The Characterization and Occurrence of an Sf 20 Serum Lipoprotein*

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SUMMARY

In the serum of patients with hyper-pre-/β-lipoproteinemia there frequently occurs in abundance an ultracentrifugally distinct low density lipoprotein component of the Sf 20 class. This study concerns the characterization of this component, present in the serum of 14 of 15 patients. The lipoprotein has been isolated ultracentrifugally; it has the electrophoretic and immunological properties of a β-lipoprotein but is distinct from very low density lipoproteins. It has a buoyant density of 1.004 g per ml which is lighter than the major low density lipoproteins of normal serum, and this observation is consistent with its higher triglyceride content. This lipoprotein has a sedimentation coefficient corrected for concentration and viscosity of -68 S, measured in a solvent of density 1.20 g per ml, and a molecular weight of approximately 4.2 million. Electron microscopy of this larger low density lipoprotein shows spherical macromolecules with a diameter approximating 256 A.

The lipoproteins which are lighter than plasma constitute a heterogeneous population of macromolecules with molecular diameters varying from 250 to over 5000 A (5). The chylomicrons and very low density lipoproteins constitute the major components of this mixture, but some components of the LD lipoprotein1 class also have densities lighter than serum. One such component is the subject of this study.

In several previous ultracentrifugal studies of serum lipoproteins a lesser component of the 1.1 Density class was observed, which floated with an Sf rate of 10 to 20, as compared to the major (Sf 4 to 8) LD lipoprotein component (6–8). This lipoprotein was generally present in greatest quantity in lactescent serum and was frequently associated with diabetes and arterial vascular disease. In 1951 isolation of this lipoprotein was reported (9); however, its characterization has apparently never been described.

The present study is concerned with re-evaluating the occurrence of this Sf 20 lipoprotein (Sf 20 LD lipoprotein) and with its physical, immunological, and gross lipid characterization.

METHODS

Serum total lipids and cholesterol determinations were performed by standard methods (10, 11). Lipoprotein electrophoresis was performed by the method of Lees and Hatch (2). Total fasting lipoproteins from 2 ml of serum were isolated in a 1-ml volume by ultracentrifugation at density 1.20 by a method similar to that of Del Gatto, Lindgren, and Nichols (12). The isolated lipoproteins were subjected to analytical ultracentrifugation, as described by these authors, at a known solvent density approximating 1.20 g per ml in double sector Epon aluminum-filled cells at 42,040 rpm and 25⁰C. Flotation rates for lipoproteins under these conditions have been compared to the Sf system of classification by the above authors.¹

¹ The abbreviations used are: LD lipoproteins, low density lipoproteins of d 1.006 to 1.063; VLD lipoproteins, very low density lipoproteins of d < 1.006; HD lipoproteins, high density lipoproteins of d 1.063 to 1.20; LD apolipoproteins, VLD apolipoproteins, and HD apolipoproteins, the lipid-free protein residues of LD lipoproteins, VLD lipoproteins, and HD lipoproteins, respectively.

¹ The analyses were performed by the Clinical Laboratories of the Shands Teaching Hospital of the University of Florida.

¹ In comparing lipoprotein flotation rates in solutions of density 1.063 and 1.20 g per ml, these authors report flotation coefficients

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Isolation of the Sf 20 LD lipoprotein fraction was accomplished by a method similar to that previously reported (9). Pasting serum was separated by centrifugation at 1500 rpm. Sodium EDTA buffered to pH 7.0 was added in a concentration of 1 mg/10 ml of serum, and all solutions used in this purification also contained EDTA. In Beckman No. 30 preparative ultracentrifuge tubes 24 ml of serum were overlaid with 5 ml of NaCl solution of density 1.006. After centrifugation at 30,000 rpm for 1 hour at 5° in a Beckman model L-2 centrifuge, the top 5 ml of solution from each tube were removed with a syringe and discarded. By using this initial centrifugation as described by Redbell (13), a considerable amount of the VLD lipoprotein could be removed. The infranatants from this centrifugation were then combined and centrifuged for 24 hours at 5° in the Ti-50 rotor at 50,000 rpm. The top 2 ml of solution from each tube were discarded and the infranatants were recombined and adjusted to a density of 1.020 g ml-1 by dialysis against a KBr solution of the same density. Following recentrifugation at 50,000 rpm for 24 hours, the Sf 20 LD lipoprotein was isolated in a 1-ml aliquot from the top of the tube. Further purification was achieved by two recentrifugations at 50,000 rpm for 24 hours each, first to eliminate traces of contaminating VLD lipoprotein at density 1.006, and then to concentrate the Sf 20 LD lipoprotein by refloating at density 1.020. The purified lipoprotein formed a clear, pale yellow solution which showed no significant change in its sedimentation or electrophoretic properties measured over a period of 1 month when stored at 4° in the presence of EDTA buffered to neutrality.

