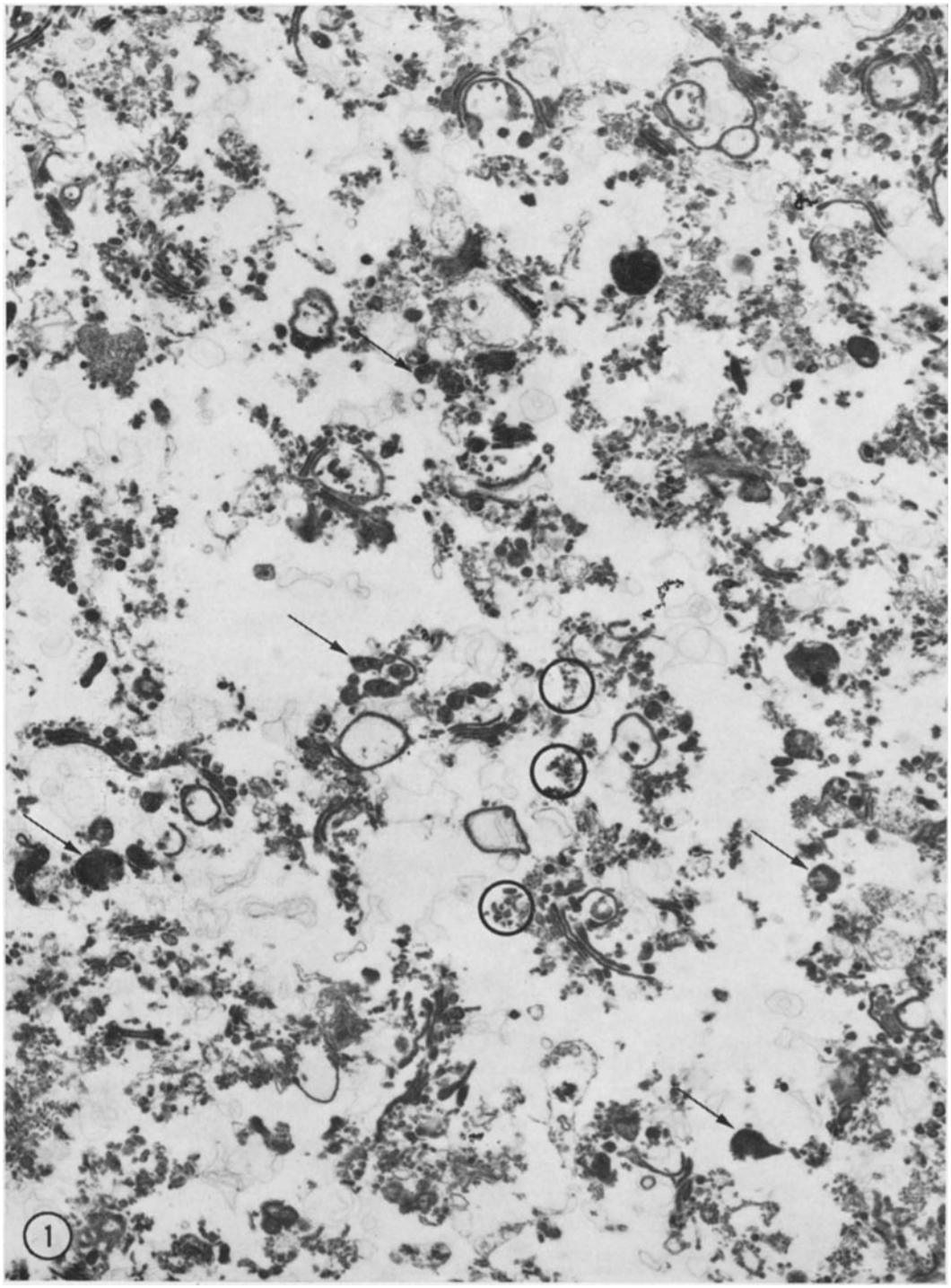


FIGURE 1 Thin section of Golgi apparatus–rich fraction from rat liver showing stacks of parallel cisternae, secretory vesicles (arrows), and numerous small vesicular profiles (circled). × 14,000.

