Title

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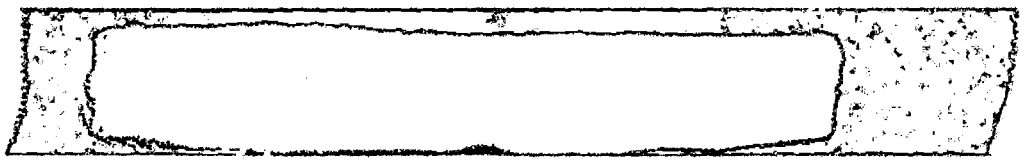

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Abstract

A determination of the molecular weight of the S_f^0 6-8 class human serum lipoprotein was carried out by direct particle count in the electron microscope. The methods for the isolation and viewing of the lipoprotein are described.

The results suggest that a dimerization occurs during the procedures used in preparation for electron microscopy.

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Introduction

The physical properties of the serum lipoprotein macromolecules have been studied by a variety of tools including the ultracentrifuge,[1,2,3] electrophoresis,[4] light scattering,[5] and electron microscope.[6] The particles as visualized in the electron microscope agreed in general with the predicted particle size of the centrifugally isolated fractions, but certain anomalies were obvious. Light-scattering data[5] had indicated a molecular weight of 3×10^6 molecular weight units and the shape of a prolate ellipsoid with axial ratio 2:1 and dimensions of 350A by 160A. These values agreed very well with ultracentrifugal data. The particles as visualized in the electron microscope[6] appeared to be oblate ellipsoids, with a diameter of 350A, flattened to a height of about 175A (see Fig. 1). From volume and density measurements the molecular weight was estimated to be about 6×10^6. One explanation of these results was that the particle seen in the electron microscope was a dimer of the molecule studied by light scattering and the ultracentrifuge.[7]

A method for determining macromolecular weights by direct particle counting was first developed by Williams and Backus,[8] and has been used extensively for quantitative electron microscopy.[9,10] This work was an attempt to apply this method to the serum lipoprotein macromolecules.

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Methods

The low-density serum lipoproteins consist of a whole spectrum of closely similar and related macromolecules. It would be desirable, therefore, in any molecular-weight determination of certain of the low-density lipoproteins by electron microscope, to obtain as homogeneous a preparation as possible.

For this purpose, a method of isolation yielding a very narrow S_f^0 6-8 lipoprotein band utilizing a sedimentation equilibrium salt gradient has been employed. First, the broad class of low-density lipoproteins was isolated from a single serum specimen by preparative flotation in a medium of ρ_{20} 1.046 g/ml.[11] This density was achieved by adding solid anhydrous NaBr to the serum. Following preparative ultracentrifugation in the Spinco 40.3 rotor at 40,000 rpm and 18°C for 24 hours, a top fraction of background density 1.042 g/ml was collected which contained most of the S_f^0 6 and higher S_f^0 serum lipoproteins originally present in the serum. This drop in density from 1.046 to 1.042 in the resulting top fraction reflects the shift toward equilibrium of the salts (mostly NaBr) present in the initial serum mixture. Approximately half of this top fraction was diluted with mock serum (NaCl solution of density 1.006 g/ml) to a final small-molecule density (exclusive of lipoproteins) of 1.031 g/ml. Three-ml aliquots of this 1.031-g/ml fraction were placed in 6-ml preparative tubes, and 3 ml of the more dense 1.042-g/ml fraction was layered in the bottom of each of the preparative tubes with a syringe and a blunted spinal needle. Following ultracentrifugation for 63 hours, sedimentation equilibrium of both the salts present as well as of the lipoprotein macromolecules had been closely approached. Initially, by layering and slight agitation, a salt gradient approximating the equilibrium salt gradient was established in the middle region of the preparative tube before the ultracentrifuge was started. After the conclusion of the 63-hour run successive fractions were pipetted from each of the preparative tubes. The fraction used for this study was the third milliliter (from the top), which corresponded to a density region on the equilibrium salt gradient of from approximately 1.033 g/ml to 1.035 g/ml. Thus, the isolated lipoprotein fraction represented a narrow band of physically similar lipoproteins of hydrated density closely approximating this narrow density range. Lipoproteins in the broad band of S_f^0 2-20

exhibit a range of estimated hydrated density of from 1.050 g/ml to 1.006 g/ml. By comparison, ultracentrifugal analysis of this narrow lipoprotein band revealed that it consisted of S_f^0 6-8 class lipoproteins (see Fig. 2).

