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BIOCHEMICAL CHARACTERIZATION OF THE GOLGI COMPLEX OF MAMMALIAN CELLS

Becca Fleischer, Fernando Zambrano,* and Sidney Fleischer

Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

We have developed methods for the isolation of Golgi apparatus from a number of mammalian tissues. The Golgi is distinct both chemically and enzymatically from the other membranes of the cell. For both liver and kidney, galactosyltransferase has been found to be a useful marker enzyme for Golgi membranes. This enzyme is involved in the modification of glycoproteins during secretion. In addition to lipoproteins and glycoproteins, the Golgi apparatus of liver is involved in the secretion of albumin, a simple protein. It does not, however, take part in the synthesis of sphingomyelin, lecithin, or triglycerides which are present in the secreted lipoproteins. These lipids appear to be synthesized predominantly by the endoplasmic reticulum. In kidney, which is rich in glycolipids, 3′-phosphoadenosine 5′-phosphosulfate, an enzyme which converts cerebroside to sulfatide, is localized predominantly in the Golgi apparatus. Thus, Golgi functions to modify glycolipids as well as mucopolysaccharides and proteins. Sulfatide constitutes a significant fraction of the total lipid of both Golgi and plasma membranes of kidney. When ^{35}S-sulfate is injected into rats, it is incorporated first into the sulfatides of the Golgi apparatus and later appears in the sulfatides of the plasma membrane. The data are consistent with the view that sulfatides are formed in the Golgi apparatus of kidney and then transported to the plasma membrane.

INTRODUCTION

In highly differentiated cells such as are present in liver or kidney, many of the cell processes which we are interested in as biochemists are mediated by membranous organelles. These membranes form compartments in which the biosynthetic machinery of the cell is organized (Fig. 1). Much research effort in the past 20 years has been devoted to the isolation of these organelles in as pure a form as possible in order that they can be characterized both chemically and enzymatically. Such studies, together with metabolic and histochemical studies on whole cells and tissues, form the basis of our understanding of how cells function.

*Permanent address: Universidad de Chile, Facultad de Ciencias, Casilla 6635 Correo 4, Santiago, Chile

