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The Golgi Complex

I. Isolation and Ultrastructure in Normal Rat Liver

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Summary. The ultrastructural arrangement of membranes of the Golgi complex has been characterized in Golgi fractions isolated from rat liver. Procedures for isolation of these fractions have been modified to provide a good yield of Golgi membranes (60 to 70%) with greater than 50-fold purification of sialyl transferase, an enzyme specific for the Golgi complex. The isolated membranes appear well preserved and both the dimensions and appearance of the Golgi complex observed by negative staining and in sections of the isolated membranes correlate well with that in liver sections.

The Golgi complex consists of a series of platelike structures, each consisting of a central sac or cisterna from which a network of fine tubules arises. The tubules increase in diameter towards the periphery of the plate and are associated with the formation of vacuoles or secretory vesicles. The structure of the Golgi complex has been related to its role in glycoprotein biosynthesis.

The Golgi complex represents an important component of most cell types functioning both in biosynthesis and in control of transport of secretory materials. Within one cell, the organelle is involved in numerous roles such as packaging of secretory proteins (Caro & Palade, 1964; Jamieson & Palade, 1966), secretion of lipoproteins (Hamilton, Regen, Gray & Lequire 1967; Mahley, Hamilton & Lequire, 1969), participation in formation of lysosomes (DeDuve & Wattiaux, 1966), biosynthesis of glycoproteins (Neutra & Leblond, 1966*a*, *b*) important in secretions and also a possible role in membrane formation (Palade, 1959; Sjöstrand, 1968).

A close relationship exists between the Golgi apparatus and the endoplasmic reticulum and these membranes possess a functional continuity and possibly some form of structural continuity (Claude, 1970; Morré, Mollenhauer & Bracker, 1971). This is important not only in synthesis and transport of material in the cell but also in the differentiation and development of cell membranes.

Our present knowledge of the arrangement of these membranes is based mainly on studies with thin sections of plant and animal tissues. In addition, freeze-etched replicas (Northcote, 1971) and negatively stained preparations (Mollenhauer & Morré, 1966; Beams & Kessel, 1968; Morré, Hamilton, Mollenhauer, Mahley, Cunningham, Cheetham & Lequire, 1970; Northcote, 1971) have provided more detailed information of the Golgi apparatus in plant cells (Beams & Kessel, 1968; Morré *et al.*, 1971). To understand the functional organization of these membranes in the normal cell and to investigate dysfunction of this organelle in disease, a detailed knowledge of the structural arrangement of the Golgi complex is essential. An abnormality either in glycoprotein biosynthesis or in transport of secretory materials may be associated with structural changes in the Golgi apparatus.

With the recent development of techniques for isolation of the Golgi apparatus from rat liver (Fleischer & Fleischer, 1970; Leelavathi, Estes, Feingold & Lombardi, 1970; Morré *et al.*, 1970) it is possible to study in more detail the fine structure and arrangement of the membranes. However, many studies of Golgi fractions have been concerned with biochemical characterization of the membranes (Fleischer, Fleischer & Ozawa, 1969; Mahley *et al.*, 1969; Schachter, Jabbal, Hudgin, Pinteric, McGuire & Roseman, 1970; VanGolde, Fleischer & Fleischer, 1971; Moscarello, Kashuba & Sturgess, 1972) while morphological studies have been used only as a parameter to judge purity of the fractions.

In the present study a modified method for isolation of Golgi apparatus-rich fractions from rat liver has been used to characterize the ultrastructure and arrangement of the Golgi complex. The arrangement of isolated membranes has been correlated with that seen in sections of the liver cell.

Materials and Methods

Male Wistar rats, 200 to 220 g were fasted for 15 hr and then anesthetized with ether and exsanguinated. The liver was resected quickly from each rat and, after removing a slice of liver from the median lobe for electron-microscopy, the remainder was used for preparation of the Golgi-rich fraction.

Isolation of Golgi-Rich Fraction from Rat Liver

Golgi membranes were isolated from each rat liver using a method based on that described by Morré *et al.*, 1970. The homogenization procedure was modified by the use of a stainless steel press and defined speed with a Potter-Elvehjem homogenizer. The liver was cleared of adhering connective tissue fragments and passed through a liver press consisting of a stainless steel plunger and a 3-cm steel plate perforated with 35 holes, 1 mm in diameter. The liver mash was suspended in 0.5 M sucrose containing 1 % dextran-

Table 1. Concentration of marker enzymes for various subcellular fractions (% of total homogenate)

Sample	Malic dehydrogenase[a]	Uricase[b]	Glucose-6-phosphatase[c]	Acid phosphatase[d]	5′-nucleotidase[e]
Supernatant[f]	17.5	58.0	6.0	32.3	—
Lower sediment[g]	36.0	15.3	35.0	31.8	—
Golgi fraction	not detected	not detected	0.7	0.26	0.24
Mitochondrial fraction	39.0	1.5	11.6	10.0	—

[a] Change in OD_{340} after 10-min incubation.
[b] Change in $OD_{282.5}$ after 1 hr.
[c] μM phosphorous liberated in 15 min.
[d] Change in OD_{410} in 30 min.
[e] μM phosphorous liberated in 15 min.
[f] $10{,}000 \times g$ supernatant.
[g] Lower sediment after removal of Golgi layer.

