ULTRACENTRIFUGAL STUDIES OF MAJOR NON-LIPIDE ELECTROPHORETIC COMPONENTS OF NORMAL HUMAN SERUM

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In the ultracentrifuge, normal human serum shows, in addition to the lipoproteins, three distinct components with sedimentation rates of approximately 4, 7, and 19 Svedberg units (1, 2). Other materials known to be present are obscured by the high concentrations of these major substances and the high diffusion spreading of the boundaries. For example, Edsall (3) has reported a number of additional components in fractions separated by alcohol and salt precipitation. Likewise, several crystalline proteins have been isolated which show sedimentation rates different from the three mentioned above (4, 5).

Electrophoresis reveals at least five major fractions, with probably a continuum of mobilities from the slowest γ-globulin through the albumin. Since the transport behavior of a protein in an electric field is in general independent of its properties in an ultracentrifugal field, it becomes necessary to determine the distribution of the various sedimentation groups within each mobility fraction. The technique of moving boundary electrophoresis has not permitted the isolation of amounts of intermediate components sufficient to answer this question conveniently. However, with the introduction of various techniques of preparative zone electrophoresis, this type of approach has become feasible. Such studies on a number of fractions isolated from normal and pathological sera have been previously reported (6, 7). Recently, Brattsten has presented data on the sedimentation rates of some representative fractions of normal serum obtained by continuous zone electrophoresis (8).

The present report attempts to ascertain the mobility distribution of all the major non-lipide proteins identified by their sedimentation rates. Because of the small number of sera studied, it has not been possible to specify exact "normal" levels of each of the serum components. However, the results may be useful, at least as a starting point, for more precise measurements of each mobility or sedimentation coefficient distribution, for

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elucidating certain problems of protein-protein interaction, and as a back-
ground for clinical studies.

EXPERIMENTAL

Preparative Ultracentrifugation—The serum lipoproteins less dense than
1.06 gm. per ml. were removed by ultracentrifugation as follows: The non-
protein density of the serum was increased to 1.06 gm. per ml. by the addi-
tion of solid NaCl in the proportion of 1.066 gm. of NaCl to 13 ml. of serum. The Spinco prepares preparative rotor No. 40 was employed at 40,000 r.p.m. for
19 hours, which speed and time are sufficient to sediment or to float pro-
teins with an s rate of ±7 Svedberg units through the 12 ml. tube. The
top 3.5 ml. layer was carefully removed according to standard lipoprotein
methods (9). Some lipoprotein of high density class and some albumin
are lost in the top layer, but negligible lipoprotein of the low density class
remains in the bottom layer. This was verified by inspection of the ve-
locity schlieren pattern of the top layer obtained from a subsequent prepa-
ratative ultracentrifugation of this bottom layer increased to a density of
1.20 gm. per ml. (9).

For complete quantitative fractionation into lipide and lipide-free frac-
tions, a preparative ultracentrifugation initially layered at 1.20 gm. per ml.
density was used. For each 6 ml. tube of a Spinco rotor No. 40.3, 3.0 ml.
of serum and 1.5 ml. of stock-saturated KBr, NaN0₃ solution of density
1.58 gm. per ml., were mixed. Then 1.5 ml. of 1.20 gm. per ml. of solvent
were carefully layered on top. The rotor was accelerated at 2000 r.p.m.
per minute by manually advancing the speed control dial on a Spinco model
L ultracentrifuge.¹ Centrifugation at 40,000 r.p.m. for 24 hours at 20°
was employed prior to pipetting the top 1 ml. of lipide layer.

The bottom layers of the preparations of 1.06 or 1.20 gm. per ml. density
were dialyzed at 4° against distilled water for 2 hours and barbital buffer
for 24 hours with three changes of buffer. After dialysis, the samples
were concentrated to serum level by ultrafiltration.

In order to obtain a relative enrichment of the higher molecular weight
components of the serum, 1000 ml. of pooled plasma from healthy blood
donors were repeatedly centrifuged, and the fluid above the gel-like pellet
was poured off. The net effect of the preparative ultracentrifugation was
to give approximately a 10-fold enrichment of the 19 S component and a
4-fold reduction of the albumin.

