Lipoprotein Particles from the Golgi Apparatus of Guinea-Pig Liver

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1. A cell fraction has been isolated from guinea-pig liver and shown to be rich in Golgi apparatus by electron microscopy. The activity of UDP-D-galactose-N-acetylglucosamine galactosyltransferase was over 100-fold greater in this cell fraction than in the liver homogenate. These data support the conclusion that the fraction was enriched in Golgi apparatus. 2. The Golgi cisternae and secretory vesicles contained electron-dense particles of 10–80 nm diameter. Disruption of the Golgi apparatus cell fraction released these particles, which were separated into VLD (very-low-density) and LD (low-density) species on the basis of their density. 3. The Golgi VLD particles possessed morphological, flotation, chemical and immunochemical properties which closely resembled those of the serum VLD lipoproteins from the same animals. 4. The Golgi LD particles were rich in phospholipid, containing 48.1 % by weight. The chemical composition of these particles was quite distinct from that of the serum LD lipoproteins, but did, however, show some similarity to that of the serum VLD lipoproteins. A marked resemblance was noted in the chemical characteristics of the Golgi LD and VLD particles (with the exception of triglyceride content). In addition, these two species of Golgi particles possessed the same antigenic determinant. 5. The results suggest that the Golgi VLD particles are the precursors of the serum VLD lipoproteins. On the basis of similarities in gross chemical composition and in the antigenic determinant of the Golgi LD and VLD particles, we conclude that the LD particles are probably the precursors of the VLD particles. In view of the marked differences in gross chemical composition of the Golgi LD particles and serum LD lipoproteins, it appears unlikely that the LD particles are directly secreted into the serum pool.

Extensive morphological observations (Jones *et al.*, 1967; Hamilton *et al.*, 1967; Stein & Stein, 1967; Claude, 1970) have provided evidence to suggest that the Golgi apparatus may play a fundamental role in the secretion of serum VLD lipoproteins† from the liver. Nascent VLD lipoproteins were seen in these studies as electron-dense particles, 30–120 nm in diameter, within the Golgi apparatus of hepatic parenchymal cells. The appearance of these particles, first within the cisternae of the smooth endoplasmic reticulum, and subsequently within the Golgi apparatus, could be correlated with the biosynthesis and secretion of serum VLD lipoproteins (Jones *et al.*, 1967; Hamilton *et al.*, 1967; Stein & Stein, 1967). Mahley *et al.* (1969) have reported the isolation and characterization of such particles from a rat liver-cell

fraction rich in Golgi apparatus. The lipid composition, protein content, and immunological behaviour of the electron-dense particles resembled those of the VLD lipoproteins of rat serum. Moreover, these workers subsequently established that the rat liver Golgi apparatus both contained and secreted a VLD lipoprotein particle that possessed the same major apoproteins as those present in the circulating serum VLD lipoproteins in this animal (Mahley *et al.*, 1970).

Despite these findings, there is as yet scant information on the possible active role(s) which the Golgi apparatus may play in the biosynthesis of the nascent hepatic VLD lipoproteins. The studies of Lo & Marsh (1970) in rat liver do, however, indicate that the Golgi apparatus may be involved in the attachment of the carbohydrate moiety to the nascent LD (density <1.04 g/ml) and high-density (density 1.04–1.21 g/ml) lipoproteins.

In the present paper we describe the isolation and characterization of VLD and LD particles from Golgi-rich fractions of guinea-pig liver. The physical and chemical properties of these particles have been compared with those of the serum lipoproteins. These studies therefore give an indication of the role

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† Abbreviations: VLD lipoproteins, very-low-density lipoproteins, density <1.007 g/ml. LD lipoproteins, low-density lipoproteins, density 1.007–1.063 g/ml. VLD particles, very-low-density particles from the Golgi apparatus. LD particles, low-density particles from the Golgi apparatus.

of the hepatocyte Golgi apparatus in the formation and secretion of serum lipoproteins in the guinea pig.

