The roles of Golgi and endoplasmic reticulum in the synthesis and assembly of lipoprotein lipids in rat hepatocytes

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Abstract Microsomes, derived from the endoplasmic reticulum and Golgi fractions, were isolated from rat liver and separated into content and membrane subfractions by treatment with sodium carbonate or by passage through the French pressure cell. The microsomal content contained particles, which had a triglyceride molar concentration tenfold greater than that of phospholipid, cholesterol, and cholesteryl ester. In contrast, the Golgi content contained particles approximately 50% larger which contained proportionally less triglyceride. [^{3}H]palmitic acid was initially incorporated into a pool of triglyceride, associated with the microsomal membrane, subsequently sequestered in the cisternal space, and then transferred to the Golgi region. [^{3}H]palmitic acid was incorporated more slowly into phospholipid. The specific activity of the microsomal content phospholipid reached a level approximately twice that of the membrane, suggesting that newly synthesized phospholipid is preferentially sequestered. However, the specific activities of the Golgi membrane and content phospholipid were similar to that of the microsomal membrane. These observations suggest that the triglyceride to be secreted is sequestered into the cisternal space of the endoplasmic reticulum together with only small amounts of other lipids. The bulk of the phospholipid and cholesterol to be secreted is apparently added to the triglyceride-rich particles when these reach the Golgi cisternae.—**Higgins, J. A., and J. L. Hutson.** The roles of Golgi and endoplasmic reticulum in the synthesis and assembly of lipoprotein lipids in rat hepatocytes. *J. Lipid Res.* 1984. **25:** 1295–1305.

Supplementary key words membrane • particles • triglyceride • phospholipid • cholesterol • plasma lipoproteins

The liver is a major site of synthesis, assembly, and secretion of plasma very low density lipoproteins (VLDL) and high density lipoproteins (HDL) (1–3). The apoproteins are apparently synthesized by bound ribosomes and the lipid components are synthesized by enzymes located in the endoplasmic reticulum (4–6). Particles resembling secreted lipoproteins are observed in the cisternae of the smooth endoplasmic reticulum and close to the junction of rough and smooth membranes (4, 7–9). Morphological studies including radioautography have suggested that these particles move within membrane vesicles to the Golgi region, where they accumulate within the *trans* elements and the dilated ends of the *cis* elements (7–9). The *trans* vesicles, which are the hepatocytes equivalent of secretory granules, apparently move to the sinusoidal surface of the cell, where the vesicle membrane fuses with the plasma membrane, releasing the lipoprotein particles into the blood plasma at the space of Disse.

Although the events involved in the formation and secretion of plasma lipoproteins are understood in outline, many questions remain concerning the details of the processes. The mechanism of sequestration of lipids in the cisternal space after synthesis by enzymes located at the cytoplasmic side of the endoplasmic reticulum, the sequence of events in the packaging of lipoprotein components, and the site or sites of this assembly remain to be elucidated. In order to examine some of these questions, we have isolated endoplasmic reticulum and Golgi membrane fractions from liver and separated the vesicle membranes and their particulate contents for analysis. These experiments have indicated that, although triglyceride appears to be sequestered in the endoplasmic reticulum, much of the phospholipid and cholesterol found in secreted lipoproteins is not packaged with the triglyceride until this reaches the Golgi membranes.

METHODS

Isolation of cell fractions

Animals. For all investigations, male Wistar rats, 150–200 g body weight, were used. These were fed normally until they were killed which was always between 9 AM and 10 AM.

Microsomes were isolated from rat liver as described previously (10, 11). Livers were removed from male rats and homogenized (five or six strokes) in ice-cold 0.25 M sucrose to give a 20% weight volume homogenate

Abbreviations: VLDL, very low density lipoproteins; HDL, high density lipoproteins.

using a Potter-Elvejhem homogenizer. The homogenates were centrifuged at 12,500 rpm for 20 min in the 8 × 35 ml rotor of an MSE Pegasus DP ultracentrifuge. The supernatant was centrifuged at 40,000 rpm for 45 min in the same rotor. This method gave a yield of approximately 40% of the endoplasmic reticulum measured in terms of glucose-6-phosphatase and had less than 4% of the protein contributed by plasma membrane and less than 2% contributed by Golgi membranes, based on determination of 5′-nucleotidase and galactosyltransferase, respectively (11). The microsomes consisted of both rough and smooth vesicles. However, some vesicles of both types contained putative lipoprotein particles when examined in the electron microscope (11).

Golgi subfractions were isolated by a method based on that of Bergeron (12) which is a modification of that described by Ehrenreich et al. (13). Rat livers were homogenized in ice-cold 0.25 M sucrose, using a Potter-Elvejhem homogenizer, at a concentration of 20 g wet weight of liver to 100 ml final volume. The homogenate was centrifuged at 10,000 rpm (7500 *g*-avg) for 10 min in the 8 × 35 ml rotor of an MSE Pegasus DP ultracentrifuge. The pellet was resuspended in half the volume of 0.25 M sucrose and recentrifuged at the same speed. The supernatants were pooled and centrifuged at 40,000 rpm (150,000 *g*-avg) for 35 min in the 8 × 35 ml rotor. The pellets were resuspended, using the Potter-Elvejhem homogenizer, in 1.15 M sucrose at a concentration of less than 7 g of the starting weight of liver per 10 ml of final volume. Ten ml of the suspension was layered beneath 10 ml of 0.86 M sucrose and 12 ml of 0.25 M sucrose. The gradient was centrifuged at 24,000 rpm (85,000 *g*-avg) for 140 min in a 6 × 38 ml swing-out rotor. Bands formed at the 0.25 M/0.86 M interface and the 0.86 M/1.15 M interface. These were removed, diluted to 0.25 M sucrose, and isolated by centrifugation at 40,000 rpm (150,000 *g*-avg) for 45 min. The lighter fraction was enriched with *trans* elements on the basis of morphology in the electron microscope, and the heavier fraction was enriched with *cis* elements based on the same criterion. Details of the specific activities and distribution of enzyme markers in these fractions have been published elsewhere (14). The *trans*-enriched fraction was enriched 33-fold with galactosyltransferase compared with the homogenate and contained very low levels of 5′-nucleotidase and glucose-6-phosphatase. The *cis*-enriched fraction was enriched 14-fold with galactosyltransferase compared with the homogenate, but contained significant amounts of 5′-nucleotidase and glucose-6-phosphatase, suggesting at least some contamination of this fraction with plasma membrane and endoplasmic reticulum.