250, 37.5 mM Tris maleate and 5 mM magnesium chloride, pH 6.4, and homogenized at 80 rpm by one up-and-down stroke of a loose-fitting Potter-Elvehjem homogenizer (clearance 0.008 inches). The homogenate was filtered through three layers of cheese cloth and centrifuged at $14{,}000 \times g$ for 60 min in a SW 25.1 rotor. The upper, slightly yellowish portion of the pellet was homogenized in 0.5 M sucrose as above, layered on a 3-step discontinuous buffered sucrose gradient (1.25, 1.5 and 1.6 M) and centrifuged at $175{,}300\,g$ minutes in a swinging bucket 50.1 rotor. The Golgi fraction, sedimenting between the two top sucrose layers was removed and pelleted by centrifugation. The yield was approximately 0.5 mg dry weight of Golgi fraction per gram of liver.

The purity of the Golgi fractions was monitored by electron-microscopy and by assays for enzyme markers for various subcellular organelles. Sialyl transferase was measured both in the liver homogenate and in the Golgi fraction. The average specific activity (cpm/mg protein) of the homogenate was 24,600 while the Golgi fraction was 1,305,000. The mean purification factor, calculated as the ratio of these specific activites was 53.

Contamination with other subcellular material was assessed from the ratio of activity of specific enzymes in the Golgi fraction, compared with the liver homogenate. The results are compared in Table 1. The Golgi fraction showed no appreciable contamination with either mitochondria, microbodies, rough endoplasmic reticulum or lysosomes, as judged by the low levels of malic dehydrogenase, uricase, glucose-6-phosphatase, acid phosphatase and 5′-nucleotidase, respectively.

Electron-Microscopy

Golgi-Rich Fraction. The fraction containing the isolated Golgi membranes was examined by negative staining and in ultrathin sections. The method used for the negative staining procedure combined both surface spreading and negative staining so that a thin layer of membrane was obtained on the specimen grid. A Beam capsule was filled with

negative staining solution and 100 μliters of the Golgi fraction in sucrose were dropped gently onto the surface so that the membranes spread as a surface film while sucrose dispersed through the solution. After 60 sec, a carbon-coated formvar specimen grid was touched to the surface and, after removing excess stain with filter paper, the grid was allowed to dry in air.

For most studies, 1.5% sodium or potassium phosphotungstate, pH 7.0, was used since it provided the best contrast and definition of Golgi membranes. Other negative stains were tested including 1 to 2% sodium silicotungstate, pH 7.0, 1% ammonium molybdate, pH 6.7, and 1% uranyl acetate, pH 4.5. While sucrose in the Golgi sample facilitated spreading of stain, the addition of 0.002% bovine serum albumin aided uniform penetration of stain, particularly for higher resolution microscopy.

For ultrathin sections, the Golgi fraction was centrifuged at 15,000 rpm for 20 min and the pellet was covered with cold 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, and cut into blocks approximately 1 mm^3 in size which were allowed to fix for a further $2^1/_2$ hr at 4 °C. Blocks were rinsed at least three times in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide in veronal acetate buffer, pH 7.4, dehydrated in alcohol solutions of graded concentration, and embedded in Spurr low viscosity epoxy resin (Spurr, 1969). A slow polymerization procedure of 24 hr at 45 °C followed by 24 hr at 60 °C was found to improve the cutting properties of the resin compared to the polymerization method recommended by Spurr (1969). This embedding medium was preferred for the Golgi-rich fractions since it allowed rapid penetration of pelleted membranes due to its low viscosity and had low background density in the electron-microscope.

Liver Samples. Each liver slice taken for electron-microscopy was covered with cold 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, and cut into blocks, 1 mm^3 in size. Approximately 20 blocks were selected from different regions of the slice and these were fixed and dehydrated as described above. Impregnation of tissue was made through propylene oxide to Epon epoxy resin, according to Luft (1961).

Sections with silver interference color were cut with a diamond knife on a Porter-Blum ultramicrotome and transferred to 200 mesh copper grids. Contrast was enhanced with lead citrate and uranyl acetate. All samples were examined at 60 KV in a Philips EM 200 electron-microscope.

Results

Electron-microscopic examination of the Golgi apparatus from fractions isolated from rat liver and in liver sections, reveal platelike structures as the predominant component. Figs. 1 through 9 illustrate characteristic features of the Golgi apparatus which have been selected from micrographs obtained by examination of Golgi fractions from more than 80 normal rats.

Structure of the Golgi Apparatus in Sections of Liver

In sections of the normal rat liver, the Golgi apparatus presents a characteristic structure of smooth surface membranes arranged in stacks of parallel flattened sacs or cisternae, small vesicles and larger vacuoles (Fig. 1). The Golgi complex has both a forming and maturing face, polarized towards the plasma membrane. A stack of 3 or 4 cisternae are usually seen in the rat liver; each cisterna extends from 500 to 800 nm in length, while