Preparative and Analytical Zone Electrophoresis—Barbiturate buffer, pH
8.6, ionic strength 0.1, was used for the zone electrophoresis in supporting
media. The procedure in which potato starch was used was similar to
that described previously (10). The block used for the analysis presented

¹ Spinco Division, Beckman Instruments, Inc., Palo Alto, California.
in Fig. 2 was 28 inches long, 8½ inches wide, and ½ inch thick, and was kept cool with a fan during the 30 hours electrophoresis at +4°, with 350 volts which gave approximately 110 ma. The length of the initial band in the direction of migration was ½ inch. For the study of the equivalent of 20 ml. of serum shown in Fig. 4, a block 18 inches long, 24 inches wide, and ½ inch thick was used. The initial band was 5/6 inch wide.

Polyvinyl chloride powder was also employed as a supporting medium in a manner analogous to starch for certain experiments. This non-swelling medium shows very low adsorption of serum proteins and does not contribute soluble products that might interfere with protein analysis (11).

The electrophoresis blocks were cut into 1 inch segments, which were suspended in test tubes with 10 ml. of buffer. Protein analysis was performed on a portion of the supernatant fluid of these well mixed tubes according to a modified Folin method (10). The remaining protein and supporting medium in the tubes were resuspended, and the protein-buffer solution was recovered by displacement filtration on a sintered glass filter under suction.

Ultrafiltration—The eluates were filtered through collodion bags2 with a vacuum of about 260 mm. of mercury. The filter bags were submerged in buffer during filtration, which was continued until a final volume of 1 to 1.5 ml. remained. This was recovered and used in the experiments in analytical ultracentrifugation.

Analytical Ultracentrifugation—A Spinco model E ultracentrifuge1 was used to obtain the distribution of the proteins of each electrophoretic fraction among the various ultracentrifugal groups. The following terms are used in this study: "s rate," the sedimentation coefficient in general; $s = s_{20,w}$, the sedimentation coefficient at infinite dilution at 20° calculated to the density and viscosity of water; and $S$, the unit of s rate (1 Svedberg $S = 10^{-13}$ sec.). The procedure for operation at 20.0°, the use of double sector centerpieces, and the measurement of the schlieren patterns by use of a micrometer screw comparator have been described elsewhere (12). The barbiturate buffer, pH 8.6, $\Gamma/2 0.1$, used in electrophoresis was retained as solvent in the experiments in analytical ultracentrifugation. Observed s rates were multiplied by 1.10 to convert to the "20, w" reference.

No two peaks of the more than 200 photographed in this study had the same observed s rate. This does not mean that each one represents a significantly different substance, since, in addition to experimental errors within ±5 per cent, the concentration dependence will prevent mixtures, which vary in composition but are of the same total concentration, from exhibiting the same s rates. In order to correct for concentration dependence, the following scheme was used. The literature value, $k = 0.005$

2 Schleicher and Schuell Company, Keene, New Hampshire.
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ml. per mg. for γ-globulin and for albumin (13, 14), was provisionally used to correct the s rate of the maximal ordinate of a representative group of peaks for the concentration of all slower components, according to the relation:

\[ s_i = s^{ob}_i \left(1 - \frac{\sum c_i}{k} \right) \]

The rate \( s_i \) corresponds to the s rate which would have been observed if the \( i^{th} \) component were the only component present. The \( s_i \) was obtained from the slope of the best line on a log x versus t plot and the \( c_i \) from the average concentration during the experiment. A composite graph was made of all the \( (s_i, c_i) \). The data were concentrated into six groups, each characterized by a slope greater than 0.005 ml. per mg., namely 0.007 ml. per mg., except for the fastest component, “19 S.” Here the \( k \) was 0.017 ml. per mg. Hence, for the main study, the observed (obs) s rates were corrected according to

\[ s_i^0 = s^{obs}_i \left(1 - \sum k c_i \right) \]

where all \( k_j = 0.007 \) ml. per mg., except when \( j = 19 \) S, in which case \( k = 0.017 \) ml. per mg. Johnston-Ogston corrections (15, 12) for superimposed gradients of slow components at faster boundaries were applied to the patterns with a concentration of the 19 S component above 6 mg. per ml. The extrapolation to infinite time and infinite dilution of the apparent distribution with respect to s rate was in accordance with the procedure of Baldwin (16, 17, 12).