Separation of membranes and contents of isolated subfractions

Microsomes were opened by one of two methods. *a*) The pellets were resuspended in 100 mM sodium carbonate, pH 11.0, at concentrations of less than 1 mg of protein/ml and kept for at least 30 min at 0–4°C (15). In either case the suspensions were centrifuged at 40,000 rpm (150,000 *g*-avg) for 2 hr in the 8 × 35 ml or 8 × 14 ml rotor of the MSE Pegasus DP ultracentrifuge. The long centrifugation step was used to ensure separation of the pelleted membranes and the released contents. *b*) The pellets from the final centrifugation step were resuspended at a concentration of 3.5 mg/ml in 0.14 M sodium chloride adjusted to pH 7.4 with sodium bicarbonate and the suspension was passed twice through the French pressure cell at 20,000 lb/sq in (16).

Golgi subfractions were opened by method (*a*) above (14, 17, 18).

Incorporation of labeled precursors into cell fractions

All isotopically labeled precursors were administered by injection into the portal vein of rats anesthetized with Nembutol. The secretory protein contents of either microsomes or Golgi subfractions were labeled by injection of [^{14}C]- or [^{3}H]leucine (5 μCi/100 g body weight) 30 min prior to killing (15, 19). Membrane and content lipids were labeled by injection of [^{3}H]palmitic acid bound to 1% bovine serum albumin (2.5 μCi/100 g body weight) at a range of times prior to removal of the liver and isolation of cell fractions.

Electron microscopy

Microsomes, Golgi subfractions, and membrane fractions were fixed, embedded, sectioned, and stained as described previously (14, 20). Content fractions were examined after negative staining with 2% phosphotungstic acid, pH 6.4. The size distribution of particles was determined as described previously (21).

Lipid extraction and separation

Lipids were extracted from cell fractions in suspension using 20 volumes of chloroform–methanol 2:1, as described previously (10, 15) and from content fractions, which contained a small amount of lipid in a large volume of aqueous medium, by the method of Bligh and Dyer (22). Lipid extracts were taken to dryness under vacuum. Aliquots were taken for counting or analysis, or were separated by thin-layer chromatography (21, 23) using a solvent system containing petroleum ether–ether–glacial acetic acid 60:40:2 for separation of

neutral lipids or containing chloroform–methanol–glacial acetic acid–water 60:50:1:4 for separation of phospholipids.

Analytical methods

Phospholipids were determined by the method of Bartlett (24) using a modification described previously (15); cholesterol and cholesteryl esters were determined as described by Kates (25). Triglyceride, diglyceride, and monoglycerides were estimated as the esters by the method of Snyder and Stephens (26) after separation by thin-layer chromatography and elution of the lipid-containing areas with chloroform–methanol 1:1. Protein was determined by the method of Lowry et al. (27).

RESULTS

Separation of endoplasmic reticulum membrane and contents

Treatment of microsomes with sodium carbonate or two passages through the French pressure cell abolished the latency of mannose-6-phosphatase (**Table 1**). Approximately 50% of the total protein and more than 70% of the labeled protein contents were lost with either treatment. On these criteria, therefore, either procedure appears effective as a technique for releasing the microsomal contents.

Rat liver microsomes consist of small vesicles of mean diameter 100 nm (21). Many of the vesicles contain small particles similar to those observed in the endoplasmic reticulum cisternae in situ (11). Sodium carbonate-treated microsomes consisted of open sheets of membrane and large vacuoles lacking bound ribosomes (**Fig. 1**). The morphology of this preparation was similar to that described by Fujiki et al. (16), who reported sodium carbonate treatment as a procedure for opening microsomal vesicles. Ribosomes were also lost from French press-treated microsomes (**Fig. 2**). However, this treatment produced small flattened vesicles, suggesting that after the contents leak the vesicles reseal although they remain permeable to mannose-6-phosphate.

TABLE 1. Loss of components of microsomes on treatment with sodium carbonate or the French pressure cell

	Sodium Carbonate Treatment	French Pressure Cell Treatment
Loss of labeled proteins (%)	78.8 ± 3.5	71.3 ± 1.2
Loss of total proteins (%)	51.0 ± 5.3	48.9 ± 7.5
Latency of mannose-6-phosphatase[a]	0.5 ± 0.6	3.2 ± 1.4

Microsomes were treated with sodium carbonate or the French pressure cell as described in Methods. The membranes and contents were separated by centrifugation at 40,000 rpm for 2 hr; the lipids were extracted and analyzed as described in Methods.

[a] Mannose-6-phosphatase latency of the membrane fraction was determined as described previously (15). The results given are averages ± SD for four determinations.

