Measurements of the Molecular Weight Variability of Plasma Low Density Lipoproteins among Normals and Subjects with Hyper-β-lipoproteinemia. Demonstration of Macromolecular Heterogeneity*

Waldo R. Fisher,† Mary Granade Hammond, and Germaine L. Warmke

ABSTRACT: The structural variability of human plasma low density lipoprotein (LDL) has been evaluated using hydrodynamic methods. The study was undertaken to determine whether there were macromolecular structural differences among the LDL of normals and of hyper-β (type II)-hyperlipoproteinemic subjects. Accordingly, LDL has been isolated and characterized from the serum of eight subjects with the familial disease hyper-β (type II)-lipoproteinemia and from three normal subjects. The sedimentation coefficients of the LDL ($s^0_{25,\rho 1.20}$) vary between −34 and −45, and the hydrated densities, measured by *s vs.* density plots, range from 1.030 to 1.040 g per cm^3. Calculated molecular weights of these LDL fall between 2.4 and 3.5 million. The lipoproteins have been banded in linear density gradients in a preparative ultracentrifuge to define the degree of density heterogeneity, and these studies complement the sedimentation velocity observations indicating that there is a single major LDL in the serum of these subjects which may exhibit a moderate density heterogeneity. In none of the physical parameters measured can a difference be demonstrated between the LDL of normal subjects and of subjects with hyper-β (type II)-lipoproteinemia, nor is there a difference in the lipid composition of these lipoproteins. Immunologically, the apoproteins are only precipitated by anti-LDL antibody. In nine subjects the LDL have been isolated and characterized on more than one occasion and in all but one instance possessed virtually identical molecular weight and hydrated density on each occasion, a finding which contrasts with the considerable variation in these parameters observed among the subjects. When the measured lipid composition of these LDL is calculated as grams of each constituent per mole of lipoprotein, each of the LDL contains the same amount of apoprotein, and differences in their molecular weights are determined by differences in the amount of bound lipid. These findings indicate that the LDL apoprotein from a given subject shows striking consistency in its lipid binding properties but that the apoprotein from various individuals differ in their capacity to bind lipid and hence in their molecular weights. It would appear that structural variations in the plasma LDL may be a frequent finding, possibly reflecting genetic variability among individual subjects.

The continuing interest in the structure of the plasma lipoproteins has recently been advanced by a series of reports examining the macromolecular properties of low density lipoproteins (LDL) from normal subjects (Del Gatto *et al.*, 1959; Lindgren *et al.*, 1969; Adams and Schumaker, 1969a,b, 1970; Mauldin and Fisher, 1970; Fisher *et al.*, 1971). The medical literature also abounds with studies of human hyperlipemic diseases in which various plasma lipoproteins circulate in high concentration. Two of these diseases, hyperpre-β (type IV)-lipoproteinemia and hyper β (type II)-lipoproteinemia, occur commonly and are genetic disorders (Fredrickson *et al.*, 1967). It, therefore, seemed appropriate to ask whether structural variability occurs in human plasma lipoproteins, perhaps as a consequence of genetic variability. Accordingly, the LDL from groups of subjects with these two common familial hyperlipemic diseases have been characterized with respect to a number of their properties in an effort to answer this question, and the findings in the group of subjects with hyperpre-β (type IV)-lipoproteinemia have recently been reported (Fisher, 1970; Hammond and Fisher, 1971). The observations strongly suggest the occurrence of structurally altered low density lipoprotein in these individuals.

In the present study the molecular properties of hydrated density, sedimentation coefficient, and molecular weight have been measured for the LDL from eight subjects with hyper-β (type II)-lipoproteinemia and in three normal subjects utilizing hydrodynamic methods which have recently been reevaluated (Fisher *et al.*, 1971). During the course of this study it became appropriate to measure these parameters from nine of the subjects on at least two occasions. It was thus feasible to compare the degree of variability of the major LDL component of a specific individual over a period of time. Furthermore, the variations in these molecular parameters which were observed among the eleven subjects are documented. In this report the structural variability of LDL from individual subjects is recorded.

Methods

The total lipoprotein fraction was isolated from serum by ultracentrifugation after adjusting the density of the serum to 1.20 g/cm^3 by the addition of solid KBr (Del Gatto *et al.*, 1959), while isolation of the LDL fraction was performed by differential density flotation in the preparative ultracentrifuge as previously described (De Lalla and Gofman, 1954; Mauldin and Fisher, 1970). In these studies the serum was initially

* From the Departments of Medicine and Biochemistry, College of Medicine, University of Florida, Gainesville, Florida. *Received August 30, 1971.*

† Recipient of a USPHS Research Career Development award, supported by American Heart Association Grant 67-732 and 70-851, USPHS Grants HE-10316 and 5-M01-RR00082-09; to whom to address correspondence.

