Proteins of S_f 10–400 Lipoproteins from Lipemic Human Plasma*

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ABSTRACT: The relationship of the S_f 10–400 lipoproteins to chylomicrons and other low-density lipoproteins in human lipemic plasma was investigated by comparing the protein portion of the lipoproteins. The S_f 10–400 lipoprotein class was divided into five fractions, each with a different lipid composition and encompassing a different S_f range. N-Terminal amino acid analyses of these fractions showed little variation in their protein composition. Serine and threonine were the major N-terminals and aspartic and glutamic acids the minor N-terminals in all fractions. The same four amino acids also were found, but in different ratios, in the chylomicron and S_f 0–10 classes. The S_f 10–400 lipoproteins from different plasmas were exhaustively extracted with cold ether, and the protein–phospholipid residues were compared by analytical ultracentrifugation. One of the major components of the residue from S_f 10–400 lipoproteins from lipemic plasma appeared to be identical with the major component of the residue from S_f 0–10 lipoproteins from nonlipemic plasma. This same component was present in small amounts in the chylomicron residue. The relative amount of this component in the S_f >10 classes increased with the degree of lipemia. These studies indicate that, under the conditions in which the liver is required to form large amounts of lipoprotein, some of the protein which normally is found in S_f 0–10 lipoproteins is incorporated into S_f 10–400 lipoproteins and chylomicrons.

The four major classes of lipoproteins in human plasma have different chemical and physical properties. Although each class is usually defined in terms of a specific range of densities or a corresponding range of flotation rates, the transition from the properties of one density class to those of another is gradual and continuous (Lindgren and Nichols, 1960; Searcy and Bergquist, 1962). Such a continuous spectrum of different lipoproteins suggests either that the classes are transformed from one into another in the plasma, or that the classes are unrelated but occupy overlapping density ranges. A knowledge of the extent to which these two possibilities occur is necessary for understanding the metabolic interrelationships, if any, that exist between the various lipoproteins.

Characterization of the protein components of various lipoproteins is a useful method of identifying different lipoproteins. The evidence indicates that a small number of different proteins serves as structural elements for the entire density range of human plasma lipoproteins. Only four amino acids have been reported as major N-terminals and four amino acids as major C-terminals (Rodbell, 1958; Shore, 1957). Exhaustive extraction of plasma lipoproteins with ether produces a limited number of discrete protein-phospholipid complexes (Avigan, 1957; Grundy *et al.*, 1959, 1961; Scanu and Page, 1959). The relative amounts of the four N-terminal amino acids and the nature of the extraction products differ in each of the four major density classes. Determination of the distribution of these proteins throughout the lipoprotein spectrum thus provides a means of comparing the different classes of lipoproteins.

The application of these amino acid analysis and extraction procedures has led to different interpretations of the relationship between the chylomicrons (S_f >400), which appear in the plasma shortly after fat is eaten, and the triglyceride-rich S_f 10–400 lipoproteins. Early studies with clearing factor lipase (reviewed by Robinson, 1963) suggested that the S_f 10–400 lipoproteins were produced from chylomicrons by the intravascular removal of triglyceride. Lindgren *et al.* (1959) showed that the interconversion was a more complex process and proposed a model system to account for the transformation of chylomicrons into S_f 10–400 and higher density lipoproteins. A number of recent reports, summarized by Dole and Hamlin (1962) and Robinson (1963), indicates that the S_f 10–400 lipoproteins are not degraded chylomicrons but rather are synthesized in the liver. Data from studies of the protein components of these lipoproteins appear to support this concept. Rodbell (1958) found an N-terminal amino acid in the S_f 10–400 lipoproteins which was not found in chylomicrons. Grundy *et al.* (1961) found after exhaustive extraction with ether that the products were different for

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the two classes. On the other hand, amino acid analyses made in this laboratory (Levy and Lynch, 1962) showed a close similarity between the protein of chylomicrons and that of the S_f 10–400 lipoproteins.

An abundance of evidence can be presented to support both hypotheses: that the S_f 10–400 lipoproteins are degradation products of chylomicrons which presumably are of intestinal origin or that they are a separate lipoprotein class synthesized by the liver. The present investigation into the nature of the S_f 10–400 lipoprotein protein was carried out in an attempt to identify further the metabolic source of this lipoprotein through a better understanding of its structure.

