Title

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Publication Date

1957-02-12

UCRL 3681

UNIVERSITY OF
CALIFORNIA

Radiation
Laboratory

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Printed for the U.S. Atomic Energy Commission

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ABSTRACT

Visualization of individual serum lipoprotein molecules has been accomplished by use of the electron microscope. A description of the ultracentrifugal and electron-microscopic technique is given as well as data on molecular dimensions.

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INTRODUCTION

One of the most promising studies of biological macromolecules has been the study of serum lipoproteins carried on by Gofman and associates in this laboratory. Highly significant correlations have been shown to exist between certain classes of serum lipoproteins as characterized in the ultracentrifuge and several disease states, notably atherosclerosis.[1,2] Whole-body x-irradiation has also been shown to profoundly influence lipoprotein metabolism.[3]

These lipoprotein macromolecules are large enough to be clearly resolvable in the electron microscope, and their direct visualization would be of great help in the evaluation of molecular dimensions and in the study of the interaction of these macromolecules with biochemical substances such as clearing factor.

A study of the serum lipoproteins by means of the electron microscope was therefore undertaken.

METHODS

Preparation of Serum Lipoproteins

The lipoprotein fractions were isolated by use of a preparative ultracentrifuge (Spinco Model L) and characterized by their flotation rate (S_f^0 rate) in an analytic centrifuge (Spinco Model E). Three classes of human serum lipoproteins were studied:

1. Lipoprotein material of density less than 1.007 g/ml (Gofman low-density lipoprotein of $S_f^0 17$ and greater). This material was prepared by spinning unaltered serum for 24 hours at 30,000 rpm in a preparative ultracentrifuge. The top 1 ml is then pipetted off, and contains all the lipoprotein material of density 1.007 g/ml or less. This class of lipoproteins is characterized by a flotation rate of $S_f^0 17$ or greater in a solution of density 1.063 g/ml.

2. Lipoprotein material of density between 1.007 g/ml and 1.063 g/ml (Gofman low-density L P $S_f^0 6$). Serum that was freed of material of density less than 1.007 g/ml by the above procedure was raised to a density of 1.063 g/ml by the addition of a NaCl solution. The 1.063 solution was now spun at 30,000 rpm for 13 hours in a preparative ultracentrifuge, and the top 1 ml containing material of density less than 1.063 g/ml was pipetted off. This material is characterized by a flotation rate in a solution of density 1.063 g/ml between $S_f^0 4$ and $S_f^0 17$, with the majority of molecules in the $S_f^0 4$ to $S_f^0 8$ range.

3. Lipoprotein material of density 1.20 g/ml and less (Gofman high-density lipoprotein HDL-2 and HDL-3 and low density lipoprotein). Serum is raised to a density of 1.20 g/ml by the addition of a NaCl-D_2O solution. This mixture is now spun at 40,000 rpm for 24 hours in a preparative ultracentrifuge, and the top 1 ml containing the high-density lipoproteins is pipetted off.

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[1] O. DeLalla, and J. W. Gofman Ultracentrifugal Analysis of Serum Lipoproteins, in Methods of Biochemical Analysis (Interscience, New York, 1954), Vol. 1, 459.

[2] Gofman, Glazier, Tamplin, Strisower, and DeLalla, Physiol. Rev. 34, 589 (1954).

[3] John E. Hewitt and Thomas L. Hayes, Am. J. Physiol. 185, 257 (1956).

Electron Microscopy

The lipoprotein fractions were fixed with a buffered osmic acid solution;[4] 0.02 ml of the lipoprotein solution as it was pipetted from the preparative tube was placed in 1 ml of buffered 1% osmic acid. After standing at room temperature for 24 hours the lipoprotein solution was further diluted with distilled water until a concentration of approximately 5 mg% of lipoprotein was reached. A small drop of this solution was then placed on a previously prepared parlodion membrance and the drop immediately taken up with a corner of an absorbent tissue (Baywipe). The specimen was then shadowed by means of a platinum-palladium-gold alloy (Usine Genevoise De Degrossisage D'Or, Geneva, Switzerland).

