Title

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Abstract

The S_f20-400 and the high-density lipoproteins of human sera have been degraded by partially extracting their lipid content with ethyl ether. The resultant degradation products have been analyzed both chemically and ultracentrifugally. In the case of the S_f20-400 class lipoproteins the ether extracted more than 60% of the total lipids. The composition of the extracted lipids (mostly glycerides) was approximately constant for each of four successive extractions. On the other hand, the main lipid constituent of the lipoprotein fragments was phospholipid. In the high-density lipoproteins, the ether extracted only a very small amount of lipids. In spite of this resistance to ether degradation an essentially lipid-free protein fragment was produced, the molecular weight of which was calculated to be approximately 40,000.

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Introduction

Lipoproteins of human sera exhibit considerable differences in such properties as density, molecular size, and chemical composition.[1, 2] Generally, these lipoproteins have been fractionated on the basis of their densities by ultracentrifugal flotation.[3] In these procedures the lipoproteins have been fractionated into three broad density classes: (a) lipoproteins with densities less than 1.007 g/ml, (b) lipoproteins with densities between 1.007 and 1.063 g/ml, and (c) lipoproteins with densities between 1.063 and 1.20 g/ml. These lipoprotein classes have been characterized and designated S_f20-400, S_f0-20, and the high-density lipoproteins, respectively. Ultracentrifugal studies indicate that each of these lipoprotein classes exhibits a distribution in density and molecular size. Further, chemical analysis of the lipid composition of each of these broad lipoprotein classes indicates gross composition differences between the classes as well as variations in composition within each class.[4, 5]

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[1] Lindgren, Elliott, and Gofman, J. Phys. and Colloid Chem. 55, 80 (1951).

[2] Oncley, Walton, and Cornwell, J. Amer. Chem. Soc. 79, 4666 (1956).

[3] O. F. deLalla and J. W. Gofman, Methods of Biochemical Analysis, Vol. I, D. Glick, ed. (Interscience, N. Y., 1954), p. 459-478.

[4] Lindgren, Nichols, Freeman, J. Phys. Chem. 59, 930 (1955).

[5] Hillyard, Entenman, Feinberg, Chaikoff, J. Biol. Chem. 214, 79 (1955).

These data have suggested possible structural relationships in the various lipoprotein groups.[6] However, formulation of the actual structure and molecular associations present in each of the lipoprotein classes requires additional information. This report describes efforts directed toward obtaining such information by studying the degradation of lipoprotein molecules and the products formed during such degradation.

The early work of Macheboeuf on horse blood lipoproteins was one of the first studies bearing on the structure of lipoproteins.[7] He found these lipoproteins to be chemically stable to repeated precipitation by ammonium sulfate and solubilization in an aqueous medium. These lipoproteins, when shaken with ether, were found to partially liberate their constituent lipids. A more complete extraction of lipids was found to require a critical concentration of alcohol in the ether. Similar observations with the chemically fractionated human sera lipoprotein, made by Oncley et al., indicated that partial extraction of lipids with ether produced water-soluble degraded lipoproteins.[8] More recently similar findings have been reported by Avigan.[9]

In the experiment presently described, ethyl ether was used in the degradation of lipoproteins of both the S_f20-400 class and the high-density class. The degradation products arising from each of these classes of lipoproteins have been isolated into the following density groups: those less dense than 1.007 g/ml, those between 1.007 and 1.065 g/ml, those between 1.065 and 1.20 g/ml, and those more dense than 1.20 g/ml. The degradation products so isolated were analyzed ultracentrifugally and chemically.

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[6] Lindgren, Freeman, Nichols, and Gofman, "The Blood Lipids and the Clearing Factor," Third International Conference on Biochemical Problems of Lipids, Brussels, July 1956, p. 224.

[7] M. A. Macheboeuf, Bull. chim. biol. 11, 268 (1929).

[8] Oncley, Gurd, and Melin, J. Am. Chem. Soc. 72, 458 (1950).

[9] J. Avigan, J. Biol. Chem. 226, 957 (1957).

Experimental Procedure

Preparation of the S_f20-400 Class and the High-Density Lipoproteins

The source of the lipoproteins was blood of a 45-year-old healthy male drawn 5 hours after a meal consisting of 85 gr of corn oil blended in skim milk. The serum lipoproteins were fractionated into the S_f20-400 class, the S_f0-20 class, and the high-density lipoproteins by preparative ultracentrifugation (Spinco Model L Centrifuge, 30.2 rotor, 30,000 rpm). There were three consecutive ultracentrifugations each of 24 hours in duration. In the first stage, serum was centrifuged which yielded the S_f20-10^5 class lipoproteins in the top 1-ml fraction of the preparative tubes. Lipoproteins of flotation rates higher than 400 S_f units were removed later from this fraction by several low-speed centrifugations. The bottom 3-ml fraction of the first-stage solution, after alteration of its solution density[10] to 1.065 g/ml was centrifuged in the second stage. This time, the top 1-ml fraction contained the S_f0-20 class lipoproteins. In similar fashion the bottom 3-ml fraction of the second-stage run, after its solution density was brought to 1.20 g/ml, was centrifuged in the third stage. The top 1-ml fraction of the final run contained the high-density lipoproteins (designated[4] as HDL-2 and HDL-3).

