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Value of serum electrophoresis as a screening procedure

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THE VALUE OF SERUM ELECTROPHORESIS AS A SCREENING PROCEDURE

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ELECTROPHORESIS

I. History and Background

The term 'electrophoresis' was first used in the early nineteenth century. In 1807, the passage of water through clay by electro-osmosis was noted after passage of an electric current into the solution. Later, it was found that egg albumin showed specific migration patterns in an electric field.

The first apparatus designed for the electrophoretic analysis of plasma proteins was reported by Tiselius[46] in 1937. This was essentially a U tube, in which plasma was added to a buffer solution at pH 8.6, and to which were applied positive and negative electrodes. The variation in mobility of the electrically charged particles was photographically recorded on the basis of differences in the refractive indexes of protein-containing phases. In the alkaline medium, the particles become negatively charged and migrate toward the anode. Molecules of albumin, being smaller and more highly charged, migrate fastest while the larger molecules of globulin move more slowly. Boundaries, due to difference in refractive indexes, are established after suitable time and the photographic record of these variations is termed the electrophoretic pattern of the specimen. Analyses of many samples of plasma from normal subjects reveal six distinct boundaries identified as albumin, alpha-1 globulin, alpha-2 globulin, beta globulin, phi fraction (chiefly fibrinogen), and gamma globulin in

order of their mobility. Many modifications of the Tiselius method have been worked out, and are much simpler and more practicable than the original.

Paper was first used as the supporting medium in electrophoresis in 1939. Its use, however, was not widespread until 1950 when six investigators independently reported using paper.[6]

In this method, the electrophoretic migration takes place on filter paper, as the particles of a solution are completely separated in what amounts to a micro-method. Histochemical staining is proportional, to some degree, to the amount of substance present. Densitometry can be applied to these calculations as components are separated according to their rates of migration in an electric field. The obvious advantages associated with this method over free electrophoresis include stabilization of the various protein migratory elements, simplicity of the aparatus, and escape from the convection currents set up by the electrical heat in the latter method.[6] For all practical purposes, paper electrophoresis carefully performed and controlled is at least as good as other methods technically, and far superior in terms of cost, convenience, and simplicity.

In addition to strips of filter paper, starch gel, agar, cellulose acetate, and movie film have been used in an effort to effect clean separation of fractions. Immuno-electrophoresis, utilizing antigen-antibody reactions is being studied. In this method, paper slips

are soaked in antibody, and individual protein fractions are identified as precipitate lines in the antigen solution.[5] This has promise in the determination of genetic abnormalities involving haptoglobins that combine with hemoglobin in serum. In addition, electrophoretic analysis has contributed to the knowledge of hematologic disorders by the identification of several new forms of hemoglobin. More precise separation by acrylimide gel may make possible the analysis of cerebral spinal fluid components with accuracy.[42]

Electrophoretic patterns are, at present, admittedly an incomplete finding for the unequivocal diagnosis of disease. It is, however, a useful adjunct in the study of the dysproteinemias which are assuming such an important role in the understanding of the pathology of many obscure diseases, particularly the 'collagen' diseases and certain reticulo-endothelial and lymphatic system disorders. As the function of the plasma cells and lymphocytes are better understood in the production of these proteins, the 'patterns' of today may be translatable to a more exact scientific interpretation.

Speculation suggests that this situation may parallel electrocardiography which was dependent a few years ago on a myriad of patterns, but now has become more precise and predictable in the light of vector analysis and the newer understanding of ion transfer and membrane permeability change to create 'ionic currents'.

II. General Discussion

A. Physical and Chemical Considerations

A review of the physical and chemical basis of electrophoresis is necessary for the proper scientific interpretation of the final pattern.

Since amino acids, peptides, and proteins are amphoteric (ampholytes), their total electrical charge may be dictated by their environs. By placing proteins in a buffer solution which makes them all negative or positive and then establishing an electric field, they will migrate either to the anode or cathode. The total migratory result depends on numerous factors. First to be considered is the protein molecule itself. The sign and magnitude of its net charge are dependent on the span between its isoelectric point and the pH of the buffer solution. The greatest magnitude, and therefore mobility, occurs when the difference in these two entities is greatest. Of equal and related importance is field current and intensity. Time required for the procedure is reduced by increased voltage and subsequent increased electrical energy per square centimeter of paper. However, this produces heat which endangers the constancy of buffer concentration (by evaporation) and even may denature the proteins.[6] A fine balance may be achieved by use of cell walls of high heat conductivity or addition of such heat-dispersing substances as glycerine, propylene glycol, or even utilizing a helium atmosphere.

The filter paper used is of importance mainly in that its grade and composition must be constant because minor fluctuations may greatly affect migration resistance. The paper may be considered a capillary system offering the proteins a highly tortuous course to travel. If manufacturing procedures are uniform, the paper may be considered a constant in the system.

Also to be considered are the physical properties of the protein molecule. Large size and any degree of anisotrophy are both factors which tend to slow migration. The former affects mobility and the latter viscosity. Very large molecules such as fibrinogen and macroglobulin present as very discrete spikes in the final pattern. This is in contradistinction to low molecular weight, high motility molecules such as albumin which 'tail' and leave less discrete back boundaries.

Another factor of importance is the ionic quality of the buffer system. It should be remembered that the buffer ions may be absorbed on the surface of the protein molecule.[6] Considering the different sizes and electrostatic forces of the various protein moeities, it can readily be seen that protein charge alteration could be a problem. Therefore, the ideal buffer is one which affords a maximum of buffering capacity while contributing a minimum of ionic strength to the system. At present, the most nearly ideal combination is sodium diethylbarbiturate and diethylbarituric acid with a pH of 8.6 and ionic strength in the range of 0.06 - 0.08.

B. The Plasma Proteins

A basic understanding of the plasma proteins themselves is also necessary in considering the value of electrophoresis. As Gamble's graphic depiction of plasma constituents illustrates, the proteins are ionically of little significance, but in total mass they are the major constituent.[6] About 85 per cent of total solids in plasma are protein. As this has come to be realized, interest in them has increased. A convenient classification of the major types of plasma proteins is:

1. Albumin
2. Globulin
3. fibrinogens
4. lipoproteins, seromucoids, and nucleoproteins.

Albumin is unique in that its main function is probably fluid balance. It is small (molecular weight=69, 000), and abundant (3×10^{17} molecules/milliliter). It thereby exerts great colloid osmotic pressure. Another important function of albumin is its relatively great binding capacity. A partial list includes: toxins of many types, penicillin and sulfoamide derivatives, fatty acids, and aromatic carboxylic and acetylated amino acids.[6] A third and less extensively explored area of albumin function is that of several enzyme inhibitors which apparently travel with albumin.

The origin of albumin is chiefly the liver.[8] Clinically, this theory is substantiated by the sensitivity and reliability of a decreased albumin fraction associated with liver damage. Experimentally, Miller et al[31] showed by means of radioactive amino

acids that nearly all of the albumin, as well as fibrinogen and a significant amount of globulin, is formed in the liver.

It has been shown that there are actually two distinct types of albumin in human plasma. In cirrhosis and the nephrotic syndrome, the faster migration phase is much reduced and it has been shown that different sites of production probably exist.[6]

The alpha-1, alpha-2, and beta fractions are rather more difficult to discuss as to function and origin. There is much overlaping in their composition and the sites of their formation are largely unknown except that the liver has been definitely implicated as to origin. Alpha-1 and beta are the fractions richest in lipoproteins. The alpha-1 globulin is also the mobility range of most isolated mucoproteins. However, the clinically most significant portion of the mucoproteins is the hexosamine content. This is found in the following concentration: alpha-1 = 17.5 milligrams per cent, alpha-2 = 25 milligrams per cent, beta = 17.4 milligrams per cent, gamma = 24.7 milligrams per cent.[6] In terms of total amount per total fraction of globulin, it can readily be seen that it is an important constituent of the alpha globulins.

Properdin, the protein which, in the presence of complement and magnesium, is capable of lysing bacteria and erythrocytes and neutralizing viruses, travels with the beta globulin fraction.

Glycoproteins are found distributed throughout all the globulins in approximately the same proportion in each fraction. It is found

in only insignificant amounts in albumin.

The functions of the alpha and beta globulins are not entirely understood. It is known that they are responsible for the transportation of many substances not soluble in water or physiologic saline solutions. Fats and numerous fat-soluble substances are carried by these globulins. Fatty acids, cholesterols, and phospholipids are examples of the former, while certain hormones and vitamins fall into the latter category. Clinically, many observations and hypotheses have been offered concerning the alpha and beta globulins. Hexosamine, for instance, has been found to be elevated in cirrhosis, rheumatoid arthritis, and pneumonia.[6] The main elevation in the former disease, however, is in the gamma globulin hexosamine, as compared to an increase in the alpha-1 content in the latter two diseases. Glycoprotein elevation has been found in a porpourri of diseases including lupus erythematosis and idiopathic thrombocytopenic purpura. Much work is being done on this constituent in reference to the 'collagen diseases'.

