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The Distribution and Partial Characterization of the Serum Apolipoproteins in the Guinea Pig

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1. Very-low-density (VLD), low-density (LD) and high-density (HD) lipoproteins were isolated by sequential ultracentrifugation from the serum of male guinea pigs fed on a diet containing 3–4% fat. The apoproteins of these lipoproteins (apo-VLD, apo-LD and apo-HD lipoproteins) were studied after delipidation with organic solvents or extraction with tetramethylurea. 2. The major apolipoprotein of LD lipoprotein isolated by gel filtration was found to closely resemble apolipoprotein B of human serum in its chemical and physical properties. Electrophoresis in sodium dodecyl sulphate–polyacrylamide gel showed that this apoprotein consisted of a number of polypeptides. 3. Tetramethylurea precipitated an apoprotein from guinea-pig serum lipoproteins that is probably the apolipoprotein B-like component. This apoprotein accounted for about 80% of the apo-LD lipoprotein, about 55% of the apo-VLD lipoprotein and about 50% of the apo-HD lipoprotein. 4. The distribution of apolipoproteins soluble in tetramethylurea was determined by densitometric scanning of stained polyacrylamide disc gels. 5. A glycine-rich component of high electrophoretic mobility (band I) and a triplet of soluble apolipoproteins (bands II–IV) were present in both VLD and LD lipoprotein classes. These components constituted a higher proportion of the tetramethylurea-soluble apoproteins of VLD lipoprotein (60–80%) than of LD lipoprotein (40–55%). 6. Small amounts (10–15%) of a component of intermediate mobility, which contained traces of half-cystine, were also present in both VLD and LD lipoproteins. 7. A group of soluble components of basic character (bands VI-X), present as minor components of VLD lipoprotein (10–20%), constituted a major proportion (30–45%) of the soluble apoproteins of LD lipoprotein. Two of these apoproteins were rich in lysine, and two of lower electrophoretic mobility were rich in arginine. 8. The pattern of tetramethylurea-soluble apoproteins in HD lipoprotein was distinguished by the presence of two polypeptides of low electrophoretic mobility as its predominant components. One of these components, band VI, resembled the A-I apolipoprotein of man in both its amino acid profile and in its electrophoretic mobility. The second major component, band VI-B, was rich in lysine and resembled the C-I apoprotein of man in amino acid composition. 9. The soluble components of bands I and IX were analogous in physicochemical properties to the R-X_1 and R-X_2 (high-arginine polypeptide) peptides of human serum lipoproteins respectively.

Extensive studies of the protein components of the serum lipoproteins have been carried out primarily in two species, man (Brown *et al.*, 1969; Fredrickson *et al.*, 1972; Shore & Shore, 1972) and rat (Camejo, 1967; Bersot *et al.*, 1970; Koga *et al.*, 1971; Herbert *et al.*, 1974). Some information is also available on the serum apolipoproteins of the pig (Janado *et al.*, 1966; Fidge, 1973; Jackson *et al.*, 1973), monkey (Edelstein *et al.*, 1973), cow (Jonas, 1972), rabbit (Shore *et al.*, 1974) and dog (Solyom *et al.*, 1971). Such investigations, particularly those in man and rat, have enabled substantial advances to be made in our understanding of the metabolism and the molecular structure of the serum lipoproteins.

The investigations of Puppione *et al.* (1971) established that the principal serum lipoprotein of the guinea pig (*Cavia porcellus*), unlike that of the rat, is a member of the low-density class. Moreover, this animal also differs significantly from the rat and many other species in possessing only trace amounts of HD* lipoproteins (Sardet *et al.*, 1972; Bohmer *et al.*, 1972). Our own studies have been mainly concerned with

* Abbreviations: VLD lipoproteins, very-low-density lipoproteins of $d<1.007$ g/ml; LD lipoproteins, low-density lipoproteins, of density as defined in the text; HD lipoproteins, high-density lipoproteins of density as defined in the text. Apolipoprotein nomenclature is according to Alaupovic (1972).

the effect of a lipid-enriched diet on the composition and distribution of guinea-pig serum lipoproteins (Mills *et al.*, 1972) and on the role of the hepatic Golgi apparatus in their formation and secretion (Chapman *et al.*, 1972, 1973).

To define more precisely the metabolism of the serum lipoproteins in *Cavia* and to determine the manner in which this is altered in diet-induced hypercholesterolaemia, qualitative and quantitative studies of their apoprotein components (apo-VLD, apo-LD and apo-HD lipoproteins) were required.

The technique of electrophoresis in polyacrylamide gel after fractional precipitation with tetramethylurea, introduced by Kane (1973), has provided a simple way of separating apolipoproteins on a scale that is well adapted to the relatively small amounts of material available from guinea pigs, but which also allows their partial characterization. This method has been used extensively in the studies that we describe here.

Experimental

Materials

1,1,3,3-Tetramethylurea, redistilled in glass, was purchased from Burdick and Jackson Inc., Muskegon, Mich., U.S.A., or from Schuchardt, Munich, West Germany. The chemicals and solvents used in amino acid analysis were obtained from Pierce Chemical Co., Rockford, Ill., U.S.A. Crystalline 'ultrapure' urea, Coomasie Brilliant Blue and a set of purified proteins, for use as molecular-weight markers in sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, were supplied by Schwarz–Mann, Orangeburg, N.Y., U.S.A. Schiff's reagent was purchased from Fisher Scientific, Fair Lawn, N.J., U.S.A. Acrylamide, *NN'*-methylenebisacrylamide and *NNN'N'*-tetramethylethylenediamine were purchased from Canal Industrial Corp., Rockville, Md., U.S.A. Mercaptoacetic acid and sodium dodecyl sulphate were obtained from Matheson, Coleman and Bell, Norwood, Ohio, U.S.A., and 2-mercaptoethanol was from Eastman Organic Chemicals, Rochester, N.Y., U.S.A. Anhydrous diethyl ether was supplied by Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A., and by Prolabo, Paris, France. Potassium persulphate was purchased from J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.; all other chemicals and solvents, of analytical grade or the purest available, were obtained from this company or from E. Merck, Darmstadt, Germany. 'Spectrapor' membrane tubing (mol.wt. cut-off approx. 3500) for use in dialysis was the product of Spectrum Medical Industries Inc., 60916 Terminal Annex, Los Angeles, Calif., U.S.A. Sephadex G-100 was purchased from Pharmacia, Uppsala, Sweden. Silica-gel G plates and the molybdatophosphoric acid spray reagent for t.l.c. were obtained from E. Merck, Darmstadt, Germany. The rabbit pellet diet of the guinea pigs was manufactured and supplied by Spillers Ltd., London E.6, U.K.

Animals and diet

Guinea pigs were obtained from the closed colony of the Imperial Cancer Research Fund at the National Institute for Medical Research, Mill Hill, London, U.K.; the pedigree of this colony was 40 years. A small number of these animals were allowed to breed in our laboratory, and were fed on a diet of rabbit pellets (containing 3–4%, w/w, fat; 16%, w/w, protein and 13%, w/w, fibre) supplemented with hay and green vegetables. Males weighing more than 700g were used.

Methods

Blood samples. Samples were drawn from animals starved overnight. The procedure used has been described (Mills *et al.*, 1972).

Isolation of serum lipoproteins. Lipoproteins were isolated from serum by sequential ultracentrifugation. In all cases, the solvents contained 1mM-EDTA and 0.01% (w/v) sodium azide, and their non-protein solvent densities were determined pycnometrically. Ultracentrifugations were performed at 12°C.

Native serum (6ml samples) was overlaid with NaCl solution (3ml) of *d* 1.007g/ml, and centrifuged in the 30.2 rotor of the Spinco L2 ultracentrifuge at 30000rev./min (79420*g*) for 16h. On completion of ultracentrifugation, the substances of density less than 1.007g/ml (VLD lipoproteins) were aspirated off in the top 1.0ml. The next 2.0ml of solution was removed and discarded. After addition of 3ml of NaCl solution (*d* 1.007g/ml) to the remixed contents of the tube, the centrifugation was repeated. The top 3ml of solution was subsequently removed and the density of the remainder raised to 1.100g/ml by addition of the appropriate volume of NaBr solution (*d* 1.341g/ml). Ultracentrifugation was then repeated under the same conditions. The lipoproteins of density 1.007–1.100g/ml (LD lipoproteins) were aspirated off in 1ml, and a further 2ml was removed and discarded. After repeating the ultracentrifugation at density 1.100g/ml, the density of the serum was raised to 1.21g/ml by dialysis against NaBr solution and the mixture ultracentrifuged in the Spinco 40.3 rotor at 40000rev./min (114500*g*) for 48h. The lipoproteins of density 1.100–1.21g/ml (HD lipoproteins) were then removed by aspiration.