The flotation patterns of the isolated S_f20-400 class and the high-density lipoprotein preparations are shown in Figs. 1 and 2, respectively.

One-ml fractions of the solution immediately below the top 1-ml fraction following each 24-hour ultracentrifugation were collected for the purpose of determining the nonprotein nitrogen (NPN) values of each lipoprotein preparation. This NPN content was assumed to be in the form of such small-molecular substances as urea, uric acid, and free amino acids. The possibility was tested that discrepancies might arise from using the second-milliliter NPN value owing to sedimentation of these small-molecular substances. Following 24-hour ultracentrifugation (as used for the lipoprotein isolation) 2% urea and 2% leucine showed average decreases in concentration in the first milliliter of 1.4% and 2.9% respectively.

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[10] All densities are referred to 20° C and represent the small-molecule (background) density, excluding the macromolecular contribution to over-all solution density by proteins or lipoproteins.

Correspondingly, the decrease observed in the second milliliter were 0.7% and 2.6% respectively. Thus, if one uses the NPN value of the second-milliliter fraction as an estimate of the NPN concentration in the top milliliter, an error of approximately 1% is involved.

Ether Degradation of the S_f20-400 Class Lipoproteins

The isolated preparation of the S_f20-400 class lipoproteins was diluted with a 1.007-g/ml solution of NaBr so that the final solution density remained 1.007 g/ml and the lipoprotein concentration was reduced to 2.02%. Four 6.0-ml aliquots of this solution were made in 25 × 15-mm screw-cup culture tubes provided with Teflon gaskets.

Thirty ml of peroxide-free ethyl ether was layered over each lipoprotein aliquot.[11] The aliquot tubes, after capping, at room temperature, were placed nearly horizontally on a rocking platform which provided a gentle stirring motion, without turbulent mixing of the ether and the aqueous phases. After 30 minutes of rocking, the aliquot tubes were centrifuged at low speed in a Size 2 International Centrifuge, and the ether phases were removed. The tubes were refilled with fresh ether and the lipid extractions were repeated three more times. The extraction time for each of the last three steps was 15 minutes. Finally, the extracting solvent was evaporated under a stream of nitrogen and the extracted lipids were further dried in vacuum over P_2O_5.

After the extraction, nitrogen was streamed over the aqueous aliquot phases for 12 hours to remove the solubilized ether. To minimize the evaporation of these aqueous phases, the nitrogen was first water-saturated by bubbling through a column of water. A slight loss in volume occurred, but this was restored by addition of distilled water.

The degraded lipoproteins produced by the extraction process were isolated in three density groups by preparative ultracentrifugation (Spinco Model L centrifuge in a type 40.3 rotor at 40,000 rpm) in three successive 24-hour runs. In the first run the aliquot solutions were centrifuged; this yielded top 1-ml fractions containing those degradation products of densities less than 1.007 g/ml. The bottom-3-ml contents of the preparative tubes of this run were mixed with an equal volume of NaBr solution, which increased

[11] The ether was equilibrated prior to use with aqueous ferrous sulfate to remove peroxides.

the solvent density to 1.065 g/ml. This solution was centrifuged in the second preparative run, which yielded top 1-ml fractions containing those degraded lipoproteins of densities less than 1.065 g/ml but greater than 1.007 g/ml. The bottom 3-ml contents of these preparative tubes of the second run were mixed with an equal volume of solution of NaBr, which increased the solvent density to 1.20 g/ml, and this mixture was centrifuged in the final run, which yielded top 1-ml fractions containing those degradation products of densities less than 1.20 g/ml but greater than 1.065 g/ml.

Ether Degradation of the High-Density Lipoproteins.

The isolated preparation of high-density lipoproteins was diluted with distilled water so that the final solvent density was 1.065 g/ml. The lipoprotein concentration was reduced to 1.37%. The formation of aliquots of this solution, the partial extraction of lipids by ether, and the subsequent removal of the dissolved ether from the aliquot solutions corresponded exactly to those steps used in the treatment of the S_f20-400 class lipoproteins (see preceding section).

