Heterogeneity of Human Plasma Very Low Density Lipoproteins. Separation of Species Differing in Protein Components†

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ABSTRACT: The heterogeneity of human plasma very low density lipoproteins (VLDL) with respect to the proteins associated with the lipoprotein particles was demonstrated by fractionation of VLDL by affinity chromatography. The proteins associated with the VLDL fractions were determined by disc electrophoresis in polyacrylamide gels containing 8 M urea and by immunodiffusion assay. The identities of the various components in the electrophoretic patterns were established by amino acid composition of the corresponding proteins isolated by ion exchange chromatography. These studies suggest that most VLDL species contain more than one kind of protein and that some species are deficient in one or more of the proteins found in unfractionated VLDL. No absolute deficiences in any of the major VLDL proteins were seen among a number of fasting individuals, both normal and hyperlipemic, but the proportions of the proteins varied, with some hyperlipemic VLDL proteins showing a relative deficiency or excess of one or other of the proteins. It is suggested that differences among VLDL species may reflect a dynamic or changing composition of the plasma VLDL. Individual differences in the proportions of the VLDL proteins, and presumably the VLDL species, may reflect differences in the homeostasis of lipid metabolism.

In human plasma, the very low density lipoproteins (VLDL),[1] the triglyceride-transport particles, contain a variety of proteins (Fredrickson *et al.*, 1972; Shore and Shore, 1972) and are related by common proteins to low density and high density lipoproteins (Shore and Shore, 1970; Gotto *et al.*, 1972). The VLDL are heterogeneous in size and composition (Lossow *et al.*, 1969; Oncley and Harvie, 1969); they contain large but variable amounts of lipids (predominantly triglycerides), small amounts of carbohydrates, and the proteins, which make up 5–12% of the VLDL mass. The proteins are important as determinants of structure and properties of VLDL and for transport, exchange, and metabolism of lipids. Increasing evidence indicates cofactor roles for lipoprotein proteins in enzyme reactions involving the lipid moieties as substrates (Brown and Baginsky, 1971; Fielding *et al.*, 1972; Havel *et al.*, 1970; LaRosa *et al.*, 1970).

In the present study, the following aspects of human plasma VLDL composition were investigated: (1) the heterogeneity of VLDL protein and further characterization of the proteins isolated from VLDL, (2) the variability of VLDL from one individual to another with respect to the component polypeptides of the protein moieties, and (3) the possibility that the VLDL fraction of plasma contains a mixture of VLDL species differing in the kinds of proteins associated with the lipoprotein particle. The protein moieties were subjected to polyacrylamide gel electrophoresis, gel filtration, and ion-exchange chromatography. The fractions were characterized by disc electrophoresis, amino acid composition, and immunodiffusion against antisera from rabbits injected with LDL or the isolated HDL proteins. The lipoproteins were fractionated on the basis of their glycoproteins by affinity chromatography on concanavalin A Sepharose.

Materials and Methods[2]

The lipoproteins, VLDL (d <1.007 g/cm^3), LDL_1 (d 1.029–1.040 g/cm^3), LDL_2 (d 1.007–1.019 g/cm^3), and HDL (d 1.080–1.195 g/cm^3) were prepared from serum by ultracentrifugation at the appropriate density as described previously (Shore and Shore, 1969). Serum was separated from the clot about 3 hr after drawing the blood and lipoprotein isolation was initiated within a day or two after drawing the blood. The lipoproteins, kept at 4° in the solvent (containing EDTA) in which they were isolated, were stable over a period of weeks with respect to polyacrylamide gel electrophoresis pattern, but the patterns shown here were obtained soon after isolation of the lipoproteins. The sera from fasted persons (none of whom were taking clofibrate) were analyzed individually. The VLDL proteins of 20 individuals, both males and females ranging in age from 20 to 43 years, with normal plasma lipoprotein concentrations were subjected to polyacrylamide gel electrophoresis. Of these, 10 representative patterns are shown here. The hyperlipemic individuals ranged in age from 33 to 46 years.