Each lipoprotein fraction was washed by centrifugation in NaCl solution of the nominal limiting density.

Characterization of serum lipoprotein fractions. Immunochemical and chemical studies showed that

the general characteristics of the serum lipoprotein preparations closely resembled those reported (Mills *et al.*, 1972) for the corresponding fractions isolated from animals fed on a diet of low (3–4%) fat content. The techniques used in immunodiffusion analyses were the same as those described in that report. Thus immunodiffusion experiments using antiserum to guinea-pig serum albumin showed the absence of this protein in the lipoprotein fractions. In addition, use of anti-(guinea pig) whole serum indicated the absence of any other serum proteins as contaminants of these fractions.

The percentage weight chemical composition of representative preparations of each of the lipoprotein fractions studied were as follows. VLD lipoprotein: cholesteryl ester, 8.0; free cholesterol, 3.9; triacylglycerol, 64.8; phospholipid, 12.6; and protein, 10.7. LD lipoprotein: cholesteryl ester, 35.7; free cholesterol, 5.4; triacylglycerol, 14.3; phospholipid, 12.5; and protein, 32.1. Insufficient material was available for accurate analysis of HD-lipoprotein preparations.

Delipidation of lipoproteins. The whole lipoprotein fractions, or precipitates prepared by extraction with tetramethylurea (see under 'Extraction with tetramethylurea'), were delipidated by extraction with ethanol–diethyl ether (3:1, v/v); the ratio of this solvent to the lipoprotein fraction was 25:1 (v/v). The tetramethylurea-insoluble residue derived from each lipoprotein fraction was delipidated with a volume of solvent equal to that required to extract the equivalent volume of the native lipoprotein fraction from which it was precipitated. Extractions were carried out at room temperature for 16–24h. The precipitated protein was sedimented by centrifugation at 3000*g* for 10min, and after aspiration of the supernatant, was re-extracted under the same conditions.

To determine whether any loss of apoproteins into the solvent phase occurred during delipidation, the protein content of this phase was estimated in the following manner. The ether content of ethanol–diethyl ether extracts was removed by evaporation under N_2 at room temperature; chloroform (2vol.) was added to the remaining ethanol. Water (2ml) was then added, and after shaking, the water phase was separated by centrifugation at 2000*g* for 15min. This extraction procedure was repeated four times, and the aqueous extracts were combined and freeze-dried. The total protein content of the freeze-dried residue was then determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. In this way, the proportion of the total (apo)protein present in the solvent phase amounted to not more than 1% by weight.

To determine the extent of delipidation of the protein residues, samples (about 400μg) were taken to detect and estimate lipid content by t.l.c. After solubilization in detergent solution (see under 'Gel-filtration chromatography'), these samples were applied to silica-gel G plates and chromatographed with a solvent of light petroleum (b.p. 40–65°C)–diethyl ether–acetic acid (75:24:1, by vol.); portions of ethanol–diethyl ether extracts of guinea-pig serum LD lipoprotein, containing known amounts of each lipid class, were run simultaneously. This chromatographic system is essentially that described by Gloster & Fletcher (1966). Plates were stained with either I_2 vapour or molybdatophosphoric acid (Randerath, 1966). Neither triacylglycerol nor cholesterol could be detected in the apoprotein residues. However, traces of phospholipid were present; by comparison with spots corresponding to known amounts of lipoprotein phospholipid, we estimate that this amounted to no more than 1% by weight of the protein residue.

The final apoprotein residues, dried under a stream of N_2, were taken either for hydrolysis before amino acid analysis, or for solubilization in detergent solution in preparation for electrophoresis (or gel chromatography).

Extraction with tetramethylurea. The procedure used for extraction with tetramethylurea was that described by Kane (1973), who has reported that this solvent specifically extracts and precipitates the apoprotein B component from human serum lipoproteins with the liberation of the other apoproteins in soluble form. We have used this technique to remove an apoprotein B-like component from the serum lipoproteins of the guinea pig. Data in the Results section validate the application of tetramethylurea to guinea-pig serum lipoproteins.

Before extraction, LD- and HD-lipoprotein fractions were dialysed against 0.05M-NaCl containing 0.01% (w/v) sodium azide for 24–48h at 4°C. Extraction was performed by the addition of an equal volume of 8.4M-tetramethylurea to a sample (containing about 1mg of lipoprotein) of each fraction in a volume of approx. 1ml. After thorough mixing the solutions were left at room temperature for 15–30min. The precipitate was separated by centrifugation at 3000*g* for 10min and the supernatant, containing the tetramethylurea-soluble apolipoproteins, was carefully removed with a pipette. After dilution with water, the protein content of the soluble extract was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. The amount of the 'apoprotein B-like' protein, insoluble in tetramethylurea, was then determined as the difference between the total protein content of each sample and that of its soluble extract.

The insoluble residue was subsequently washed with a solution of 4.2M-tetramethylurea (about 3ml). After separation by centrifugation at 3000*g* for 10min, the final residue was extracted as described under 'Delipidation of lipoproteins'.

Gel-filtration chromatography. Each sample of

apo-LD lipoprotein (3–10mg), dissolved in 0.5–3ml of 0.01M-sodium phosphate buffer (pH8.0) containing 1% (w/v) sodium dodecyl sulphate, 1% (v/v) 2-mercaptoethanol and 0.01% sodium azide (solution A), was applied to a column (1.2cm×130cm) of Sephadex G-100. The column was equilibrated and eluted with 0.15M-sodium phosphate buffer (pH8.0), containing 0.01% sodium azide, at a flow rate of 8–10ml/h. The effluent was monitored continuously for absorbance at 280nm with the use of an LKB Uvicord II u.v. absorptiometer; fractions (2.0ml) were collected on an LKB Ultrorac fraction collector. After determination of protein content in all fractions, the material eluted in the peak at the void volume was freeze-dried, dissolved in a minimum volume of solution A and dialysed for 48–72h at 4°C against 0.01M-sodium phosphate buffer (pH8.0) containing 0.1% sodium dodecyl sulphate, 1% 2-mercaptoethanol and 0.01% sodium azide. Samples of this material (fraction I), containing 200–300μg of protein, were subsequently electrophoresed in sodium dodecyl sulphate–polyacrylamide gels.

Electrophoresis. The extracts of tetramethylurea-soluble apoproteins from each lipoprotein fraction were electrophoresed in 7.5% (w/v) polyacrylamide gels containing 8M-urea by the modification of the procedure of Davis (1965) described by Kane (1973). Gels were made up at pH8.91 and consisted of a stacking gel 1cm in length, together with a running gel of 10cm. To eliminate the possible contribution of glycine to the amino acid content of the polyacrylamide-gel bands that were subsequently cut from the stained gels, α-amino *n*-butyric acid was substituted for glycine in the upper tank buffer at a final concentration of 0.046M (J. P. Kane, personal communication). The maximum sample volume applied to each gel was 0.5ml. In some instances, portions of the same extract were electrophoresed simultaneously on paired gels, one in the presence of reducing agents, the other in their absence. Electrophoresis was continued until the tracking dye had run to within 1–2cm of the end of the running gel.

On completion of electrophoresis the gels were removed from their respective tubes and fixed in 12.5% (w/v) trichloroacetic acid for 1h. Staining was then carried out by immersion in a solution of 0.1% Coomasie Brilliant Blue in 12.5% trichloroacetate for 2h. The gels were destained in 10% trichloroacetate for at least 48h, with several changes of the destaining solution during this period. It was found that Coomasie Brilliant Blue was superior in several respects to Amido Schwarz in its ability to stain the tetramethylurea-soluble apoprotein components of guinea-pig serum lipoproteins. In particular, this stain consistently gave more intense, well-resolved apoprotein bands and a lower, more evenly distributed background than could be obtained with Amido Schwarz. Densitometric scanning of the gels was performed either with a Clifford Densicomp electrophoresis densitometer (Clifford Instruments, Natick, Mass., U.S.A.) or with a Joyce–Loebl double-beam recording microdensitometer (Joyce–Loebl Ltd., Gateshead-on-Tyne, Co. Durham, U.K.). The values computed by the Clifford scanner were used in calculating the percentage of densitometric area of each stained band, and the areas corresponding to stained bands were cut from scans made on the Joyce–Loebl densitometer and their weights used for calculation of the densitometric areas.