The degraded lipoproteins arising from the high-density lipoproteins were isolated in three density groups by preparative centrifugation in two 24-hour runs. In the first run the ether-treated lipoprotein solution, whose density was 1.065 g/ml, was centrifuged and yielded top 1-ml fractions containing those lipoproteins whose densities were less than 1.065 g/ml. The bottom 3-ml fractions of these preparative tubes were then mixed with an equal volume of NaBr solution, raising the solution density to 1.20 g/ml. This mixture was centrifuged, yielding two fractions, namely, the top 1-ml fraction, containing those products of densities less than 1.20 g/ml but greater than 1.065 g/ml, and the bottom fraction, containing those degradation products of density greater than 1.20 g/ml.

Extraction and Analysis of the Total Lipids.

The extractions of the total lipids from the lipoproteins were made according to the method described by Freeman et al.[12]

The total lipid extracts were analyzed by silicic acid chromatography combin with infrared absorption spectrophotometry.[12] The infrared

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[12]Freeman, Lindgren, Ng, and Nichols, J. Biol Chem. 227, 449 (1957).

absorption measurements were made with a Baird Associates, Model B recording spectrophotometer.

Determination of Protein

The Kjeldahl nitrogen values were determined for the delipidized portion of the lipoproteins according to the method of Ma and Zuazaga.[13] The nonprotein nitrogen values of the lipoprotein solutions were determined after centrifugation from the second ml fractions of the solutions just below the lipoprotein-containing fractions. Any lipoproteins contaminating the NPN background solutions were removed by precipitation with 10% trichloroacetic acid.

The Kjeldahl nitrogen values were converted to total protein by the factor 6.67 mg protein per mg. nitrogen.[14]

Ultracentrifugal Analyses

The ultracentrifugal analyses were made with a Spinco Model E analytical ultracentrifuge. Flotation coefficients were measured from the slope of the plots of $\ln x$ vs $\omega^2 t$ where x indicates the radial distance of the maximum ordinates of the flotation pattern.

The estimates of the hydrogen densities of the lipoproteins were made from the extrapolated values of the solution densities at which zero sedimentation velocity would occur on the plots of ηS vs ρ, where η is the viscosity of the lipoprotein-free solvent relative to water (calculated from data in the International Critical Tables), S the measured flotation rate, and ρ the solution density.

Results and Discussion

Degradation Products of the S_f20-400 Class Lipoproteins.

The S_f20-400 lipoprotein solution initially contained 440.8 mg of lipids. Following the partial extraction with ether, 167 mg of lipids (40% of the total lipids) remained in soluble form within the aqueous phase. A total of 273.9 mg of lipids was extracted. Table I presents the weights and

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[13] T. S. Ma and G. Zuazaga, Ind. Eng. Chem. Anal. Ed. 14 280 (1942).

[14] B. Shore, Arch. Biochem. and Biophys. 71, 1 (1957).

compositions of these extracted lipids. Within the limits of experimental errors, the compositions of each of the successively extracted lipids are indistinguishable from one another. However, these lipid compositions are significantly different from the total lipid composition of the initial S_f20-400 class lipoproteins (see Table II).

Table I

Weights and compositions of lipids extracted by ethyl ether from the aqueous solution of S_f (20-400) class lipoproteins initially containing 440.8 mg of lipid.

Extraction step	Weight of lipids extracted (mg)	composition of Lipid[a] (wt %)				
		CSE	CS	PL	TG	FA
1st	47.8	13	9	13	64	2
2nd	78.8	15	9	13	62	2
3rd	92.2	15	8	13	63	1
4th	55.3	15	9	13	62	1

[a]CSE denotes cholesteryl esters (as oleate); CS, cholesterol; PL, phospholipids (as lecithin); TG, triglycerides (as triolein); and FA, unesterified fatty acids (as oleic acid). The values are averages of two determinations.

Table II

Composition of the initial and the degraded S_f (20-400) class lipoproteins

	Lipoprotein[a, b] composition (mg)		Lipid composition[b c] (wt %)				
	Protein	Lipid	CSE	CS	PL	TG	EA
Initial S_f (20-400) Lipoproteins	40.6	440.8	11	8	23	58	1
Degraded S_f (20-400) $d_4^{20} < 1.007$ g/ml	4.3	56.9	15	7	18	57	2
Degraded S_f(20-400) $1.007 < d_4^{20} < 1.065$ g/ml	12.6	14.6	5	13	61	18	4
Degraded S_f (20-400) $1.065 < d_4^{20} < 1.20$ g/ml	18.2	21.6	5	9	63	17	6

[a] In amounts actually isolated for each density range.

[b] Averages of at least two determinations.

[c] Abbreviations as in Table I.

The soluble degraded lipoproteins or lipoprotein fragments resulting from the ether extraction of the S_f20-400 lipoproteins were also found to exhibit a wide distribution with regard to both density and molecular size.