Water-soluble, lipid-free proteins of VLDL were prepared by extractions at 0–4° of VLDL (1–2 mg of protein/ml) in 0.5 M NaCl, 0.01 M Tris, and 0.0008 M EDTA at pH 7.6 with diethyl ether and subsequent extraction with ether and ethanol mixtures (with increasing ethanol concentration from 10 to 30% by volume) of the phospholipid–protein complexes in water at pH 4. Several extractions with a solvent-to-water phase ratio of about 1–2 to 1 were used. The organic extracts were washed with a small volume of water to recover the small amount of protein carried over in them. After ether extraction and again after ether–ethanol extraction, the aqueous phase was dialyzed against water (degassed and bubbled with prepurified N_2) at 4° in size 18 cellulose casing (Union Carbide,

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[1] Abbreviations used are: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

[2] Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Atomic Energy Commission to the exclusion of others that may be suitable.

Chicago, Ill.) that had been precleaned by soaking in 1% $NaHCO_3$, 8 M urea, and finally water. Protein solutions were concentrated by ultrafiltration and by selective removal of water from the dialysis bag by Dextran 20.

Disc gel electrophoresis was carried out essentially as described by Davis (1964). The polyacrylamide gels, usually 10% (although 7.5 and 12% gels were also found to give satisfactory patterns), contained 8 M urea. The protein samples were preincubated in 8 M urea at room temperature for about 1 hr. Tris–glycine buffer, 0.05 M, pH 8.3 (no urea), and a current of 2.5 mA/tube were used. The lipid-free protein, obtained by ether and ether–ethanol extraction or by hexane and chloroform–methanol (3:1) extraction of VLDL, gave satisfactory patterns. However, chloroform–methanol rendered insoluble in urea the component of VLDL protein that does not enter the separating gel. Phosphorus analyses on the ether–ethanol-extracted protein indicated that <3% phospholipid remained in the preparation. However, complete extraction of lipids by solvents probably is not essential for electrophoresis or chromatography in the presence of urea because urea can dissociate lipid from protein. The HDL and its lipid-free protein give the same disc electrophoresis patterns, and the pattern of the phospholipid–protein complex from extraction of VLDL is similar to that of the lipid-free protein. However, solvent extraction of most of the phospholipids from VLDL is needed for optimal yield and resolution of the proteins seen in the upper part of the separating gel in polyacrylamide electrophoresis; the lower band proteins are dissociated from lipid in good yield by urea.

The VLDL proteins or the smaller (<20,000) molecular weight components obtained by gel filtration on Sephadex G-150 were fractionated by ion-exchange chromatography. Columns of DEAE-cellulose (0.9 × 26–30 cm) were equilibrated at 8° with 0.005 M Tris-HCl in 8 M urea (pH 8.2). A pump delivered eluting buffer at 15 ml/hr to the column from a closed, 250-ml mixing flask (initially filled with equilibrating buffer) connected to a reservoir of limiting buffer. The limiting buffer was 0.1 M Tris-HCl in urea at pH 8.2 during the first 30 hr, at which time it was changed to 0.2 M Tris-HCl in urea at pH 7.5. The last component was eluted with 0.4 M Tris-HCl, pH 7.5. Addition of NaCl (1 M) to the last buffer did not elute additional components. Conductivity, temperature, and absorbance at 240, 260, and 280 nm of the column effluent were monitored continuously. The fractions were dialyzed for about 2 hr on rapid dialyzers, concentrated by ultrafiltration, and dialyzed further against water. All the fractions yielded water-soluble proteins.

Amino acid analysis was carried out as before (Shore and Shore, 1968) except that mercaptoethanol (1 μl/2 ml) was added to the constant-boiling HCl for hydrolysis of the proteins. Protein concentration was obtained from the amino acid analysis; the per cent protein in the lipoproteins was estimated from the protein concentration, the volume, and the weight of extracted lipids.

Double immunodiffusion assay (Ouchterlony, 1968) was carried out in 1% agar–agar no. 3 (Oxoid, Consolidated Laboratories, Chicago Heights, Ill.) in 0.075 M sodium barbital buffer (pH 8.3) and in commercially available plates of 0.9% agarose in borate–saline (μ = 0.175) at pH 8.5 from Miles Laboratories (Kankakee, Ill.). Antisera were obtained from three rabbits, each of which had been injected subcutaneously three times over a period of 1 month with one of the following antigens: LDL (d 1.029–1.040 g/cm^3) and the two major HDL proteins isolated by ion exchange chromatography (Shore and Shore, 1968), all at an injection dose of 10 mg of protein in 1 ml of saline mixed with 1 ml of Freund's adjuvant. The VLDL were extracted with ether before immunodiffusion.

