tion in calcium binding (17, 9 and 4 M per 10^5 grams, respectively, with 10 mmoles of free Ca^{++} per liter at pH 7.4). Thus, factors other than net charge also must be important. Study[4] of the viscosity of β-lactoglobulin heated with calcium chloride at various pH values suggested that certain groups ionizing in the pH range of 6 to 7.5, perhaps α-amino or histidine groups, might be involved in calcium binding. These groups, however, as a rule coördinate poorly with calcium, and carboxyl–hydroxyl (serine) chelation with calcium has been suggested[12] as specific binding sites, with net charge an important determining factor. The binding of calcium to casein measured by Carr[15] is shown in Fig. 4 for comparison with β-lactoglobulin. The pH region in which the binding is changing most rapidly is the range in which the secondary acid group of the phosphate esterified to the casein molecule is ionizing. Studies in this Laboratory (unpublished) show that the binding parallels the net charge as well.

The dialysis results in Fig. 5 show that although the calcium in the aggregate is apparently mechanically trapped, this calcium is freely dialyzable when the aggregates are dissolved. The results also illustrate the long periods of dialysis required to free a protein of ions that have an affinity for it.

The calculations shown in Fig. 6 support the conclusion that the aggregation of β-lactoglobulin in the presence of calcium is an isoelectric aggregation. Thus, isoelectric precipitation of β-lactoglobulin solutions can be obtained by lowering the pH to the isoelectric region, or at constant pH by the addition of calcium or any other cation that is bound to it to a similar degree. Actually an even better agreement than shown is likely since electrophoretic studies[20] of β-lactoglobulin in the presence of calcium have shown that the charge is not reduced to the extent expected from the calcium binding. It was surmised that simultaneously with the binding of calcium some other positive ion, perhaps hydrogen ion, might be dissociated from the molecule to some extent. These conclusions suggest that calcium ion, or any other cation that is bound, would be expected to precipitate a negatively charged protein if the protein is insoluble in dilute salt solution at its isoelectric point. Casein is insoluble at its isoelectric point in its native state and it is aggregated or precipitated by calcium ions. Myosin, too, is an example of such a protein and it too is precipitated by cations.[21] The precipitation of proteins by metal ions has recently been discussed.[12] The importance of the net charge was emphasized although with certain metals other factors are important as well.

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PHILADELPHIA, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

A Rapid Method for the Bulk Isolation of β-Lipoproteins from Human Plasma[1]

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A simple and rapid method for the concentration and isolation of β-lipoproteins from human serum or plasma is described. The method involves the use of a dextran sulfate of large molecular weight as a specific complexing agent for the lipoproteins. Dissociation of the complex by ultracentrifugation in a salt density gradient yields β-lipoproteins of varying density and of high purity. The chemical and physical properties of material prepared in this fashion from pooled normal human plasma are described in detail. The analyses of lipoprotein flotation patterns by means of a distribution function is proposed.

Introduction

The β-lipoproteins, because of their relatively low concentration in normal human plasma (*ca.* 7% of the total plasma proteins)[4] and their instability and marked tendency to undergo oxidation,[5] present special problems when attempts are made to recover them in their native state.

The lipoproteins of human plasma have been separated by differential ultracentrifugal flotation[6] without preliminary concentration but this procedure has not yielded material in sufficient amount to allow full chemical analysis and has led to their characterization mainly in terms of their ultracentrifugal flotation constants. The concentration of material effected by preliminary cold ethanol fractionation followed by ultracentrifugal flotation[7] has allowed more detailed chemical characterization of β-lipoproteins but it was suspected that the manipulations involved in the preliminary fractionation procedure might have exposed the material to some degree of oxidative change. The need has remained therefore for a method sufficiently rapid and involving sufficiently little manipulation, to allow recovery of the β-lipoproteins in a natural state and yet in sufficient yield to allow detailed chemical and physical examination.

It had been observed previously that a sulfate ester of the macromolecular polysaccharide, dextran, formed insoluble complexes with fibrinogen when added to normal human plasma under controlled conditions.[8] The present study originated

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(2) Rockefeller Travelling Fellow in Medicine, 1954–1955.