After fractionation these degraded lipoproteins were studied by analytical ultracentrifugation. Figure 3 shows a comparison of the ultracentrifugal distribution of the initial S_f20-400 class lipoprotein with that of the fractionated degradation products. To facilitate comparison, this figure was drawn by replotting the ultracentrifugal flotation patterns with reference to a straight baseline. The areas in this figure are proportional both to the weight of the initial material used and the weights of the fractionated degradation products.

The flotation pattern for the product S_f20-400 lipoproteins of density less than 1.007 g/ml has a peak with approximately the same flotation rate as that of the initial S_f20-400 class lipoproteins. However, the increased skewness of the product flotation pattern indicates the production of some lipoproteins of higher S_f rates not initially present. Alternatively, of course, this increase in S_f rate could also result from a lowered molecular density. Except for this additional skewness, which represents a small portion of the total distribution, these low-density degraded lipoproteins appear to be ultracentrifugally equivalent to the initial S_f20-400 class lipoproteins. Comparison of the lipid composition of this low-density fraction of the degraded lipoproteins with the initial S_f20-400 class lipoproteins also shows very little difference. Since the extraction of lipids from the initial lipoproteins had not been carried to completion,[15] it is reasonable to expect that some lipoproteins of the S_f20-400 class might remain relatively unchanged.

The degraded lipoproteins fractionated into the two density ranges of from 1.007 to 1.065 g/ml and from 1.065 to 1.20 g/ml have ultracentrifugal distributions as presented in Fig. 3. Table II presents the chemical compositions found for these fractions. Table III shows the estimates of some

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[15] In a preliminary experiment with a similar system it was found that ether extracted approximately 80% of the total lipids of the S_f20-400 class lipoproteins in about 90 minutes. Further extraction for several hours yielded only a negligible amount of additional lipid.

of the physical properties for these distributions of lipoproteins. Molecular weights were calculated from Svedberg's equation for sedimentation velocity assuming spherical particles.

Table III

Some physical properties of the degradation products from the S_f 20-400 and the high-density lipoproteins.

	Density range (g/ml)	Hydrated density (g/ml)	Mol. Wt.	Wt. % Lipid
Degraded S_f 20-400 Lipoproteins				
	1.007 - 1.065	1.05	1.6×10^6	85
	1.065 - 1.20	1.11	8.2×10^5	54
Degraded HDL				
	1.065	1.04	1.1×10^6	79
	1.065 - 1.20	1.09	5.2×10^5	61
	1.20	1.32	4.3×10^4	1

The naturally occuring serum lipoproteins fractionated in the density ranges from 1.007 to 1.063 g/ml and from 1.063 to 1.20 g/ml have been characterized previously.[4] Comparison of the chemical and physical properties of each of the degraded lipoprotein fractions with the natural serum lipoprotein fractions of the corresponding density ranges reveals similarities in hydrated density and lipid-to-protein ratio. However, the calculated molecular weights are comparable only in order of magnitude and the lipid chemical compositions are grossly different. In contrast to the natural lipoproteins, the degraded lipoproteins have a high content of phospholipids and a low content of cholesteryl esters.

Inspection of Table II shows only a slight variation in the lipid composition for the two fractions of the degraded lipoproteins whose density ranges are from 1.007 to 1.065 g/ml and from 1.065 to 1.20 g/ml. On the other hand, the lipid-to-protein ratios differ by a factor of five.

A closer inspection of the differences between these two groups of degraded lipoproteins can be made by comparing the weights of the lipid and protein per mole of lipoproteins. From data in Tables II and III it is calculated that a mole of degraded lipoproteins within the density range from 1.007 to 1.065 g/ml has approximately 1.4×10^6 grams lipid and 380,000 grams protein. Between these two groups of degraded lipoproteins of closely similar lipid composition the weights of lipid per mole lipoproteins differ by a factor of three while the weights of protein per mole lipoproteins differ by a factor of only one and a half.

It is significant that in each of the successive ether extractions performed on the S_f20-400 class lipoproteins the composition of the lipid was essentially constant. Also, the lipid content of the degraded lipoproteins varies from particles of one density to another, yet the composition of these lipids remains relatively constant. These observations suggest that ether extraction of lipids from lipoproteins may be a selective process that involves particular combinations of lipid constituents. Since these lipid constituents are displaced from the lipoproteins in these particular combinations they may also exist in this same or similar combination in the intact lipoprotein. This may be indicative of some structural features of the lipid moiety of lipoproteins. Similarly, the lipids of the degraded lipoproteins may also exist as particular combinations of lipids, mutually bound together, associated with the protein moiety.