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Experimental

Materials

UDP-[U- 14 C]galactose (specific radioactivity 289mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All reagents and solvents were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K., and were of analytical grade or the purest available. Maleic acid was recrystallized once from water before use. The 2-mercaptoethanol was purchased from Ralph N. Emanuel Ltd., Alperton, Middx., U.K. Bio-Rad anion-exchange resin AG1-X2 (200–400 mesh; Cl⁻ form) was supplied by Micro-Bio Laboratories, London, U.K. Silicic acid for use in lipid chromatography was obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Agarose was purchased from Seravac Laboratories (Pty.) Ltd., Maidenhead, Berks., U.K. Bovine serum albumin (fraction V from bovine serum) was supplied by Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. The components of the scintillation medium were obtained from Thorn Electronics Ltd., Tolworth, Surrey, U.K., and were of scintillation grade.

Animals and diet

The guinea pigs and their diet have been previously described (Mills *et al.*, 1972). These animals were killed at about the same time of day (0900–1100h). A blood sample was first taken by cardiac puncture under light ether anaesthesia; while the guinea pig remained under anaesthesia, a midline abdominal incision was made, and the liver was perfused via the hepatic portal vein with 50ml of a 0.15M-NaCl. After dissecting out the gall bladder, the liver was excised. The livers were approx. 40g in weight.

Methods

Preparation of the Golgi apparatus. A cell fraction rich in Golgi apparatus was prepared from each liver essentially by the method of Mahley *et al.* (1969). The final pellet containing Golgi apparatus was resuspended in 5.0ml of 0.15M-NaCl. This suspension constituted the Golgi-rich fraction. For the determination of UDP-D-galactose-N-acetylglucosamine galactosyltransferase activity, the final pellet was resuspended in 2.0ml of a 0.5% (w/v) Triton X-100 solution.

Electron microscopy. After the final ultracentrifuga-

tion of the Golgi-rich fraction at 25000rev./min for 20min, the pellet was resuspended in 30ml of one-half strength Karnovsky fixative solution (Karnovsky, 1965), and allowed to fix for a period of 3 to 3.5h at room temperature. On completion of fixation, the Golgi-rich fraction was sedimented by centrifugation at 1900g for 20min, and the pelleted material was washed by gentle resuspension in 20ml of 0.1M-cacodylate buffer at pH7.4. After treatment with OsO₄ for 1h, the material was dehydrated and embedded in Araldite resin. Thin sections of the embedded specimens were cut on a Cambridge ultratome, stained with Reynolds' lead citrate solution (Reynolds, 1963) on uncoated grids, and examined with a Phillips EM.300 electron microscope.

Assays of enzymic activity. UDP-D-galactose-N-acetylglucosamine galactosyltransferase (UDP-galactose-N-acetylglucosamine 1-galactosyltransferase, EC 2.4.1.22.) was assayed in the homogenate of guinea-pig liver (after filtration) and in the Golgi-rich fraction by the method of Morré *et al.* (1969). In each series of assays, controls (in triplicate) were set up to correct for the presence of any free [14 C]-galactose. The mean radioactivity (d.p.m.) for these controls was then subtracted from the mean radioactivity obtained for five assay samples, giving a net radioactivity that represents transferase activity. The quantity of UDP-[U- 14 C]galactose added to each reaction mixture was 25nmol, and was equivalent to 55500d.p.m.

The 14 C-labelled reaction product, N-acetylaminolactose, together with any free galactose, was washed directly into glass scintillation vials containing 10ml of Bray's (1960) medium. Radioactivity was determined by the method of Felts & Mayes (1967), employing a Packard Tri-Carb scintillation spectrometer (model 3320). Samples were corrected for quenching by the channels-ratio procedure. The efficiency of 14 C counting in unquenched samples under these conditions was 65.98%.

The protein content of each fraction was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Isolation of Golgi particles. Electron microscopic studies suggested that the Golgi-rich fraction contained several kinds of smooth-surfaced tubules and vesicles. The type of vesicles predominating in the preparations possessed contents which appeared to be particulate. Release of these particles was effected by subjecting the Golgi-rich fraction to disruptive treatment, and was confirmed by electron-microscopic examination of the fraction after such treatment. Subsequently, the particles were divided into two fractions according to their density.

Disruption of Golgi membranes. The disruptive procedure was that of Mahley *et al.* (1969), with the exception that the Golgi-rich fractions were frozen to -15°C.

Preparation of Golgi particle fractions I and II. The isolation of the Golgi particle fractions involved the use of two general procedures, which are summarized as follows.

Procedure A. Samples were ultracentrifuged at 30000 rev./min (79420g) for 16h in the no. 30.2 rotor of the Spinco L2 ultracentrifuge at a running temperature of 13°C.

