Unusual Electrophoretic Patterns of Plasma Proteins in Human Subjects

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Study of 313 plasma electrophoretic patterns from 158 hospitalized subjects revealed that 30 per cent of these patients exhibited a heterogeneity marked by occurrence of double peaks in the alpha-2 and 6 per cent in the beta globulin fraction. Occurrence of double peaks could not be related to any specific disease. Similar study of plasma from 18 normal adult subjects did not reveal double peaks in any of the protein fractions. Existence of prealbumin components, with mobilities greater than that of the albumin, has been observed in 6 of 158 pathological subjects. Mobilities and concentrations of components x₁, x₂, and x₃ are reported.

Study of plasma protein electrophoretic patterns from 158 patients and 18 normal subjects reveals that the majority of these patterns conform to observations reported earlier by other investigators (1–9). A considerable number, however, show additional peaks on the electrophoretic pattern. These differences include the appearance of double peaks where a single peak normally occurs and the presence of components that migrate faster than albumin. Similar observations have been made by others (10–28); however, no extensive survey for these peculiar boundaries has been made in a variety of diseased individuals.

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I am indebted to Professor Smith Freeman, Biochemistry Department, Northwestern University Medical School, Chicago, and Research Consultant for the Veterans Administration Hospital, Hines, Ill., for his comments and suggestions in preparation of this manuscript; to Professor Virgil L. Koenig, Department of Biochemistry, Northwestern University Medical School, Chicago, for doing the ultracentrifugal studies and for other assistance; and Mrs. J. X. Wheeler, B.S., Research Section of the Veterans Administration Hospital, Hines, for the urinary steroid and plasma lipid data.

Received for publication June 10, 1960.
Methods and Material

Fasting blood samples were obtained from 158 hospitalized patients suffering from various diseases and disorders. A total of 313 blood samples were collected from these patients. Additional blood was collected from 18 apparently normal male hospital employees. Plasma total protein determinations were made by the micro-Kjeldahl method (29) and corrected for the nonprotein constituents. The nitrogen-to-protein conversion factor used was 6.25.

The plasma electrophoretic separation was carried out in the Klett apparatus (30) after a 19-hr. dialysis against veronal buffer at pH 8.6 and ionic strength 0.1. The electrophoretic analysis was carried out at 1-2° for 180-200 min., using 10 mAm. and 110 v. The patterns were enlarged 2-4 times, traced, and the area of each component was compared to the total area of the pattern and recorded as the percentage of the total area. Mobilities of the components were computed from the distance between each peak and the center of the boundary anomaly according to the following equation:

\[ u = \frac{d A K}{t I R m} \]

where \( u \) is mobility expressed in centimeters² per volt per second; \( d \), distance boundary traveled in centimeters; \( A \), cross sectional area of cell; \( K \), conductivity cell constant; \( t \), time in seconds; \( I \), current in amperes; \( R \), resistance of buffer in ohms; and \( m \), magnification factor of optical system.

Results

The findings of this study are presented in Table 1 and graphically illustrated in Fig. 1 and 2. A total of 313 electrophoretic separations were made on 158 patients. Similar separations were made on plasma of 18 apparently normal male hospital employees.

Because of the great variety of diseases studied, it became necessary to group the patients under general classification of diseases. The number of subjects in each group and the number of those exhibiting the double peaks were as follows.

1. Endocrine group (hypo- and hyper-adrenalism, hypo- and hyperpituitary states): 29 different subjects (13), with double peaks
2. Rheumatic or collagen group: 16 subjects, 4 with double peaks
3. Granuloma group (16 with tuberculosis, 2 with Hodgkin’s disease, etc.): 21 subjects, 7 with double peaks
4. Osteoporosis group: 5 subjects, 3 with double peaks
5. Liver diseases group (biliary cirrhosis, biliary xanthomatosis, xanthoma tuberosum, familial hyperlipemia and hepatitis): 13 subjects, 5 with double peaks
6. Kidney group: 16 subjects, 4 with double peaks
7. Miscellaneous group (21 with multiple myeloma, 12 with ulcerative colitis, 5 with carcinoma, 3 with cardiac failure, etc.): 58 subjects, 21 with double peaks
The fast-moving components and their relation to other protein fractions are illustrated in Fig. 2. Table 1 shows plasma protein concentrations and mobility of normal subjects and those patients whose plasma contained a moiety migrating faster than albumin.