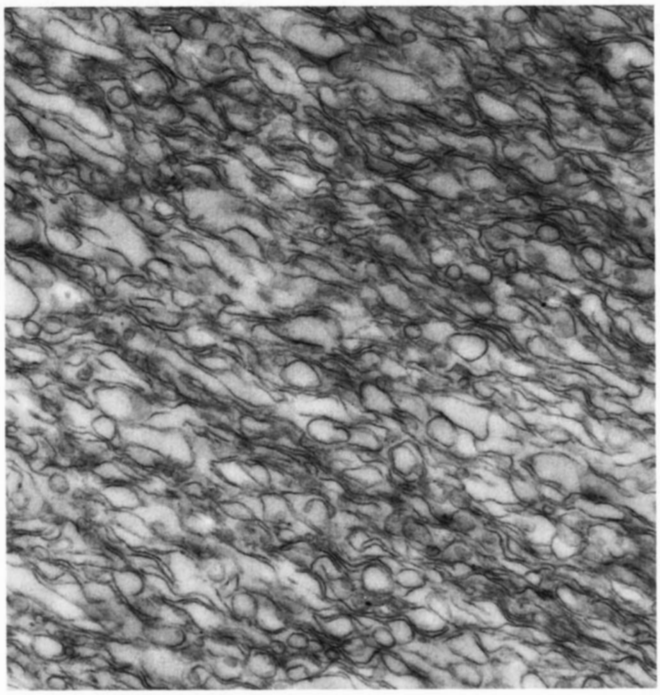

Fig. 1. Morphology of sodium carbonate-treated microsomes. Microsomes were isolated, treated with sodium carbonate, and prepared for electron microscopy as described in Methods. Ribosomes are lost from the membranes, which appear as sheets or large empty vacuoles (×44000).

Loss of lipid from sodium carbonate-treated microsomes

The lipids of untreated microsomes contained 0.08 mol of triglyceride, 0.016 mol of diglyceride, 0.11 mol of free cholesterol, and 0.17 mol of cholesteryl ester per mol of phospholipid with no detectable monoglyceride (**Table 2**). Phospholipid is therefore the major lipid and microsomes contain only low concentrations of neutral lipid. Similar compositions have been reported by others (28–30). Treatment of microsomes with sodium carbonate followed by centrifugation resulted in loss of 70% of the triglyceride, 16% of the diglyceride, 5% of the cholesterol, 9% of the cholesteryl ester, and 0.6% of the phospholipid. In control experiments microsomes were resuspended in 0.25 M sucrose in place of sodium carbonate. In this case no detectable phospholipid or triglyceride appeared in the content fraction. Repeated prolonged centrifugation of the supernatant did not pellet any of the lipid and repeated treatments of the membrane pellet with sodium carbonate did not remove

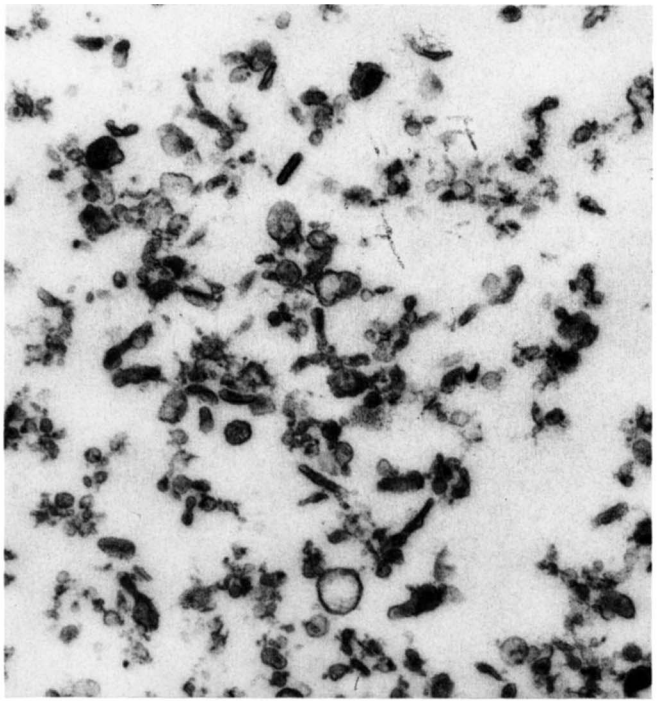

Fig. 2. Morphology of French pressure cell-treated microsomes. Microsomes were isolated, passed through the French pressure cell, and prepared for electron microscopy as described in Methods. Ribosomes are lost from the membranes, which appear as small vesicles many of which are flattened in profile (×44,000).

more lipid from the membrane. A significant amount of neutral lipid appears to be associated with the membrane. This is an unexpected finding as triglyceride and cholesteryl ester are not usually membrane bilayer components. The concentrations relative to phospholipid are low, however, and it is possible that the membrane-associated neutral lipid is newly synthesized and yet to be sequestered in the cisternal space.

The lipid of the content fraction contained 10 mol of triglyceride, 0.6 mol of diglyceride, 1 mol of cholesteryl ester, and 0.14 mol of cholesterol per mol of phospholipid. The mole ratio of triglyceride to phospholipid of VLDL isolated from rat Golgi membranes, rat plasma, or secreted by isolated hepatocytes range from 2.0 to 3.5 (18, 31–35). The reported cholesterol to phospholipid mole ratios and cholesteryl ester to phospholipid ratios of VLDL from these sources varies from 0.16 to 0.65 and 0.4 to 1.35, respectively (31–35). Thus, the triglyceride of the content fraction is three- to fivefold greater than that of VLDL while the cholesterol and cholesteryl ester content relative to phospholipid is of the same order as that of the VLDL.

Density of lipids released from microsomes by sodium carbonate treatment

The content fraction from sodium carbonate-treated microsomes was adjusted to pH 7.4 with HCl and to a density of 1.21 g/ml with sodium potassium bromide, and centrifuged at 115,000 *g* beneath a discontinuous gradient of steps of densities 1.006, 1.02, and 1.063 g/ml of potassium bromide for 20 hr. The triglyceride, phospholipid, and cholesteryl ester were distributed in a parallel way on the gradient with approximately 50% of each lipid in the density 1.006 g/ml layer and 30% in the density 1.02 g/ml layer (**Fig. 3**). In contrast, cholesterol was more evenly distributed. However, this lipid is at a low concentration in the content fraction and very small amounts are assayed in the gradient fractions.