(3) Lilly Research Laboratories Fellow in the Medical Sciences; National Academy of Sciences–National Research Council, 1955–1957.

(4) J. L. Oncley and F. R. N. Gurd, "Blood Cells and Plasma Proteins: Their State in Nature," ed. J. L. Tullis, Academic Press, Inc., New York, N. Y., 1953, p. 337.

(5) B. R. Ray, E. O. Davisson and H. L. Crespi, *J. Phys. Chem.*, **58**, 841 (1954).

(6) F. T. Lindgren, H. A. Elliott and J. W. Gofman, *ibid.*, **55**, 80 (1951).

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from the observation that dextran sulfate is also capable of forming a loose complex with the β-lipoproteins in serum. When this complex was redissolved, layered into a saline density gradient tube,[9] and centrifuged at 105,000 *g*, it was found that the complex dissociated. The dextran sulfate sedimented while the β-lipoproteins distributed themselves in the upper portion of the tube as three density classes.

Experimental

Methods.—*Concentration* was determined from dry weight determinations. β-Lipoprotein solutions were dialyzed with intermittent stirring for from 3 to 4 days against a 10- to 30-fold excess of 0.15 *M* sodium chloride. Triplicate aliquots of lipoprotein solution and dialysate were dried at 76–77° and then dried *in vacuo* at 76–77° to a constant weight. A salt correction was made by subtracting the dry weight of the dialysate from the dry weight of the lipoprotein sample.

Total phosphorus was determined by the method of Lowry, *et al.*[10] Phospholipid was estimated by multiplying the total phosphorus value by the factor 25.0. Lipid nitrogen was estimated by multiplying the total phosphorus value by the factor 14/31.

Total nitrogen was determined by Nesslerization[11] or Conway diffusion.[12,13] Peptide was estimated by subtracting lipid nitrogen from total nitrogen and multiplying by the factor 6.25.

Total cholesterol was determined by the method of Abell, Levy, Brodie and Kendall.[14] Reproducibility was improved by incubating the alcoholic potassium hydroxide–lipoprotein mixture at 44° rather than 37°.

Density of various gradient layers was measured by placing small drops in a xylene–chlorobenzene gradient tube.[15,16] The anhydrous density of lipoproteins with a density less than water was calculated as the reciprocal of the apparent partial specific volume obtained from measurements with a Sprengel–Ostwald pycnometer.[17]

Dextran sulfate[18] was prepared by the method of Ricketts from a dextran derived from the fermentation of sucrose by the "β-coccus arabinosaceus (Birmingham)"[19] strain of *Leuconostoc mesenteroides*. The intrinsic viscosity of the parent dextran, as measured in a B.S.S. No. 1 viscometer in 0.15 *M* sodium chloride was $[\eta] = 0.67$. Two preparations derived from the same dextran and containing, respectively, 16.5 and 17.6% sulfur were used. Both preparations gave similar results. Dextran sulfate concentration was estimated by a modification of the McIntosh toluidine blue procedure.[20]

Electrophoretic examination on filter paper was performed by the method of Hardwicke.[21] Filter paper strips were stained with Wool Black for protein and Sudan Black for free electrolysis lipid.

Ultracentrifugal flotation analyses were made with an air-driven ultracentrifuge[22] in a sodium chloride solution with a density of 1.063 g./ml. Low density β-lipoproteins were centrifuged at 300 r.p.s. while the higher density β-lipoproteins were centrifuged at 900 r.p.s. The average temperature was about 25°. The conventional Svedberg unit, S_f, was used to signify the flotation rate.[23] A distribution function, developed by Williams and Saunders[24] for heterogeneous high polymer systems, was used to describe the flotation patterns. Here

$$g(S_f) = \chi \frac{dn}{d\chi} \times \left(\frac{\chi}{\chi_\infty}\right)^2 \times \frac{\omega^2 t}{n_1 - n_0}$$

Where

S_f = experimentally detd. flotation constant
t = time in seconds corrected for acceleration
χ = position in cell from reference bar for material of S_f at time t
$dn/d\chi$ = height at χ
$n_1 - n_0$ = area = limiting area obtained by plotting the area of various pictures *vs.* time and extrapolating to zero time

Lipoproteins were floated at concentrations of 0.5 to 1%. In this range, concentration dependence was negligible.[25]

Preparation

Concentration of Lipoproteins.—The starting material was plasma or serum pooled after collection from normal human volunteers. No attempt was made to restrict observations to samples taken from groups selected on the basis of age or sex.