Procedure B. On completion of ultracentrifugation, the fraction present at the top of the sample in each tube was pipetted off in a known volume by the method of DeLalla & Gofman (1954).

The densities of all NaCl solutions employed in the isolation of the various fractions were determined

by pycnometry, and were accurate to within ± 0.0005 g/ml of that specified. Each solution contained 1 mM-EDTA, 50 mM-NaHCO₃ and 0.5% NaN₃ at pH 7.0.

Golgi particle fraction I. Golgi particle fraction I was isolated from the Golgi-rich fraction, after disruptive treatment, as outlined in Scheme 1.

The Golgi suspension (5 ml) was layered under the NaCl solution of density 1.007 g/ml, to a final volume of 9 ml. The suspension was then ultracentrifuged by procedure A. After ultracentrifugation, the fraction with density <1.007 g/ml was taken off in a volume of 0.1 ml as outlined in procedure B; 8.0 ml of infranatant remained in the tube. The top fraction was

Scheme 1. Procedure for the isolation and purification of two fractions of Golgi particles from the Golgi apparatus-rich fraction of guinea-pig liver

For details see the Experimental section.

washed twice by ultracentrifugation under the same conditions after addition of 8.0 ml of NaCl solution of density 1.007 g/ml. The top 1.0 ml fraction, obtained by application of procedure B after ultracentrifugation, constituted Golgi particle fraction I (fraction GP I). This fraction contained Golgi particles of density <1.007 g/ml, and amounted to $346.1 \pm 61.5 \mu\text{g}$.

Golgi particle fraction II. The isolation of Golgi particle fraction II from the density 1.007 g/ml infranatant, obtained after the initial ultracentrifugation of the Golgi suspension, is outlined in Scheme 1.

Before transfer of the infranatant, the top 2.0 ml was taken off and discarded, leaving a bottom portion of 6.0 ml. The pellet at the base of the tube was then resuspended in this bottom portion of infranatant, and the total volume was divided into two portions of 3.0 ml. To each of the portions was added 2.0 ml of NaCl solution of density 1.007 g/ml; the solvent density of each portion (5.0 ml) was subsequently adjusted to 1.063 g/ml by addition of 1.0 ml of a NaCl solution of density 1.342 g/ml. NaCl solution of density 1.063 g/ml was then layered upon each portion to a final vol. of 9.0 ml, and the samples were ultracentrifuged by procedure A.

After ultracentrifugation, the top fraction was taken off in a vol. of 1.0 ml from each tube by procedure B. NaCl solution of density 1.063 g/ml was then layered upon the combined volumes of the top fractions to a final vol. of 9.0 ml, and the whole was ultracentrifuged by procedure A.

Upon completion of ultracentrifugation, the top fraction was taken off in a vol. of 1.0 ml by procedure B. This fraction was then washed twice by ultracentrifugation under the same conditions after addition of 8.0 ml of NaCl solution of density 1.063 g/ml.

The top 1.0 ml fraction, obtained by procedure B after the final ultracentrifugation, constituted Golgi particle fraction II (fraction GP II). This fraction contained Golgi particles of density 1.007–1.063 g/ml, and amounted to $118.2 \pm 22.9 \mu\text{g}$.

Isolation of serum lipoproteins. Serum was prepared from each blood sample as outlined by Mills *et al.* (1972). VLD (density <1.007 g/ml, $S_r < 20$) and LD (density 1.007–1.063 g/ml, $S_r 0-20$) lipoproteins were isolated from serum by preparative ultracentrifugation as described by the above authors.

Analytical methods. The lipid and protein components of the Golgi particle and serum lipoprotein fractions were separated after overnight extraction in 20 ml of ethanol–diethyl ether (3:1, v/v) as described by Mills *et al.* (1972). The procedures outlined by these authors were employed to fractionate and quantitatively determine the lipid components in each extract. Protein was determined by the method of Lowry *et al.* (1951).

Gas-liquid chromatography. Samples of the hexane extracts of the alkaline hydrolysates from the chole-

steryl ester, triglyceride and phospholipid fractions were taken for analysis by g.l.c. The methyl esters of each fraction were prepared by esterification with diazomethane, and analysed on columns of polyethylene glycol succinate as stationary phase, employing an argon detector at about 160°C . The areas of the peaks on each chromatogram were automatically integrated by a Honeywell precision integrator linked to the chromatograph (Pye, Cambridge, U.K.). The identification of the peaks was confirmed by comparison with standard mixtures (Applied Science Laboratories, State College, Pa., U.S.A.).

