The Effect of a Lipid-Rich Diet on the Properties and Composition of Lipoprotein Particles from the Golgi Apparatus of Guinea-Pig Liver

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1. A cell fraction rich in Golgi apparatus was isolated from the livers of guinea pigs fed on a lipid-rich diet (1.6% cholesterol, 15% corn oil). 2. The Golgi cisternae and secretory vesicles contained electron-dense particles which were tentatively identified as VLD (very-low-density) and LD (low-density) lipoproteins. Particles of moderate electron density, 150–500 nm in diameter, were seen associated with membranous elements of the Golgi-apparatus cell fraction. Disruption of this cell fraction permitted the release of these three species of particles, which were separated into particulate lipid, and VLD and LD lipoproteins. 3. The large particles of moderate electron density, isolated as particulate lipid, were distinct from both species of Golgi particles in their chemical composition and in possessing an immunochemically unreactive apolipoprotein(s). Morphological observations suggest that the particulate lipid arose from cytoplasmic lipid droplets which were present as contaminants of the Golgi-rich fraction. 4. The chemical and immunochemical results are consistent with the suggestion that the Golgi LD particles are precursors of the VLD particles, into which they may be transformed by the addition of both triglyceride and cholesteryl ester. The present results provide further support for the proposal that the Golgi VLD particles are precursors of the serum VLD lipoproteins in the guinea pig. 5. Hepatic Golgi VLD particles isolated from guinea pigs fed on the lipid-rich diet contained significantly higher molar amounts (relative to protein) of both cholesteryl ester and triglyceride than similar particles from animals fed on a normal diet. These results suggest that the type of Golgi VLD particle produced from the LD particle is a direct consequence of the amount and composition of the dietary lipid. 6. Hepatic Golgi LD particles isolated from guinea pigs fed on different diets were similar in chemical composition and contained approx. 50% by weight of phospholipid. We conclude that the Golgi LD particle is normally present in the Golgi-apparatus cell fraction from guinea-pig liver, and may represent the end product of lipoprotein biosynthesis in the smooth endoplasmic reticulum. 7. The serum LD lipoproteins and Golgi LD particles were quite distinct in chemical composition. However, these two lipoprotein species were immunochemically identical and exhibited a similar range of flotation rate. It appears unlikely that the Golgi LD particles are secreted as the precursors of the serum LD lipoproteins.

As part of an investigation of lipoprotein metabolism in relation to atherogenesis, we have recently reported the effects of a lipid-rich diet on the distribution and composition of serum lipoproteins in the guinea pig (Mills *et al.*, 1972). Marked differences were noted in both the distribution and composition of the serum lipoproteins in animals fed on the lipid-rich diet compared with those fed on a normal diet. In particular, the serum lipoproteins from guinea pigs fed on the enriched diet were not unlike those found in some human subjects with Fredrickson's type II hyperlipoproteinaemia, a lipid disorder

known to be associated with an increased risk of ischaemic heart disease (Slack, 1969).

To evaluate the role of the hepatic Golgi apparatus in the maintenance of the serum lipoprotein profile in guinea pigs fed on the lipid-rich diet, hepatocyte Golgi LD[†] and VLD particles were isolated and subjected to chemical and immunochemical analyses. The physical and chemical properties of these two Golgi-particle species were compared with those of the corresponding serum lipoproteins, and in addition, with those of similar species of Golgi particles from guinea pigs fed on a normal diet (Chapman *et al.*, 1972). In this way it was possible to determine the

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[†] Abbreviations: LD, low-density, density 1.007–1.063 g/ml; VLD, very-low-density, density <1.007 g/ml.

effects of a lipid-rich diet on nascent lipoprotein formation in the hepatic Golgi apparatus. Further, the validity of the hypothesis relating the two species of Golgi particle to the serum VLD lipoproteins, which was proposed on the basis of studies in guinea pigs fed on a normal diet, is discussed in the light of the present report. A preliminary account of this investigation has been presented (Chapman & Mills, 1972).

Experimental

Materials

Refined edible corn oil was purchased from Loders and Nucoline Ltd., London E.C.4, U.K. Cholesterol, for inclusion in the semi-synthetic diet, was supplied by Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.

The additional materials have been previously detailed (Chapman *et al.*, 1972).