Fibrinogen was removed from freshly drawn acid citrate dextrose[26] plasma or resin collected[26] plasma, either by the addition of commercial human thrombin (fraction III-2, method 9)[27] or by cold ethanol precipitation (method 6).[28] All operations were performed in the 0° cold room. Five ml. of 0.5% (w./v.) dextran sulfate solution in distilled water per 100 ml. of original plasma volume were added to the serum with stirring.[29] A cloudy lipoprotein–dextran sulfate complex formed immediately. The precipitate was allowed to stand for from 2 to 4 hours and then centrifuged at about 3,000 *g* in an International Centrifuge at 0°. The precipitate was a soft greasy orange-yellow paste. In several experiments the serum was first diluted with an equal volume of 0.15 *M* sodium chloride[30] before the addition of the dextran sulfate. The dilution appeared to effect a more complete separation of the lipoprotein.

As an alternative procedure, dextran sulfate in similar concentration was added to a lipid-rich protein fraction III prepared by a modification[31] of the cold ethanol method 10.[26] The fraction III paste was dissolved in one or two plasma

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(29) Since precipitation depends on both lipoprotein and dextran sulfate concentration the optimal dextran sulfate concentration was estimated by titrating aliquots of the sera to maximum precipitation. Precipitation was measured turbidimetrically. The lipoprotein–dextran sulfate complex redissolves in a large dextran sulfate excess. The figure given is the average value found suitable for normal serum.

(30) All solutions added in the preparation of β-lipoproteins contained 0.1 g. per liter of the disodium salt of ethylenediaminetetraacetic acid adjusted to *p*H 7.0 ± 0.2 with 1 *N* sodium hydroxide. Chelation of trace divalent metallic cations was shown to increase the stability of β-lipoproteins by Ray, *et al.*[5]

(31) D. M. Surgenor, R. B. Pennell and M. Melin, unpublished experiments.

volumes of a solution containing 0.15 *M* sodium chloride and 0.01 *M* sodium bicarbonate at *p*H 6.8 ± 0.2 and further diluted to two plasma volumes with 0.15 *M* sodium chloride. Dextran sulfate was added and the complex isolated as described. The final β-lipoprotein, isolated by the action of dextran sulfate in these various procedures, appeared to be identical.

Purification of β-Lipoproteins.—The lipoprotein–dextran sulfate paste was suspended in 5 ml. of 2.0 *M* sodium chloride per 100 ml. of original plasma volume. In each of several 13.5-ml. capacity lusteroid centrifuge tubes (Spinco Model L, Rotor No. 40) 5.0 ml. of this suspension was layered on top of 3 ml. of 2.0 *M* sodium chloride. The tube was filled by layering 0.15 *M* sodium chloride above the lipoprotein–dextran sulfate suspension and the tubes were then centrifuged with refrigeration at 105,000 *g* for 18 hours.

Following centrifugation, the material in the gradient-tube was found to have separated into visible layers which were numbered from top to bottom, I through IV. Lipoproteins were concentrated in layer I to III while non-lipid-carrying protein impurities were concentrated in layer IV. However, immunochemical studies with β-lipoprotein antisera showed some β-lipoprotein in layer IV.[32] The layers were separated with the aid of a tube-cutter[33] and analyzed for nitrogen, phosphorus and total cholesterol (Table I).