![Electrophoretic patterns](image)

**Fig. 2.** Electrophoretic patterns of abnormal plasma proteins with the fast-moving components: multiple myeloma and multiple sclerosis (A); carcinoma (B); and undiagnosed (C).

**Discussion**

Several reports appeared in the literature relating occurrence of double peaks in the alpha₂ and beta globulins to various diseases. Kunkel *et al.* (5) in the study of patients with primary biliary cirrhosis reported an elevated beta globulin consisting of 2 components. These investigators were able to indicate a correlation between the total lipid level and the beta globulin peaks determined electrophoretically. Sternberg (9) also observed 2 fractions in the beta globulin in patients with biliary cirrhosis, with obesity and in 2
patients with myocardial infarction. Fischer et al. (7) have shown that the beta globulin of nephrotic plasma is composed of 2 distinct peaks. Bernsohn and Cochrane (11) reported double peaks in the alpha₂ globulin in 15 of 27 patients with multiple sclerosis.

Table 1. DISTRIBUTION OF PLASMA PROTEINS AND FAST-MOVING COMPONENTS

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fibrinogen</th>
<th>Beta</th>
<th>Alpha₂</th>
<th>Alpha₃</th>
<th>Albumin</th>
<th>X-1</th>
<th>X-2</th>
<th>X-3</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. myeloma and m. leukemia</td>
<td>7.92</td>
<td>0.43</td>
<td>0.71</td>
<td>0.41</td>
<td>1.44</td>
<td>0.21</td>
<td>0.34</td>
<td>0.40</td>
<td>12.38</td>
</tr>
<tr>
<td>m. sclerosis</td>
<td>2.4</td>
<td>3.3</td>
<td>4.8</td>
<td>5.3</td>
<td>8.5</td>
<td>6.5</td>
<td>11.1</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>Carcinoma with metastasis</td>
<td>0.81</td>
<td>1.08</td>
<td>0.93</td>
<td>0.53</td>
<td>3.60</td>
<td>0.08</td>
<td>0.08</td>
<td>7.07</td>
<td>1.09</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>0.67</td>
<td>0.88</td>
<td>0.81</td>
<td>0.59</td>
<td>2.87</td>
<td>0.22</td>
<td></td>
<td></td>
<td>6.34</td>
</tr>
<tr>
<td>Hypocalcemia due to absorptive failure</td>
<td>1.44</td>
<td>0.90</td>
<td>1.17</td>
<td>0.73</td>
<td>3.34</td>
<td>0.10</td>
<td>0.12</td>
<td></td>
<td>7.60</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.6</td>
<td>3.4</td>
<td>4.5</td>
<td>5.7</td>
<td>11.6</td>
<td>11.8</td>
<td>35.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, with congest. heart failure</td>
<td>1.11</td>
<td>2.3</td>
<td>3.1</td>
<td>4.3</td>
<td>5.6</td>
<td>6.6</td>
<td>12.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.3</td>
<td>0.44</td>
<td>0.54</td>
<td>1.09</td>
<td>0.48</td>
<td>0.07</td>
<td>0.32</td>
<td></td>
<td>6.77</td>
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<tr>
<td></td>
<td>1.5</td>
<td>2.6</td>
<td>3.5</td>
<td>4.4</td>
<td>5.6</td>
<td>6.5</td>
<td>8.0</td>
<td></td>
<td>1.04</td>
</tr>
</tbody>
</table>

Electrophoresis of plasma from subject C, collected 10 and 30 days after initial observation revealed that x-component was still present. Dialysis against phosphate buffer at pH 7.7 and ionic strength 0.1 with subsequent electrophoresis revealed also existence of this component. The rapid component appeared about twice as great in concentration on the descending boundary. Ultracentrifugal study of the same plasma did not show the presence of any unusual plasma protein component.*

Electrophoretic separations of plasma proteins of 158 hospitalized patients in this study show that 30 per cent of these patients exhibited the double peak in the alpha₂ globulin and 6 per cent in the beta fraction.