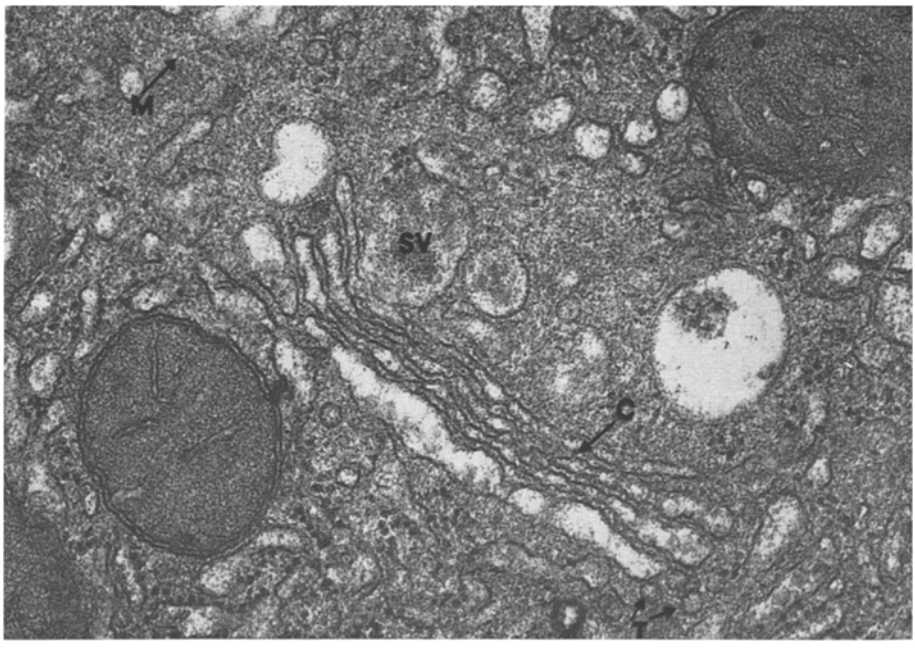

Fig. 1. Electron-micrograph of a section through rat hepatocyte. The Golgi complex consists of a series of parallel cisternae (*C*) with small vesicles (*T*) towards the periphery of the cisternae which correspond to the network of tubules. Secretory vesicles (*SV*) are also seen. The Golgi complex is isolated from other organelles by a zone of cytoplasm; microtubules (*M*) are frequently seen in this zone. 52,000 ×

the width varies according to the position in the stack. At the forming face, cisternae are 25 to 30 nm wide, roughly similar to adjacent cisternae of rough endoplasmic reticulum while at the maturing face, the cisternae are irregular and often distended. Cisternae in one stack are separated by a space of 15 to 20 nm.

Towards the edge of each plate, the cisternae tend to dilate so that their width approaches 50 nm. Small vesicles or tubules are found at the periphery of the cisternae; these appear spherical and have a diameter in the range of 40 to 80 nm. Larger oval or round vacuoles, localized mainly towards the maturing face, vary in diameter from 150 to 400 nm and contain some osmiophilic granules, 40 to 50 nm in diameter.

The membranes of the Golgi complex are separated from other intracellular membranes by a more densely granular zone of cytoplasm. Tubular structures are frequently observed towards the periphery of this zone oriented in a direction parallel to the long axis of the cisterna (Fig. 1). These resemble microtubules since they are long straight tubules, 20 to 25 nm in diameter with a hollow core.

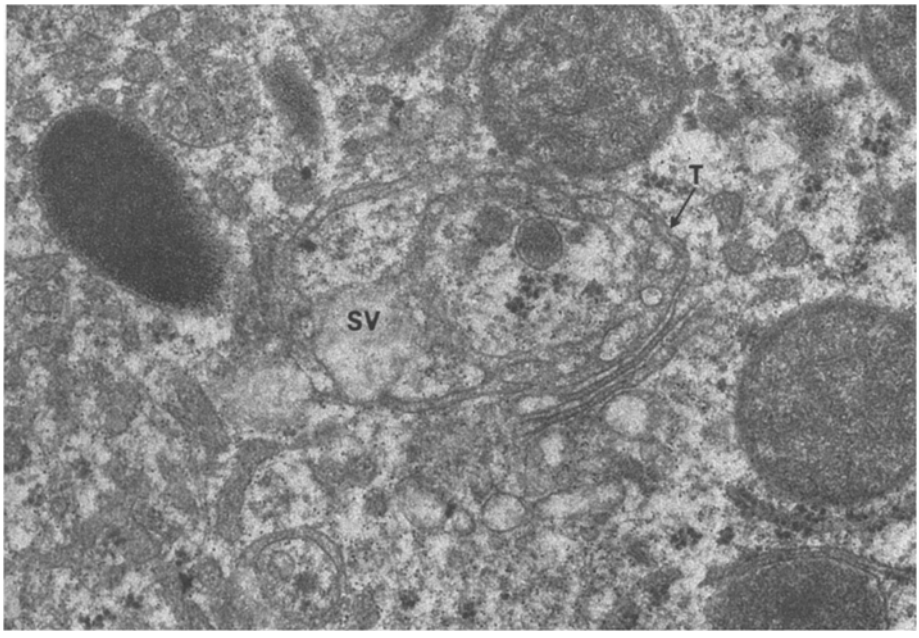

Fig. 2. Section through the Golgi apparatus of the rat hepatocyte. This plane of section has transected the Golgi plate and shows the network of tubules. Secretory vesicles (*SV*) arise from these tubules. 52,000 ×

In certain sections through the liver cell, the relationship between the different structures of the Golgi complex is revealed. Fig. 2 represents a rare plane of section which passes longitudinally through a plate of the Golgi complex. Although the central cisterna is not shown, the network of fine tubules and their circular arrangement are demonstrated. Large vacuoles, containing granules, are continuous with the peripheral tubules. This appearance resembles that seen in the isolated Golgi complex (compare Fig. 5).

Composition of the Isolated Golgi Fractions

The Golgi fractions isolated from rat liver consist of a series of platelike structures. Each plate is roughly 1 μ in diameter and consists of a central sac or cisterna, surrounded by a network of tubules and by vacuoles containing granules, referred to here as secretory vesicles (Fig. 3). Sections of the pelleted Golgi fractions contain smooth-surfaced membranes arranged in a form typical of the Golgi complex of rat liver with stacks of 3 or 4 parallel cisternae associated with small and large vesicles (Fig. 4).