TABLE I: Summary of Clinical Data for Hyperlipemic Subjects.

Subjects	A	B	C	D	E	F	G	H
Age	42	40	46	43	29	68	38	29
Sex	F	F	F	F	M	F	M	M
Known duration of Hyperlipemia (years)	5	3	5	5	4	25[a]	2	3
Initial cholesterol[b]	412	513	507	417	390	680	590	435
Initial total lipids[b]	1140	1110	1000	1280	950	1550	1550	1040
Lipoprotein Electrophoretic Pattern[c]	Type II	Type II	Type II	Type II	Type II	Type II	Type II	Type II
Coronary artery disease	0	0	0	0	0	0	+	0
Xanthomata	0	0	0	0	0	+	0	0
Arcus senilia	0	+	0	0	0	+	0	+
Family history of hyperlipemia	+	+	+	+	+	?	+	?
Premature vascular disease	+	+	0	0	0	0	0	+

[a] This figure is based upon the length of time this subjects has had Xanthomata. [b] Lipid assays were performed in the Clinical Laboratory of the Shands Teaching Hospital of the University of Florida by standard methods (Pearson *et al.*, 1953; Bragdon, 1960). [c] Lipoprotein electrophoresis was performed by the method of Lees and Hatch (1963).

TABLE II: Physical Measurements on LDL from Study Subjects.

Subjects and Date of LDL Prepn	$s^0_{25,\rho 1.20}$[a]	Hydrated Density (g/cm^3)	$\bar{V}$ (ml/g)	Hydrated Density from Gradient Banding Expt	Mol Wt, $\times 10^{-6}$	Serum Cholesterol (mg/100 ml)
			Hyperlipemic Subjects			
A (3/69)	−45	1.033	0.968		3.5	510
(3/70)	−46	1.032	0.969	1.033	3.5	492
B (7/68)	−42	1.031	0.970		3.1	498
(10/70)	−41	1.032	0.969	1.033	3.0	229
C (5/58)	−42	1.030	0.971		3.0	425
(5/70)	−42	1.032	0.969	1.032	3.1	355
D (6/68)	−41	1.034	0.968		3.0	417
(5/70)	−42	1.034	0.968	1.031	3.0	424
E (5/69)	−40	1.033	0.968		2.9	383
F (11/70)	−39	1.036	0.965	1.037	2.8	548
(6/70)	−39	1.034	0.967		2.9	580
G (10/68)	−38	1.036	0.965		2.7	590
(11/70)	−38	1.036	0.965	1.035	2.7	336
H (9/68)	−39	1.035	0.966		2.9	479
(11/68)	−34	1.036	0.965		2.4	435
(3/70)	−34	1.040	0.962	1.037	2.5	385
			Normal Subjects			
I (5/68)	−39[b]	1.035	0.966		2.9	
(7/69)	−39	1.034	0.967		2.7	
(6/70)	−40[b]	1.032	0.969	1.033	2.9	
(11/70)	−39	1.035	0.966	1.033	2.9	
J (7/69)	−38	1.034	0.967		2.7	
K (3/70)	−44	1.036	0.965	1.038	3.4	
(6/71)	−44	1.032	0.969	1.031	3.3	

[a] Sedimentation coefficient at infinite dilution measured in a solvent of density 1.20 g/cm^3 at 25°. The value is corrected for the relative viscosity of the solvent (Fisher, 1970). [b] This value of s was not corrected to infinite dilution and hence the calculated molecular weight is a less accurate estimation.

centrifuged at a density of 1.006 g/cm^3, and, following removal of traces of very low density lipoprotein, the density of the serum was adjusted to 1.06 with solid KBr prior to the second centrifugation. Following their isolation, lipoprotein fractions were dialyzed against buffered KBr solutions in order to adjust the solvent density as previously described (Mauldin and Fisher, 1970). Lipoprotein protein concentration was measured by a modification of the Lowry method, using bovine serum albumin as a standard, and the concentration of LDL was calculated from the ratio of the specific extinction coefficients LDL/bovine serum albumin (Fisher *et al.*, 1971). Sedimentation velocity experiments and measurement of the diffusion coefficient of the lipoprotein samples were performed in a Spinco Model E ultracentrifuge using schlieren optics, and the hydrated density of the lipoprotein samples was evaluated by interpolation of s *vs.* ρ data to an s value of zero by methods which have been described previously (Fisher *et al.*, 1971). Viscosity values of the KBr-containing solutions were obtained from the International Critical Tables.