The foregoing discussion may be extended by considering the mole ratios of the lipid constituents. The mole ratios with respect to cholesteryl ester (as oleate): cholesterol: phospholipid (as lecithin): triglyceride (as oleate) of the lipids extracted by ether are approximately 1.3 : 1.4 : 1: 4.3. For the degraded lipoprotein fractions of the density ranges from 1.007 to 1.065 g/ml and from 1.065 to 1.20 g/ml the ratios are approximately 0.10 : 0.43 : 1 : 0.26 and 0.10 : 0.29 : 1 : 0.23, respectively. The ratios between cholesteryl esters, phospholipid, and triglyceride are approximately the same for the two fractions of the degraded lipoproteins. The variable component is cholesterol, which is higher for that fraction with the higher lipid-to-protein ratio.

It is of interest to know what mole ratios can be obtained by extraction with solvents other than ethyl ether. In this study it is interesting to note that the numbers of hydroxyl, acidic, and basic groups in the combination of lipid

constituents extracted by the ether are fewer than in the combination of lipid constituents extracted by the addition of a more polar solvent, such as methyl alcohol, to the ether. If these mole ratios can be obtained with extracting solvents other than ether, then such mole ratios may suggest some of the lipid-lipid molecular associations involved in the structure of lipoproteins.

Degradation Products of the High-Density Lipoprotein

The ether in contact with the aqueous solution of the initial high-density lipoproteins extracted only 3.05 mg from 152.2 mg of lipids originally present. The weights of the lipids extracted in each of the four extraction steps were 1.08, 0.69, 0.48, and 0.80 mg. Although a negligible amount of lipid was extracted, a considerable alteration of the physical properties of the lipoproteins was effected by the ether, as can be seen in Fig. 4.

The Inspection of Table IV shows no distinguishable differences in lipid composition between the initial material and the isolated degradation products. However, the average lipid-to-protein ratios show significant changes for each of the isolated degradation products from that of the initial material.

The degradation product which sedimented in the solution of density 1.99 g/ml was found to contain only 1.2% lipid by weight. The sedimentation patterns, Fig. 5, suggest that its degree of homogeneity is higher than that of the initial high-density lipoprotein molecules. The hydrated density was estimated (by study of $\eta \int \underline{vs}\ \rho$) to be 1.32 g/ml, and the calculated molecular weight (assuming spherical particles) was 40,000. It is of interest that in a similar study on centrifugally isolated high-density lipoproteins, Scanu et al. obtained by prolonged alcohol-ether and ether extraction at -20°C a soluble protein containing only 0.5% lipid.[16] The molecular weight of this protein, calculated by sedimentation and diffusion, assuming a partial specific volume of 0.729, was 75,000. However, it is not possible to compare their results directly with ours, since the conditions of extraction as well as the manner of characterizing the soluble protein degradation product were different.

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[16] Scanu, Lewis, and Bumpus, Arch. Biochem. and Biophys. 74, 390 (1958).

Table IV

Compositions of the initial and the degraded high-density lipoproteins

	Lipoprotein composition[a] (mg)		Lipid composition[b] (wt %)				
	Protein	Lipid	CSE	CS	PL	TG	FA
Initial HDL	162	152	32	6	44	16	3
Degraded HDL $d_4^{20} < 1.065$ g/ml	13.3	51.5	34	7	41	16	3
Degraded HDL $1.065 < d_4^{20} < 1.20$ g/ml	37.6	59.8	31	6	46	13	4
Degraded HDL 1.20 g/ml $< d_4^{20}$	48.8	0.6	--	-	--	--	-

[a] In amounts actually isolated for each density range.

[b] Averages of two determinations.

Utralcentrifugal analyses show that the appearance of the degradation products was accompanied by a significant reduction in HDL-3 lipoprotein concentration.[17] This observation together with the fact that a negligible amount of lipid was extracted by the ether, suggest the dissociation of the HDL-3 lipoproteins into an essentially lipid-free protein product and a lipid or lipoprotein product. The latter apparently is involved in the formation of the lower-density, higher-molecular-weight products. Table III shows that the amount of protein per molecule of product (expressed as grams protein per mole lipoprotein) is comparable to that calculated for the HDL-2 lipoprotein. A reasonable mechanism for the above degradation process might

[17] R. N. Hazelwood in his thesis (University of California, Berkeley, 1957) calculated the mol wt of HDL-2 lipoproteins of hydrated density of 1.105 g/ml to be 400,000 and the HDL-3 lipoproteins of hydrated density of 1.153 g/ml to be 175,000.

be the dissociation of HDL-3 lipoproteins into at least two products, (a) a lipid-free protein unit and (b) a lipid-containing unit. In the degradation process one or more of the latter units might then associate with a HDL-2 lipoprotein to form a larger low-density macromolecular complex.