Animals and diet

Male guinea pigs (approx. 750 g in wt.) from a randomly bred laboratory colony were fed on the semi-synthetic diet *ad libitum* for a period of 6 days. The composition of this diet has been outlined by Mills *et al.* (1972). Immediately before the animals were given the semi-synthetic diet, a blood sample (8 ml) was taken by cardiac puncture under ether anaesthesia. A further blood sample was taken after the 6-day period; the liver was then perfused and excised as described by Chapman *et al.* (1972).

Methods

Preparation of the Golgi apparatus. A Golgi-apparatus cell fraction was prepared from the liver of each guinea pig essentially as outlined by Mahley *et al.* (1969). The procedures employed in the electron-microscopic and enzymic studies of this cell fraction have been described (Chapman *et al.*, 1972).

Disruption of Golgi membranes. Release of the electron-dense particles from the Golgi cisternae was effected by disruptive treatment (Mahley *et al.*, 1969), except that the Golgi-rich fraction was frozen to -15°C .

Isolation of Golgi particles. Golgi-particle fractions I (density $<1.007\text{ g/ml}$) and II (density $1.007\text{--}1.063\text{ g/ml}$) were prepared from the Golgi-apparatus cell fraction, after disruptive treatment, by ultracentrifugal flotation (Chapman *et al.*, 1972).

Since Golgi-particle fraction I contained fatty particles, which were absent from similar fractions isolated from guinea pigs fed on the normal diet, these particles were separated from the soluble components of fraction I as follows; NaCl solution of

density 1.007 g/ml was layered on to fraction I (in a volume of 1.0 ml) to a final volume of 5.0 ml in a cellulose acetate tube [5.1 cm (2 in) $\times$ 1.3 cm ($\frac{1}{2}\text{ in}$) diam.]. The sample was then centrifuged at 39000 rev./min for 20 min in the SW 39 rotor of the Beckman L2 preparative ultracentrifuge, at a running temperature of 13°C (i.e. $120734g_{\text{av.}}$). On completion of ultracentrifugation, the base of the tube was punctured with a mounted needle, and the infranatant dripped out. The fatty pellicle, formed during ultracentrifugation, adhered to the wall of the tube during this process and was subsequently resuspended in NaCl solution (1.0 ml) of density 1.007 g/ml . This suspension contained material of $S_r > 1000$, which will be referred to as particulate lipid. [S_r is defined as the flotation velocity in S^{-1} measured in an NaCl solution of density 1.063 g/ml at 26°C].

The infranatant was then layered under 4.0 ml of NaCl solution (density 1.007 g/ml) and centrifuged for 16 h at 30000 rev./min in the 30.2 rotor of the Beckman L2 ultracentrifuge at a running temperature of 13°C (i.e. $79420g_{\text{av.}}$). After ultracentrifugation, the top fraction was removed in a volume of 1.0 ml by the method of de Lalla & Gofman (1954). This fraction constituted Golgi-particle fraction I, and contained particles of $S_r 20\text{--}1000$.

The mean amounts ($\pm\text{s.d.}$) of Golgi-particle fractions I and II isolated from four animals were $1905 \pm 1885\text{ }\mu\text{g}$ and $727 \pm 611\text{ }\mu\text{g}$ respectively. The mean amount of particulate lipid isolated from two animals was 12.6 mg .

For ultracentrifugal analysis, Golgi low-density (density $<1.100\text{ g/ml}$) substances were prepared from the disrupted Golgi-apparatus cell fraction as follows.

The suspension of the cell fraction, in a volume of 6.5 ml and at a density of 1.007 g/ml , was adjusted to a solvent density of 1.100 g/ml by addition of 2.5 ml of an NaCl solution of density 1.342 g/ml . The resultant mixture was then ultracentrifuged at 30000 rev./min for 16 h in the 30.2 rotor at a running temperature of 13°C (i.e. $79420g_{\text{av.}}$). After ultracentrifugation, the Golgi low-density substances were removed in a volume of 1.0 ml by the procedure of de Lalla & Gofman (1954). These substances were subsequently subjected to ultracentrifugal analysis at a density of 1.100 g/ml in the Spinco model E ultracentrifuge (Mills *et al.*, 1972).

Isolation of serum lipoproteins. Serum VLD (density $<1.007\text{ g/ml}$, $S_r > 20$) and LD (density $1.007\text{--}1.063\text{ g/ml}$, $S_r 0\text{--}20$) lipoproteins were isolated from each blood sample as described by Mills *et al.* (1972).

Analytical methods. Chemical analysis of the particulate lipid and particle fractions from the Golgi-rich fraction, and of the serum lipoproteins, was carried out as described by Mills *et al.* (1972).