The question as to whether Coomasie Brilliant Blue permits an accurate quantitative determination of each of the apoprotein components by densitometry may be considered. In a detailed study of the ability of this dye to stain urease quantitatively in polyacrylamide-gel slabs, Fishbein (1972) has shown that for bands containing up to about 12μg of protein, quantitative staining may occur at distances of migration as low as 2.2cm. However, at this distance the staining of bands containing more than 12μg of protein was decreased by almost 40% on a densitometric basis, and thus deviated considerably from linearity. Detailed consideration of our data (see the Results section, Fig. 1) suggests that at low migration distances (1–2cm) most of the apoprotein components (bands VI-X) each contained not more than 10μg of protein; these bands might therefore be expected to fall within the range of linearity predicted by Fishbein (1972). Components of higher mobility (2–10cm) fell well within the limits of quantitative staining observed for urease. The data suggest that band compaction is probably of minor importance to the quantification of apolipoprotein components. In contrast, however, substantially larger errors could arise from variations in the amounts of dye bound by each apolipoprotein. Since Coomasie Blue binds electrostatically to positively charged groups in proteins (Fazekas de St. Groth *et al.*, 1963), it is probable that the apoproteins of basic character (bands V-X) will bind more dye molecules/mol than the more acidic components, thereby enhancing their chromogenicity. In the absence of determinations of the absolute chromogenicities of the individual soluble apolipoproteins, it has not been possible to correct the data (see Fig. 1) for such variations in dye binding. The results presented are therefore semi-quantitative in nature.

The sodium dodecyl sulphate–polyacrylamide-gel electrophoretic procedure of Weber & Osborn (1969) was used in the determination of the molecular weights of the serum apolipoproteins. Apoprotein samples were dissolved in solution A as described for gel-filtration chromatography and were not dialysed before electrophoresis. To obtain a dye front that was readily measurable on completion of electrophoresis, the concentration of Bromophenol Blue

solution was raised to 0.5%. The gels were fixed in 20% (w/v) sulphosalicylic acid for 15–20h, and subsequently stained in a solution of 0.05% Coomasie Brilliant Blue in 12.5% (w/v) trichloroacetic acid for 2h. Destaining was carried out in 10% trichloroacetate for not less than 72h, after which time the distance of migration of each band was measured.

The periodic acid–Schiff staining procedure of Kapitany & Zebrowski (1973) was used to detect the presence of carbohydrate moieties in the apolipoproteins after electrophoresis in polyacrylamide gel.

Amino acid analysis. The dried apoprotein residues, in ampoules containing between 2 and 5mg of protein, were dissolved in 6M-HCl and frozen. They were subsequently connected to a freeze-drier and allowed to thaw and then de-gas under a vacuum of $<20\mu$ for 15–20min. A crystal of phenol was added to protect tyrosine, since it has been shown that the addition of small amounts of readily halogenated or oxidized substances will decrease losses of this amino acid (Hill, 1965). The ampoules were then sealed under vacuum and hydrolysed for 22h at 110°C. The hydrolysates were freeze-dried and the dried amino acids dissolved in 0.2M-sodium citrate buffer, pH2.2.

Determination of the amino acid composition of individual stained apolipoprotein bands in the 7.5% (w/v) polyacrylamide gels was based on the procedure of Houston (1971). The amino acid composition of a sample of ovalbumin, sectioned from polyacrylamide gel after Coomasie Blue staining, did not differ from that of the original protein preparation. The use of Coomasie Blue in the Houston (1971) procedure was therefore validated. Individual apoprotein bands were identified by their respective electrophoretic mobility values (see Table 5). Normally, several samples of the same tetramethylurea-soluble fraction were electrophoresed on different gels; corresponding bands from separate gels were then pooled before hydrolysis. After hydrolysis for 22h at 110°C in the presence of a crystal of phenol, the hydrolysates were cooled to 4°C and left for about 12h, during which time the bulk of the acrylamide was coagulated. The hydrolysate solution was then removed with a Pasteur pipette and any residual acrylamide was sedimented by centrifugation at 3000*g* for 10min. The clear supernatant was separated and freeze-dried. The dried residue was dissolved in water (approx. 5ml) and the pH of the solution raised to between 9 and 10 by the addition of a few drops of 5M-NaOH, after which it was freeze-dried. This procedure was repeated at least four times to remove residual NH_3. The final residue was dissolved in 0.2M-sodium citrate buffer, pH2.2.

Amino acid analyses were performed on a Beckman model 121 amino acid analyser (Beckman Instruments Inc., Fullerton, Calif., U.S.A.) equipped with an expanded-range recorder, and on a JEOL JLC 5AH amino acid analyser (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan). Analyses were carried out as described by Moore *et al.* (1958). Neither the tryptophan nor the half-cystine contents of the apoproteins were determined.

Results

Amino acid composition of guinea-pig serum lipoproteins

The amino acid compositions of apo-VLD lipoprotein, apo-LD lipoprotein and apo-HD lipoprotein are presented in Table 1. Glutamic acid was the predominant amino acid, and the dicarboxylic acids together accounted for about 25% (27.1, 24.4 and 26.9% in apo-VLD, apo-LD and apo-HD lipoproteins respectively) of the total protein mass of each class. The content of leucine was also uniformly high. In contrast methionine content was low (about 0.5%) in all lipoprotein fractions. Apo-VLD lipoprotein possessed rather higher contents of glutamic acid and serine (16.2 and 11.3mol/100mol respectively) and a lower leucine content (9.7mol/100mol) than the other apoproteins.

Table 1. *Amino acid composition of guinea-pig serum lipoproteins*

The number of separate preparations of each lipoprotein fraction studied is given in parentheses. Two or three analyses of the hydrolysate of each preparation were made; the composition of each preparation was the mean of these analyses. Values are expressed as mol of each amino acid/100mol of amino acid residues and are means ±S.E.M. Results for apo-HD lipoprotein are expressed as the mean ± the range between preparations. LD lipoproteins were isolated in the density range 1.007–1.100g/ml, whereas HD lipoproteins were of density 1.100–1.210g/ml.

Amino acid	Apo-VLD lipoprotein (3)	Apo-LD lipoprotein (3)	Apo-HD lipoprotein (2)
Lysine	7.9±0.45	7.9±0.64	8.6±0.10
Histidine	1.4±0.06	1.9±0.07	2.2±0.05
Arginine	4.1±0.25	3.9±0.20	5.1±0.40
Aspartic acid	10.9±0.27	11.4±0.24	11.5±0.25
Threonine	5.7±0.17	6.2±0.22	4.7±0.40
Serine	11.3±0.77	9.2±0.35	7.1±0.30
Glutamic acid	16.2±0.28	13.0±0.18	15.4±0.90
Proline	2.4±0.32	3.3±0.46	4.2±0.25
Glycine	7.1±0.45	5.8±0.18	7.2±1.30
Alanine	8.4±0.46	7.6±0.16	8.8±0.45
Valine	5.0±0.24	5.2±0.12	5.4±0.20
Methionine	0.4±0.22	0.6±0.36	0.5±0.25
Isoleucine	4.0±0.36	5.6±0.15	2.6±0.80
Leucine	9.7±0.36	11.2±1.54	11.1±1.20
Tyrosine	2.3±0.34	2.6±0.50	1.9±0.20
Phenylalanine	3.0±0.18	4.4±0.05	3.6±0.15

Table 2. *Apoprotein composition of guinea-pig serum lipoproteins: contents of tetramethylurea-insoluble protein*

Values are the mean wt. (%) ± S.E.M. of tetramethylurea-insoluble protein in each of the number of preparations given in parentheses. Each preparation was isolated from the pooled sera of two or more animals. Protein insoluble in tetramethylurea is that akin to apolipoprotein B in man (Kane, 1973).

Serum lipoprotein class	Density (g/ml)	Tetramethylurea-insoluble protein (%)
VLD (6)	<1.007	55.6±3.9
LD (5)	1.007–1.100	79.6±2.6
HD (3)	1.100–1.210	49.4±1.3

The polarity indices of each of the apoprotein species were compared, the polarity index being defined as the sum of the residue mol percentages of polar amino acids (Capaldi & Vanderkooi, 1972). The polar residues were classified by these authors as Asp, Asn, Glu, Gln, Lys, Ser, Arg, Thr and His. The index values are 57.5, 53.5 and 54.6% for apo-VLD lipoprotein, apo-LD lipoprotein and apo-HD lipoprotein respectively.

Solubility characteristics of guinea-pig serum apoproteins

The total apoprotein fraction of each class of guinea-pig serum lipoprotein was only partially soluble in aqueous buffers (e.g. 0.01 M-sodium phosphate, pH 8.0) but was completely soluble in the presence of 1% (w/v) sodium dodecyl sulphate. The apoprotein fractions could not be dissolved by titration to pH 11.5 as reported for rat apolipoproteins (Koga *et al.*, 1969); the effects of dissociating agents such as urea were not determined.