Lipoprotein concentration was determined by the Lowry technique (14) with a purified preparation of which Sf 20 LD lipoprotein as a standard. The concentration of the standard was determined by lyophilizing the solution and weighing the dried, unextracted lipoprotein. The extinction coefficient of this whole lipoprotein was compared to that for bovine serum albumin. Assuming that the Lowry reagent reacts only with the peptide moiety of the lipoprotein, then on the basis of the albumin extinction coefficient, the protein content of Sf 20 LD lipoprotein is approximately 16%. This value is a reasonable approximation of the value measured gravimetrically (Table II and as discussed subsequently).

Sedimentation velocity measurements were performed in a Spinco analytical ultracentrifuge with an An-D rotor, with the use of two double sector cells with Epon aluminum-filled centerpieces. One of the cells was fitted with a positive wedge window. Thus, each cell contained solvent and lipoprotein solutions, and two separate samples could be run simultaneously. Centrifugation was performed at 42,040 rpm and 25°, and the sedimentation coefficient was calculated from the slope of the line derived by plotting the log of the distance from the center of rotation to the lipoprotein peak as a function of time.

In the determination of the buoyant density of Sf 20 LD lipoprotein, solutions of the lipoprotein were dialyzed against KBr solutions of known density. The densities of these dialyzing solutions were determined in triplicate at 25°, with the use of calibrated 10-ml flasks. The viscosities of the KBr solutions were obtained from the International Critical Tables based on the known salt concentrations.

which are not corrected for the viscosity of the solutions. The flotation coefficients reported in the present study are all corrected for viscosity, with the result that the values differ somewhat from those reported by Del Gatto et al., as will be discussed subsequently.

Immunological studies were performed by the use of specific rabbit antihuman HD lipoprotein serum obtained from Hoechst Pharmaceuticals, Inc. The specificity of this antiserum was confirmed in this laboratory. Antihuman LD lipoprotein goat serum was prepared using a composite LD lipoprotein antigen. This was obtained by combining 1-ml serum samples from 100 hospital patients and purifying the LD lipoprotein ultracentrifugally. The purity of the LD lipoprotein was established by acrylamide gel electrophoresis. An antigen consisting of 1.8 mg of lipoprotein in 0.6 ml of dilute KBr solution was homogenized with 1.2 ml of Freund’s complete adjuvant (Difco H37-Ra) and injected intradermally at multiple sites in a young goat. After 2 weeks the serum from the goat, containing a single, specific precipitating antibody, was harvested.

Immunodiffusion and immunoelectrophoresis were performed by standard techniques, with an agar medium containing 2% special Nobel agar (Difco) in a Veronal buffer, pH 8.6. The precipitin lines were photographed directly or after staining with Amido black.

In order to study the immunological properties of the apoprotein, the lipoprotein was gently delipidated and resolubilized in a manner adapted from Gotto, Levy, and Fredrickson (15). Specifically, 1 ml of a solution containing 5 mg of lipoprotein per ml, which had been dialyzed against 0.15 M NaCl, was added dropwise with vigorous agitation to 50 ml of ethanol-diethyl ether (1:3) at -21°. After standing for 4 hours, the suspension was centrifuged at the same temperature, and the solvent was discarded. The precipitate was washed once with cold diethyl ether, recentrifuged, and evacuated. The apoprotein was readily dissolved in 0.5 ml of 0.13 M Tris-HCl buffer, pH 8.6, containing 0.06 M sodium decyl sulfate. The apoproteins remained soluble when dialyzed at 4° against the same buffer but in the absence of the sodium decyl sulfate, and these solutions were used for immunodiffusion studies.