The potassium bromide gradient was selected to compare the density of the content lipid with that of plasma lipoproteins, which may be separated on this gradient (36). VLDL moves to the top of the gradient and HDL remains at the top of the load layer. The content lipids therefore tend to behave like VLDL, although their composition differs from that of this class

TABLE 2. Loss of lipids from sodium carbonate-treated microsomes

		Composition (mol/mol of Phospholipid)		
	Lipid in Contents	Original Microsomes	Membranes	Content
	%			
Triglyceride	69.6 ± 7.9	0.082 ± 0.026	0.026 ± 0.007	9.76 ± 1.56
Diglyceride	15.9 ± 3.2	0.016 ± 0.004	0.0135 ± 0.002	0.60 ± 0.13
Free cholesterol	4.75 ± 2.87	0.11 ± 0.015	0.14 ± 0.05	0.14 ± 0.02
Cholesteryl ester	9.0 ± 4.85	0.17 ± 0.069	0.10 ± 0.03	0.95 ± 0.37
Phospholipid	0.65 ± 0.38			

Microsomes were isolated and treated with sodium carbonate as described in Methods. The lipids were extracted from aliquots of the original microsomes, the membrane fraction, and the content fraction, and analyzed as described in Methods. The % lipid in the content fraction and the mole ratio of lipid to phosholipid were determined. Results given are average ± SD for four determinations.

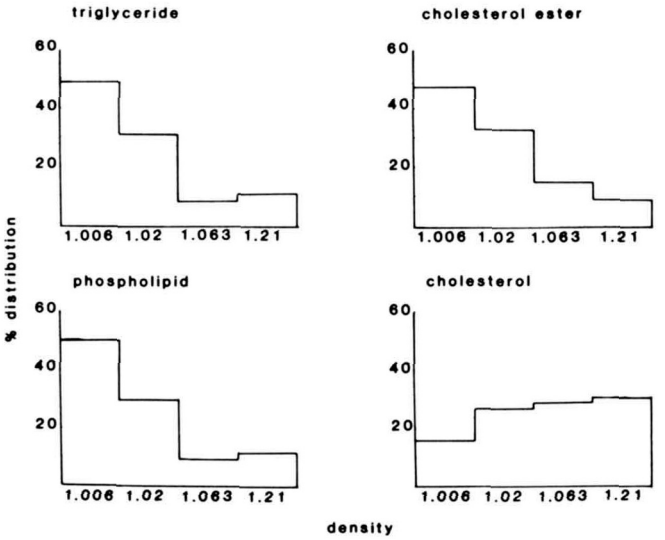

Fig. 3. Density distribution of lipids of microsomal contents released by sodium carbonate treatment. Microsomes were separated into membrane and content subfractions by sodium carbonate treatment as described in Methods. The pH of the content supernatant was adjusted to 7.4 and to a density of 1.21 g/ml with potassium bromide. Ten-ml aliquots of the content fraction were layered beneath 9-ml steps of density 1.063, 1.02, and 1.006 g/ml potassium bromide containing 10 mM EDTA. These were centrifuged at 115,000 *g* for 20 hr in the 8 × 38 ml swing-out rotor of the MSE Pegasus DP ultracentrifuge. The layers were removed, the lipid was extracted by the method of Bligh and Dyer (22) and separated by thin-layer chromatography, and individual lipids were determined as described in Methods. The % distribution of each lipid in the gradient is plotted against the density. Each value is the average of three experiments.

of lipoprotein. In a separate series of experiments the content fraction was adjusted in density 1.17 g/ml with sucrose and centrifuged for 34 hr beneath a sucrose gradient made up of steps of density 1.11 and 1.008 g/ml. The lipids also distributed on this gradient in a parallel fashion, confirming that the content lipid constituents are associated. These observations suggest that the lipid composition of microsomal contents is uniform and that these do not separate into classes comparable with VLDL and HDL isolated from plasma.

Morphology of content fractions

A thin turbid layer was apparent at the top of the potassium bromide gradient, while the remaining fractions were clear. Samples were taken of the turbid layer and of the density steps from the gradient and examined in the electron microscope after negative staining with phosphotungstic acid. The turbid layer contained many small spherical particles (**Fig. 4a**). These were mainly single, although profiles of two associated particles were seen frequently. The single particles had a size distribution of 10–34 nm with a peak diameter of 20 nm (**Fig. 5**). Particles of similar size and appearance were

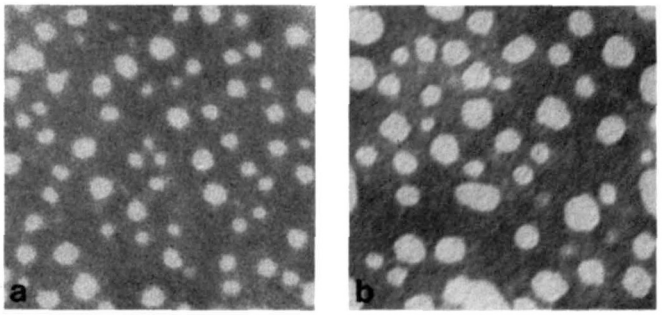

Fig. 4. Morphology of particles of density of 1.006 g/ml isolated from microsome and Golgi contents. Microsomes and *trans*-enriched Golgi subfractions were isolated as described in Methods. These were separated into membranes and content subfractions using sodium carbonate and the contents were layered on potassium bromide gradients as in the legend to Fig. 3. The floating lipid from the top of the density 1.006 g/ml layer was removed and examined in the electron microscope after negative staining with 2% phosphotungstic acid, pH 6.4. (a), Microsome-derived particles; (b), Golgi-derived particles (×138,000).

also observed in the layers of density 1.006 and 1.021 g/ml, although the concentration was low. The denser layers from the gradient contained no comparable particles, although the load layer contained smaller particles consistent with the presence of protein molecules.