Experimental Procedures

Chylomicrons. Pooled lipemic plasma[1] was centrifuged at 9500 × *g* (10,400 rpm) for 30 minutes in a Beckman Spinco type 30 rotor.[2] The chylomicrons which floated were removed by aspiration and resuspended in 10 volumes of 0.15 M NaCl, *d* 1.003.[3] Solid NaCl was added to increase the density of the chylomicron solution to *d* 1.005. The solution was layered under 10 ml of 0.15 M NaCl in type 30 rotor tubes and centrifuged at 9500 × *g* for 30 minutes to float the chylomicrons. This washing process was repeated five to ten times until the infranatant solution was free of turbidity.

S_f 10–400 Lipoproteins. The chylomicron-free plasma, *d* 1.02, was centrifuged in the type 30 rotor at 50,000 × *g* (24,000 rpm) for 36–40 hours. The S_f 10–400 lipoproteins collected in a turbid layer at the top of the tubes and appeared to be separated from the yellow S_f 0–10 lipoproteins and plasma proteins by a centimeter of clear, colorless solution. The solution containing the S_f 10–400 lipoproteins was removed by aspiration and adjusted to *d* 1.06 by the addition of NaCl. Adjusted solution (25 ml) was layered under 10 ml of NaCl solution, *d* 1.019, in type 30 rotor tubes and centrifuged at 50,000 × *g* for 36 hours to pack the lipoproteins at the top of the tube. The packed material was resuspended in *d* 1.019 NaCl solution and centrifuged at 60,000 × *g* (26,200 rpm) for 28 hours. The refloated lipoproteins were resuspended in the appropriate salt solution.

S_f 0–10 Lipoproteins. The remaining plasma, free of S_f 10–400 lipoproteins, was adjusted to *d* 1.063 by the addition of NaCl and centrifuged in the type 30 rotor at 50,000 × *g* for 38 hours. The solution containing the lipoproteins which had floated was removed and adjusted to *d* 1.063 with NaCl. This solution was layered under an equal volume of *d* 1.019 NaCl solution in Beckman Spinco type 40 rotor tubes and centrifuged at 68,000 × *g* (32,000 rpm) for 28 hours. Lipoproteins with *d* 1.019–1.063 (S_f 0–10) collected in an orange zone at the interface of the two solutions and were removed with the aid of a tube slicer.

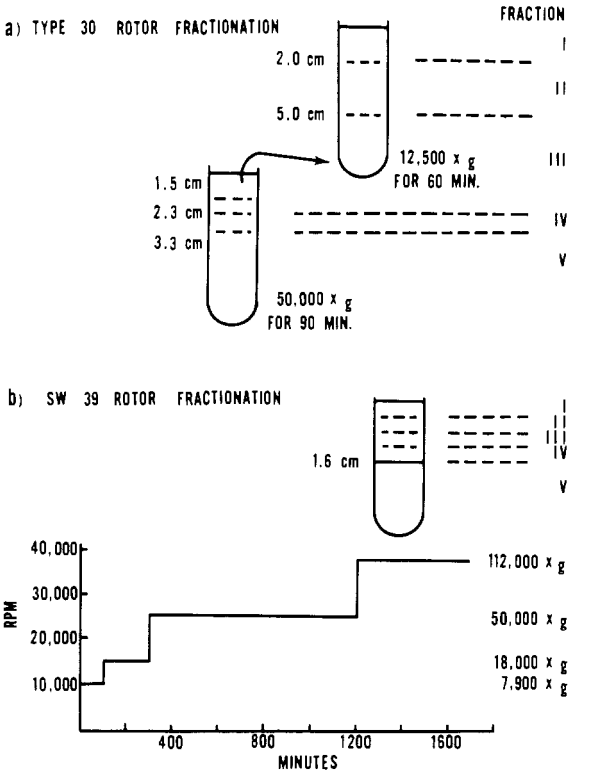

FIGURE 1: Centrifugation procedures used in fractionating S_f 10–400 lipoproteins. (a) Procedure A; (b) procedure B.

Each class of lipoproteins was examined by analytical ultracentrifugation and by electrophoresis on paper and was found to be free from contamination with other proteins or lipoproteins. Ultracentrifuge patterns of the classes showed that the use of salt solutions of the appropriate densities accurately defined the S_f limits of 0 and 10. The value of S_f 400 for the upper limit of S_f 10–400 could only be estimated, owing to the turbidity in this region. There was no appreciable variation in the ultracentrifuge patterns for S_f 10–400 preparations from different pools of plasma. All patterns showed a broad main peak at S_f 80 ± 10 with a distinct shoulder at S_f 25 ± 5.

