A Method of Scanning Paper Electrophoresis Strips and Its Application to the Study of Plasma Proteins*

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Although paper electrophoresis has many distinct advantages over the free boundary method, the most important being that a very small amount of material is required, there is difficulty in the quantitative determination of the concentrations of the separated components. The most commonly used method of detection at the present time involves staining the protein with a dye such as bromphenol blue or amido black, and then either scanning with a densitometer (1, 2) or else cutting out the stained areas and eluting them for determination in a spectrophotometer. Although they provide quantitative results, these methods have several shortcomings. First, they require considerable time; second, there is no selectivity, so that if two components of a mixture have the same or nearly the same mobility, they are not separable; and third, the optical methods have limited resolution.

In 1955 Sharpsteen discovered that paper electrophoresis strips of blood plasma could be scanned with a movable electrode (a fixed electrode being maintained at one end of the wet paper strip) and a voltage record of the separated components obtained. This very significant observation seemed to offer, even in its crudest form, the possibility of a higher resolution of components in such a protein mixture than had heretofore been achieved by optical methods. The voltages obtained by scanning the strip in the manner indicated were later shown to arise from electrochemical reactions, i.e. the paper strip is essentially a series of concentration cells arising from the reduced activity of free metal ion (due to binding to the protein in specific locations on the paper strip), related the observed electromotive force to the concentrations of the various protein components, and demonstrated the high degree of selectivity in the detection of several components in a mixture of proteins by using, in turn, various metal ions.

EXPERIMENTAL PROCEDURE

Materials

The salts used were all analytical reagent grade. Crystalline bovine serum albumin (BSA) was an Armour product, Lot No. P67580. Bovine fibrinogen was prepared from plasma Fraction I (Armour Lot No. S3904) by a modification of the method of Laki (3), as described by Sturtevant et al. (4). The human γ-globulin was obtained as a 16.5% solution (Lederle, Lot No. 2175-26). Veronal buffers for electrophoresis were prepared from commercial packets of Spinco B-2 buffer. The electrodes of the scanner were made from platinum, silver, and copper, respectively.

The following solutions of the various metal ions were used: (a) 0.05 M cupric sulfate; (b) 0.03 M silver nitrate; (c) a mixture of Fe++ and Fe+++ ions prepared by pipetting 25 ml of 0.25 M sodium thiosulfate into 500 ml of 0.1 M ferric ammonium sulfate with vigorous stirring. When the initial dark red color had faded, the solution was diluted to 1 liter; (d) a mixture of Hg2++ and Hg+++ ions prepared by dissolving 0.025 mole each of mercuric and mercuric nitrates in water, a minimal quantity of 5 M HNO₃ being added dropwise until the material just dissolved. The solution was then diluted to 1 liter.

Apparatus

Electrophoresis was carried out in a Durrum-type Spinco model R paper electrophoresis apparatus, equipped with a Duo-
The paper strip scanner was constructed by the Cambridge Instrument Company. It consisted of a plexiglass sheet with a depression for holding the wet paper strip, and a covering plexiglass sheet to prevent evaporation. A stationary platinum electrode was attached to one end of the paper strip, and a movable, scanning electrode made contact with the paper through a slot cut in the upper plexiglass sheet. The scanning electrode was moved along the length of the paper strip by means of a constant-speed motor at a rate of 2.44 inches per minute. Two different instruments were utilized for measuring and recording the electromotive force between the fixed and scanning electrodes as a function of the distance which the scanning electrode traveled along the paper strip. Qualitative records were obtained with a Versascribe electrocardiograph (Cambridge Instrument Company) modified to function as a D.C. oscillograph. It was mechanically coupled to the scanner so that the scanning electrode moved at the same rate as the chart paper of the recorder. All patterns reported in this paper were obtained with this instrument. Quantitative measurements were made on records obtained with a Cambridge Instrument Company dye-dilution curve recorder, modified to obtain a chart speed of 7.5 inches per second, and with variable but accurately reproducible sensitivity of from 1.1 to 22 mm per mv (maximal peak height = 180 mm). The areas under the peaks on these records, measured with a Coradi planimeter, could be determined with a precision of better than 1%. Although not done here, it would be worthwhile to record automatically the area under the peaks together with the voltage record itself. Similarly, an automatically recorded derivative of the voltage curve would be useful in quantitative interpretation.

The metal electrodes were in the form of thin ribbons, mounted in plexiglass, with beveled, chisel-type tips. The long edge of the chisel tip was perpendicular to the direction of scanning. The tip was sufficiently rounded to prevent abrasion of the paper, and was held against the paper with the minimal pressure required to maintain good contact.

Absolute magnitudes of some values of electromotive force were checked independently with a Leeds and Northrup type K potentiometer. A Beckman model G pH meter was used for the determination of pH.

**Methods**

**Striping Experiments**—Initial quantitative studies were carried out using paper strips on which protein solutions of various concentrations were simply placed in various locations with the Spinco sample stripper, but not subjected to electrophoresis.

In this manner, the effect of protein concentration on the voltage record could be investigated without introducing possible additional problems which arise during electrophoresis.