Quantitative distribution of an apolipoprotein B-like component of guinea-pig serum lipoproteins

The quantitative distribution of that part of the protein moiety of each class of guinea-pig serum lipoprotein that was insoluble in 4.2 M-tetramethylurea is summarized in Table 2. These data show this component to comprise a major portion of the total apoprotein in all of the lipoprotein classes of serum in this species. In the six different preparations of VLD lipoproteins studied, the proportion was more variable than that in either the LD- or HD-lipoprotein preparations. Thus the range in the VLD-lipoprotein samples was 40.4–69.1%, and that in LD lipoprotein was 72.4–89.4% and in HD lipoprotein 46.4–52.3%. This variation probably arises from differences in the density profiles of the individual VLD-lipoprotein samples, which would lead to differences in their average composition. Thus it is relevant that Eisenberg *et al.* (1972) and Quarfordt *et al.* (1972) found the proportion of apolipoprotein B in human apo-VLD lipoprotein to increase markedly as the size of the parent lipoprotein molecule decreased.

Table 3. *Amino acid composition of the tetramethylurea-insoluble apoprotein from guinea-pig serum lipoproteins*

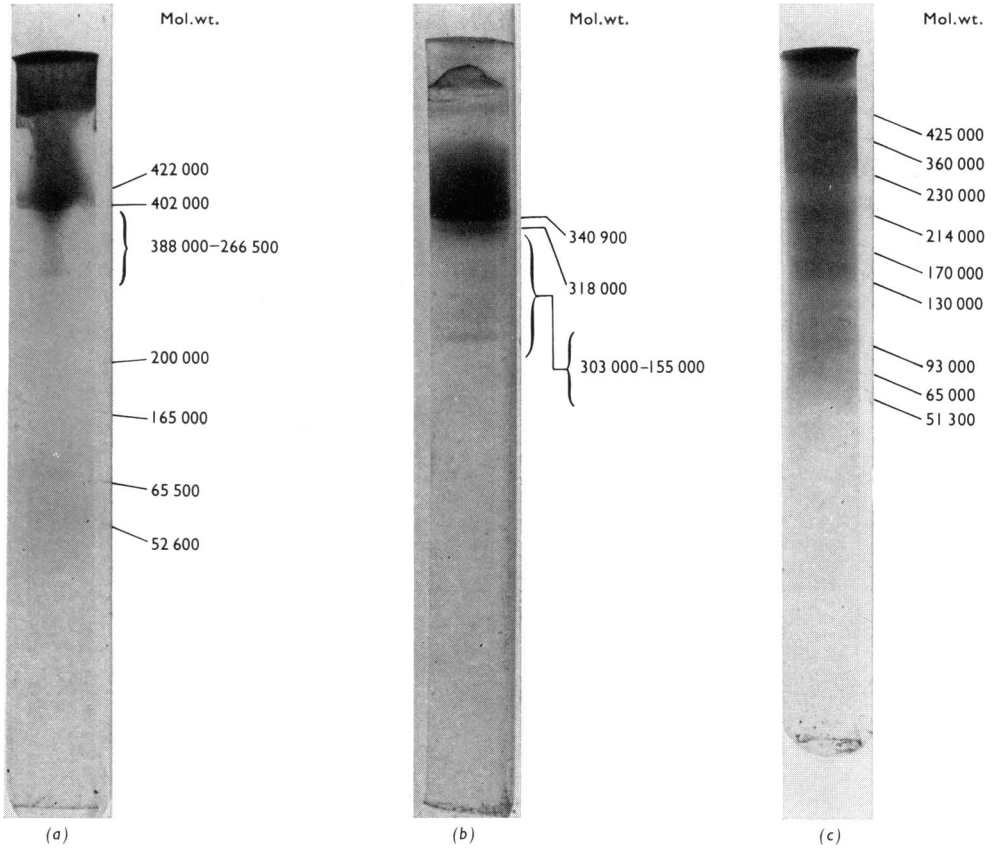
Tetramethylurea-insoluble apoprotein fractions were isolated as described in the Experimental section. Values are the mean for hydrolysates from three separate preparations of each apoprotein fraction, and are expressed as mol of each amino acid/100 mol of amino acid residues, ±S.E.M. Each hydrolysate was analysed in duplicate. The amount of the tetramethylurea-insoluble fraction obtained from HD lipoprotein was insufficient for accurate analysis. VLD lipoprotein, d < 1.007 g/ml; LD lipoprotein, d 1.007–1.100 g/ml.

Amino acid	Tetramethylurea-insoluble fraction: Apo-VLD lipoprotein	Apo-LD lipoprotein
Lysine	8.0±0.29	7.6±0.33
Histidine	2.0±0.07	2.1±0.04
Arginine	3.9±0.28	3.6±0.18
Aspartic acid	10.9±0.22	10.9±0.09
Threonine	6.4±0.30	6.2±0.09
Serine	8.2±0.38	7.8±0.12
Glutamic acid	12.8±0.26	12.1±0.11
Proline	3.0±0.26	3.5±0.73
Glycine	5.9±0.57	4.9±0.18
Alanine	7.8±0.64	7.0±0.06
Valine	5.3±0.07	5.5±0.18
Methionine	0.3±0.14	1.0±0.17
Isoleucine	5.2±0.54	5.7±0.13
Leucine	12.8±1.06	13.7±0.55
Tyrosine	2.7±0.04	3.5±0.06
Phenylalanine	4.6±0.28	4.6±0.23

The tetramethylurea precipitation procedure has been validated by comparing the data obtained by its application with those obtained by gel-filtration chromatography of the delipidated, detergent-solubilized apoprotein. Thus in three experiments in which sodium dodecyl sulphate-solubilized apo-LD lipoprotein (d 1.007–1.100 g/ml) was chromatographed on Sephadex G-100, 80–88% of the total protein applied was eluted at the void volume; this material will be referred to as fraction I.

Physicochemical characterization of the apolipoprotein B-like component

Amino acid composition. The amino acid compositions of the insoluble apoprotein fractions obtained by tetramethylurea extraction of the VLD and LD lipoproteins are shown in Table 3. The compositions markedly resembled each other, both fractions possessing high contents of aspartic acid, glutamic

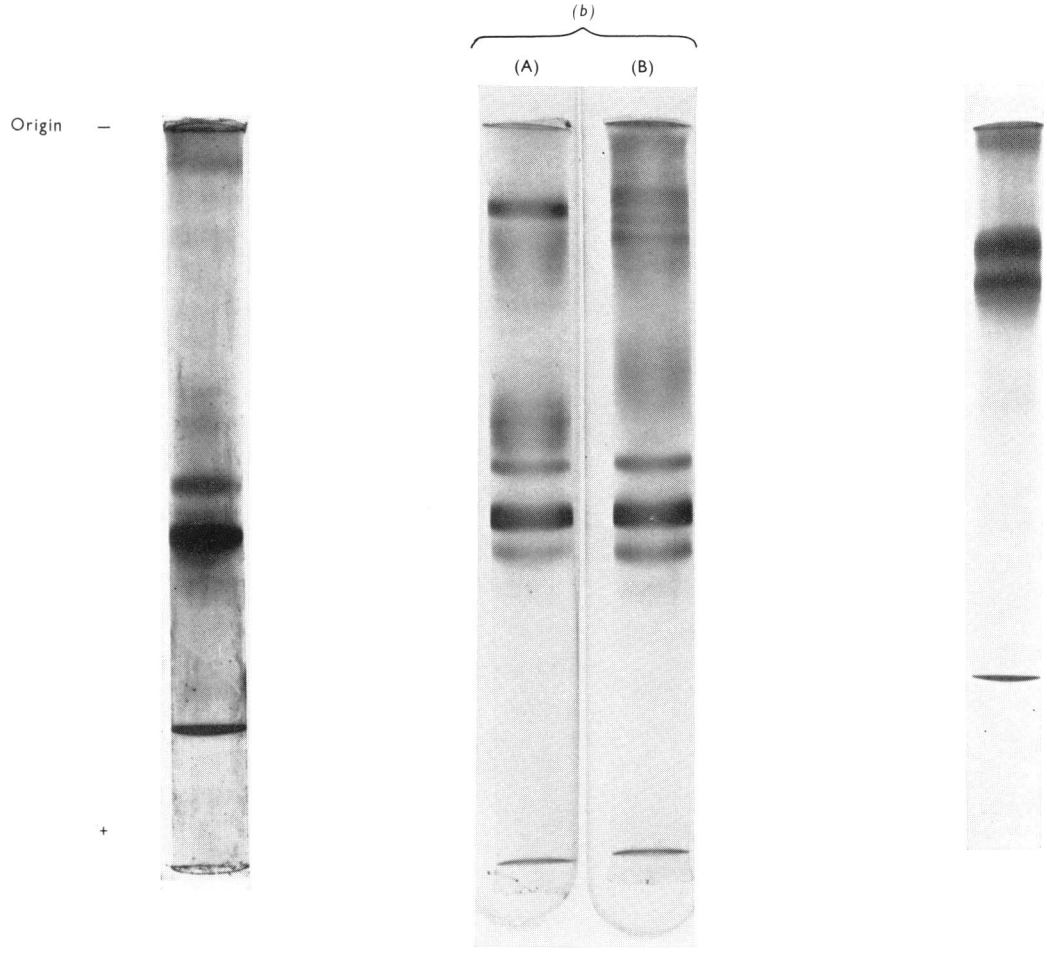


EXPLANATION OF PLATE 1

Sodium dodecyl sulphate–polyacrylamide-gel patterns of apo-LD lipoprotein from guinea-pig and human serum and of fraction I from guinea-pig apo-LD lipoprotein