Fractionation of S_f 10–400 Lipoproteins. Two methods were used to fractionate the S_f 10–400 lipoproteins. In procedure A, separation was effected in the type 30 fixed-angle rotor on the basis of differences in flotation rate. In procedure B, using the Beckman Spinco SW-39 swinging bucket rotor, separation resulted from differences both in flotation rate and hydrated density of the lipoproteins.

In procedure A, a solution of S_f 10–400 lipoproteins was adjusted to *d* 1.20 by the addition of NaBr. Ten ml of this solution, containing 60 mg protein, was layered

[1] Lipemic plasma was obtained from blood collected in acid-citrate-dextrose solution by the Louisville Regional Blood Center of the American Red Cross. Plasma from six units was pooled for the isolation procedures.

[2] All isolation and fractionation procedures which made use of the type 30, type 40, and SW-39 rotors were carried out at 8°.

[3] All solutions used in the isolation of lipoproteins contained 0.01% ethylenediaminetetraacetate sodium salt, pH 7.5. The symbol *d* is used to represent density in g/ml at 20°.

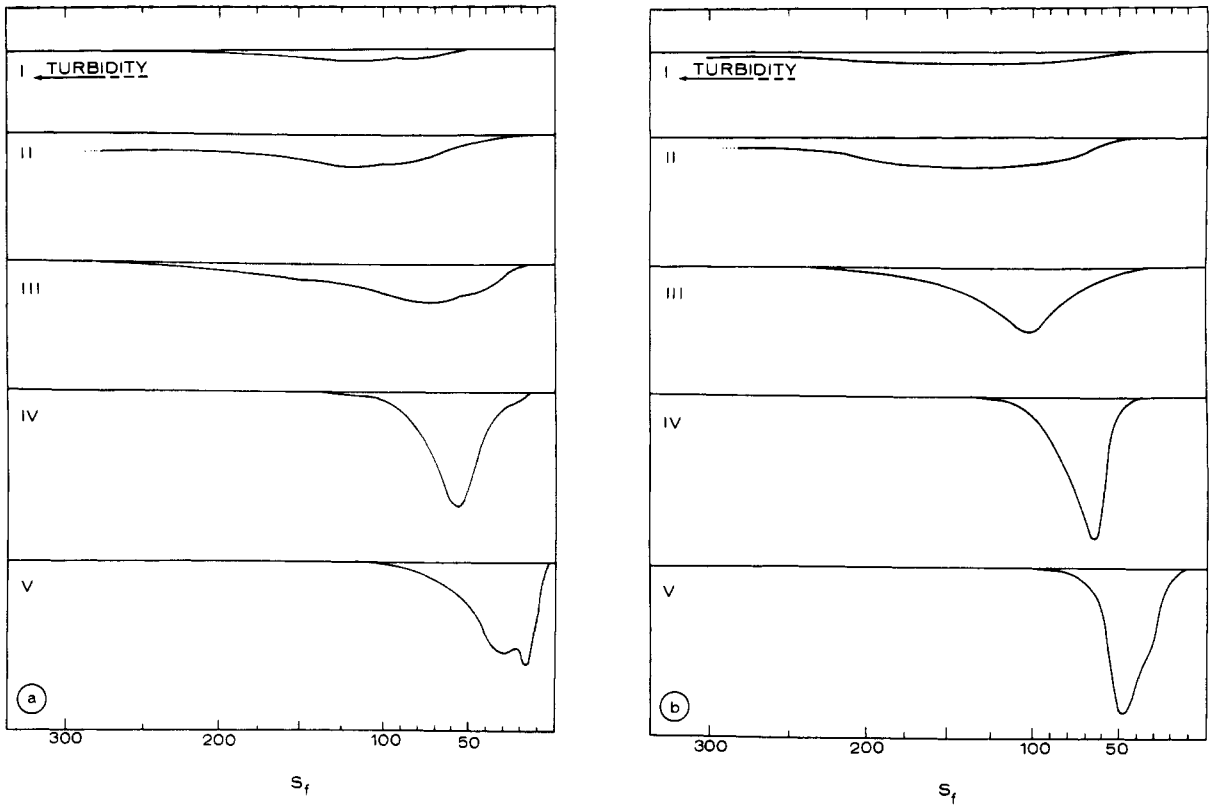

FIGURE 2: Approximation of S_f distribution of lipoproteins in S_f 10–400 fractions. The curves are tracings of the flotation patterns obtained under identical conditions of centrifugation and redrawn with a straight base line on a common abscissa. The tracings approximate the patterns observed after an equivalent of 5.7 minutes of centrifugation at 39,460 rpm. No correction for the influence of one component on another has been applied. (a) Fractions from procedure A; (b) fractions from procedure B.

