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Characterization of the Serum Lipoproteins and their Apoproteins in Hypercholesterolaemic Guinea Pigs

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1. Hypercholesterolaemia was induced in male guinea pigs after 6 days on a chow diet supplemented with 1.6% (w/w) cholesterol and 15% (w/w) corn oil. Both the VLD (very-low-density) and LD (low-density) lipoproteins were increased in cholesterol-fed animals, although the low concentrations of HD (high-density) lipoproteins remained essentially unchanged. LD lipoproteins of *d* 1.019–1.100 were the major class, accounting for 74% of the total substances of $d<1.100$. 2. Both VLD and LD lipoproteins exhibited alterations in their chemical composition, physical properties and apolipoprotein content. The VLD lipoproteins in cholesterolaemic animals were rich in cholesterol (25.9%), deficient in protein (4.9%) and exhibited electrophoretic mobility greater than that of β-globulin; their average particle size (64.5 nm) was larger than that in controls (46.3 nm). The LD lipoproteins in animals fed on the experimental diet were also richer in cholesterol (53.1%) and of larger diameter (24.3 nm) than in the control group (41.1% and 21.4 nm respectively). 3. The apolipoprotein-B content of both VLD and LD lipoproteins was elevated in cholesterolaemic animals, particularly in the VLD class, where it represented 74.8% of the total protein moiety. 4. Apo-VLD lipoprotein exhibited an increase from 6 to 19% in its complement of tetramethylurea-soluble apolipoproteins with low electrophoretic mobility (relative mobility <0.29); this was primarily accounted for by apolipoproteins characterized by high arginine (7.2 and 6.4% respectively) and glutamic acid (20.1 and 20.0% respectively) contents. 5. By contrast, there was little change in the soluble apolipoproteins of LD lipoproteins in hypercholesterolaemic animals. 6. These studies show the response of the guinea pig to dietary fat and cholesterol to be distinct from that elicited by similar stimuli in the rabbit, rat, pig and dog.

The earliest detailed study of the serum lipoproteins and their apoproteins in diet-induced hypercholesterolaemia was that of Shore *et al.* (1974) in the rabbit. These investigators observed a rapid and dramatic change in the serum lipoprotein distribution in animals fed on a 1% cholesterol diet, which was characterized by the occurrence of large amounts of β-VLD lipoproteins.‡ These lipoproteins contained elevated proportions of both cholesteryl esters and an arginine-rich apolipoprotein.

It now appears that the formation and accumulation of serum β-VLD lipoproteins in diet-induced hypercholesterolaemia is characteristic not only of the rabbit but also of miniature pigs (Hill *et al.*, 1975; Mahley *et al.*, 1975) and of hyper-responding dogs (Mahley & Weisgraber, 1974; Mahley *et al.*, 1974).

‡ Abbreviations: VLD lipoprotein, very-low-density lipoprotein ($d<1.007$); LD lipoprotein, low-density lipoprotein; HD lipoprotein, high-density lipoprotein. Apolipoprotein nomenclature is that of Alaupovic (1972). β-VLD lipoprotein is defined as VLD lipoprotein with mobility equal to that of β-globulin.

It should, however, be noted that an abnormal type of HD lipoprotein, 'HDL_c', which is enriched in cholesterol and an arginine-rich apolipoprotein, also characterizes the hypercholesterolaemia of the latter species.

In man, however, β-VLD lipoproteins have only been observed in the comparatively uncommon disease of primary dysbetalipoproteinaemia (Fredrickson's Type III hyperlipoproteinaemia) (Havel & Kane, 1973). These β-VLD lipoproteins were believed to represent an accumulation of the degradation products ('remnants') of chylomicrons and VLD lipoproteins, a hypothesis that has been substantiated in supradiaphragmatic rats by Mjos *et al.* (1975).

In contrast with these animals, the guinea pig typically responds to acute administration of a diet supplemented with cholesterol (and unsaturated fat) almost exclusively by increasing its LD lipoproteins (Chapman, 1970; Mills *et al.*, 1972). Some aspects of the metabolism and structure of the serum LD lipoproteins in guinea pigs fed on cholesterol for a

short period closely resemble those occurring in certain cases of familial hypercholesterolaemia in man (Mills *et al.*, 1972; Mills & McTaggart, 1974; Mills *et al.*, 1976). This condition is distinct from that which follows prolonged (10–12 weeks) intake of a semisynthetic diet containing 1% cholesterol, since such guinea pigs exhibit a haemolytic anaemia which is accompanied by the appearance of several species of abnormal lipoproteins (Sardet *et al.*, 1972).

In view of our continuing interest in the hypercholesterolaemic guinea pig (*Cavia porcellus*) as an experimental model for the human disease, and as part of a study of the metabolism of the serum lipoproteins in the diet-induced hypercholesterolaemia of this animal, we undertook a qualitative and quantitative investigation of the serum lipoproteins in these animals, the results of which form the subject of this report.

A preliminary communication of this work was presented at the International Conference on the Biochemistry of Lipids in Graz (Chapman & Mills, 1975).

Experimental

Materials

Sets of purified proteins, for use as molecular-weight markers in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, were supplied by Boehringer Mannheim G.m.b.H. (Mannheim, W. Germany) and by Schwarz/Mann (Orangeburg, NY, U.S.A.). Phosphotungstic acid (analytical grade) was obtained from E. Merck, Darmstadt, Germany. Refined edible corn oil and cholesterol, for inclusion in the lipid-rich diet, were purchased from Loders and Nucoline, London E.C.4, U.K., and BDH, Poole, Dorset, U.K., respectively. All additional materials used have been previously detailed (Chapman *et al.*, 1975).

Animals and diets

Male guinea pigs (approx. 500–750g), bred randomly in the laboratory colony at the Courtauld Institute, were fed on a diet of rabbit pellets *ad libitum*. This diet, whose composition has been previously described (Chapman *et al.*, 1975), was supplemented with hay and green vegetables. Animals fed on this dietary regimen are referred to as the 'controls' or 'normals' and their diet in the same terms.

The lipid-rich or experimental diet consisted of the rabbit pellets to which was added 1.6% cholesterol and 15% corn oil (by wt.). Before introduction of the lipid-rich diet, a blood sample was removed from each animal by cardiac puncture under light ether anaesthesia after overnight starvation. The animals, normally a group of two to five, were then fed on the lipid-rich diet *ad libitum* for 6 days. After this period, they were starved overnight and a second blood sample was taken on the following morning by the same procedure.

Methods

Blood samples. Serum was separated by low-speed centrifugation (50000*g*-min) after clotting at room temperature (20–23°C). Merthiolate (0.001%, w/v) and NaN_3 (0.01%, w/v) were then added to the serum. Samples shipped to France were normally refrigerated in ice. Isolation of lipoproteins was normally commenced immediately on arrival. Only when serum samples were intended for isolation of apolipoproteins were they pooled.

Quantitative ultracentrifugal analysis of serum. The lipoprotein content of serum samples was quantitatively determined by ultracentrifugal analysis at solvent densities of 1.063 for detailed determination of VLD lipoproteins, and otherwise at *d* 1.100, as described earlier (Mills *et al.*, 1972). For the purpose of the calculations, it was assumed that the physical characteristics of the guinea-pig serum lipoproteins were the same as those of their human counterparts. Flotation rates at a solvent density of 1.100 are denoted by F; calculation showed that F values of 0, 22 and 33S correspond to hydrated densities of 1.100, 1.019 and 1.007 respectively.

Isolation of serum lipoproteins. Lipoprotein fractions were separated from the sera by sequential ultracentrifugation (Havel *et al.*, 1955), and were as follows: VLD lipoproteins of $d<1.007$; LD lipoproteins of *d* 1.007–1.100; HD lipoproteins of 1.100–1.21. The exact procedure used was outlined previously (Chapman *et al.*, 1975); the use of a higher limiting density of 1.100 for complete isolation of LD lipoprotein has been explained elsewhere (Chapman *et al.*, 1975).

Purity of lipoprotein fractions. The purity of each of the lipoprotein fractions was evaluated in two ways. (1) Agarose-gel electrophoresis, by the method of Noble (1968), showed LD lipoproteins with mobility equal to that of β-globulin, and VLD lipoproteins of higher mobility. No contamination of either of these fractions with HD lipoproteins could be detected. Additional data are provided in the Results section. (2) Immunological studies [immunodiffusion (Ouchterlony, 1953) and immunoelectrophoresis (Grabar & Williams, 1955)] with rabbit antisera to guinea-pig albumin and to guinea-pig whole serum (Nordic Immunological Laboratories, Maidenhead, Berks, U.K.) showed the absence of contaminating serum proteins from the various lipoprotein fractions.