Acrylamide gel electrophoresis of the isolated lipoproteins was accomplished on a 3.5% gel with a Tris-borate buffer at pH 8.7 (16). The lipoproteins were not previously stained but instead were localized following electrophoresis by staining the gel with Amido black.

Lipid analyses were performed on isolated LD lipoprotein obtained from nonpeneic individuals and on the isolated Sf 20 LD lipoprotein. The lipoproteins were dialyzed exhaustively against water to remove salt. The solution, containing considerable precipitated lipoprotein, was lyophilized, and the lipoproteins were further dried in a vacuum over P205. Weighed aliquots of about 10 mg of desiccated lipoprotein were extracted overnight with chloroform-methanol, 2:1 (v/v) by suspending them in 5 ml of solvent. The solvent was separated, and the protein was washed with four additional portions of solvent. The combined solvent extract was evaporated; the recovered lipid was then dried over P205 in a vacuum at 5°, and was weighed to determine the lipid content of the lipoprotein. The apoprotein content was calculated from the difference in the weight of the total lipoprotein and the weight of the extracted lipid.

The composition of the lipid was measured by the method of Amenta (17) that involved a thin layer chromatographic separation of the cholesterol esters, glycerides, and cholesterol on silica gel followed by acid dichromate oxidation of these lipids. The reduction of dichromate was measured photometrically. Phospholipid was determined by measuring total phosphorus on an aliquot of the lipid extract, by the method of Bartlett (18), and
FIG. 1 (left). Selected schlieren patterns during analytical ultracentrifugation of total serum lipoproteins. The lipoproteins from 2 ml of serum were concentrated to a 1-ml volume. Centrifugation was at 42,040 rpm with a KBr solvent of density 1.17 g per ml and temperature 25°. Column 1, photographs taken when centrifuge reaches maximum speed and showing variable concentration of very low density lipoproteins; Column 2, photographs at 4 min showing low density lipoproteins; Column 3, photographs at about 12 min again showing low density lipoproteins and initial appearance of high density lipoproteins at bottom (right) of cell. Top row, normal patient; middle row, patient with hyper-pre-β-lipoproteinemia showing the characteristic increase of LD lipoprotein; bottom row, patient with hyper-pre-β-lipoproteinemia showing increased VLD lipoprotein and the presence of an additional, faster floating, LD lipoprotein component (Sf 20 LD lipoprotein).

FIG. 2 (right). Analytical ultracentrifugation of total lipoproteins from three patients with elevated levels of very low density (pre-β) lipoproteins. Cases were selected to show variations in both low density lipoprotein components. Photographs taken at approximately 4 and 8 min after centrifuge reached top speed. Conditions of lipoprotein isolation and centrifugation were the same as in Fig. 1.

Subjects

The subjects for this study were classified, with respect to their lipemic status, on the basis of the total lipid and cholesterol content of their fasting sera and their lipoprotein electrophoresis patterns. Twenty individuals with normal values and with no metabolic disorders known to affect lipoprotein metabolism were selected as a control group. Fifteen patients with hyper-β-lipoproteinemia were chosen on the basis of an elevated serum cholesterol (with an associated increase in serum lipids) and a lipoprotein electrophoresis pattern showing an absence of pre-β-staining lipoproteins. Fifteen patients with hyper-pre-β-lipoproteinemia were classified on the basis of elevated serum lipids...
and a clearly increased pre-β-band on electrophoresis of their lipoproteins. Any mildly lipemic patients with equivocal increases in pre-β-lipoproteins were rejected from the study.

The Sf 20 LD lipoprotein samples used in the lipoprotein characterization studies were obtained from four subjects whose serum contained large quantities of pre-β-lipoproteins. All four subjects had diabetes, and two had sustained previous myocardial infarctions.