Avigan[9] found that the high-density lipoproteins were stable against ethyl ether extraction at 4°C and that their delipidization with an alcohol-ether mixture yielded soluble protein molecules. However, in our investigation extraction with ether above 25°C yielded an appreciable amount of soluble, essentially lipid-free protein molecules. These similar experiments at different temperatures suggest that stability of the high-density lipoprotein against ether is strongly temperature-dependent.

Acknowledgments

The authors wish to acknowledge helpful discussions with Drs. John W. Gofman and N. K. Freeman.

This work was supported in part by the U. S. Atomic Energy Commission.

Figure Captions

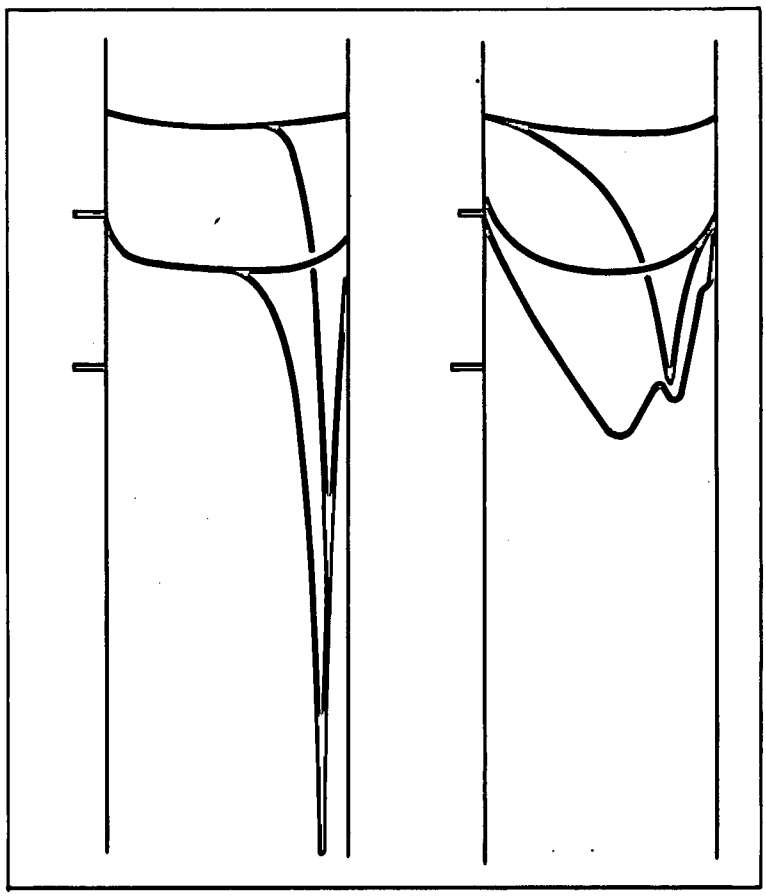
Fig. 1. Ultracentrifugal flotation patterns of the initial S_f20-400 class lipoproteins. The first frame was taken when rotor reached full speed (52,640 rpm); the second frame 6 minutes later. The solution density, at 20°C, for the upper pattern - 1.0072 g/ml, for the lower pattern - 1.0638 g/ml. Flotation proceeds toward the left. For all analytic runs double-sectored analytical cells were used allowing accurate positioning of the base line. A wire angle of 45° was used.

Fig. 2. Ultracentrifugal flotation patterns of the initial high-density lipoproteins (HDL-2 and HDL-3) taken 32 minutes after rotor reached full speed (52,640 rpm). The solution densities, at 20°C, for the upper and lower patterns are 1.1964, and 1.3061 g/ml respectively. Flotation proceeds toward the left.

Fig. 3. Comparison of the ultracentrifuge flotation diagrams of the initial S_f20-400 class lipoproteins and their degradation products.

