Protein Electrophoresis

E. F. Wilt

N. C. Brady
PROTEIN ELECTROPHORESIS

E. F. WILT, M.D.* AND N. C. BRADY, M.D.*

Some background and clinical applications of serum electrophoresis are presented with particular emphasis upon technic of filter paper electrophoresis as performed in the laboratory of Henry Ford Hospital. The ability to separate the components of a protein mixture by means of electrophoresis is based upon the fact that on the individual protein molecule, in either an acid or alkaline solution, is carried an electrical charge. Therefore, it is possible to cause the migration of these molecules in an electrical field to either the positive or negative pole. The rate at which migration occurs is to a large extent determined by the size of the individual molecular charge. Furthermore, it is known that the size of the individual electrical charge on the protein molecule varies from one protein species to another. This basic principle underlying all of clinical electrophoresis was well known as early as the beginning of the 19th century. At that time physicists and chemists noted that it was possible to cause the migration of a colloidal solution through a clay filter in the presence of an electrical current. During the early years of the present century numerous investigations utilizing the technic were carried out on microscopic size particles such as red blood cells and bacteria as well as the separation of the components of colored colloidal solutions. However, the problem of separating sub microscopic sized particles such as protein molecules as well as the separation of the components of colorless colloidal solutions remained.

As a solution to the problem Arne Tiselius in 1937 introduced a method which made use of a complicated optical system and took advantage of the differential refractive indices of the separated proteins. He was able to photograph the individual bands of optical density caused by the protein segments as they migrated through solution. This technic proved to be an extremely effective one, allowing for the rather exact separation of individual proteins in solution. Unfortunately, it was limited in its clinical application because of the time consumed in individual analyses and the expense of the apparatus involved.

A method for measuring serum albumin and globulin known as the “salt fractionation” method had been used by clinicians for many years. With this technic the globulin fraction of the serum is chemically precipitated from solution and measured separately from the albumin which remains in solution. The technic is simple but subject to some definite errors. For instance, a portion of the globulin fraction frequently fails to precipitate from solution, particularly the alpha 1 globulin fraction which remains in solution with the albumin. The globulin fraction is determined along with the albumin, giving rise to falsely elevated levels of serum albumin and conversely low values for serum globulin.

The method of determining the distribution of serum protein by means of filter paper electrophoresis which was developed about 1950 represents a compromise between the relatively precise but tedious and expensive method of Tiselius and the more simple but inaccurate “salt fractionation” method. Because of its simplicity and accuracy, filter electrophoresis has had wide clinical use.

*Department of Medicine
Figure I
Normal pattern with report.

Figure I is a representation of a fairly normal distribution of adult serum proteins. You will note the strip of filter paper at the bottom with the dark staining bands of separated protein material, while above we have the graphic representation of these segments of protein. On the far right, representing the most rapidly migrating serum protein is the serum albumin, normally representing between 45 to 62 per cent of the total protein. Next is the alpha 1 globulin, normally between 3 and 7 per cent of the total. Next, the alpha 2, approximately 6 to 13 per cent. Then the beta globulin, between 9 to 18 per cent. And finally, the slow moving gamma globulin, ranging between 11 and 22 per cent.

In actual practice the strip of filter paper is impregnated with an alkaline buffered solution and a carefully measured amount of serum protein is applied to the paper. The filter paper is again immersed in the alkaline solution and an electrical current is passed through the solution. The protein is allowed to migrate across the filter paper over a period of 16 hours, at the end of which time a bromphenol dye is applied to the filter paper. This dye is bound fast to the free amino groups of the protein molecule, thereby affording a visual representation of the separated protein increments. The filter paper is then dried and subjected to a photoelectric cell analysis which measures the optical density of the various portions of the paper and constructs the linear graph which you see in Figure I. Finally the various segments of the graphic curve are separated by the pencilled lines which you see, the area beneath the curve is computed and the values reported as percentage of total protein. The total protein
Protein Electrophoresis

itself is determined by a colorimetric method entirely separate from the electrophoretic analysis. As you can see, the filter paper technique itself is subject to some error. In some cases it may be quite difficult to decide at what point the lines separating each segment of the graph should be made and the selection is often quite arbitrary. Also it can be noted that there is some blue staining protein material on the filter paper lying between the more definite bands of protein, and it is well known that a certain portion of the protein is absorbed on the filter paper during its migration. This is particularly true of the serum albumin. This source of error is to some extent being obviated by the introduction of a thinner filter paper, to which less of the protein material is absorbed.