Owing to the very low concentrations of HD lipoproteins in the sera of guinea pigs fed on either the normal or the lipid-rich diet, insufficient amounts

were obtained for evaluation of purity or for chemical analysis.

Electron-microscopic studies. Preparations of VLD and LD lipoproteins containing up to 0.2mg of protein/ml were negatively stained with 2% (w/v) phosphotungstate at pH7.4 (Forte *et al.*, 1968), as outlined previously (Chapman & Goldstein, 1976). Grids were examined with a Philips EM 300 electron microscope; photomicrographs were taken at magnifications ranging from 31000 to 75500.

Delipidation of lipoproteins. The lipoprotein fractions were delipidated with ethanol/diethyl ether (3:1, v/v) essentially by the method of Brown *et al.* (1969) as described previously (Chapman *et al.*, 1975). The final residues were washed in peroxide-free diethyl ether at 4°C, dried under N_2 and dissolved in either solution A, which contained sodium dodecyl sulphate (1%, w/v), 2-mercaptoethanol (1%, v/v), NaN_3 (0.01%, w/v) and sodium phosphate (0.01M) buffer, pH8.0, or a 0.01M-Tris/HCl buffer containing 8M-urea at pH8.0, in preparation for polyacrylamide-gel electrophoresis; guinea-pig apolipoprotein B was insoluble in the latter solution. Final protein concentrations varied from 1 to 5mg/ml; protein contents were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as calibration standard. Controls were included to correct for any contribution of the solubilization buffer to colour yield at 750nm.

Extraction with tetramethylurea. The tetramethylurea procedure of Kane (1973) was used under the conditions of Chapman *et al.* (1975) to determine the proportion of apolipoprotein B in guinea-pig serum lipoproteins.

Chemical analyses of lipoprotein fractions. The chemical composition of each lipoprotein fraction was determined by the procedures outlined by Mills *et al.* (1972).

Gel-filtration chromatography. Samples (3–5mg) of apo-LD lipoprotein (*d* 1.007–1.100), solubilized in solution A, were chromatographed on a column of Sephadex G-150 (55cm×0.9cm). The column was equilibrated and eluted with a buffer containing Tris/HCl (0.01M), sodium decyl sulphate (0.002M), NaN_3 (0.01%, w/v) and merthiolate (0.001%, w/v) at pH8.0; flow rates varied from 10 to 15ml/h. The effluent was continuously monitored at 280nm with a Gilson Spectrochrom MD; fractions (2–3ml) were collected on a Gilson micro-fractionator. The fractions in each peak were subsequently pooled and freeze-dried; the dried material from each peak was then dissolved in a volume of water which resulted in final protein and sodium decyl sulphate concentrations of 1–3mg/ml and 2–10mM respectively. Complete and rapid solubilization of the apolipoprotein-B component was achieved with this procedure. It is noteworthy that excess unbound sodium dodecyl sulphate was separated from the apoprotein by this method, since it was eluted at the lower limit of the included volume of the column.

Samples of the solubilized peak materials were taken for determination of protein content by the method of Lowry *et al.* (1951), and for determination of amino acid composition.

Electrophoresis. The protein components of guinea-pig serum lipoproteins were studied by (1) electrophoresis in sodium dodecyl sulphate/polyacrylamide gel (Weber & Osborn, 1969), (2) the modification by Kane (1973) of the procedure of Davis (1965) and (3) by the procedure of Davis (1965), by using stacking and separating gels at acrylamide concentrations of 3.5 and 7.5% (w/v) respectively; the gels contained urea at a final concentration of 8M. Samples studied by method (2) were extracted with tetramethylurea (Kane, 1973), and those studied by method (3) were dissolved in 0.01M-Tris/HCl/8M-urea as described under 'Delipidation of lipoproteins'. Gels were stained with Coomassie Brilliant Blue and scanned densitometrically by methods detailed previously (Chapman *et al.*, 1975).

Amino acid analysis. Samples (200–500μg) of the apoprotein residues were hydrolysed in 6M-HCl as described earlier (Chapman *et al.*, 1975). Samples of apoproteins dissolved in solution A were freeze-dried before dissolution and hydrolysis under the same conditions.

A modification (Chapman *et al.*, 1975) of the procedure of Houston (1971) was used for the determination of the amino acid composition of individual stained apolipoprotein bands cut from 7.5% (w/v) polyacrylamide gels.

Amino acid analysis was performed on a JEOL JLC 5 AH amino acid analyser (Japan Electron Optics Laboratory Co., Tokyo, Japan), by using a two-column procedure (Moore *et al.*, 1958).

Results

Distribution and concentrations of the serum lipids and lipoproteins

The total serum cholesterol and triacylglycerol contents in guinea pigs fed on the normal and experimental diets are shown in Table 1. The lipid-rich diet effected a significant increase in the total serum concentrations of both triacylglycerol and cholesterol ($P<0.001$ for cholesterol, $0.01>P>0.001$ for triacylglycerol, as determined by Student's *t* test). The serum cholesterol and triacylglycerol concentrations were positively correlated in the experimental animals ($r=0.8228$, $P<0.01$).

The alterations that occurred in the quantitative distribution of the serum lipoproteins when animals were fed on the experimental diet are summarized in Table 2. Thus the concentrations of the VLD lipoproteins ($d<1.007$) were some 10-fold elevated ($P<0.001$), and those of the LD lipoproteins

(d 1.019–1.100) were more than doubled, after administration of the lipid-rich diet ($P<0.001$). It is noteworthy that LD lipoproteins of F 22–33 (i.e. d 1.007–1.019) were only detected in one control animal, and that as a result of the lipid-rich diet the concentration of these lipoproteins rose, but only to amount to 10.2% of the total LD (d 1.007–1.100) substances. Thus, as previously described (Mills *et al.*, 1972), the increase in the LD lipoproteins after cholesterol feeding was primarily in the density interval 1.019–1.100 (i.e. F 0–22) and not in that of d 1.006–1.019 (i.e. F 22–33).

The concentrations of HD lipoproteins (d 1.100–1.21) in the control animals were very low and typically did not amount to more than 5mg/100ml of serum. Their concentrations were not significantly altered by the lipid-rich diet.

Of the 23 animals originally fed on the experimental diet for the 6-day period, six were hyper-responders. Thus they exhibited more than a 60-fold elevation in their VLD-lipoprotein concentrations and a 100-fold increase in their F 22–33 lipoproteins as compared with the controls (Table 3). Further, the F 0–22 lipoprotein concentrations in the hyper-responders (244.3mg/100ml of serum) were rather higher than those in animals considered as giving a typical response (192.6mg/100ml of serum).

Agarose-gel electrophoresis of guinea-pig serum lipoproteins

The electrophoretic mobilities of isolated VLD lipoproteins from normal and experimental guinea pigs were essentially identical in agarose gel (Plate 1*a*). These lipoproteins were seen as a sharp band on lipid staining with Sudan Black. The mobility of such VLD lipoproteins closely resembled that of VLD lipoproteins from human serum (i.e. greater than β-VLD).

The LD-lipoprotein fraction (d 1.007–1.100) from cholesterol-fed animals displayed a similar mobility to that from controls (Plate 1*b*), although it was occasionally present as a rather diffuse band. The mobilities of LD lipoproteins from animals fed on the normal and lipid-rich diets were always greater than those of their human counterpart (Plate 1*b*).

Chemical composition of guinea-pig serum lipoproteins

We have previously described the detailed chemical compositions of serum lipoproteins in guinea pigs fed on the normal and lipid-rich diets (Mills *et al.*, 1972; Chapman *et al.*, 1973).

For the present purposes, the mean chemical compositions (%, by wt.) of preparations of VLD and LD lipoproteins from a (representative) guinea pig fed on the normal and subsequently on the experimental diet are shown in Table 4. It is particularly noteworthy that the proportions of both esterified and free cholesterol were markedly increased in the VLD lipoproteins after the experimental diet; only the free cholesterol component of the LD lipoproteins displayed a similar change. Moreover, the proportions of both protein and triacylglycerol were diminished in these lipoproteins.