G.l.c. analyses of the cholesteryl ester, triglyceride and phospholipid fractions were performed as outlined by Chapman *et al.* (1972).

Immunological analyses. The procedures employed in the preparation of rabbit antisera to guinea-pig serum lipoproteins, and in immunodiffusion, have been previously described (Chapman *et al.*, 1972).

Results

Electron microscopy and enzymic activity of the Golgi-apparatus cell fraction

Large numbers of Golgi cisternae and secretory vesicles were visible in the Golgi-apparatus cell fraction on examination by electron microscopy (Plate 1). Moreover, both the Golgi cisternae and the secretory vesicles contained numerous electron-dense particles whose staining characteristics suggested them to be lipoprotein in nature. These particles were essentially spherical in shape, with diameters ranging from 10 to 60nm; the majority of such particles were within the range 20–55 nm. Since the minimum diameter of a serum VLD lipoprotein molecule is 30nm in both rat (Hamilton *et al.*, 1967) and man (Nichols, 1969), and the diameters of serum LD lipoproteins range from 17 to 26nm in man (Forte *et al.*, 1968; Nichols, 1969), the results are consistent with the presence of particles possessing both VLD- and LD-flotation characteristics in the Golgi-rich fraction.

Large particles of moderate electron density, spherical in shape and with diameters from approx. 150 to 500nm, were also present in the Golgi-apparatus cell fraction. The staining properties of these particles indicated them to be primarily lipid in nature. The particles did not exhibit a limiting membrane, but did appear to be loosely associated with membranous elements of the fraction (Plate 1, inset).

The principal contaminants of this cell fraction were elements of the rough endoplasmic reticulum; contamination was estimated at <5%.

The activity of UDP-galactose-*N*-acetylglucosamine galactosyltransferase, a marker enzyme for the Golgi apparatus (Fleischer, 1969), was significantly higher in the Golgi-rich fraction than in the liver homogenate. Thus, in a representative experiment, the specific activity (nmol of *N*-acetylaminolactose synthesized/h per mg of protein at 37°C) of the enzyme in the Golgi-apparatus cell fraction (25.0) was some 55-fold greater than that in the liver homogenate (0.45).

These results support the conclusion that the Golgi-apparatus cell fraction was substantially enriched in Golgi apparatus and associated vesicular profiles and moreover, that this fraction contained numerous electron-dense particles tentatively identified as VLD and LD lipoproteins. In addition, larger particles of lower electron density, which appeared to consist primarily of lipid, were seen in association

Table 1. Composition of hepatocyte Golgi particles and particulate lipid, and of serum lipoproteins, from guinea pigs fed on a lipid-rich diet

Values are the mean weight % of each component $\pm$ s.d. for the numbers of preparations given in parentheses. Golgi-particulate lipid indicates the particulate lipid isolated from the Golgi-apparatus cell fraction. The quantity of cholesterol ester was calculated as $1.67 \times$ the amount of ester cholesterol.

Fraction	Density (g/ml)	S _r	Composition (% w/w)				Ratio of esterified/free cholesterol
			Cholesteryl esters	Free cholesterol	Triglycerides	Phospho- lipids	
Golgi-particulate lipid (2)	<1.007	>1000	8.3	1.6	79.7	3.2	3.2
Golgi-particle fraction I (4)	<1.007	20–1000	17.8 $\pm$ 8.3	2.8 $\pm$ 1.8	67.2 $\pm$ 19.4	9.7 $\pm$ 8.5	4.4
Serum VLD lipoprotein (4)	<1.007	>20	23.4 $\pm$ 8.0	9.4 $\pm$ 1.3	49.1 $\pm$ 9.8	12.9 $\pm$ 1.5	1.5
Golgi-particle fraction II (4)	1.007–1.063	0–20	9.0 $\pm$ 2.4	12.4 $\pm$ 1.5	13.7 $\pm$ 7.3	50.1 $\pm$ 12.9	0.4
Serum LD lipoprotein (4)	1.007–1.063	0–20	47.9 $\pm$ 3.5	11.9 $\pm$ 0.7	3.6 $\pm$ 1.8	18.2 $\pm$ 2.1	2.4

with membranous elements of this fraction. To determine the possible origin of the large particles of moderate electron density that were present in the Golgi-rich fraction, thin sections of intact liver tissue were examined by electron microscopy.