under 25 ml of 0.5 M NaCl in type 30 rotor tubes. The tubes were centrifuged at 50,000 × g (24,000 rpm) for 90 minutes in the type 30 fixed-angle rotor. After centrifugation lipoproteins were found distributed throughout the tube with the greatest accumulation in the top 2 cm of the solution. Four fractions were obtained by using a tube slicer to cut the tubes as indicated in Figure 1. The top fraction was further fractionated by a similar process, using centrifugation at 12,500 × g (12,000 rpm) and making cuts as indicated.

In procedure B, the S_f 10–400 lipoproteins from another solution were suspended in 0.5 M NaCl and packed into a thin gelatinous layer by centrifugation at 50,000 × g for 36 hours. The packed lipoproteins were removed with a spatula and approximately 5 g of the packed lipoproteins, containing 400 mg of protein, was resuspended in 0.5 M NaCl to give a final volume of 16 ml. Five ml of this suspension was placed in each of the three tubes of the SW-39 swinging bucket rotor, and the rotor was accelerated according to the schedule depicted in Figure 1. Preliminary studies using the analytical ultracentrifuge indicated that this procedure yielded a better fractionation than the procedure in which the rotor was accelerated directly to top speed. At the end of the centrifugation the lipoproteins were found to be packed in a transparent orange plug 1.6 cm thick. The top of the plug contained white opaque material. The plugs were sliced laterally into four equal segments, and the corresponding segments from each of the three tubes were combined and brought to a final volume of 10 ml with 0.5 M NaCl. The fractions were numbered as indicated in Figure 1. The turbid infranatant solutions from the three tubes were combined to form fraction V.

Analytical Ultracentrifugation. Analyses were made with a Beckman Spinco Model E analytical ultracentrifuge. Flotation rate measurements were made under the conditions described by DeLalla and Gofman (1954) at 26.0° in 1.748 m NaCl, d_{26} 1.063. A rotor centrifuge speed of 52,640 rpm was used in characterizing the classes of lipoproteins, but flotation measurements of the S_f 10–400 fractions were made at 39,460 rpm. The lipoprotein solutions were adjusted to approximately d_{26} 1.063 by addition of appropriate amounts of a higher density NaCl solution and were then dialyzed against 1.748 m NaCl. Identical lipoprotein concentrations (1%) and rotor acceleration times were used with all fractions of S_f 10–400 lipoproteins to facilitate comparison of S_f distribution in the fractions.

Analytical Methods. Phospholipid was determined

TABLE I: Summary of Flotation Coefficients and Composition of S_f 10–400 Fractions.

	Fraction	S_f of Maximum Ordinate	Total S_f 10–400 Lipoprotein (%)	Composition (%) TG	Chol.[a]	PL	Protein	TC/PL Ratio
Procedure A	I	>250	23	61	19	15	5.3	0.94
	II	100	33	55	22	18	5.2	0.86
	III	76	23	53	19	20	7.8	0.70
	IV	45	13	51	20	20	8.3	0.70
	V	20, 44[b]	8	46	23	21	10	0.76
Procedure B	I	>250	27	66	19	13	3.2	0.98
	II	130	29	59	20	16	4.7	0.86
	III	99	23	47	25	21	7.0	0.87
	IV	62	14	35	30	26	10	0.81
	V	47, 25[b]	7	22	34	31	13	0.77

[a] Cholesterol plus cholesterol esters. [b] Minor component.

by the method of Lowry *et al.* (1954). Triglycerides were determined by the method of Van Handel (1961). Protein was measured by the method of Lowry *et al.* (1951). Total cholesterol was measured by the method of Bloor (1916) and converted to weight cholesterol plus cholesterol ester by multiplying by a factor of 1.41. This conversion factor was determined by measuring the unesterified cholesterol/total cholesterol ratio of a number of typical S_f 10–400 preparations by the method of Sperry and Webb (1950) and assuming the molecular weight of cholesterol ester to be 650.