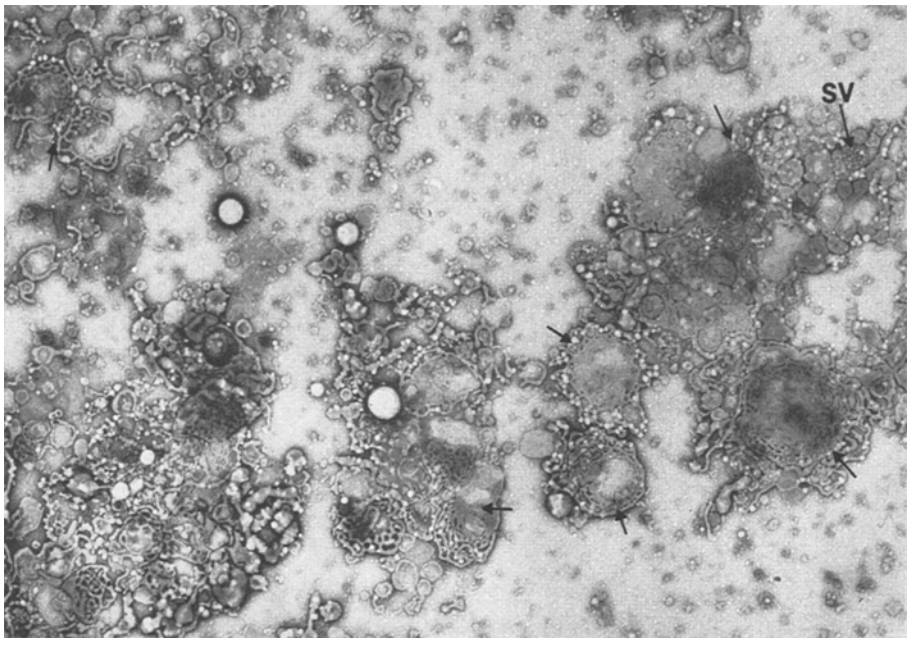

Fig. 3. Negatively stained preparation of the Golgi fraction isolated from rat liver. Numerous platelike structures (e.g., at arrows) are seen each with central cisterna surrounded by a fine tubular network. Secretory vesicles (*SV*) are associated with the peripheral tubules. 12,000 ×

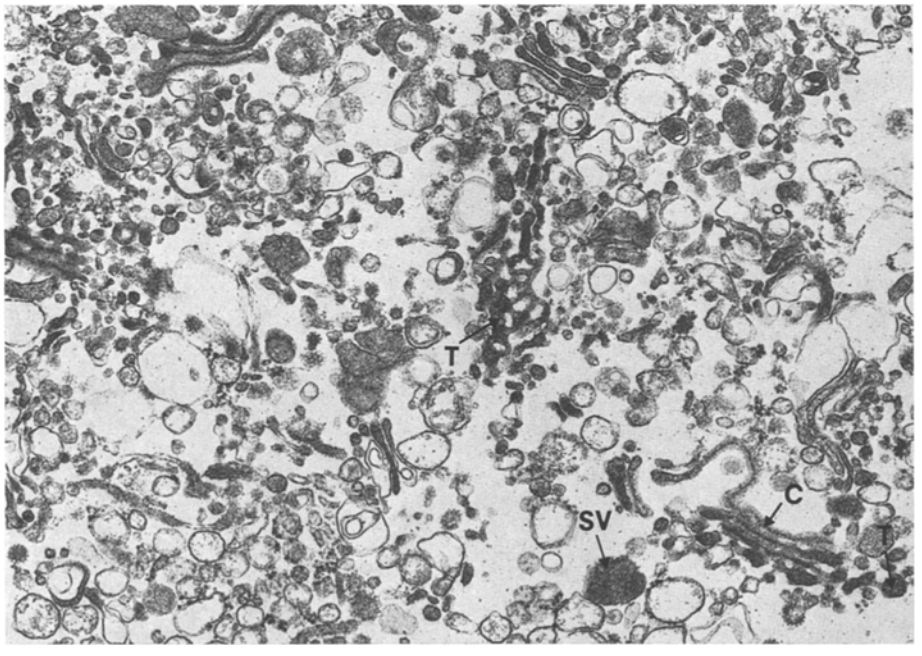

Fig. 4. Section through the pelleted Golgi fraction isolated from rat liver. The fraction mainly shows stacks of Golgi apparatus consisting of cisternae (*C*), tubules (*T*) seen in cross-section as small vesicles and occasionally as tubular networks. Secretory vesicles (*SV*) are associated with the peripheral tubules. 18,000 ×

Morphometric analysis using a point counting system (Weibel, Stäubli, Gnagi & Hess, 1969) on randomly sampled sections through isolated fractions revealed that 70% of membranes are typical of Golgi complex. Smooth-surfaced vesicles derived from damaged Golgi membranes and from smooth endoplasmic reticulum contributed approximately 20% of the fractions but contamination with rough endoplasmic reticulum was less than 10% and with mitochondria was minimal (<2%). Analysis of the different structures of the Golgi complex showed that the cisternae contribute roughly 20 to 25%, tubules 40 to 45%, and secretory vesicles 30% of the isolated Golgi complex.

Ultrastructure of Isolated Golgi Complex

In negatively stained preparations, the individual plates frequently appear separate but close together on the specimen grid (Fig. 5) with linkage between tubules of adjacent plates by means of smooth-surfaced tubules. The displacement of plates probably results from surface tension forces during the negative staining procedure.

The Golgi plates are usually either flat or slightly curved so that they appear with central round cisternae surrounded by an even layer of tubules.