Results

Dextran sulfate of large molecular size precipitated a lipoprotein-rich fraction from serum rapidly and without requiring significant modification in *p*H, ionic strength or the dielectric constant of the medium. The total cholesterol to nitrogen ratio of a dextran sulfate precipitate from sera was 1.5, while the same ratio for a dextran sulfate precipitate from fraction III was 3.7. Fraction-III-O prepared by cold alcohol method 9 had a total cholesterol to nitrogen ratio of 1.2.[27]

Paper electrophoresis of the material initially precipitated by dextran sulfate showed two principal components, a lipid-rich (sudanophilic) β-globulin (showing some trailing) and albumin. The precipitate also contained approximately 80% of the dextran sulfate originally added to the serum. After ultracentrifugation in the salt density gradient tube, no dextran sulfate was detectable in layers I to III. Layer IV contained dextran sulfate in an amount corresponding, within the limits of experimental error, with that present in the complex before centrifugation. A sensitive biological test has been developed to assay dextran sulfate in lipoprotein solutions.[34] Since dextran sulfate shows heparin-like anticoagulant activity,[35] it markedly alters the "prothrombin time" in a one-stage prothrombin system.[36] No dextran sulfate was found in lipoprotein layers I to III by this test, although the test is sensitive to dextran sulfate added to lipoprotein solutions in concentrations as low as 0.5 μg./ml.

It thus appeared that suspension in the strong salt medium had brought about dissociation of the complex and that ultracentrifugation had allowed separation of the components on the basis of their very different densities.

Characteristics of the Lipoproteins.—Layers I to III presented marked differences in appearance. These were reflected in equally marked differences in physico-chemical characteristics.

Layer I consisted of a translucent or semi-opaque "cream" floating on the surface of the liquid in the tube. The density[37] of the salt medium in this portion of the tube was 1.022 g./ml., but this density did not correspond with that of the lipoproteins. When layer I pools (Table I) were dialyzed and refloated in the ultracentrifuge at a density near 1.005 g./ml., the pools did not show any marked change in chemical composition. The anhydrous density of layer I lipoproteins was found to be 0.98.

When examined by paper electrophoresis layer I lipoproteins often remained at the point of ap-

TABLE I

THE PERCENTAGE COMPOSITION OF β-LIPOPROTEINS

Peptide	Phospholipid	Total cholesterol	Cholesterol phospholipid
Layer I		*Sf* 10–100	
9.1[a]	20.9	16.2	0.77
9.3	19.3	14.2	.73
11.6	19.6	13.7	.70
8.9	15.1	17.9	1.19
10.6	18.5	14.3	0.77
10.9	20.4	16.6	.81
10.5[a]	20.8	17.8	.85
9.7[a]	19.1	15.1	.79
9.6[a]	17.6	17.9	1.02
10.0[c]	19.0[c]	16.0[c]	0.85[c]
Layer II		*Sf* 5–15	
19.6[b]	26.1	32.6	1.25
21.0	24.6	29.7	1.21
20.5	21.7	27.6	1.27
16.5	23.4	25.2	1.08
14.1	23.3	27.7	1.19
18.3[c]	23.8[c]	28.6[c]	1.20[c]
Layer III		*Sf* 3–9	
22.4	25.1	34.1	1.36
21.6	22.0	29.8	1.35
20.1	21.1	30.0	1.42
21.9	22.0	30.0	1.36
21.6	23.1	31.0	1.34
23.9	22.5	31.5	1.40
20.2	21.1	28.7	1.36
23.6	22.3	31.5	1.41
21.9[c]	22.4[c]	30.8[c]	1.38[c]
Layer III-A		*Sf* 6–10	
17.6	24.0	33.3	1.39
19.1	23.6	35.2	1.49
18.4[c]	23.8[c]	34.3[c]	1.44[c]
Layer III-B		*Sf* 4–8	
20.6	22.7	34.2	1.51
21.2	25.2	34.9	1.39
20.9[c]	24.0[c]	34.6[c]	1.45[c]
Layer III-C		*Sf* 3–6	
22.6	21.9	31.5	1.44
24.6	22.6	33.3	1.48
23.6[c]	22.3[c]	32.4[c]	1.46[c]

[a] Layer re-floated at a density near 1.05 g./ml. [b] Layer sedimented at a density near 1.05 g./ml. [c] Average values.