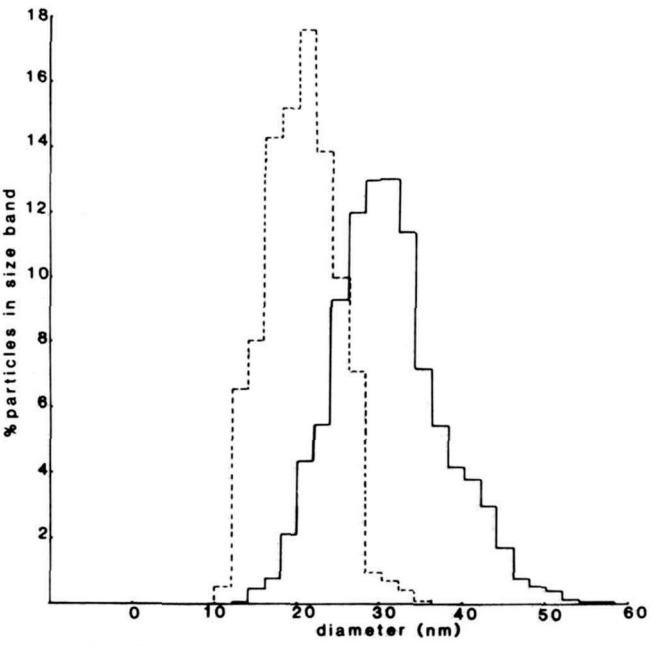

Fig. 5. Size distribution of particles of density 1.006 g/ml isolated from microsomes and Golgi fractions. Particles were isolated from microsomes and *trans*-enriched Golgi subfractions and examined in the electron microscope as described in the legend to Fig. 4. The size distribution was determined as described in Methods. The diameters of 600 particles were determined in 2-nm steps using micrographs of two preparations. The particles within each size band as a percentage of the total counted are plotted against diameter; – – – microsomal particles; —— Golgi particles.

Loss of lipid from French pressure cell-treated microsomes

The French pressure cell is an effective method for opening microsomal vesicles (Table 1). This treatment resulted in loss of 64.76 ± 7.0% of the triglyceride of the microsomes measured in four experiments. Loss of phospholipid was 2.97 ± 0.9% however, considerably greater than the phospholipid of the content fraction of sodium carbonate-treated microsomes. In view of the morphology of French pressure cell-treated microsomes, it is possible that small vesicles which did not pellet on centrifugation could account for some of the phospholipid in the content fraction of microsomes treated in this way. Consistent with this, when the contents of French pressure cell-treated microsomes were centrifuged on a discontinuous potassium bromide gradient, the distribution of triglyceride was the same as that found for the content of sodium carbonate-treated microsomes. The phospholipid showed a different distribution, however, with 75% remaining in the denser layers of 1.063 and 1.21 g/ml. The mole ratio of triglyceride to phospholipid of the fraction of density 1.006 g/ml was 10. This fraction contained particles similar in size and appearance to those found in preparations from sodium carbonate-treated microsomes.

Sodium carbonate or French pressure cell treatment of microsomes produce different morphological results and would be expected to cause different perturbation, if any, of the membranes or released contents. However, the lightest fractions on the potassium bromide gradient were similar in density, composition, and appearance of either method of opening microsomal membranes. The French pressure cell has the disadvantage that membrane fragments apparently remain in the content fraction. This limits the usefulness of this method for investigations of the assembly of lipoproteins. However, these results are presented here because they are consistent with the observations using sodium carbonate to open the microsomal vesicles that the bulk of the triglyceride in the content fraction is in a component which contains very little phospholipid.

Sodium carbonate treatment of Golgi preparations

Sodium carbonate treatment of *trans*-enriched Golgi subfractions resulted in loss of 66% of the labeled triglyceride contents and 82% of the labeled protein contents (**Table 3**). In the electron microscope, sodium carbonate-treated Golgi fractions appeared as flat sheets of membrane comparable with those reported by Howell and Palade (17) for Golgi preparations treated in this way.

Seventy-seven percent of the triglyceride, 50% of the diglyceride, 30% of the free cholesterol, 54% of the cholesteryl ester, and 20% of the phospholipid were lost from sodium carbonate-treated Golgi preparations (**Table 4**). The composition of the content fraction was 0.99 mol of triglyceride, 0.24 mol of diglyceride, 0.45 mol of cholesterol, and 1.1 mol of cholesteryl ester per mol of phospholipid. Thus, the Golgi contents differ from those released from microsomes and contain proportionally less triglyceride. As with the microsomes, a fraction of the triglyceride remained associated with the Golgi membranes. This was not removed by repeated treatment with sodium carbonate.

TABLE 3. Loss of content fractions on treatment with sodium carbonate

	Trans Fraction	*Cis* Fraction
Loss of total protein (%)	33.9 ± 5.0	52.8 ± 3.9
Loss of labeled protein content (%)	82.4 ± 1.4	74.5 ± 0.4
Loss of labeled triglyceride content (%)	65.8 ± 1.6	49.0 ± 2.2

Golgi subfractions enriched with *trans* elements and with *cis* elements were prepared as described in Methods. These were resuspended in 100 mM sodium carbonate, pH 11.0, at a concentration of less than 1 mg of protein/ml. The samples were left overnight at 4°C and centrifuged at 40,000 rpm for 2 hr to separate the content and membrane fractions. The % loss of protein, % loss of labeled protein, and % loss of labeled triglyceride were determined as described in Methods. Results given are average ± SD of four determinations.