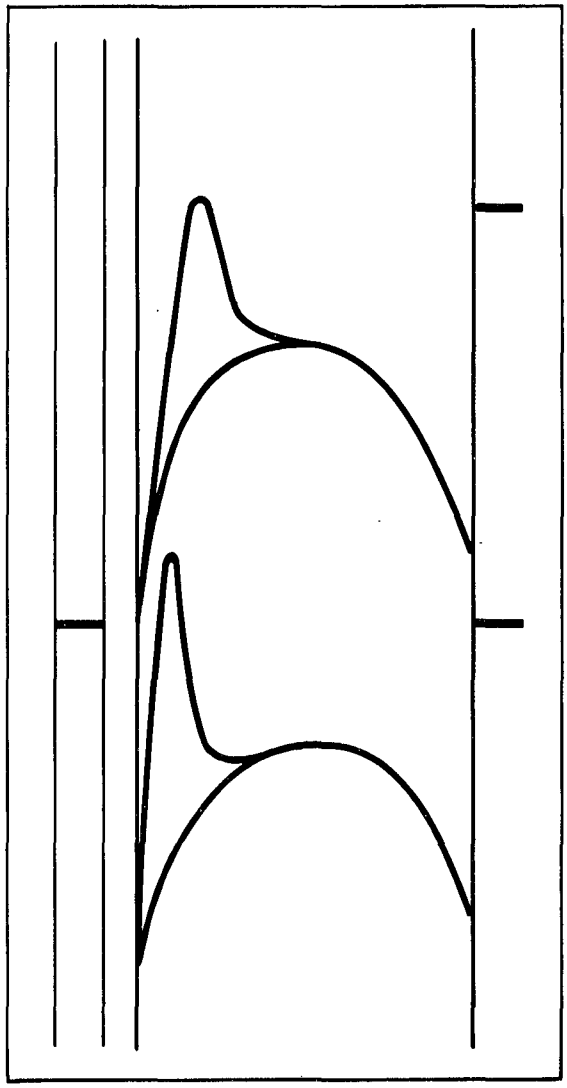
Fig. 4. Comparison of the ultracentrifugal diagrams of the initial high-density lipoproteins (HDL-2 and HDL-3) and their degradation products. The initial distribution contains predominantly HDL-3 lipoproteins. After extraction there is a pronounced reduction of the HDL-3 class, resulting in a lipoprotein distribution principally within the HDL-2 class.

Fig. 5. Ultracentrifugal sedimentation patterns of the essentially lipid-free degradation products from the high-density lipoproteins taken 108 minutes after rotor reached full speed (52,640 rpm). Solution density, at 20°C, of upper pattern, 1.098 g/ml; of lower pattern, 1.199 g/ml. Sedimentation proceeds toward the right.



MU-15945

Fig. 1.



MU-15946

Fig. 2.

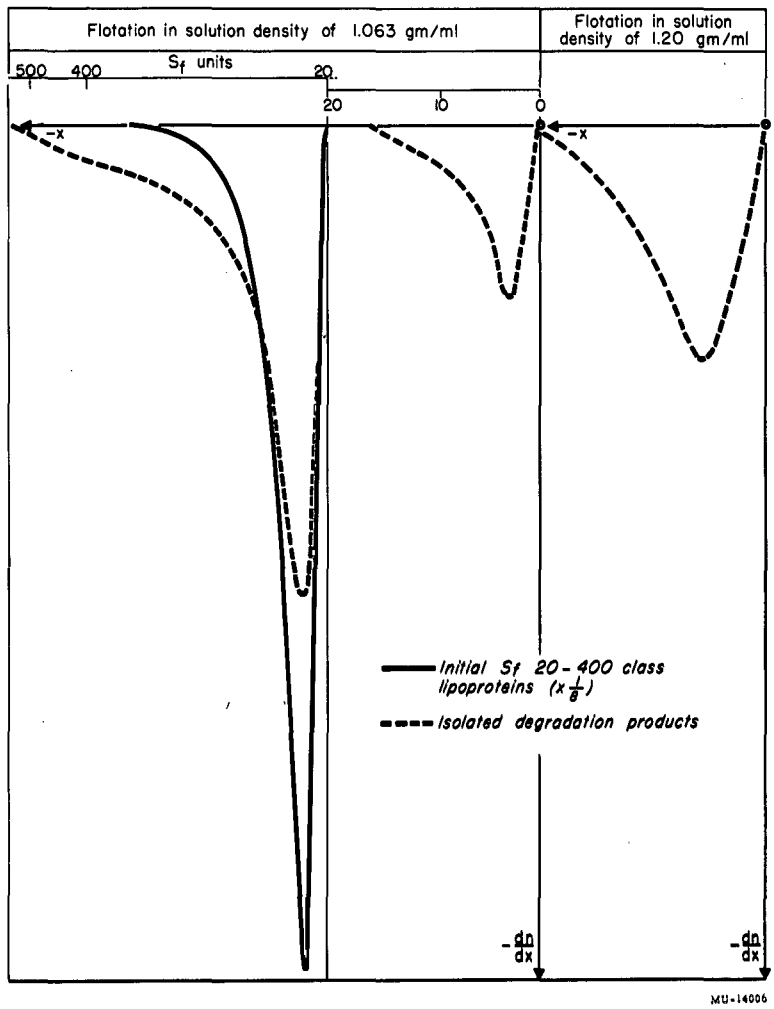

Flotation in solution density of 1.063 gm/ml
Flotation in solution density of 1.20 gm/ml
500
400
Sf units
20
20
10
0
-x
-x
Initial Sf 20-400 class lipoproteins (x 1/8)
Isolated degradation products
-dp/dx
-dp/dx
MU-14006

Fig. 3.