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(37) The average density of a gradient tube section at a temperature of about 15°. The boundaries of a lipoprotein class within the gradient tube are not well defined. It is difficult both to establish the position of greatest lipoprotein concentration within a layer and to be certain that the distribution of lipoprotein above and below the layer is uniform. The gradient tube section has an average density near but not necessarily equivalent to the average density of the lipoprotein class.

plication, giving a faint protein stain with Wool Black but an intense lipid stain with Sudan Black. In some samples the material migrated as a β-globulin but showed heavy lipid "trailing" from the origin to the front. With starch block electrophoresis, iodinated layer I lipoproteins migrated as a distinct band with the mobility of an α_2-globulin.[32] The layer I lipoprotein band was distinct from the α_1-lipoprotein band. In this layer, lipoproteins are distributed over the S_f 10–100 range; however, a typical distribution function (Fig. 1) shows the predominant molecular species to lie between S_f 20–50.

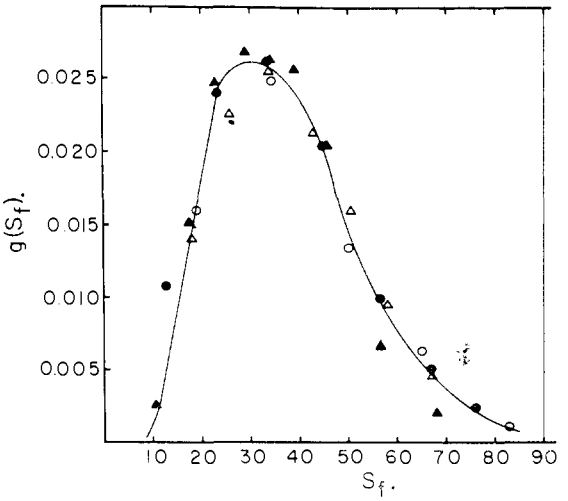

Fig. 1.—$g(S_f)$ *vs.* S_f distribution curve for S_f 10–100 lipoproteins (layer I): O, 20 min.; ●, 30 min.; △, 40 min.; ▲, 60 min.

Density, chemical composition and the cholesterol/phospholipid ratio all corresponded with β-lipoproteins in the S_f 10–100 range. For example, Havel, Eder and Bragdon[38] and Lindgren, Nichols and Freeman,[39] on analysis of lipoproteins with densities less than 1.019 and 1.006 g./ml., respectively, obtained by other preparative methods, found values corresponding closely with those shown in Table I for the material in Layer I.

Layer II consisted of a slightly pigmented (yellowish) transparent zone with an average density[37] of 1.026 g./ml. Paper electrophoresis showed the material to consist of a lipid-rich (sudanophilic) β-globulin. The lipoprotein concentration of this layer was consistently low. The material was of lower density and correspondingly higher flotation rate distribution, S_f 5–15, than the main bulk of the lipoproteins which occurred in layer III (Figs. 2 and 3). The chemical composition resembled that of the material in layer III, but the total cholesterol/phospholipid ratio was significantly lower (Table I). It is probable that the chemical composition reported for this layer is influenced by contamination from layer I and layer III lipoproteins.

Layer III consisted of deeply-pigmented orange-yellow material in clear solution. The average density[37] of this layer was near 1.038 g./ml. The lipoprotein concentration in this layer was high and comprised the major portion of the material derived from the original complex. Paper electrophoresis showed a single sudanophilic β-globulin. Examination by Tiselius electrophoresis showed a single peak (Fig. 4) with the mobility of a β-globulin.[40] Iodinated Layer III lipoproteins migrated as α_2-globulins on starch block electrophoresis. Samples examined in the analytical ultracentrifuge had flotation distributions in the S_f 3–9 range (Fig. 3). The density, electrophoretic mobility, flotation distribution, chemical composition and total cholesterol/phospholipid ratio of this material are all in general agreement with