Some of the generalizations which have been made in the literature regarding this subject follow. In a large series of cases reported in 1956 from the Walter Reed Army Hospital, the following general reports were noted. The most common alteration in serum proteins were:

1. A depression of serum albumin. This was seen most frequently in chronic wasting diseases, inanition, cirrhosis, malignant disease — particularly wide spread metastatic cancer, and in the various forms of nephrosis.

2. An elevation of alpha 2 globulin. This was seen most frequently in acute infectious or inflammatory states, such as pneumonia, infectious hepatitis, and acute thrombophlebitis.

3. An elevation in gamma globulin. This was noted most frequently in diseases in which one would expect an elevation of antibody titre. It was seen in tuberculosis, sarcoidosis, and in chronic inflammatory diseases of connective tissues such as lupus erythematosis, rheumatoid arthritis and periarteritis nodosum.

Protein patterns seen in most disease states are non-specific. Changes in the serum and plasma proteins usually manifest themselves by increases or decreases in the concentrations of the normal components, or by the appearance of the proteins not seen under normal conditions. These changes can almost be predicted without the electrophoretic analysis, if the common denominators of pathophysiologic conditions are reviewed. Thus, in infectious and inflammatory diseases, the decrease in albumin and increase in globulins have been demonstrated indirectly by the sedimentation rate, variations in the albumin-globulin ratio and by Rouleaux formation.

Some serum proteins are not seen under normal conditions. MUCOPROTEINS are normally occurring proteins which must be isolated at a low pH of 2-4.5. They are present in increased amounts in plasma of patients with pneumonia, bronchogenic and gastric carcinoma, lymphatic leukemia and Hodgkin’s disease. CRYOGLOBULINS are characterized by reversible cold precipitation. Electrophoretically, they migrate between beta and gamma globulins. They may be related to Raynaud’s syndrome and cold purpura; and are most often—though not commonly—associated with multiple myeloma; and are not diagnostic of any disease. There is no direct relationship between the amount of cryoglobulins, and the presence or severity of symptoms. The electrophoretic pattern of severe macroglobulinemia is indistinguishable from that of
multiple myeloma — this differentiation must be made by ultracentrifuge techniques. Marked macroglobulinemia is frequently associated with a definite syndrome "Waldenstrom's macroglobulinemia" — characterized by dyspnea, mucous membrane bleeding, and bone marrow infiltration of small atypical lymphocytes.

Serum protein values in ambulatory subjects are significantly higher than in patients or normal subjects at bed rest, owing to hemodilution when recumbent, resulting from return into the circulation of edema fluid which collects in the legs on standing. The highest plasma albumin levels occur in healthy subjects. In anorexia nervosa and in volunteers on a starvation diet—the plasma protein concentration and electrophoretic pattern is within normal range.

Each of the five component peaks represents 90-95% of that component, with small amounts of the others. The main limitation of electrophoretic protein analysis is the fact that the separation of protein components depends upon a single property — their mobility in an electric field.

In disease states associated with wasting or malnutrition, serum albumin is lower. It is especially pronounced when there is marked loss of albumin in the urine (nephrotic syndrome), when there is a disturbance in albumin formation (cirrhosis of the liver) or with extravasation of proteins (burns).

The following ten case abstracts illustrate these changes in electrophoretic patterns, and each pattern is given in Table I.