Gamma globulin origin and function has been intensively studied and presents a more understandable picture than its other globulin compatriots. Much of our present knowledge of gamma globulins has been gathered by use of immuno-electrophoresis. This technique, in brief, utilizes preliminary fraction dispersion in agar. When this has been completed, specific anti-human gamma globulin

serum is allowed to diffuse perpendicularly in the agar. Visible precipitate lines are seen where the protein fractions and sub-fractions lie. This technique produced the evidence that three distinct gamma globulins exist.[16] These gamma globulins are divided into two gamma-one and one gamma-two components. A less confusing and more universal terminology is derived from their sedimentation constant values. The major portion of the fraction is comprised of gamma-two globulin which has a sedimentation value of seven Svedberg units (7S) and molecular weight of 160, 000. These globulins account for 90-95 per cent of the entire gamma globulin fraction. A high (1, 000, 000) molecular weight of globulin exists as one of the gamma-one constituents and has a sedimentation constant of 19S. The third type is also a gamma-one, but has a 7S value and molecular weight of 160, 000. This fraction is quite insignificant in terms of total amount, but the 19S macroglobulins constitute from 5-10 per cent of the total fraction.

The gamma-two globulins contain the majority of antibacterial and antiviral antibodies. The 19S macroglobulins include among others, the Wassermann, saline-agglutinating Rh, and Forssman antibodies. Also present are the antityphoid "O" agglutinins. An example of the related yet independent reactions possible within the gamma fraction is the response to diphtheria toxoid in which diphtheria antitoxin is found as a 7S gamma-two globulin, and skin-

sensitizing antibodies are found in the gamma-one component.[16]

Probably all gamma globulin is functionally antibody. Research techniques at present allow us only to identify a very small percentage of total gamma globulin as specific antibody. However, the practically infinite number of theoretical antibodies easily accounts for the remainder of gamma globulin. In strong support of the idea that gamma globulin is entirely antibody is the theory that all gamma globulin arises from antigenic stimulation of various components of the reticuloendothelial system.[6] It has been shown that animals in a germ-free environment develop only approximately one-third the gamma globulin that control animals do.

The origin of gamma globulin was long thought to be from lymphocytes and lymph nodes. Although this may to a certain extent be ture, recent investigation has pointed to plasma cells and undifferentiated cells which resemble plasma cells. Harris et al[20] in 1949 removed antigenically stimulated lymph nodes and made saline extracts of them. These extracts failed to show increased gamma globulin. 7S gamma-one globulins are apparently from not only plasma cells but also a transitional type of plasma cell. The average normal person has about 300-600 milligrams per kilogram of circulating gamma globulin and approximately 0.6-1.2 grams per kilogram of total body gamma globulin. The average half life of gamma globulin is 20-35 days,

so a fairly great turnover is usually in effect.

Infants are born with a normal level of gamma-two globulin because of placental transfer. However, synthesis does not begin until 4-12 weeks of age, with normal adult level being reached by approximately 6 months to 1 year. It can be seen, considering the half life of gamma globulin, that the individuals lowest level is at 1-3 months of age.

C. Specific Considerations of Disease

The usefulness of electrophoresis is by no means limited to research problems. In diagnostic problems of medicine, the changes in serum proteins are an important facet in the complete picture of various diseases and in following their progress. The albumin-globulin ratio has long been recognized as a valuable index of liver function and in various kidney disorders. Low values for albumin may result from impaired synthesis or increased catabolism in the liver or from increased elimination by the kidney. Rise in globulin may indicate accumulation of abnormal proteins, as in myeloma, or reticulo-endothelial activity increase.

Variations in albumin-globulin ratio, while a simple calculation, may signal quite complex patterns of protein change. Changes in serum protein values are found in many pathologic conditions. An early indication of these changes was the finding of Bence Jones protein in multiple myeloma in 1845. Many aberrations are known now, and these are often manifested by increase or decrease of normally occurring protein molecules or dysproteinemias. Synthesis of abnormal proteins, or paraproteinemia, is reflected often by different type peaks, occurring somewhere between the albumin and gamma globulin peaks. Some have mobility similar to normal proteins and appear as a concentration of a normal protein. The other two general categories of protein abnormality, hypoproteinemia and hyperproteinemia, are self-explanatory.

The remainder of this section will be devoted to a literature review of electrophoretic findings in a number of major disease categories.

1. Hypogammaglobulinemia

This entity was first described in 1952 by Bruton[9] and as a primary disease exists in three forms. These are:

1. Congenital - a sex-linked recessive found mostly in males.
2. Acquired - mostly in adults of both sexes
   a. idiopathic
   b. secondary - usually granulomatous or neoplastic lymphoid disease.
3. Transient or Physiologic.

Gitlin[16] described another defect in gamma globulin synthesis in which one or more fractions (gamma-two, 19S gamma-one, or 7S gamma-one) are lowered in the face of at least one normal component. Also included in his classification are the generalized hypoproteinemias found in the nephrotic syndrome, exudative gastroenteropathies, and in idiopathic hypercatabolic hypoproteinemia.

The hypogammaglobulinemias are associated with repeated infection (frequently upper respiratory in type), decreased gamma globulin production, and pavcity of antibody formation. When treatment is necessary, maintenance may be carried out by infusion of pooled gamma globulin.[17]

True agammaglobulinemia is probably quite rare. This has been confirmed by Gitlin[16] with immunoelectrophoresis. Paper electrophoresis probably gives an inaccurate picture of the total gamma globulin in that much of the projected gamma fraction may be other proteins which did not migrate to their expected positions.[38]

Barett et al[4] suggest that immunoprecipitation methods are best for study and diagnosis of low gamma globulin levels. Amounts as small as 1-5 milligrams per cent may be accurately measured. Puckett[38] points out that the gamma decrease should be out of proportion to any decrease in other fractions. A uniform decrease is more suggestive or prolonged dietary deficienty of protein, malabsorption syndromes, pernicious anemia, cancer of the stomach, celiac syndrome, ulcerative colitis, or cystic fibrosis.

Sunderman and Sunderman[44] present four cases of hypogammaglobulinemia with concentrations of 0.0-0.5 grams per cent of gamma globulin. The mean was 0.3 grams per cent.

Transient hypogammaglobulinemia refers to the period when the newborn's supply of maternal gamma globulin is depleted, and he is yet unable to produce any of his own. Angelopoulos and Bechratis[1] showed that Total serum protein and globulin levels were lowest (means of 5.85 and 2.09 respectively) at ages 1-3 months in their series. It is also known that skin reaction to skin testing is weakest when gamma globulin is lowest.[11]

A relationship between hypogammaglobulinemia and the 'collagen diseases' is quite possible. Wolf et al[51] reports acquired agammaglobulinemia followed by rheumatoid arthritis, idiopathic thryombocytopenic purpura, systemic lupus erythematosis, and hemorrhagic tendency. Page reports two cases of lymphocytic leukemia preceded by hypogammaglobulinamia; this raises the

interesting question of the importance of decreased host resistance and leukemia.

2. Acute and Chronic Infections

Into this category fall many of the so called 'non-specific' patterns. It is generally agreed that large series of cases will show a decreased albumin fraction, increased alpha (particularly alpha-2) fraction and equivocal to slightly increased beta and gamma globulins. Brackenridge and Crillag[7] emphasize the consistency of alpha globulin increase, particularly in pneumonia (the majority of their series falling into the third to fourteenth standard deviation elevation) and acute bacterial infections. The increased alpha globulins are felt by the authors to represent increased hexosamine concentration in the serum, which they chemically cinfirmed in the majority of their cases. The findings in chronic bacterial infections are much the same but frequently not as pronounced. Viral infections are often associated with raised gamma globulin.[7]

Tuberculosis and Boeck's sarcoid are more specific and represent somewhat of an electrophoretic differentia. Minimal tuberculosis infection presents a reciprocal albumin decrease and gamma globulin increase. More severe cases characteristically develop a markedly decreased albumin, increased gamma globulin, and significantly raised alpha-2 globulin. A sharply peaked, increased beta globulin is highly indicative of caseation (33). Alpha-1 is characteristically unchanged. The best indication of serious disease is markedly increased gamma and alpha-2 globulin (this is seen most frequently in pleuritis with effusion) while

remission is immediately reflected in alpha-2 return to normal.[33] This is felt by these authors to be the most sensitive of all prognostic indicators in tuberculosis.

The "sarcoid stair-step" pattern is easily recognized. Sunderman and Sunderman[44] report a series of nine sarcoidosis patients in which the alpha-2 through gamma globulin and Total serum protein are all significantly elevated. McQuiston and Hudgen[29] report that these findings are present in at least 70 per cent of Boeck's sarcoid. In their series, they found that 66 per cent of tuberculosis patients had typical tuberculosis patterns. Only 7 per cent of sarcoid patients had tuberculosis-like patterns whereas 28 per cent of tuberculosis patients had stair-step findings. None-theless, they conclude that electrophoresis is a useful adjunct in the laboratory differential diagnosis between these two diseases.

