ULTRACENTRIFUGAL STUDIES OF LIPOPROTEINS OF HUMAN SERUM

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In spite of several extensive ultracentrifugal studies of human sera by Mutzenbecher, McFarlane, and Pedersen, the interpretation of the patterns observed has remained in doubt. Specifically, the greatest difficulties encountered by previous workers have arisen in their efforts to study undiluted human sera. McFarlane (1) referred to marked distortions in the pattern observed with undiluted human serum and suggested a trial and error dilution of the serum with salt solution to minimize such distortions. Pedersen (2) recommended diluting sera with various salt or buffer solutions to 40 per cent of the initial concentration, since under these conditions "adequate" resolution of the albumin and so called "X protein" peaks in the sedimentation diagram could be made. Both these workers found the apparent concentration of the X protein to vary considerably with respect to both over-all protein and salt concentration. The variations in concentration ranged from a value of X protein constituting up to 30 per cent of the serum proteins when studied in concentrated serum to an immeasurably small value when the serum was greatly diluted. Pedersen has explained this variation in X protein concentration by assuming this molecule to be a labile complex of albumin, globulin, and lipides which dissociates with increasing dilution of the serum.

On the basis of ultracentrifugal studies of human sera by the present authors, a wholly different interpretation of the ultracentrifugal patterns observed is given herewith. This interpretation indicates that the X protein concentration in human sera is vastly smaller than reported by Pedersen or McFarlane, but more consistent with electrophoretic data for the low density B1 lipoproteins. Further, the ultracentrifugal pattern observed for human serum with increasing dilution can be explained without assuming that any dissociation of X protein occurs.

EXPERIMENTAL

An electrically driven ultracentrifuge designed by E. G. Pickels was employed, the Thovert-Philpot-Svensson cylindrical lens-refractive index method for the observation of migrating boundaries being utilized (3). All runs were made between 25 and 30° at rotor speeds of 59,780 r.p.m.,
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giving centrifugal fields between 240,000g and 300,000g, at the meniscus and base, respectively. Blood was obtained from presumably normal individuals in the postabsorptive state.

A large number of undiluted sera were studied ultracentrifugally. Typical patterns obtained approximately 2 hours after reaching full speed are shown in Fig. 1. The vertical bar seen in Fig. 1, b, c, and d in the albumin complex represents a region of refractive index gradient in the cell so great that an entering light beam is completely thrown out of the optical system. We have determined that this bar has no bearing on the symmetry or asymmetry of the albumin peak. Of great interest is the “dip” below the base-line characteristically associated with the asymmetric boundary of the albumin complex. Pedersen accounted for albumin boundary asymmetry as being due to the presence of a density-sensitive lipoprotein (X protein), the $S_{20, w}$ value of which is very close to that of albumin. However, a two-component resolution, assuming a protein of this nature, even if present in the high concentration which Pedersen described,

![Fig. 1. Ultracentrifugal patterns of normal undiluted human sera obtained approximately 2 hours after the rotor attained full speed.](image)

could not conceivably give rise to the “dip” phenomenon which we have observed. An explanation is possible if we assume that the asymmetry and “dip” result from a piling up of the lipoprotein along the albumin concentration gradient at the sedimenting albumin boundary. Fig. 2, a gives the concentration diagram for a lipoprotein which for any reason has completely piled up in the region of the albumin gradient. The theory of the diagonal bar-cylindrical lens method of recording refractive index boundaries reveals that such a pile up must give rise to a biphasic curve (Fig. 2, b). In Fig. 2, c is given the concentration diagram for lipoprotein, in process of piling up, and for albumin, and in Fig. 2, d, the separate corresponding optical patterns expected. Fig. 2, e shows the albumin and lipoprotein pile up patterns in a single composite picture, which is the net result observed with the ultracentrifuge. A comparison of Fig. 2, e with the experimental observations of Fig. 1 demonstrates the plausibility of our hypothesis of the origin of the “dip” phenomenon.

It is of interest to consider how the observed ultracentrifugal pattern will vary with slight displacement of the lipoprotein pile up relative to the
position of the albumin concentration gradient. Fig. 3, a shows the separate albumin and lipoprotein patterns with such relative displacements. Fig. 3, b shows the corresponding composite patterns. All these types of distortions of the albumin boundary complex have been observed by altering serum density (see Fig. 3, c). Sucrose, sodium chloride, or magnesium sulfate added in quantities sufficient to give equivalent density increments produces the same type of pattern distortion.