In contrast to the endoplasmic reticulum contents, the *trans*-enriched Golgi contents distributed heterogeneously on a potassium bromide gradient. Triglyceride and cholesteryl esters tended to be at higher concentrations in the light fractions, while phospholipids and cholesterol were more evenly distributed. The composition of the $d < 1.006$ g/ml fraction was 52.9% triglyceride, 23.2% phospholipid, 21.2% cholesteryl ester, and 6.1% cholesterol; that of the $d > 1.006 < 1.02$ g/ml fraction was 17.6% triglyceride, 29.8% phospholipid, 40.5% cholesteryl esters, and 12.2% cholesterol; that of the $d > 1.02 < 1.063$ g/ml fraction was 35.2% triglyceride, 35.3% phospholipid, 21.3% cholesteryl ester, and 8.0% cholesterol; and that of the $d > 1.21$ g/ml fraction was 36.4% triglyceride, 46.6% phospholipid, 2.6% cholesteryl ester, and 46.6% cholesterol. A similar heterogeneous distribution of lipids of Golgi contents released by sodium carbonate (18) or by the French pressure cell (35) has been observed by others. The compositions of the fractions of different density are comparable with those reported by Dolphin (35) from similar investigations. Dolphin (35) and Howell and Palade (18) have demonstrated that Golgi content fractions also contain the appropriate apoproteins relative to their density. In our investigations only lipids were investigated. However, it is clear that the lipid compositions of the Golgi content fractions differ from those of the endoplasmic reticulum content and that the Golgi content lipids do not separate

TABLE 4. Loss of lipids from sodium carbonate-treated Golgi fractions

	% Lipid in Content	Composition (mol/mol of Phospholipid)		
		Original Golgi Fraction	Membranes	Contents
Trans fraction				
Triglyceride	76.56 ± 6.27	0.54 ± 0.09	0.32 ± 0.064	0.99 ± 0.18
Diglyceride	50.00 ± 4.32	0.156 ± 0.02	0.099 ± 0.02	0.24 ± 0.03
Free cholesterol	30.33 ± 6.15	0.42 ± 0.24	0.33 ± 0.14	0.45 ± 0.18
Cholesteryl ester	54.33 ± 2.31	0.40 ± 0.24	0.32 ± 0.04	1.1 ± 0.48
Phospholipid	19.47 ± 5.49			
Cis fraction				
Triglyceride	43.85 ± 6.83	0.17 ± 0.07	0.096 ± 0.02	1.01 ± 0.13
Diglyceride	48.4 ± 5.81	0.07 ± 0.01	0.027 ± 0.01	0.23 ± 0.01
Free cholesterol	3.00 ± 1.73	0.32 ± 0.11	0.40 ± 0.06	0.48 ± 0.4
Cholesteryl ester	33.33 ± 7.61	0.14 ± 0.08	0.08 ± 0.05	0.68 ± 0.2
Phospholipid	3.88 ± 2.72			

Golgi subfractions were prepared as described in Methods and treated with sodium carbonate as described in Table 3. Lipids were extracted from aliquots of the original Golgi fractions, the membrane fraction, and the content fraction, and analyzed as described in Methods. The % lipid in the content and the mole ratios of each lipid to phospholipid were determined. Results given are averages ± SD for four determinations.

clearly into fractions corresponding to HDL or VLDL of plasma.

The fraction of density 1.006 g/ml from Golgi contents has a size distribution of 18–50 nm with a peak of 32 nm (Fig. 4b, Fig. 5) and therefore differs from particles released from the microsomal fraction measured under similar conditions.

A similar series of experiments was performed using the Golgi subfraction enriched with lamellae *cis* elements. A proportionally lower amount of lipid was lost into the supernatant on sodium carbonate treatment compared with *trans* secretory elements (Tables 4, 5). However, the morphology of the membrane fraction, the lipid composition, density distribution, and appearance of the particles released were similar to those released from the fractions enriched with *trans* elements.

Incorporation of [^{3}H]palmitic acid into microsomal and Golgi triglyceride

[^{3}H]palmitic acid bound to albumin was injected intraportally and was rapidly incorporated into triglyceride of microsomal and Golgi fractions. The specific activity of the triglyceride associated with the microsomal membrane rose rapidly to peak at 1 min after injection of the labeled fatty acid followed by an increase in the specific activity of the triglyceride of the content fraction which peaked at 5 min after injection (**Fig. 6**). The activity of both microsomal pools of triglyceride fell, while the specific activity of the Golgi triglyceride pools rose. In contrast to the microsomal triglyceride, the specific activity of the Golgi membrane and content were similar at all times investigated (Fig. 6).

During isolation of microsomes from rat liver a variable amount of material, presumed to be cytoplasmic triglyceride (37, 38), floats to the surface of the centrifuge tube. The specific activity of the triglyceride of this fraction rose slowly and was only a fraction of that of the membrane and content triglyceride at all times investigated (Fig. 6). It is unlikely, therefore, that the floating lipid contributes to the content fraction or to the specific activity of its triglyceride.

[^{3}H]palmitic acid is preferentially incorporated into triglyceride of liver, however the labeled fatty acid was also incorporated into the phospholipids (**Fig. 7**). The specific activity of the microsomal membrane phospholipid rose initially. However, except at early times, the specific activity of the content phospholipid was greater than that of the membrane, suggesting that the small amount of phospholipid in the content is enriched with newly synthesized phospholipid. The specific activities of both Golgi membrane and content subfractions reached a level similar to that of the microsomal membrane phospholipid. These observations suggest that the microsomal membrane is a precursor of both Golgi membrane and content phospholipid and that in the Golgi elements the specific activity of the content is diluted by the addition of phospholipid from the Golgi membranes.

Incorporation of [^{3}H]palmitic acid into the membrane and content subfractions of the *cis*-enriched Golgi fraction was also investigated. At all times the specific activities of the phospholipid and triglyceride of these fractions were similar to those of the *trans*-enriched Golgi subfractions. These data are not included in Figs. 6 and 7.