### RESULTS

**Occurrence of Sf 20 LD Lipoprotein: Study of Lipoproteins from 50 Subjects**

Total serum lipoproteins, isolated from the serum of individual subjects, were submitted to analytical ultracentrifugation as previously described. The appearance of the lipoprotein patterns observed are recorded in Fig. 1 and reflect the variation in amount of lipoprotein present in 2-ml volumes of serum for the three groups of subjects. The control patterns were, in general, as expected, with a mere trace of very low density lipoprotein, a sharp low density lipoprotein peak which frequently possessed a trailing shoulder of high density lipoprotein (HDL lipoprotein), and the major high density lipoprotein component which did not separate from the bottom of the analytical cell during the brief centrifuge run. The lipoproteins from hyper-β-lipoproteinemic sera were very similar in appearance except for a marked increase in size of the low density peak. By contrast, lipoproteins from hyper-pre-β-lipoproteinemic patients contained an increased quantity of very low density lipoproteins, which, in some cases, was quite striking. An additional consistent finding was the presence of a rapidly floating, minor LD lipoprotein component, which is Sf 20 LD lipoprotein. The total amount of this minor component varied considerably in different patients, appearing as a faster moving, small shoulder on the major LD lipoprotein peak in some and as a discrete, separate component in others. The relative amounts of the Sf 20 LD lipoprotein and of the major LD lipoprotein components seemed unrelated. Selected patterns showing these variations are shown in Fig. 2. An average viscosity-corrected flotation rate for a solvent of density 1.20 g per ml has been calculated from measurements made of each of the low density lipoproteins from these subjects. For the slow component (Sf 4 to 8) this value is -35 S with a standard deviation of 2.3, and for Sf 20 LD lipoprotein the value is -60 S with a standard deviation of 2.9. The lipoproteins in this sample population thus appear to be relatively homogeneous.

The association of Sf 20 LD lipoprotein with increased quanti-

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**TABLE I**

| Occurrence of Sf 20 LD Lipoprotein | Number of Subjects | Number of subjects with Sf 20 component |
|-----------------------------------|--------------------|----------------------------------------|
| Control                           | 20                 | 1                                      |
| Hyper-β-lipoproteinemia           | 15                 | 2                                      |
| Hyper-pre-β-lipoproteinemia       | 15                 | 14                                     |
| Total                             | 50                 | 17                                     |

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4 These subjects conformed clinically to the type II and type IV class, respectively, as described by Fredrickson, Levy, and Lees (4).

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**Fig. 3.** A, paper electrophoresis of purified serum lipoprotein. Patterns from left to right; total serum lipoproteins (showing β and pre-β components), isolated LD lipoprotein (β lipoprotein), and Sf 20 LD lipoprotein from patients F.T., H.V., and M.H., respectively. B, acrylamide gel electrophoresis of isolated serum lipoproteins. From left to right: Sf 20 LD, LDL, Sf 20 LDL lipoproteins, and VLD lipoprotein, which remains at the origin.

**Fig. 4 (upper).** A, immunoelectrophoresis. Left, Sf 20 LDL lipoprotein; right, LDL lipoprotein. Antihuman LDL lipoprotein goat serum in trough. B, immunodiffusion. 1, HD lipoprotein; 2, LDL lipoprotein; 3, Sf 20 LDL lipoprotein (H.V.); 4, Sf 20 LDL lipoprotein (F.T.); 5, Sf 20 LDL lipoprotein (M.H.). Center well; top, anti-LDL lipoprotein serum; bottom, anti-HD lipoprotein serum.

**Fig. 5 (lower).** Immunodiffusion of delipidated lipoproteins. Top well, VLD lipoprotein; lower left well, Sf 20 LDL lipoprotein; lower right well, LDL lipoprotein. Left, anti-LDL lipoprotein serum in center well; right, anti-HD lipoprotein serum in center well.
Studies with Isolated S\textsubscript{f} 20 LD Lipoprotein

S\textsubscript{f} 20 LD lipoprotein was isolated from four patients, F.T., H.V., M.H., and E.W. The data obtained from the study of the lipoproteins from each of these patients will be identified.

Figure 3 shows the results of electrophoresis of the purified lipoprotein on paper and in acrylamide gel. By this paper technique, which is generally used in separating the serum lipoproteins in classifying hyperlipoproteinemic disease states (4), S\textsubscript{f} 20 LD lipoprotein moves with the LD lipoprotein (β-lipoprotein) fraction rather than with VLD lipoprotein (pre-β-lipoproteins). In acrylamide gel the S\textsubscript{f} 20 LD lipoprotein preparations moved as single bands with a mobility slightly less than that of the major LD lipoprotein class.

Figure 4 illustrates that in double agar diffusion studies S\textsubscript{f} 20 LD lipoprotein precipitates with anti-LD lipoprotein serum.