3. Renal Disease

One of the more characteristic of all abnormal electrophoretic patterns is found in association with nephrosis. In all cases, alpha-2 globulin is greatly increased as contrasted to greatly decreased albumin, alpha-1 globulin, and gamma globulin. Longsworth and MacInnes[27] report that beta globulin is frequently greatly increased in lipid nephrosis. These peculiar changes are the result of lipid and cholesterol elevation. Diuresis following treatment with corticosteroids results in a return to a normal pattern. The reason for these findings is probably the result of a changed glomerular membrane permeability which allows wasting of smaller proteins in combination with selective catabolic increases involving all but alpha-2 globulin.[10] Wakim and McKenzie[49] have shown that no abnormal protein is associated with the pattern alteration and support the above theory of glomerular damage.

Sunderman,[44] Brackenridge,[7] and Rosenthal[40] all agree that acute nephritis, chronic glomerulonephritis, and pyelonephritis are associated only with the immediate response pattern of slightly decreased albumin, increased alpha globulin, and slightly or no gamma globulin change.

In all reports, it was stated that return to normal electrophoretic pattern was associated with an improved prognosis.

4. Hepatic Diseases

In the area of hepatic disease, electrophoresis may be highly characteristic or might be at its most confusing. Since the liver is the organ of production of albumin and much of the globulins, any alteration of its function is likely to be mirrored quickly in plasma protein abnormality. Herein lies the basis for much of the confusion in interpreting abnormal electrophoretic patterns, for any disease associated with even the most subtle secondary involvement of the liver is likely to produce an abnormal pattern. Even when marked liver disease is present, care must be taken in interpretion before ascribing the protein alteration entirely to the liver damage. Many authors have written regarding the differentiation of the 'pure liver disease pattern' from that association with diffuse disease. Popper and Schaffer[36] claim that in pure hepatocellular damage hypoalbuminemia and a decrease of either or both of the alpha globulin fractions will always be found, whereas in any other disease associated with decreased albumin, normal or elevated alpha globulins are the rule.

A good correlation between liver damage severity and the amount of albumin decrease exists; however, chronicity is also a factor in the degree of hypoalbuminemia.[48] By the same token, protein return to normal is a very sensitive and reliable indication as to prognosis.

The pattern seen with Laennec's cirrhosis is the most charac-

teristic in this group. Albumin is greatly and consistently lowered. In Brackenridge's[7] series of 20 cases, all the albumin values were within the range of minus three to eight standard deviations from the normal mean. Both he and Sunderman[44] report low to low-normal alpha globulins and normal beta globulin. Gamma globulin characteristically appears as a high, broad, often jagged peak. Brackenridge's series has all 20 patients in the range of plus three to fourteen standard deviations from normal. The breakdown of the hepatic mesenchymal cells is thought to stimulate the Kupffer cells and histiocytes of both hepatic and extrahepatic tissues to produce gamma globulin which forms a heterogenous gamma peak consisting of many globulins of slightly different origin, composition, and mobility.[48] Hence, the degree of this phenomenon as reflected by the gamma peak is of highly significant prognostic value. Another very unfavorable protein change late in cirrhotic disease is decreased beta globulin. When massive enough parenchymal destruction has taken place, globulin production is impaired to the extent that insufficient beta globulin may be found in xanthomatous biliary cirrhosis.[40] Because of the multi-mobility gamma globulins, this peak and the beta peak may appear to be fused (tear drop pattern) which is quite characteristic in cirrhosis. For this reason, many errors are made in separation of the fractions, making the beta often times artifactually elevated.[24] Brackenridge reports that the above changes are not found in cardiac

cirrhosis or 'precirrhosis' (hepatomegaly and chronic alcoholism). In this series of eight such cases, the patterns were essentially normal.

The changes in viral hepatitis are more in question. Sunderman and Sunderman[44] report that the Total serum proteins and albumin values are normal; alpha-1 decreased, and gamma globulin is equivocally increased. Brackenridge[7] found in his series that albumin is lowered, alpha-1 increased, and gamma globulin ranged from high normal in mild cases to markedly increased in severe cases. Rosenthal[40] suggests that an immediate response pattern is the rule with alpha-2 increase being the primary alteration. Quite possibly all are correct in that the immediate response is simply one to infection and any changes past that are reflections of the severity of liver damage as the disease progresses.

Rosenthal and Wagner[40] report the findings in several other liver disorders. In fatty metamorphosis, they report increased beta globulin levels. Mild cholecystitis is likely to produce an immediate response pattern, whereas, severe gallbladder disease produces decreased albumin and moderately increased alpha-2 and gamma globulin. They also report that common duct obstruction produces little change until liver damage is caused in which case gamma globulin begins to rise. Finally, they point out that electrophoretic changes may be absent in some diseases associated with

liver damage. Most consistently the following are examples:

1. acute hepatitis
2. hepatic amyloidosis
3. fatty liver in hepatic failure
4. some metastatic carcinomas and lymphomas.

5. Collagen Diseases

One of the more important areas of disease as regards electrophoresis is that of the so-called collagen diseases. There has been much speculation as to the relation between these diseases and hyperimmunity. Should such a relationship exist, the value of the entire scope of electrophoretic procedures may rightly be expected to increase greatly. Much study of the plasma proteins has already been done, particularly of lupus erythematosis. Grigsby, Bullock, and Fuertes[18] have reported six cases and reviewed the literature extensively. They believe that there is a typical form to the gamma globulin peak of the protein pattern of the serum, and have observed the same configuration in the pericardial fluid of a patient with effusion due to lupus erythematosis. The peak was observed to rise sharply on the cathode side and to slope granually on the anode sied. This has been found to be presnet before the lupus erythematosis cell test could be demonstrated. An additional source of information in the treatment of these patients is the demonstration of marked disturbance of gamma globulin. It has been suggested that these patients would benefit from administration of pooled gamma globulin as protection against infections which often are fatal in this condition. The configuration of the peak in gamma globulin has been noted to change from peaked to broad after adrenal cortical steroid therapy. The L-E cell phenomenon is apparently due to an antibody related to the alpha globulins. This is an antibody for nucleoprotein, and it forms an amorphous

mass of nuclear material which is recognized as the L-E cell.

Detection of early renal involvement in systemic lupus erythematosis is very difficult by ordinary means, and pathologic changes have been found repeatedly in patients with normal urinary findings. The report by Stevens and Knowles[43] on electrophoretic analysis of urinary gamma globulin in lupus nephritis is of considerable importance. Their study was made on 22 patients with diagnosis established by positive L-E cell preparations. Control groups included 28 patients with other renal disease and 10 without nephropathy. Disproportionately high urinary gamma globulin was found consistently in early lupus nephritis and follows roughly the degree of activity of the disease. Total protein seemed to correlate with renal damage rather than with stage of activity. Theoretical basis for the expectation that the urine might show increase in gamma globulin lies in the finding by electron microscopy that the basic 'wire-loop' lesion results from deposition of protein material in the glomerular basement membrane. Further studies are necessary to verify the consistency of the elevation of urinary gamma globulin in the early active phase of lupus nephritis and its decrease as activity lessens.

Pollack et al[35] discuss extensively the various protein abnormalities in different phases of the disease. In their overall series of 49 patients, they found significantly lowered Total serum proteins and albumin. The alpha-1 and beta globulins were essentially normal; but the alpha-2 and gamma globulins were significantly elevated,

especially the latter. Of additional and perhaps more significant interest was the finding of a gamma to albumin ratio of .55 in systemic lupus erythematosis patients compared to .28 in normal subjects. The authors feel that this ratio is the most valuable finding in assessing prognosis and progress in the disease. The alpha-2 globulin was highest in cases of active disease with proteinuria and often normal in inactive or treated disease. All the proteins tend more toward normal levels when the patient is usine Prednisone. Very severe, terminal cases sometimes have no increase in gamma globulin, but generally a greatly increased, heterogenous-type gamma peak is seen. Grigsby et al[18] suggest the possibility of this representing a paraproteinemia.

The changes in rheumatoid arthritis, although not particularly specific, are very sensitive indicators of the activity of the disease. Sunderman[44] and Brackenridge[7] both found the primary changes to be decreased albumin, slight to moderately increased beta and gamma globulin and marked increases in the alpha globulin, particularly alpha-2. The Total serum protein was esentially normal. The increased alpha globulins (mostly of glycoproteins) correlated well with the degree of inflammatory activity. Steroid treatment is associated with normal gamma levels.[19] Acute rheumatic fever has a very similar pattern to that observed in rheumatoid arthritis.

Sunderman and Sunderman[44] report 5 cases of diffuse scleroderma in which the Total serum protein, albumin, and each of the globulin fractions are proportionately decreased. These patients all had

significant visceral involvement.

Brackenridge[7] reports 4 cases of polymyositis and one case of polyarteritis nodosa. The former showed slightly decreased albumin, markedly elevated alpha-1 and alpha-2 globulins and essentially normal gamma globulin. The latter pattern was different in that a marked gamma globulin elevation was present.