Immunological analyses. Antisera to guinea-pig VLD and LD serum lipoproteins were prepared in New Zealand white rabbits. The antigen preparations of serum VLD lipoproteins and LD lipoproteins contained approx. 0.3 and 2.5 mg of protein respectively. Pooled guinea-pig serum (50 ml) was employed in the isolation of these preparations. Each lipoprotein preparation (3–5 ml) was concentrated in Visking bags to approx. 1.5 ml by pervaporation; this involved passing a stream of air over the bag for about 1 h at room temperature. The preparation was then emulsified with 2 ml of Freund's adjuvant. This emulsion was then injected intramuscularly. Booster injections of the preparations were administered during the second and third weeks after the initial injection. One week after the last injection, 20 ml of blood was collected from the ear vein of each rabbit, and subsequently at intervals of 2–3 weeks.

Immunodiffusion was performed in gels of 1% agarose by the method of Ouchterlony (1968). The gels were made up in a solution containing 25 mm-phosphate and 0.15 M-NaCl, at pH 8.0 and an ionic strength of 0.23.

Results

Electron microscopy and enzymic activity of the Golgi apparatus cell fraction

Electron microscopy of the Golgi apparatus cell fraction from guinea-pig liver showed it to contain numerous Golgi apparatus, together with large numbers of associated smooth-surfaced vesicular and saccular profiles. Several of the larger dilated Golgi cisternae contained electron-dense granules, 10–80 nm in diameter (Plate 1). At least 60% of these granules had diameters in the range 35–60 nm. The degree of contamination of this cell fraction by other hepatocyte organelles was estimated as being less than 5%.

The activity of a marker enzyme for the Golgi apparatus, UDP-galactose-*N*-acetylglucosamine galactosyltransferase (Fleischer, 1969), was substantially enriched in the Golgi apparatus-rich cell

Table 1. *Weight % chemical compositions of hepatocyte Golgi particles and serum lipoproteins from the guinea pig*

Values are the mean wt. % of each component $\pm$ S.D. The number of preparations analysed is given in parentheses. The quantity of cholesteryl esters was calculated as $1.67 \times$ the quantity of ester cholesterol.

Fraction	Density (g/ml)	Cholesteryl esters A	Free cholesterol B	Triglycerides	Phospholipids	Protein	A/B
GP I (4)	<1.007	2.8 ± 0.9	3.0 ± 0.2	66.2 ± 1.8	19.9 ± 1.5	8.1 ± 2.9	0.93 ± 0.2
Serum VLD lipoproteins (3)	<1.007	1.5 ± 0.7	4.2 ± 0.1	73.1 ± 0.2	13.7 ± 0.2	7.4 ± 0.7	0.36 ± 0.1
GP II (4)	1.007–1.063	4.8 ± 1.3	7.9 ± 1.3	18.5 ± 6.0	48.1 ± 8.3	20.7 ± 8.0	0.61 ± 0.04
Serum LD lipoproteins (3)	1.007–1.063	37.5 ± 5.5	4.2 ± 0.3	18.1 ± 2.3	16.8 ± 0.5	23.4 ± 3.0	8.93 ± 0.6

Table 2. *Chemical compositions of hepatocyte Golgi particles and serum lipoproteins from the guinea pig*

The data 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. The molecular weight of the protein moiety is that of the human LD apolipoprotein (Pollard *et al.*, 1969), and the molecular weights of the lipid esters were calculated from their respective mean fatty acid distributions.

Component	Golgi particle fraction I	Golgi particle fraction II	Serum VLD lipoproteins	Serum LD lipoproteins
Cholesteryl esters (647)	14	9	9	65
Free cholesterol (387)	25	27	39	13
Triglycerides (863)	256	27	311	24
Phospholipids (800)	83	75	63	24
Protein (27000)	1	1	1	1

fraction, as compared with that in the homogenate of guinea-pig liver. Thus, the specific activity (units of specific activity are nmol of *N*-acetylaminolactose synthesized/h per mg of protein at 37°C) of the preparation of Golgi apparatus (32.8) was some 100-fold greater than that of the liver homogenate (0.3).