The technique of gradient banding of LDL has been described in detail as have the immunologic methods which were utilized (Hammond and Fisher, 1971). Briefly, linear density gradients were preformed by mixing equal volumes of a buffered solution containing either 12 or 15% sucrose, thus creating a gradient extending from 1.006 to approximately 1.06 g per cm^3. The gradients were centrifuged in swinging-bucket rotors, either the Spinco SW 25.1 rotor at 25,000 rpm for 65 hr or the SW 41 rotor at 41,000 rpm for 24 hr at 5° after being overlayered with LDL which was dialyzed against the low density solvent. Maximally concentrated LDL (3 ml) was used to overlayer the gradient for the SW 25.1 rotor and 1 ml for the SW 41 rotor. Samples were recovered by collecting drops after puncturing the bottom of the tubes, and the fractions were assayed at 280 nm to estimate relative lipoprotein concentration.

Immunologic studies were performed on lipoprotein samples recovered in the peak fractions from the density gradient banding experiments, this LDL having initially been purified by differential density flotation. The lipid composition of the lipoproteins was determined by a previously described method (Fisher, 1970).

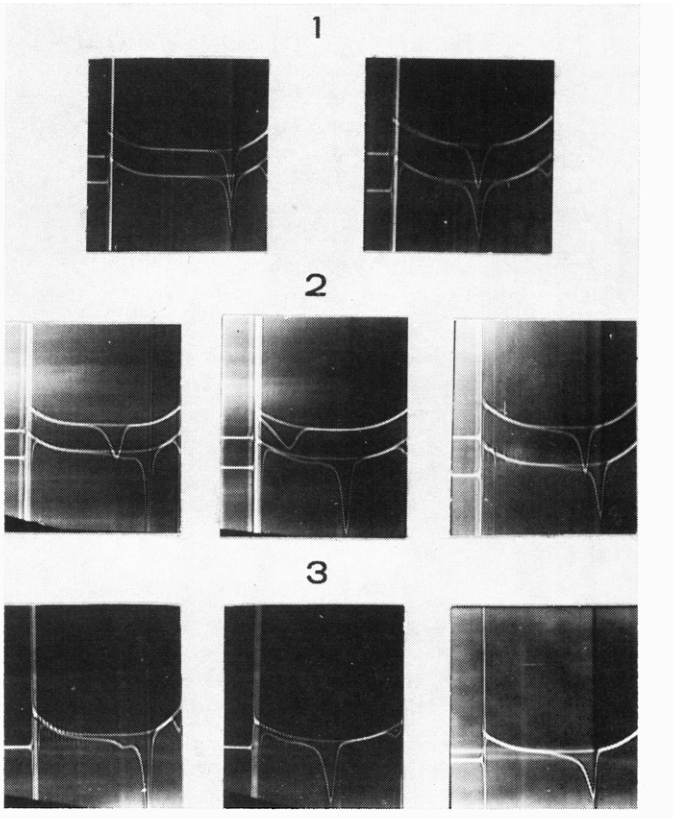

FIGURE 1: Analytical ultracentrifugation of total lipoproteins and of LDL fraction from three hyperlipemic subjects. Centrifugation at 42,040 rpm at 25°. Times are after centrifuge reaches operating speed. Total lipoprotein samples are from 2 ml of serum concentrated to a 1-ml volume. Solution density 1.20 g/cm^3 unless otherwise specified. (1) Subject E: wedged cell (top) LDL fraction, concentration 7 mg/ml. Standard cell (bottom) total lipoprotein. Photographs at 6 and 15 min at bar angles of 70 and 75°. (2) Subject G: left and middle, standard cell, total lipoproteins. Wedged cell, unrelated sample. Photographs at 6 and 14 min at bar angle 75°. Right, LDL fraction. Wedged cell solution density 1.20 g/cm^3, standard cell density 1.16 g/cm^3. Photograph at 10 min. LDL concentration 7 mg/ml. Bar angle 70°. (3) Subject H: left and middle, total lipoprotein. Photographs at 6 and 15 min. Bar angle 70 and 75°. Right, LDL fraction. Photograph at 10 min. LDL concentration 13 mg/ml. Bar angle 80°.

Subjects

Subjects for this study were either normal individuals or individuals who had the diagnosis of hyper-β (type II)-lipoproteinemia established on the basis of published criteria (Fredrickson *et al.*, 1967). The subjects were all Caucasian, and none of them were diabetic, as determined by glucose tolerence testing. Aside from the hyperlipoproteinemia and the associated vascular disease, the patients were all in good health. A summary of the clinical data of each hyperlipemic subject is presented in Table I. The study extended over several years, and in two normal and seven hyperlipemic subjects lipoproteins were isolated and characterized on two or more occasions.