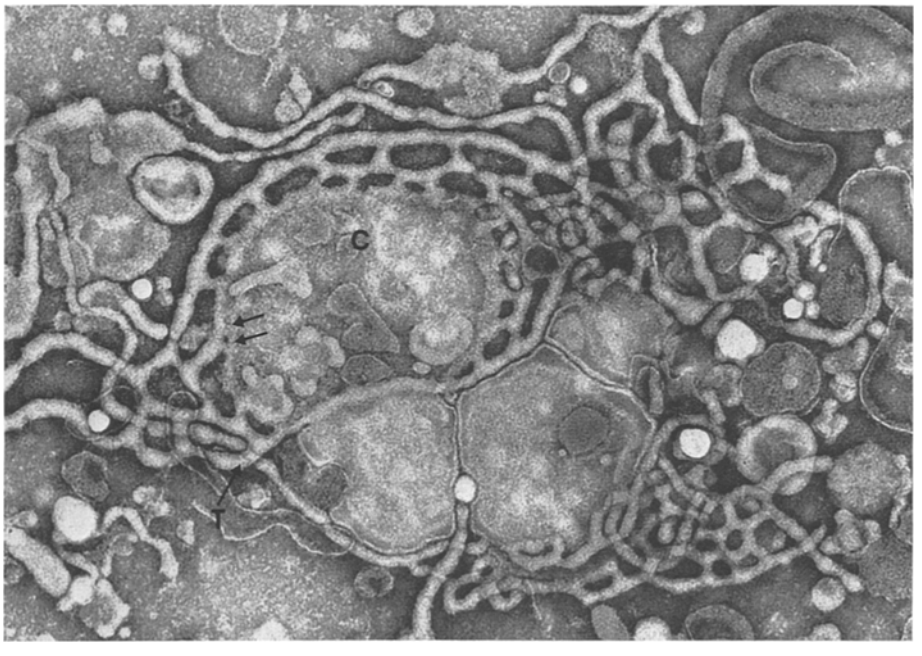

Fig. 5. Negatively stained preparation of the Golgi complex to show detailed structure of the central flattened cisternae (*C*) and its continuity with the tubules (*T*). In this preparation the individual plates of the Golgi complex are displaced relative to one another. Depressions at the periphery of the cisterna are shown at arrows. 50,000×

Occasionally, cisternae are more cup-shaped so that in negatively stained preparations the plate is folded and the tubules are seen only at one border of the cisternae.

Cisternae. The cisternae of the Golgi apparatus are flattened sacs which constitute the central portion of each plate. The diameter of a cisterna is usually in the range of 400 to 600 nm so that it occupies approximately half the overall plate diameter. While the structure of the cisterna appears homogenous in some plates (Fig. 5) higher resolution studies reveal differentiation of the cisterna into regions of varying electron density. The cisternae are flattened so that in section, they present an elongated profile roughly 30 nm wide and bounded by a double membrane, 70 Å thick. The intercisternal space is approximately 15 to 20 nm. The disc-shaped form and the continuity with tubular structures of the cisterna seen in negatively stained preparations is similar to certain planes of section through the liver which pass through the longitudinal axis of a cisterna (Fig. 2).

Tubules. Tubules are found at the periphery of each Golgi plate arising from between 10 and 30 points around the circumference of the cisterna. The tubules and cisternae appear to be related structures evidenced both by the continuity of the boundary membrane and by the similar electron density of these adjacent structures. Tubules are arranged in a regular network (Figs. 2–5) characterized by rows of similar diameter tubules arranged concentrically with the cisternal circumference and joined by tubules perpendicular to the circumference. In the normal liver cell, 1 to 3 rows of tubules are usually found which, together with the intertubular spaces, occupy a border roughly 200 nm wide. Smaller tubules are adjacent to the cisterna and widen towards the plate periphery. With increase in tubule diameter, the intertubular spaces also enlarge (Fig. 5). Towards the edge of the cisterna, minute depressions, 5 to 10 nm in diameter appear in the membrane; these widen and segregate tubular structures. The intertubular spaces enlarge to a diameter of 80 to 100 nm between the outer tubule rows. Negative stain accumulates in the intertubular spaces and either obliterates structural detail or reveals some granular or vesicular material (Fig. 6). The granular material has a lamellar form with alternate light and dark bands, 40 Å wide, typical of lipoprotein or phospholipid materials (Glauert, 1965). Occasionally, a single or a group of secretory granules bounded by membrane are seen in the interstices of tubules. The origin of these vesicles is not known.

Interconnection of plates in the Golgi apparatus exists through smooth-surfaced tubules of similar diameter to the tubule network, which join the outer tubules of adjacent plates. While the tubular network of one plate

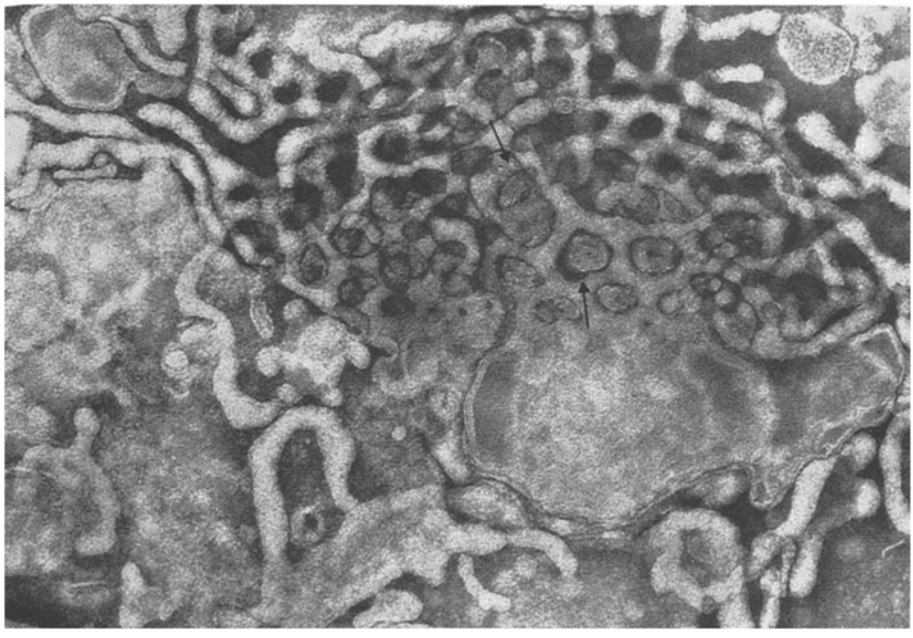

Fig. 6. Negatively stained preparation of isolated Golgi plate showing the detailed structure of the tubular network. Lamellar structures are seen in the interstices of the tubules (at arrows). 74,000 ×