Electron-microscopic studies of lipoproteins from animals fed on the normal and lipid-rich diets

Results obtained in electron-microscopic studies of negatively stained VLD ($d<1.007$) and LD (d 1.007–1.100) lipoproteins from guinea pigs fed on the normal

Table 1. *Serum cholesterol and triacylglycerol concentrations in guinea pigs fed on the normal and lipid-rich diets*

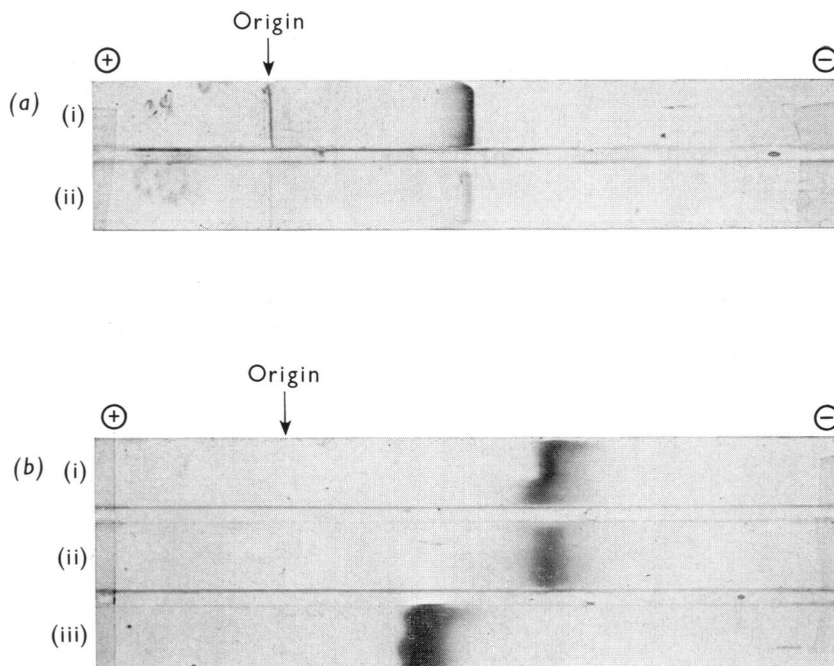
Lipid concentrations were determined on individual serum samples taken from 13 animals before and after feeding of the lipid-rich diet. Values are means±S.D.

	Total serum cholesterol concentration (mg/100ml)		Total serum triacylglycerol concentration (mg/100ml)	
Diet ...	Normal	Lipid-rich	Normal	Lipid-rich
Mean	44±18	214±104	47±21	203±165
Range	12–74	111–488	19–90	76–704

Table 2. *Concentrations of serum low-density lipoproteins in guinea pigs fed on normal and lipid-rich diets*

Values are means±S.D. and are expressed as mg of lipoprotein/100ml of serum within the defined intervals of flotation rate (F); values for density intervals are derived from flotation rates (F) at a solvent density of 1.100 (see under 'Methods' for details). The numbers of animals examined are given in parentheses.

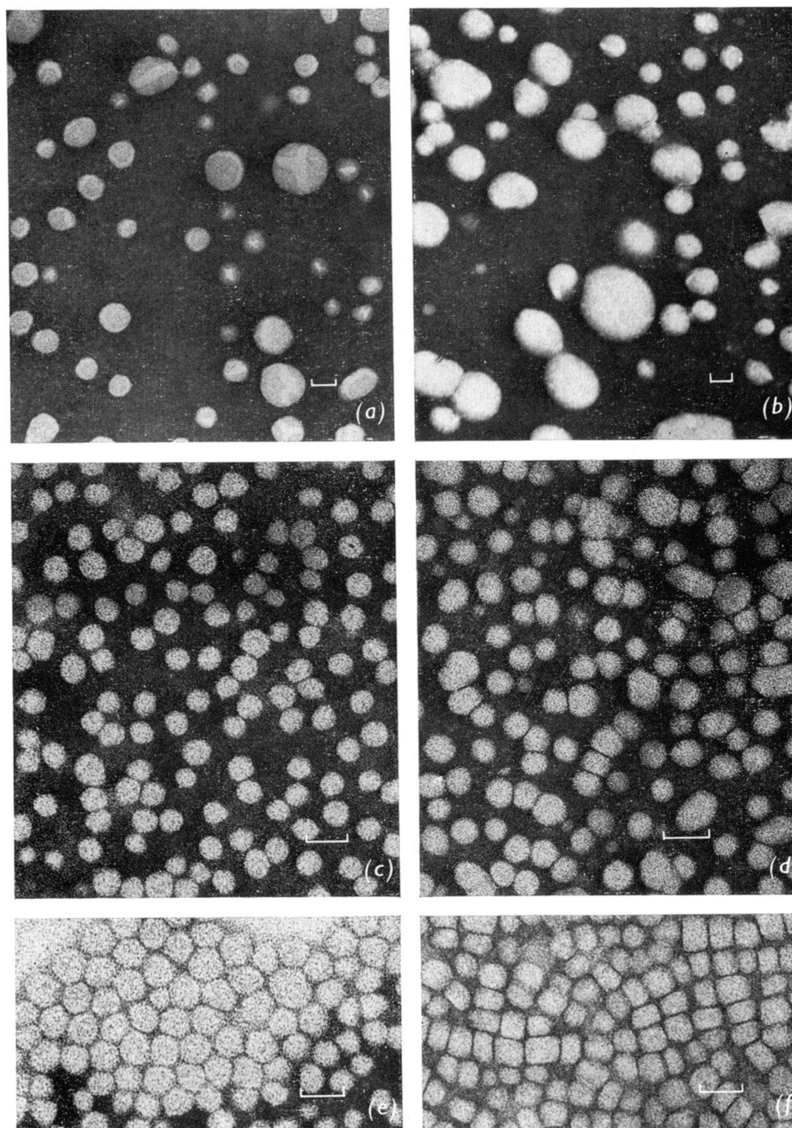
	Lipoprotein concentration					
Diet ...	Normal			Lipid-rich		
$F_{1.100}$ range ...	0–22	22–33	>33	0–22	22–33	>33
Density interval ...	1.019–1.100	1.007–1.019	<1.007	1.019–1.100	1.007–1.019	<1.007
Mean	90±27 (19)	1±4 (19)	4±6 (19)	193±14 (17)	22±4 (17)	44±6 (17)
Range	50–143	0–18	1–24	78–333	0–50	8–108



EXPLANATION OF PLATE I

Electrophoretic patterns in agarose gel of VLD and LD lipoproteins from guinea pigs fed on the normal and lipid-rich diets

Electrophoresis was performed in 1% agarose gel by the procedure of Noble (1968); gels were stained for lipid with Sudan Black. Gel (*a*); samples are VLD lipoproteins (i) from animals fed on the normal diet and (ii) from those fed on the lipid-rich diet. Gel (*b*); samples are LD lipoproteins (*d* 1.007–1.100) from guinea pigs fed on (i) the lipid-rich and (ii) the normal diet; and (iii) LD lipoprotein (*d* 1.024–1.045) from normal human serum.



EXPLANATION OF PLATE 2

Electron photomicrographs of VLD and LD lipoproteins from guinea pigs fed on the normal and lipid-rich diets
Lipoprotein preparations were negatively stained with 2% (w/v) sodium phosphotungstate; the samples shown are considered representative of those studied. Photomicrographs are of VLD lipoproteins from animals fed on (*a*) the normal and (*b*) the lipid-rich diet, and of LD lipoproteins from animals fed on (*c*) the normal and (*d*) the lipid-rich diet; (*e*) and (*f*) illustrate the typical formations seen in preparations of LD lipoproteins from control and experimental animals respectively when stained at protein concentrations greater than about 0.3 mg/ml. Each bar represents 40 nm.