There is no precipitin line formed with anti-HD lipoprotein serum. On immunoelectrophoresis all three preparations behaved similarly in that they formed a precipitin line with anti-LD lipoprotein serum which was similar to that formed by the major lipoprotein fraction. The native S\textsubscript{f} 20 LD lipoprotein component thus apparently possesses the antigenic properties of low density lipoprotein, but native VLD lipoprotein has similar properties. Partial delipidation of VLD lipoproteins, however, exposes an HD lipoprotein antigenic site which reacts with anti-HD lipoprotein serum, while similar treatment of LD lipoprotein does not.

Immunodiffusion was performed with...
In Fig. 8, it is indicated that there is a small concentration dependence of a series of lipoprotein concentrations. The results, shown in Fig. 8, have been corrected for the relative viscosity of the solution, and the mean of four separate determinations each performed in duplicate, ± one standard deviation.

In Table II, the lipid composition of LDL and Sf 20 LD lipoproteins is presented. The total lipid content, cholesterol ester, cholesterol, triglyceride, and phospholipid content are shown for each of the four patients. The recovery of lipid is also shown for each sample.

In order to determine the concentration dependence of the sedimentation coefficient, flotation measurements were made at a series of lipoprotein concentrations. The results, shown in Fig. 8, indicate that there is a small concentration dependence for the lipoproteins isolated from the four subjects.

The sedimentation coefficient corrected for concentration and viscosity for Sf 20 LDL lipoprotein, as determined in a solvent of density 1.20 g per ml at 25°C ($s_{20}$), is $-68 S$. This is a mean value from the determinations shown in Fig. 8. If one mathematically adjusts this value from that obtained in a solvent of density 1.20 g per ml to that expected in a solvent of density 1.063 g per ml, then an $s_{20,1.063}$ value of $-20 S$ is obtained. Alternatively, by inspecting Fig. 7, it may be observed from the plot of $s_{20}$ against density, which was derived from experimentally measured flotation determinations, that at a solution density of 1.063 g per ml, the viscosity-corrected sedimentation coefficient is $-20 S$. Thus, this lipoprotein may be classified as an Sf 20 lipoprotein, hence, its designation as "Sf 20 LDL lipoprotein" distinguishing it from the major low density lipoprotein of normal serum with an $s_{20,1.063}$ value of 4 to 8.

Electron microscopy of a purified Sf 20 LDL lipoprotein preparation from patient F.T. showed the presence of macromolecules which were generally round and fairly uniform in size, as seen in Fig. 9. Moderate aggregation is present; however, the diameters of 100 unaggregated molecules were measured. A mean diameter of 250 Å with a standard deviation of 24 Å was calculated.

The results of lipid analyses of the lipoproteins are recorded in Table II. The total lipid content of the lipoprotein and the percentage composition of the extracted lipid are shown. The results of duplicate assays on one sample of normal LDL lipoprotein are given in order to document the agreement of these analyses with those from seven other laboratories as recorded by Oncley (22). The value for lipid content and percentage composition found in this study and recorded in Table II agree closely with the average values reported in the review by Oncley.

Four separately isolated samples of Sf 20 LDL lipoprotein were similarly analyzed in duplicate. In contrast to normal LDL lipoprotein, Sf 20 LDL lipoprotein apparently has a higher total lipid content, and the composition differs strikingly in terms of the greater triglyceride content and decreased cholesterol esters.

**DISCUSSION**

The ultracentrifugal survey of the lipoproteins of 50 individuals which was undertaken to determine the occurrence of Sf 20 LDL lipoprotein indicates that this lipoprotein is a common component of the lipoprotein spectrum. The finding that it is most commonly associated with increased concentrations of VLD lipoprotein is an observation consistent with the findings of previous investigators (6-9).

The isolation of Sf 20 LDL lipoprotein, as initially reported by Lindgren, Elliott, and Gofman (9) and as undertaken in this study, utilizes its unique hydrated density to separate it from other serum lipoproteins. Thus, by means of differential density flotation, it is possible to obtain an ultracentrifugally discrete lipoprotein which has the electrophoretic mobility and immunological properties of a $\beta$-lipoprotein.