Electrophoresis was performed in 3.3% (w/v) polyacrylamide gels containing 0.1% sodium dodecyl sulphate for about 4h at 8mA/gel. The molecular weights of the stained protein bands indicated on the plates were calculated as described by Weber & Osborn (1969). Purified proteins of known molecular weight were electrophoresed in each set of gels for calibration purposes. (*a*) Apo-LD lipoprotein (*d* 1.007–1.100g/ml) from guinea-pig serum; this pattern is representative of that obtained from three separate samples of apo-LD lipoprotein, each of which were electrophoresed in duplicate. (*b*) Apo-LD lipoprotein (*d* 1.024–1.045g/ml) from human serum, representative of twelve preparations, each from separate donors. (*c*) Fraction I, isolated from guinea-pig apo-LD lipoprotein after chromatography on Sephadex G-100; this pattern is typical of that obtained from two preparations of fraction I, each electrophoresed in duplicate.

(*Facing p.* 428)



EXPLANATION OF PLATE 2

Polyacrylamide-disc-gel patterns of tetramethylurea-soluble apolipoproteins of guinea-pig serum VLD, LD and HD lipoproteins

Samples of tetramethylurea-soluble extracts of each lipoprotein preparation were electrophoresed in 7.5% (w/v) polyacrylamide gel containing 8M-urea at pH 8.91 and in the presence of reducing agents. Gels were stained with Coomasie Blue. (*a*) VLD lipoprotein, $d < 1.007$ g/ml; some 90μg of tetramethylurea-soluble protein was applied. The pattern shown is representative of that seen in five preparations of VLD lipoprotein, samples of which were electrophoresed in triplicate in eight separate experiments. (*b*) LD lipoprotein, d 1.007–1.100 g/ml; some 100μg of tetramethylurea-soluble protein was applied. Gel (A) shows the sample electrophoresed in the absence of reducing agents and gel (B) the same sample electrophoresed in their presence. The pattern shown is illustrative of that seen in five preparations of LD lipoprotein, electrophoresed in duplicate or triplicate in ten separate experiments. (*c*) HD lipoprotein, d 1.100–1.210 g/ml; some 100μg of tetramethylurea-soluble protein was applied. The pattern is representative of that seen in two preparations of HD lipoprotein, electrophoresed in duplicate or triplicate in two separate experiments.

Table 4. *Comparison of the amino acid composition of fraction I from the serum apo-LD lipoprotein of the guinea pig with the corresponding apoprotein fraction in other species*

Fraction I is the material eluted at the void volume after chromatography of sodium dodecyl sulphate-solubilized apo-LD lipoprotein (*d* 1.007–1.100g/ml) on Sephadex G-100. Values are the mean of three preparations of fraction I, each of which was isolated from separate preparations of (apo)-LD lipoprotein from pooled sera. Hydrolysates were analysed in triplicate; the mean composition of each fraction I preparation was the mean of triplicate analyses of its hydrolysate. Values are expressed as mol of each amino acid/100mol of amino acid residues, ±S.E.M. The human apolipoprotein data are taken from Gotto *et al.* (1972) and represent the amino acid composition of chromatographically purified apo-LD lipoprotein (i.e. apolipoprotein B) from human serum; the units have been converted from mol of amino acid/100000*g* of protein into mol of amino acid/100mol of amino acid residues. The data for the rat are taken from Koga *et al.* (1971) and represent the amino acid composition of fraction PI from the serum apo-LD lipoprotein (*d* 1.019–1.040g/ml) in this species. Fraction PI was isolated by gel-filtration chromatography on Sephadex G-200; values have been converted from mol of amino acid/100000g of protein into mol/100mol of amino acid residues.

Amino acid	Guinea-pig apo-LD lipoprotein fraction I	Human apolipoprotein B	Rat fraction PI
Lysine	7.9±0.14	8.0	7.2
Histidine	2.3±0.04	2.2	2.0
Arginine	3.7±0.06	3.1	3.7
Aspartic acid	10.9±0.06	10.9	11.1
Threonine	6.4±0.27	6.7	6.5
Serine	7.7±0	8.7	8.9
Glutamic acid	11.7±0.24	10.8	11.8
Proline	4.0±0	4.2	4.5
Glycine	5.0±0.20	5.5	5.0
Alanine	7.0±0.15	7.0	6.0
Valine	5.5±0.31	5.6	6.1
Methionine	0.9±0.24	1.8	1.4
Isoleucine	5.7±0.20	5.0	5.6
Leucine	13.6±0.48	12.3	12.0
Tyrosine	3.0±0.43	3.3	3.3
Phenylalanine	4.7±0.16	4.9	4.9

acid and leucine. The fractions were deficient in methionine residues.

The material isolated as fraction I by gel-filtration chromatography in sodium dodecyl sulphate was freeze-dried and its amino acid composition determined (Table 4). An overall similarity in the amino acid compositions of this fraction and that of the tetramethylurea-insoluble apoprotein of both guinea-pig apo-LD and apo-VLD lipoproteins (Table 3) is evident. These data suggest that the apoprotein fraction(s) precipitated by tetramethylurea from apo-LD lipoprotein and from apo-VLD lipoprotein closely resembles that isolated as fraction I.

Molecular-weight determination. The molecular weights of the various apoprotein preparations were determined by electrophoresis in sodium dodecyl sulphate–polyacrylamide gel; we found the reproducibility of molecular-weight estimates with this technique to be within the range of 2–4%. Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of sodium dodecyl sulphate-solubilized apo-LD lipoprotein resulted in the band pattern seen in Plate 1. The primary component of guinea-pig apo-LD lipoprotein (Plate 1*a*) was resolved as a diffuse, intensely stained area, the leading boundary of which was split into two bands. The molecular weights of these components were 422000 and 402000 respectively. Immediately below was a series of seven faint bands whose molecular weights were 388500, 372000, 352800, 328000, 309900, 289000 and 266500 respectively. Apolipoproteins were also identified with molecular weights of 200000 and 165000 (faintly stained) and of 65500 and 52600.

The principal component of apo-LD lipoprotein from human serum, i.e. apolipoprotein B, exhibited a slightly lower molecular weight than that of guinea-pig apo-LD lipoprotein (Plate 1*b*). Thus a doublet of bands, of molecular weights 340900 and 318000, was seen at the leading boundary of this component. A further series of six faint bands, whose molecular weights ranged from 303000 to 155000, was also detected. The result of sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of the fraction I material isolated from guinea-pig apo-HD lipoprotein is shown in Plate 1(*c*). Several (about 10) components were detected, whose overall molecular-weight range was 425000–51300. The most prominent bands were those with molecular weights of 230000, 214000 and 130000.

Distribution of the soluble apolipoproteins. The electrophoretic patterns of polypeptides that may be extracted from VLD, LD and HD lipoproteins by 4.2M-tetramethylurea are shown in Plate 2. As indicated by their similar electrophoretic mobilities, several of the soluble apolipoproteins were present in each lipoprotein class (Table 5).