The lipoprotein macromolecules were fixed by adding 0.1 ml of the lipoprotein solution to 0.9 ml of buffered (pH 7.3) 1% osmium tetroxide and allowing to stand at room temperature for 1/2 hour. The stained material was then diluted 1:100 in distilled water. The solution that was sprayed contained human serum albumin (0.1%), 2640A-diameter polystyrene latex (3.4×10^{10} particles per ml), and stained lipoprotein (approximately 2×10^{-6} g/ml.) Spraying was done with a DeVilbiss hand nebulizer, onto screens coated with Parlodion or Formvar, which were then shadowed with uranium or platinum-palladium-gold alloy. Entire drops were photographed in an RCA-2 electron microscope, and the number of polystyrene and lipoprotein particles in each drop was determined. The concentration of lipoprotein in the original solution was determined by ultracentrifugal analysis.

To determine the lipoprotein molecular weight, the volume of a drop is calculated by counting the number of polystyrene latex spheres present in the drop. This volume is used to determine the weight of lipoprotein present in the drop from the known lipoprotein W/V concentration. The weight of lipoprotein present is then divided by the number of lipoprotein particles present to give the weight per particle, and this is converted to molecular weight.

Results and Discussion

A typical drop pattern in shown in Fig. 3 along with an enlarged inset. The large polystyrene particles and the smaller lipoprotein macromolecules can be seen. The edge of the drop is outlined by the human serum albumin that was included in the sprayed mixture.

Table I is a compilation of the particle counts obtained. The average value for the molecular weight is 6.9×10^6. This value would tend to support the hypothesis that the particle seen in the electron microscope is a dimer or higher-multiple unit of the macromolecule observed in the ultracentrifuge and light scattering.

The reason for this apparent dimerization is not known, but it seems probable that it takes place either during fixation or during drying of the specimen for the electron microscope. Similar instances of specific aggregation prior to viewing in the electron microscope have been noted in other macromolecules.[12] Research is in progress to determine the properties of this aggregation and its relationship to lipoprotein structure.

Table I

Particle counts obtained

S_f^0 6-8 (g/ml)	2640-A PSL particles 1 ml	Number of drops counted	Total PSL counted	Total S_f^0 6-8 counted	Molecular weight
2.21×10^{-6}	3.4×10^{10}	16	577	3501	6.5×10^6
2.88×10^{-6}	3.4×10^{10}	8	572	3146	8.3×10^6
2.88×10^{-6}	4.0×10^{10}	5	198	1372	6.0×10^6

FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Human serum lipoprotein, S_f^0 6-8. Fixed in 1% buffered O_sO_4. 880-A-diameter polystyrene latex (PSL) marker molecules. x 54,230.

Fig. 2. Analytic flotation of S_f^0 6-8 low-density serum lipoproteins. Schlieren patterns were obtained at 14 minutes after rotor reached full speed (52,640 rpm) in a Spinco Model E ultracentrifuge. Flotation proceeds from left to right and occurs here in 0.195 M NaCl solutions to which has been added solid NaBr to yield resultant solution densities (26°C) of 1.057 g/ml, 1.077 g/ml, and 1.192 g/ml for the top, middle, and bottom patterns, respectively.

Fig. 3. Spray drop pattern. S_f^0 6-8 human serum lipoprotein. 2640-A-diameter polystyrene latex marker molecules. x 7,800. Enlarged inset x 13,500.