N-Terminal Analyses. N-Terminal amino acids were determined by the fluorodinitrobenzene method (Fraenkel-Conrat *et al.*, 1955). The lipoproteins were coupled with 1-fluoro-2,4-dinitrobenzene in an aqueous ethanol–bicarbonate medium as described by Shore and Shore (1960). The lipid was removed with a series of organic solvents after precipitating the dinitrophenylated protein with acid. All processes of coupling, washing, and hydrolysis for each sample were performed in a single tube to minimize losses. Chromatography of the dinitrophenyl amino acids was carried out in a 0.05 M phthalate buffer, *p*H 5.6, *t*-amyl alcohol system on Whatman No. 1 paper. Corrections for losses during hydrolysis and chromatography were made by subjecting standard mixtures of the amino acids to the same procedures. Triplicate samples containing 20 mg or more of protein were used for each determination whenever possible. Satisfactory analyses could be made on samples containing as little as 12 mg of protein.

Extraction of Lipoproteins with Ether. Partial delipidation of lipoproteins was accomplished by the method described by Avigan (1957). The lipoproteins were suspended in 0.5 M NaCl and adjusted to *p*H 8.6. The lipoprotein suspension and 20 volumes of peroxide free ether were placed in a screw-cap container which was then rotated slowly about a horizontal axis to produce a constantly changing interface between the two solutions. Extraction took place at 4° for a total of 90 hours. The ether phase was replaced with fresh ether 18, 42, and 66 hours after starting the extraction.

Results

Analytical Ultracentrifugation. Ultracentrifuge patterns showed that the two methods of fractionating the S_f 10–400 lipoproteins produced fractions with different S_f maxima (Figure 2). The degree of overlap between fractions was less when procedure B (SW-39 rotor fractionation) was used. Recombination of nonadjacent fractions (II and IV, or III and V) gave ultracentrifuge patterns with the two distinct peaks. Similar studies were not made with procedure A fractions. The turbidity observed in fractions I from both procedures resulted from the high concentrations of lipoproteins with S_f >250.

Lipid Analyses. The composition of various S_f 10–400 preparations consistently fell within the following ranges: 50–53% triglyceride, 21–23% cholesterol and cholesterol ester, 17–19% phospholipid, and 6.5–8.5% protein. These ranges are similar to those reported in the literature (Cornwell and Kruger, 1961). The composition of the various fractions varied markedly with flotation rate (Table I). The variations were greater in fractions from procedure B, indicating again the smaller overlap between fractions. In both sets the fractions with the lower flotation rates contained more protein, cholesterol, and phospholipid, and less triglyceride.

N-Terminal Amino Acid Analyses. The amounts of

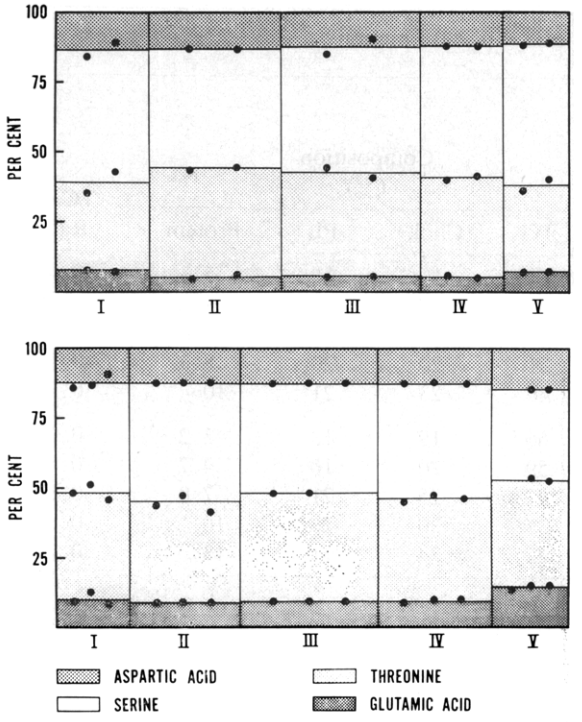

FIGURE 3: N-Terminal amino acids in S_f 10–400 fractions. Each dot represents a single determination. Width of column proportional to the amount of protein in fraction. Upper graph: fractions obtained by procedure A. Lower graph: fractions obtained by procedure B.