In control gels, a component of high mobility was present which ran at the dye front and stained for both protein and carbohydrate. This has been regarded as an artifact, since attempts to determine an amino acid composition brought to light only traces of glycine. However, on electrophoresis of polypeptides soluble in tetramethylurea, at least sixteen amino acids could be detected in this band, suggesting that a rapidly migrating peptide was present; this has been called band I. It should be noted that densitometric measurements of this band in stained gels, on which apoprotein samples had been electrophoresed,

Table 5. *Electrophoretic mobilities of the tetramethylurea-soluble apolipoproteins from guinea-pig serum VLD, LD and HD lipoproteins*

Electrophoretic mobility is expressed as the ratio of the distance of migration of the individual apolipoprotein to that of the dye front. Mobility values are means ± S.E.M. for the number of different preparations of each lipoprotein fraction given in parentheses; triplicate samples of each preparation were electrophoresed, and the average mobility value for each apolipoprotein from these gels was taken for calculation of the fraction mean for that apolipoprotein. All samples were electrophoresed in 7.5% (w/v) polyacrylamide gels containing 8M-urea and in the presence of reducing agents. Soluble apolipoproteins are denoted by their corresponding band number in the stained gel, as shown in Fig. 1. n.d., Band not detectable; n.m., bands not measurable owing to their diffuse nature. *, †, ‡ indicate bands detected in one, two or three separate preparations respectively. LD lipoproteins were isolated in the density interval 1.007–1.100g/ml, whereas HD lipoproteins were of density 1.100–1.210g/ml.

Tetramethylurea-soluble apolipoprotein	Electrophoretic-mobility value		
	VLD lipoprotein	LD lipoprotein	HD lipoprotein
Band number	(5)	(4)	(2)
I	1.0	1.0	1.0
II-B	0.66†	0.65 ± 0.006	n.d.
II	0.60 ± 0.006	0.60 ± 0.012	n.d.
III	0.56 ± 0.006	0.55 ± 0.008	0.55
IV	0.49 ± 0.005	0.47 ± 0.010	0.50
V	n.m.	0.41 ± 0.008	n.d.
VI-B	0.29*	n.d.	0.28
VI	0.25 ± 0.003‡	0.22† ± 0.013	0.23
VII	0.19 ± 0.005	0.18 ± 0.008	0.19*
VIII	0.14 ± 0.005‡	0.15† ± 0.006	0.15*
IX	0.10 ± 0.005‡	0.12 ± 0.007	0.10*
X	0.07‡	0.07‡	0.06

have been corrected for the contribution of this artifact (see Fig. 1).

Above band I, a triplet of components of intermediate mobility was typically seen in preparations of both LD and VLD lipoproteins. These components have been denoted as bands II, III and IV and exhibited mean electrophoretic mobilities of 0.61, 0.56 and 0.49 respectively. Of these bands, III was generally the most prominent of the tetramethylurea-soluble apolipoproteins of VLD lipoprotein. A faint band was occasionally visible immediately below band II in the tetramethylurea-soluble fraction of LD-lipoprotein preparations, and is denoted as band II-B: this band was also detected in a single preparation of VLD lipoprotein.

The presence or the absence of reducing agents had no effect on the electrophoretic mobilities of bands I–IV, as may be seen from the paired gels (A) and (B) (Plate 2*b*) of the tetramethylurea-soluble fraction of LD lipoprotein. The presence of reducing agents markedly affected the component(s) denoted as band V, since this stained as a diffuse area under such conditions (Plate 2*b*, gel B). Under non-reducing conditions however, the material was condensed into a thin band, surrounded on each side by a narrow area of diffuse stain (Plate 2*b*, gel A). This effect was only discernible in tetramethylurea-soluble fractions of LD lipoprotein, in which the relative content of band V material was greater than in VLD or HD lipoprotein (see Fig. 1). The band V component(s) of the tetramethylurea-soluble fraction of VLD lipoprotein was poorly resolved, and was detected as a diffuse area of faint stain.

A group of components of low mobility (bands VI–X, with mean electrophoretic mobilities of 0.23, 0.19, 0.15, 0.11 and 0.07 respectively) were particularly prominent in the soluble apolipoproteins of LD lipoprotein, and were sharply resolved under reducing conditions. In the absence of reducing agents, band X was not detectable; under such conditions, the component of lowest mobility corresponded to band IX (see Fig. 1 and Plate 2*b*, gel A). Beneath this band was a region of diffuse stain in which distinct bands could not be differentiated; this region corresponded to that of the bands VI–VIII in gels of samples electrophoresed under reducing conditions.

The components of low electrophoretic mobility (i.e. bands VI–X) were less prominent in preparations of VLD lipoprotein, and indeed it was necessary to apply a minimum quantity of about 100μg of tetramethylurea-soluble apoprotein to detect them. Of these apoproteins, those migrating as bands VII, IX and X were usually present.

The appearance and variable position of the band denoted as VI in preparations of apo-LD lipoprotein suggested that this band may in fact represent more

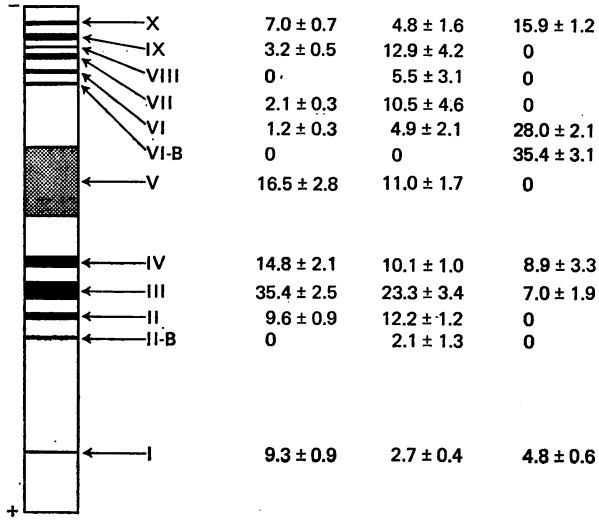


Fig. 1. *Percentage distribution of tetramethylurea-soluble apolipoproteins in guinea-pig serum lipoproteins*

The lipoprotein fractions were isolated from a single representative sample of pooled serum. Values are the mean % densitometric 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. All gels contained 8 M-urea; samples were electrophoresed in the presence of reducing agents. The densitometric areas of band I in gels of each lipoprotein class were corrected for the contribution of 'band I' in control gels on a gravimetric basis, after the band I peaks had been cut from each scan and weighed. Two or more control gels, on which only the marker dye, reducing solution and sucrose had been electrophoresed, were included in each experiment.

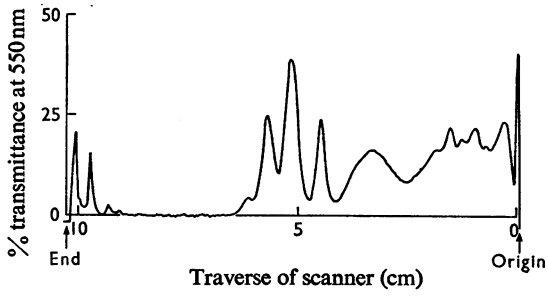


Fig. 2. *Representative densitometric scan of the tetramethylurea-soluble apolipoprotein pattern of LD lipoprotein electrophoresed in 7.5% polyacrylamide gel*

The spikes at each end of the scan correspond to the origin and end of the gel respectively. The gel was stained with Coomasie Brilliant Blue and scanned at 550nm.

than one component. Band VI-B was seen in one sample of VLD lipoprotein, and was positioned slightly below band VI itself.

Whereas the serum VLD and LD lipoproteins had patterns of tetramethylurea-soluble apoproteins that were basically alike, the HD lipoprotein pattern was different. Thus its predominant components were resolved as two broad bands of low electrophoretic mobility (0.23 and 0.28 respectively). The former component probably corresponds to band VI in VLD and LD lipoproteins, and the latter was present in trace amounts in VLD lipoprotein where it was denoted band VI-B. Each of these bands appeared as a doublet in lightly loaded gels (to which approx. 50 μg of tetramethylurea-soluble protein had been applied).

The proportions of the other components in HD lipoprotein were variable. However, components with electrophoretic mobilities corresponding to bands I, III, IV and X could be identified.

Of the tetramethylurea-soluble apoproteins of guinea-pig serum lipoproteins, only the band V component(s) stained for carbohydrate.

The semi-quantitative distribution of the soluble apolipoproteins in each of the lipoprotein classes is presented in Fig. 1. A representative densitometric scan of a sample of LD lipoprotein is shown in Fig. 2. The major soluble apoprotein of both the VLD and LD lipoproteins was band III, which represented about 25–35% of the total in each lipoprotein class.