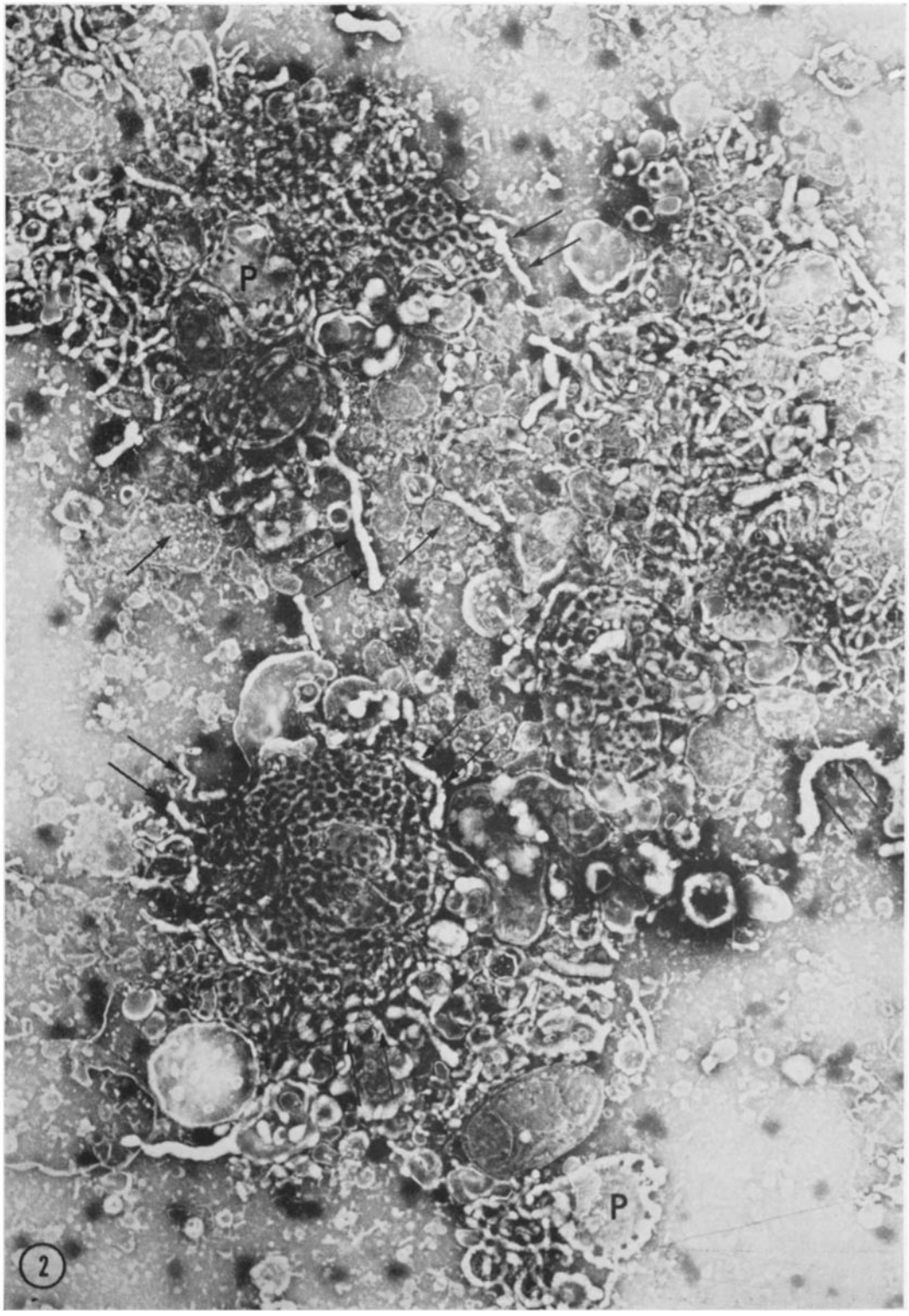