Columns of concanavalin A Sepharose (Aspberg and Porath, 1970) (0.5 × 13 cm or 0.9 × 9 cm) were used for affinity chromatography of VLDL. The column was equilibrated at room temperature with 0.2 M NaCl–0.1 M sodium acetate solution adjusted to pH 6.8 with acetic acid. Sodium azide was used as a preservative. In two cases, the VLDL was dialyzed against the equilibrating solution; in three others 2 ml of undialyzed VLDL (~20 mg) was added to the larger column and the column was washed with several bed volumes of the salt solution, followed sequentially by salt solutions containing 0.05 M glucose, 0.1 M glucose, 0.05 M mannose, 0.1 M mannose, 0.1 M methyl α-D-glucopyranoside, 0.1 M methyl α-D-mannopyranoside, and 0.2 M NaCl containing 0.1 M Tris base (pH 9.5). All solutions and the column matrix were degassed. The column effluent was monitored continuously at 280 nm. The fractions were concentrated by ultrafiltration and dialyzed. For disc electrophoresis, a sample was lyophilized, extracted with hexane and then chloroform–methanol (3:1, vv), and taken up in a small volume of 8 M urea, or extracted with ether and ether–ethanol as described above.

Results

VLDL Protein Heterogeneity in Polyacrylamide Gel Electrophoresis. Disc electrophoresis of VLDL protein in polyacrylamide gels containing urea usually gives patterns containing five to seven major bands plus several minor bands that represent only part of the proteins, and considerable protein that remains in the sample gel (Figure 1). Variations in the proportions of the protein components are seen among VLDL proteins from normal plasmas (patterns 1–10, Figure 1) and from plasmas in which VLDL was well above normal concentration (patterns 11–21, Figure 1). The proportions of components that enter the separating gel in VLDL, relative to each other and to the protein remaining in the sample gel, vary.

Frequently band H is the most abundant component entering the sample gel, but bands B and C sometimes predominate, as in patterns 1, 7, and 15 of Figure 1. The ratio of band H to band I (two forms of the same protein) also varies considerably in both normal and hyperlipemic VLDL. In hyperlipemic persons, the distribution of the VLDL proteins can resemble that in normal persons (*e.g.*, in Figure 1, patterns 11, 12, 20, and 21 are similar to patterns 1–10), but occasionally a relative deficiency or excess of one or more components is seen. Patterns 17 and 18 show relatively little of bands B and C, respectively; patterns 15, 16, and 17 are low in band F. The proportions of the bands to each other in the separating gel do not appear to be related in any consistent way to the percentage of lipid of the VLDL fraction, but differences in the proteins would be expected to be associated with differences in content of the various lipid species (not investigated). The VLDL contained 8–10% protein, except sample 21 of Figure 1 (apparently normal electrophoretic pattern), which was derived from a hyperlipemic (280 mg of triglycerides/100 ml of serum) individual whose VLDL was largely S_f 100–400 and low in the S_f 20–100 lipoproteins usually present.

In general, the denser fractions of VLDL contain less of the components in the separating gel and LDL contain relatively very small amounts. The small amounts of these VLDL proteins (*i.e.*, those seen in the separating gel) that do occur in LDL occur mainly in the less dense LDL_2 (d 1.007–1.019