Results

Figure 1 shows photographs of the schlieren patterns taken during analytical ultracentrifugation of the total lipoprotein fraction (d <1.20 g/cm^3) and of the LDL fraction (d 1.006–1.060 g/cm^3) isolated from three of the hyperlipemic subjects. The patterns have been selected to demonstrate the degree of heterogeneity seen in the LDL from these subjects, and aside from the magnitude of the LDL peak, there is no obvious difference from the patterns observed with the lipoproteins of normal subjects. Part 1 of this figure shows an apparently homogeneous lipoprotein while in parts 2 and 3 variable heterogeneity in the LDL fraction is evident. The preparation from subject H demonstrates the greatest degree of heterogeneity observed in this study; however, even in this preparation there is clearly a major fraction, and only a small proportion of the total lipoprotein floats up at a more rapid rate. In all instances the LDL fractions from the normal and the hyper-β-lipoproteinemic subjects have been observed to consist of one major component which appears either homogeneous or slightly heterogeneous upon analytical ultracentrifugation.

Following its isolation, LDL from the various subjects was studied by sedimentation–velocity ultracentrifugation to define the sedimentation coefficient, and these values are recorded in Table II. In each instance the recorded sedimentation coefficient has been derived by the extrapolation to infinite dilution of a minimum of three sedimentation determin-

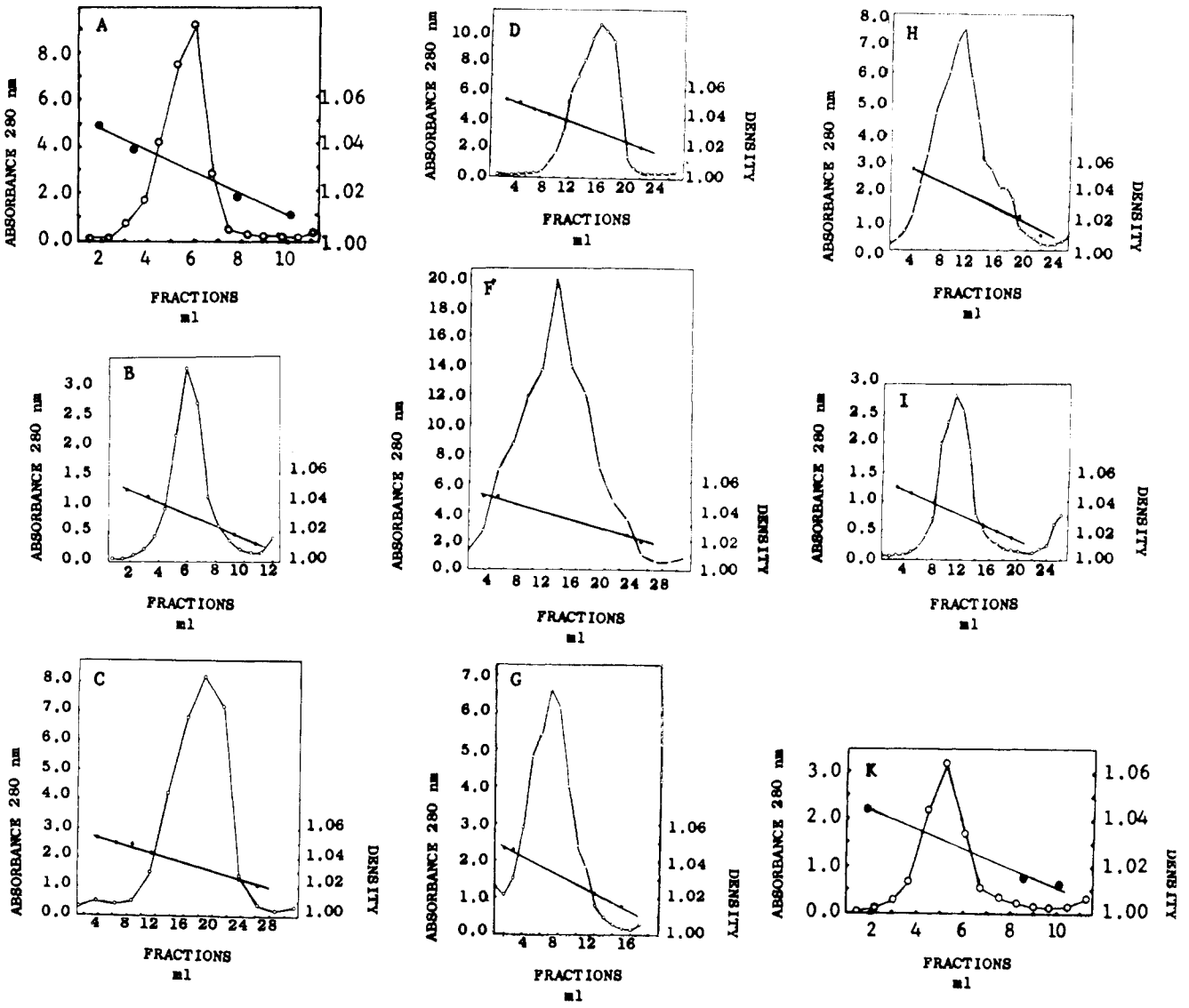

FIGURE 2: Equilibrium banding of LDL in preformed density gradients. For details, see Methods. (●) Density gradient measured in eluted fractions. (○) Absorbance at 280 nm of eluted fractions. Capital letters identify the subjects whose LDL have been banded.

ations at differing lipoprotein concentrations. From sedimentation *vs.* density plots, constructed utilizing a minimum of three sedimentation measurements at different solvent densities, the hydrated density of the lipoproteins has been determined. These values, also reported in Table II, were measured in KBr solutions and thus represent true values for the hydrated density as neither preferential hydration or salt binding occur with LDL in this solvent system (Fisher *et al.*, 1971).