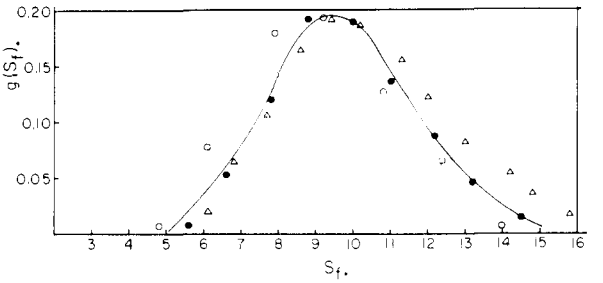

Fig. 2.—$g(S_f)$ *vs.* S_f distribution curve for S_f 5–15 lipoproteins (layer II): O, 20 min.; ●, 30 min.; △, 40 min.

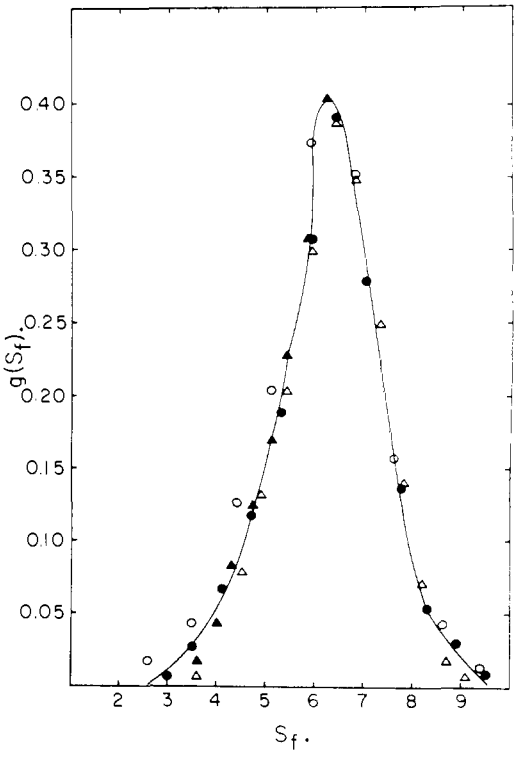

Fig. 3.—$g(S_f)$ *vs.* S_f distribution curve for S_f 3–9 lipoproteins (layer III): O, 40 min.; ●, 60 min.; △, 80 min.; ▲, 100 min.

(38) R. J. Havel, H. A. Eder and J. H. Bragdon, *J. Clin. Invest.*, **34**, 1345 (1955).

(39) F. T. Lindgren, A. V. Nichols and N. K. Freeman, *J. Phys. Chem.*, **59**, 930 (1955).

(40) S. H. Armstrong, Jr., M. J. E. Budka and K. C. Morrison, THIS JOURNAL, **69**, 416 (1947).

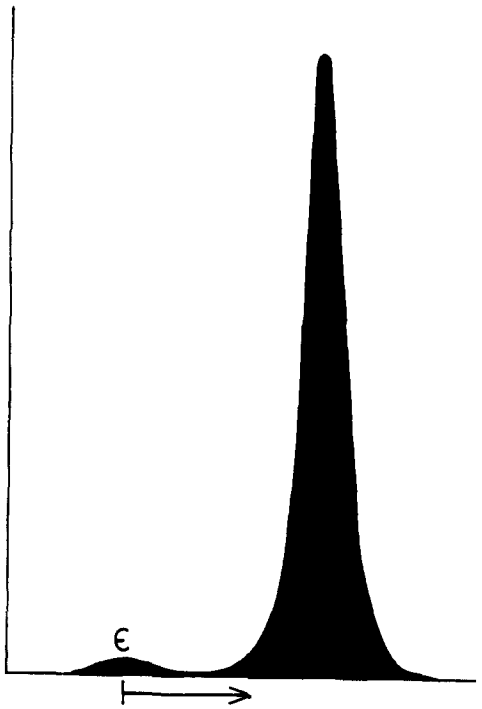

Fig. 4.—Electrophoresis of S_f 3–9 lipoproteins (layer III). Descending boundary, $\mu = -3.2 \times 10^{-5}$, *p*H 8.6, sodium diethylbarbiturate, $T/2 = 0.1$.