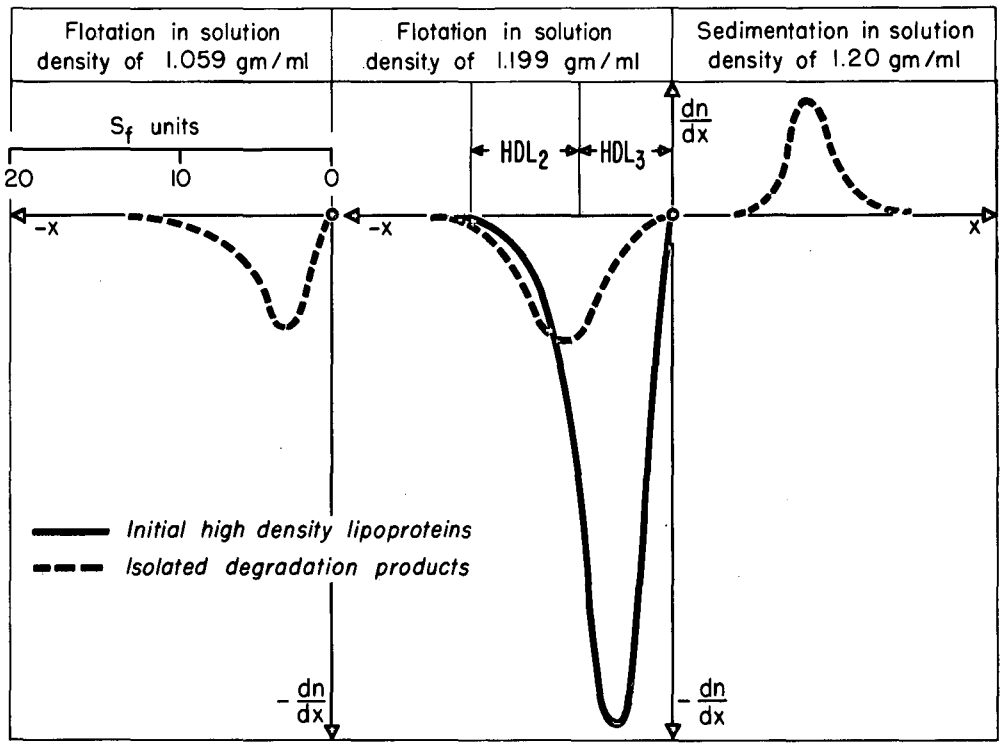
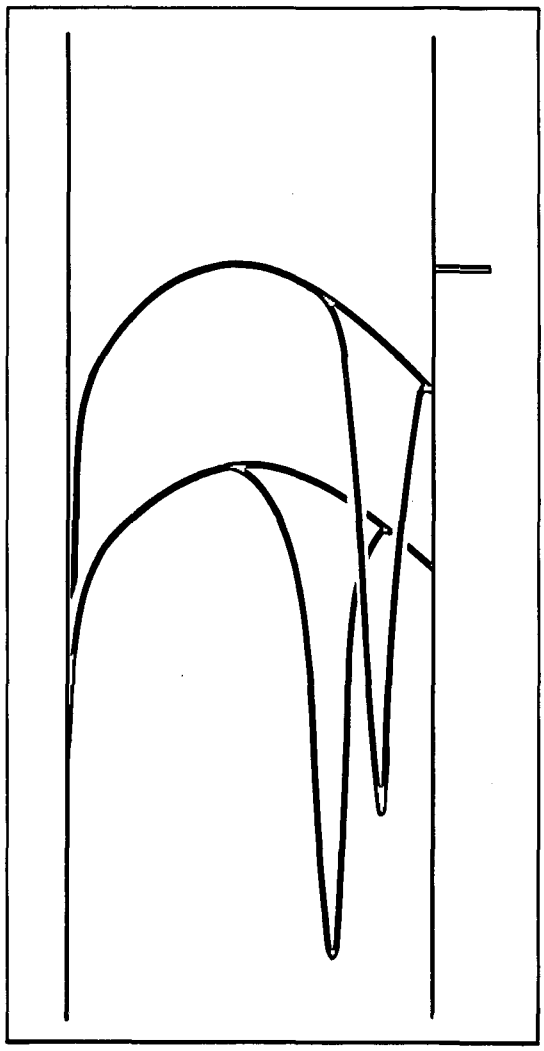

Flotation in solution density of 1.059 gm/ml
Flotation in solution density of 1.199 gm/ml
Sedimentation in solution density of 1.20 gm/ml
S_f units
20
10
0
HDL_2
HDL_3
$\frac{dn}{dx}$
-x
-x
x
Initial high density lipoproteins
Isolated degradation products
$-\frac{dn}{dx}$
$-\frac{dn}{dx}$
MU-14005

Fig. 4.



MU-15947

Fig. 5.