6. Hematologic Diseases

Of all disease types, probably none has been investigated as much as hematologic diseases in terms of their associated electrophoretic patterns. This interest has been justified because many of the more predictable and spectacular electrophoretic patterns are found associated with this disease group. Much study, some of it dating back to Tiselius' original work, has been done on especially multiple myeloma, macroglobulinemia, idiopathic paraproteinemias, and Hodgkin's disease.

a. Myeloproliferative disorders

Much disagreement is found as to the characteristic pattern in chronic granulocytic and monocytic leukemias. Sunderman and Sunderman[44] report that gamma globulin is very elevated, with slight alpha-2 increase and slight albumin decrease, all being in the presence of a normal Total serum protein. Fahey and Boggs[12] concur with this finding. Brackenridge[7] found a slight alpha-2 increase, slight albumin decrease; but the remaining fractions were normal. Each author cites reports in agreement with his findings. The only consistent abnormality is alpha-2 increase which is nicely explained by Miller and Sullivan[30] who found that increased vitamin B_{12} was bound and circulating in chronic granulocytic leukemia and that the B_{12} binding protein travelled with the alpha-2 fraction, thereby effecting a consistent increase in the fraction. Brackenridge[7] reports no significant change in polycythemia

vera, myelofibrosis, or erythroleukemia.

b. Lymphomas and Lymphocytic Leukemia

There is little argument as to the findings in chronic lymphocytic leukemia.[7, 12, 40, 44] Most prominent is the markedly decreased gamma globulin, often to levels of clinical hypogammaglobulinemia. Sunderman and Sunderman[44] found a mean gamma globulin value of 0.4 grams per cent. This low level is often associated with the onset of severe infection. Fahey and Boggs[12] feel that this is a good method of predicting prognosis. They also note that treatment with antimetabolites and adrenal corticosteroids showed no direct effect on the serum proteins in their series. As mentioned earlier, agammaglobulinemia preceeding leukemia may represent more than a chance relationship.[34]

In Hodgkin's disease, the most consistent finding was that of increased alpha-1 and alpha-2 globulins. Albumin is decreased and gamma globulin generally increased until the terminal state was reached, at which time hypogammaglobulinemia often was manifest. Gaulian and Fahey[15] attribute the alpha increases entirely to protein-bound hexose, which is also much increased when chemically determined.

Lymphosarcomas, follicular lymphomas, and reticulum cell sarcomas are all most prominently associated with increased gamma globulin, often suggestive of paraproteinemias.[5] In all these diseases and also Hodgkin's disease, a correlation between plasma cell concentration in the bone marrow and amount of gamma globulin

could be made.

Plasmocytosis was associated with increased gamma globulin and even Bence Jones protein was demonstrated in one case.[18]

c. Multiple Myeloma

A tremendous amount of material concerning this disease and its protein changes may be found in the literature. Multiple myeloma produces some of the most definitive dysproteinemias and paraproteinemias. Longworth,[28] in 1939, reported a homogeneous peak in the beta region of two cases. Kekwick[25] found changes in gamma and beta globulins. Atamer[2] reports an alpha-2 myeloma protein.

There have been many reports of myeloma patterns, with the so-called 'M' proteins being found as peaks ranging from beta to slower than gamma in electrical mobility. Recently, a report from the Mayo Clinic[26] in 165 patients with multiple myeloma showed diagnostic 'myeloma proteins' in 126 and abnormal patterns in 30 more. In addition, it was reported that in 6051 serum electrophoretic patterns in non-myeloma patients, only 15 had resemblance to myeloma. These included macroglobulinemia, amyloidosis, and lymphoma. The ratio of height to width of the abnormal proteins as calculated by desitometers is found to be useful in delineating the true myeloma proteins. Other diseases which might simulate multiple myeloma in these patterns are cirrhosis, hepatitis, osetoarthritis, and metastatic carcinoma to bone. These, however, should present sufficient diagnostic evi-

dence in other respects to prevent difficulty here. It is felt that the electrophoretic patterns in multiple myeloma are definitive enough to be useful, particularly since the presence of Bence Jones proteins in the urine is not consistent, and bone marrow diagnosis is not universally available. Electrophoresis of urinary proteins show the same 4:1 height-width ratio of the abnormal component in 36 per cent of the Mayo Clinic series reported.

The origin of the abnormal protein and whether or not there was significant difference between the Bence Jones protein, M-protein, and the alpha, beta, gamma peaking types has been extensively studied; the general consensus being that the proteins are produced by the malignant "myeloma cells"[21, 39, 50]. Sedimentation and molecular studies indicate that the urinary Bence Jones protein is markedly different from normal serum protein in that its molecular weight is small, its sedimentation constant different (2.8-3.7X), its halflife extremely short (1.5 days), and its nitrogen concentration greater[6]. In addition, it has been shown that these characteristics are quite unlike those of myeloma gamma globulins which have a sedimentation constant of 7S. Furthermore, some investigators feel that the myeloma globulin found associated with alpha and beta globulins or those which migrate independently may well be unlike either the Bence Jones or gamma proteins[21, 26].

Reiner and Stern[39] studied the sera of 91 multiple myeloma

patients and found that 78 per cent of these presented major protein anomalies while 22 per cent were associated with minor changes. Fifty-five per cent of the major abnormal patterns showed the pathologic protein to migrate with or in the vicinity of gamma globulin. Fifteen and four-tenths per cent traveled with or near beta globulin and 6.6 per cent with alpha globulin.

Grigsby et al[18] found that relapse of multiple myeloma was usually associated with a decreased albumin and increased alpha-1 and gamma globulins. However, with spontaneous or urethane-induced remission, alpha, beta, and gamma globulins all fell with albumin increasing.

d. Macroglobulinemia

Since Waldenstrom first described macroglobulins in 1944 and then reported a disease characterized by idiopathic macroglobulinemia in 1948, this subject has been intensively studied and reported on.[37]

Macroglobulins are large proteins (molecular weight=1,000,000 or more) with sedimentation rates in the vicinity of 20-22S, and are normally in the plasma at concentrations of 0.2-0.4 grams per cent. They travel with alpha-2 or gamma globulin.[40, 18] They are frequently seen secondarily in association with a wide spectrum of diseases such as liver cell damage, nephrosis, leukemia, rheumatoid arthritis, carcinomas, lupus erythematosis, or even arteriosclerotic heart disease.[40]

Waldenstrom's macroglobulinemia, in which the abnormal

protein is generally thought to be causative, is characterized by an increased erythrocyte sedimentation rate, pallor, edema, decalcification, Raynaud's phenomena, and generalized "lymph node enlargement" and hepatosplenomegaly.[37] A prominent bleeding defect may be included, in which case, the disease may be known as Waldenstrom's purpura.[50] In either case, the etiology of the condition stimulates much conjecture. It is probably most closely related to lymphocytic leukemia or multiple myeloma, although the prognosis is much better than in either of these diseases. It has also been seen prominently in association with lymphosarcomas.[37] In the case of the purpuric manifestation, it has been shown that the macroglobulins interfere with fibrin formation by absorbing some of the clotting factors.[50]

e. Other Abnormal Proteins

Cryglobulins, as the name implies, precipitate at 5°C. They are generally found migrating with gamma globulin. Clinically, they can be 'essential', the details of which are obscure. Most frequently, however, and of great prognostic or early diagnosis potential, they are associated with such varying disease states as multiple myeloma, lupus erythematosis, subacute bacterial endocarditis, polyarteritis nodosa, rheumatoid arthritis, cirrhosis, polycythemia vera, lymphocytic leukemia, and lymphosarcoma.[40] Chronic purpura is a frequent manifestation in the presence of cryoglobulins.[50]

Another little known entity is pryoglobulinemia. This protein

appears at 40-60°C. It is most often associated with multiple myeloma, but Varges and Wenzel[47] report two cases associated with lymphomas, one being a lymphosarcoma, the other a reticulum cell sarcoma.

7. Endocrine and Metabolic Diseases

Only a limited amount of work has been done on diseases in this category and, as might be expected, the protein changes in most cases are slight or none at all.

Brackenridge[7] and Jencks et al[23] report a sharply peaked, elevated beta globulin peak in severe diabetes mellitus. This is explained by an increased lipoprotein. Sunderman and Sunderman[44], however, found no beta globulin abnormality in their series of 12 cases. They did find that the Total serum protein and albumin were significantly decreased with slight alpha-2 increase.

More interest has been placed on the serum proteins of patients with thyroid disease. In cases associated with any significant increase in thyroid autoantibody titer such as is often found in Hashimoto's disease or chronic non-specific thyroiditis, there is invariably a large increase in gamma globulin and Total serum protein, a significant elevation of alpha-2 globulin with slight or no change in albumin, alpha-1 and beta globulin[41]. In cases of these diseases where the thyroid autoantibody titer was normal, the protein changes were also absent.

In gout, Brackenridge[7] found that the alpha and beta globulins were slightly increased.

8. Neoplasms

In the area of neoplasia, many diverse and at time bizarre electrophoretic patterns have been reported. It can be generally concluded that no specific changes may be expected and that the general health of the patient as regards his tumor is most consistently reflected in the protein pattern at any given time. Many factors come into play such as metastasis, degree of liver involvement, diet, and ascites.