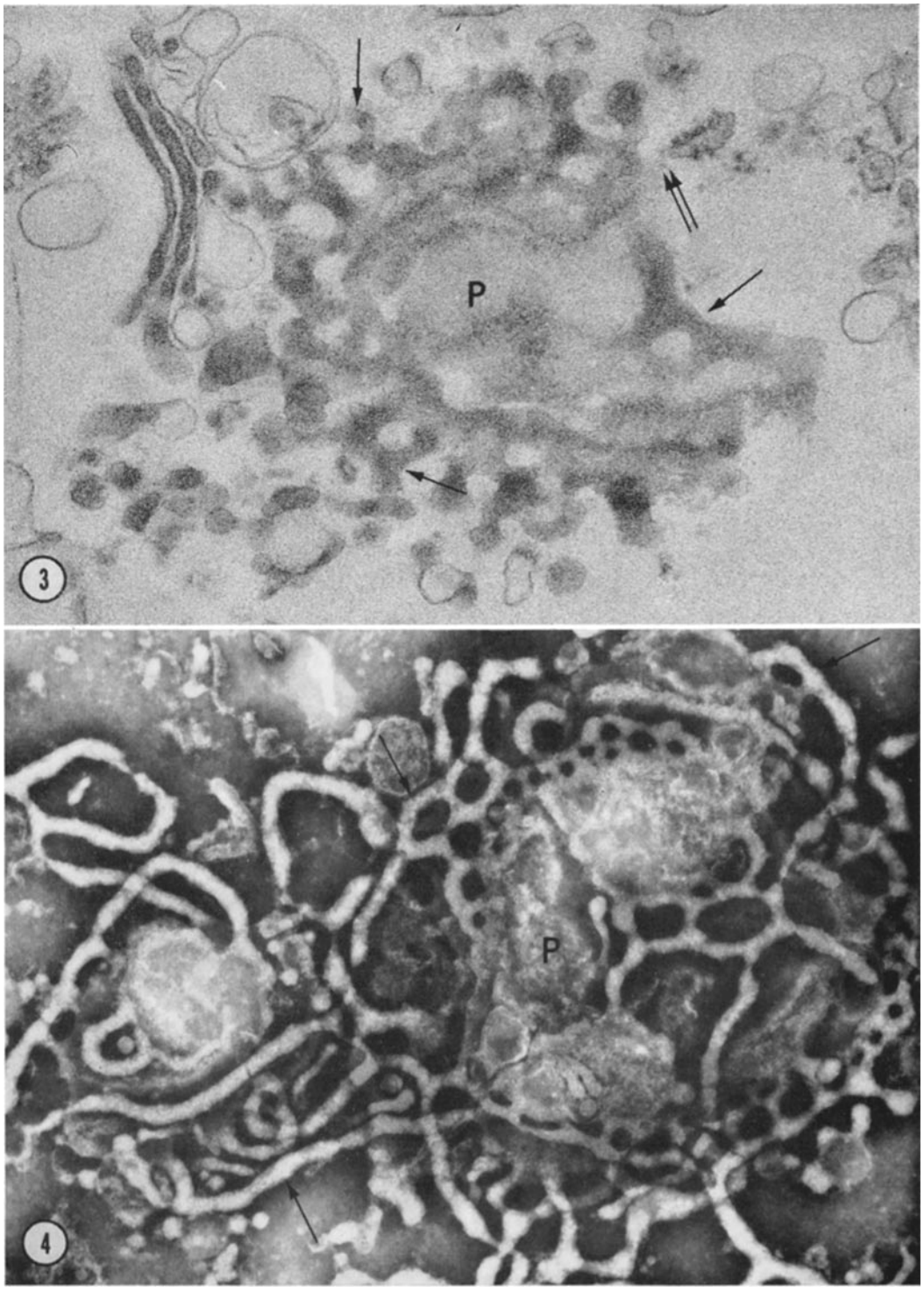