usually remains discrete from tubules of other plates, a close relationship is found in some cases between the tubules evidenced by the interweaving of tubular networks between successive plates (Fig. 7). This suggests that some interaction or inter-relationship occurs between adjacent plates within the Golgi apparatus.

In sections through the Golgi fraction, tubules are found in association with the periphery of the cisternae (Fig. 4). The tubules appear as oval or spherical vesicles (40 to 80 nm in diameter) in cross-section with portions of the tubular network revealed occasionally. The continuity between cisternae and tubules has been confirmed in thin sections of liver as well as in isolated Golgi fractions.

Associated with the more peripheral tubules of each plate are fragments and lengths of smooth-surfaced tubules of the smooth endoplasmic reticulum. In contrast to the tubule network of the Golgi plates, these are wider, 80 nm, less uniform in diameter and in their branching pattern and frequently are distended with electron-lucent material. Interconnections between the two types of tubules are present at the periphery of the plates but these are less marked and more susceptible to breakage than the tubular branches of the

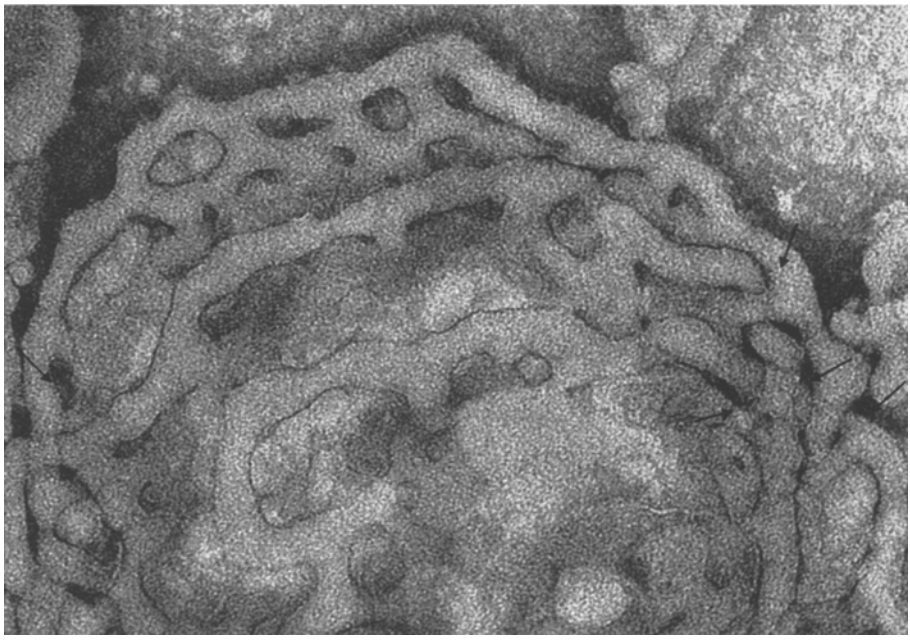

Fig. 7. Negatively stained preparation of portion of isolated Golgi complex. Three plates are superimposed and the tubules of successive plates appear to be interwoven (at arrows). 117,000 ×

Golgi plate. This is the most common site at which the Golgi plate becomes separated from other intracellular membranes. The outer tubules and the associated smooth endoplasmic reticulum contain individual membrane-bounded granules, 40 nm in diameter, which are highly electron lucent and presumably lipoprotein in nature (Hamilton *et al.*, 1967; Mahley *et al.*, 1969).

Secretory Vesicles. The term secretory vesicle has been used in this study to include all collections of particles or granules grouped together and bounded by membrane to form a discrete structure. Two main types of vesicles are observed in negatively stained preparations of the Golgi apparatus (Fig. 8). First, rounded or oval sacs, 300 to 400 nm in diameter are found associated with the more peripheral tubules: these contain diffuse, highly electron-lucent particles, and the membrane is not penetrated by negative stain. Similar electron-lucent granules in the outer tubules of the Golgi plate appear to have a spatial separation from the tubule membrane, suggesting that some granules may have a boundary membrane (Fig. 9). Second, vesicles of varying sizes, usually round or oval, in the range of 150 to 400 nm in diameter are found associated with the tubule network.