Results

Ultracentrifugal Analysis of Whole Serum—The resolution of a moving boundary or zone pattern can be increased by extrapolation to infinite time to diminish the blurring from diffusion relative to the separation due to the field. Such an analysis was performed on the patterns obtained in the analytical ultracentrifuge for a normal lipide-free serum (bottom fraction of 1.20 gm. per ml. of density preparation) at total protein concentrations of approximately 35, 17, 10, and 5 mg. per ml. Fig. 1 shows the distribution for the 10 mg. per ml. sample. Assuming that the 4 S and 7 S components are really symmetrical, then the 5 S intermediate can be resolved. Thus, five non-lipide components are visible in this plate-analysis procedure. The further extrapolation to infinite dilution indicates that each is homogeneous to within a standard deviation of 10 per cent of the nominal s rate, which is the limit of the resolving power for this method (16). The composition of this serum is given in Column A, Table I. The mobility
distribution of each of these components cannot be determined from this data and the electrophoretic pattern alone.

![Ultracentrifuge schlieren pattern from normal lipide-free serum extrapolated to infinite time.](image)

**Ultracentrifugal Analysis of Electrophoretic Fractions**—The electrophoretic distribution obtained in the starch medium of a normal serum, free from lipides less dense than 1.06 gm. per ml., is given in Fig. 2, upper part. The usual five main components are identified just below the segment numbers, and their relative concentrations are indicated. Representative patterns of analytical ultracentrifugation of the separated fractions from this type of experiment are given in Fig. 3. The use of two double sector cells simultaneously permits a visual demonstration of the various sedimentation
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rate components obtained from the electrophoretic fractions because relative displacements can be seen by inspection. Thus, in Fig. 3, left-hand column, which represents 24 minutes of ultracentrifugation, the presence of the 19 S component in γ1 and α2 regions is evident. The patterns in Fig. 3, right-hand column, at 96 minutes show resolution of the large slow com-

### Table I

**Mobility Distribution of Ultracentrifugal Components in Various Ultracentrifugally Prepared Normal Serum Samples**

The values are given in per cent; 0.0 per cent means less than 0.1 per cent.

<table>
<thead>
<tr>
<th>Component</th>
<th>Column A*</th>
<th>Column B†</th>
<th>Column Ct</th>
<th>Column D†</th>
<th>Column E‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 S-α1</td>
<td>1.2</td>
<td>4.0</td>
<td>3.6</td>
<td>3.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4 S-alb</td>
<td>58.4</td>
<td>56.3</td>
<td>46.4</td>
<td>54.8</td>
<td>12.6</td>
</tr>
<tr>
<td>4 S-α2</td>
<td>9.3</td>
<td>6.2</td>
<td>7.6</td>
<td>4.7</td>
<td>0.2</td>
</tr>
<tr>
<td>5 S-β</td>
<td>26.1</td>
<td>5.7</td>
<td>1.8</td>
<td>2.8</td>
<td>8.5</td>
</tr>
<tr>
<td>7 S-α2</td>
<td>16.6</td>
<td>7.6</td>
<td>1.5</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>7 S-γ</td>
<td>16.6</td>
<td>7.6</td>
<td>1.5</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>12 S-α2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>2.8</td>
</tr>
<tr>
<td>12 S-β</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>2.8</td>
</tr>
<tr>
<td>19 S-α2</td>
<td>5.0</td>
<td>2.0</td>
<td>2.5</td>
<td>2.6</td>
<td>23.6</td>
</tr>
<tr>
<td>19 S-γ</td>
<td>1.0</td>
<td>1.3</td>
<td>2.4</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>28 S-γ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
<td>44 S-γ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Column A, analytical ultracentrifuge patterns extrapolated to infinite time of whole lipoprotein-free serum (no electrophoresis).
† Columns B, C, and D, combined zone electrophoresis and analytical ultracentrifugation of lipoprotein depleted sera.
‡ Column E, combined zone electrophoresis and ultracentrifugation of preparation enriched in the faster sedimenting components.

ponent noted at the earlier time, and demonstrate the progressive increase in the s rate of the major peak from 3 S to 4 S, 5 S, and 7 S in proceeding from α1 to α2, β, and γ1 regions.