Electron micrographs were taken on an RCA EMU-2-E electron microscope.

Results

Electron micrographs of the three lipoprotein fractions described above are shown in Figs. 1, 2, and 3.

Figure 1 shows lipoprotein molecules of density less than 1.007 g/ml ($S_f^0 17$ and greater). The molecules appear to be spheres with diameters ranging from 320 Å to 800 Å.

Figure 2 shows lipoprotein molecules of density from 1.007 g/ml to 1.063 g/ml ($S_f^0 6$). These molecules also appear to be spherical and of a fairly uniform diameter. Average diameter is 350 Å (standard deviation ± 25 Å).

Figure 3 shows the high-density lipoprotein with densities of 1.20 g/ml and less. The HDL-2 and HDL-3 molecules appear to be spherical with an average diameter of 150 Å (standard deviation ± 25Å).

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[4] M.G. Farquhar, Lab. Investigation 5, 317 (1956).

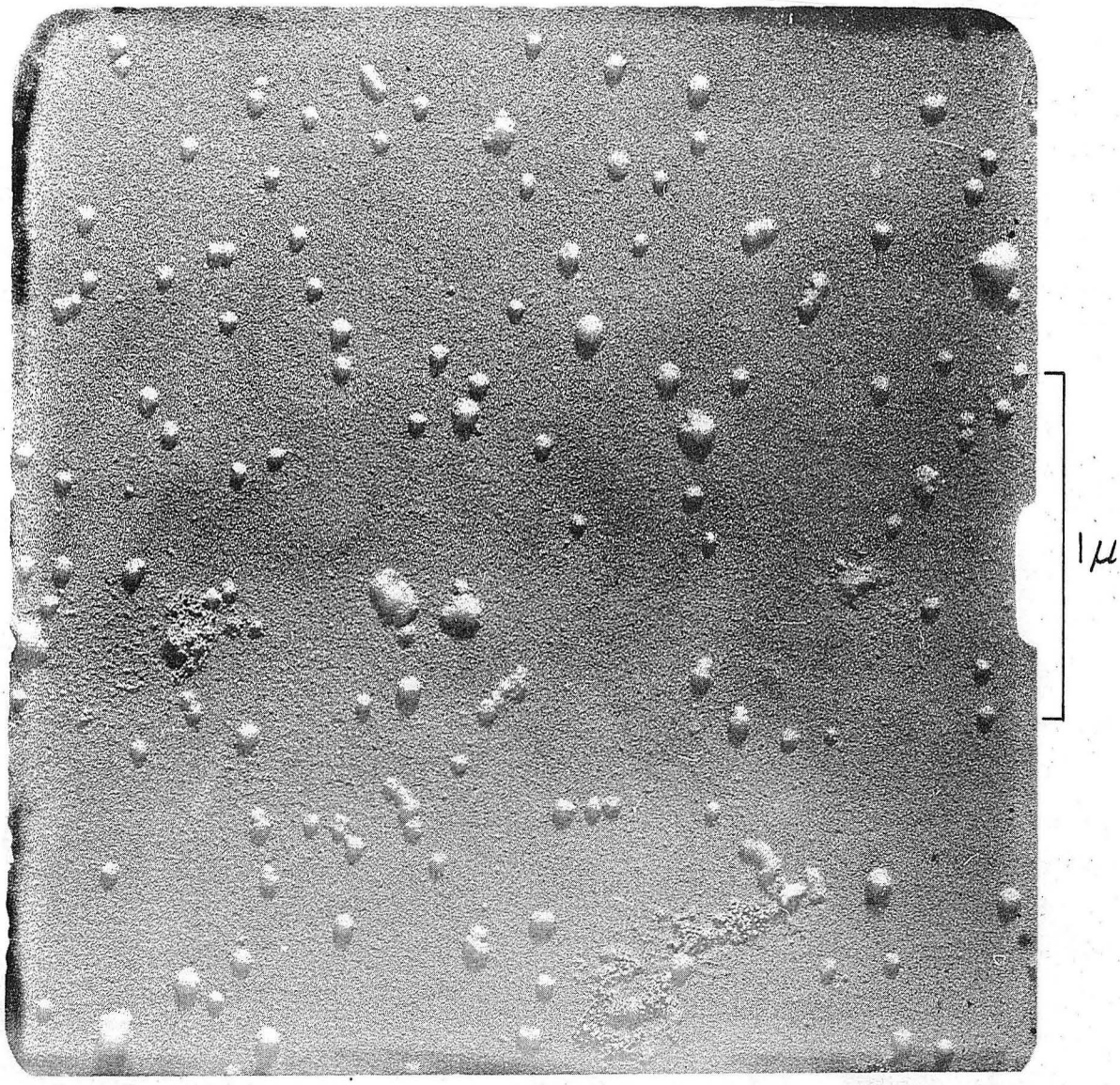
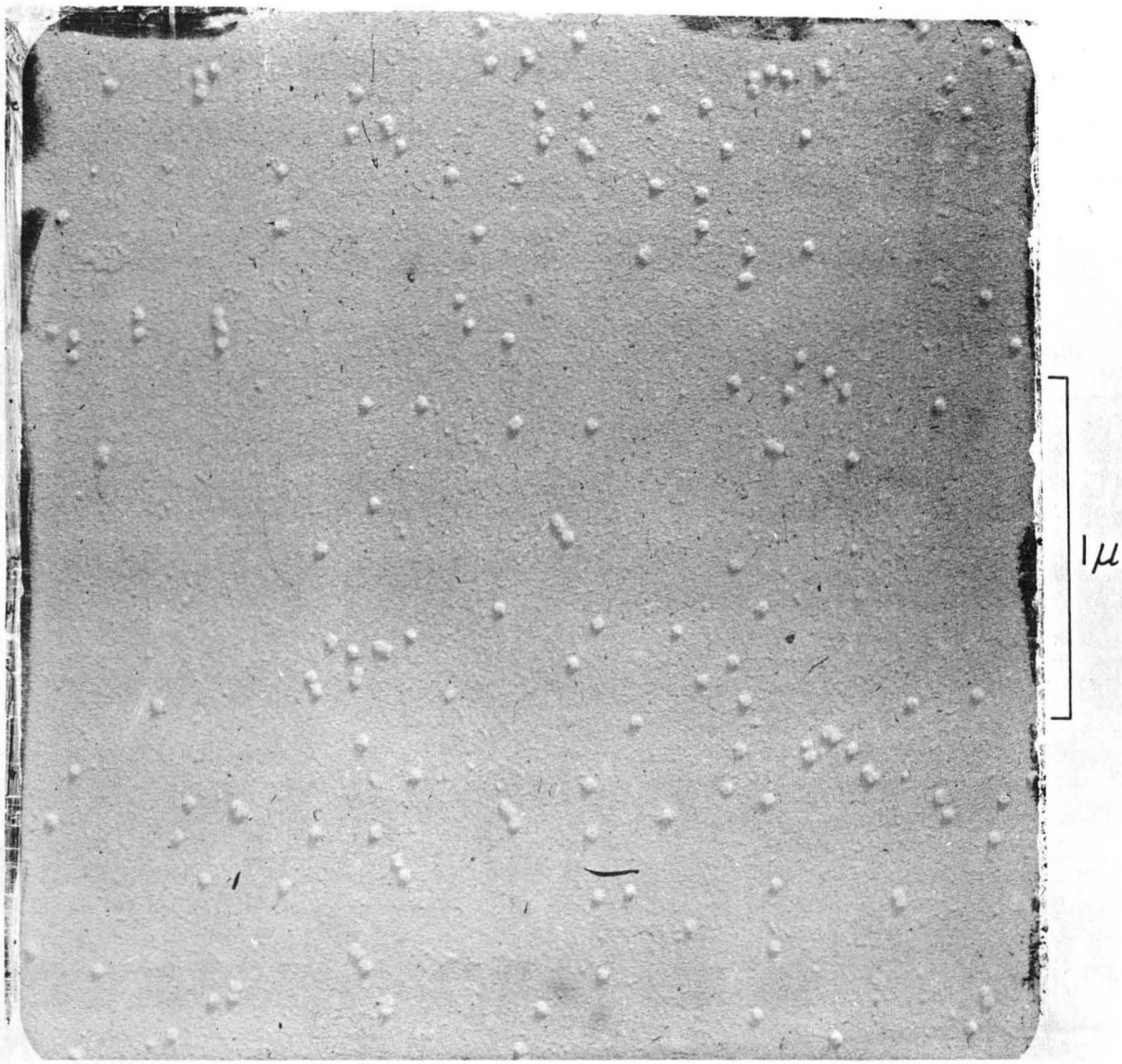
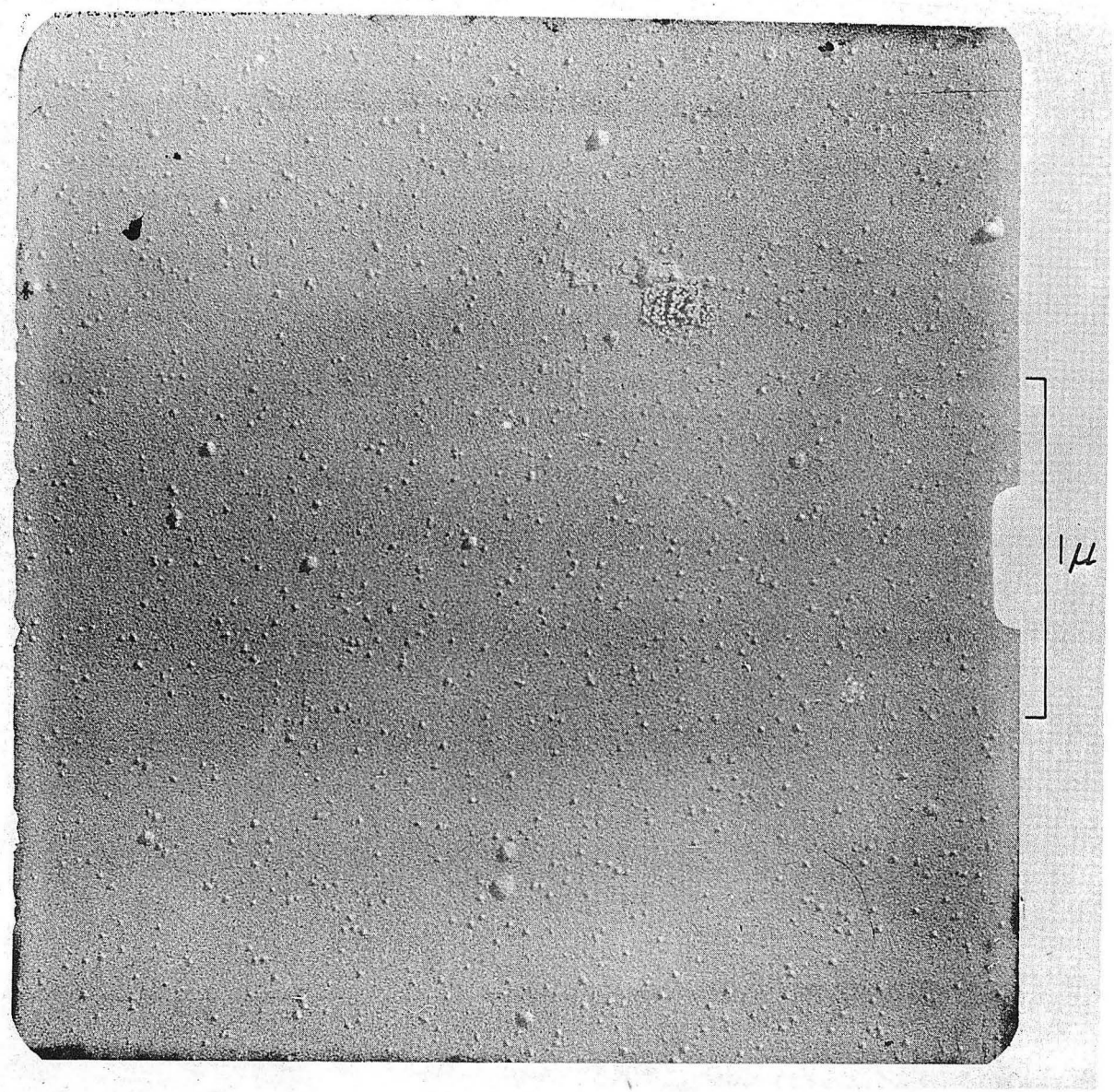