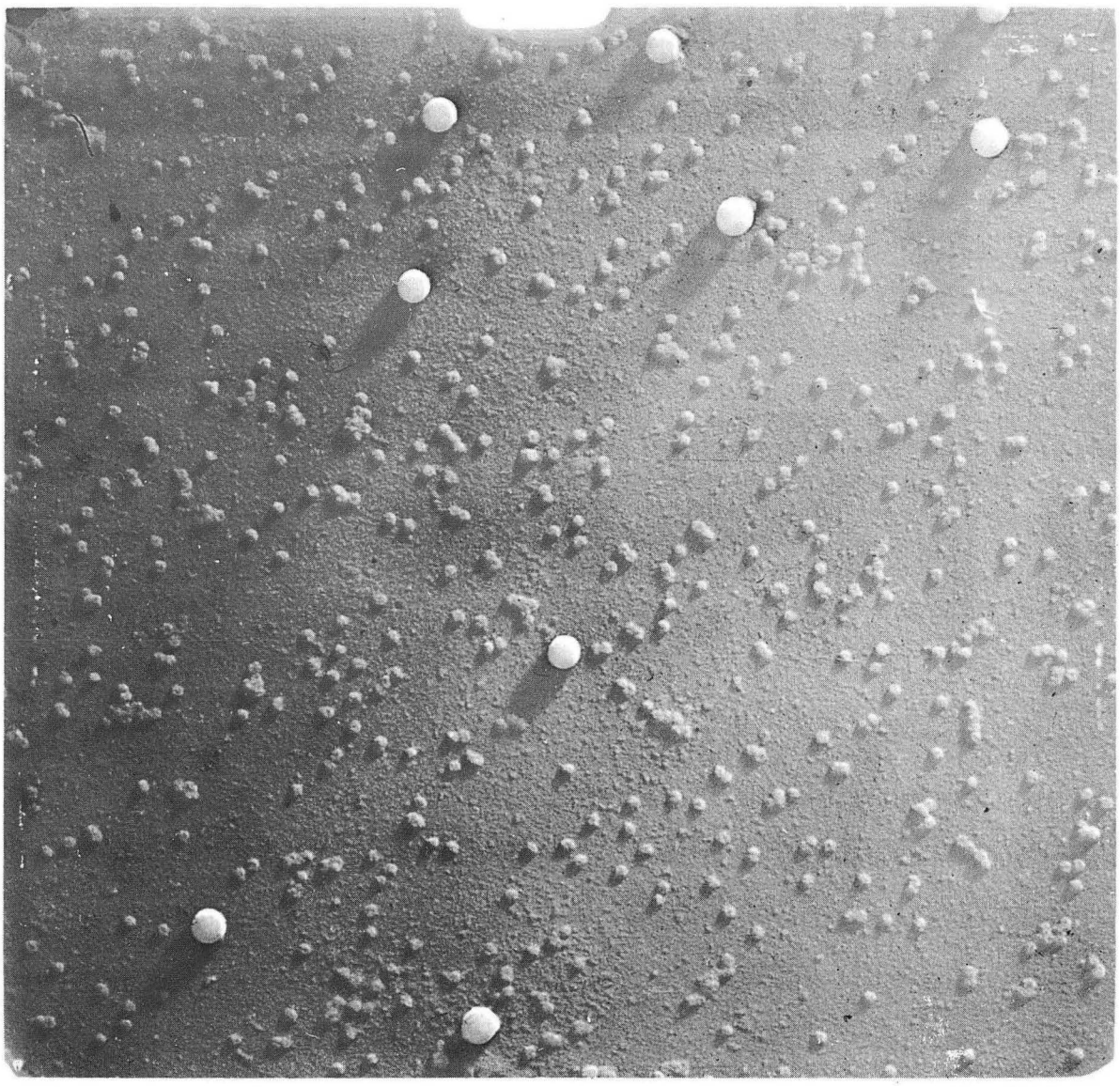

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Fig. 1.

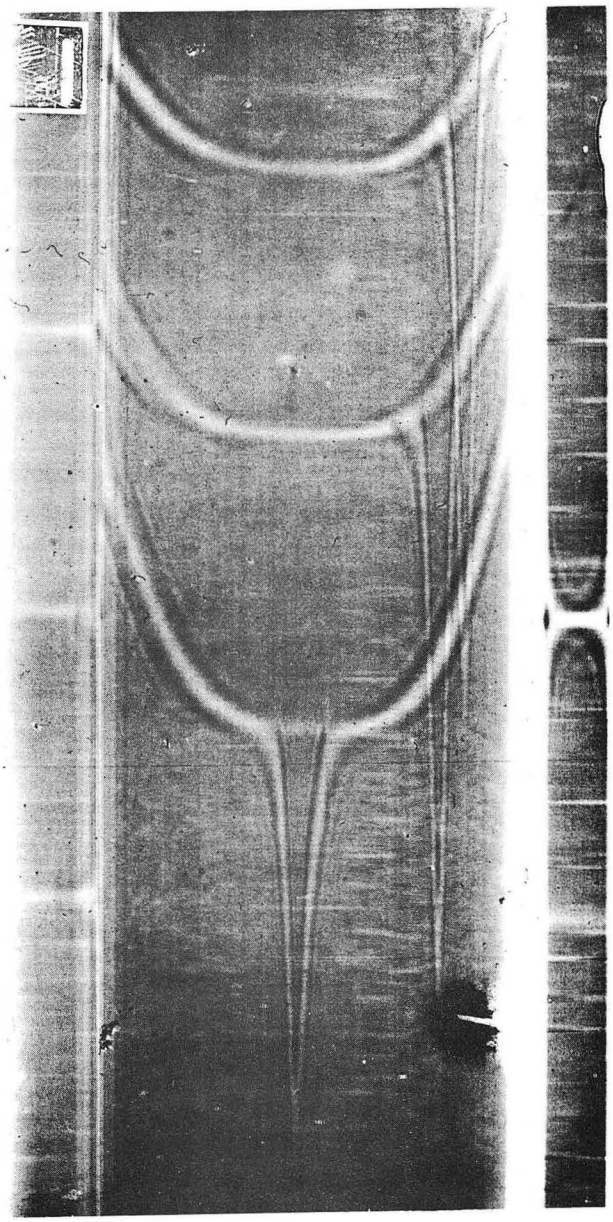

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Fig. 2.