In principle, the distribution with respect to s rates of the protein of each segment could be obtained for the patterns in Fig. 3 by a double extrapolation to infinite time and zero concentration. This was done only for one of the γ-globulin fractions and for albumin, showing, as in Fig. 1, that the standard deviation of the sedimentation rate distribution was within 10 per cent of the mean s rate. This is in accordance with the results
of Cann for γ-globulin (14). Alternatively, the components of Fig. 3 were grouped by their calculated infinite dilution sedimentation rates. The actual $s_{20, w}^0$ values in barbiturate buffer, pH 8.6, $\Gamma/2 = 0.1$, were 3.0 S, 3.7 S, 4.2 S, 6.0 S, 11 S, and 16.8 S. The $s_{20, w}^0$ is 10 per cent greater. Hence, the following six sedimentation rate groups in normal human serum were identified and denoted by using the nearest integer to the $s_{20, w}^0$ as 3 S, 4 S, 5 S, 7 S, 12 S, and 19 S groups. The 12 S was the only group that exhibited a very broad $s$ rate distribution (9 to 15 S).

The relative composition of each electrophoretic segment with respect to these $s$ rate groups was determined. The results are shown in Fig. 2, lower part. Each separate shaded area can be interpreted as the mobility distribution that would have been obtained if each $s$ rate group could have been isolated in the ultracentrifuge prior to electrophoresis. The points
to observe are that the $7 \, S$ component has a very broad mobility distribution. The $4 \, S$ group is mostly albumin but one-tenth of this is of $\alpha_2$-globulin mobility. The $19 \, S$ component also exhibits two main mobility groups; about one-third of the total is in the fast $\gamma$-globulin region and two-thirds in the $\alpha_2$-globulin region. The $12 \, S$ component is broad, both in mobility and $s$ rate. The $3 \, S$ and $5 \, S$ components are both sharply defined as $\alpha_1$- and $\beta$-globulins, respectively. The detailed composition is given in Column B, Table I.

The entire electrophoresis and ultracentrifugal experiment was repeated on a different sample of serum from the same donor. However, this time, after determination of the protein curve, eluates from the segments were pooled to give five fractions corresponding to $\gamma$, $\beta$, $\alpha_2$, $\alpha_1$-globulin and albumin. It should be noted that the preparative ultracentrifugation to remove the low density lipoproteins was not quantitative as performed.

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FIG. 3. Selected ultracentrifugal patterns of four electrophoretic fractions at 24 and 96 minutes. The phaseplate angles and $s$ rate groups are indicated.
In this repeated experiment, a slightly different preparative procedure was employed which resulted in only about 90 per cent recovery of the albumin in the bottom fraction. It can be seen from Table I, Column C, that all the components of Fig. 2 are again found, and that the relative mobility distribution of each s rate group is reproduced. Also, the recovery of components of s greater than 4 S is duplicated within experimental error.

Completely lipide-free material obtained by the preparative ultracentrifugation of the initially layered serum at 1.29 gm. per ml. of density was also investigated. The results are given in Column D, Table I. The use of the salt solution of high density (25 per cent) for 24 hours apparently did not adversely affect the results, but may have increased the amount of 12 S material. Since this fractionation was performed in a quantitative manner (as the low density fractionation could have been), this set of data may eventually be used in determining normal levels. The low density lipoproteins, at least, should be removed because of probable interference in the analytical pattern owing to floating material (2), and to simplify analysis by merely reducing the number of components in the samples. The sera listed in Table I, Columns B and C, contain the α₁-lipoproteins, whereas those in Columns A and D do not.

Recovery Experiments—As controls on the procedures involved in this study, the following experiments were performed. First, in the study represented in Fig. 2, some slowly sedimenting material from 0 to 4 S was detected in the γ-globulin region. This was at most 0.3 per cent of the total protein. Inasmuch as this might be amylase breakdown products of the starch, two portions of the same whole serum were separated, both on starch and on the polyvinyl chloride supporting medium. The γ-globulin regions were eluted, concentrated, and analyzed in the analytical ultracentrifuge at the same total concentration of 26 mg. per ml. The polyvinyl chloride fraction showed less than 0.1 per cent of the total protein to be slow material, whereas the protein eluted from starch contained less than 0.3 per cent. In a subsequent experiment, indicated in Column D, Table I, all the procedures in the presence of starch were carried out in a cold room at 4°. This, together with the exposure to the salt solution of high density, apparently reduced the enzymatic action, for none of this slow material was detected. Both the starch and the polyvinyl experiments revealed that γ-globulin contained the 19 S component, and appeared in general to yield comparable distributions of the proteins even with the high electroosmotic flow in the polyvinyl medium which makes the α₂-globulin remain at the origin.