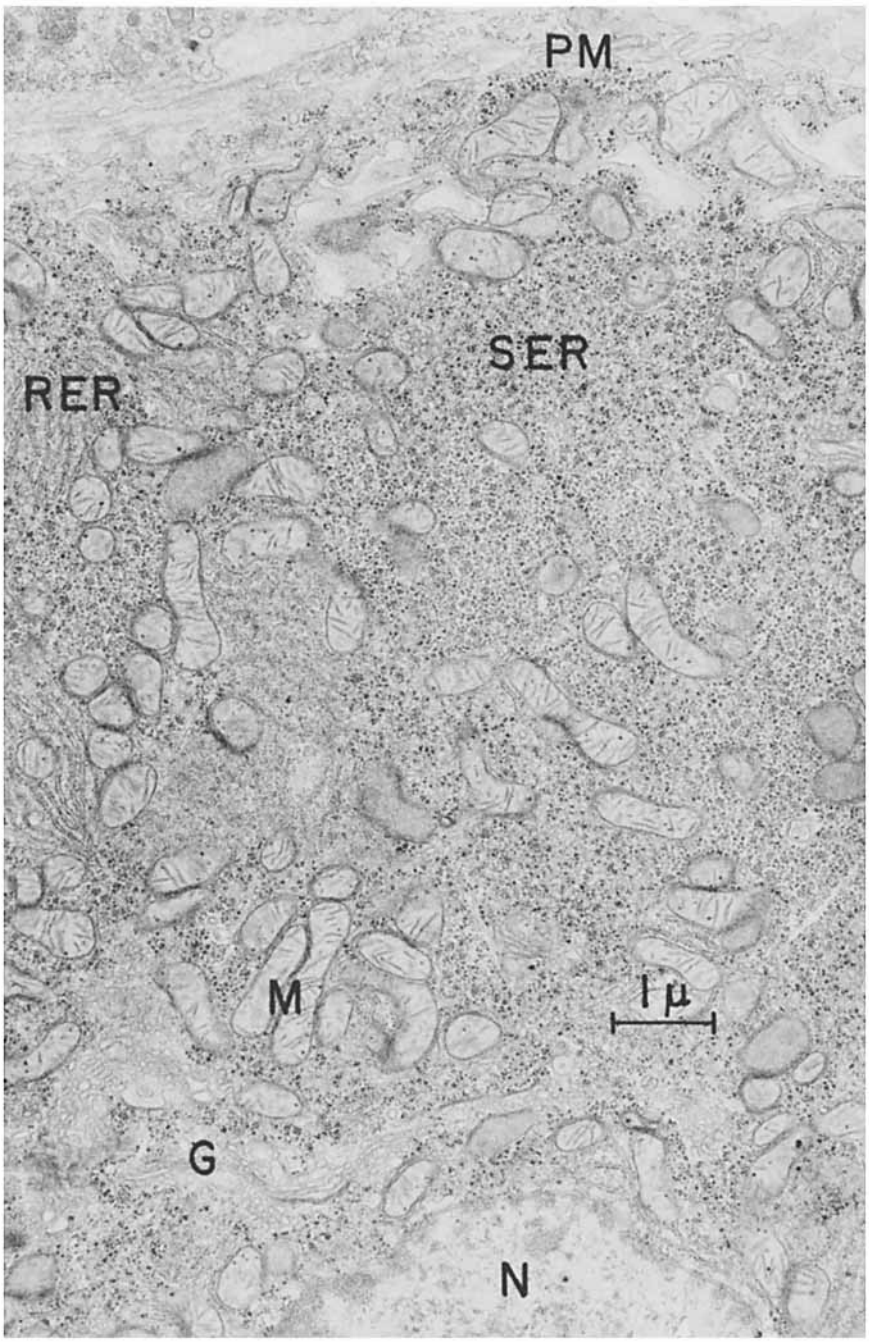

Fig. 1. Electron micrograph of a section of a rat liver cell (× 12,500). The organelles of the cell are designated N = nucleus, G = Golgi apparatus, M = mitochondria, RER = rough endoplasmic reticulum, SER = smooth endoplasmic reticulum, PM = plasma membrane.

The Golgi apparatus is present in the cytoplasm of most mammalian cells. Morphologically, it consists of a series of flattened, smooth membrane-bound cisternae usually surrounded by what appear in sections to be small veiscles and by large secretory vesicles. Even in the early days of its recognition as a cell organelle, the Golgi apparatus was known to be involved in the synthetic and secretory capacities of cells. (For a comprehensive review of the cytological and histochemical evidence pertaining to the structure and function of the Golgi apparatus, see Ref. 1.). Autoradiographic studies on the incorporation of radioactive leucine into proteins secreted by the pancreas (2), by liver (3), and by plasma cells (4), have shown that the Golgi apparatus mediates the intracellular transfer of newly synthesized proteins between the rough endoplasmic reticulum and the plasma membrane. In parenchymal cells of rat liver, the Golgi complex participates in the secretion of serum very low density lipoproteins (5, 6), and albumin, a simple protein (7, 8). Rough and smooth endoplasmic reticulum and Golgi apparatus thus form a complex network within the cell through which materials are passed until they finally exit through the plasma membrane, presumably by a kind of reverse pinocytosis. These membranes are thus functionally, and perhaps physically, contiguous.

ISOLATION OF GOLGI APPARATUS

We became interested in isolating the Golgi complex in the course of our studies on the other membranous cell organelles of liver. Since morphologically identifiable Golgi did not appear in any of our fractions, we concluded that, like endoplasmic reticulum, Golgi apparatus vesiculated under our usual conditions of homogenization and would probably be found as a minor constituent of the microsomal fraction. We therefore looked for an enzymic marker that would help us identify Golgi membranes in such a mixture. By means of autoradiography, LeBlond and his coworkers have shown that ^{3}H-galactose is preferentially incorporated into glycoproteins or mucopolysaccharides in the Golgi of most cells (9).

One of the most common terminal sequences of sugars found in serum glycoproteins, most of which are secreted by the liver, is sialic acid $\longrightarrow$ galactose $\longrightarrow$ N-acetylglucosamine attached to protein or to an inner core of sugars. The incorporation of these sugars into glycoproteins has been shown to proceed by the stepwise addition of sugars, each mediated by a specific glycosyltransferase (10). The reactions below illustrate the manner in which galactose is incorporated into glycoproteins.

$$\text{(1)}\quad \text{UDP–Gal} + \boxed{\text{Protein}}\text{–N–acetylglucosamine} \xrightarrow[\text{transferase}]{\text{galactosyl}} \text{UDP} + \boxed{\text{Protein}}\text{–N-acetylglucosamine–Gal}$$

$$\text{(2)}\quad \text{UDP–}{}^{14}\text{C–Gal} + \text{N-acetylgluosamine} \xrightarrow[\text{transferase}]{\text{galactosyl}} \text{UDP} + {}^{14}\text{C–N–acetyllactosamine}$$

TABLE I. Distribution of Protein and Galactosyltransferase After Centrifugation of Rat Liver Homogenate in a Sucrose Step-Gradient*

Fraction	Position in gradient (% sucrose)	Total protein (mg)	Galactosyltransferase Specific activity	Galactosyltransferase % total activity	NADH-cytochrome c reductase
Original homogenate (3.3 gm liver)	bottom (52%)	291	1.9	100	--
Gradient fraction					
1	interface (29%/33%)	0.51	235	22	0.196
2	interface (33%/36%)	0.42	129	10	0.419
3	interface (36%/38.7%)	0.55	109	11	0.994

*Galactosyltransferase is expressed as nmoles galactose transferred/mg protein/hr at 37°. NADH-cytochrome c reductase is expressed as μmoles cytochrome c reduced per min per mg protein at 32°. 4 μg rotenone were added to the assay before addition of the substrate.