Table 6. *Amino acid composition of the tetramethylurea-soluble apolipoproteins from guinea-pig serum lipoproteins*

Values are the means of single analyses of each of the number of hydrolysates of each component given in parentheses. These values are expressed as mol of each amino acid/100mol of amino acid residues, ± S.E.M.; values for components for which only two hydrolysates were analysed are expressed as the mean ± the range. Hydrolysates were derived from between 6 and 12 stained bands sectioned from a group of gels on which samples (each containing 100–200 μg of protein) of the same lipoprotein fraction had been electrophoresed. Insufficient material was obtained to permit accurate analysis of the apoproteins of bands II-B and VIII. The numbering of the individual apolipoprotein bands is illustrated in Fig. 1.

	Apolipoprotein band number									
Amino acid	I (3)	II (2)	III (3)	IV (2)	V (3)	VI-B (2)	VI (2)	VII (3)	IX (3)	X (2)
Lysine	6.1±0.52	4.0±1.50	4.1±0.49	5.3±1.70	5.8±0.99	11.5±3.60	7.1±2.70	7.3±1.80	6.3±0.70	5.4±1.30
Histidine	0.4±0.07	0.8±0.10	0.2±0.06	1.3±0.75	1.0±0.44	1.8±0.90	1.1±0.15	1.9±0.19	0.4±0.12	1.2±0.05
Arginine	2.7±0.41	2.0±0.25	2.6±0.55	2.1±0.30	4.0±0.40	2.9±0.10	5.7±1.70	2.8±0.74	10.4±0.65	7.5±4.30
Aspartic acid	10.0±1.13	11.3±1.80	9.1±0.75	10.8±1.05	16.0±1.44	9.5±0.05	12.0±0.10	13.2±1.04	16.2±1.64	14.8±1.40
Threonine	5.3±0.80	8.1±0.70	8.4±0.39	5.9±0.40	7.7±0.49	5.8±0.05	5.3±0.30	5.6±0.12	4.6±0.42	3.9±1.15
Serine	8.3±0.96	15.9±1.65	14.5±1.63	12.6±2.50	10.5±1.40	10.5±3.50	9.0±0.85	12.8±1.61	8.7±0.39	7.5±0.55
Glutamic acid	19.0±1.56	19.6±0.85	21.5±0.62	19.5±1.05	14.9±1.88	14.4±2.30	21.4±0.95	19.1±1.41	13.7±0.24	15.1±2.25
Proline	1.0±0.39	0.3±0.35	0.2±0.07	0.4±0.20	2.0±0.69	0	0	0.6±0.52	0.7±0.73	0
Glycine	14.9±1.15	7.4±0.07	6.8±0.31	10.4±3.10	7.4±1.89	9.1±0.05	6.3±0.80	7.5±0.97	10.2±0.61	7.7±4.00
Alanine	8.0±0.40	9.2±0.90	7.9±0.28	11.8±3.05	7.3±0.38	9.5±0.15	10.2±1.10	10.3±0.61	8.8±0.25	7.9±4.10
Valine	5.3±0.34	5.0±0.75	4.5±0.75	4.7±0.10	4.8±0.55	3.6±2.45	5.0±0.80	3.0±0.72	2.8±0.93	4.0±1.20
Methionine	1.3±0.32	1.1±1.05	3.0±0.80	1.9±0.10	0.5±0.29	0.8±0.70	0.3±0.30	0.4±0.30	0	2.3±0.85
Isoleucine	2.1±0.57	2.4±0.50	3.0±0.37	2.3±0.40	4.4±0.49	2.5±0.40	2.4±0.40	1.2±0.72	2.9±1.19	2.0±0.40
Leucine	10.9±0.44	8.9±0.55	11.0±0.40	7.6±0.15	8.7±0.88	10.6±2.00	9.4±4.20	7.6±0.74	7.2±0.89	9.0±0.65
Tyrosine	1.8±0.35	2.9±0.40	2.2±0.25	1.0±0.60	2.0±0.57	3.0±0.40	2.0±0.50	1.9±0.78	2.7±0.42	5.0±2.00
Phenylalanine	2.7±0.15	1.0±0.30	1.0±0.24	2.2±0.35	3.0±0.71	4.0±0.80	2.6±0.60	4.6±0.45	4.4±0.48	6.3±3.10

The distribution of the remaining apoproteins in these two lipoprotein classes was similar, although the more slowly migrating components (electrophoretic mobilities <0.29) always represented a higher proportion of the tetramethylurea-soluble apoproteins of LD (30–45%) than of VLD lipoproteins (10–20%). As noted above, the predominant soluble apoproteins of HD lipoprotein were bands VI and VI-B, which comprised more than 60% of the total on a densitometric basis. The component of slowest mobility, band X, was present in this lipoprotein class in substantial amounts, representing about 15% of the total.

Amino acid composition of the soluble apolipoproteins

The amino acid compositions of the peptides isolated from polyacrylamide gels are presented in Table 6. To obtain the largest amounts of these apolipoproteins for amino acid analysis, each component was isolated from the lipoprotein class in which it was most abundant. Thus bands VI-B and VI were isolated from HD lipoprotein, and band X was isolated from both HD and LD lipoproteins. All other components were obtained from LD lipoprotein, except for band I, which was isolated from all three lipoprotein classes. The soluble apolipoproteins uniformly exhibited high contents (10mol/100mol or more) of glutamic acid and aspartic acid, and their proportions of serine, leucine and alanine tended to be only slightly less. By contrast, these polypeptides were deficient in histidine, proline and methionine residues, with proline falling below the limits of detection in bands VI, VI-B and X, whereas methionine appeared to be absent from band IX. Band VI-B could also be distinguished by its unusually high lysine content (11.5mol/100mol) and band IX by its high arginine content (10.4mol/100mol). The rapidly migrating peptide of band I was distinguished by a mean content of 14.9mol/100mol of glycine. The amino acid profiles of bands II and III were alike, and in addition, were unique among the soluble polypeptides in exhibiting tyrosine/phenylalanine ratios greater than 1. The similarity in composition and electrophoretic mobility of these two peptides suggests that they may be polymorphic forms of a single protein.

For technical reasons, it was difficult to determine accurately the half-cystine content of any of the peptides isolated from polyacrylamide gel. However, traces of this amino acid were detected in hydrolysates of band V, an observation which may be relevant to the marked effect of reducing agents on its definition (Plate 2*b*, gels A and B).

On determination of the polarity indices (Capaldi & Vanderkooi, 1972) of the individual apolipoprotein bands, it was found that band I exhibited a lower index (51.8%) than any of the other components, whose indices ranged from 55.4 to 62.7%.

Discussion

Our previous investigations (Mills *et al.*, 1972) of guinea-pig serum lipoproteins indicated that particles characteristic of the LD-lipoprotein family were distributed throughout the density range 1.007–1.100g/ml in animals fed on a normal diet. We therefore adopted this density interval for isolation of LD lipoproteins in the present study.

Sardet *et al.* (1972) have reported studies of guinea-pig apolipoproteins in sodium dodecyl sulphate–polyacrylamide gel, but were unable to obtain apoprotein bands that were well resolved in either VLD or LD lipoproteins. These workers used a high gel concentration (10%, w/v) followed by Amido Black staining. Our preliminary experiments showed these conditions to be unfavourable for the study of guinea-pig apolipoproteins.

The apoprotein fractions precipitated by tetramethylurea from guinea-pig serum LD and VLD lipoproteins exhibited amino acid compositions that were similar, and which resembled that of the fraction I material isolated by gel-filtration chromatography of sodium dodecyl sulphate-solubilized apo-LD lipoprotein (Tables 3 and 4). Moreover, both the tetramethylurea-insoluble fraction of LD lipoprotein and fraction I accounted for about 85% of the total apoprotein moiety of LD lipoprotein. These data are consistent with the conclusion that the apoprotein fraction precipitated by tetramethylurea from apo-LD lipoprotein is the same as the fraction I material isolated by gel-filtration chromatography. Not only is this component the principal apoprotein of guinea-pig serum LD lipoproteins, but it is also the major component (55%) of the VLD lipoproteins. Its distribution is therefore similar to that of apolipoprotein B in man (Gotto *et al.*, 1972) and to that of the corresponding protein in the rat (Bersot *et al.*, 1970; Koga *et al.*, 1971). Further, the guinea-pig apoprotein is similar to human apolipoprotein B in its solubility, elution characteristics on gel-filtration chromatography, and in its (apparent) molecular weight and amino acid composition. In addition, it resembles the rat counterpart of apolipoprotein B (fraction PI; Koga *et al.*, 1971) in both amino acid composition and in its behaviour on gel-filtration chromatography. We therefore propose to refer to this protein as guinea-pig apolipoprotein B.