The sedimentation rates of the lipoproteins isolated from the four patients are nearly identical, as are their hydrated densities, which approximate the density of serum. Thus, during the initial centrifugation at density 1.066 g per ml, this component remains distributed throughout the tube and is not removed with the VLD lipoprotein which accumulates at the meniscus. It is readily separable from the major LDL lipoprotein components which are more dense.

The possibility that Sf 20 LDL lipoprotein is an artifically produced lipoprotein resulting from the gradual decomposition...
of VLD lipoprotein during purification is considered unlikely, for when VLD lipoprotein is purified by ultracentrifugation and then studied in the analytical ultracentrifuge, there is no Sf 20 LD lipoprotein present. This suggests that VLD lipoprotein is not degraded to Sf 20 LD lipoprotein during the isolation procedure.

On the other hand, the question of whether Sf 20 LD lipoprotein actually consists of VLD lipoprotein which has simply lost some of its lipid, and thus has a slightly higher density so that it is not removed by flotation with the bulk of the VLD lipoprotein, has been more troublesome. Recently Brown, Levy, and Fredrickson (23) have demonstrated that when VLD lipoprotein delipidated by ethanol-ether was suspended in Tris-HCl buffer in the absence of sodium deoxycholate, approximately 30% of the protein dissolved. The soluble polypeptides apparently are HD apolipoprotein and two polypeptides characteristic of VLD lipoprotein, while the insoluble protein is predominantly LD apolipoprotein, and this could be solubilized upon addition of sodium deoxycholate to the solution. A sample of Sf 20 LD lipoprotein was similarly delipidated and suspended in Tris-HCl buffer without detergent. Of the 2.9 mg of apoprotein only 4% was solubilized, as measured by the Lowry reaction, in the absence of sodium deoxycholate; however, complete solubilization occurred upon the addition of detergent. This solubilized apoprotein was immunologically reactive with anti-LD but not with anti-HD lipoprotein serum (Fig. 5). It would thus appear that the apoprotein of Sf 20 LD lipoprotein consists predominantly of LD apolipoprotein and contains few, if any, of the additional readily soluble polypeptides found in VLD apolipoprotein.

On the basis of the hydrodynamic data measured in these studies it is possible to calculate a molecular weight for Sf 20 LD lipoprotein. Before considering this, however, it seems appropriate to discuss the most likely source of error in such a calculation, namely, the measured value of the buoyant density. The figure of 1.004 g per ml was obtained from a standard Sf versus density plot, the values being determined in KBr solutions. The linearity of the plot (Fig. 7) indicates, as discussed by Cox and Schumaker (24) and Hill and Cox (25), that the various parameters determining this plot do not vary as a function of salt concentration. However, by using solutions of different salts or D2O, these authors have demonstrated variations in the values of the measured buoyant densities of several proteins. They postulate that these variations reflect differences in the amounts of water preferentially bound to the hydrodynamic unit in the presence of the different salts, thus giving rise to the observed salt-dependent differences in the measured buoyant densities of these proteins.

Unfortunately, because of the insolubility of dried lipoproteins, it is impossible to measure their partial specific volume by dissolving the lipoprotein in a solvent and measuring the volume increase. The approach of measuring buoyant density in D2O has never been reported for lipoproteins, and apparently all the published figures on the buoyant densities of lipoproteins have been measured in salt solutions. If these figures prove to be erroneous, due perhaps to preferential water binding, then lipoprotein molecular weight determinations made by hydrodynamic methods will need correction. A modification of the value of the calculated molecular weights, necessitated by an improved determination of \( \bar{v} \), would probably be of a small order of magnitude, especially if the measurement of the sedimentation coefficient and the calculation of the molecular weight are made under conditions of high solvent density. For this reason measurements of the sedimentation coefficient have been made in solutions of density 1.20 g per ml, as suggested by Del Gatto, Lindgren, and Nichols (12); however, the values recorded in this study differ from theirs by being corrected for the relative viscosity of the solvent. In this way sedimentation-floatation data obtained in various laboratories, using different salts to prepare the high density solvents, may be compared. This correction is important, for the relative viscosity of concentrated salt solutions may differ considerably from one to another. In these studies KBr solutions have been used in order to minimize this viscosity correction.

Although it remains possible that the calculated molecular weight of Sf 20 LD lipoprotein may need slight modification in the future, the conclusions of this study should remain essentially unaltered.