On the basis of these data molecular weights of the various lipoproteins may be calculated, providing the frictional coefficient is known. A frictional ratio of 1.11 has recently been determined for normal LDL (Fisher *et al.*, 1971). On the assumption that the frictional ratio for LDL from different individuals does not vary appreciably, one may calculate molecular weights of the lipoproteins from each of the subjects. These values are also shown in Table II.

In order to assess the validity of the assumption that the frictional ratio measured in normal subjects may be utilized in calculating molecular weights of LDL from the hyperlipemic subjects, measurements of the diffusion coefficient were performed on single preparations of the lipoproteins from four of these subjects, and the values measured for these lipoprotein solutions are as shown in Table III. These single measurements of the diffusion coefficients of the LDL from four hyperlipemic subjects agree closely with the value of $D^0_{25} = 2.2 \times 10^{-7}$ g²/sec previously reported for the LDL from the two normal subjects, I and J (Fisher *et al.*, 1971). These observations suggest that the LDL from the hyperlipemic sub-

TABLE III: Diffusion Coefficients of LDL from Hyperlipemic Subjects.[a]

Subject	$D_{25} \times 10^7$ (cm²/sec)	LDL Concn (mg/ml)
A	1.9	13.6
B	2.0	5.0
C	2.1	7.1
D	2.1	4.2

[a] Values are single measurements at the indicated LDL concentrations.

TABLE IV: Lipid Composition of LDL.

A. Lipid Composition of LDL from Hyperlipemic Subjects

Subject	% Total Lipid	Lipid Composition[a] Cholesterol Ester[b]	Cholesterol	Triglyceride	Phospholipid	% Recov of Lipid[c]
A	81 ± 0.5	55 ± 0.5	14 ± 0.3	3 ± 1.0	28 ± 0.3	96 ± 1.8
B	81 ± 0.5	48 ± 0.1	14 ± 0.1	10 ± 0.1	28 ± 0.2	98 ± 0.1
C	71 ± 0.3	48 ± 0.1	16 ± 0.1	13 ± 0.0	24 ± 0.1	100 ± 0.5
E	78 ± 0.2	53 ± 0.5	11 ± 0.7	10 ± 0.6	27 ± 0.3	94 ± 1.4
G	76 ± 1.1	50 ± 0.1	12 ± 0.1	12 ± 0.1	27 ± 0.0	96 ± 0.1
H	77 ± 0.3	47 ± 0.1	17 ± 0.2	10 ± 0.1	26 ± 0.0	97 ± 0.0
Average	79	50	14	9	27	
Normal LDL[d]	79	47	10	14	28	

B. Composition of LDL from Subjects Expressed as Quantity of Each Constituent per Mole of Lipoprotein ($g \times 10^{-6}$)

Subject	Mol Wt of LDL	Protein	Cholesterol Ester	Cholesterol	Triglyceride	Phospholipid
A	3.5	0.66	1.53	0.40	0.07	0.78
B	3.1	0.60	1.20	0.34	0.25	0.70
C	3.0	0.60	1.14	0.37	0.30	0.57
E	2.9	0.63	1.20	0.25	0.23	0.61
G	2.7	0.66	1.02	0.24	0.24	0.56
H	2.9	0.67	1.05	0.38	0.22	0.57

[a] In expressing percentage composition the sum of the amounts of each component from a given subject was taken as 100%. [b] Measured as cholesterol palmitate. [c] Per cent Recovery of Lipid records the sum of the amounts of the individual lipid components recovered as a percentage of the total lipid in the initial sample. [d] From Oncley, the average of seven values reported by various investigators (Oncley, 1960).

jects have frictional coefficients similar to those of normal LDL and provide a justification for assuming the same frictional ratio in the molecular weight calculation.[1]

In observing the sedimentation coefficients, hydrated densities, and molecular weights of the LDL from the various subjects, it is obvious that there is variation from one subject to the next. By contrast, for a given subject repeated measurements of these same parameters are virtually identical in all instances, except for Subject H, even though many months often elapsed between determinations. The small variation in the molecular weights of the LDL observed for each of the other subjects suggests that differences recorded in LDL molecular weight from one subject to another may well be significant.