Galactosyltransferase (reaction 1) was shown to be present in liver microsomes, and it was also shown that the enzyme could use free N-acetylgluocosamine as an acceptor for the galactose (reaction 2) to form lactosamine (11). Reaction 2 is the basis of our assay of this enzyme (12, 13). Since there was reason to suspect that galactosyltransferase might be localized in Golgi apparatus, we first isolated a subfraction of bovine liver microsomes which was highly enriched with respect to galactosyltransferase activity, and poor in enzymes known to be present in purified preparations of endoplasmic reticulum, mitochondria, and plasma membranes. In isolated cell organelles of bovine liver, there was an 80-fold concentration of galactosyltransferase in the presumed Golgi fraction as compared to the homogenate (12). There was appreciable activity in smooth microsomes while that in rough microsomes was small. This is generally an indication that the activity resides not in the membranes of endoplasmic reticulum but in a contaminant of the smooth fraction. Since little or no transferase activity was found in the isolated plasma membrane fraction, it seemed likely that this activity reflected the level of contamination of the smooth microsome fraction by membranes derived from the Golgi complex. The presumed Golgi fraction was distinct from plasma membrane in its low ATPase, 5′-nucleotidase, and galactosidase activities. Unlike endoplasmic reticulum it had a low glucose-6-phosphatase activity.

Our idea that we had indeed isolated Golgi membranes was strengthened by the unique morphology of this fraction. Unlike any other of our cell fractions, our presumed Golgi-rich fractions appeared in negative staining as large sacs with attached tubules. Thin sections of OsO_4-fixed and embedded samples also showed tubular profiles and large sacs (Fig. 2). The fraction also reacted heavily with OsO_4 on prolonged treatment, a characteristic property of Golgi apparatus in situ.

Although as biochemists we were convinced that we had indeed isolated Golgi membranes from liver, we set out to modify our methods to both improve our yields and to obtain a more intact preparation of the Golgi complex. The modification consisted primarily of fractionating the Golgi apparatus directly from a homogenate of rat liver using a sucrose step-gradient (13, 14). Analysis of the material in the upper three fractions is shown in Table I. About 20–25% of the galactosyltransferase activity of the homogenate

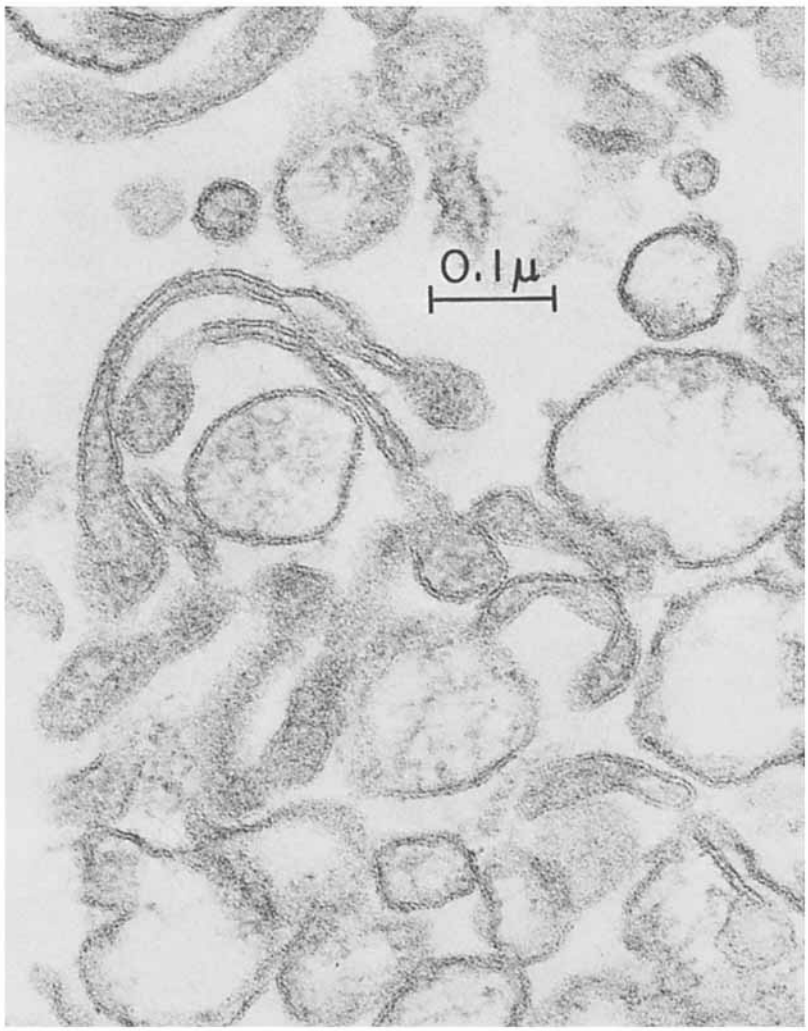

Fig. 2. Electron micrograph of a section of isolated Gogli vesicles from beef liver (× 160,000). The preparation consists of smooth-surfaced vesicles often associated with tubular profiles. Dense material appears to be present in the tubules whereas the sacs contain a light network of granular material.

is recovered in the fraction from the 29%/33% sucrose interface with about a 100-fold concentration over the activity of the homogenate. As judged by its content of various marker enzymes for other organelles, the fraction appeared to be 80–90% pure. In addition, there is a striking preservation of the structure of the Golgi apparatus during the isolation (Fig. 3A). A higher magnification illustrates the characteristic features of the Golgi apparatus from liver which are found in this preparation (Fig. 3B). It consists of 3 to 4 flattened cisternae arranged in parallel rows, a number of small vesicles surrounding the cisternae, which are probably cross sections of tubules attached to the