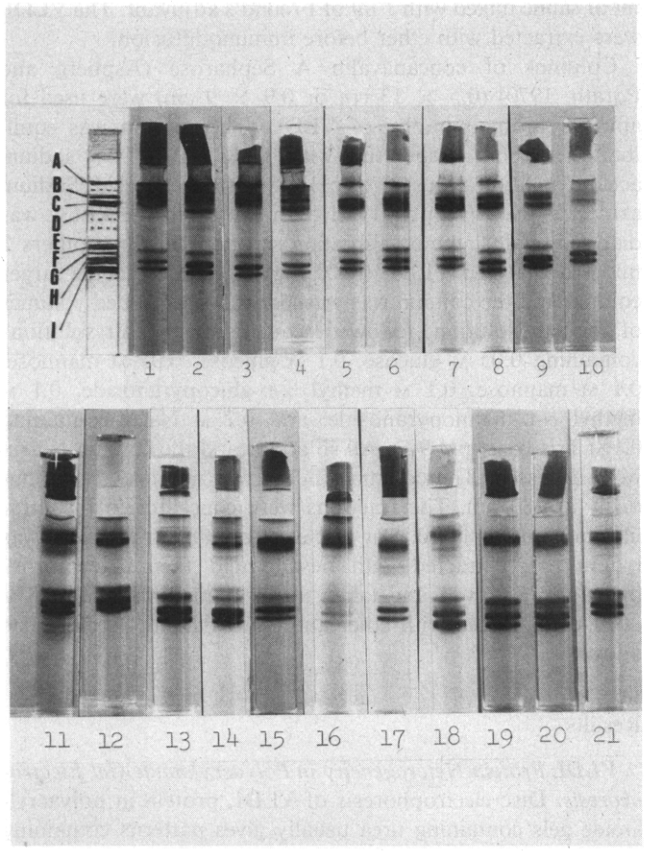

FIGURE 1: Disc electrophoresis patterns of VLDL protein in 10% polyacrylamide gels containing 8 M urea. Patterns 1–10: VLDL proteins from young male and female individuals with normal plasma lipoprotein concentrations. Patterns 11–21: VLDL proteins from plasmas with elevated VLDL.

g/cm³), which constitute normally a small fraction of the total plasma LDL, in HDL_1 (Shore and Shore, 1972), and not in the major LDL fraction (d 1.029–1.040 g/cm³). Figure 2 shows some LDL patterns from polyacrylamide gel electrophoresis. The LDL sometimes give a small band near the top of the separating gel (pattern 5), but this protein (Shore and

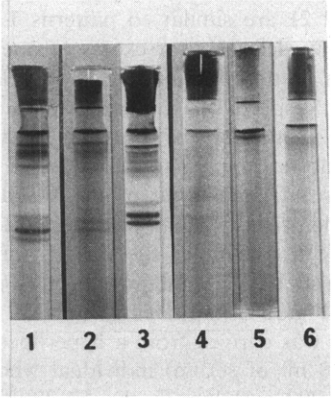

FIGURE 2: Disc electrophoresis patterns of VLDL and LDL proteins in 10% polyacrylamide gels containing 8 M urea. Pattern 1 corresponds to VLDL; 2 to LDL_2 (d = 1.007–1.019 g/cm³); 3 to LDL_2 in which a much larger amount of protein was taken for electrophoresis; and patterns 4 and 5 correspond to LDL_1 (d = 1.029–1.040 g/cm³); pattern 6 corresponds to the higher molecular weight fraction from gel filtration of VLDL protein.

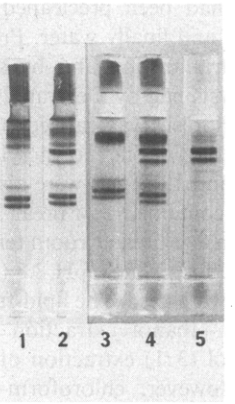

FIGURE 3: Disc electrophoresis patterns of VLDL (patterns 1 and 3), VLDL plus HDL (patterns 2 and 4) and HDL (pattern 5).

Shore, 1972) differs from the VLDL proteins corresponding to bands B and C of Figure 1. All LDL and most VLDL contain proteins that remain in the sample gel and that localize at the top of the separating gel. The higher molecular weight fraction from gel filtration of VLDL protein resembles LDL in disc electrophoresis (pattern 6 of Figure 2) and in amino acid composition. The LDL proteins are probably made up of smaller subunits that are not dissociated by urea alone (Shore and Shore, 1972).