Numerous large lipid droplets were frequently observed free in the cytoplasm of hepatic parenchymal cells. The droplets did not appear to possess a limiting membrane, and exhibited a considerable variation in size, ranging from 150 to 500 nm in diameter. These observations are therefore consistent with the conclusion that the large particles of lipid present in the Golgi-rich fractions are derived from the lipid droplets seen free in the hepatocyte cytoplasm.

Chemical composition

The mean weight % chemical compositions of the particulate-lipid and Golgi-particle fractions, and of the serum VLD and LD lipoproteins, are shown in Table 1. The serum lipoproteins were isolated from the blood samples of the four animals whose livers had been taken for preparation of the Golgi-apparatus cell fraction.

The particulate lipid, isolated from the Golgi-apparatus cell fraction after disruptive treatment, was distinct from the other fractions of similar density, i.e. Golgi-particle fraction I and serum VLD lipoprotein, in containing almost 80% by weight of triglyceride. In addition, its protein content was higher than that of either of these fractions. Although there was an overall similarity in the composition of Golgi-particle fraction I and the serum VLD lipoproteins, there was a higher proportion of triglyceride in fraction I (67.2%) than in the serum VLD lipoproteins (49.1%). However, the proportion of this component was particularly variable in both

lipoprotein species, as witnessed by the high standard deviations (19.4 and 9.8 respectively). The free cholesterol content of the serum VLD lipoproteins (9.4%) was significantly higher ($0.01 > P > 0.005$) than that of Golgi-particle fraction I (2.8%). Thus the ratio of esterified to free cholesterol was some three-fold lower in the serum VLD lipoproteins than in fraction I.

Golgi-particle fraction II was characterized by its high phospholipid content (50.1%). This fraction was further distinguished from the serum counterpart, i.e. the LD lipoproteins, by its substantially lower proportion of cholesteryl ester ($P < 0.001$) and by its low ratio of esterified to free cholesterol.

The chemical compositions of the various fractions were subsequently expressed on a molar basis (Table 2). The molar composition of the particulate lipid was distinct from that of the other fractions. The molar proportions of both free cholesterol and of phospholipid were similar in the two Golgi-particle fractions. However, the amounts of cholesteryl ester and triglyceride in fraction I were some 12- and 29-fold greater respectively than those in the particles of higher density, i.e. fraction II.

When allowance was made for the statistical variance in the protein contents of fraction I and the serum VLD lipoprotein, it was found that their molar compositions were not significantly different.

The serum LD lipoprotein was characterized by its low contents of both triglyceride and phospholipid.

Thus there was an overall similarity between the chemical compositions of Golgi-particle fraction I and the serum VLD lipoproteins. The molar compositions of the two species of Golgi particle displayed similar amounts of phospholipid and free cholesterol. The composition of fraction II was distinct from those of the other fractions.

Table 2. *Chemical composition of hepatocyte Golgi particles and particulate lipid, and of serum lipoproteins, from guinea pigs fed on a lipid-rich diet*

The results are expressed as the molar amount of each component relative to that of protein; all values are thus molar ratios. The molecular weight of each component is shown in parentheses; that taken for the protein moiety is that suggested for the human LD apolipoprotein by Pollard *et al.* (1969); those of the lipid esters were calculated from their respective mean fatty acid distribution. Golgi-particulate lipid indicates the particulate lipid isolated from the Golgi-apparatus cell fraction.

Component	Golgi-particulate lipid	Golgi-particle fraction		Serum VLD lipoproteins	Serum LD lipoproteins
		I	II		
Cholesteryl esters (647)	49	297	25	185	112
Free cholesterol (387)	16	77	59	124	47
Triglycerides (863)	352	839	29	292	6
Phospholipids (800)	15	130	115	83	34
Protein (27000)	1	1	1	1	1

EXPLANATION OF PLATE I

Electron micrograph of a section from the pellet of the Golgi-apparatus cell fraction from the liver of a guinea pig fed on the lipid-rich diet

Several Golgi cisternae (GC) and secretory vesicles (SV) are recognizable. The secretory vesicles contain numerous electron-dense particles 10–60nm in diameter. A small amount of contamination with elements of the rough endoplasmic reticulum (RER) is evident. The bar represents 200nm. The inset is a selected area of the field showing electron-opaque lipid particles (LP) with loosely associated membranous elements (arrows). The bar represents 200nm.