Chemical composition of Golgi particles

The weight % chemical composition of the two species of Golgi particles is presented in Table 1. The composition of these species is compared with that of the serum lipoproteins of corresponding density, i.e. VLD lipoproteins and LD lipoproteins, from the same animals.

The proportions of both cholesteryl esters and free cholesterol were similar in fraction GP I and in the serum VLD lipoproteins. In addition, the protein content of these fractions was alike (8.1 and 7.4% respectively). However, the proportion of triglyceride was significantly lower, and that of phospholipid

higher, in fraction GP I as compared with the serum VLD lipoproteins.

The weight % composition of fraction GP II differed markedly from that of the Golgi particles of lower density, and moreover, from that of both serum lipoprotein species. In particular, its phospholipid content was remarkably high (48.1%). Further, the phospholipid and protein moieties of this species accounted for almost 70% (68.9) of its weight.

The composition of the serum LD lipoproteins was clearly distinguished from that of both fractions of Golgi particles by its high content of cholesteryl esters (37.5%).

Thus, a comparison of the chemical composition of fraction GP II with the other fractions on the basis of weight gave little indication of any relationship. However, some similarities between the composition of fraction GP II and fraction GP I became evident when the data were expressed on a molar basis (Table 2). In addition, the molar composition of fraction GP II resembled that of the serum VLD lipoproteins.

Table 3. *Weight % fatty acid composition of the lipid esters of hepatocyte Golgi particles and serum lipoproteins from the guinea pig*

Values are the mean wt. % of each fatty acid $\pm$ s.d. The number of preparations analysed is given in parentheses. The total % of fatty acids in each lipid ester of the various fractions may not total 100 due to the omission of fatty acids present in trace amounts (generally $<1\%$).

Lipid ester	Fraction	Density (g/ml)	Fatty acids							
			16:0	16:1	18:0	18:1	18:2	18:3	20:4	
Triglyceride	GPI (4)	<1.007	22.2 ± 2.1	5.8 ± 4.2	10.1 ± 3.0	23.6 ± 5.7	20.6 ± 10.9	8.7 ± 6.1	1.5 ± 0.9	
	Serum VLD	<1.007	19.7 ± 1.5	2.4 ± 0.7	6.1 ± 1.2	12.0 ± 9.6	32.6 ± 9.6	15.9 ± 4.0	1.1 ± 1.0	
	lipoproteins (3)									
	GP II (4)	$1.007-1.063$	26.3 ± 6.1	11.1 ± 3.5	13.4 ± 1.3	26.5 ± 3.2	9.0 ± 3.3	6.5 ± 4.3	0.7 ± 1.4	
Cholesteryl ester	Serum LD	$1.007-1.063$	21.3 ± 3.2	3.3 ± 0.1	11.2 ± 3.2	23.8 ± 4.0	24.7 ± 5.8	9.4 ± 3.3	1.9 ± 1.2	
	lipoproteins (3)									
	GPI (4)	<1.007	23.3 ± 1.7	14.1 ± 4.2	16.0 ± 5.8	26.6 ± 4.4	7.4 ± 5.1	2.2 ± 4.3	1.9 ± 3.8	
	Serum VLD	<1.007	29.9 ± 2.1	4.9 ± 1.0	21.1 ± 5.7	23.1 ± 5.7	13.2 ± 9.8	2.7 ± 2.4	0.3 ± 0.6	
Phospholipid	lipoproteins (3)									
	GP II (4)	$1.007-1.063$	22.9 ± 4.5	13.1 ± 2.5	17.4 ± 3.3	25.5 ± 3.3	4.2 ± 1.1	9.4 ± 9.1	0.3 ± 0.5	
	Serum LD	$1.007-1.063$	9.2 ± 1.6	2.7 ± 1.3	6.5 ± 6.6	15.2 ± 1.3	60.9 ± 10.8	2.5 ± 0.9	1.3 ± 0.9	
	lipoproteins (3)									
Phospholipid	GPI (4)	<1.007	25.5 ± 5.1	6.3 ± 4.8	24.9 ± 8.1	23.7 ± 3.9	9.9 ± 5.3	1.6 ± 1.1	3.4 ± 1.3	
	Serum VLD	<1.007	11.7 ± 4.6	2.0 ± 1.1	27.4 ± 1.5	16.5 ± 1.9	30.3 ± 2.6	4.5 ± 0.9	4.1 ± 0.7	
	lipoproteins (3)									
	GP II (4)	$1.007-1.063$	23.8 ± 3.3	8.0 ± 1.3	23.3 ± 3.2	22.4 ± 2.9	10.6 ± 2.6	1.5 ± 0.8	2.2 ± 1.1	
Phospholipid	Serum LD	$1.007-1.063$	15.0 ± 1.1	3.2 ± 0.5	27.7 ± 0.2	16.6 ± 1.3	25.0 ± 1.0	2.5 ± 1.0	4.6 ± 0.8	
	lipoproteins (3)