Fig. 1. Lipoprotein molecules of density less than 1.007 g/ml (S_f^0 17 and greater). x 63,000.



ZN-1677

Fig. 2. Lipoprotein molecules of density from 1.007 g/ml to 1.063 g/ml (S_f^0 6). x 63,000.



ZN-1678

Fig. 3. Lipoprotein molecules of density less than 1.20 g/ml (HDL-2 and HDL-3). x 63,000.

Because in some of the previous attempts at electron microscopy of lipoproteins unfixed material had been used, a fraction of density less than 1.063 g/ml was prepared without the fixation in osmic acid. Figure 4 shows that without fixation the lipoprotein molecules no longer show a spherical shape but have been reduced to a ringlike structure. The one solid particle is a polystyrene latex (880 Å). This would seem most likely to be due to the loss of a more volatile fraction of the molecule during the high-vacuum procedures necessary for viewing in the electron microscope. Osmic acid fixation apparently prevents this effect.

Finally, a synthetic emulsion (Lipomul, Upjohn) was fixed with osmic acid and observed in the electron microscope. One of the lipid particles is shown in Fig. 5. The size of these particles (approximately 0.5μ) agreed very well with that stated by the manufacturer.

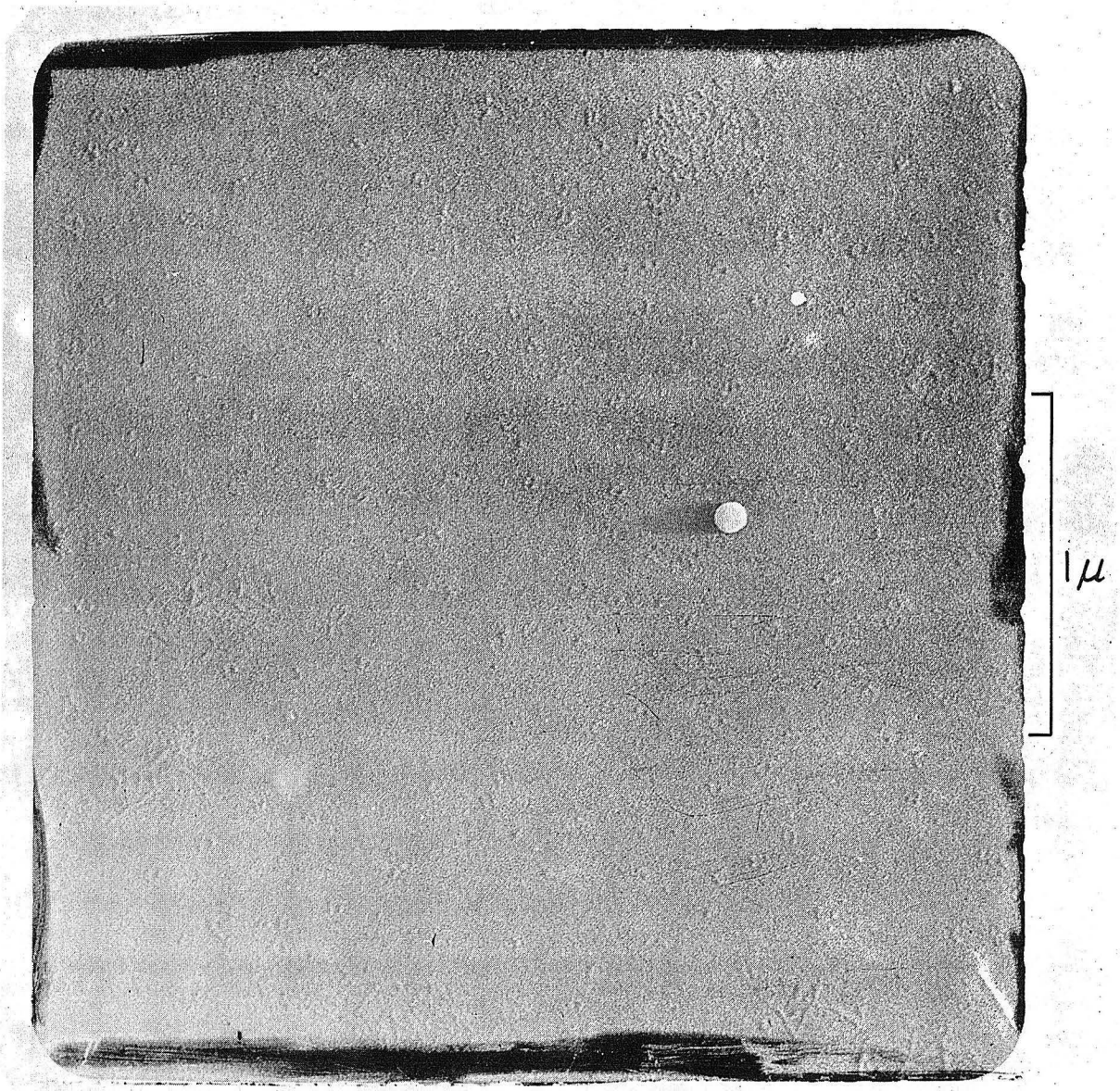
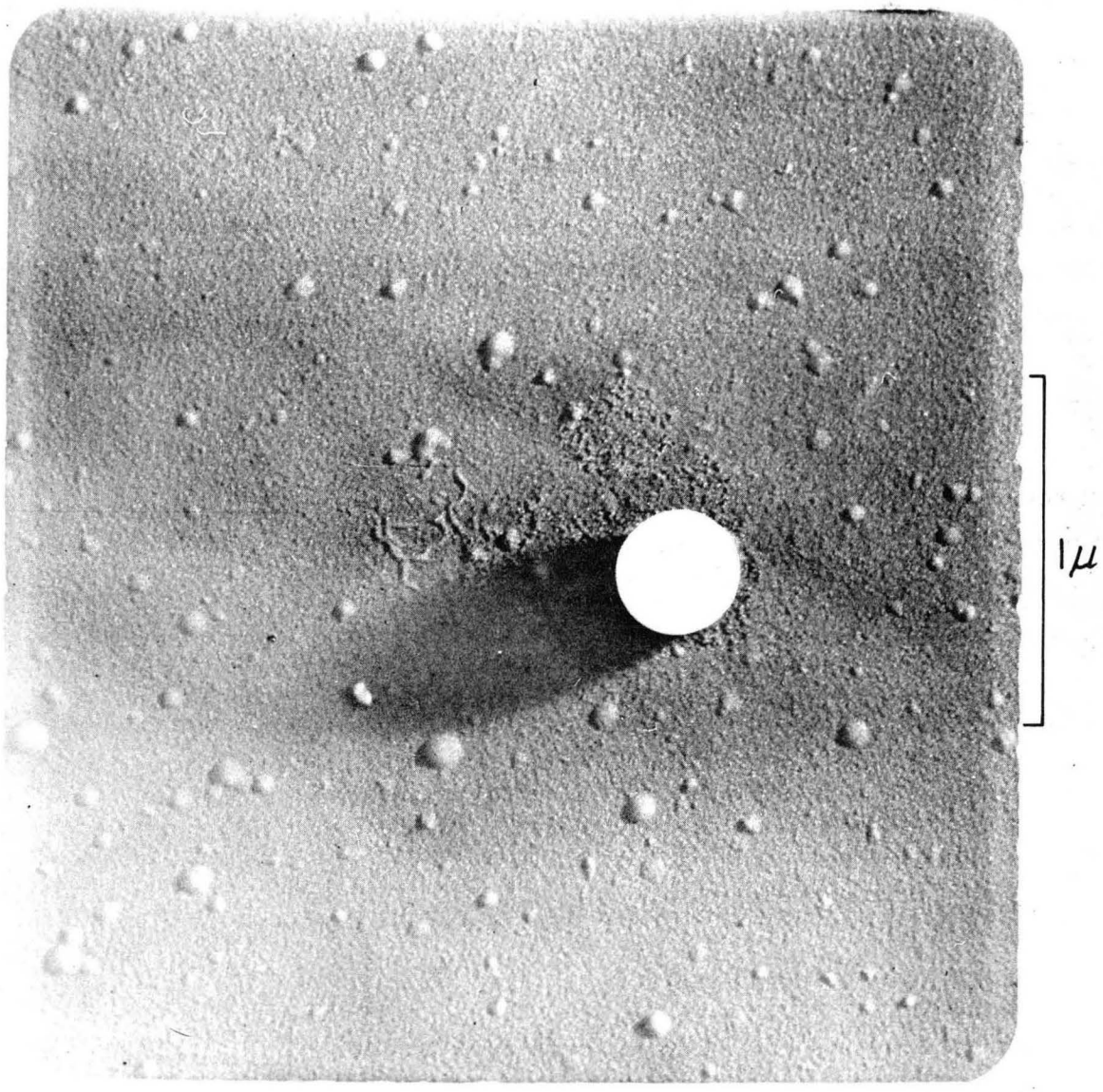