In general, it can be said that the Total serum protein and albumin will be markedly lowered.[7, 23, 24, 40, 44] After this finding, the globulin levels will vary extremely from report to report. Fairweather[13] reports beta globulins ranging up to 43 per cent. Fahey and Boggs[12] report a case of carcinoma of the ovary with alpha-2 and beta globulins each displaying two distinct bands. They also cite a squamous cell carcinoma with the same findings.

Jenck's et al,[23] in a series including 76 malignant and 33 benign neoplasms, present a definitive analysis of expected changes. For malignant tumors they found most had low albumin and high alpha-2 and gamma globulin concentration in order of decreasing frequency. They found that the majority of benign tumors were associated with no change.

9. Gastrointestinal Disease

The liver has already been discussed. Of the remainder of gastrointestinal disorders, most are electrophoretically rather non-specific and reflect protein depletion primarily.

The etiology of this depletion has been the subject of many papers. Jeffries, Holsted, and Habman[22] recently completed studies very suggestive of protein leakage through gastrointestinal mucosa as being the primary cause of protein depletion. They suggest that decreased synthesis or lessened absorption probably plays little part in the hypoproteinemia seen with most gastrointestinal disorders.

Brackenridge[7] found that many gastrointestinal disorders were associated only with the changes seen with infection. As may be expected, these included cholelithiasis and pancreatitis. He also reports that no consistent changes, if any at all, were found with peptic ulcer disease per se, but complications such as bleeding may be associated with minor protein abnormalities. Ulcerative colitis in an active state generally shows a marked depletion pattern with albumin levels as low as 0.87 grams per cent opposed to a generally much increased gamma globulin. This may be in the absence of edema. Awwad[3] studied cases of marked protein loss in kwashiorkor and found no relationship between hypoalbuminuria and edema or the severity of either. Malabsorption syndrome is also generally associated with marked depletion patterns; however,

in this case, as may be expected, no gamma globulin increase is found.

10. Obstetric Conditions

Normal pregnancy is associated with one of the most consistent electrophoretic changes. The Total serum protein and albumin decrease fairly steadily as pregnancy progresses.[23] At the same time, all the globulins tend to increase. The most notable of these, however, is the beta fraction which usually reaches 17-18 per cent.[7, 31, 23] This is attributed to beta lipoprotein increase with the phospholipid/cholesterol ratio remaining constant in spite of the total elevation.[7]

Miles and his co-workers[32] made an interesting study comparing electrophoretic patterns of sera from normal pregnancies and from early and late preeclamptics. In preeclampsia, they found conclusively that beta globulin tended to be even more elevated and that a concurrent elevation of alpha-2 globulin existed. Brackenridge[7] found much the same results and further showed an increased hexosamine content in the blood of preeclamptics. This tends to explain and confirm the alpha-2 increase. Miles[32] further states that the finding of increased beta and alpha-2 globulins even in asymptomatic pregnant women is presumptive evidence of impending preeclampsia.

11. Miscellaneous Disease

Mental Disease - Much work has been done in trying to equate mental diseases with protein change as reflected in elecrophoretic patterns. Most such attempts have been notably unsuccessful. Brackenridge[7] included 48 various mental disorders in his series and found no significant change except in those in which acute organic brain disease was present. This organic disease was associated with the typical immediate response 'tissue injury' protein pattern. However, in a study conducted by Fessel[14] on the sera of 40 psychotics, 37 showed hypergammaglobulinemia and split beta globulin peaks. These patients were carefully screened as to the possibility of liver or other disease. Further investigation may show significant and predictable electrophoretic anomalies.

Cardiovascular - The relationship between plasma protein abnormalities and cardiovascular system disease has been extensively dealt with in the recent literature, but as yet the results of this investigation have been generally equivocal. Hence, this subject is included in the miscellaneous discussion and reports only the findings of two fairly large series. Brackenridge[7] found only a tissue injury response in angia pectoris, myocardial infarction, and cerebrovascular accidents. However, he consistently found high normal to elevated beta globulin in his patients classified as having 'cardiovascular disease with generalized

atherosclerosis'. He explains this as being due to increased lipoprotein content. Jencks et al[23] did not find the beta globulin elevation but did find generally decreased albumin and slightly increased globulin fraction.

Dermatologic Diseases - Tickner[45] reported on several dermatologic diseases. His conslusion was that the protein changes were either slight or quite non-specific in the majority of instances. He did find exfoliative dermatitis associated with significantly decreased Total serum protein and albumin. In addition, pemphigus vulgaris exhibited marked changes consisting of decreased Total serum protein, albumin, and gamma globulin while the alpha globulins were both increased. Pemphigoid disease, however, had a normal gamma globulin level.

Bronchial asthma - Brackenridge[7] studied 18 cases of asthma with great interest. His untreated cases displayed low normal albumin, increased alpha-2 and gamma globulins, and slightly raised alpha-1 and beta globulins. This he attributes to "recently isolated beta 2A globulins and fast moving antibodies". The significance of this finding is thought to be that it represents a delayed reaction to tissue necrosis.

Allergy - Dees and Grunt[11] studied electrophoretic patterns in allergic children and found that no specific changes could be expected in such diseases as eczema or allergic rhinitis. They did find, however, that a correlation exists between gamma globulin level and the intensity of skin reaction to various allergens. As

may be expected, low gamma globulin predisposes to weak skin reaction.

III. A Study of 237 Electrophoretic Patterns at the University of Nebraska Hospital and Clinics

A. Purpose and Intent

The vast majority of material in the literature that deals with paper electrophoresis of the plasma proteins consists of carefully screened and selected patterns demonstrating specific pathology. Even though these reports have conclusively proved what pattern might be expected in such diseases as multiple myeloma, cirrhosis, nephrotic syndrome, etc., much criticism has been directed at electrophoresis because it is supposedly "unreliable"; i.e., it is abnormal or normal when such findings are unexpected. Therefore, the value of the procedure is frequently questioned when its use is proposed in any but the "classic" protein diseases. Much of the basis for the "unreliable" description of the procedure stems from the interpreters' lack of basic knowledge concerning the plasma proteins and the pathophysiology of the diseases reflected in the electrophoretic patterns.

The purpose of this paper is to review a consecutive number of electrophoretic patterns and their associated clinical findings with no advance regard as to diagnosis, reason for ordering or what results were expected. In view of the lack of preliminary criteria, it is reasonable to expect that a fair measure of the reliability of the technique as both a screening and specific procedure might be obtained.

Because it is felt that serum protein electrophoresis is not only reliable but also a laboratory test as useful as the blood count, sedimentation rate, or urinalysis in assessing a patient's general health, this study attempted to demonstrate impartially that a significant majority of results are entirely predictable. To achieve this end, all available electrophoretic patterns run in the University of Nebraska clinical laboratory on our hospital and clinic patients from November 15, 1959, to May 25, 1961 were reviewed.

This comprises a truly random sampling as no charts were unduly included or excluded for any reason. All patterns were studied with the patient's chart so as to place the electrophoretic findings in proper clinical perspective. Two hundred and thirty-two patients were reviewed. This included two hundred and thirty-seven electrophoretic patterns because in five individuals one other pattern was significantly different to merit its inclusion for purposes of contrast.

Paramount to any such project is an extensive review of the literature which should include broad consideration of the chemistry and physics involved as well as the findings in various disease states. Such a review is found in the two preceeding sections.

B. Materials and Normals

The electrophoretic patterns to be discussed were all run in a Spinco model R, series D, Durrum type cell. The power supply is a Spinco Duostat by Beckman. Its specifications are: volts, 115; cycles, 50/60; watts, 100. Constant current is set at 2-30 milliamperes while the ammeter is adjusted to read 3 milliamperes.

The paper strips are Whatman number 300-028 and the wicks used are number 319329. The buffer used is a combination of sodium barbiturate and barbituric acid with a pH of 8.6.

The strips are run in the cell for 15 hours and then removed and dried in a drying oven. They are then rinsed, after which they are placed in bromophenol blue dye. After final rinsing and drying, they are photoelectrically projected on paper in terms of a continuous line. A Spinco Analytrol by Beckman, model RB, is used for this procedure.

Anyone familiar with the literature concerning electrophoresis will realize that a disparity exists where normal values are concerned. Each author presents his own normals, and the wide variance that the reader encounters is somewhat distressing. It is apparent that each laboratory must seek its own normal values, and one must assume that in each reported instance the values are statistically significant for that equipment. In our case, the normal values used for patterns run were determined on the sera from fifty supposedly healthy medical students, nurses, and medical

technologists at University of Nebraska Hospital and seventy-five nurses at Bishop Clarkson Memorial Hospital. The normal Total serum protein is 7 grams per cent with a range of 6-8 grams per cent. The individual protein values are:

	Mean per cent	Standard Deviation	Range per cent	Range per cent grams
albumin	64.1	±4.3	55.5-72.7	3.89-5.09
alpha-1	2.9	± .75	1.4-4.4	.10-.31
alpha-2	6.5	±1.5	3.5-9.5	.25-.67
beta	10.6	±1.0	8.6-12.6	.60-.98
gamma	18.1	±2.25	13.6-22.6	.95-1.58

C. Discussion of Specific Findings

The data recorded and analyzed on each patient consisted of the following:

1. Name and hospital number
2. Age, sex, race
3. Total serum protein, albumin, globulin, and albumin/globulin ratio
4. Albumin, alpha-1 globulin, alpha-2 globulin, beta globulin, and gamma globulin in per cent of total and grams per cent
5. Hemoglobin and Bromsulfalein - in many instances thymol turbidity, cephalin flocculation, transaminase levels, white blood count, and other pertinent lab tests were recorded.
6. Diagnosis
7. Comment - Any pertinent history, physical, or laboratory findings tending to elucidate the electrophoretic findings were included.