It is noteworthy that although the principal component of apo-LD lipoprotein exhibited a high molecular weight (>400000) on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, this same component (i.e. apolipoprotein B) was resolved into several bands of lower molecular weight after its isolation by gel-filtration chromatography. Such observations are consistent with the presence of a number of different apolipoprotein subunits in guinea-pig apolipoprotein B. Evidence for hetero-

Table 7. *Comparison of the amino acid compositions of some serum apolipoproteins from guinea pig, man, rabbit and rat*

Values are expressed as mol of each amino acid/100 mol of amino acid residues. The compositions of the guinea-pig apoproteins are taken from Table 6. Human apolipoproteins R-X_1, R-X_2 ('high-arginine peptide'), C-I, A-I and A-II values are from Shore & Shore (1972) for apolipoproteins isolated from the serum VLD and HD lipoproteins; values for the rabbit R1 and R2 apolipoproteins are from Shore *et al.* (1974), and that for the rat C-I is from Herbert *et al.* (1974).

	Amino acid composition											
Amino acid	Guinea-pig band I	Human R-X_1	Guinea-pig band VI-B	Human C-I	Rabbit R1	Rat C-I	Human A-II	Guinea-pig band VI	Human A-I	Guinea-pig band IX	Human R-X_2	Rabbit R2
Lysine	6.1	6.1	11.5	15.4	14.9	16	11.6	7.1	7.9	6.3	4.9	4.4
Histidine	0.4	1.5	1.8	0	1.6	2	0	1.1	2.2	0.4	1.1	1.0
Arginine	2.7	3.0	2.9	5.4	3.3	4	0	5.7	6.7	10.4	10.6	10.1
Aspartic acid	10.0	7.4	9.5	9.0	6.3	10	4.1	12.0	9.5	16.2	4.8	5.0
Threonine	5.3	5.2	5.8	4.5	11.4	8	8.1	5.3	3.8	4.6	4.9	3.1
Serine	8.3	14.1	10.5	12.6	10.2	6	8.1	9.0	6.2	8.7	6.0	5.8
Glutamic acid	19.0	18.3	14.4	15.9	16.1	14	19.8	21.4	18.3	13.7	22.8	22.7
Proline	1.0	3.0	0	1.2	2.3	2	5.2	0	3.8	0.7	2.9	3.2
Glycine	14.9	16.1	9.1	3.2	2.3	2	4.3	6.3	4.5	10.5	5.9	5.9
Alanine	8.0	7.1	9.5	5.4	4.2	9	6.7	10.2	7.6	8.8	10.6	11.8
Valine	5.3	4.6	3.6	3.5	1.6	0	7.6	5.0	5.2	2.8	6.7	6.2
Methionine	1.3	0.8	0.8	1.5	1.6	4	1.5	0.3	1.5	0	2.3	3.0
Isoleucine	2.1	2.5	2.5	4.8	8.1	6	1.5	2.4	0	2.9	1.2	1.0
Leucine	10.9	6.3	10.6	10.4	7.0	9	10.2	9.4	14.9	7.2	10.8	10.9
Tyrosine	1.8	1.6	3.0	0	0	0	4.6	2.0	2.8	2.7	1.4	1.5
Phenylalanine	2.7	2.5	4.0	4.8	8.1	6	5.1	2.6	2.3	4.4	1.4	1.5

geneity of subunit polypeptides in the human apoprotein was first provided by the studies of Shore & Shore (1969) and by those of Kane *et al.* (1970). These observations were further supported by electrophoretic studies of the detergent-solubilized apoprotein (Lipp & Wiegandt, 1973).

The analyses summarized in Table 2 show that tetramethylurea-insoluble protein accounts for up to 50% of the protein moiety of the HD lipoproteins, suggesting the presence of a high proportion of apolipoprotein B. Thus Sardet *et al.* (1972) have observed significant numbers of LD-lipoprotein-sized particles in the guinea-pig HD-lipoprotein fraction of density 1.090–1.21 g/ml. However, the amounts of HD lipoprotein available for our analyses were very small, and it has not been possible to establish that tetramethylurea precipitates the same protein from both the HD and LD lipoproteins of the guinea pig. It may therefore be premature to conclude that guinea-pig HD lipoproteins differ from those of man in having a high content of apolipoprotein B (Kostner & Alaupovic, 1972; Kane, 1973).

The tetramethylurea-soluble polypeptides from guinea-pig serum lipoproteins yield an electrophoretic pattern on polyacrylamide gel that is superficially like that obtained from those of man. Thus the mobilities of bands II, III and IV were close to those of the human C-III and C-II polypeptides in basic polyacrylamide gel. In their amino acid compositions, however, they bore no resemblance to any of the human C (or A) apolipoproteins. Similarly, the band V component, which was prominent in the soluble fractions of both apo-VLD and apo-LD lipoproteins, was distinguished by its composition from the human apolipoproteins, particularly in its high content of aspartic acid. By contrast, the amino acid composition of band I resembled in several respects that of the R-X_1 peptide (Table 7), which has been isolated from human serum apo-VLD lipoprotein by ion-exchange chromatography (Shore & Shore, 1972).

Bands VI-B and VI displayed a relative mobility on polyacrylamide gel very like that of the human A apoproteins and were, moreover, prominent components of the tetramethylurea-soluble polypeptides of guinea-pig HD lipoproteins. Band VI-B (which was occasionally resolved into a doublet and may therefore consist of more than one polypeptide or may contain disulphide bonds) was also sometimes detected in the VLD lipoproteins in trace amounts. This polypeptide resembles the human A-II component in its high lysine content, but appears also to contain both histidine and arginine, which are absent from the human peptide (Table 7). These and other compositional differences are less marked when band VI-B is compared with the human A-I polypeptide. In this respect therefore band VI-B seems more akin to human polypeptide A-I than to polypeptide A-II. There is, however, an even closer degree of similarity between the amino acid compositions of band VI-B and the human C-I polypeptide, although the latter has a higher lysine content and possesses substantially less glycine and alanine (Table 7). Differences between the proportions of certain amino acids in the rabbit R1 apolipoprotein and that of its apparent human analogue, the C-I peptide, have been reported by Shore *et al.* (1974). In contrast, the differences in composition between the human C-I peptide and its rat analogue are rather less marked.

In a similar manner to that described for band VI-B, the amino acid profile of the band VI component displayed features in common with both human A-I and C-I polypeptides; in this case it resembled the A-I polypeptide more closely than the C-I.

In the light of the present evidence it has not been possible to determine whether the peptides of bands VI and VI-B are structural components of guinea-pig HD lipoproteins, as are the A apoproteins of man, or whether they are in dynamic equilibrium between the VLD and HD lipoproteins as the human C peptides are thought to be (Havel *et al.*, 1973*b*).

The polypeptides of both bands IX and X are rich in arginine. Of these, band IX is similar in arginine content and relative mobility in basic polyacrylamide gel to the 'arginine-rich peptide' of both man and rabbit, but nonetheless differs from both of these apoproteins (Table 7) in its content of other amino acids. The most notable of these are the proportions of aspartic acid and glutamic acid; however, these two together account for 29.9 mol/100 mol of the guinea-pig peptide and for 27.6% and 27.7% of the human and rabbit peptides respectively. This suggests that these amino acids are interchangeable between species, a supposition that is possible in view of the likeness between the triplet codons for aspartic acid (GAU or GAC) and glutamic acid (GAA or GAG) (Nirenberg & Matthaei, 1961).

Although rich in arginine, band X is distinguished by its high content of the aromatic acids tyrosine and phenylalanine, which accounted for some 11 mol/100 mol. This peptide does not appear to have a counterpart among the apoproteins of man.

The polarity indices of the soluble polypeptides of guinea-pig lipoproteins are in the range 55.4–62.7% (except for band I), indicating that they are of rather more polar character than most soluble proteins, many of which have a polarity index close to 47% (Capaldi & Vanderkooi, 1972). It may be postulated therefore that these peptides associate less strongly with the hydrophobic constituents of the lipoproteins than band I and apolipoprotein B, whose polarity indices are 51.8 and 50.6% respectively.

These investigations have established that the guinea pig has an apolipoprotein that in its solubility behaviour, amino acid composition and (apparent)

molecular weight is a counterpart of human apolipoprotein B. On the other hand, although a number of the soluble polypeptides, particularly those of bands I, VI-B, VI and IX, partially resemble some of the human apolipoproteins, the available evidence does not establish their equivalence. However, the apparent lack in the guinea pig of an analogue of the human C-II polypeptide, which is the activator of lipoprotein lipase (Havel *et al.*, 1973*a*), is noteworthy in view of the deficiency in activator capacity of guinea-pig post-heparin plasma for lipoprotein lipase (Whayne & Felts, 1970*a*,*b*; Bier & Havel, 1970).

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