On the basis of the measured values of \( s \) and \( \bar{v} \) and accepting the apparent sphericity of these macromolecules, as indicated by electron microscopy, one may estimate the molecular weight of Sf 20 LD lipoprotein. By assuming a spherical particle, with a calculated radius equaling

\[
\left(\frac{3M}{4\pi N}\right)^{1/3}
\]

one may obtain (26) an expression for the molecular weight as equal to

\[
\left[\frac{6\bar{v}}{1 - \bar{v}\bar{v}}\right]^{1/3} (38/4) N \bar{v}
\]

The calculated molecular weight by this method is \( 4.2 \times 10^6 \) g per mole.

Alternatively, by utilizing the buoyant density of the particles and their apparent diameter as measured in the electron micrograph, from which the volume of an assumed sphere may be calculated, an additional crude estimation of the molecular weight may be obtained. This value is \( 5.8 \times 10^6 \) g per mole. This somewhat greater value might be anticipated, for during drying on the grid it seems likely that the molecules lose their spherical shape and become somewhat flattened, with an artificial increase in diameter. The resulting value for the molecular weight is about 20% greater than that determined by hydrodynamic methods; however, it probably represents a reasonable confirmation of the \( 4.2 \times 10^6 \) figure.

\( \bar{v} \) By accentuating the difference between the buoyant density of the protein and the density of the solution in which the sedimentation coefficient is measured, the effect of an error in \( \bar{v} \) upon the term \( (1 - \bar{v}) \) is minimized.

Three salts are commonly used to adjust the density of lipoprotein solutions prior to analytical ultracentrifugation. The relative viscosities of these solutions at 25°C, as calculated from the data in the International Critical Tables, are as follows.

| Solution | Density | Relative Viscosity at 25°C |
|----------|---------|---------------------------|
| NaCl     | 1.063   | 1.16                      |
| KBr      | 1.063   | 0.977                     |
| NaBr     | 1.063   | 1.06                      |
| KBr      | 1.20    | 0.966                     |
| NaBr     | 1.20    | 1.23                      |

\( \bar{v} \) = sedimentation coefficient \( (s, \rho, 1/\eta) \), \( \eta \) = absolute viscosity of the solution, \( \bar{v} \) = partial specific volume of lipoprotein = 1/buoyant density, \( \rho \) = density of the solution, \( N \) = Avogadro's number and \( M \) = molecular weight of lipoprotein.
The molecular weight, determined from hydrodynamic measurements, of the major LD lipoprotein component isolated from normal subjects was reported by Adams and Schumaker to be 2.3 to 2.5 x 10^6 g per mole (27). They also found a hydrated density of 1.030 g per ml. It is apparent, therefore, that the Sf 20 LD lipoprotein isolated in these studies is both larger and of lighter density than the major LD lipoprotein component of normal serum. The composition studies recorded in Table II indicate that it more closely resembles LD than VLD lipoprotein in its composition; however, it differs significantly in having triglycerides rather than cholesterol esters as its major component. This finding, of course, is consistent with its lighter density. The protein content of Sf 20 LD lipoprotein, determined gravimetrically, was 18%. Reference to the data of Lindgren, et al. (28) indicates that a lipoprotein of hydrated density 1.004 g per ml should have a protein content of approximately 16 to 17%, and this value could vary slightly depending on the lipid composition.

In the present study several properties of Sf 20 LD lipoprotein have been defined, and the common occurrence of this lipoprotein has been reaffirmed, especially in association with increased concentrations of VLD lipoprotein. Further investigation of the structural relationship of this lipoprotein to other low density lipoproteins is in progress (29).

The metabolic relationships of Sf 20 LD lipoprotein are not clearly defined at present; however, the observations of Graham et al. (30), of Anfinsen, Boyle, and Brown (31), and more recently, of Shore and Shore (32) are certainly pertinent, for their evidence suggests that Sf 20 LD lipoprotein may well be a product of lipoprotein lipase-induced cleavage of VLD lipoprotein. The study of Shore and Shore reports the isolation of a lipoprotein liberated from VLD lipoprotein, and its properties resemble those of Sf 20 LD lipoprotein isolated in the present study from hyperlipemic individuals in a fasting state. These relationships need future clarification.

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