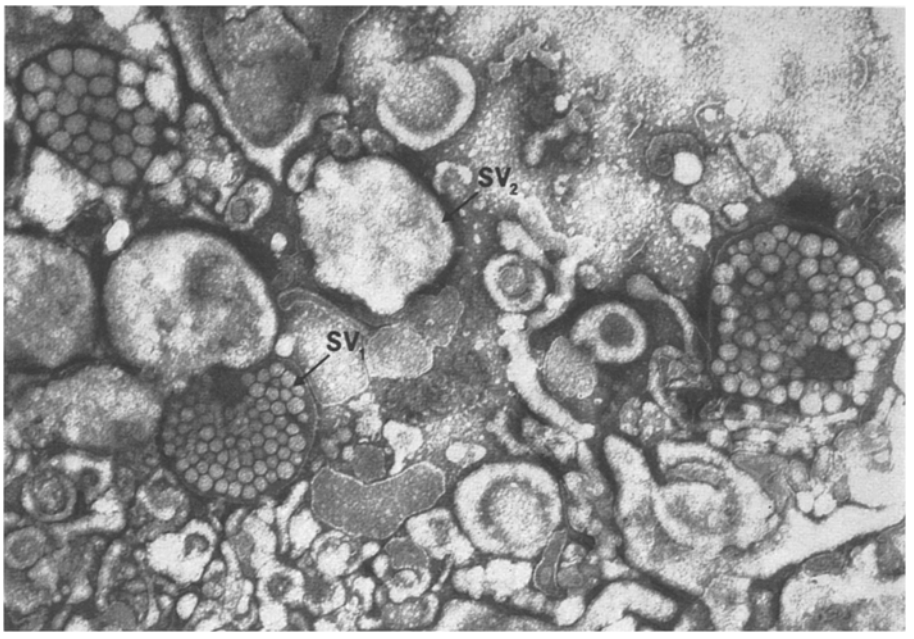

Fig. 8. Negatively stained preparation of isolated Golgi fraction showing secretory vesicles. Two types of vesicles are shown: (1) vesicle not penetrated by stain and containing diffuse electron-lucent granules (SV_1), and (2) vesicles penetrated by stain and with electron-lucent granules of varying size (SV_2). 50,000 ×

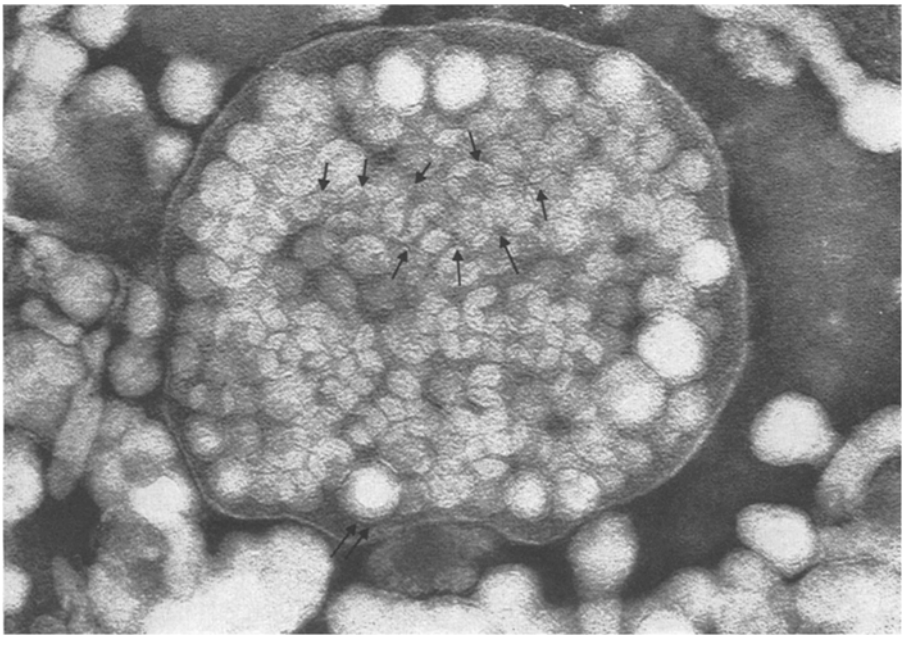

Fig. 9. Negatively stained secretory vesicles from the isolated Golgi fractions showing tubular orientation of granules. Note the wide variation of granule size within the vesicle. Some granules appear to have a boundary membrane (double arrow). 143,000 ×

These vesicles are always penetrated by the negative stain and the edge of the sac appears as an unstained band. Oval or spherical granules, 40 to 80 nm in diameter, are found within these vesicles. Even within one vesicle, the range of granule size is wide. Orientation of the particles is observed in the form of linear or tubular arrays (Fig. 9). A close relationship exists between the tubules and the secretory vesicles (Fig. 2), and direct continuity between these structures is observed rarely. This suggests that these vesicles originate from the Golgi tubules.

Abnormally large vesicles, up to 1 μ in diameter, are occasionally found. These consist of extensive folded areas of membrane similar to those of the secretory vesicles and contain clusters of granules. It seems likely that these large vesicles are probably derived from plates in which membrane has been damaged and separated. The incidence of secretory vesicles and particularly the large vesicles increases after mechanical homogenization or treatment of Golgi fractions with detergent which damages the peripheral tubules.

In sections, oval or rounded membrane-bounded vacuoles containing osmiophilic granules are associated with the tubules or the Golgi apparatus. Larger oval sacs, containing granules 40 to 80 nm in diameter are found towards the periphery of the Golgi plate and have a structural continuity with more peripheral tubules. In addition, collections of osmiophilic granules are found in rounded vesicles which originate directly from the tubules. These correspond to the two types of secretory vesicles observed in negatively stained preparations.

Discussion

The structure of the rat hepatic parenchymal cell has been reported in detail previously (Bruni & Porter, 1965) and has included the general description of the Golgi complex as a series of parallel or concentric cisternae continuous with a system of both small and large vesicles. More recent studies concerning the ultrastructure of the Golgi apparatus have been made from sections of the liver cell and these have been involved mainly with its role in lipid and lipoprotein secretion (Hamilton *et al.*, 1967; Claude, 1970).

In the mature rat liver, the membranes of the Golgi complex occupy approximately 2 to 3% of the cytoplasmic volume, that is roughly 12% of volume reported for the total endoplasmic reticulum, although at earlier stages of development the Golgi complex occupies a much greater proportion of the intracellular membranes, suggesting that it has a role in differentiation (Sturgess & de la Iglesia, 1972).