After each pattern was studied and considered in view of primary pathology, age, sex, any secondary disease, and treatment, it was classified in one of the following ways:

I. Normal pattern, normal pattern expected

II. Normal pattern, abnormal pattern expected

III. Abnormal pattern, normal pattern expected

IV. Abnormal pattern, abnormal pattern expected

- A. "Diagnostic" pattern
- B. Pattern highly suggestive of disease present
- C. Non-specific change, marked
- D. Non-specific change, slight

V. Pattern abnormal, diagnosis not established.

Categories I and IV are those in which the majority of cases should fall if one assumes that good correlation may be drawn between serum proteins and the patient's clinical condition.

Categories II and III represent, of course, failure of this correlation to exist. Category IV is subdivided into five divisions. Included in A are those patterns which are so characteristic of and so consistently associated with a certain disease process that they are generally referred to in the literature as 'diagnostic'. These are:

1. Laennec's cirrhosis
2. Multiple myeloma
3. Hypogammaglobulinemia
4. Sarcoidosis
5. Nephrotic syndrome

The patterns found in IV (B) could not be used to make a diagnosis; but in conjunction with a clinical impression, they are highly consistent with and/or suggestive of a certain disease being present. Subdivisions C and D include all non-specific abnormal patterns in cases in which protein abnormality is expected. D includes those patterns in which at least one protein fraction is 3 to 4 standard deviations above or below its mean. Class C is for those patterns which are 5 standard deviations or more from the mean. Category V is self-explanatory in that an abnormal pattern is found in a case in which the patient is clinically sick, but no diagnosis has been established.

Presentation of Findings

I. Normal pattern, normal pattern expected

Sixty-eight patterns fell into this group. As would be expected, all of these patterns were ordered on people who were suspected of having some disease present. It was not difficult to assess the various protein fractions in light of the normal values; however, judgement on the part of the author had to be exercised in deciding whether or not these normal patterns were consistent with what pathology, if any, was present. In order that the reader may be able to agree or disagree with my opinion, Tables I and I_A list the diagnosis for each patient. In all these, the Bromsulfalein, sex, age, history, and hemoglobin were carefully weighed before his inclusion in this category. The table needs little explanation except that the 'mental diseases' include four patients with depression, two with cardiac neurosis, and one each with a diagnosis of conversion reaction, hypochondriasis, and psychogenic polydipsia. Of the 68 patterns in Category I, 53 are found in Table I. Table I_A includes the remainder which numbers 15. These are patients who may once have had abnormal patterns but are presently under treatment or whose disease has spontaneously subsided. Many of these are follow-up studies, ordered to assess progress. This group, needless to say, was carefully studied in light of additional laboratory studies and description of condition. One patient included in Table I_A had a pattern done before the onset of multiple myeloma. A subsequent

pattern after the disease began is found on a following page (Patient FD).

Table I

Diagnosis	Number of Patients
Mental Disease	9
Mild Peptic Ulcer disease	6
Mild controlled Diabetes	5
Arteriosclerotic heart disease, early	4
Epilepsy	3
No diagnosis.	3
Urinary tract calculus.	2
Benign prostatic hypertrophy	2
Melena, etiology undetermined.	1
Osteosrthritis.	1
Veruccae on back.	1
Hemorrhoids, chronic brain syndrome	1
Cavernous hemangioma	1
Ulcer on leg, second degree burn.	1
Chronic sinusitis.	1

Table I (continued)

Chorioretinitis	1
'Preleukemia'	1
Allergic vascular purpura	1
Multiple sclerosis, in remission	1
Ophthalmic artery aneurysm	1
Guillian-Barre Syndrome	1
Anemia secondary to pregnancy	1
Myoma, rectal mass, cor pulmonale	1
Retarded growth	1
Non-toxic goiter	1
Pulmonary fibrosis	1
Rheumatic heart disease, old	1
Total patients	53

Table I_A

Diagnosis	Number of patients
Hepatitis follow-up	3
Post-cholecystectomy	2
Post-gastrectomy	1
Lupus Erythematosis (on steroids)	1
Polyarteritis nodosa (on steroids)	1
Adenocarcinoma of endometrium (treated surgically and with irradiation)	1
Obstructive jaundice (conservative treatment, follow-up)	1
Chronic meningitis, treated	1
Ulcerative colitis, in remission	1
Acute rheumatic fever, subsided	1
Cirrhosis, one year strict dietary treatment	1
Before onset of multiple myeloma	1
Total patients	15

II. Normal pattern, abnormal pattern expected

These normal patterns are unexplainable in view of the patients' clinical condition and are found in detail in Table II. In each case, there was no reason to feel that a remission was in progress or that treatment was sufficient to account for the normal proteins. Patient GS had a liver biopsy which showed marked, advanced cirrhosis. Her Bromsulfalein retention was 18 per cent. JK was clinically quite sick with hepatitis at the time of the electrophoretic pattern. Her thymol turbidity was 19.6 units; cephalin-cholesterol flocculation was 3+, and serum glutamic pyruvic transaminase was 310 units.

III. Abnormal pattern, normal pattern expected

In this group are seven patients whose abnormal electrophoretic patterns cannot be explained by the description of their symptoms or other laboratory work in their charts. It is felt that this group (i.e. - abnormal pattern, no reason) is the one in which inconsistencies in the electrophoretic technique would most frequently be found. It may be that the procedure is not so untrustworthy in some of these cases as is the diagnosis which does not correlate well with it. An example of this is illustrated by patient LM in Table III whose grossly abnormal pattern is not even commented upon in his chart. The physical indicates that his liver was 'enlarged and tender', but no further work-up was initiated as regards his hepatic status. Patient JC

was reluctantly included in this group because no sound evidence that her pathology comprised an 'exudative enteropathy' could be found in her chart, although she was edematous and had a Bromsulfalein retention of 14.7 per cent. It may be noted that the remainder of the patients in this group are placed in it because of "immediate response" (decrease albumin, increased alpha globulin) patterns. These are seen often with acute infections and such may well be present but undiagnosed in any of these patients. Infection is very likely in disease states such as imcomplete abortion (EM) and diabetes (VR).

IV. Abnormal patterns, abnormal expected

There were 15 patients with so-called "diagnostic" (group A) electrophoretic patterns.

Nephrotic syndrome - (See Table IV) - Each of these patients exhibits a greatly increased alpha-2 globulin, patient LB having 40.3 per cent which is higher than any report noted in the literature. As may be expected with an albumin level of 0.46 grams per cent, she was admitted with severe edema.

Boeck's Sarcoid - (See Table V) - Both of these young women, one of whom is a Negro and the other Caucasian, had lymph node biopsies diagnostic of Sarcoidosis. Also, both were carefully studied for the possibility of tuberculosis. In these cases the electrophoretic pattern should have been quite helpful in the differential diagnosis (see Page 18).

Hypogammaglobulinemia - (See Table VI) - This was the only case of primary hypogammaglobulinemia. The patient, a 23 month old white male, had been hospitalized six times for bronchitis and/or bronchopneumonia. The diagnosis had been made elsewhere at age 6 months, and he had been receiving injections of gamma globulin. In spite of this, the pattern exhibited a diagnostically low level of gamma globulin. Another interesting finding is the increased alpha-2 globulin which is undoubtedly in response to the current infection. Alpha-hemolytic Streptococcus was cultured from his throat.

Multiple Myeloma - (See Table VII) - These patients each had multiple electrophoretic patterns on their charts. FD, interestingly enough, happened to have a perfectly normal pattern (see Table I_A) and Total serum protein one year prior to the onset of his multiple myeloma. It was as follows: Total serum protein=7.08 grams per cent, Albumin=63.8 per cent, alpha-1=3.6 per cent, alpha-2=7.2 per cent, beta=10.6 per cent, gamma=14.7 per cent. It is interesting that the change to the myeloma protein findings are so sudden as contrasted to most malignancies which are generally more insidious and gradual in development. Serial patterns on CM are also interesting (see Table VII_A). The patient's well-being was directly related to serum protein findings. It is also interesting to note that Bence Jones protein was at all times absent indicating that his malignant cells were producing only one type of abnormal protein. On his

November, 1962, admission his bone marrow showed complete replacement by malignant myeloma cells.

Cirrhosis - (See Table VII) - Eight patients exhibited classical cirrhosis patterns in conjunction with physical, historical, and laboratory findings equally diagnostic of cirrhosis. Little comment is necessary except to mention that two patterns are included on BK because one is so characteristically "teardrop" shaped that beta and gamma could not be differentiated. Two patterns were included for patient GG to illustrate the value of following patients with electrophoresis. The second was done 18 months (April, 1961, October, 1962) after the first during which time she observed a regimen of alcohol abstinence and good dietary intake.