The major VLDL bands do not correspond to either of the major HDL proteins as shown by comparison of the polyacrylamide gel electrophoresis patterns of VLDL, HDL, and a mixture of VLDL and HDL (Figure 3). Minor VLDL components similar in mobility to the HDL proteins are sometimes seen in polyacrylamide gel patterns, *e.g.*, band D and the minor components below it and above band F in patterns 2, 4, and 17 in Figure 1. However, band D corresponds to a protein other than either of the major HDL proteins, judging from the electrophoretic patterns of mixed VLDL and HDL (Figure 3) and from evidence presented in the following section. The electrophoresis patterns of Figure 1 indicate that major HDL proteins are present in very small amounts, less than 1% of the total protein, if they are present in VLDL.

VLDL Proteins Separated by Ion-Exchange Chromatography. A number of different VLDL proteins can be isolated by ion-exchange chromatography on DEAE-cellulose (Shore and Shore, 1970, 1972). As expected from the electrophoresis patterns, the VLDL fractions of different individuals vary in the proportions of the various protein components that are separated by ion-exchange chromatography; the chromatograms in Figure 4 show even more protein heterogeneity than do the polyacrylamide gel electrophoresis patterns of Figure 1. In patterns A and B of Figure 4, the whole, lipid-free protein of VLDL was applied to the column; in other experiments, only the low molecular weight fraction from gel filtration of samples corresponding to electrophoresis patterns 13, 14, 17, 18, and 21 were chromatographed. All the major fractions of these samples corresponded to proteins present in Figure 4; the LDL or LDL-like protein components of VLDL are mostly not eluted from the DEAE in the experiments of Figure 4.

Each of the protein fractions from the ion-exchange (DEAE) chromatography was correlated with the polyacrylamide gel bands seen in patterns of unfractionated VLDL protein and with the amino acid composition of the fraction. Three proteins occurred in multiple forms: the protein(s) corresponding

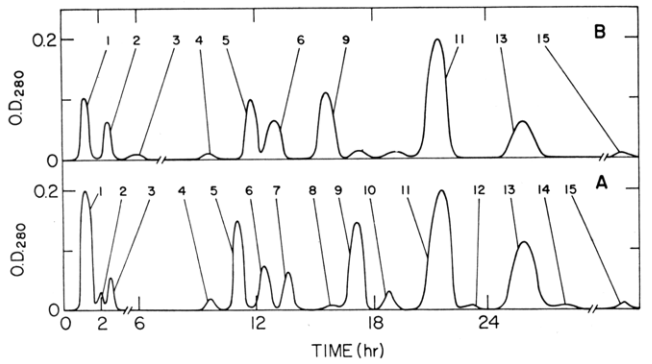

FIGURE 4: Ion-exchange chromatography on DEAE-cellulose of the whole lipid-free protein moieties of VLDL. Chromatograms A and B correspond to patterns 11 and 12, respectively, in Figure 1.

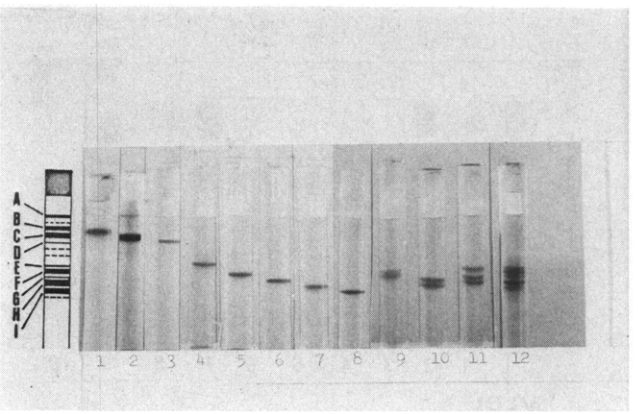

FIGURE 5: Disc electrophoresis patterns of isolated VLDL proteins in 10% polyacrylamide gels containing 8 M urea. Patterns 1–8 correspond to bands B, C, D, E, F, G, H, and I in Figure 1 and are from DEAE fractions 1, 4 + 5 + 6, 7, 8, 9, 10, 11, and 13, respectively, of Figure 4 and Table I. Pattern 9 corresponds to patterns 4 + 5; 10, to 7 + 8; 11, to 5 + 7 + 8; 12, to 4 + 5 + 6 + 7 + 8.