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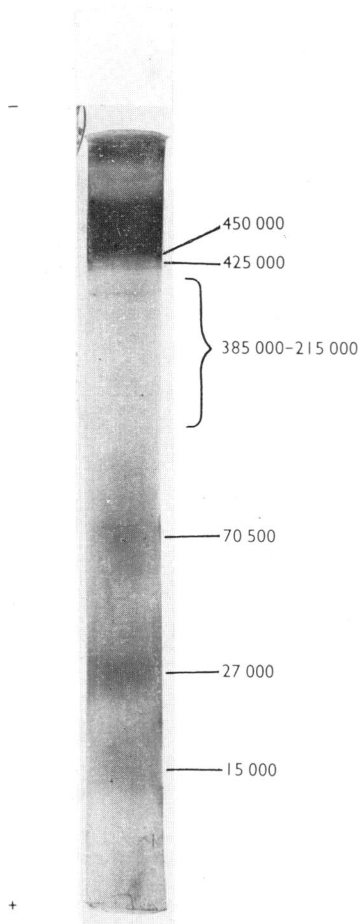

EXPLANATION OF PLATE 3

Sodium dodecyl sulphate/polyacrylamide-gel electrophoretic pattern of apo-LD lipoprotein from guinea pigs fed on the lipid-rich-diet

The pattern is representative of three separate preparations of apo-LD lipoprotein (*d* 1.007–1.100) from the serum of guinea pigs fed on the experimental diet. Samples of each apoprotein preparation were electrophoresed in duplicate and contained 100–200 μg of protein. The molecular weights of the stained protein bands were calculated as described by Weber & Osborn (1969) from a series of purified proteins of known molecular weight; such calibration standards were electrophoresed in each series of gels. Gels were stained with Coomassie Brilliant Blue.

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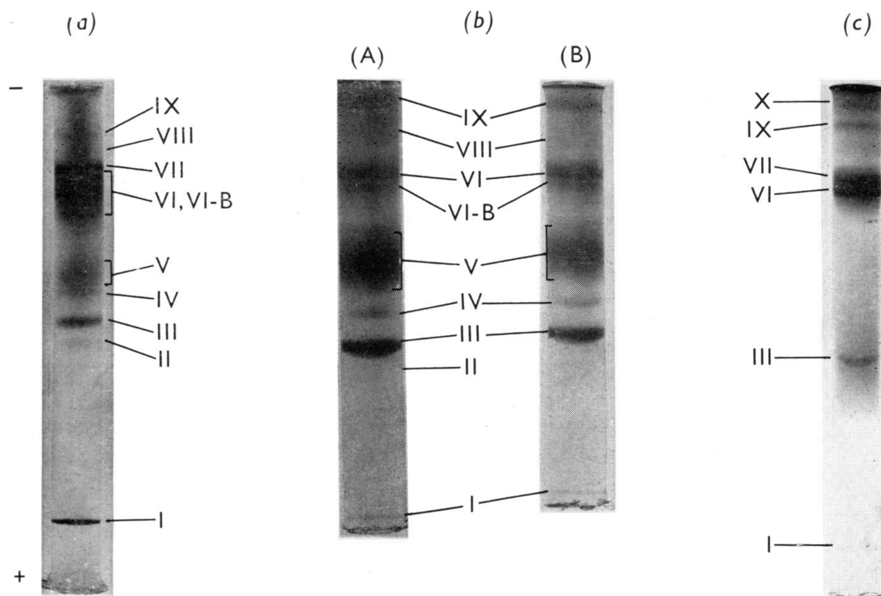

EXPLANATION OF PLATE 4

Polyacrylamide-disc-gel patterns of 'soluble' apolipoproteins from the VLD, LD and HD lipoproteins of guinea pigs fed on the lipid-rich diet

Apolipoprotein samples (100–150μg) were electrophoresed in 7.5% (w/v) polyacrylamide gels containing 8M-urea at pH8.9 and in the presence of reducing agents; gels were stained with Coomassie Brilliant Blue. The patterns are representative of five separate preparations of VLD and LD lipoproteins, and two of HD lipoproteins; samples were normally electrophoresed in duplicate or triplicate. The nomenclature used for the individual bands is that described previously for apolipoproteins from animals fed on the normal diet (Chapman *et al.*, 1975). (*a*) Tetramethylurea-soluble extract of VLD lipoprotein (d<1.007). (*b*) LD lipoprotein (d 1.007–1.100); (A) tetramethylurea-soluble extract applied; (B) Tris/urea-soluble protein applied. (*c*) Tetramethylurea-soluble extract of HD lipoprotein (d 1.100–1.21).

and experimental diets are presented in Table 5 and Fig. 1. The results are taken from a single preparation of each lipoprotein class. Two other preparations gave similar results.

In guinea pigs fed on the normal diet, the distribution of particle diameters in the VLD lipoproteins was skewed, the smaller particles of 30–50 nm accounting for some 70% of the total (Fig. 1*a*). The VLD lipoproteins in animals fed on the lipid-rich diet were substantially more heterogeneous in size, and their distribution was markedly skewed (Fig. 1*b*). Both the mean and modal diameters of the VLD lipoproteins in the experimental animals (64.5 and 51.9 nm respectively) were greater than those of the corresponding particles in the controls (46.3 and 36.0 nm respectively; Table 5). Indeed, some 24% of the VLD lipoproteins in the experimental animals were of greater diameter (80–140 nm) than the largest particles detected in the controls (77.5 nm); particles of diameter greater than 120 nm are considered as chylomicrons rather than as VLD lipoproteins (Nichols, 1969).

No structural dissimilarity could be detected by electron microscopy between the VLD lipoproteins from the two groups of animals (Plates 2*a* and 2*b*).

The LD lipoproteins in the control animals were highly homogeneous in size and appearance (Plate 2*c*), although isolated over a broad density range. Thus more than 60% of the particles had the mean diameter (21.4 nm) (Table 5, Fig. 1).

In contrast, the LD lipoproteins of animals fed on the lipid-rich diet were of larger diameter (mean 24.3 nm; Table 5) and relatively heterogeneous (Plate 2*d*). Thus some 41% of the particles were within the range 19.5–24.0 nm (Fig. 1*d*), and some 52% in the range 24.0–32.0 nm; in control animals, only 15% of the LD-lipoprotein particles were present within this latter range. A significant proportion of larger particles with diameters ranging from 24

Table 3. *Concentrations of serum low-density substances in hyper-responding guinea pigs*

Guinea pigs were fed on the lipid-rich diet for 6 days. Values are means ± S.D. from six animals, and are expressed as mg of lipoprotein/100 ml of serum within the defined intervals of flotation rate (F) at a solvent density of 1.100. Values for density intervals are calculated from such F rates.

	Lipoprotein concentration		
$F_{1.100}$ range ...	0–22	22–33	>33
Density interval ...	1.019–1.100	1.007–1.019	<1.007
Mean	244 ± 29	94 ± 10	243 ± 50
Range	174–349	60–118	113–426

Table 4. *Chemical composition of serum lipoproteins from a guinea pig fed on the normal and the experimental diet*

Values are the mean percentages (by wt.) of each component and are taken from a representative experiment. The quantity of cholesteryl ester was calculated as 1.67 [value calculated from the ratio of the molecular weight of the ester (i.e. cholesterol + fatty acid) to that of free cholesterol] × the quantity of ester cholesterol. Serum VLD lipoproteins were isolated as lipoproteins of $d < 1.007$; LD lipoproteins were separated in the density interval 1.007–1.100.

	Composition			
Diet ...	Normal		Experimental	
Component	Serum VLD lipoproteins	Serum LD lipoproteins	Serum VLD lipoproteins	Serum LD lipoproteins
Cholesteryl ester	8.1	35.7	18.2	40.9
Free cholesterol	3.9	5.4	7.7	12.2
Triacylglycerol	64.8	14.3	60.2	6.8
Phospholipid	12.6	12.5	9.1	18.6
Protein	10.7	32.1	4.9	21.5

Table 5. *Comparison of particle diameters of serum VLD and LD lipoproteins in guinea pigs fed on the normal and lipid-rich diets*

The data are taken from one preparation of each fraction, which was considered representative of three. All diameters are expressed in nm; between 100 and 200 particles were measured in each lipoprotein class.