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EXPLANATION OF PLATE 2

Schlieren photographs of the distribution of (a) Golgi low-density (density $<1.100\text{g/ml}$) substances isolated from the Golgi-rich fraction of a guinea pig fed on the semi-synthetic diet and (b) serum lipoproteins of density $<1.100\text{g/ml}$ from the same animal

Ultracentrifugal flotation took place from right to left in an NaCl solution of density 1.100g/ml , at 52640rev./min and at a running temperature of 26°C . The photograph was taken 18 min after full speed was reached. The angle of the schlieren diaphragm was approx. 50° . The lipoproteins in (b) were derived from 3 ml of serum, and those in (a) were derived from the Golgi-rich fraction prepared from the whole liver.

Table 3. Fatty acid composition of lipid esters of hepatocyte Golgi particles and particulate lipid, and of serum lipoproteins, from guinea pigs fed on a lipid-rich diet

Values are the mean weight % of each fatty acid $\pm$ s.d. for the numbers of preparations given in parentheses. The total % of fatty acids in each lipid ester may not total 100% owing to the omission of fatty acids present in trace amounts (generally <1%). Golgi-particulate lipid indicates the particulate lipid isolated from the Golgi-apparatus cell fraction (as in Table 1).

Lipid ester	Fraction	Density (g/ml)	S _r	Fatty acids									
				C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:4}			
Triglycerides	Golgi-particulate lipid (2)	<1.007	>1000	11.3	1.0	2.1	20.6	57.0	7.1	0.0			
	Golgi-particle fraction I (4)	<1.007	20-1000	10.5 $\pm$ 4.5	1.5 $\pm$ 0.5	4.8 $\pm$ 3.0	24.0 $\pm$ 3.0	50.0 $\pm$ 11.4	5.5 $\pm$ 1.8	2.6 $\pm$ 1.9			
	Serum VLD lipo-protein (4)	<1.007	>20	7.3 $\pm$ 2.0	2.5 $\pm$ 0.6	3.7 $\pm$ 2.1	29.9 $\pm$ 2.0	53.1 $\pm$ 6.7	4.2 $\pm$ 1.5	0.1 $\pm$ 0.1			
	Golgi-particle fraction II (4)	1.007-1.063	0-20	13.1 $\pm$ 7.2	3.0 $\pm$ 3.0	13.7 $\pm$ 3.6	29.6 $\pm$ 3.6	30.7 $\pm$ 12.2	3.4 $\pm$ 3.0	4.7 $\pm$ 3.2			
	Serum LD lipo-protein (4)	1.007-1.063	0-20	9.4 $\pm$ 1.4	2.0 $\pm$ 0.3	6.9 $\pm$ 2.9	26.2 $\pm$ 2.0	44.1 $\pm$ 5.7	5.2 $\pm$ 1.2	2.5 $\pm$ 1.8			
Cholesteryl esters	Golgi-particulate lipid (2)	<1.007	>1000	14.0	3.0	2.4	31.3	35.9	6.1	0.7			
	Golgi-particle fraction I (4)	<1.007	20-1000	16.7 $\pm$ 4.0	3.8 $\pm$ 2.0	8.7 $\pm$ 5.1	29.9 $\pm$ 5.9	29.2 $\pm$ 6.2	5.8 $\pm$ 4.4	1.5 $\pm$ 1.2			
	Serum VLD lipo-protein (4)	<1.007	>20	9.8 $\pm$ 7.1	1.6 $\pm$ 1.2	4.7 $\pm$ 2.2	36.3 $\pm$ 3.4	38.6 $\pm$ 8.5	6.7 $\pm$ 0.8	0.6 $\pm$ 0.2			
	Golgi-particle fraction II (4)	1.007-1.063	0-20	21.4 $\pm$ 3.6	6.7 $\pm$ 2.4	12.0 $\pm$ 4.4	31.3 $\pm$ 2.1	18.6 $\pm$ 5.8	4.0 $\pm$ 3.7	4.5 $\pm$ 3.9			
	Serum LD lipo-protein (4)	1.007-1.063	0-20	11.9 $\pm$ 7.6	1.8 $\pm$ 0.3	2.0 $\pm$ 0.5	22.9 $\pm$ 4.8	54.3 $\pm$ 7.3	4.1 $\pm$ 1.5	0.5 $\pm$ 0.6			
Phospholipids	Golgi-particulate lipid (2)	<1.007	>1000	14.5	1.6	15.1	17.4	43.4	3.7	2.6			
	Golgi-particle fraction I (4)	<1.007	20-1000	14.4 $\pm$ 6.0	3.3 $\pm$ 2.4	16.6 $\pm$ 2.4	23.8 $\pm$ 2.8	31.7 $\pm$ 10.8	4.3 $\pm$ 3.3	3.0 $\pm$ 0.8			
	Serum VLD lipo-protein (4)	<1.007	>20	9.9 $\pm$ 4.3	0.9 $\pm$ 0.5	17.6 $\pm$ 4.2	19.7 $\pm$ 3.2	43.4 $\pm$ 4.8	2.3 $\pm$ 1.1	3.4 $\pm$ 1.7			
	Golgi-particle fraction II (4)	1.007-1.063	0-20	10.4 $\pm$ 3.8	1.6 $\pm$ 1.5	25.8 $\pm$ 4.2	19.0 $\pm$ 5.5	35.7 $\pm$ 7.5	1.9 $\pm$ 1.4	4.4 $\pm$ 0.8			
	Serum LD lipo-protein (4)	1.007-1.063	0-20	10.2 $\pm$ 1.3	1.1 $\pm$ 0.4	20.1 $\pm$ 2.8	18.3 $\pm$ 3.1	40.1 $\pm$ 4.8	1.0 $\pm$ 0.3	3.0 $\pm$ 2.0			