EXPLANATION OF PLATE I

Electron micrograph of a section from the pellet of the Golgi apparatus cell fraction from guinea-pig liver

A single Golgi profile (G) is shown; the innermost cisterna is grossly dilated at its terminal end and contains numerous electron-dense granules, which are approximately spherical in shape and range from 10 to 80nm in diameter. Two vesicles (V) containing electron-dense granules of similar dimensions to those within the adjacent cisternae are prominent. Large saccular elements (S) are also present. The bar represents 200nm.

EXPLANATION OF PLATE 2

Immunodiffusion analysis of hepatocyte Golgi particles of density <1.007 g/ml

A, Antiserum to guinea-pig serum VLD lipoprotein; B, guinea-pig serum VLD lipoprotein; C, Golgi particles of density <1.007 g/ml (fraction GP I).

EXPLANATION OF PLATE 3

Immunodiffusion analysis of hepatocyte Golgi particles of density 1.007–1.063 g/ml

A, Whole guinea-pig serum; B, antiserum to guinea-pig serum VLD lipoprotein; C, Golgi particles of density 1.007–1.063 g/ml (fraction GP II); D, guinea-pig serum LD lipoprotein; E, antiserum to guinea-pig serum LD lipoprotein; F, antiserum to whole guinea-pig serum.

The data show that the molar composition of fraction GP II strongly resembles that of both fraction GP I and of the serum VLD lipoproteins in all but its triglyceride content; that of fraction GP I and the serum VLD lipoproteins are some ten- and eleven-fold greater respectively. Further, an overall similarity between the molar compositions of fraction GP I and the serum VLD lipoproteins is clearly evident.

In summary, a close resemblance between the chemical compositions of fraction GP I and the serum VLD lipoproteins has been established. The molar compositions of fraction GP II and of the serum VLD lipoproteins were markedly alike. Some similarity was evident between the compositions of the two species of Golgi particles when expressed as a molar ratio with respect to protein.

Fatty acid compositions of the lipid esters

The fatty acid compositions of the triglycerides, cholesteryl esters, and phospholipids of the Golgi particles were compared with those in the corresponding components of the serum lipoproteins (Table 3).

The distributions of fatty acids in the triglycerides of the serum VLD lipoproteins and fraction GP I resembled each other. However, whereas the proportion of the 18:1 acid was somewhat higher, and that of the 18:2 acid lower, in fraction GP I relative to the serum VLD lipoproteins, together these acids amounted to 44% of the total fatty acids in both species. Moreover, the respective proportions of these acids were particularly variable, as indicated by their high standard deviations. Although some similarity was noted between the patterns of cholesteryl ester fatty acids in these species, the phospholipid fatty acids were not alike in distribution. In particular, the proportions of the 18:2 acid in the phospholipid moiety were notably dissimilar.

The fatty acid patterns in the lipid esters of fraction GP II were not similar to those of the lipoproteins of the same density from serum, i.e. LD lipoproteins. However, the distribution of fatty acids in each lipid moiety of fraction GP II closely resembled that in the same moiety of fraction GP I.

The fatty acid patterns of the triglycerides and cholesteryl esters of the two density classes of serum lipoproteins were quite different, but there was a marked similarity between their phospholipid fatty acid distributions.

Immunochemical analyses

Golgi particles of density <1.007 g/ml (fraction GP I) produced a single strong precipitin line against antiserum to guinea-pig serum VLD lipoproteins, i.e. anti-VLD lipoproteins (Plate 2). This precipitin line fused with that formed between guinea-pig

serum VLD lipoprotein and the same antiserum in a reaction of identity. These lines stained strongly for both lipid and protein components.