protein obtained after fractionation were too small to permit accurate determination of the absolute amounts of each N-terminal per gram of protein. It was possible to estimate for the total S_f 10–400 sample used in procedure B that there were in the N-terminal positions 4.3 μmoles of aspartic acid, 13.3 μmoles of serine, 12.5 μmoles of threonine, and 3.7 μmoles of glutamic acid per gram of protein. The corresponding values calculated from data reported by Shore and Shore (1962) for a similar preparation are 5.9 μmoles of aspartic acid, 10 μmoles of serine, 10.5 μmoles of threonine, and 2.8 μmoles of glutamic acid. The relative amounts of N-terminals found in different S_f 10–400 preparations are given in Table II. Similar data for the other low-density lipoproteins in lipemic plasma are shown for comparison. Although there was some variation in the preparations from different pools of plasma, serine and threonine occurred in approximately equal amounts as the major end groups, and aspartic acid and glutamic acid were present in smaller amounts.

The relative amounts of each N-terminal in the fractions are shown in Figure 3. The compositions were identical within the range of experimental error. All four amino acids were found in all fractions in approximately the same proportion that they were found in the unfractionated samples. Appreciable differences were found only when the narrow range of S_f 10–20 lipoproteins (maximum at S_f 12) was obtained and when comparisons were made between classes of lipoproteins (Table II). No class lacked any one N-terminal amino acid.

TABLE II: N-Terminal Amino Acids of Lipoproteins from Lipemic Plasma.

Lipoproteins	N-Terminal Amino Acids (moles per 100)			
	Aspartic Acid	Serine	Threonine	Glutamic Acid
Chylomicrons	35	26	16	23
(two preparations)	30	35	17	18
S_f 10–400 A	13	46	35	6
B	13	39	37	11
Range of six preparations	12–18	32–52	31–41	5–13
S_f 10–20	11	37	31	22
S_f 0–10	8	25	34	33

Two experiments were conducted to determine whether or not the presence of N-terminal glutamic acid was the result of the aggregation of S_f 0–10 lipoproteins with other low-density lipoproteins. Preparations of S_f 10–400 and S_f 0–10 lipoproteins containing approximately equal amounts of protein were mixed together, the combined lipoproteins in the solution were packed together by centrifugation after adjusting the solution to d 1.063, and the two classes were separated by the usual procedures. Analyses of the N-terminals of each class were made before and after the attempt to cross-contaminate the classes. No significant changes in the composition of the S_f 0–10 lipoproteins were caused by the mixing. The minor changes which occurred in two different S_f 10–400 preparations are shown in Table III. There was no increase in the amount of N-terminal glutamic acid which would have been found had aggregation taken place. The loss of some N-terminal glutamic acid and the decrease in the serine/threonine ratio in both preparations indicate that some components of S_f 10–400 lipoproteins can be lost during repeated handling. No attempt was made to analyze the small amount of material produced with density greater than 1.063.

Partially Extracted S_f 10–400 Lipoproteins. The material remaining after extracting S_f 10–400 lipoproteins for 90 hours with cold ether typically contained 53% protein, 44% phospholipid, 3% triglyceride, and less than 1% cholesterol. It contained much less cholesterol and triglyceride than did the high-density material obtained by Hayashi *et al.* (1959) after extracting S_f 20–400 lipoproteins with ether for 1.25 hours. Examination by ultracentrifugation of our material from lipemic

TABLE III: N-Terminal Amino Acids of S_f 10–400 Lipoproteins before and after Mixing with S_f 0–10 Lipoproteins.

	N-Terminal Amino Acids (moles per 100)			
	Aspartic Acid	Serine	Threonine	Glutamic Acid
Preparation One				
Before	14.8	37.0	43.4	4.85
After	12.5	42.5	40.6	4.36
Preparation Two				
Before	13.4	32.4	41.4	12.9
After	15.4	40.2	38.7	5.74

plasmas consistently showed two major components with sedimentation coefficients ($s_{20,w}$) of 12–13 and 4–5 S and one or two minor components of about 6 and 16 S (Figure 4). The 6 S component was not always clearly resolved from the more abundant 4–5 S component, particularly in samples containing greater amounts of lipid.

One sample was centrifuged in sodium chloride solutions of d 1.0025, 1.0197, 1.0389, and 1.0771, and estimates of the hydrated densities of the components were made from plots of ηs versus d. Densities of 1.135 and 1.102 were found for the 12.4 and 4.71 S components, respectively. A trace component of 15.7 S had an estimated density of 1.168. Avigan (1957) reported values of 12.8 S and d 1.137 for the single extraction product which he obtained from S_f 0–10 lipoproteins. Additional experiments in this laboratory confirmed the finding of the 13 S component as the only major residue in the S_f 0–10 and 10–20 classes from nonlipemic plasma. The similarity of the 13 S components from the different classes was verified by electrophoresis and N-terminal analyses (Bobbitt, 1964).