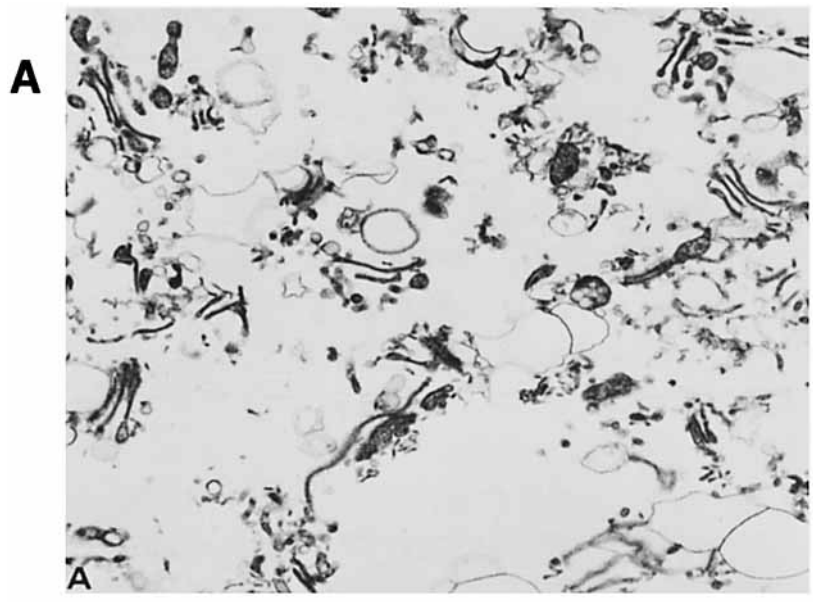

Fig. 3. (A) Electron micrographs of Golgi-rich fraction isolated from rat liver homogenate by centrifugation in a sucrose step-gradient (× 20,000). (B) Higher magnification of isolated Golgi apparatus from rat liver (× 64,000). The bars are equal to 0.5 μ.

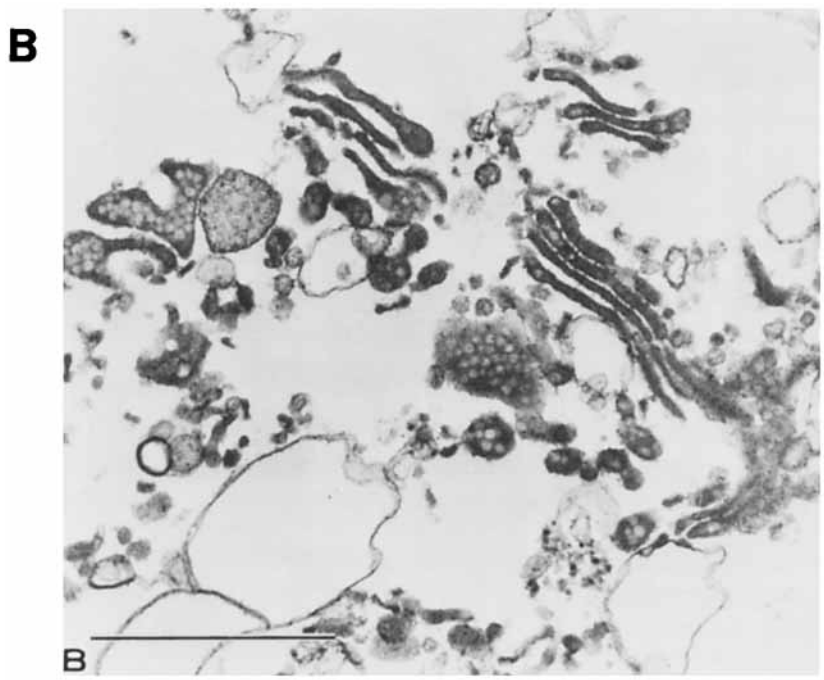

TABLE II. Lipid Content of Rat Liver Subcellular Fraction

Cell fraction	Mito	RER	Golgi	Golgi membrane	PM
Protein	1.0	1.0	1.0	1.0	1.0
NL	–	0.051	0.399	0.341	0.322
PL	–	0.374	0.649	0.825	0.672
Cholesterol	–	0.014	0.071	0.078	0.128
% free	–	–	64	86	92
% esterfied	–	–	36	14	8
Coenzyme Q	1.4	ND	5.1	6.8	0.2

All values are expressed as milligrams except the coenzyme Q which is in micrograms. ND = not detected; NL = neutral lipid; PL = phospholipid. Lipid was extracted from the fractions, using chloroform:methanol, 2:1 by volume, and was freed of nonlipid contaminants by Sephadex chromatography. The total lipid was fractionated into NL and PL by column chromatography using silicic acid (25). The amount of NL and PL was determined by drying and weighing an aliquot of the fraction. Cholesterol was determined in the NL fraction by the method of Searcy et al. (28). Free and esterified cholesterol were separated by thin-layer chromatography using a mixture of n-hexane:ethyl ether (7:3 by volume) asd estimated (28) after elution from the plates. Coenzyme Q in the NL fractions was determined from the optical density at 272 mμ minus that at 292 mμ of the oxidized vs. the reduced form.

cisternae, and large vesicles full of particles. These particles are thought to be the precursors of low density lipoproteins which are secreted by the Golgi apparatus of liver (5, 6).