similar values obtained by other workers for the major β-lipoprotein species of plasma.[4,6, 38–43]

In spite of the apparent electrophoretic homogeneity of layer III lipoproteins, the flotation distribution, boundary spreading during flotation and variation in chemical composition, all suggested a spectrum of closely related lipoproteins. For this reason, two layer III pools were recentrifuged in the sodium chloride gradient tube and then separated arbitrarily into three subfractions. These subfractions *III*-A, *III*-B and *III*-C had average densities[37] of 1.027, 1.038 and 1.047 g./ml., and contained approximately 15, 60 and 25% of the original layer III lipoprotein.

The flotation distribution (Fig. 5) of these subfractions demonstrated the heterogeneity of the β-lipoproteins in the S_f 3–9 range. As the lipoproteins increase in density, A through C, their distribution functions are shifted to a lower S_f range. The chemical analysis (Table I) of subfractions A, B and C does not indicate any major differences in composition, although the peptide content increases with increasing density.

That portion of the lipoprotein molecule unaccounted for as peptide, phospholipid, free cholesterol and cholesterol ester may be assumed to be largely triglyceride. Using a ratio for free/total cholesterol of 0.27[4] to estimate cholesterol ester and with the further assumption that the ester was cholesterol oleate, a very approximate triglyceride content may be estimated by difference from the percentage composition data in Table I. The average values obtained for layer I (47.0%), layer II (14.9%) and layer III (9.3%) agree with the triglyceride distribution found for lipoproteins of corresponding sedimentation classes by Lindgren, *et al.*,[39] in their infrared studies. The increasing percentage of triglyceride found in the lipoproteins as one progresses from layer III to layer I is consistent with the accompanying decrease in density and change in flotation distribution.

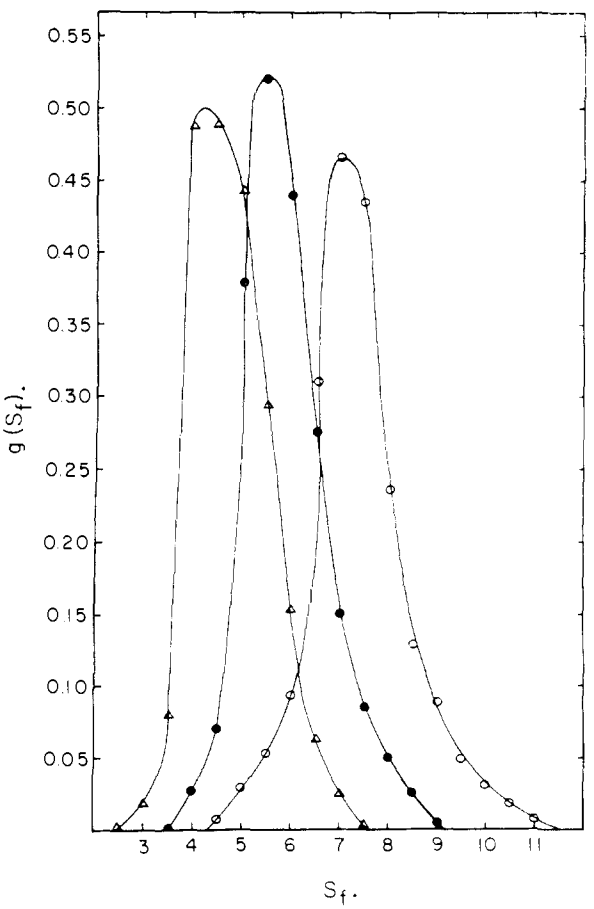

Fig. 5.—$g(S_f)$ *vs.* S_f distribution curves for S_f 6–10 (layer III-A), S_f 4–8 (layer III-B), S_f 3–6 (layer III-C) lipoproteins: ○, S_f 6–10; ●, S_f 4–8; △, S_f 3–6.