			Particle diameter (nm)		
Diet	Lipoprotein class	Density	Mean ± S.D.	Mode	Range
Normal	VLD	<1.007	46.3 ± 8.4	36.0	30.6–77.5
Lipid-rich	VLD	<1.007	64.5 ± 20.9	51.9	34.6–130.0
Normal	LD	1.007–1.100	21.4 ± 2.5	21.6	12.5–28.0
Lipid-rich	LD	1.007–1.100	24.3 ± 3.4	21.6	12.5–35.0

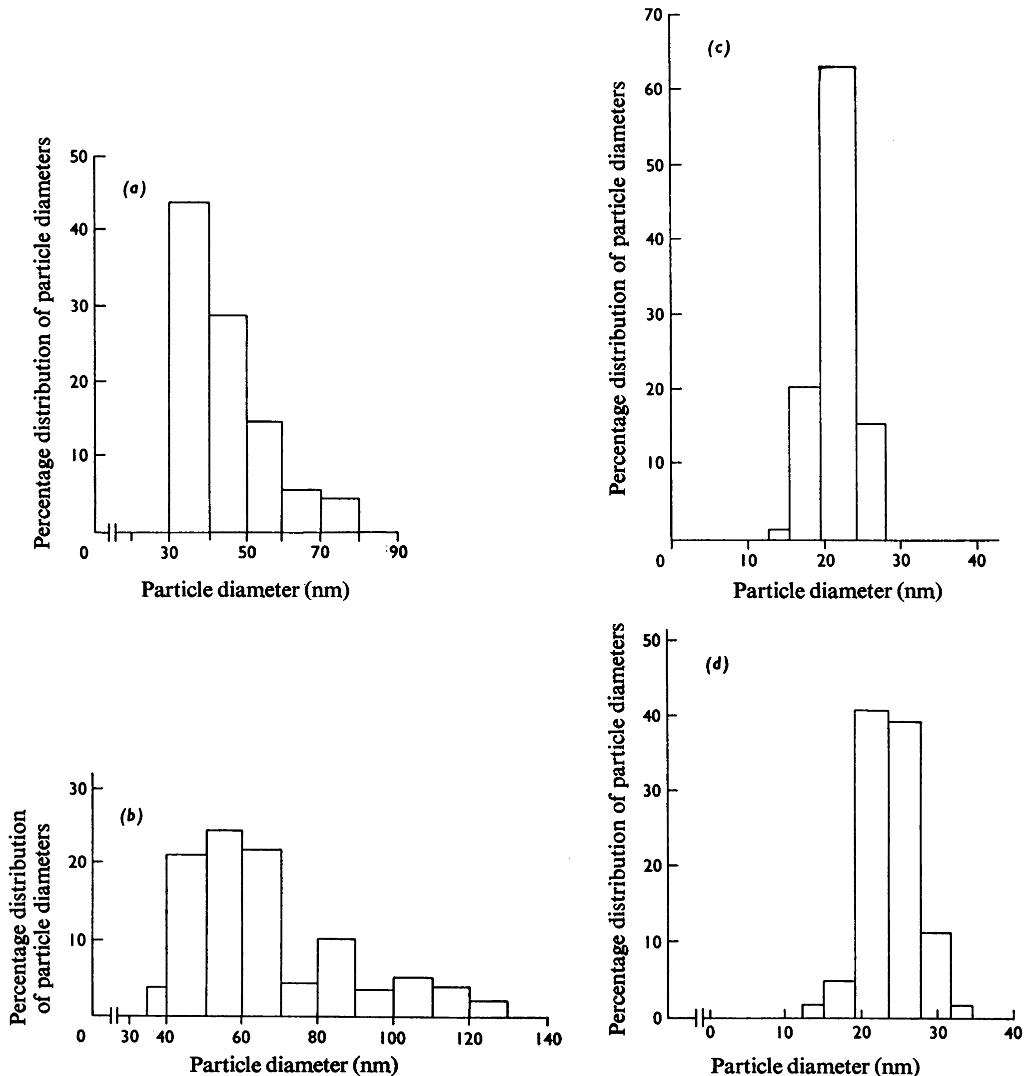

Fig. 1. *Distribution of particle diameters determined on electron photomicrographs of negatively stained VLD and LD lipoproteins from guinea pigs fed on the normal and lipid-rich diets*

(*a*) and (*b*) Distribution of VLD lipoproteins in normal and experimental guinea pigs respectively; (*c*) and (*d*) distribution of LD lipoproteins in normal and experimental guinea pigs respectively.

to 32 nm had therefore appeared in such animals in response to the experimental diet. LD lipoproteins in experimental animals were also distinct from those in the controls in their marked susceptibility to deformation when examined at (protein) concentrations between 0.3 and 0.35 mg/ml (Plate 2*f*). The typical dimensions of deformed particles were 38 and 19 nm in largest and smallest dimension respectively. Under similar conditions, LD-lipoprotein particles from animals fed on the normal diet assumed polygonal rather than rectangular shapes (Plate 2*e*).

Amino acid composition of serum lipoproteins from guinea pigs fed on the lipid-rich diet

The amino acid compositions of the total apoproteins of the VLD, LD and HD lipoproteins (i.e. apo-VLD, apo-LD and apo-HD lipoproteins) are summarized in Table 6.

Table 6. *Amino acid composition of serum lipoproteins from guinea pigs fed on the lipid-rich diet*

The numbers of separate preparations of each lipoprotein fraction analysed are given in parentheses. At least two analyses of each preparation were performed; the composition of individual preparations was the mean of these analyses. All preparations were separated from the pooled sera of two or more animals. Values are expressed as mol of each amino acid/100mol of amino acid residues and are means ±S.E.M. LD lipoproteins were isolated in the density range 1.007–1.100 and HD lipoproteins in the range 1.100–1.210.

Amino acid	Apo-VLD lipoprotein (3)	Apo-LD lipoprotein (4)	Apo-HD lipoprotein (3)
Lysine	7.5±0.07	7.9±0.18	9.0±1.08
Histidine	1.7±0.31	2.2±0.11	2.0±0.21
Arginine	6.0±0.27	3.8±0.20	5.7±1.04
Aspartic acid	9.5±0.28	10.8±0.24	10.7±0.09
Threonine	5.0±0.47	6.2±0.15	5.2±0.26
Serine	8.6±0.81	8.5±0.44	7.2±0.96
Glutamic acid	18.9±0.61	13.0±0.36	18.4±1.46
Proline	3.4±0.49	3.7±0.34	3.3±0.88
Glycine	7.3±0.85	5.1±0.10	5.2±0.96
Alanine	7.5±0.68	6.8±0.11	7.3±0.87
Valine	5.7±0.50	5.4±0.18	4.9±0.49
Methionine	0.6±0.35	1.5±0.16	0.4±0.43
Isoleucine	3.0±0.49	5.3±0.07	2.5±0.18
Leucine	9.7±0.58	12.7±0.33	12.2±1.18
Tyrosine	2.4±0.88	2.4±0.46	2.4±0.14
Phenylalanine	3.2±0.46	4.6±0.25	3.5±0.55

When compared with the (total) apoprotein compositions of the serum lipoproteins in guinea pigs fed on the normal diet (Table 1 of Chapman *et al.*, 1975), it is evident that the apo-LD lipoproteins are the most alike. In apo-VLD lipoprotein from experimental animals, the proportions of both arginine and glutamic acid (6.0 and 18.9% of the total respectively) were increased compared with their values (4.1 and 16.2% respectively) in the corresponding apoprotein from controls. By contrast, the proportions of aspartic acid and serine were rather lower (9.5 and 8.6% respectively) than those found in apo-VLD lipoprotein from guinea pigs fed on the control diet (10.9 and 11.3% respectively). In apo-HD lipoprotein, the largest change in the proportion of any amino acid was in glutamic acid, which increased from 15.4% in the controls to 18.4% of the total in experimental animals.

These observations suggested that the relative proportions of the various apolipoproteins had changed in both the VLD and HD lipoproteins after feeding of the lipid-rich diet.

Molecular-weight determination of apoprotein components

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was used to determine the molecular weights of the various components of the apo-VLD and apo-LD lipoproteins from guinea pigs fed on the experimental diet. The electrophoretic patterns typical of apo-VLD and apo-LD lipoproteins were very similar; their major component was of high molecular weight and was generally seen as a doublet band of mol.wts. 400000–425000 and 450000–465000 (Plate 3; apo-VLD lipoprotein not shown). Such behaviour is typical of guinea-pig apolipoprotein B (Chapman *et al.*, 1975). On a densitometric basis, this component amounted to some 80% of the total material.

Immediately below this component was a series of three to six fainter bands whose mol.wts. ranged from 215000 to 385000. Three bands of rather lower molecular weight were consistently detected in both apo-LD and apo-VLD lipoprotein; these stained diffusely and were of 70500, 27000 and 15000 mol.wt. Neither the 27000- nor the 15000-mol.wt. components were detected in apo-LD lipoprotein from control animals.