to band C in Figure 1 occurs in two or three DEAE fractions (4, 5 and 6 of Figure 4) that have the same amino acid composition and similar if not identical electrophoretic mobilities; fractions 2 and 15 are very similar in amino acid composition; fractions 10, 11, 12, 13, and 14, multiple forms of another protein, differ in mobility and correspond to bands H and I and to minor bands including band G that show up poorly in photographs of gels of the unfractionated protein. The correlation confirmed the relative deficiency of proteins corresponding to bands B and F in the VLDL of electrophoretic pattern 13 in Figure 1; amino acid analyses of all the DEAE fractions from this deficient VLDL indicated no variant forms of these proteins with altered electrophoretic mobilities. Figure 5 shows some polyacrylamide gel electrophoresis patterns of isolated proteins and mixtures of isolated proteins.

The amino acid compositions of all the major DEAE fractions found in the VLDL samples of Figure 4 are given in Table I. These major proteins are in composition very similar to if not identical with the major proteins of all the VLDL samples analyzed by DEAE chromatography. Thus, the arginine-rich protein corresponding to band C in polyacrylamide gel electrophoresis, which has been found previously in three individuals (Shore and Shore, 1970) appears to be a major component of VLDL of most normal and hyperlipemic individuals. The proteins corresponding to electrophoretic bands B, F, H, and I, which have been isolated by several investigators (Brown *et al.*, 1969; Shore and Shore, 1970) are usually major VLDL components with some individual exceptions as noted above. Also included in the table are the compositions of proteins occasionally found in considerable amount; these include the proteins corresponding to electrophoretic bands D and E in Figure 1. None of the protein fractions corresponded in composition to albumin or other known plasma proteins (Schultze and Heremans, 1966) or to the major HDL proteins (Shore and Shore, 1968). The proteins corresponding to electrophoretic band D in patterns 2, 11, and 14 of Figure 1 were isolated; their compositions differed from those of the two major HDL proteins. Nor are the HDL proteins present together with other proteins in some of the fractions. There were no precipitation reactions between fractions 4, 5, 6, 7, 8, or 9 of Figure 4A and antisera to either of the major HDL proteins. Both lipid-free HDL proteins, isolated in the same way as these VLDL fractions, reacted with the corresponding antiserum.

These data on the composition of isolated VLDL proteins, together with the polyacrylamide gel electrophoresis results, show that the major HDL proteins are only trace components at most of VLDL (contrary to reports based on immunochemical assay and the compositions of fractions from Pevikon zone electrophoresis (Gustafson *et al.*, 1966)). The HDL carboxyl-terminal group glutamine was not found in the carboxypeptidase digests of VLDL proteins (in lipid-free, soluble form) or the phospholipid complex. Digest (10 mg of VLDL protein plus 0.1 mg of DFP-treated carboxypeptidase A at 25 or 35°) samples taken for amino acid analysis contained no

TABLE I: Amino Acid Composition (mol/10^3 mol of Amino Acids) of Some VLDL Proteins.

	DEAE Fraction							
	1	4, 5, 6	7	8	9	10, 11, 12	2, 13	3
Electrophoretic band (Figure 1)	B	C	D	E	F	G, H, I		
Lysine	154	48	67	82	70	76	67	67
Histidine	0	13	16	0	0	15	28	11
Arginine	54	106	55	34	14	29	14	17
Aspartic acid	90	48	67	99	69	88	70	110
Threonine	45	38	49	81	102	65	41	33
Serine	126	54	86	130	116	139	203	151
Glutamic acid	159	233	202	130	176	131	188	289
Proline	12	27	36	33	43	27	21	19
Glycine	32	58	36	34	30	40	186	110
Alanine	54	108	128	126	85	124	70	50
Half-cystine	0	0	0	0	0	0	7	
Valine	35	68	66	98	56	73	29	27
Methionine	15	24	37	0	26	24	7	6
Isoleucine	48	13	9	0	13	0	14	19
Leucine	104	109	87	55	96	65	36	37
Tyrosine	0	14	24	17	54	25	7	11
Phenylalanine	48	14	25	48	26	48	14	19
Tryptophan	24	28	>10		23	34		