Upon immunodiffusion of antisera to guinea-pig serum lipoproteins against Golgi particles of density 1.007–1.063 g/ml, no precipitin reactions were observed. The lack of reaction may have been due to the small quantities of Golgi LD particles that were isolated (approx. $100\mu\text{g}$), of which less than $10\mu\text{g}$ were available for immunochemical analysis. Thus, subsequent investigations in guinea pigs fed a high lipid diet supplemented with cholesterol have enabled larger quantities of this species to be isolated (approx. $700\mu\text{g}$) (Chapman, 1970). Golgi LD particles (fraction GP II) from such animals produced a single precipitin line against anti-VLD lipoprotein, which fused in a reaction of identity with that formed between the same antiserum and whole guinea-pig serum (Plate 3). In addition, a single precipitin reaction was observed between fraction GP II and antiserum to guinea-pig serum LD lipoproteins (anti-LD lipoproteins). This precipitin line fused with that produced between the same antiserum and guinea-pig serum LD lipoprotein. Reaction of fraction GP II against antiserum to whole guinea-pig serum resulted in production of a single precipitin line, which fused in a reaction of identity with that formed between fraction GP II and anti-LD lipoprotein. All of these lines stained for both lipid and protein components. The additional precipitin reactions produced between antiserum to whole guinea-pig serum and its antigen stained only for protein.

We have reported (Mills *et al.*, 1972) that one principal apolipoprotein is present throughout the whole range of lipoproteins of density <1.100 g/ml in guinea-pig serum. The present data show that Golgi VLD particles are immunochemically identical with serum VLD lipoproteins and possess the same apolipoprotein. Further, it has been established that the Golgi LD particles possess the same antigenic determinant as that characteristic of both the VLD and LD lipoproteins of guinea-pig serum. This evidence supports the conclusion that the Golgi VLD and LD particles share a common antigenic determinant, and therefore probably contain the same apolipoprotein.

Discussion

The morphological and enzymic characteristics of the Golgi apparatus cell fraction from guinea-pig liver indicate it to be enriched in Golgi apparatus. Morphologically, this fraction closely resembled similar preparations from both rat (Mahley *et al.*, 1969; Fleischer & Fleischer, 1970; Morré *et al.*, 1970), and bovine liver (Fleischer *et al.*, 1969). Enzymically, the fraction was similar to Golgi apparatus-rich preparations from rat and bovine

liver in exhibiting a high UDP-galactose-*N*-acetylglucosamine galactosyltransferase activity (Fleischer & Fleischer, 1970; Morré *et al.*, 1970; Fleischer *et al.*, 1969).

As in the rat (Mahley *et al.*, 1969), our studies in the guinea pig have shown that VLD particles from the hepatic Golgi apparatus exhibited a gross chemical composition which resembled that of the serum lipoproteins of similar density, i.e. VLD lipoproteins. In addition, these particles were immunochemically indistinguishable from the serum VLD lipoproteins. Further, the range of diameters of the electron-dense particles within the Golgi cisternae (10–80 nm) was similar to serum VLD lipoproteins in the rat (30–60 nm) (Hamilton *et al.*, 1967; Ockner & Jones, 1970) and in man (30–70 nm) (Oncley, 1963; Lindgren & Nichols, 1960; Nichols, 1969).

The chemical compositions of the Golgi VLD particles and serum VLD lipoproteins exhibited some small differences, principally in the proportions of phospholipid and triglyceride. A difference in the overall density distributions of the Golgi VLD particles and serum VLD lipoproteins may partially account for this observation. Morphological examination of the Golgi apparatus cell fraction indicated the existence of a wide range of particle sizes, which would give rise to such a density distribution (Lindgren & Nichols, 1960). Differences in the fatty acid patterns of the lipid components of the Golgi VLD particles and serum VLD lipoproteins were also seen, although an overall similarity was noted in the fatty acid distributions of both the triglyceride and cholesteryl ester components. As the VLD lipoproteins present in the serum pool originate in the intestine (Ockner *et al.*, 1969*a,b*; Windmueller *et al.*, 1970) as well as the liver (Schumaker & Adams, 1969), some differences in the fatty acid distributions of the two VLD populations might be expected. However, the lipid moieties of the nascent lipoproteins secreted from these organs become subject to modification and exchange reactions upon entering the circulation (Nichols, 1969; Schumaker & Adams, 1969; Lopez-S, 1971). These reactions may therefore facilitate the transformation of the Golgi VLD particles to a lipoprotein species resembling that characteristic of the serum compartment.