Fatty acid composition of the lipid esters

The weight % fatty acid compositions of the triglycerides, cholesteryl esters and phospholipids of the Golgi particles and particulate lipid, and of the serum lipoproteins, are presented in Table 3. The test of similarity employed in determining whether a given fatty acid distribution was similar to another was the Student's unpaired *t* test (Snedecor & Cochran, 1967). A significant difference between each pair of distributions was indicated by a value for *P* of less than 0.05.

The fatty acid distribution of each lipid component of the particulate-lipid fraction was similar to that of the same component of both Golgi-particle fraction I and the serum VLD lipoproteins.

The triglyceride fatty acid patterns of fraction I and the serum VLD lipoprotein showed a marked similarity; both exhibited a particularly high proportion of the $C_{18:2}$ acid (50.0 and 53.1 % respectively). The cholesteryl ester and phospholipid fatty acid distributions of these two lipoprotein species were also similar. The fatty acid distributions of the triglycerides and cholesteryl esters in Golgi-particle fractions I and II resembled each other. Only the proportions of the $C_{18:0}$ acid were significantly different ($0.05 > P > 0.025$) in the phospholipids of these fractions.

The distributions of fatty acids in the triglycerides of fraction II and the serum LD lipoproteins were alike. Moreover, their phospholipids showed very similar distributions. In contrast, the cholesteryl ester fatty acids of these two fractions displayed significantly different patterns, with the exception of the $C_{16:0}$, $C_{18:3}$ and $C_{20:4}$ acids, whose proportions were similar.

Fatty acid composition of dietary lipid

The weight % fatty acid composition of a lipid extract of the dietary corn oil was as follows: $C_{16:0}$ acid, 8.3%; $C_{16:1}$ acid, 0.5%; $C_{18:0}$ acid, 2.0%; $C_{18:1}$ acid, 31.5%; $C_{18:2}$ acid, 56.7%; $C_{18:3}$ acid, 0.9%; $C_{20:4}$ acid, 0%. These results show that unsaturated C_{18} fatty acids constituted almost 90% of the total fatty acids of the corn oil, with the $C_{18:2}$ acid accounting for over 55%.

Distribution of Golgi low-density substances

The ultracentrifugal distribution of low-density substances (density < 1.100 g/ml) isolated after disruptive treatment of the hepatic Golgi-apparatus cell fraction is presented in Plate 2.

The distribution of Golgi low-density substances (7.2–30.7S) was almost identical with that observed in the serum lipoproteins of the same density (9.8–32.6S). However, although the Golgi low-density

substances were evenly distributed over their flotation range, a well-defined asymmetrical peak of 15.4S was seen in the profile of serum lipoproteins.

Immunological analyses

Immunodiffusion of the particulate lipid from the Golgi-rich fraction against antisera to guinea-pig serum lipoproteins and to whole guinea-pig serum did not produce any precipitin reactions.

The Golgi VLD particles (fraction I) were immunochemically indistinguishable from the serum VLD lipoproteins on immunodiffusion against antiserum to guinea-pig serum VLD lipoproteins. The Golgi VLD particles and serum VLD lipoproteins therefore share the same principal antigenic determinant. We have previously reported that hepatic Golgi LD particles (fraction II), isolated from guinea pigs fed on the lipid-rich diet, possess this same antigenic determinant (Chapman *et al.*, 1972) and therefore probably contain the same apolipoprotein as the VLD particles.