A second general control on recovery was performed as follows: A sample of the starting material for the experiment listed in Column D, Table I, obtained after preparative ultracentrifugation and prior to electrophoresis,
was compared with one eluted from starch and concentrated after standing without electric field for a period of time equal to the duration of the electrophoresis. The two samples were adjusted to the same two total protein concentrations of 10 mg. per ml. and 30 mg. per ml. and analyzed in the analytical ultracentrifuge. The analytical patterns were precisely superimposable, thus indicating no selective loss of any ultracentrifugal component in the procedure.

Special Analysis of 19 S Ultracentrifugal Component—In order to determine more clearly the distribution of the faster sedimenting components than is possible from Fig. 2, two separate special studies were made. The first was to use the pooled normal serum enriched in heavy component by repeated preparative ultracentrifugations for combined electrophoretic and ultracentrifugal analysis. The results are given in Table I, Column E. In addition to an accentuation of the 12 S material, two components even faster than the 19 S, one of 28 S, and the other of 44 S were visible. In this experiment it was observed that even the first low density preparative ultracentrifugation packed the 19 S component into a pellet. This component was not as easily resuspended as the other serum components and is perhaps more labile (18).

The second special experiment avoided these limitations. The lipoproteins were removed without packing the 19 S component, by taking advantage of the reduced sedimentation rate in the high density medium (1.20 gm. per ml.). Then, to avoid the repeated centrifugation necessary to concentrate the 19 S material, a very broad starch block which accommodated 24 ml. of solution in an initial zone only 5/6 inch wide was used.
for electrophoresis. The electrophoretic distribution of the 19 S component, compared to the lipoprotein-free whole serum, is given in Fig. 4. The very small amount of 19 S material in the β-globulin region was shown to be an overlap of the 19 S-α2 component by subjecting this one fraction to a second electrophoresis. Neither the center nor the region on the γ-globulin side showed any of the 19 S material on subsequent analytical ultracentrifugation, but the material on the α2 side did. In this serum, the 19 S-γ was almost as concentrated as the 19 S-α2 component. No deleterious effect could be attributed to the exposure of the material to 20° for approximately 24 hours and to the salt concentration at high density (about 25 per cent).

DISCUSSION

Through the combined use of electrophoretic and ultracentrifugal separation, the number of major components observed in normal serum is increased considerably over that seen with either procedure alone. The five electrophoretic fractions subdivide into ten major groups or families of proteins. This can be visualized more clearly in a three-dimensional map (Fig. 5) with the sedimentation rate along the z axis, the electrophoretic mobility along the y axis, and the relative distribution g(s, w) along the x axis. It should be noted that the distribution along the mobility axis was obtained from a zone pattern while that along the sedimentation rate axis was obtained by the moving boundary technique. Fig. 5 has been drawn as if the effect of the initial zone width and diffusion spreading of the zones and boundaries had been removed. In this respect the picture is an idealization aimed only to give a pictorial idea of the different s rate-mobility groups present in normal serum. Since reelectrophoresis of fractions from adjacent segments yields protein peaks separated by the same amount, the zone electrophoresis as employed with a narrow starting band gives practically the true mobility distribution. The analytical ultracentrifuge patterns extrapolated to infinite time and infinite dilution indicate a heterogeneity in the s axis of 10 per cent of the mean s rate, for all components except the 12 S. The sensitivity of the schlieren optical system is relatively low, which makes it probable that discrete components that are present only in very low concentration (less than 100 mg. per 100 ml. of serum) will not be detected. The picture given thus presents only the major serum protein groups. This applies only in part to Fig. 5, lower section, where the data from the experiments on the preparation enriched in heavy components are summarized. By using an enlarged scale, the high molecular weight groups detected in these experiments have been plotted at the approximate relative concentration present in the original serum sample.