Occurrence of double peaks in relation to specific diseases such as Addison's disease, nephritis, ulcerative colitis, multiple myeloma, pulmonary tuberculosis, biliary cirrhosis and others comprising the total number studied made it evident that the occurrence of double peaks could not be assigned to one specific disease. It was also evident that the plasma protein concentration and percentage distribution in each of the diseased groups agreed generally with observations reported by other investigators (1-9). For this reason the actual protein distribution in the diseases studied was omitted, and only examples of the double peaks in the alpha₂ and beta globulin were graphically illustrated (Fig. 1).

The occurrence of this double peak moiety was not persistent. Subsequent analysis of plasma of subjects who exhibited the double peak did not always result in appearance of a double peak. An
attempt was made to relate these occurrences to lipid changes in patients with kidney and various liver disorders, but the data did not reveal any correlation. An attempt was also made to relate this phenomenon to steroid excretion levels in subjects with various endocrine disorders but again the data did not reveal any correlation. Examination of the remainder of 313 electrophoretic patterns confirmed further lack of specificity for this phenomenon to any particular group of disorders.

Existence of a protein component with mobility greater than albumin has been observed in the plasma of normal subjects (10, 12-15). Reports have also been made of similar component in cerebrospinal fluid (16-19). Intravenous infusion of heparin into adult subjects resulted in appearance of a fast-moving component (21-28).

In their study of blood protein response in tuberculosis, Seibert and Nelson (10) reported x-component as a break in the leading side of the albumin curve. In their study, plasma was dialyzed in a heavy cellophane sack for 3 days against a phosphate buffer of pH 7.7 and ionic strength 0.1. Hoch and Chanutin (12) demonstrated a protein component in normal and pathological serum and plasma that migrated 18 per cent faster than the albumin. These authors also reported that 4 of 43 pathological sera contained 2 fast components with mobilities variable from patient to patient. These mobilities were 26, 33, 50, and 300 per cent greater than albumin. Smithies (14), using starch electrophoresis, was able to demonstrate repeatedly 2 fast-moving components in normal human serum. Gavrilesco et al. (20) demonstrated this also and concluded that the first fraction was identical to the fraction observed in the cerebrospinal fluid, while the second fraction was rich in lipids. Grabar (13) and Schultze et al. (15) reported one fast-moving component in normal serum and plasma. Schultze et al. (15) isolated this component by precipitation, adsorption, and zone electrophoresis. Their study revealed that this fraction, normally found in a concentration of 0.5 per cent of total protein, has a mobility when purified 50 per cent greater than albumin, is richer in carbohydrates (hexoses, glucosamine) and tyrosine and tryptophane, and has a molecular weight of 61,000. These authors have also shown that the prealbumin component normally found in plasma is not a lipoprotein, since it did not stain with Sudan black B or a protein denaturation product. Component x-2 as reported by Smithies (14) was rich in lipid.
Interesting observations have been made with regard to appearance of prealbumin component in plasma of subjects infused with heparin (21-28). In all instances reported, only one fast-moving component was evident. Mobility of this fraction was similar to the mobility of x-component observed in normal serum (12, 15). The changes in the serum lipoprotein pattern observed during the clearing reaction of heparin were followed by liberation of free fatty acids and appearance of the fast-moving component (26-28). The factor (C.F.) responsible for these changes has been localized by zone electrophoresis in the beta globulin fraction and has been shown to lower the beta and increase the alpha globulin concentrations (26).

It is evident from the foregoing statements that there may be several fast-moving components. These fractions have been found in plasma of normal individuals, in hospitalized subjects, in plasma of subjects receiving heparin, and in the cerebrospinal fluid. The fast-moving components reported in this paper have been found in the plasma of hospitalized, critically ill subjects. One patient’s plasma had 3 of these fractions with mobilities 58, 82, and 123 per cent greater than the albumin. Two fractions were found in the plasma of each of the 2 patients, with mobilities for x-1 component 58 and 70 per cent, and for x-2 component 550 and 406 per cent greater than albumin. One component was found in each of the remaining 3 patients with mobilities 65, 74, and 26 per cent greater, respectively. Average percentage concentrations of these fractions in terms of total protein present were 2.2, 2.4, and 3.2 for components x-1, x-2, and x-3, respectively.

Since no fast-moving component was observed in the plasma of 18 normal subjects examined for these, and only 6 of 158 hospitalized patients exhibited these prealbumin components, it must be concluded that occurrence of these fractions, as studied by the method presented in this paper, is not common and that their significance with respect to diseases in which they occurred still remains obscure.

References