Table I

<table>
<thead>
<tr>
<th>Normal Percentages</th>
<th>GAMMA</th>
<th>BETA</th>
<th>ALPHA₂</th>
<th>ALPHA₁</th>
<th>ALBUMIN</th>
<th>TOTAL PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Average</td>
<td>11-24</td>
<td>9-19</td>
<td>6-14</td>
<td>3.8</td>
<td>45-62</td>
<td>6.3-8 Gm.</td>
</tr>
<tr>
<td>CASE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nephrosis</td>
<td>16</td>
<td>32</td>
<td>27</td>
<td>6</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>2. Diabetic Nephropathy</td>
<td>32</td>
<td>23</td>
<td>38</td>
<td>7</td>
<td>ABSENT</td>
<td>3.1</td>
</tr>
<tr>
<td>3. Laennec's Cirrhosis</td>
<td>44</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>28</td>
<td>5.4</td>
</tr>
<tr>
<td>4. Postnecrotic Cirrhosis</td>
<td>50</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>21</td>
<td>7.4</td>
</tr>
<tr>
<td>5. Thrombophlebitis</td>
<td>15</td>
<td>22</td>
<td>19</td>
<td>8</td>
<td>35</td>
<td>6.1</td>
</tr>
<tr>
<td>6. Rheumatoid Arthritis</td>
<td>40</td>
<td>14</td>
<td>8</td>
<td>4</td>
<td>34</td>
<td>8.1</td>
</tr>
<tr>
<td>7. Infectious Hepatitis</td>
<td>44</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>33</td>
<td>5.8</td>
</tr>
<tr>
<td>8. Carcinomatosis</td>
<td>22</td>
<td>24</td>
<td>17</td>
<td>14</td>
<td>23</td>
<td>3.7</td>
</tr>
<tr>
<td>9. Multiple Myeloma</td>
<td>39</td>
<td>13</td>
<td>12</td>
<td>6</td>
<td>30</td>
<td>8.9</td>
</tr>
<tr>
<td>10. Hypogammaglobulinemia</td>
<td>4</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td>64</td>
<td>6.3</td>
</tr>
</tbody>
</table>

CASE NO. 1.

The serum was obtained from a five year old white female who had a history of pyelitis at age 2, and was admitted with an 8 month history of periorbital edema. She was found to have 4 plus albuminuria and a few fatty casts. NPN was 35. Hgb. 12.5, WBC 16,150, cholesterol 550. Note here the elevated beta and alpha 2 globulins, with a markedly reduced albumin — a fairly consistent and diagnostic finding in nephrosis. After treatment with ACTH, the serum albumin may rise and the beta and alpha 2 globulins revert to normal concentrations.
Protein Electrophoresis

CASE NO. 2

A white male, 55 years old, with a five year history of diabetes mellitus. He had been known to have a high renal threshold for glucose, but had been well controlled by 28 units NPH insulin daily. Seven months prior to this serum protein study, he had developed peripheral edema resistant to therapy, and also subsequently pleural effusion and thrombophlebitis involving both legs. There was some question as to exposure to hepatotoxins in connection with his work in sales of cement sealers, disinfectants, etc. Liver biopsy showed fibrosis only, and an iron stain was negative. A renal biopsy yielded only a small piece of tissue and the patient would not allow it to be repeated. NPN 38 mg %. 24 hour urine contained 6 grams of protein. Hgb. 12.8 mg %. Bence Jones protein was absent. An LE test was negative. Note here particularly the elevated gamma and alpha 2 globulins and the total absence of albumin. The percentages here are undoubtedly altered by the markedly abnormal absolute concentrations. In this patient with diabetes mellitus with nephropathy, treatment with safl pure albumin over a one month period of time effected only an elevation of the albumin concentration to 5%.

CASE NO. 3

A 54 year old white male with a liver biopsy diagnosis of Laennec's cirrhosis who was admitted with bronchopneumonia in 1956, and in 1957 with bleeding varices, which eventually led to his demise. Serum bilirubin, total 9 mg %, direct 3 mg %, CO2 18.3 mg %, BUN 25 mg %, hgb. 11.5 gms %, cephalin cholesterol 4 plus, thymol turbidity 16 units, thymol flocculation 3 plus. Again note the markedly elevated gamma globulin and depressed albumin. Although it is certainly not diagnostic, in two thirds of cirrhotics the gamma globulin is found to be elevated.

CASE NO. 4

The serum was obtained from a 52 year old, obese, white female who had jaundice 12 years ago, followed by evidence of liver disease. Five years ago she had a splenectomy for hepatosplenomegaly, at which time a shunt was not possible due to technical reasons. A liver biopsy at that time revealed postnecrotic cirrhosis. In 1957 she first developed peripheral edema and two months later died with bronchopneumonia and lung abscesses. Note the markedly elevated gamma globulin and depressed albumin again on this patient. On a later study macroglobulins were found to be present by ultracentrifuge study.