We have done extensive studies on the chemistry and enzymology of the Golgi preparation from rat liver in comparison with the other membranous organelles of that cell. A comparison of the major protein bands after polyacrylamide gel electrophoresis of purified rat liver cell fractions showed that each organelle had a characteristic pattern (14). Rough and smooth microsomes were similar, but the Golgi fraction was different from both endoplasmic reticulum and from plasma membranes. The P_{450} and cytochrome b_5 content of our liver Golgi fractions have also been investigated (15). The P_{450} level was about that expected from the level of contamination of the Golgi preparations with endoplasmic reticulum. The cytochrome b_5 level, however, was higher than predicted from contamination with ER and thus is probably present in the Golgi membranes.

The lipid composition of the Golgi complex compared to those of rough endoplasmic reticulum (RER) and plasma membranes (PM) is shown in Table II. The Golgi complex has the highest content of neutral lipids of any of the fractions isolated. Removal of about 75% of the contents of the Golgi complex by treatment in a Parr bomb (13) decreases the neutral lipid content somewhat but the membrane preparation is still very rich in neutral lipids. As first reported by Nyquist et al. (16) the Golgi preparations from rat liver also contain coenzyme Q, whereas endoplasmic reticulum does not. The coenzyme Q is membrane-bound. Table III summarizes the distribution of phospholipids in the Golgi apparatus in comparison with other membranes of the rat liver cell. Except for its content of sphingomyelin, the Golgi complex most closely resembles ER membranes in its phospholipid composition. In sphingomyelin content and in its content of cholesterol, however, the Golgi complex seems to be intermediate between ER and plasma membranes. It is interesting to note that Golgi which has been disrupted to remove at least 75% of its contents (13) still has essentially the same phospholipid pattern as intact Golgi.

TABLE III. Phospholipid Composition of Rat Liver Subcellular Fractions

	Mito	RER	Golgi	Golgi membrane	PM
Origin	0.9	0.9	1.0	0.8	0.9
PE	34.6	21.8	23.5	19.6	23.3
PC	40.3	58.4	54.0	49.6	39.3
Sph	0.5	2.5	7.8	7.6	16.0
DPG	17.8	1.1	1.0	1.2	1.0
PI	4.6	10.1	8.6	12.2	7.7
PS	0.7	2.9	3.0	5.6	9.0
LPE	0.6	1.1	0.3	1.6	1.3
LPC	ND	0.5	0.4	1.4	1.0
PA	ND	0.8	0.4	0.4	0.5

All values expressed as % of total phospholipid phosphorus. PE = phosphatidylethanolamine; PC = phosphatidylcholine; Sph = sphingomyelin; DPG = diphosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidyl serine; LPE = lysophosphatidylethanolamine; LPC = lysophosphatidylcholine; PA = phosphatidic acid.

ROLE OF LIVER GOLGI IN SECRETION

The liver is active in the secretion of a large number of serum proteins, the most abundant of which is serum albumin. In collaboration with Theodore Peters, Jr., we have measured the amount of serum albumin present in Golgi apparatus as compared to rough and smooth microsomes of rat liver (7). The vesicles were disrupted using deoxycholate and the amount of serum albumin present was determined after precipitation with specific antibodies. Albumin was present in smooth and rough microsomes but was most abundant in the Golgi apparatus where it constituted 3–6% of the total protein. The Golgi vesicles can be freed of their contents and the membranes recovered using nitrogen decompression (Table IV): This treatment releases about 37% of the protein and 30% of the phosphorus present in the preparation. Galactosyltransferase remains bound to the membrane whereas serum albumin, presumably contained in the lumen of the vesicles, is released to the extent of 74%, The phosphorus released is probably a measure of the amount of serum lipoproteins also present in the lumen.

We have also followed the time course of the appearance of newly synthesized albumin in endoplasmic reticulum and Golgi apparatus of rat liver after pulse labeling with ^{14}C-leucine (7). Maximum levels of activity appeared at about 5 min in the rough endoplasmic reticulum, at 15 min in the smooth fraction, and at 20 min in the Golgi. Release of radioactive albumin into the blood began after about 15 min and proceeded steadily until 55 min concurrently with the decline in the Golgi fraction. These data support the view that albumin passes through the Golgi apparatus during the secretion process. Similar results were also reported independently by Glaumann and Ericsson (17). It is interesting to note that recent studies by Redman have shown that colchicine administered in vivo blocks the secretion of serum albumin by the liver in rats and that the block appears to be specifically in the release of albumin by the Golgi apparatus (18). This process is thought to involve fusion of Golgi vesicles with the plasma membrane. A similar effect of colchicine on the secretion of serum lipoproteins in rats has also been reported (19).