The bright orange-yellow color of freshly-isolated β-lipoprotein is due to carotenoids. The spectrophotometric absorption curve of several preparations prepared by the present procedure closely resembled that published by Oncley, *et al.*,[7] except that the extinction coefficient at the absorption maximum (460 mμ) was greater. Correspondingly, the material isolated by the present procedure was noted to be more deeply pigmented than samples obtained by the previous cold ethanol fractionation technique. This observation suggested that the rapidity and relative simplicity of the present procedure allowed isolation of the material with minimal oxidative degradation, since autoxidation is the most likely cause of carotenoid decomposition.[4] The carotenoids present in layer

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III were found to be β-carotene, lutein and lycopene.[44]

No attempt was made to estimate the total lipoprotein recovery by the dextran sulfate procedure. However, it was found that dextran sulfate precipitates material containing from 50–70% of the plasma cholesterol (the range found for the cholesterol distribution in β-lipoproteins by cold ethanol method 10[26]). The concentration of β-lipoproteins in the plasma of normal adults varies over a wide range. When lipoprotein concentrations were estimated from cholesterol distribution studies[9,38,43–46] and the percentage composition data of the present investigation, the average β-lipoprotein concentration of plasma was found to be 520 mg./100 ml. (470–570 mg./100 ml.); of this, 25 to 35% (130 to 180 mg./100 ml.) was in layer I, 5 to 10% (25 to 50 mg./100 ml.) in layer II, and 55 to 65% (290 to 340 mg./100 ml.) in layer III.

Stability of β-Lipoprotein.—The changes reported to occur with the aging of β-lipoproteins[4] (*i.e.*, decrease in optical density at wave lengths from 410 to 540 mμ; increase in optical density from 240 to 410 mμ; decrease in flotation rate and increased boundary spreading; increase in electrophoretic mobility) were confirmed in the present study. These changes occurred more slowly in material stored as a concentrated solution, or as a paste, at 0°.

The destruction of β-lipoprotein solubility by lyophilization was also confirmed. It was found that considerable stability was conferred by lyophilization in the presence of 10% sucrose, a method advocated by Keltz and Lovelock.[47]

Discussion

The lipoproteins isolated by the dextran sulfate procedure are closely related in structure, solubility antigenic specificity and metabolism, and have been classified as β-lipoproteins. Nevertheless their heterogeneity in chemical composition, density and their flotation rate distribution, makes it evident that this class of proteins, even within a narrow density range, consists of a continuum of closely similar compounds rather than a discrete entity of invariable composition. Lipoproteins are not distributed uniformly throughout this continuum, but are concentrated in two regions, S_f 3–9 and S_f 10–100. It has been suggested[48] that the lipoprotein distribution found in plasma represents the sequence of molecules in the metabolic chain which accomplishes lipid transport, and that components in the high S_f range are progressively transformed into those of lower S_f classes. Experiments with iodinated lipoproteins indicate that lipoproteins in the S_f 10–100 distribution range are converted by an unidirectional process to lipoproteins in the S_f 3–9 distribution range.[45,49] Immunochemical studies show that the β-lipoproteins all have similar peptide moieties.[49]

The mechanism of interaction between the large molecular weight dextran sulfates and β-lipoproteins is not understood at present. It should be stressed that dextran sulfate introduced as a clinical anticoagulant,[50] and which is of considerably lower molecular weight, has been found to be ineffective in producing the type of separation described here. For example, a dextran sulfate prepared from low molecular weight dextran (intrinsic viscosity $[\eta] = 0.04$), but containing the same proportion of sulfate groups, did not precipitate β-lipoproteins from serum. Complexes between many sulfated polysaccharides and β-lipoproteins which change both electrophoretic mobility and solubility have been reported.[51,52] Since high molecular weight dextran sulfates precipitate fibrinogen and the β-lipoproteins, while low molecular weight dextran sulfates form soluble complexes, it appears that size is one determining factor in the solubility of the complex. Recent investigations by Bernfeld, Donahue and Berkowitz[53] suggest that the structure of the polysaccharide is closely related to the properties of the complex formed.

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