The Bromsulfalein difference was 35.5 per cent in April of 1961 as compared with 11.7 per cent in October of 1962. Two patterns are also shown for AR with an interval of 6 months treatment. It will be noted that the second pattern is normal. At this time, his jaundice and ascites had completely subsided and clinically he was in good condition.

The mean figures, in per cent of total and grams per cent, agree with the literature as do the appearances of each pattern. The second pattern for patients GG, AR, and BK are not included in calculating the means.

The remaining patients in Category IV break down as follows:

B. Pattern highly suggestive of disease present. . .35

C. Non-specific change, marked. 29

D. Non-specific change, slight. . . . . .73.

The patients included in these groups will not all be presented or discussed. Each was studied carefully before being placed in each group. A large number of the patients in D suffered infections, and quite a large number of those in C were patients with malignancies of one type or another. Some of the more interesting patterns or groups of patterns in these sub-divisions (mostly in group B) will be discussed.

In Table IX are found the secondary hypogammaglobulinemias. The mean gamma globulin concentration in the three chronic lymphocytic leukemias was 0.42 grams per cent. Patients LL and HW had an infection of the arm and bronchopneumonia respectively. Patient CS was admitted for cancer of the prostate with multiple bony metastases. A bone marrow examination showed extensive myelofibrosis. Patient DF is a 6 year old, white male who was hospitalized for numerous infectious episodes. The electrophoretic pattern was not commented on in the chart. The gamma globulin level is obviously low enough so that primary hypogammaglobulinemia should have been considered.

Table X includes 4 cases which resemble nephrosis electrophoretically, but the other findings are not such that they could

be placed in the "diagnostic" category. Patient LL is the most likely of the 4 to truly be a nephrotic. The alpha-2 globulin concentration is 1.1 grams per cent. She is said to have "chronic nephrosis", but no definitive work-up is present. She was pregnant at the time this patern was done, and it is felt that this partially explains the increased beta globulin fraction. Patient VH is a poorly controlled diabetic who was admitted with 3/ proteinuria and edema. Patients LP and PK were admitted with multiple pathology in addition to renal failure, and in each it is likely that the patterns reflect more than their renal difficulties.

In Table XI are three severe diabetics whose electrophoretic patterns were thought to be representative of advanced, uncomplicated diabetes mellitus (see Page 35). In all three, the beta fraction is sharply peaked and discrete. The mean beta globulin per cent of total is 19.7, and the mean concentration in grams per cent is 1.18. Patient MB had an abscess on admission; and RS was admitted with bronchopneumonia, leg ulcers, and gangrene. This accounts for the increased alpha-2 globulin in each case. Patient ES had no infection.

Table XII lists the "collagen" diseases encountered in this study with at least one pattern run when the patient was not being treated. Patients ES and SS are clinically proven cases of lupus erythematosis. ES presented with, among other findings, hepatosplenomegaly. A liver biopsy was normal.

The pattern is extremely abnormal. The large gamma peak is irregular and jagged. A second pattern is presented for SS, run 10 months after the first. At the time of the second pattern, she was on Prednisone and clinically in very good condition. Patient LD was an interesting differential problem. She was a 15 year old white female with consistently negative lupus erythematosis preparations. Her Antistreptolysin-O titer was 833 units, and liver studies were all normal. The differential was between 'lupus sine lupus' and rheumatoid arthritis with the staff split evenly between the two entities. The electrophoretic pattern should have elucidated the problem as it is classic for lupus erythematosis (see Page 24) and not at all like that expected in rheumatoid arthritis (see Page 26). Patient MP presented with difficulty walking. Muscle biopsy proved polyarteritis nodosa. The marked gamma globulin increase is expected in this disease (see Page 27). The second pattern is obviously normal. The eosinophil count at the time of the first pattern was 1,000 per cubic millimeter, and 590 per cubic millimeter at the time of the second. Finally, patient DK is a good example of the type pattern found in patients suffering from rheumatoid arthritis. Note the marked decrease in albumin and increase in alpha-2 globulin along with significant alpha-1 globulin increase (see Page 25). An electrophoretic pattern on joint fluid from this patient showed a much decreased albumin

and increased globulin.

Table XIII demonstrates an unusual case in which the diagnosis was finally made on the basis of the electrophoretic finding itself. Numerous patterns were run, all with decreased albumin levels, and many attempts were made to find primary pathology. The 21 year old white female whose chief complaint was anasarca, was finally diagnosed as "idiopathic hypoalbuminemia". The pattern was helpful in ruling out nephrosis, an original impression, but even at dismissal it was felt that a "collagen'picture" would develop eventually. The sharply peaked paraprotein-like gamma fraction supports this impression.

Table XIV demonstrates the typical findings in carcinoma (see Page 36). In all cases, the albumin is decreased and alpha-2 and gamma globulins increased. In addition, alpha-1 globulin is elevated in these patients. Liver studies were normal in all cases except EW who had a Bromsulfalein retention of 10.5 per cent.

In Table XV are a few miscellaneous cases which proved interesting or typical enough to merit inclusion. Patient ET exhibits typical findings for Hodgkin's disease (see Page 29). Liver studies were normal. Patient RD shows fairly typical changes expected considering she had common and hepatic duct obstruction with bilirubin levels of 10.7 milligrams per cent direct and 16 milligrams per cent total. The second pattern was 7 months

after treatment when her bilirubin was 0.24 milligrams per cent direct and 0.68 milligrams per cent total. She was clinically much improved. Patient RH was diagnosed as having pericholangiolitic hepatitis which was subsiding at the time of the electrophoretic pattern. His total lipids were 1,500 milligrams per cent and cholesterol 335 milligrams per cent. The pattern is unusual only in that two distinct beta globulin bands are seen, as might be expected. Patient BS was pregnant at the time the pattern was run, explaining the increased beta globulin. Also, she was suffering from hepatitis. This is also well reflected in the final pattern as the alpha-1 globulin is definitely increased (see Page 22).

V. Pattern abnormal, diagnosis not established

There were seven patients in this group. In all cases it was a matter of no diagnosis having been arrived at, rather than the patient not having any disease at all. An example of the patients found in this category is patient DF in Table IX. His chart had no definite diagnosis on it.

Table II

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
GS	Laennec's cirrhosis	8.58	57.5	3.9	8.2	11.1	19.3
WB	Scleroderma, Thorazine-induced jaundice	7.32	58.4	3.7	6.6	12.6	18.8
JK	Infectious Hepatitis	6.25	63.6	4.0	6.5	10.9	15.0

Table III

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
JC	Diverticulitis with abscess form	6.86	24.1	7.5	12.8	15.0	40.6
MR	Pathologic compression - Fracture of T-12, L-2	6.42	42.1	7.7	15.0	12.9	22.3
EC	Anovulatory bleeding - probable polycystic ovary	6.43	47.0	6.4	12.7	13.5	20.2
VR	Well controlled diabetic	6.64	51.0	6.9	12.4	13.4	16.3

Table III (continued)

LM	Pneumonitis, congestive heart failure, arteriosclerotic heart disease, pulmonary emphysema	7.8	22.0	7.9	9.1	14.0	47.0
EB	Anxiety Reaction	7.83	50.7	5.1	11.9	13.4	18.9
EM	Incomplete abortion	5.5	56.2	5.7	14.9	12.4	10.8

Table IV

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
LB	Juvenile nephrosis	4.04	11.3	6.5	40.3	25.8	16.1
FH	Diabetic Nephropathy	5.4	36.1	6.7	22.2	16.4	18.6

Table V

Patient	Total serum protein grams	Albumin %	Albumin grams%	Alpha-1 %	Alpha-1 grams%	Alpha-2 %	Alpha-2 grams%	Beta %	Beta grams%	Gamma %	Gamma grams%
JK	7.45	41.7	3.1	5	1.4	12.1	0.9	13	1.0	27.9	2.1
EC	6.5	35.8	2.3	8	0.5	11.1	0.72	20.4	1.3	24.7	1.6

Table VI

Patient	Total serum protein grams	Albumin %	Albumin grams%	Alpha-1 %	Alpha-1 grams%	Alpha-2 %	Alpha-2 grams%	Beta %	Beta grams%	Gamma %	Gamma grams%
DB	6.5	63.8	4.1	3.6	0.2	12.9	0.8	11.2	0.7	8.5	0.5

Table VII

Patient	Total serum protein grams%	Albumin %	Alpha-1 %	Alpha-2 %	Beta %	Gamma %	Gamma grams%
FD	9.2	32.2	3.4	7.5	12.2	44.7	4.11
CM	13.26	21.5	1.5	3.5	2.7	70.8	9.42

Table VII A

Patient - CM

Date	Total serum protein per cent	Albumin per cent	Gamma per cent	Bence Jones protein	Hemoglobin grams	Clinical
3-24-61	13.5	22.4	70.3	negative	5.8	weakness, dyspnea, anemia, bone marrow
3-29-61	13.26	21.5	70.8	negative	6.2	showed multiple myeloma on Urethane.
8-28-61	9.4	53.6	35.2	negative	13.6	clinic visit, feeling good, discontinues Urethane.
4-2-62	10.5	37.0	50.0	negative	13.8	clinic visit, not feeling good, Urethane and Cytoxan.
11-2-62	11.5	27.2	60.8	negative	8.6	admitted in congestive heart failure, nausea, vomiting, weight loss, expired 11-7-62.