Discussion

A marked resemblance was noted in the apolipoprotein, free cholesterol and phospholipid content of the LD and VLD particles isolated from the Golgi-rich fraction. [The term LD is used specifically to indicate material isolated from the Golgi-apparatus cell fraction with low-density (density 1.007–1.063 g/ml) flotation characteristics, and is not synonymous with (serum) β -lipoprotein.] In addition, the fatty acid distributions of each of the lipid esters of these two particle species were similar. The possibility therefore arises that the LD particle may be transformed into a VLD particle within the Golgi apparatus by addition of both triglyceride and cholesteryl ester. This situation is in contrast to that in guinea pigs fed on a normal diet, where such a transformation may be accomplished solely by the addition of triglyceride. These observations do, however, support the conclusion that the type of transformation undergone by the Golgi LD particle is a direct consequence of the amount and composition of the dietary lipid. In this way, the composition of the nascent VLD lipoprotein particle secreted from the liver may be closely related to the metabolic environment. This conclusion is supported by the studies of Heimberg & Wilcox (1972) in a perfused-rat-liver system, which showed that the type of VLD lipoprotein secreted by the liver was prescribed by the nature of the infused free fatty acid. Similar observations have been reported by Ockner *et al.* (1969) in investigations of intestinal lipoprotein biosynthesis. These workers established that dietary long-chain fatty acids differ significantly in their effects on the transport of cholesterol and triglyceride by the lipoproteins of

intestinal lymph. Further, their results implied that the type of fatty acid fed directly influenced the composition and properties of the lipoproteins by which it was transported.

On comparison of the chemical composition of the Golgi LD particles isolated in the present study with that of similar particles from animals fed on a normal diet (Table 4), it was found that these two samples of LD particles did not differ in composition to a statistically significant extent, and that they both contained 50% of phospholipid. The amount of the Golgi-LD-particle fraction isolated from guinea pigs fed on the lipid-rich diet ($727\mu\text{g}$) was some sixfold greater than that from animals fed on a normal diet ($118\mu\text{g}$). This observation is consistent with the morphological appearance of the Golgi-rich fraction isolated in the present study. Thus this cell fraction contained greater numbers of lipoprotein-staining particles than similar fractions from animals fed on the normal diet. Many of these particles exhibited apparent diameters of less than 20nm. These results are consistent with the conclusion that the Golgi-rich fraction contains a lipoprotein particle species with low-density flotation characteristics, which is not an artifact of the disruption procedure.

Some indication of the possible origin of the phospholipid-rich LD particle may be derived from consideration of the cytomembrane content of the Golgi-apparatus cell fraction. Thus the procedures employed in the preparation and characterization of this cell fraction do not exclude the possibility that it may contain vesicles derived from the smooth endoplasmic reticulum. Moreover, electron-microscopic studies in rat liver have shown that continuities may exist between the smooth endoplasmic reticulum and membranes of the Golgi apparatus (Claude, 1970). Since the smooth endoplasmic reticulum is involved in the hepatic biosynthesis of nascent VLD lipoproteins (Jones *et al.*, 1967; Stein & Stein, 1967; Hamilton, 1969; Claude, 1970), precursor LD-lipoprotein particles, possibly characteristic of the smooth endoplasmic reticulum (Chapman *et al.*, 1972), may

therefore be present in vesicles of smooth endoplasmic reticulum contained in the Golgi-rich fraction. Morphological observations did not indicate any differentiation in the distribution of the LD and VLD particles in vesicles of this cell fraction. We therefore conclude that the proposed cisternal transformation of LD into VLD particles may occur as a continuous process during the transport of precursor LD particles both to and within the Golgi apparatus.

Although the chemical composition of the Golgi LD particles was quite distinct from that of the serum LD lipoproteins, these two lipoprotein species possessed the same principal apolipoprotein and exhibited similar ultracentrifugal distributions. The possibility that some of the Golgi LD particles are secreted from the liver as the precursors of the serum LD lipoproteins cannot therefore be excluded. Should the hepatic Golgi LD particles be secreted into the circulation, however, they must subsequently undergo extensive modification. The results appear therefore to be consistent with the proposal that the serum LD lipoproteins are produced primarily as a result of the metabolic transformation of the VLD lipoproteins in the circulation (Levy *et al.*, 1971).