Since the Golgi apparatus of liver evidently takes part in the secretion of serum

TABLE IV. Recovery of Golgi Membranes after Disruption of Golgi Apparatus with a Parr Bomb

Component	Original	Pellet	Supernatant	Recovery
Total protein (mg)	25	13.7	7.5	85
μg P/mg protein	24.7	34.0	14.4	93
Galactosyltransferase	594	1059	157	105
Rat serum albumin (μg)	0.90	0.27	0.65	102

Galactosyltransferase is expressed as nmoles galactose transferred/hr/mg protein at 37°. μgP = micrograms total phosphorus. Rat serum albumin was determined by precipitation with specific antibody (7, 13).

lipoproteins, we investigated the possibility that the Golgi apparatus might be involved in the biosynthesis of lecithin, sphingomyelin, or triglycerides, the major lipid components of serum lipoproteins. Together with Dr. van Golde we studied the subcellular distribution of a number of enzymes known to be involved in the biosynthesis of lecithin and triglycerides in liver (20, 21). Table V summarizes briefly some of our findings. It can be seen that the Golgi fraction from rat liver is not capable of synthesizing lecithin, sphingomyelin, or triglycerides. The levels of activity found are within the range expected from our estimates of contamination of the preparation by endoplasmic reticulum. We obtained similar results with bovine liver fractions except that bovine liver Golgi are capable of acylating lysolecithin. These results confirm the in vivo work of Stein and Stein, who showed by means of autoradiography that labeled glycerol and palmitic acid are incorporated first in rough and smooth endoplasmic reticulum in rat liver and later appear in the Golgi apparatus (22). It should also be pointed out that lecithin and sphingomyelin are components of the membranes of both the Golgi complex and the plasma membrane. This means that these organelles must depend on the endoplasmic reticulum for their supply of new sphingomyelin and lecithin, as well as for new protein components.

TABLE V. Relative Specific Activities of Lipid Synthesizing Enzymes in Golgi Apparatus from Rat Liver

	Microsomes		Golgi	PM
Formation of lecithin	Smooth	Rough		
CDP-chol + diglyceride	100	64	9.6	6.1
Acyl-CoA + lysolec	100	48	5.6	–
N-Methylation	100	149	5.3	10.0
Formation of triglycerides				
Acyl-CoA + diglyceride	100	186	3.4	–
Formation of sphingomyelin				
CDP-choline + ceramide	100	110	4.1	9.9
% Contamination with smooth microsomes	–	–	8	9

The relative specific activity is expressed as the specific activity of that fraction divided by the specific activity of that enzyme in smooth microsomes × 100 (20, 21).

TABLE VI. Fractionation of Rat Kidney Homogenate in the Zonal Rotor

Fraction	Cerebroside sulfotransferase*	Galactosyl transferase*	Glucose-6 phosphatase	Ouabain-sensitive ATPase
1	28.0	579	.055	0.00
2	22.0	467	.077	0.05
3	12.1	347	.102	0.13
4	7.9	158	.151	0.26

*Activities expressed as nmoles sulfate or galactose transferred/hr/mg protein. All others in μmoles substrate hydrolyzed/min/mg protein. The fractions are from the upper portions of the gradient only. Fraction 1 was obtained by combining tubes 4–9, 31–34% sucrose; fraction 2 from tubes 10–11, 34–35% sucrose; fraction 3 from tubes 12–13, 36% sucrose; and fraction 4 from tubes 14–15, 36–37% sucrose.

ROLE OF KIDNEY GOLGI IN SULFATIDE FORMATION

We have recently become interested to see if our methods of preparation of the Golgi apparatus from liver could be applied to other tissues such as kidney or lung where little or nothing is known about the role of the Golgi apparatus. We also wished to investigate the possible role of the Golgi apparatus in glycosphingolipid formation, and kidney was particularly suited for such a study.

When we applied this procedure with some modification to the fractionation of rat kidney, we found a very interesting distribution of enzymatic activities in fractions obtained in the upper parts of the gradient between 30 and 37% sucrose (Table VI). There is a close correlation between the cerebroside sulfotransferase activity (the ability to transfer ^{35}S-sulfate from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to added mixed bovine brain cerebrosides to form sulfatides) and the galactosyltransferase activity of these fractions. There is no correlation between these activities and glucose-6-phosphatase activity, which is predominantly an endoplasmic reticulum activity, or with ouabain-sensitive ATPase, which is predominantly a plasma membrane activity. Figure 4A shows an electron micrograph of a typical field observed when fractions rich in cerebroside-sulfotransferase are combined, fixed in suspension in glutaraldehyde, stained, embedded, and sectioned (23, 24). About 70% of the field consists of morphologically identifiable Golgi apparatus. The major contaminants are very large smooth vesicles probably derived from plasma membranes. Figure 4B is a higher magnification of the predominant structure present in this fraction. It consists of a number of flattened cisternae with attached anastomizing tubules. In contrast to liver, secretory vesicles are not commonly seen in kidney Golgi preparations.