FIGURE 2 Negative contrast of Golgi apparatus–rich fraction from rat liver (same pellet as Fig. 1) showing the complex assortment of fenestrated plates (*P*), secretory vesicles (single arrows), and several kinds of tubules which characterize the isolated Golgi apparatus. The larger distended tubules (double arrows) contain secretory product and appear translucent. A relatively intact stack of cisternae (dictyosome) is seen at the lower left with several such tubules at its periphery. × 21,000.



FIGURES 3 and 4 Peripheral tubules (single arrows) are continuous with the platelike central portions of cisternae (*P*) as shown in thin section (Fig. 3, × 68,000) and in negative contrast (Fig. 4, × 78,500). In Fig. 3, a fragment of endoplasmic reticulum (double arrow) is seen adjacent to the system of peripheral tubules.

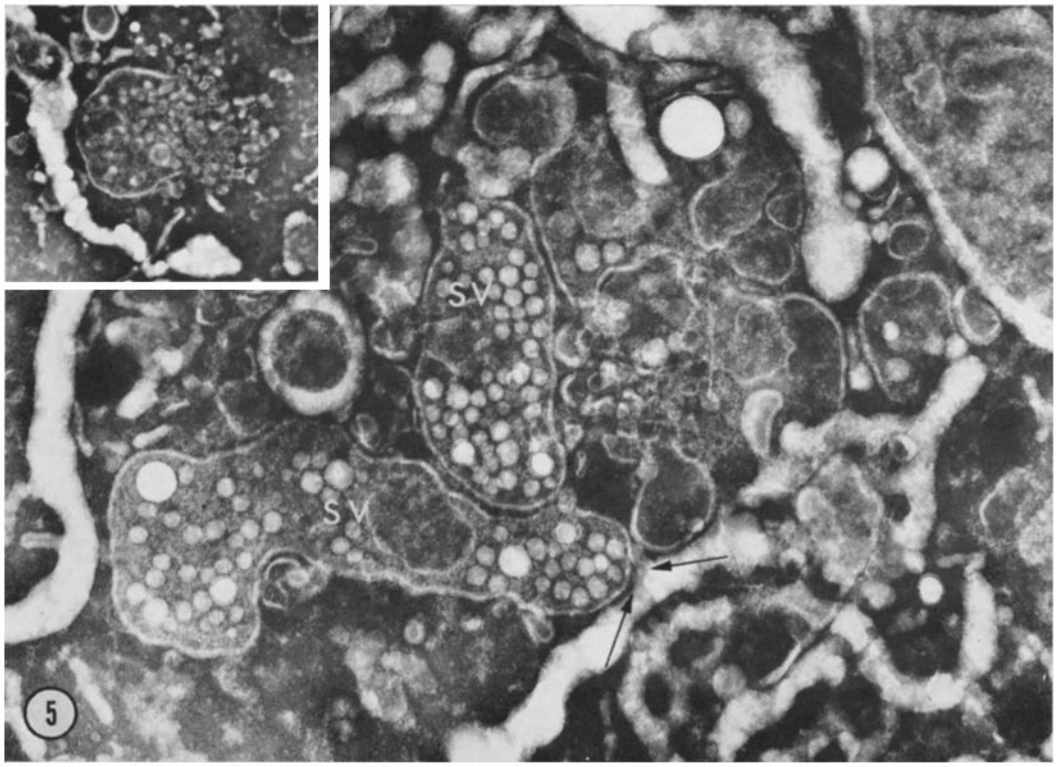


FIGURE 5 Translucent particles of the secretory vesicles (*SV*) and distended peripheral tubules are morphologically similar to plasma very low density lipoproteins (VLDL) (5). Short tubules suspected of connecting the particle-laden tubules with the vesicle interior are shown by arrows. If we assume a functional relationship between tubules and vesicles, the tubules adjacent to vesicles which are filled with translucent particles might be visualized as input tubules. × 71,000. Insert shows a ruptured vesicle adjacent to one of the input tubules. × 23,000.

have been characterized by clusters of 300–1000 A particles (3, 4, 6) which appear identical with those in the tubular network (5). These particles are precursors of the very low density lipoproteins of plasma (5, 8, 9) and serve as one criterion for recognition of Golgi apparatus in isolated cell fractions from rat liver. Secretory vesicles filled with these particles appear to be attached to the platelike portions of the cisternae by means of tubules (Figs. 5 and 6), as described previously for plant cells (13). Structures comparable to secretory vesicles are observed by negative staining (Fig. 5), but many apparently are disrupted by this procedure and release particles onto the grid surface (Fig. 5, *insert*).

When fragments of rough-surfaced endoplasmic reticulum were found in the pellets, they were often closely associated with the tubular peripheries of the stacks (Fig. 6). This close topographical relationship in pellets of isolated Golgi apparatus may indicate sites of continuity between rough-surfaced endoplasmic reticulum and the peripheral system of Golgi apparatus tubules in intact hepatocytes.