Thus, the morphological, flotation, chemical and immunochemical characteristics of the Golgi VLD particles support the concept that they may be the precursors of serum VLD lipoproteins in the guinea pig.

The presence of an LD lipoprotein particle in a liver-cell fraction rich in Golgi apparatus has not been previously reported, and the significance of this Golgi lipoprotein species is less clear than that of the Golgi VLD particles. Thus, the gross chemical composition of the Golgi LD particles (density 1.007–

1.063 g/ml) was markedly dissimilar to that of the serum LD lipoproteins in these animals. In particular, the Golgi particles were much richer in phospholipid, which amounted to 48.1% of their weight, in direct contrast with the serum LD lipoproteins, which had a threefold lower content (16.9% by weight). The Golgi LD particles were, however, immunochemically indistinguishable from the serum LD lipoproteins. The distributions of fatty acids in the cholesteryl ester and phospholipid moieties of the Golgi LD particles and serum LD lipoproteins were quite distinct. The high content of cholesteryl esters in the serum LD lipoproteins (37.5%) also distinguished it from the Golgi LD species, whose content was sevenfold lower (4.8%). It therefore seems improbable that the Golgi LD particles might be secreted directly into the serum pool as the precursors of the serum LD lipoproteins.

The gross chemical compositions of the Golgi LD and VLD particles were similar in a number of ways. Thus, the fatty acid patterns of the lipid moieties of these two species of Golgi particles were remarkably alike, and the molar proportions of the chemical components of the two particle species were similar in all but the triglyceride moiety. In addition, immunochemical analysis of the Golgi LD particles supports the conclusion that they share a common antigenic determinant with the VLD particles, and probably a common apolipoprotein. In view of the overall similarity of the Golgi LD and VLD particles, it may be postulated that the LD particles are precursors of the VLD particles within the Golgi apparatus, into which they are transformed by the addition of triglyceride. The LD particle species might therefore represent a fundamental lipoprotein particle, corresponding to the terminal product of lipoprotein synthesis in the smooth endoplasmic reticulum. Evidence for a sequential formation of lipoprotein particles has been derived from electron microscopic studies (Jones *et al.*, 1967; Hamilton *et al.*, 1967; Claude, 1970).

However, on the present evidence, it seems equally possible that the LD particles arise as a result of the removal of triglyceride from the VLD particles. Loss of triglyceride from these particles might occur during the disruptive procedure used to release them from the Golgi cisternae. Thus, Zilversmit (1965) has shown that freezing and thawing chylomicrons dissociates them into a phospholipid-rich membrane pellet and an oil layer of triglyceride. In the present studies, a triglyceride-containing lipid layer did not appear during the ultracentrifugal separation of the nascent lipoprotein particles from the disrupted Golgi membranes. It therefore seems unlikely that the LD particles arose from the VLD particles as a result of their mechanical disruption.

Removal of triglyceride from the VLD particles might also result from the action of a triglyceride

lipase. Such lipase activity has not yet been found localized in hepatocyte Golgi membranes, although these membranes do contain phospholipase activity (Van Golde *et al.*, 1971). Triglyceride lipases are, however, present in hepatic microsomal elements and lysosomes (Guder *et al.*, 1969), which are present as contaminants (<5%) of the Golgi apparatus cell fraction. The Golgi particles might therefore be accessible to such lipase action during the period of the disruption procedure.

Evidence in support of the conclusion that the LD particles are normally present within the Golgi apparatus of guinea-pig hepatocytes derives from the observation that the range of particle diameters within the Golgi cisternae was approx. 10 to 80nm. Thus lipoproteins of the same density (i.e. 1.007–1.063g/ml) from human serum possess diameters in the range from 17 to 26nm (Forte *et al.*, 1968), whereas those of lower density (<1.007g/ml) have a minimum diameter of 30nm (Lindgren & Nichols, 1960; Nichols, 1969). These data are therefore consistent with the conclusion that lipoprotein particles of low density (1.007–1.063g/ml) are present within the Golgi cisternae before their disruption.

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