CASE NO. 5

This serum was obtained from a 39 year old white male who had thrombocytopenic purpura 12 years ago, followed 7 years ago by a splenectomy. He was admitted with thrombophlebitis. Hgb. 18.5 gms %, WBC 15,450 with an increased number of polymorphonuclears. Platelets 130,000. Note here the moderate increase in the alpha 2 globulins characteristic of infectious states.

CASE NO. 6

Serum was obtained from a 56 year old negro female with painful swelling of her left knee which was diagnosed clinically as rheumatoid arthritis. Bence Jones
protein was negative. Note here the three-fold elevation of the gamma globulin and the moderately depressed albumin.

CASE NO. 7

The serum was obtained from a 30 year old white female who clinically had infectious hepatitis with a two month history of jaundice, malaise, dark urine, light stools and a distaste for cigarettes. She followed a rapid downhill course, terminating in coma and death three weeks after admission. Transaminase 416, bilirubin: total 13 mg %, direct 6 mg %. Alkaline phosphatase 4.4, heterophile negative. Again note the markedly elevated gamma globulin and depressed albumin in this patient with infectious hepatitis.

CASE NO. 8

Electrophoretic pattern of serum obtained from a patient who had had endometrial carcinoma for 2 years with abdominal carcinomatosis for 9 months. Although there is a definite tendency toward lower albumin and elevated alpha 2 globulin in carcinoma and sarcoma, this tendency is largely dependent upon the presence of widespread disease, and probably does not represent a specific abnormality due to the presence of malignancy. Note here that both the alpha 2 and alpha 1 globulins are slightly elevated and the albumin markedly depressed.

CASE NO. 9

The serum was obtained from a 60 year old colored male who had a pyarthrosis of his knee in 1955 when a peripheral blood smear revealed target cells, a left shift, Rouleaux formation, and 1 myelocyte. In 1957 he was found to have a blue mass growing on the lateral tongue margin, a liver 6 cm. below the costal margin and arthritic complaints. A bone marrow aspiration revealed myeloma cells and a diagnosis of multiple myeloma was established. Cryoglobulins were found to be present in his serum. The other findings as noted were a marked elevation of gamma globulin and a depression of the albumin with a high serum protein.

CASE NO. 10

The serum was obtained from a 9 year old negro boy with a long history of septic infections, draining ears, meningitis twice, and pneumonia three times. He has rheumatoid arthritis, has had one eye and his spleen removed. In the past year he has had 5 hospitalizations for infections. Six electrophoretic patterns have been almost identical to this one. A brother is also known to demonstrate a similar protein abnormality. Note here the marked hypogammaglobulinemia. Administration of regular doses of intramuscular gamma globulin has not altered the protein picture in this patient, for the gamma globulin has varied only from 4 to 6%.

Figure 2 touches upon the usefulness of electrophoretic study of the hemoglobins. The first filter paper strip represents 100% “A” hemoglobin, as found in normal adults. In sickle cell anemia, 85 to 100% of the hemoglobin is of the “S” type. The second filter paper strip represents the hemoglobin pattern found in sickle cell trait
Protein Electrophoresis

Figure II

Electrophoretic pattern of normal hemoglobin compared with that of hemoglobins S, and C.

in which approximately 35% of the hemoglobin is of the “S” type and 65% is of the “A” type. In hemoglobin “C” disease, 100% of the hemoglobin is of the “C” type. The third filter paper strip represents a mixture of the “A” and “C” types of hemoglobin as seen in a negro child with hemoglobin “C” disease who had received blood transfusions. Thus, we can see that electrophoretic study, while not necessary, is helpful in separating sickle cell trait from sickle cell anemia. It is also of definite value in establishing the diagnosis of hemoglobin “C” disease.

We have tried to point out some of the helpful clinical applications of the electrophoretic protein analysis. As you have seen pointed out on the chart and by the case summaries, many of the alterations are non-specific. The study of protein patterns by electrophoresis is fascinating, is not intended to be a substitute for good clinical judgment, and should be particularly correlated with the physician’s own estimate of the patient’s status.

BIBLIOGRAPHY