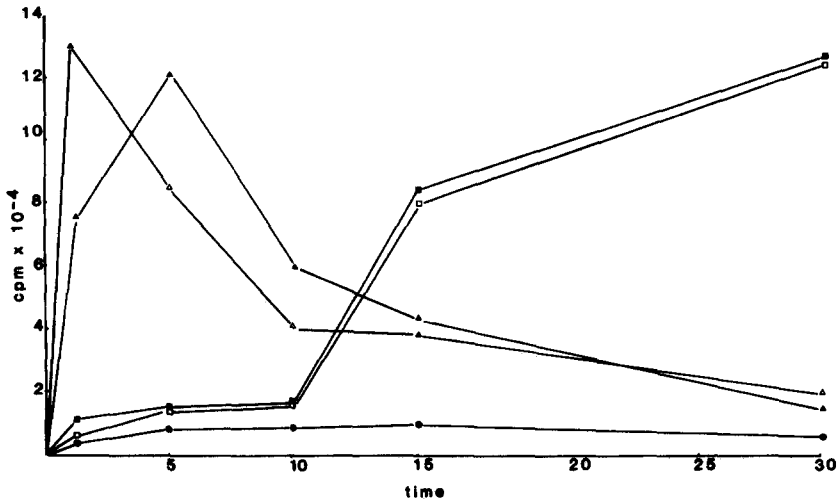

Fig. 6. Incorporation of [^{3}H]palmitic acid in triglyceride of subfractions of rat liver. [^{3}H]palmitic acid bound to 1% albumin was injected into the portal vein (5 μCi/150 g body weight). Livers were removed at intervals after injection and microsomal membrane and content subfractions of *trans*-enriched Golgi membrane and content subfractions were isolated as described in Methods. The lipids were extracted, separated by thin-layer chromatography, and the specific activities of the triglyceride fractions were determined as described in Methods. The floating turbid layer which forms at the top of the centrifuge tube after centrifugation of the total microsomal pellet was also collected, extracted, and the specific activity of the triglyceride was determined. Points plotted are the averages of three experiments performed in duplicate. (▲), Microsomal content triglyceride; (△), microsomal membrane triglyceride; (■), Golgi content triglyceride; (□), Golgi membrane triglyceride; (●), floating turbid layer triglyceride.

DISCUSSION

Rat hepatocytes secrete both VLDL and HDL (1–3). Both forms of lipoprotein, characterized by flotation density and apoprotein content, have been isolated from the incubation medium of cultured hepatocytes (34). There have been a number of investigations of the nature of Golgi contents from normal and hypercholesterolemic rat liver (18, 33, 35, 39, 40). These investigators have used sodium carbonate, osmotic shock and the French pressure cell, or ultrasonic treatment to open isolated Golgi fraction. Although there are differences in the size and composition of the particles released by these treatments, they have all indicated that Golgi preparations contain VLDL. It is clear, therefore, that complete packaging of the lipoproteins can take place by the time the lipids reach the Golgi region. It has also been considered that the particles observed in the cisternae of the endoplasmic reticulum are lipoproteins. This is suggested by the observations that apoproteins

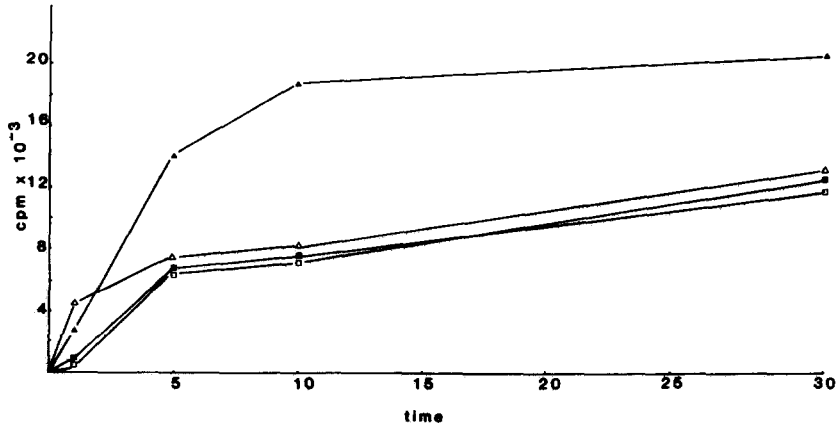

Fig. 7. Incorporation of [^{3}H]palmitic acid into phospholipids of subfractions of rat liver. Experiments were performed as described in the legend to Fig. 6. The specific activities of the phospholipid fractions were determined. Points plotted are the averages of three experiments performed in duplicate. (▲), Microsomal content phospholipid; (△), microsomal membrane phospholipid; (■), Golgi content phospholipid; (□), Golgi membrane phospholipid.

and lipids are synthesized in the endoplasmic reticulum and is consistent with morphological observations (4–9). In conflict with this, however, are recent reports that in chick liver cells apoA-I of HDL is synthesized in the endoplasmic reticulum but is not combined with lipid until the components reach the Golgi membranes (41). Investigations of estrogen-treated chick liver cells have indicated that syntheses of protein and lipid components of VLDL are not tightly linked (42). The density distribution of lipid and apoproteins released from Golgi contents suggest that these contain precursors in addition to completely assembled lipoproteins (18, 35). These observations emphasize the importance of Golgi elements as a site of lipoprotein assembly and raise questions concerning the nature of the particles observed in the endoplasmic reticulum cisternae.