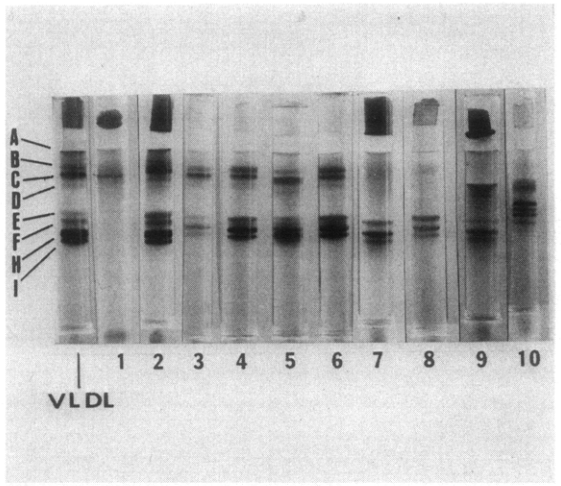

FIGURE 6: Disc electrophoresis patterns of VLDL fractions from affinity chromatography on concanavalin A Sepharose. Fraction 1 was not retarded and fractions 2–10 are in order of elution (10 was most tightly bound).

more glutamine (0.004 μmol/10 mg) than the enzyme blank.

While the polyacrylamide gel electrophoresis pattern is a convenient means of assessing the protein composition of VLDL (rapid and requiring little protein), it does not give as complete a picture as does ion-exchange chromatography. The latter gives much better resolution, separates the components that remain unrecognized as gel bands because they do not fix and/or stain well, and allows unequivocal identification of the components seen in the electrophoresis patterns (*i.e.*, mobility is not an infallible clue to the protein identity).

The variability among VLDL from different individuals with respect to the proportions of protein components in polyacrylamide gel electrophoresis patterns and in DEAE-cellulose chromatographs indicates that VLDL vary with respect to the protein moiety. There are two possible reasons. First, VLDL may be a mixture of lipoproteins differing distinctly in protein content, *i.e.*, some lipoprotein particles may contain one or more, but not all, of the proteins present in unfractionated VLDL. Second, most VLDL particles may contain all of the major VLDL proteins in variable proportions (a VLDL particle of mol wt $1–5 \times 10^7$ could contain as many as 50 or more polypeptide subunits). The experiment described in the following section indicates that the plasma VLDL fraction contains a number of particulate (molecular ?) species differing in the kinds of protein associated with them.

Fractionation of VLDL on Concanavalin A Sepharose. Elucidation of the nature of VLDL heterogeneity necessitated a means of lipoprotein fractionation based on the protein moiety rather than on size and density. (VLDL subfractions differing in density contain the same proteins.) Procedures used for fractionation of the protein are not suitable because they can dissociate lipids and disrupt the noncovalent bonds that hold the particles together. Concanavalin A Sepharose was selected for VLDL fractionation because (1) it could selectively bind some of the VLDL glycoproteins but not others, and (2) VLDL apparently are not adversely affected by agarose (Sata *et al.*, 1970). Concanavalin A selectively binds glycoproteins and polysaccharides that contain a terminal, nonreducing α-D-glucopyranosyl, α-D-mannopyranosyl, or D-fructofuranosyl residue (Goldstein, 1972).