Fig. 4. Lipoprotein molecules of density less than 1.063 g/ml prepared without osmic acid fixation. The spherical particle is a polystyrene latex marker molecule. x 63,000.



ZN-1676

Fig. 5. A single lipid particle from a synthetic emulsion. x 63,000.

DISCUSSION

Certain of the above results are in contrast to previously published studies of the electron microscopy of lipoproteins. Macheboef[5] attempted to photograph horse lipoproteins, but the techniques of electron microscopy at that time (1949) made visualization of individual molecules very difficult.

Prendergast and Teague[6] in 1951 looked at human serum lipoproteins in the electron microscope, using unfixed material. They describe the lipoproteins as solid ellipsoidal particles. This seems to be in contradiction to the results reported in this paper, which show that unfixed molecules appear as ringlike structures (Fig. 4). It seems possible that the particles described as lipoproteins by Prendergast and Teague may be small particles of salt. Similar particles have been produced in this laboratory by submitting salt solutions to the preparative procedures of Prendergast and Teague.

Beisher[7] in 1954 used osmic acid to stain preparations of certain lipoproteins. The staining procedure was carried on after the lipoprotein had been allowed to dry on the collodion membrane. Such a technique apparently produces uneven and incomplete staining. Also, the preparations used were so concentrated that visualization of individual particles was very difficult.

As reported in results above, Figs. 1 and 2 show electron micrographs of two solutions that differ only in the centrifugal properties of the lipoproteins they contain. The techniques used in the preparation of the electron micrographs are essentially the same, yet the micrographs show particles that differ significantly in size. It follows therefore that the particles observed are in fact the lipoprotein macromolecule and not an artifact of the preparative procedure such as salt.

Further work should lead to valuable information on the size and shape of isolated classes of serum lipoproteins and their fragments and degradation products.

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[5] M. A. Macheboef, Discussions of the Faraday Society 6, 62 (1949).

[6] M. D. Prendergast and Maxwell Teague Circulation 4, 23 (1951).

[7] D. E. Beisher, Circulation Research 2, 164 (1954).

This work was supported in part by the U.S. Atomic Energy Commission and the Albert and Mary Lasker Foundation.