In order to assess the density homogeneity of low-density lipoproteins from nine of these subjects, the lipoproteins of the LDL fractions were concentrated, dialyzed against buffered KBr of density 1.006 g/cm^3, and overlayered on a linear density gradient extending from 1.006 to 1.06 g per cm^3. The distribution of the lipoproteins following ultracentrifugation is depicted for a number of the subjects in Figure 2. The hydrated density of these various lipoproteins may be estimated from these plots, based upon the position of the major lipoprotein peak. These densities are recorded in Table II, and it may be observed that the hydrated density measured in this manner compares very favorably with that calculated from the *s vs.* ρ plots derived from sedimentation measurements in the analytical ultracentrifuge.

In order to evaluate more precisely the density heterogeneity of these lipoproteins, the solution densities from the plots in Figure 2 were recorded on either side of each LDL peak at that point where the absorbance of the solution measured 10% of the maximum peak absorbance. Density heterogeneity, defined by this density range, varied from 0.019 to 0.038 g per cm^3 with a mean of 0.024 g/cm^3. Subjects A, C, D, and I were among those with the least heterogeneity, while subjects F and H had the greatest heterogeneity. Subject H is also the only individual whose LDL values vary appreciably from one occasion to the next in terms of their weight average molecular weight and hydrated density as shown in Table II.

The lipid composition of the lipoproteins of six hyperlipemic subjects is recorded in Table IV A, and though there are variations among these LDL, no particular pattern is evident. The mean values for the composition of the LDL from these subjects is also recorded, and when compared to the compositional data reported for normal LDL, there is no appreciable difference (Oncley, 1960).

Table IV B records the composition of these LDL expressed as grams of each constituent per mole of lipoprotein. The striking observation emerging from these data is that the protein content of the lipoproteins is essentially invariant, even though the lipoproteins vary in molecular weight from 2.7 to 3.5 million. Rather, the differences in the molecular weights of the lipoproteins is a result of differences in the amount of the various lipid constituents. These data thus indicate that the polypeptide content of each of these lipoproteins is the

[1] The following equation was used in calculating molecular weights: Fisher *et al.*, 1971

$$M = \left(\frac{6.66\eta s}{1 - \bar{v}\rho}\right)^{3/2} \left(\frac{3\bar{v}}{4}\right)^{1/2} \pi N$$

same but that the amount of lipid associated with the polypeptide may vary. Apparently, it is the difference in the amount of lipid which determines the variations in molecular weight of the LDL among the subjects.

The immunologic characterization of the LDL from several of these subjects was performed on lipoproteins obtained from the peak fractions following banding of the LDL in a density gradient. This lipoprotein was then delipidized and resolubilized as previously described (Hammond and Fisher, 1971), and immunodiffusion of the apoprotein was then performed against the appropriate antiserum. The apoproteins of subjects A, B, C, G, and H were studied, and all reacted with the anti-LDL serum giving a ring of identity. By contrast, no precipitation occurred with an antiserum prepared against the very low density lipoprotein peptides present in the SF-3 fraction (Brown *et al.*, 1970). Immunodiffusion against the high density lipoprotein antiserum revealed a faint line of precipitation with the lipoproteins of several of these subjects; however, this precipitation line did not coincide with the strong line of precipitation formed with the apoprotein from high density lipoprotein. As this HDL antiserum has also been shown to react with apo-LDL, it is presumed that this line is a precipitate of the apoprotein and the anti-LDL component in the antiserum. On the basis of the immunologic studies it would appear that the LDL from these hyperlipemic subjects has the immunologic properties of normal LDL.[2]

Discussion

The presence of structurally altered low density lipoproteins circulating in the plasma of subjects with the disease hyperpre-β (type IV)-lipoproteinemia has been reported (Hammond and Fisher, 1971). The present studies were undertaken to answer the question of whether structural variability of LDL also occurs among normal subjects and individuals with the familial disease, hyper-β (type II)-lipoproteinemia, a genetic disorder which probably is inherited as an autosomal dominant (Fredrickson *et al.*, 1967). Accordingly, the LDL from eight hyperlipemic subjects was isolated and characterized. In a similar manner the LDL from three normal subjects was studied, the smaller number being chosen as the characterization of normal LDL has been reported from other laboratories (Lindgren *et al.*, 1969; Adams and Schumaker, 1969a,b, 1970; Bjorklund and Katz, 1956; Toro-Goyco, 1958). To determine the changes occurring in the macromolecular properties of the LDL from a specific subject over a period of time, it was elected to restudy nine subjects.

In compiling the data from this study a number of interesting features regarding the structure of LDL have become evident. In all but one instance the molecular weights of the LDL for a given subject are identical upon repeated isolation. On the other hand, molecular weights of the LDL from different subjects may vary over a range of from 2.4 to 3.5 million. The observation that the molecular weight of LDL from various individuals may differ over a broad range has been reported by other observers (Lindgren *et al.*, 1969; Adams and Schumaker, 1970); however, the invariant molecular weight of LDL from a given individual over a period of many months apparently has not been well documented.