In order to verify that cerebroside sulfotransferase is localized predominantly in Golgi apparatus, we also prepared purified nuclei, mitochondria, rough and smooth microsomes, plasma membranes, and cell supernatant from rat kidney and measured a number of possible marker enzymes including galactosyltransferase and sulfotransferase in these fractions. Table VII summarizes the sulfotransferase and galactosyltransferase activities found for purified subcellular fractions from rat kidney. Both activities are enriched to about the same extent in the Golgi-rich fraction as compared to the homogenate. Little or no activity is present in any other fractions studied other than smooth microsomes. The activity of this heterogeneous fraction is probably due to the presence

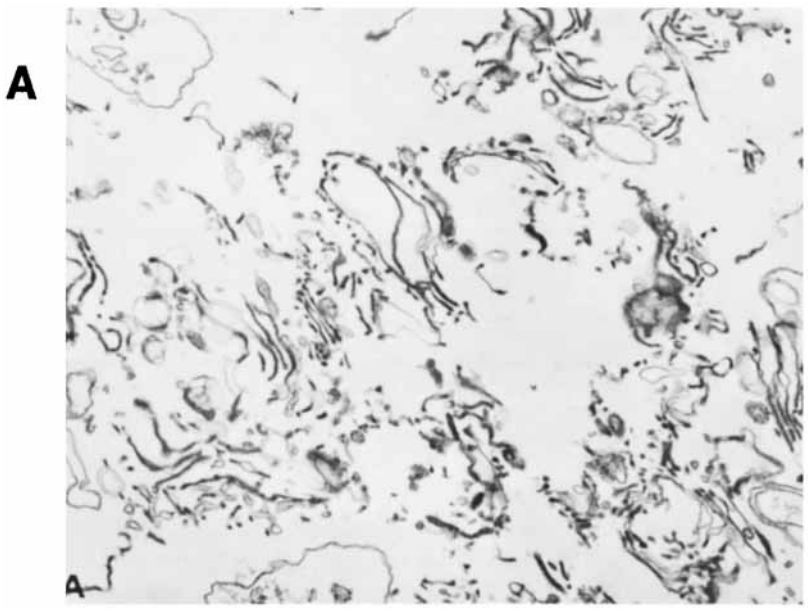

Fig. 4. (A) Electron micrographs of Golgi-rich fraction isolated from rat kidney by centrifugation in a sucrose step-gradient (× 20,000). The fraction is equivalent to combined fractions 1 + 2 described in Table VI. An average of about 70% morphologically identifiable Golgi was observed in a number of fields. (B) A higher magnification of the isolated kidney Golgi apparatus (× 64,000). The bars are equal to 0.5 μ.

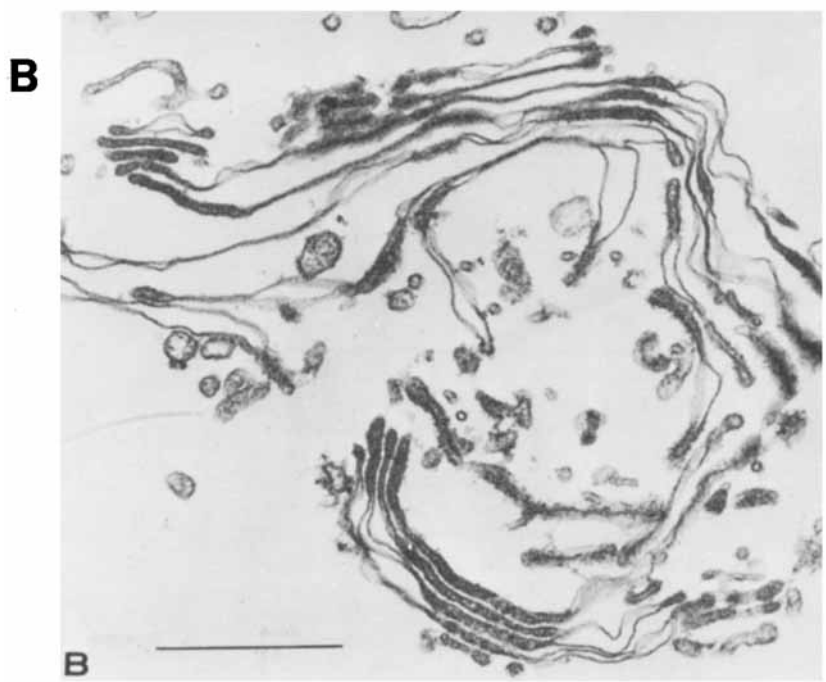

TABLE VII. Distribution of "Marker Enzymes" in Purified Subcellular Fractions of Rat Kidney

Fraction	Cerebroside sulfotransferase	Galactosyl transferase	Glucose-6 phosphatase	Ouabain-sensitive ATPase	Succinate-cyt. c reductase
Homogenate	0.31	10.9	0.055	0.05	0.27
Nuclei	0.19	3.4	0.103	–	0.04
Rough microsomes	0.39	8.2	0.191	0.00	0.01
Smooth microsomes	2.6	54.5	0.309	0.00	0.01
Golgi apparatus	25.6	553	0.077	0.03	0.05
Mitochondria	0.16	1.7	0.013	0.00	0.81
Plasma membranes	0.32	6.1	0.031	0.52	0.13
Supernatant	0.00	0.0	–	–	–

Enzyme activities expressed as in Table VI. Supernatant is the fraction of the homogenate which is not sedimentable at 104,000 × g for 1 hr. The Golgi apparatus fractions used were equivalent to combined fractions 1 + 2 shown in Fig. 4A and Table VI.

of Golgi membranes to the extent of about 8% of the protein. The Golgi apparatus fraction from rat kidney can be distinguished from endoplasmic reticulum by its low glucose-6-phosphatase activity, from plasma membranes by its low ouabain-sensitive ATPase activity, and from mitochondria by its low succinate-cytochrome c reductase activity. Isolated Golgi apparatus from rat liver, although very active in galactosyl-transferase activity, contains no detectable cerebroside sulfotransferase activity.