The basis for the pile up phenomenon is the difference in sedimentation rates of the lipoprotein on either side of the albumin boundary gradient. A related type of anomaly occurring in mixtures of proteins, without a pile up but due to the same fundamental cause, has been previously described and mathematically treated by Johnston and Ogston (4). Two main factors contribute to this change: The viscosity of the albumin-containing solution is higher than the viscosity of its own supernatant so-

![Diagram](http://www.jbc.org/

Fig. 2. Pile up analysis and the resulting "dip" phenomenon that is observed ultracentrifugally (see the text for a complete explanation).

solution, and the effective buoyant force on sedimenting molecules is not the same in the albumin solution as in the supernatant solution, since the density difference between sedimenting particle and sedimenting medium is not the same in the two solutions. This is particularly important for the lipoprotein, the density of which is very close to that of the serum itself.

Two major types of situations can in general result in the pile up of lipoprotein: (a) The lipoprotein sediments in the same direction as the albumin. Here buoyancy difference and viscosity difference are additive in slowing lipoprotein sedimentation in the albumin solution relative to that in supernatant solution. Now, if lipoprotein has a sedimentation rate in supernatant solution greater than the albumin sedimentation rate, whereas the lipoprotein in albumin solution sediments more slowly than albumin itself, then the effect will be to produce a progressive pile up of lipoprotein somewhere in the albumin concentration gradient. Since in
this instance the lipoprotein sediments with the albumin boundary, it cannot be seen as an independently sedimenting component.

(b) The lipoprotein sediments toward the albumin boundary from both sides. This is mandatory if the lipoprotein density falls between the density of albumin solution and that of the supernatant solution. Here, if the lipoprotein sediments more rapidly in supernatant solution than does albumin, an appreciable pile up will be expected. It is this situation which, we believe, usually exists in undiluted serum. Further, small density increments produced by salt or sucrose addition to serum may be expected to shift the pile up along the albumin concentration gradient and thus give rise to a variety of bizarre patterns described in Fig. 3.

Fig. 3. Variations in ultracentrifugal patterns with variations in the location of the lipoprotein pile up on the albumin concentration gradient.

In the situation described in section b, a lipoprotein boundary migrating toward the center of rotation may or may not be observed. If the migration is slow, the boundary may be poor or may be lost entirely, due to the factors usually operative in producing diffuseness of boundaries.

A third situation may arise when the density of the solution is raised to the point where lipoprotein migrates in the direction opposite to albumin both above and below the albumin boundary. In this case, the analysis of Johnston and Ogston applies and predicts the possibility of some distortion of the albumin boundary. However, no pile up phenomenon will be expected along the albumin concentration gradient. It is to be noted here that, for lipoprotein, the viscosity and buoyancy effects operate in opposite directions, so that distortions will tend to be minimized.

It is thus evident that the classical method of boundary resolution ap-
plied to the albumin boundary is not applicable. Determinations such as those of Pedersen and McFarlane measure, therefore, some function of the concentration of lipoprotein that has piled up in the albumin boundary, but by no means the true serum lipoprotein content.

In view of the low density of the lipoprotein (specific volume 0.97), a method capable of measuring its concentration in human serum is available; namely, flotation. The necessary requirement that the density of the serum be greater than that of the lipoprotein may readily be achieved by the addition of small quantities of sodium chloride. Once the lipoprotein has moved a small distance away from the base of the cell in flotation, it then moves in an essentially homogeneous medium. Fortunately, the rate of flotation can be made sufficiently rapid so that the lipoprotein concentration measurements can be made before its boundary is obscured by meeting the sedimenting protein components. Fig. 4, a, b, and c shows the progressive flotation of the low-density lipoprotein, as studied in serum containing 7.8 per cent of added sodium chloride. The lipoprotein appears as an "inverse" peak, which is fully expected from the theory of the optical system. Since the specific refractive increment of the lipoproteins is very close to that for other proteins (5), the area under this peak is a measure of the concentration of the lipoprotein. At the density chosen for these studies, the albumin peak is essentially symmetrical (Fig. 4, d). A comparison of the area under the lipoprotein peak with that under the albumin peak gives a measure of the abundance of low density lipoprotein relative to those substances measured ultracentrifugally as albumin.

Ten sera from normal male and female young adults were studied by the method of flotation. The results tabulated in Table I indicate the lipoprotein concentration to be of the order of 5 per cent of total serum proteins, an abundance far lower than that quoted in the literature on the basis of previous interpretation of sedimentation diagrams.