The Golgi complex has an important role in glycoprotein synthesis in the liver, contributing 95% of the total plasma glycoprotein fraction (Sar-

cione, 1962) and in packaging of proteins such as albumin (Ashley & Peters, 1969; Glaumann & Ericsson, 1970; Peters, Fleischer & Fleischer, 1971).

In the present study, methods for isolation of the Golgi complex have been modified to provide morphologically well-preserved membranes in a good yield. The structure of the Golgi complex has been characterized by negative staining and ultrathin sections of isolated Golgi membranes and related to the appearance in liver sections.

By negative staining, the Golgi apparatus is found to consist mainly of platelike structures which are either superimposed in stacks of 3 or 4 plates or arranged separately on the specimen grid. The latter arrangement has been attributed to an artifact of the negative staining procedure which displaces plates relative to one another. In the plant cell, the presence of intercisternal elements or fibers has been proposed to explain the binding of plates in the Golgi apparatus (Mollenhauer & Morré, 1966; Morré *et al.*, 1970). While no such structures have yet been identified in the rat liver cell, it seems likely that some binding between individual plates maintains the intercisternal spaces and enables the isolation of the complete stacked Golgi apparatus.

The Golgi plate consists of a central cisterna with a disc-like shape. In some plates the cisterna appaears homogeneous while in others the appearance of tubular and vesicular structures in the cisterna suggests that differentiation of structure occurs at some stage in development.

A network of tubules is continous with each cisterna; their arrangement and the progressive increase in diameter of both the tubules and the intertubular spaces suggests that these arise from membrane at the periphery of the cisternae.

Associated with peripheral tubules of the Golgi plate are tubules derived from the smooth endoplasmic reticulum. These contained material similar to the low density and very low density lipoproteins as reported previously, Hamilton *et al.*, 1967; Mahley *et al.*, 1969; Claude, 1970. The pathway of lipoprotein synthesis in the endoplasmic reticulum with formed granules appearing later in the Golgi apparatus was shown by Stein and Stein (1967) and confirmed by recent studies on subcellular fractions by Van Golde *et al.* (1971). Morphological studies of rat liver, (Claude, 1970) have shown lipoprotein first in rough endoplasmic reticulum, then in vesicles of smooth endoplasmic reticulum which fuse with peripheral fenestrated tubules of the Golgi apparatus to form secretory vesicles.

Evidence from the present study suggest that smooth endoplasmic reticulum is associated with peripheral Golgi tubules and shows occasional

connections. In contrast to the Golgi tubules, smooth endoplasmic reticulum has a greater tendency to vesiculate, is more irregular both in diameter and in branching pattern, and contains more lipoprotein material. Connection between the two types of tubules is tenuous and this is a common site at which Golgi plate is segregated from other membranes, suggesting that this linkage is more susceptible to homogenization than other parts of the Golgi complex. The smooth endoplasmic reticulum has been reported as more fragile than other intracellular membranes and rapidly disintegrated into chains of vesicles during shearing (Trump, Goldblatt & Stowell, 1962). This substantiates the view that the nature and perhaps the development of Golgi tubules and smooth endoplasmic reticulum is distinct.

The secretory vesicles of the Golgi fraction consist of two main types; one associated with smooth endoplasmic reticulum and containing lipid granules and the other associated with peripheral tubules. The irregular form and the penetration of stain into the latter type of vesicle suggests that they may originate from damaged tubules, particularly since mechanical homogenization and treatment of Golgi fractions with detergent results in an increased proportion of these vesicles. Granules within the vesicles tend to be arranged in a linear pattern. In section, corresponding vesicles were found to be continous with tubular structures which suggests that the vesicles represent cross-sections of dilated tubular structures.

Although the Golgi complex has been shown to have a major role in the synthesis of oligosaccharide chains of glycoprotein, the site of glycoprotein synthesis has not been established. Using isolated Golgi fractions, carbohydrates have been found to bind first to the membranes of the Golgi complex, where they are assembled by specific transferase enzymes onto the oligosaccharide chain before release as glycoprotein (Moscarello *et al.*, 1972; Sturgess, Mitranic & Moscarello, 1972).

In relationship to the structure of the Golgi complex, the tubules appear to be the most likely site for assembly of carbohydrates into the glycoprotein molecule with the newly synthesized glycoprotein stored or transported by the secretory vesicles. This has been suggested by studies on incorporation of radioactively labeled carbohydrates and from morphological changes in the Golgi complex. In electron-microscope autoradiographs of Golgi membranes, isolated from rat liver at different times after injection of *L*-(^{3}H) fucose, radioactivity was localized initially in the cisternae, appeared rapidly in tubules and later in secretory vesicles. The appearance of fucose in the tubules corresponded to the time of incorporation of fucose into glycoprotein (Moscarello, Minaker, Mitranic & Sturgess, 1973).

Indirect evidence for the tubules as the site of glycoprotein biosynthesis has been obtained from the proliferation of tubules associated with increased incorporation of carbohydrate in the Golgi apparatus (Sturgess, Katona & Moscarello, 1971). Although electron-microscope cytochemistry must be interpreted with caution, a recent study has indicated that more carbohydrate-rich material is associated with the small vesicles (tubules) than either cisternae or secretory vesicles in sections of intestinal cells, whereas lipid material tends to be concentrated in larger vesicles (Sage & Jersild, 1971). Since these small vesicles correspond to the network of tubules it seems likely that macromolecular carbohydrate materials pass through the tubules. The relationship between structural organization of the Golgi apparatus and glycoprotein synthesis are being further investigated both in the normal liver and in different experimental conditions.

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