Although sulfatides are known to be present to a significant degree in kidney, little is known of the subcellular distribution of this lipid. We therefore determined the glycolipid and sulfatide content of our fractions (Table VIII). The lipid/protein ratio

TABLE VIII. Lipid Content of Rat Kidney Subcellular Fractions

	Mito	RER	Golgi	PM
NL	.040	.116	.162	.317
PL	.215	.395	.537	.858
GL	.012	.020	.147	.071
Sulfatide	.016	.022	.120	.055
Total Lipid	.267	.531	.848	1.25

All values expressed as mg/mg protein. NL = neutral lipid, PL = phospholipid, GL = glycolipid. Lipid was extracted from the fractions using chloroform: methanol, 2:1 by volume and was freed of non-lipid contaminants by chromatography on Sephadex. The total lipid was fractionated into NL, PL, and GL by column chromatography using silicic acid (25). The amount of lipid in the NL, PL, and total lipid fractions was determined by drying and weighing an aliquot of the fraction. The amount of glycolipid in the GL fraction was determined by analysis for sphingosine (26). Sulfatide was determined in the GL fraction by the method of Kean (27).

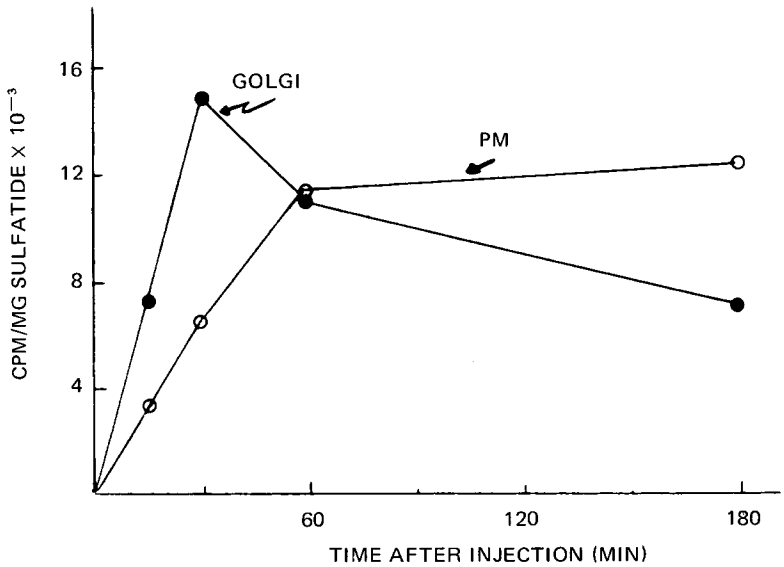

Fig. 5. Appearance of radioactive sulfatide in isolated cell fractions from kidney after i.v. injection of ^{35}S-sulfate into rats. The dose was 0.5 mCi/rat. Golgi-rich and plasma membrane-rich fractions were isolated from the same sample of kidney. Each point represents pooled kidneys from 2 rats.

varies from a low of 0.3 for mitochondria to about 1.1 for the plasma membrane fraction. These ratios reflect the amount of membrane present in these fractions. Kidney mitochondria have a large amount of matrix protein present, rough endoplasmic reticulum vesicles have attached ribosomes, and the Golgi apparatus probably has considerable amounts of secreted materials in its cisternae. The amount of phospholipid present is probably a good measure of the amount of membrane present in these preparations. On the basis of either protein or phospholipid, however, it is clear that the Golgi fraction is the richest in glycolipid and that sulfatide is the major glycolipid present. Sulfatide is also the major glycolipid in the other subcellular fractions (29).

If our results on the localization of the cerebroside sulfotransferase are correct, it follows that in vivo, sulfatide should be formed first by the Golgi apparatus and later transferred to the plasma membrane. We have recently looked at the rate of incorporation of radioactive sulfate into sulfatide of Golgi apparatus and of plasma membranes of kidneys after injection of the rats with a single i.v. pulse of ^{35}S-labeled inorganic sulfate. Figure 5 shows that, in vivo, sulfatide in the Golgi apparatus is labeled before that in plasma membranes. It is maximally labeled in about 30 min while the sulfatides of plasma membrane reach a maximum in about an hour. After 3 hours, the activity of the sulfatide in Golgi drops while that in the plasma membrane appears to plateau. These data are compatible with the idea that sulfatide is formed in vivo first in the Golgi apparatus and is then transferred, by some as yet undefined mechanism, to the plasma membrane. This is the first direct evidence for the synthesis of a plasma membrane component by the Golgi apparatus.

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