Table VIII

Patient	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
PK	6.7	36.4	5.7	8.6	18.2	31.1
HC	6.53	28.2	6.3	20.5	13.7	36.3
RV	5.8	41.0	7.2	8.6	9.4	33.8
AJ	5.4	33.5	5.1	13.7	13.8	33.9
WG	6.6	30.7	1.1	3.2	9.5	55.6
AR	7.8	37.3	4.5	6.4	16.4	35.4
	7.16	66.4	2.8	6.7	11.6	12.6
BK	7.1	19.5	4.7	6.6	15.3	54.0
	6.87	25.7	5.4	7.2		61.7
GG	6.75	31.3	5.6	9.6	17.7	35.8
	8.1	50.5	4.8	8.7	14.7	21.4
Mean per cent total		32.2	5.0	9.65	13.6	39.5
Mean gram per cent	6.58	2.04	0.31	0.61	0.91	2.61

Table IX

Patient	Diagnosis	Total serum protein %	Albumin %	Alpha-1 %	Alpha-2 %	Beta %	Gamma %	Gamma grams%
LL	Chronic lymphocytic leukemia	6.11	57.3	6.3	14.2	12.6	9.6	.59
HW	Chronic lymphocytic leukemia	4.51	61.5	6.5	10.1	13.6	8.3	.37
OK	Chronic lymphocytic leukemia	6.62	68.4	3.8	10.0	10.0	7.9	.32
DF	Hypogammaglobulinemia	7.32	74.5	2.4	8.0	7.1	8.0	.58
CS	Hypogammaglobulinemia	5.3	65.6	6.8	8.3	12.1	7.3	.39

Table X

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
LL	chronic nephrosis questionable	6.0	45.7	5.7	18.4	17.1	13.2
VH	nephrosis, secondary to diabetes	6.8	48.6	4.2	15.8	13.7	17.8
LP	acute renal failure, possible viral nephritis	4.8	43.1	9.9	15.2	14.6	17.2
PK	renal failure, cirrhosis	5.8	43.1	6.2	16.4	8.2	26.0

Table XI

Patient	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta %	Beta grams%	Gamma per cent
MB	6.64	49.2	4.5	12.4	17.5	1.16	16.4
ES	5.6	53.4	5.2	7.5	17.3	0.97	16.5
RS	5.8	30.1	7.3	14.6	24.4	1.42	23.6

Table XII

Patient	Diagnosis	Total serum protein %	Albumin %	Alpha-1 %	Alpha-2 %	Beta %	Gamma %	grams%
ES	lupus erythematosis	7.56	11.6	9.9	11.1	6.7	60.8	4.6
LD	lupus erythematosis or rheumatoid arthritis	8.55	37.3	3.7	8.3	9.8	41.0	3.5
SS	lupus erythematosis	8.62	38.2	3.5	6.4	9.6	42.3	3.64
		8.38	61.5	3.1	6.5	15.4	13.4	
MP	polyarteritis	7.0	26.7	5.8	15.4	15.0	37.1	2.6
		7.43	63.0	3.0	3.8	14.5	14.8	
DK	rheumatoid arthritis	7.44	38.3	7.4	18.5	13.6	22.2	

Table XIII

Patient	Total serum protein per cent	Albumin/ Globulin	Albumin %	Albumin grams%	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
LG	6.0	0.47	31.9	1.91	4.9	11.1	9.7	42.4

Table XIV

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
RL	cancer of prostate	5.4	40.0	10.9	___	49.1	___
HP	basal cell cancer of scalp with osteomyelitis	6.64	30.0	6.5	13.9	23.5	26.1
EW	bronchogenic cancer	7.33	37.7	6.0	12.6	19.2	24.6
LK	cancer of splenic flexure	6.75	21.2	9.1	18.2	13.6	37.9

Table XV

Patient	Diagnosis	Total serum protein grams	Albumin per cent	Alpha-1 per cent	Alpha-2 per cent	Beta per cent	Gamma per cent
ET	Hodgkin's disease	8.1	33.7	6.1	11.7	12.2	36.2
RD	Obstructive jaundice	5.2	37.7	7.8	11.4	18.0	25.2
		6.75	67.1	3.4	6.0	8.2	15.3
RH	Pericholangiolitic jaundice	6.75	61.7	3.2	6.0	B_1 5.4 B_2 10.1	13.6
BS	Hepatitis-pregnancy	6.42	46.7	7.1	12.0	19.4	14.8

D. Statistical findings

Table XVI

Category	Number	per cent of total
I. Normal pattern, normal pattern expected	68	28.69
II. Normal pattern, abnormal pattern expected	3	1.26
III. Abnormal pattern, normal pattern expected	7	2.95
IV. Abnormal pattern, abnormal pattern expected	(152)	(64.15)
A. diagnostic pattern	15	6.32
B. pattern highly suggestive	35	14.76
C. non-specific change, marked	29	12.24
D. non-specific change, slight	73	30.80
V. Pattern abnormal, diagnosis not established	7	2.95
Totals	237	100.00

Table XVII

	Number	per cent of total
Total in which result seen was expected	227	95.79
Total in which result seen was not expected	10	4.21
Total patterns	237	100.00

IV. Summary

The thesis is advanced that electrophoresis of the plasma or serum proteins is a valuable laboratory adjunct to diagnosis and further, that the procedure is accurate to the degree that it may be utilized properly as a screening procedure in health and disease. In the latter regard, it is felt that electrophoresis is certainly as reliable and consistent in reflecting the patient's general status as such time honored procedures as the complete blood count or sedimentation rate and further, that in most instances it is probably more specific than either.

In attempting to prove the above statements to be ture, it was first necessary to gather a literature review which included the electrophoretic findings in a broad and inclusive cross-section of diseases or disease groups that are commonly encountered. Discussion of several less common entities is also included because electrophoretic changes are either diagnostic or specific.

After such a background was established, a review of 237 electrophoretic patterns was begun. These were taken strictly in sequence, covering 18 consecutive months. Because the charts were selected without any advance regard as to diagnosis or electrophoretic results, it is felt that accurate appraisal of the degree of correlation between protein changes and status of health could be drawn.

In this reveiw of patterns, the correlation between disease

(absence or presence of) and the electrophoretic pattern was very good. In 227 instances (or 95.79 per cent) the electrophoretic change or lack of change was as expected. In only 10 (4.21 per cent) was there failure of correlation between the patient and the pattern. In some of this latter group, it is strongly suspected that the diagnosis was more inaccurate than were the protein changes.

All patterns reviewed were classified into five general categories. The criteria for inclusion in each category were as scientific and strict as was possible. Table XVI shows the results of the entire study broken down into groups. Normal patterns, when such were expected, comprised 29 per cent of the study. These diagnoses are found in Tables I and I_A. Sixty-four per cent of the total patterns were abnormal in the face of a disease expected to produce such a result. Of these 152 abnormal patterns, 50 were "diagnostic" or very suggestive of the disease present. The remaining 102 patterns in the abnormal group were non-specific in their changes. Patterns placed in this last group were done so strictly on the basis of number of standard deviations from the mean that at least one protein fraction exhibited. As to the decision that an abnormal pattern was expected in these cases, the literature review in Part II was at all times consulted. Three per cent of the total were patterns on charts with no definitive diagnosis, but in which sufficient pathology was present to explain the protein changes.

In addition to the literature review and statistical findings,

many illustrations of protein changes and several interesting patterns or groups of findings are presented.

In summary, it is felt that electrophoresis of the serum proteins is a sensitive and reliable means of demonstrating both the general health of a patient and, on some occasions, specific pathology. Statistically, it is felt that the correctness of this assumption has been proved. The use of electrophoresis in diagnosis is quite obvious. An additional use is in assessing the patient's progress. It has been demonstrated that serum proteins are sensitive indicators of the presence of disease not only at the onset but also through the subsidence of the disease process.

V. Conclusions

1. A review of the literature concerning electrophoretic patterns in health and disease is presented.
2. Two hundred and thirty-seven electrophoretic patterns are reviewed and interpreted in light of the patients clinical condition
3. Two hundred and twenty-seven patterns (95.79 per cent) exhibited changes or a lack of changes as would be expected.
4. Ten patterns (4.21 per cent) were normal or abnormal when the converse is expected.
5. Of 152 predicted abnormal patterns, 50 (twenty-seven per cent of total patterns) were diagnostic or highly suggestive of the disease present.
6. In this study of a large number of disease entities, it has been conclusively proved that the electrophoretic patterns accurately reflect the pathology or lack of it in the majority (96 per cent) of instances.
7. It is concluded that electrophoresis of the serum proteins is a reliable and sensitive indication of the presence of disease in general.

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