In terms of their molecular weights and hydrated densities, the LDL in this small sample of patients with hyper-β-lipoproteinemia cannot be said to differ from normal LDL.[3] Immunologically, the apoprotein of these LDL react only with LDL-specific antiserum. The lipid content and composition (Table IV A) of the LDL isolated from six hyperlipemic subjects shows very little difference from that reported for normal LDL (Oncley, 1960). Recently Slack and Mills (1970) have reported a decrease in the per cent triglyceride present in the LDL fraction of subjects with hyper-β-lipoproteinemia. The data in the present study also suggest that a decrease in triglycerides in these LDL might be present; however, the differences are not impressive. It would appear reasonable to conclude that if differences between normal and hyper-β-lipoproteinemic LDL do occur, these differences are small and are less than the variations observed among individuals within each group. The failure to demonstrate differences in total amino acid composition of LDL between normal and hyper-β-lipoproteinemic subjects also suggests that there are not significant differences between these groups of subjects (Pinon and Laudat, 1969).

In order to evaluate further the differences in the molecular parameters of the LDL isolated from subjects in this study, the density heterogeneity of the lipoproteins was measured following banding in linear density gradients using the preparative ultracentrifuge. In 1969 Adams and Schumaker reported their studies on the equilibrium banding of LDL from normal subjects in the analytical ultracentrifuge and demonstrated in single subjects a heterogeneous population of macromolecules with hydrated densities which on occasion extend over a range of 0.008 g/cm^3 (Adams and Schumaker, 1970). Figure 2 documents similar observations on the density heterogeneity of LDL, thus confirming the findings of Adams and Schumaker. The lipoproteins from subjects F and H demonstrate a significantly greater degree of density heterogeneity than for the other subjects. Apparently LDL is so constituted that there is some variance permitted in the amount of lipid which is bound; however, all of these apoproteins bind lipid in a manner which produces a lipoprotein continuum existing within a relatively narrow density range.

By contrast a family of low density lipoproteins of differing hydrated densities and molecular weights, which span the entire LDL range, have been isolated from the serum of five subjects with the disease hyperpre-β (type IV)-lipoproteinemia (Hammond and Fisher, 1971). There appears to be a fundamental difference in the lipid binding properties, reflected in the degree of density heterogeneity, of LDL in this previously reported group of subjects.

In attempting to define further the structure of these lipoproteins, one may analyze the relationship of their molecular weight to their molecular volume, as recently proposed (Adams and Schumaker, 1969a,b). These investigators have derived an expression relating the molecular weight and molecular volume of a family of lipoproteins by the following equation: $V_0' = V_0 - \bar{v}\ \mathrm{av}\ M_0 + \bar{v}\ \mathrm{av}\ M_0'$, where V_0 = the partial molar volume of the initial lipoprotein and V_0' that of the newly formed lipoprotein; M_0 and M_0' are the molecular weights of the initial and new lipoprotein; and $\bar{v}$ av is the par-

[2] The authors express their appreciation to Drs. Sam Lux and Robert I. Levy from the National Heart Institute for the gift of the very low density lipoprotein and high density lipoprotein antisera.

[3] The molecular weights of LDL reported in this study differ from previously reported values from a number of laboratories This difference arises from the utilization of the recently reported frictional coefficient in calculating the molecular weights of LDL. Previous investigators have either assumed this lipoprotein to be an anhydrous sphere in molecular weight calculations or have assigned arbitrary values for the frictional ratio (Fisher *et al.*, 1971).

tial specific volume of the increment of material added in generating the second lipoprotein from the first (this may either be lipid or lipid plus protein). Using this equation Adams and Schumaker have plotted measurements of the molecular weight and molecular volume for several samples of normal LDL, very low density lipoprotein, and both of these lipoproteins which were restudied following treatment with lipoprotein lipase. The resulting plot was linear with a slope of 1.03.

This equation has been applied to the family of LDL components isolated from a group of subjects with hyperpre-β (type IV)-lipoproteinemia, and again a linear plot was defined with a slope of 1.03 (Hammond and Fisher, 1971). In like manner the points defined by the molecular weight and molecular volume for the 21 low density lipoproteins characterized in the present study have also been plotted, and these points all fall precisely on the previously defined line. One may assume then that these different lipoproteins could theoretically be generated from each other by the addition of an increment of material having a partial specific volume of 1.03 ml/g or a density of 0.970 g/cm^3. As observed by Adams and Schumaker, this density is consistent with the added increment of material having a mixed lipid composition and containing very little, if any, protein.