The rat liver Golgi apparatus *in situ* is composed of heterogeneous elements, i.e., platelike regions, several kinds of tubules, intercisternal regions, a variety of secretory vesicle types, and cisternae (16). All these elements have been identified in the isolated preparations and the preparations obtained appear to be representative of the Golgi apparatus of the intact cell. Occasional mitochondria, fragments of rough endoplasmic reticulum, and smooth membranes suspected of being membranes from ruptured secretory vesicles (Fig. 6) were observed in Golgi apparatus prepara-

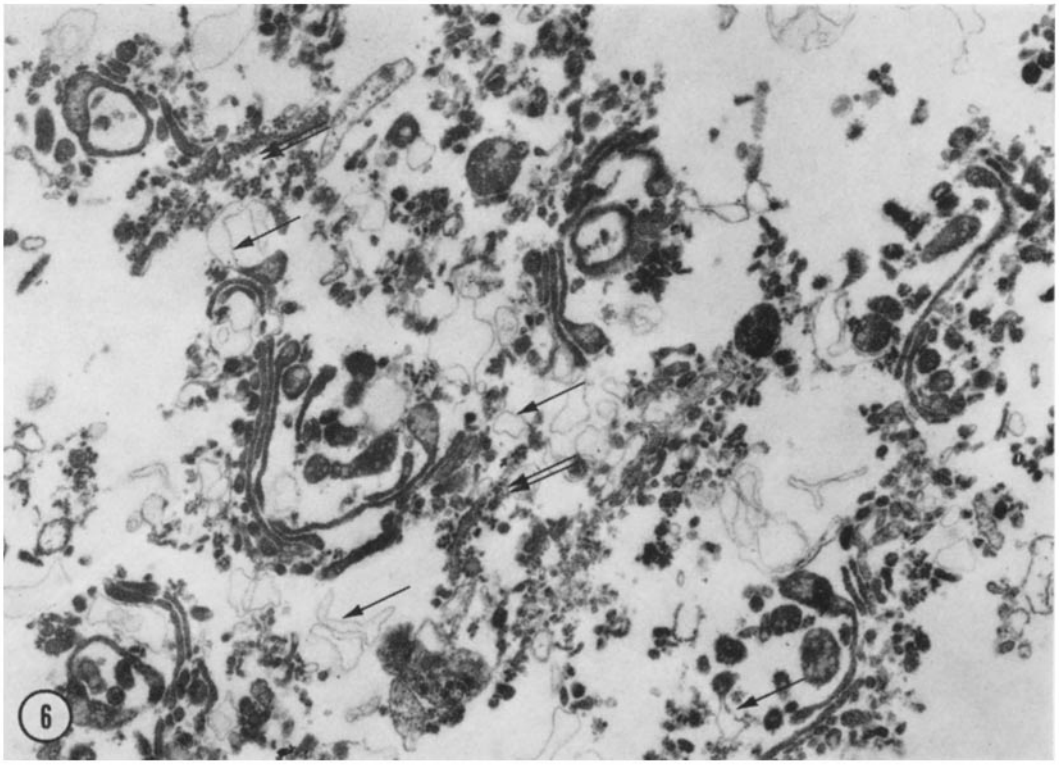


FIGURE 6 Thin section of Golgi apparatus-rich fraction showing tubular connections between secretory vesicles and platelike portion of cisternae and smooth membrane thought to be derived from ruptured or partially ruptured secretory vesicles (single arrows). Double arrows show fragments of endoplasmic reticulum adjacent to the peripheral tubules of the Golgi apparatus. × 17,000.

tions by both thin sectioning and negative-staining techniques. Lysosome-like vesicles and occasional microbodies were also found. Free ribosomes, nuclei, glycogen, or identifiable plasma membrane fragments were rarely encountered.

Although the electron microscope has been, and will continue to be, indispensable in the assay of isolated Golgi apparatus fractions, this technique has been extended and amplified in Part II of this communication (see reference 1) by assays for enzyme activities known to be concentrated in the Golgi apparatus and in other cell fractions. The results emphasize that Golgi apparatus fractions isolated routinely in the manner described may exceed 80% Golgi apparatus-derived material. We anticipate that this technique for isolating Golgi apparatus from rat liver will be applicable to other tissues and will facilitate studies of the membranes, enzymes, secretory products, and functions of this cell component.

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