Proportion of apolipoprotein B in serum lipoproteins from guinea pigs fed on the lipid-rich diet

The proportion of the total apoprotein of each lipoprotein class insoluble in tetramethylurea, i.e. guinea-pig apolipoprotein B (Chapman *et al.*, 1975), is shown in Table 7. By this method, apolipoprotein B was found to be the major apoprotein of both VLD and LD lipoproteins, but was only a trace (<5%) component of apo-HD lipoprotein. Comparison of these results with those in animals fed on the normal diet showed that the proportion of apolipoprotein B in the VLD lipoproteins was significantly increased from 55.6% in control animals to 74.8% in those fed on the experimental diet ($P<0.001$). In comparison, there was only a minor increase (from 79.6 to 85.5%) in the proportion of apolipoprotein B in apo-LD lipoprotein after substitution of the experimental diet.

The proportion of the apolipoprotein-B component in apo-LD lipoprotein was also estimated by gel-filtration chromatography of its sodium dodecyl sulphate-solubilized (total) apoprotein. We have previously established that the fraction eluted in the void volume under these conditions is the guinea-pig counterpart to apolipoprotein B in man (Chapman *et al.*, 1975). In determinations on three separate preparations of apo-LD lipoprotein from animals fed on the lipid-rich diet, the amount of protein recovered in the void-volume fraction was 83.9±2.5%, a value closely resembling that obtained by using tetramethylurea (85.5%).

Table 7. *Proportion of apolipoprotein B in the serum lipoproteins of guinea pigs fed on the lipid-rich diet, as determined by content of tetramethylurea-insoluble protein*

Values are the mean percentages (w/w)±S.E.M. of tetramethylurea-insoluble protein in the numbers of preparations given in parentheses. Each lipoprotein preparation was separated from the pooled sera of two or more animals. For comparative purposes, data from animals fed on a normal diet (Chapman *et al.*, 1975) have been included.

Serum lipoprotein class	Density (g/ml)	Tetramethylurea-insoluble protein (%) Lipid-rich diet	Normal diet
VLD (3)	<1.007	74.8±2.6	55.6
LD (4)	1.007–1.100	85.5±2.0	79.6
HD (1)	1.100–1.210	4.1	49.4

Separation and physicochemical characterization of the apolipoprotein-B component

For preparative purposes, the apolipoprotein-B component was separated from the total apoprotein of LD lipoprotein (*d* 1.007–1.100) by gel-filtration chromatography on Sephadex G-150.

Guinea-pig apolipoprotein B was eluted in the excluded volume; its elution was coincident with that of Blue Dextran. The latter portion of the peak occasionally trailed, and may have been indicative of some degree of fractionation. In addition, this trailing edge typically exhibited a small shoulder; fractions of apolipoprotein B for subsequent study were normally taken from the centre of the peak.

The amino acid composition of apolipoprotein B isolated from guinea pigs fed on the lipid-rich diet is detailed in Table 8, and is compared with that of the equivalent fraction from control animals. The compositions are essentially indistinguishable, and are characteristically rich in aspartic acid, glutamic acid, lysine and leucine.

Distribution of the soluble apolipoproteins

The tetramethylurea-soluble protein components of the serum lipoproteins from guinea pigs fed on the lipid-rich diet were examined by electrophoresis in polyacrylamide gel. Patterns typical of these polypeptides from VLD, LD and HD lipoproteins of such animals are shown in Plate 4. The patterns from VLD- and LD-lipoprotein classes were qualitatively alike (Plates 4*a* and 4*b*, sample A), and were distinct from that of HD lipoprotein in containing greater proportions of apolipoproteins of high and of intermediate electrophoretic mobility (i.e. bands II, III, IV and V). Indeed such components were often present in only trace amounts in HD lipoproteins (Plate 4*c*, Fig. 2).

Table 8. *Amino acid composition of apolipoprotein B from the serum apo-LD lipoprotein of guinea pigs fed on the lipid-rich diet, and comparison with that from animals fed on the normal diet*

Apolipoprotein B was isolated by gel-filtration chromatography of detergent-solubilized apo-LD lipoprotein (*d* 1.007–1.100) on Sephadex G-150. Values are the means±S.E.M. of four separate preparations of apolipoprotein B; the hydrolysate of each preparation was analysed in duplicate or triplicate. Values are expressed as mol of each amino acid/100mol of amino acid residues. The data for apolipoprotein B from the apo-LD lipoprotein of guinea pigs fed on the normal diet are taken from Chapman *et al.* (1975).

Amino acid	Diet ...	Guinea-pig apolipoprotein B Lipid-rich	Normal
Lysine		8.0±0.09	7.9
Histidine		2.1±0.06	2.3
Arginine		4.2±0.21	3.7
Aspartic acid		10.8±0.25	10.9
Threonine		6.2±0.18	6.4
Serine		7.7±0.80	7.7
Glutamic acid		11.6±0.56	11.7
Proline		3.4±0.89	4.0
Glycine		5.2±0.26	5.0
Alanine		7.0±0.26	7.0
Valine		5.5±0.65	5.5
Methionine		1.0±0.34	0.9
Isoleucine		5.6±0.70	5.7
Leucine		13.6±0.41	13.6
Tyrosine		3.1±0.09	3.0
Phenylalanine		4.8±0.07	4.7

Determination of the electrophoretic mobilities (Table 9) indicated that the various components present in each lipoprotein class exhibited mobility values that closely resembled those previously found in the soluble apolipoproteins of control animals (Chapman *et al.*, 1975). It is noteworthy that no additional components could be detected in the apolipoprotein patterns from experimental animals; the mobilities of the band-II, -III, -IV and -V components in such patterns, however, tended to be more variable (Table 9) than those in the corresponding patterns from control animals.

Densitometric scanning permitted a semi-quantitative determination of the distribution of the soluble apolipoproteins in each lipoprotein class (Table 10), and revealed a marked increase in bands VI-B and VI in both VLD and LD lipoproteins in the experimental animals. Thus these components, which were difficult to resolve, had increased by 33-fold and 12-fold respectively in the VLD and LD lipoproteins after the lipid-rich diet. The VLD lipoproteins were distinguished by their high content of band VII, which had risen to 24.8 from 2.1 in animals fed on the

Table 9. *Electrophoretic mobilities of the tetramethylurea-soluble apolipoproteins from the VLD, LD and HD lipoproteins of guinea pigs fed on the lipid-rich diet*

Electrophoretic mobility is defined as the ratio of the distance of migration of the individual apolipoprotein to that of the dye front. Values are means±S.E.M. for the numbers of lipoprotein preparations given in parentheses. Samples were normally analysed in duplicate or triplicate. The conditions used for electrophoresis in 7.5% (w/v) polyacrylamide disc gels are described in the Experimental section (under 'Electrophoresis'). Data in control animals are taken from Chapman *et al.* (1975). Abbreviation: n.d., not detectable.

Tetramethylurea-soluble apolipoprotein band no.	Mobility value in control animals	Electrophoretic mobility		
		VLD lipoprotein (3)	LD lipoprotein (4)	HD lipoprotein (2)
I	1.0	1.0	1.0	1.0
II-B	0.66	n.d.	0.65*	n.d.
II	0.60	0.59±0.01	0.59±0.03	0.60
III	0.55	0.54±0.03	0.53±0.03	0.53
IV	0.48	0.47±0.04	0.47±0.04	0.45
V	0.41	0.42±0.03	0.41±0.04	n.d.
VI-B	0.28	0.26±0.02† (VI-B + VI)	0.27±0.02	0.29
VI	0.23		0.24±0.02	n.d.
VII	0.19	0.19±0.01	0.20*	0.20
VIII	0.15	0.14±0.01	n.d.	0.14
IX	0.11	0.11±0.01	0.11*	0.12
X	0.07	n.d.	0.05*	0.08

* Band detected in two preparations only.
† Band diffuse.

Table 10. *Distribution of soluble apolipoproteins from the serum lipoproteins of guinea pigs fed on the lipid-rich diet*

Values are the mean percentage densitometrically determined area of each soluble apolipoprotein ±S.D., and are taken from single scans of three 7.5% (w/v) polyacrylamide gels of each lipoprotein fraction. Samples were electrophoresed in the presence of reducing agents, and were isolated from a representative pool of serum. Numbers in parentheses are those reported previously for the corresponding fractions from animals fed on the normal diet (Chapman *et al.*, 1975). Band nomenclature is indicated in Plate 4.