Some of the VLDL particles applied to a column of concanavalin A Sepharose were not adsorbed; those adsorbed were eluted sequentially in fractions by D-glucose, D-mannose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, and finally salt solution adjusted to pH 9.5. The resulting lipoprotein fractions were subjected to polyacrylamide gel electrophoresis and immunodiffusion assay for comparison of their protein moieties. The VLDL fractions from the affinity chromatography varied in protein composition, as shown in Figure 6. The particles that were not bound to concanavalin A contained very little of the proteins corresponding to bands F, G, H, and I in Figure 1 and Table I. The isolated lipid-free proteins of bands G, H, and I do not bind the agglutinin (lipid-free F does); yet, they are associated with lipoprotein particles that are bound, presumably through F and/or other glycoproteins. Not all of the bound VLDL are rich in F. The bound VLDL also vary considerably in content of the proteins corresponding to bands B and C in the upper part of the gel pattern; the more tightly bound VLDL are relatively deficient in B and C. The proteins that are usually present in relatively small amounts in unfractionated VLDL are concentrated in particular VLDL species rather than being distributed evenly among all the VLDL; the protein corresponding to band E is concentrated in a VLDL fraction (pattern 2 of Figure 6) with low affinity for concanavalin A; the protein of band D is concentrated in tightly bound VLDL (patterns 9 and 10). The latter species reacted positively with antisera to isolated HDL proteins but most of the other fractions did not. The fraction shown in pattern 1 also gave a slight positive reaction, although the bands characteristic of HDL proteins are not seen. The HDL, which contain much more ceramide monoglucosides than LDL or VLDL (Skipski *et al.*, 1967), did not bind concanavalin A Sepharose. The VLDL fractions from affinity chromatography also varied in the content of LDL proteins, according to the immunodiffusion assay. Most, but not all species, gave a positive reaction with anti-LDL serum: the lipoprotein fractions corresponding to patterns 1, 5, 6, and 10 did not react and fractions 3 and 8 gave weaker reactions than the other LDL-containing species.

Discussion

Plasma VLDL are a mixture of lipoproteins differing in protein moiety as well as in size and density. Eight to ten lipoprotein fractions differing in protein composition were isolated on the basis of differences in affinity for concanavalin A. It is not certain that each of these fractions contains only one lipoprotein species. However, it appears that most VLDL species contain more than one kind of protein and that some species are deficient in one or more of the several proteins found in unfractionated VLDL. Some of the VLDL species appear not to contain LDL or β protein, and most VLDL species do not contain either of the two major HDL proteins that are at most trace components of VLDL. That these proteins were not isolated from any of a number of VLDL preparations is in agreement with the immunochemical studies of Walton (1973) and contrary to those of Gustafson *et al.* (1966) and Pearlstein *et al.* (1971).

The proportions of the several VLDL species in plasma probably vary among different individuals, accounting in part at least for differences in the relative amounts of the different VLDL proteins seen in polyacrylamide gel electrophoresis patterns and in ion exchange chromatograms. The VLDL from fasting, apparently healthy young adults vary somewhat in protein composition, but none of the "normal" and few of the hyper-VLDL samples examined showed gross deficiencies in any of the major protein components. The VLDL of some

hyperlipemic individuals appeared to be deficient in one or other of the proteins and some contained relatively more than usual of a protein rich in arginine (band C). The proportions of the VLDL species may be affected by dietary habits and physiological state, but in some instances an elevation or a deficiency in one or other of the species may be associated with abnormalities or imbalances in VLDL metabolism.

The differences among VLDL species may reflect a dynamic composition or state of the lipoproteins; that is, the VLDL particles probably are not of fixed composition in the plasma. The dynamic state of VLDL composition could be a consequence of exchange reactions of lipids (Gurd, 1960) and/or lipid–polypeptide complexes among VLDL species and between VLDL and higher density lipoproteins; it could also be a consequence of enzyme-mediated (*e.g.*, lecithin–cholesterol, acyltransferase, and/or lipoprotein lipase) reactions among the various plasma lipoproteins.

It is not yet clear what are the implications for VLDL structure and function of the existence of so many different VLDL proteins and molecular species. The existence of multiple VLDL species suggests that the metabolism of VLDL and the triglycerides is more complex than previously suspected. One of the VLDL proteins (band F in Figure 1) is a cofactor for triglyceride hydrolysis by lipoprotein lipase (Brown and Baginsky, 1972; Havel *et al.*, 1970; LaRosa *et al.*, 1970); yet some VLDL species have little or none of this protein. An alternate mechanism may exist for triglyceride utilization, perhaps by an enzyme system requiring the VLDL protein R-Ser, which corresponds to gel electrophoresis band B and which has been reported to activate lipolysis (Ganesan *et al.*, 1971), or other VLDL proteins.

Acknowledgments

We express our appreciation to Mr. Stanley Krotz and Miss Marie Laskaris for their technical assistance and to Dr. Valeska Evertsbusch for her suggestions on the manuscript preparation.

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