In the reported studies on the composition of the LDL components of several patients with hyperpre-β-lipoproteinemia, Hammond and Fisher demonstrated that though these various lipoproteins vary in molecular weight over a twofold range, each component contained essentially the same weight of apoprotein per mole of lipoprotein (Hammond and Fisher, 1971). The differences in molecular weight were found to be due to changes in the total amount of lipid associated with the apoprotein, an observation completely in keeping with the predictions from the molecular weight *vs.* molecular volume plot. Similarly, when one looks at the compositional data in Table IVB, it is evident that for the subjects reported in the present study, the weight of the protein component of the LDL of each of the subjects is constant, and the differences in the molecular weights of the various lipoproteins result from differences in the amount of lipid associated with the apoprotein.

From these data it would appear that though the molecular weights of LDL from this series of subjects vary from 2.4 to 3.5 million, the amount of the apoprotein present in the LDL from all of the subjects is invariant. Furthermore, for a given subject the molecular weight and hydrated density of the LDL remains constant, or nearly so, over the period of observation. Of special interest is the fact that, as the degree of hyperlipemia for subjects B and G changes, as reflected by changes in the serum cholesterol during dietary restriction (Table II), the macromolecular parameters of LDL still remain constant even though the concentration changes.

Several years ago it was proposed that for simple proteins the amino acid sequence contained the information necessary to determine the three-dimensional structure (Anfinsen, 1967). For complex proteins, such as LDL, one would presume that the three-dimensional structure in solution would be determined by the interactions of the polypeptide chain, the associated lipid, and the aqueous milieu.

The observations in this study cannot as yet be explained in these terms. Grossly there are no striking differences in the lipid composition of these LDL, though the lipid content clearly increases with increasing molecular weight. Quite possibly a detailed study of the types of phospholipids or perhaps the fatty acids associated with these various LDL might reveal significant compositional differences which could alter the amount of lipid bound by the apoprotein in LDL from different individuals. Alternately it is possible that individual variation occurs in the structure of the apoproteins of these subjects and that different apoproteins bind different amounts of lipid, thus accounting for the observed variations in LDL molecular weight. If so then polypeptide heterogeneity should be found when the structures of the human LDL apoproteins are delineated, and it is tempting to speculate that such structural variations might reflect genetic variability among individual subjects.

References

Adams, G. H., and Schumaker, V. N. (1969a), *Anal. Biochem. 29*, 117.

Adams, G. H., and Schumaker, V. N. (1969b), *Ann. N. Y. Acad. Sci. 164*, 130.

Adams, G. H., and Schumaker, V. N. (1970), *Biochim. Biophys. Acta 210*, 462.

Anfinsen, C. B. (1967), *Harvey Lect. 61*, 96.

Bjorklund, R., and Katz, S. (1956), *J. Amer. Chem. Soc. 78*, 2122.

Bragdon, J. H. (1960), *in* Lipids and Steroid Hormones in Clinical Medicine, Sunderman, F. W., and Sunderman, F. W., Jr., Ed., Philadelphia, Pa., Lippincott Co., p 11.

Brown, W. V., Levy, R. I., and Fredrickson, D. S. (1970), *Biochim. Biophys. Acta 200*, 573.

De Lalla, O. F., and Gofman, J. W. (1954), *Methods Biochem. Anal. 1*, 459.

Del Gatto, L., Lindgren, F. T., and Nichols, A. V. (1959), *Anal. Chem. 31*, 1397.

Fisher, W. R. (1970), *J. Biol. Chem. 245*, 877.

Fisher, W. R., Granade, M. E., and Mauldin, J. (1971), *Biochemistry 10*, 1622.

Fredrickson, D. S., Levy, R. I., and Lees, R. S. (1967), *N. Engl. J. Med. 276*, 273.

Hammond, M. G., and Fisher, W. R. (1971), *J. Biol. Chem. 246*, 5454.

Lees, R. S., and Hatch, F. T. (1963), *J. Lab. Clin. Med. 61*, 518.

Lindgren, F. T., Jensen, L. C., Wills, R. D., Freeman, N. K. (1969), *Lipids 4*, 337.

Mauldin, J., and Fisher, W. R. (1970), *Biochemistry 9*, 2015.

Oncley, J. L. (1960), *in* Brain Lipids and Lipoproteins and the Leucodystrophies, Folch-Pi, J., and Bauer, H. J., Ed., New York, N. Y., Elsevier, p 1.

Pearson, S., Stern, S., and McGavack, T. H. (1953), *Anal. Chem. 25*, 813.

Pinon, J. C., and Laudat, P. (1969), *Biochim. Biophys. Acta 187*, 144.

Slack, J., and Mills, G. L. (1970), *Clin. Chim. Acta 29*, 15.

Toro-Goyco, E. (1958), Ph.D. Thesis, Harvard University, Cambridge, Mass.