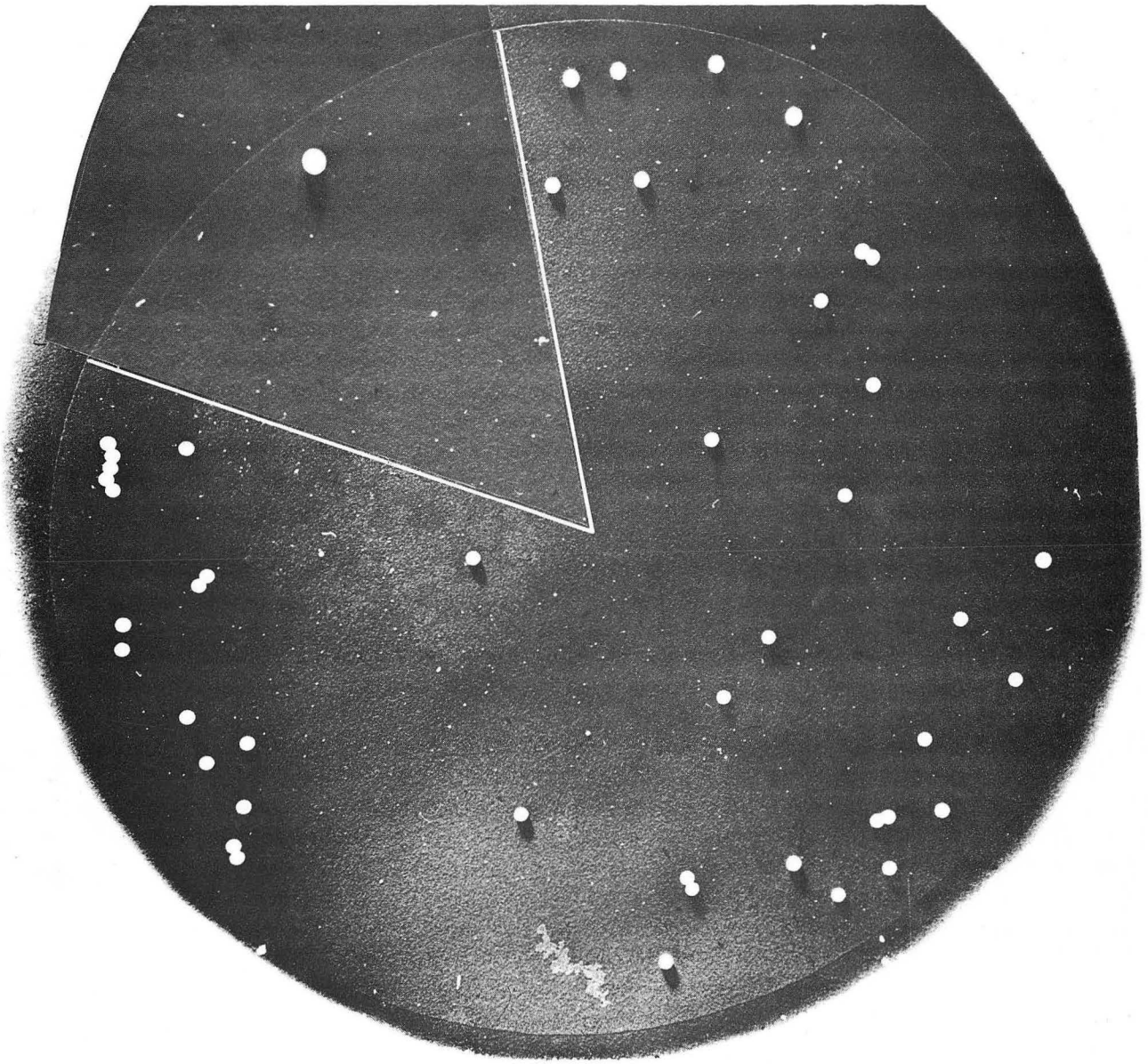

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Fig. 3.