The relative amounts of the different components varied with different plasmas. The patterns in Figure 4 were obtained with ether-extracted S_f 10–400 lipoproteins from three different individuals. The amount of 13 S component is smallest in the nonlipemic plasma and greatest in the plasma from the person subjected to fat loading. The 6 S component, which is present in extracted S_f 10–400 lipoproteins in small amounts, is a major component in extracted chylomicrons (Figure 4b, lower curve). A trace amount of a 13 S component could also be seen in this extracted chylomicron preparation.

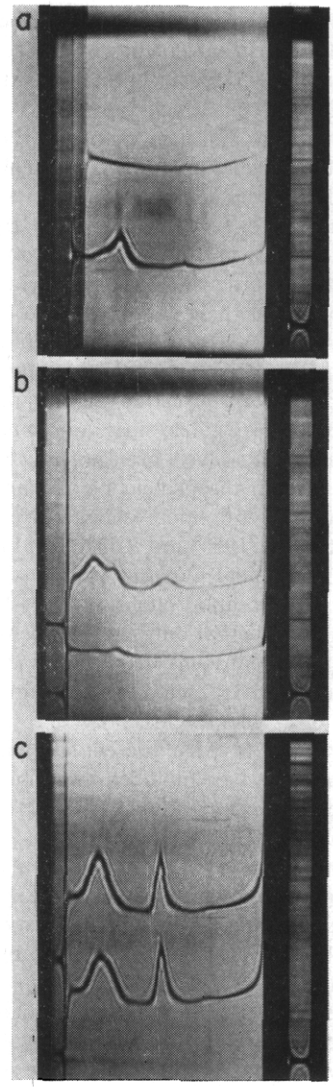

FIGURE 4: Ultracentrifuge sedimentation patterns of ether-extracted lipoproteins from different plasmas. Pictures were taken 30 minutes after attaining full speed of 59,780 rpm. The solvent was 0.5 M NaCl. (a) Normal nonlipemic plasma: upper curve, S_f 10–20 lipoproteins; lower curve, S_f 10–400 lipoproteins; (b) lipemic plasma: upper curve, S_f 10–400 lipoproteins; lower curve, chylomicrons; (c) S_f >10 lipoproteins from plasma drawn 3 hours (upper curve) and 6 hours (lower curve) after the subject had ingested 250 g of corn oil.

Discussion

The marked variation in lipid composition of the different S_f 10–400 fractions is further evidence that the size of these very low-density lipoproteins, as revealed by their flotation rates, is determined largely by the amount of lipid in the particle and especially by the amount of triglyceride. The unvarying distribution of the N-terminal amino acids throughout the class shows that within the density limits of this class the composition of the lipid does not prescribe the type of protein in the particle. The distribution of the proteins throughout the S_f 10–400 range is determined by the amount of lipid associated with the protein and not by any inherent properties of the protein. The *in vitro* production

of atypical lipoproteins by lipolysis described by Shore and Shore (1962) is in agreement with this conclusion.

The association of specific proteins with particular classes of lipoproteins which typically is observed in nonlipemic plasma indicates that under normal circumstances lipoproteins are formed with a characteristic protein and a characteristic range of amount and composition of lipid. Previous reports of large amounts of N-terminal serine and threonine and small amounts of N-terminal aspartic and glutamic acids in the S_f 10–400 lipoproteins have been used to support the hypothesis that this class chiefly consists of lipoproteins with a unique protein component synthesized in the liver for transport of triglyceride (Searcy and Bergquist, 1962; Robinson, 1963). The small amounts of aspartic acid and glutamic acid have been previously considered to be derived from the protein of overlapping chylomicrons and S_f 0–10 lipoproteins, respectively. The reports by Rodbell (1958) and Rodbell and Fredrickson (1959) that N-terminal glutamic acid was not present in chylomicrons or lipoproteins of S_f >100, and by Grundy *et al.* (1961) that only the high-density fraction of S_f 20–400 lipoproteins from nonlipemic plasma contained an ether-extracted residue similar to that found in S_f 0–20 lipoproteins, provide a basis for this proposal. The results of the present study indicate that this hypothesis must be modified to account for a different protein distribution in S_f 10–400 lipoproteins from lipemic plasma.