Apolipoprotein band no.	Area (%)			
	Tetramethylurea-soluble apolipoproteins			Tris/urea-soluble apolipoproteins of LD lipoprotein
	VLD lipoprotein	LD lipoprotein	HD lipoprotein	
X	(7.0) 0	(4.8) 4.2±1.4	(15.9) 9.3±3.3	0
IX	(3.2) 8.5±2.2	(12.9) 12.0±2.5	(0) 10.1±3.4	10.2±2.6
VIII	(0) 2.1±0.7	(5.5) 2.8±0.8	(0) 0	4.8±1.0
VII	(2.1) 24.8±3.1	(10.5) 6.2±1.2	(0) 57.2±5.1 (VII + VI)	0
VI	(1.2) 39.4±3.4 (VI + VI-B)	(4.9) 20.7±2.7 (VI + VI-B)	(28.0)	28.5±1.9 (VI + VI-B)
VI-B	(0)	(0)	(35.4) 0	
V	(16.5) 12.5±1.5	(11.0) 30.6±2.3	(0) 1.4±1.8	35.0±3.0
IV	(14.8) 3.2±0.4	(10.1) 2.1±3.0	(8.9) 6.7±2.7	3.9±0.8
III	(35.4) 5.1±0.7	(23.3) 12.4±1.9	(7.0) 11.4±5.0	13.3±2.0
II	(9.6) 1.4±0.3	(12.2) 4.5±1.8	(0) 3.7±4.6	3.0±2.6
II-B	(0) 0	(2.1) 0.8±0.8	(0) 0	0
I	(9.3) 3.0±0.4	(2.7) 4.7±2.4	(4.8) 0.2±0.4	1.2±1.4

normal diet. The proportion of this component was essentially unchanged in the LD-lipoprotein class. The LD lipoproteins of the experimental animals were, however, distinct in that their major soluble apolipoprotein was band V. Thus this component had increased almost 3-fold, to 30.6 from 11.0% in the control animals.

Because of the very low content of HD lipoproteins in guinea pig, only two (pooled) preparations were available in sufficient amounts for study of their apolipoprotein content. The results obtained (Plate 4*c*, Tables 9 and 10) suggest that band VI-B is absent from these lipoproteins in experimental animals and that the principal soluble apolipoproteins are

Table 11. *Amino acid composition of the tetramethylurea-soluble apolipoproteins from guinea pigs fed on the experimental diet*
Values are the means of duplicate analyses of each component, and are expressed as mol of each amino acid/100 mol of amino acid residues. Hydrolysates were derived from six stained bands of each component which had been sectioned from a series of gels on which samples (approx. 200 μg of total protein) of the same lipoprotein fraction had been electrophoresed. The numbering of individual apoprotein bands is shown in Plate 4 and Table 10. * indicates data obtained in normal animals taken from Chapman *et al.* (1975).

	Apolipoprotein band no.					
Amino acid	VI	VI*	VII	VII*	IX	IX*
Lysine	4.7	7.1	5.0	7.3	4.8	6.3
Histidine	0.6	1.1	0.5	1.9	0.3	0.4
Arginine	7.2	5.7	6.4	2.8	6.1	10.4
Aspartic acid	9.8	12.0	12.1	13.2	12.7	16.2
Threonine	4.1	5.3	4.4	5.6	3.9	4.6
Serine	9.3	9.0	11.0	12.8	8.6	8.7
Glutamic acid	20.1	21.4	20.0	19.1	14.6	13.7
Proline	0.3	0	0	0.6	0.9	0.7
Glycine	7.8	6.3	7.6	7.5	12.6	10.2
Alanine	12.0	10.2	11.7	10.3	13.9	8.8
Valine	8.6	5.0	7.5	3.0	6.0	2.8
Methionine	0.1	0.3	0	0.4	0	0
Isoleucine	2.6	2.4	2.1	1.2	2.6	2.9
Leucine	8.8	9.4	7.3	7.6	7.1	7.2
Tyrosine	1.9	2.0	1.6	1.9	2.4	2.7
Phenylalanine	2.1	2.6	2.8	4.6	3.5	4.4

those of bands VI and VII. Band VII was not observed in HD-lipoprotein particles from control animals, although further samples of HD lipoproteins from both groups of guinea pigs would be needed to confirm this observation.

Since preliminary experiments showed that the resolution of large amounts of the basic components with low electrophoretic mobility was unreliable when the tetramethylurea technique was used (Kane, 1973), we also examined the apolipoproteins soluble in a Tris/urea solution (see under 'Delipidation of lipoproteins' in the Experimental section). This typically resulted in the detection of greater amounts of material in the zone of the slower-migrating components (i.e. bands VI-B to X inclusive), as illustrated in Plate 4(*b*), sample B, for the fraction from LD lipoprotein. The resolution of the more basic components after solubilization in Tris/urea was not always superior, however, to that obtained by using Kane's (1973) procedure. Densitometric scanning of such gels revealed that the distribution of the various components (Table 10) was similar to that seen with the tetramethylurea technique, the principal differences concerning increases of the order of 10% in the proportions of bands VI-B/VI. Such observations suggest that the band-VI-B and -VI components may not be completely soluble in tetramethylurea. It may be relevant that band VI is a component with a higher arginine content than average (see following section) and that in this context Kane *et al.* (1975) have noted some retention of the human arginine-rich apolipoprotein in the precipitate after tetramethylurea treatment.

Amino acid composition of the soluble apolipoproteins

The LD lipoproteins were the principal source of polypeptides for amino acid analysis, since this lipoprotein class was present in greatest amount. Since the definition of zones corresponding to bands VI-B and X was typically poor, they were not subjected to analysis; insufficient amounts were obtained to allow analysis of the band-II component.

Of the basic soluble apolipoproteins (bands VI-B to X), sufficient material was obtained for analysis of gel zones with mobilities corresponding to bands VI, VII and IX. The amino acid profiles of components VI, VII and IX are presented in Table 11. The overall composition of apolipoprotein band VI from the experimental animals closely resembled that of the corresponding component from controls; their contents of lysine and valine differed to the greatest extent (by 2.4 and 3.6% respectively). The amino acid profile of band VII also resembled that of its counterpart in control animals, the most notable differences being its elevated contents of arginine (6.4%) and valine (7.5%), which were some 3.6 and 4.5% higher than those seen in the corresponding fraction from control animals.

Analysis of band IX from experimental animals failed to confirm the high arginine content (10.4%) seen in material from animals fed on the normal

diet, although the arginine content (6.1%) of this material (and also of bands VI and VII) was notably higher than that in components of greater mobility, i.e. bands I–V. Differences in the composition of band IX from the two groups of animals which were greater than 3% were also seen in the proportions of aspartic acid, alanine and valine. These discrepancies may derive from the poor resolution encountered in the gel zones corresponding to both bands IX and X, or to the presence of components that we have been unable to identify, or both. A definitive analysis of these components awaits their separation by chromatographic procedures. The amino acid compositions of the polypeptides soluble in tetramethylurea and isolated as bands I, III, IV and V on polyacrylamide gel were essentially the same as those previously reported (Chapman *et al.*, 1975) for the corresponding components from the serum lipoproteins of animals fed on the normal diet.

Discussion

The present studies have confirmed and extended those of Mills *et al.* (1972) on the effect on guinea-pig serum lipoproteins of a diet supplemented with cholesterol and corn oil. In a few days, this produces dramatic increases in the serum lipoproteins which are largely confined to the class of d 1.019–1.100 (Table 2), leaving the amounts of denser lipoproteins almost unchanged. Thus, in 17 experimental animals, this class accounted for 74% of the lipoproteins of $d<1.100$. However, the response was not uniform, the total lipoprotein content ranging from 122.8 to 500.4mg/100ml of serum after 6 days on the diet. At the same time, a rise in the total content was accompanied by an increase in the ratio of VLD to LD lipoproteins. Thus, in the six animals with the greatest response to the diet (Table 3), the VLD components amounted to an average of 41.9% of the total lipoprotein. In the experimental group as a whole, however, the proportion was only 17.1%.