The particles released from the vesicular space of the endoplasmic reticulum differ significantly from those released from Golgi fractions. The microsomal particles have a mean diameter of 20 nm compared with 32 nm for particles released from Golgi subfractions. The reported sizes of VLDL isolated from Golgi preparations and examined after negative staining include 22.7-nm particles isolated from *cis* preparations (35), 40-nm particles isolated from *trans* preparations (35), 20–60-nm particles (mean 38 nm) for VLDL released by sodium carbonate (18), and 30–100-nm particles (mean 55 nm) for VLDL released by the French pressure cell (33). Our measurements of the diameters of VLDL isolated from Golgi fall within this range. Because of possible technical differences between different laboratories, negative staining cannot be considered as giving an absolute measurement of particle size. However, using identical measurements, we found that microsomal content particles are significantly smaller than Golgi content particles. Microsomal particles appear to have a homogeneous lipid composition with a mole ratio of triglyceride to phospholipid of 10. In contrast, total Golgi contents have a mole ratio of triglyceride to phospholipid of 1.0, while the fraction of density 1.006 g/ml has the greatest triglyceride content with a mole ratio to phospholipid of 2.0. Despite these differences, however, the incorporation of [^{3}H]palmitic acid is consistent with a product precursor relationship between the content triglyceride of microsomes and of the Golgi fractions. These observations suggest that triglyceride to be secreted is sequestered in the cisternal space of the endoplasmic reticulum, but that the bulk of the phospholipid and cholesterol that are to be secreted are not added until the triglyceride particles reach the Golgi membranes.

In interpreting the observations reported here it is important to eliminate any source of contamination of the endoplasmic reticulum content that might account for its composition. Cross contamination between Golgi and endoplasmic reticulum fractions undoubtedly exists. The microsomal membranes contain less than 5% Golgi membrane measured in terms of galactosyltransferase (11). However, the Golgi vesicles have a high content relative to the membrane (14). A small contamination of the microsomal membranes with Golgi membrane could, therefore, produce a significant contribution of Golgi content to the microsomal content. However, this would tend to increase the phospholipid, cholesterol, and cholesteryl ester of the endoplasmic reticulum content rather than decrease it. The contamination of the Golgi fractions with endoplasmic reticulum is low (14) and, as the microsomes have a relatively small content, this would not add significantly to that of the Golgi fractions.

Membrane fragments or small vesicles may also contaminate the content fraction even after prolonged centrifugation. This appears to be true of French pressure cell-treated microsomes. The small amount of phospholipid in the content of sodium carbonate-treated microsomes moves parallel with the triglyceride on potassium bromide or sucrose gradients. It is unlikely, therefore, that the microsomal content is significantly contaminated with membrane, and if there is contamination this would tend to increase the phospholipid of the content.

The presence of a floating lipid layer at the top of the centrifuge tube in the centrifugation step in which microsomes are pelleted is also a potential source of contamination of the microsomes. Care was taken to remove this layer completely before resuspending the microsomal pellet. In control experiments microsomes were treated with 0.25 M sucrose in place of sodium carbonate, and recentrifuged. No detectable lipid was lost into the supernatant, suggesting that lipid released by sodium carbonate treatment is not simply trapped in the pellet. In addition, the specific activity of the triglyceride of the floating lipid layer was a fraction of that of the endoplasmic reticulum membrane and content at all times investigated. Triglyceride-rich membrane-bound particles have been isolated from liver cells in culture (37) and have been observed in livers of rats treated with drugs that inhibit lipoprotein assembly and/or secretion (43, 44). These particles have a low buoyant density and diameters of 0.5–2.0 μm (37). It is unlikely, therefore that these contribute to the triglyceride-rich particles isolated from microsomes.

In an earlier study by Glaumann, Bergstrand, and Erikson (38), the contents of microsomal and Golgi fractions were released by osmotic shock and ultrasonic treatment with a Polytron homogenizer. The content and membrane fractions were separated by prolonged centrifugation, 2 hr at 250,000 *g*, and the content

lipoproteins were isolated by flotation at d < 1.03 g/ml. These investigators concluded that the microsomal contents released in this way were rich in phospholipid relative to triglyceride compared with Golgi-derived lipoprotein particles. The major difference between these investigations and ours lies in the method used to open the microsomal vesicles. The Polytron ultrasonic homogenizer is a fairly destructive technique and might be expected to produce membrane fragments that do not pellet on centrifugation and have a density < 1.03 g/ml. Our observations of similar experiments using the French pressure cell are consistent with this possibility. Although Glaumann et al. (38) demonstrated that their content fractions did not contain active glucose-6-phosphatase or NADH cytochrome C reductase, this does not exclude the possibility of contaminating membrane fragments remaining in their lipoprotein preparations. Even a small contamination would significantly affect the composition of the endoplasmic reticulum contents.

The composition and size of the endoplasmic reticulum content particles is unusual. A high neutral lipid content is usually found in larger lipoproteins. However, we do not yet have complete details of the composition of these particles, which may contain unusual nonlipid stabilizing molecules.

The present observations indicate that triglycerides to be secreted from the liver are synthesized in association with the endoplasmic reticulum, subsequently transferred to the cisternal space, and then to the Golgi cisternae. Newly synthesized phospholipids are initially incorporated into microsomal membranes; however, in contrast to the labeling patterns of the triglyceride, the specific activities of this phospholipid pool do not peak rapidly. The specific activity of the phospholipid of the microsomal content rose to approximately twice that of the membrane. The content phospholipid is not the major precursor of the Golgi content, which has a specific activity similar to that of the Golgi membrane and the microsomal membrane. It appears probable, therefore, that the phospholipids are carried from the endoplasmic reticulum to the Golgi region as components of the membrane vesicles, which carry triglyceride-rich particles. In the Golgi elements phospholipid transfer to the particles would result in equalization of the specific activities of the phospholipids of the membranes and content. Similar processes may be involved in transport and incorporation of cholesterol into the Golgi contents. One possible mechanism for such transfer is through the action of cholesterol or phospholipid-transfer proteins. Alternatively, the pool of triglyceride associated with the Golgi membranes might be interacting with the membrane in order to accept additional phospholipid and cholesterol. Either mechanism is consistent with our observations and these are under further investigation.

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