Under conditions in which the liver is required to produce large amounts of triglyceride-bearing lipoproteins, the supply of protein could become a limiting factor. The liver would then be forced to utilize all the proteins synthesized for lipid transport in the production of larger than usual lipoproteins containing much more triglyceride. An increase in size would be particularly advantageous if the protein were situated only on the surface of the particle. These larger particles would have increased flotation rates, allowing some of the proteins normally found in the S_f 10–400 class to be isolated with the chylomicron class and proteins normally found in the S_f 0–10 class to appear in both the S_f 10–400 and chylomicron classes.

This revised hypothesis describing the kind of lipoprotein synthesized by the liver offers several advantages over those based on data obtained only with nonlipemic plasma. It successfully accounts for the earlier immunochemical and electrophoretic evidence of the presence of S_f 0–10 (β-lipoprotein) protein in chylomicrons (Scanu and Page, 1959). It also explains the numerous observed interrelationships among the different classes of lipoproteins. In this respect, this hypothesis is consonant with the theory of intravascular degradation of chylomicrons to higher density lipoproteins by the action of clearing factor lipase. Furthermore, this hypothesis, and the data on which it is based, provide a basis for explaining the occurrence of more than one kind of chylomicron (Gordis, 1962; Bierman *et al.*, 1962; Nye, 1964). Finally, it re-emphasizes that the composition and concentration of the different lipoprotein classes are a direct consequence of the over-all processes of lipid metabolism.

References

Avigan, J. (1957), *J. Biol. Chem. 226*, 957.

Bierman, E. L., Gordis, E., and Hamlin, J. T. (1962), *J. Clin. Invest. 41*, 2254.

Bloor, W. R. (1916), *J. Biol. Chem. 24*, 227.

Bobbitt, J. L. (1964), Doctoral dissertation, Univ. of Louisville.

Cornwell, D. G., and Kruger, F. A. (1961), *J. Lipid Res. 2*, 110.

DeLalla, O. F., and Gofman, J. W. (1954), *Methods Biochem. Analy. 1*, 459.

Dole, V. P., and Hamlin, J. T. (1962), *Physiol. Rev. 42*, 674.

Fraenkel-Conrat, H., Harris, J. I., and Levy, A. L. (1955), *Methods Biochem. Analy. 2*, 359.

Gordis, E. (1962), *Proc. Soc. Exptl. Biol. Med. 110*, 657.

Grundy, S. M., Dobson, H. L., and Griffin, A. C. (1959), *Proc. Soc. Exptl. Biol. Med. 100*, 704.

Grundy, S. M., Dobson, H. L., Kitzmiller, G. E., and Griffin, A. C. (1961), *Am. J. Physiol. 200*, 1307.

Hayashi, S., Lindgren, F., and Nichols, A. (1959), *J. Am. Chem. Soc. 81*, 3793.

Levy, R. S., and Lynch, A. C. (1962), *Circulation 26*, 750.

Lindgren, F. T., and Nichols, A. V. (1960), *in* The Plasma Proteins, Vol. II, Putnam, F. W., ed., New York, Academic, p. 1.

Lindgren, F. T., Nichols, A. V., Hayes, T. L., Freeman, N. K., and Gofman, J. W. (1959), *Ann. N.Y. Acad. Sci. 72*, 826.

Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., and Farr, A. L. (1954), *J. Biol. Chem. 207*, 1.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Nye, W. H. R. (1964), *Proc. Soc. Exptl. Biol. Med. 116*, 350.

Robinson, D. S. (1963), *Advan. Lipid Res. 1*, 133.

Rodbell, M. (1958), *Science 127*, 701.

Rodbell, M., and Fredrickson, D. S. (1959), *J. Biol. Chem. 234*, 567.

Scanu, A., and Page, I. H. (1959), *J. Exptl. Med. 109*, 239.

Searcy, R. L., and Bergquist, L. M. (1962), Lipoprotein Chemistry in Health and Disease, Springfield, Thomas.

Shore, B. (1957), *Arch. Biochem. Biophys. 71*, 1.

Shore, B., and Shore, V. (1960), *J. Lipid Res. 1*, 321.

Shore, B., and Shore, V. (1962), *J. Atherosclerosis Res. 2*, 104.

Sperry, W. M., and Webb, M. (1950), *J. Biol. Chem. 187*, 97.

Van Handel, E. (1961), *Clin. Chem. 7*, 249.