As Mills *et al.* (1972) pointed out, the dietary induction of hypercholesterolaemia in the guinea pig is accompanied by the substitution of an 'abnormal' LD lipoprotein for that usually present. The electron-microscopic observations presented in Table 5 and Plate 2 confirm that the 'abnormal' form is a slightly larger particle than the normal. They also show that there is a redistribution of the VLD lipoproteins towards particles of larger diameter. As would be expected, these changes in size are accompanied by a decrease in the proportion of protein in both the LD and the VLD lipoproteins. At the same time, there is a substantial increase in their proportions of cholesterol and cholesteryl ester, which is brought about at the expense of triacylglycerol. These changes in the proportions of lipid and protein are also accompanied by an increase in the relative amount of protein insoluble in tetramethylurea (i.e. guinea-pig apolipoprotein B). This effect was particularly marked in the VLD lipoproteins, in which the content of apolipoprotein B had increased from 55.6 to 74.8% of the total apoprotein. However, it was also evident, although to a lesser degree, in the LD lipoproteins of the experimental animals, which displayed a 6% increase in their apolipoprotein-B content. Alterations in the complement of tetramethylurea-soluble apolipoproteins towards components of a more basic character accompanied these changes in apolipoprotein-B content in both the VLD and LD lipoproteins (see below).

The haemolytic anaemia which is induced in guinea pigs by feeding them for 10–12 weeks on a diet containing 1% cholesterol is accompanied by the production of abnormal serum lipoproteins (Sardet *et al.*, 1972). In particular, the VLD lipoproteins produced under these conditions were enriched in cholesteryl esters and depleted of triacylglycerol to an even greater extent than in our experiments. However, the VLD compounds produced by our guinea pigs have electrophoretic mobilities greater than that of β-globulin, whereas those described by Sardet *et al.* (1972) were of mobility equal to that of β-globulin, like the VLD lipoproteins of hypercholesterolaemic rabbits (Shore *et al.*, 1974), pigs (Mahley *et al.*, 1975) and dogs (Mahley *et al.*, 1974). The main distinction between the LD lipoproteins produced by our regime and that used by Sardet *et al.* (1972) was the presence in the latter of vesicular particles with a high free cholesterol content. Such particles were entirely absent from our preparations, an observation that suggests that the diet used by Sardet *et al.* (1972) had brought about a much more profound perturbation of lipoprotein metabolism than in our experiments.

The rabbit is the only other animal that responds readily to an increase in dietary cholesterol and has been studied in detail. The most pertinent study for comparison is that by Camejo *et al.* (1973), whose rabbits were fed for 21 days on a diet supplemented with cholesterol and sesame-seed oil; this diet raised the content of LD lipoproteins to an average of 848 mg/100ml of serum. In the VLD lipoproteins, the cholesteryl ester content was 53.1% (calculated from the quoted proportion of esterified cholesterol) and that of triacylglycerol 11.9%. These compounds therefore contained more cholesteryl ester and much less triacylglycerol than the VLD lipoproteins from our hypercholesterolaemic guinea pigs (18.2 and 60.2% respectively). By contrast, the LD lipoproteins from the cholesterol-fed rabbits and guinea pigs were of almost the same composition.

The apo-VLD lipoproteins of hypercholesterolaemic rabbits have been thoroughly investigated by Shore *et al.* (1974), who found that an apolipoprotein

B-like component accounted for some 40% of their protein moiety, compared with the 75% of guinea-pig apolipoprotein B present in the apo-VLD lipoproteins of our experimental animals. This may be partly due to differences in the profiles of VLD lipoproteins in the two species. The major portion (some 50%) of the apo-VLD lipoprotein in hypercholesterolaemic rabbits consisted of polypeptide species rich in arginine (fractions R2 and R3, each containing more than 10mol of arginine/100mol; in addition, these fractions contained more than 22mol of glutamic acid/100mol; Shore *et al.*, 1974). In contrast, only a minor proportion (some 19%) of the apo-VLD lipoprotein in our experimental animals was made up of polypeptides that could be compared with the rabbit R2 and R3 species. Such polypeptides were primarily represented by bands VI and VII, which contained relatively high proportions of arginine (7.2 and 6.4% respectively) and also of glutamic acid (each greater than 20%). A 3-fold increase in the content of polypeptides of basic character (i.e. bands VI-B, VI, VII, IX and X) had, however, occurred in the apo-VLD lipoprotein of the hypercholesterolaemic animals, since such components amounted to only 6% of the total apoprotein in controls. It is noteworthy that such alterations in the protein moiety of VLD lipoprotein were detectable by amino acid analysis (Table 6), which revealed increases in its proportions of both arginine and glutamic acid.

Changes in the complement of soluble apolipoproteins in apo-LD lipoprotein mainly involved the basic polypeptides of bands VI and VI-B, which represented 1% of the total apoprotein moiety in normal animals and had risen to 3% in the experimental group. We are unaware of any detailed studies of LD lipoproteins in hypercholesterolaemic rabbits.

Relatively little information is available on the effects of a cholesterol-enriched diet on the serum lipoproteins in the rat, apart from the studies by Lasser *et al.* (1973). These investigators fed a chow diet supplemented with 1% (w/w) cholesterol and 10% (v/v) olive oil to Sprague–Dawley rats for at least 2 weeks, and noted marked changes in the relative concentrations and compositions of their serum lipoproteins. These changes were characterized by increases in the concentrations of both the VLD (3.7-fold) and the LD (*d* 1.006–1.055) lipoproteins (5.6-fold), and by a decrease in the concentrations of the HD lipoproteins. Like the guinea pig and rabbit, the cholesterolaemic-rat VLD lipoproteins were rich in cholesterol (49.2%) and poor in triacylglycerol (31.1%). The rat was distinguished from these two other species by the appearance of a new LD lipoprotein, rich in both phospholipid and cholesterol, which appeared in the density interval 1.006–1.030 in the cholesterol-fed animals; this lipoprotein constituted some 20% of the total. Unfortunately, little is known of the soluble apolipoproteins in hypercholesterolaemic rats, although Mahley *et al.* (1975) have noted a preponderance of an arginine-rich apoprotein in the β-VLD lipoproteins of such animals.

The most significant difference between the response of the guinea pig on one hand, and the rabbit and rat on the other, may lie in their contrasting contents of arginine-rich apolipoproteins. Whether this difference results from the inability of hypercholesterolaemic guinea pigs to synthesize arginine-rich polypeptides in adequate amounts remains to be established.

The induction of hypercholesterolaemia in the guinea pig may produce the 'abnormal' lipoproteins by either or both of two mechanisms; firstly, by a modification at the site(s) of synthesis, or, secondly, by an alteration of the degradative processes that occur after secretion of the lipoprotein into the bloodstream. Evidence for the formation of cholesterol-enriched VLD lipoproteins in the liver has been provided by a study of the nascent VLD particles from the hepatic Golgi apparatus of hypercholesterolaemic guinea pigs (Chapman *et al.*, 1973). The cholesteryl ester content of these particles was some 20-fold higher than that of the corresponding fraction from control animals. In addition, the proportion of protein was less than one-third of that in particles from controls. This suggests that the cholesterol-rich (serum) VLD lipoproteins are at least partly produced in the liver, and that the nascent hepatic particles are larger than those produced under normal conditions. The work of McTaggart (1974) on guinea pigs treated with Triton WR 1339 may also be significant in this context. In those experiments, two groups of animals were used, one fed on the experimental diet, the other on the normal. Chemical analysis of VLD lipoproteins obtained 8h after administration of Triton WR 1339 showed that those from the first group contained an average of 6.1% cholesteryl ester, whereas the control group contained only 0.3%. If it is accepted that the detergent inhibits the degradation of VLD particles by lipoprotein lipase (Schotz *et al.*, 1957; Scanu & Oriente, 1961; Borensztajn *et al.*, 1976), McTaggart's (1974) observations indicate that the abnormality of composition is already established at the time of secretion.

Since the VLD lipoproteins in our experimental animals were larger than those in animals fed normally, it is unlikely that they arise through the further degradation of the VLD lipoproteins found in normal guinea pigs. However, the investigations of the metabolism of guinea-pig LD lipoproteins by Mills & Weech (1976) suggest that the mechanism by which they are degraded is modified in the hypercholesterolaemic animals. If it can be established

that this is the same mechanism by which the VLD lipoproteins are metabolized, it would follow that the altered lipoproteins found in hypercholesterolaemic guinea pigs are the result of changes in the processes both of synthesis and of intravascular degradation.

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