One of the major obstacles encountered in this study, which partially
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hampers a clear cut delineation of the serum components in terms of sedimentation rate, is the problem of protein-protein interaction. The protein groups of higher s rate might be particularly suspected of being aggregates of lower molecular weight proteins. The 28 S and 44 S components were present in too low a concentration to be rendered visible in whole serum and were only identified after enrichment by preparative ultracentrifugation. The 12 S group, also difficult to visualize in whole serum, was seen clearly only after electrophoretic separation. The latter might be similar to the 9 to 12 S components noted in some preparations of γ-globulin prepared by alcohol fractionation and attributed to aggregation of the 7 S (19). The 19 S group was observed in whole serum, and the recovery experiments gave no indication of a change in the relative concentration of these proteins during the various procedures. Additional support for the identity of the 19 S material is in the recent findings that the

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**Fig. 5.** Electrophoretic and ultracentrifugal display of major non-lipide normal serum proteins.
19 S component of the γ-globulin is immunologically different from the 19 S-α and 7 S-γ components (20, 21), and that it also contains considerably more carbohydrate than the 7 S components (22).

Besides aggregation of the same protein, there can be combinations of different proteins. Thus, hemoglobin is known to combine with one of the α₂-globulins (haptoglobin) to form what has been termed the hemoglobin-haptoglobin complex (23, 24). The 7 S material in the α₂ region of Fig. 2 showed a faint reddish color when observed in the ultracentrifuge and may well be this complex. Subsequent experiments with minimally hemolyzed serum, obtained with silicone-treated syringes and tubes, showed no red color in this fraction, although definite 7 S material was still present in the α₂ region. It was apparent that the relative amounts of the various ultracentrifugal components of the α₂ fraction were considerably influenced by the amount of hemoglobin in the serum.

The multiple components charted in Fig. 5 are not necessarily homogeneous proteins. New procedures of fractionation may well subdivide some of them. For example, the 4 S component in the α₂ region probably contains both haptoglobin and ceruloplasmin. Many of the enzymes known to be present in small amounts in the α₂ fraction also may fall, in part, under this peak. Other components appear more homogeneous and are identifiable in terms of known proteins that have been isolated and crystallized from serum. The 5 S-β-globulin peak probably represents chiefly the iron-binding protein. The 19 S-α₂ component resembles closely the α₂-glycoprotein recently described by Brown et al. (25). The α₁-globulin (3 S) is probably primarily the acid glycoprotein isolated by Weimer, Mehl, and Winzler (26) and Schmid (4). A more complete bibliography on the identification of these serum components with a given sedimentation rate and mobility is given by Brattsten (8). Some of the results obtained by Brattsten for the relative composition of selected segments differ from those presented here. In particular, no 4 S material of γ mobility was found in the present study, and, even with hemolysis, the relative amount of 7 S material in the α₂ region did not attain 79 per cent. It should be noted, however, that, since the various ultracentrifugal components of an electrophoretic fraction do not have their maximal concentration at the same mobility, the relative composition in any segment can vary widely, depending on the heterogeneity and mobility resolution attained. It is only by analyses of all segments of the electrophoretic separation that the true three-dimensional display can be obtained. The recent observations of Smithies (27), indicating that normal individuals have different types of haptoglobin, also raise the possibility that the α₂ fraction and perhaps others show variation in the ultracentrifugal pattern in different normal individuals.
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SUMMARY

1. Analysis in the ultracentrifuge of the fractions obtained by zone electrophoresis of lipoprotein-free normal human serum has indicated the presence of at least ten major groups of proteins.

2. Observations on three normal sera showed that 7 S components were broadly distributed through the γ-, β-, and α2-globulin regions of the electrophoretic patterns: 4 S components, in the albumin and α2 regions; and 19 S components, in the γ and α2 fractions. In addition, 3 S and 5 S components were found in the α1 and β fractions, respectively. Analysis of a serum preparation enriched in heavy components by repeated cycling in the preparative ultracentrifuge gave similar results and showed the additional presence of 12 S, 28 S, and 44 S components.

3. The problem of protein-protein interaction in these experiments and the importance of obtaining sera free of hemolysis are discussed.

4. A three-dimensional display of the major non-lipide groups of proteins obtained by combined electrophoretic and ultracentrifugal analysis of normal serum is presented.

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