The physical, chemical and immunochemical properties of the Golgi VLD particles are consistent with the proposal that they are precursors of the serum VLD lipoproteins. The principal difference between these two lipoprotein species was in their relative proportion of triglyceride, that in the serum VLD lipoproteins being notably low (49.1%). This may be accounted for by the observation that the serum VLD lipoprotein in guinea pigs fed on the lipid-rich diet is of low triglyceride content, in contrast to the sera of animals fed on the normal diet, whose VLD lipoprotein contains a greater proportion of triglyceride (Mills *et al.*, 1972).

A comparison of the composition of Golgi VLD particles isolated from guinea pigs fed on the lipid-rich diet with that of similar particles from animals fed on a normal diet is shown in Table 5. When

Table 4. *Composition of hepatocyte Golgi LD particles from guinea pigs fed on different diets*

The results for animals fed on the normal diet are taken from Chapman *et al.* (1972). The molecular weight of each component is given in Table 2.

Component	Mean weight (%)		Molar composition	
	Normal diet	Lipid-rich diet	Normal diet	Lipid-rich diet
Cholesteryl esters	4.8	9.0	9	25
Free cholesterol	7.9	12.4	27	59
Triglycerides	18.5	13.7	27	29
Phospholipids	48.1	50.1	75	115
Protein	20.7 ± 8.0	14.7 ± 6.0	1	1

Table 5. *Composition of hepatocyte Golgi VLD particles from guinea pigs fed on different diets*

The results for animals fed on the normal diet are taken from Chapman *et al.* (1972). The molecular weight of each component is given in Table 2.

Component	Mean weight (%)		Molar composition	
	Normal diet	Lipid-rich diet	Normal diet	Lipid-rich diet
Cholesteryl esters	2.8	17.8	14	297
Free cholesterol	3.0	2.8	25	77
Triglycerides	66.2	67.2	256	839
Phospholipids	19.9	9.7	83	130
Protein	8.1	2.5	1	1

expressed as a molar amount relative to that of protein, the proportion of cholesteryl ester in VLD particles from animals fed on the lipid-rich diet was some 20-fold higher than that in similar particles from animals fed on the normal diet. Although the mean weight % composition of these two samples of VLD particles showed them to contain similar proportions of triglyceride, expression of the results on a molar basis established the amount of triglyceride to be threefold higher in particles from animals fed on the lipid-rich diet.

These results indicate that when guinea pigs are fed on a diet enriched with corn oil and cholesterol, the liver synthesises a VLD lipoprotein particle whose apolipoprotein(s) is bound to increased amounts of both triglyceride and cholesterol. The carriage of additional triglyceride and cholesterol (principally as the ester) by the VLD apolipoprotein(s), considered together with an increased rate of hepatic lipoprotein production (Mills *et al.*, 1972), may be seen as an attempt by the liver to maintain normal intracellular concentrations of these lipids. Since the most pronounced increase was in the molar amount of cholesteryl ester in the Golgi VLD particle, it would appear that the liver may have a rather limited capacity to store cholesterol in comparison with that of triglyceride.

The large triglyceride-rich particles identified by electron microscopy in the Golgi-apparatus cell fraction corresponded morphologically to the lipid droplets seen free in the cytoplasm of hepatic parenchymal cells. Similar droplets have been observed in rat liver after prolonged perfusion with free fatty acid (Jones *et al.*, 1967). Such triglyceride accumulation may be indicative of maximal rates of secretion of this lipid from the liver, as suggested by the studies of Heimberg *et al.* (1969) in rat livers perfused with free fatty acids.

The presence of the large lipid droplets in a cell fraction with density ($>1.10 < 1.19$ g/ml) substantially higher than their own (<1.007 g/ml) may have resulted from their association with membranous elements of

the Golgi-rich fraction, and also from their occlusion within it. The subsequent disruptive treatment of this cell fraction permitted the release and isolation of the particles as a lipoprotein species of $S_r > 1000$. This lipoprotein species may therefore be regarded as a cytoplasmic contaminant of the Golgi-rich fraction. Such a conclusion is consistent with the observation that the immunochemical and chemical characteristics of this species were quite distinct from those of both the LD and VLD particles. However, since the fatty acid distributions of the lipid esters of the lipid droplets were similar to those of both species of Golgi particles, it may plausibly be postulated that they represent a precursor lipid pool, which might be utilized in the transformation of the Golgi LD to VLD particles. Equally plausible is the possibility that the lipid in these three lipoprotein species may be derived from the same fatty acid pool.

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