The X protein has been suggested by Pedersen to be a labile complex of albumin, globulin, and lipides on the basis of changes in the apparent concentration of this component with changes in the serum protein and
salt concentrations. The pile up theory presented here could readily explain the apparent changes in concentration of lipoprotein observed by Pedersen without invoking any dissociation of the molecule. In Table II is given the concentration of lipoprotein at various total protein and salt concentrations for a single serum. Within the experimental

### Table I

**Low Density Lipoprotein Content of Ten Normal Human Sera**

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Sp. gr. of serum</th>
<th>Total protein (gm. per 100 ml.)</th>
<th>Lipoprotein (gm. per 100 ml.)</th>
<th>Lipoprotein (per cent of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0305</td>
<td>8.10</td>
<td>0.20</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>1.0310</td>
<td>8.26</td>
<td>0.23</td>
<td>2.8</td>
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<tr>
<td>3</td>
<td>1.0276</td>
<td>7.08</td>
<td>0.40</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>1.0298</td>
<td>7.86</td>
<td>0.35</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>1.0314</td>
<td>8.40</td>
<td>0.24</td>
<td>2.9</td>
</tr>
<tr>
<td>6</td>
<td>1.0288</td>
<td>7.50</td>
<td>0.23</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>1.0294</td>
<td>7.70</td>
<td>0.45</td>
<td>5.8</td>
</tr>
<tr>
<td>8</td>
<td>1.0278</td>
<td>7.14</td>
<td>0.43</td>
<td>6.0</td>
</tr>
<tr>
<td>9</td>
<td>1.0244</td>
<td>5.96</td>
<td>0.33</td>
<td>5.7</td>
</tr>
<tr>
<td>10</td>
<td>1.0240</td>
<td>5.82</td>
<td>0.20</td>
<td>3.4</td>
</tr>
</tbody>
</table>

### Table II

**Low Density Lipoprotein Content of a Single Human Serum Sample Obtained from Different Salt and Total Protein Concentrations**

<table>
<thead>
<tr>
<th>Preparation of serum sample</th>
<th>Cell used</th>
<th>Resultant sp. gr.</th>
<th>Lipoprotein (gm. per 100 ml.)</th>
<th>Lipoprotein (per cent of total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0% NaCl added</td>
<td>0.3</td>
<td>1.048</td>
<td>0.33</td>
<td>4.6</td>
</tr>
<tr>
<td>4.7% &quot; &quot;</td>
<td>0.3</td>
<td>1.000</td>
<td>0.33</td>
<td>4.7</td>
</tr>
<tr>
<td>6.1% &quot; &quot;</td>
<td>0.3</td>
<td>1.069</td>
<td>0.42</td>
<td>6.0</td>
</tr>
<tr>
<td>7.8% &quot; &quot;</td>
<td>0.3</td>
<td>1.081</td>
<td>0.40</td>
<td>5.6</td>
</tr>
<tr>
<td>9.4% &quot; &quot;</td>
<td>0.3</td>
<td>1.093</td>
<td>0.41</td>
<td>5.8</td>
</tr>
<tr>
<td>1 volume serum + 1 volume 8% NaCl</td>
<td>0.8</td>
<td>1.042</td>
<td>0.40</td>
<td>5.6</td>
</tr>
<tr>
<td>1 &quot; &quot; + 3 volumes 8% NaCl</td>
<td>0.8</td>
<td>1.048</td>
<td>0.46</td>
<td>6.5</td>
</tr>
</tbody>
</table>

error of measuring small areas, the data indicate no significant variation of lipoprotein content and hence the stability of this molecule, in accordance with the report of Edsall (6) on the relative stability of the low density \( B_1 \) lipoprotein to such manipulations as precipitation and resolution.

It has been further stated that a density-sensitive component is present only in the sera of humans (7). Ultracentrifugal studies reported elsewhere

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have demonstrated the presence of such a component in about 60 per cent of rabbit sera.

SUMMARY

1. The difficulties which have prevented a satisfactory interpretation of the ultracentrifugal pattern of human serum, both diluted and undiluted, have been reviewed.

2. The observation of a "dip" in the ultracentrifugal pattern of undiluted human sera has led the present authors to explain the major peculiarities of albumin boundary asymmetry as being due to a pile up of lipoprotein (X protein) on the albumin concentration gradient. The existence of the pile up phenomenon renders classical two-component resolution of asymmetrical albumin boundaries completely erroneous both in the calculation of sedimentation rates and concentration of lipoprotein.

3. A method for measuring the concentration of low density lipoproteins by flotation has been described and applied. The results of analysis of lipoprotein concentrations by this method are in much better agreement with electrophoretic and fractionation data concerning this lipoprotein than are the data in the literature up to the present. The data obtained in the present work render unnecessary the postulation of a great degree of lability of lipoprotein with variation in salt and protein concentration.

BIBLIOGRAPHY

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