The filter paper strips were first saturated with Veronal buffer (pH 8.6, ionic strength, 0.075) was used in all of the runs. The paper strip scanner was not especially designed for a quantitative transfer of solution to the paper, there was some difficulty in achieving uniform stripes but, with care, good reproducibility was obtained. After striping, the paper strips were placed in an oven at 130° for 30 minutes (as is normally done in an electrophoresis run) in order to denature the protein and fix its location on the strip.

After heat denaturation, the strips were allowed to cool and then were immersed in a solution of a particular metal ion at room temperature for an empirically determined, but critical, period of time, which differed from system to system. After removal from the treatment solution, the strip was allowed to drain for a few seconds, placed into position in the scanner, allowed to equilibrate for 2 minutes, and then scanned with an appropriate electrode. The initial scan was often found to be erratic, and as a rule only the second and third scans were used.

The second scan was usually recorded with the dye-dilution (quantitative) recorder, whereas the Versascribe was customarily used to record the third scan. It was found that with either recorder, the second and third scans were almost always nearly identical, provided there was no great time lapse between them.

In the “copper” system the strips were immersed in the 0.05 M cupric sulfate solution for 20 seconds and then scanned with a copper electrode. The fixed electrode at one end of the paper was platinum which gave similar patterns to those obtained with a fixed copper electrode except for a shift of the baseline voltage.

With the use of the fixed platinum electrode, the baseline was adjusted to any desired position by means of a constant bucking voltage from an external battery and resistors.

In the “silver” system the strips were immersed in the 0.03 M silver nitrate solution for 10 seconds and then scanned with a movable silver electrode and a fixed platinum electrode.

In the “iron” system a Fe+++ mixture was used instead of a Fe++ electrode because the latter is not easily reversible. The instability of ferrous ion in air presented no problem. Since ferric hydroxide has a low solubility, it was necessary to keep the pH low. Despite the fact that low pH is not very favorable for iron binding to the β-globulin of blood serum (8), nevertheless satisfactory results were obtained. In the “iron system” the strips were immersed in the ferrous-ferric solution for about 2 or 3 seconds and then scanned with platinum electrodes.

In the “mercury system” the strips were immersed in the mercury-mercurous solution for 10 seconds and then scanned with platinum electrodes.

**Electrophoresis Experiments**—Electrophoretic experiments were carried out in the conventional manner. In the cell of the Spinco model R apparatus, 8 runs could be carried out simultaneously, a 10-μl sample of protein solution having been applied to each paper strip. During the runs a constant current of 5 ma was allowed to pass for 18 hours. At the end of the run, the paper strips were placed in the oven at 130° for 30 minutes, and then treated in an identical manner as described above for the striping experiments.

The length of the immersion period was largely determined by the time required for the strip to become soaked through, and usually depended on the particular salt used. Significantly longer periods of soaking, i.e., more than 2 or 3 times the minimum, tended to cause suppression of the signal.
THEORY

Our first task is to account for the origin of the observed electromotive force and to relate the electromotive force to the protein concentration. Consider a filter paper strip which has a transverse stripe of denatured protein. If the strip is immersed in a solution of a salt containing metal ions \( M^{n+} \) and then withdrawn, the activity of the metal ion will be uniform throughout the paper (and equal to that of the treatment solution if interactions of \( M^{n+} \) with the paper or buffer are negligible) except in the region containing the denatured protein. If the latter can bind the metal ion to any extent, then the activity of the free metal ion will be lower in the portion of the paper containing the protein. This difference in metal ion activity can be detected by placing an electrode of the corresponding metal, \( M \), in contact with \( Mn^{+} \), each with an intrinsic binding constant \( k \), then the actions of \( Mn^{+} \) with the paper or buffer are negligible except in to compute the paper (and equal to that of the treatment solution if interaction of the protein according to Equation 1. Therefore, in order to draw, the activity of the metal ion will be uniform throughout the region containing the denatured protein. If the latter can respond to the fixed electrode (at the end of the paper strip) in our apparatus and the latter corresponds to the scanning electrode which can traverse the paper strip. Since each electrode is in contact with metal ions at different activities, the assembly constitutes an electrolyte concentration cell whose electromotive force can be computed. The portion of the paper between the two electrodes is the analogue of the liquid junction or salt bridge. As the scanning electrode moves lengthwise along the strip it contacts successive areas of varying activity of \( M^{n+} \) (depending on the concentration of protein present and its binding affinity for \( M^{n+} \)). Thus, the half-cell electromotive force, arising at the scanning electrode tip, varies as this electrode moves. In other words, the electromotive forces of a whole series of half-cells are measured as the electrode proceeds. The recorder plots the electromotive force as a function of the distance traveled by the scanning electrode. If this explanation is correct, we should be able to compute the electromotive force as a function of protein concentration and verify the computations. In the rest of this section we shall report this calculation and, in the next section, shall present experimental data to check the theory.

Ignoring liquid junction potentials, and assuming the metal to be pure and at unit activity, we may write expressions for the half-cell potentials at \( 29^\circ \) at any position of the scanning electrode and, thereby, obtain the following equation for the over-all electromotive force of the cell.

\[
E_{\text{cell}} = \frac{1}{n} \log \left( \frac{a_{M^{n+}}} {a_{M^{n+}}} \right)_{\text{fixed}} - \frac{1}{n} \log \left( \frac{a_{M^{n+}}} {a_{M^{n+}}} \right)_{\text{scanning}}
\]

where the \( a \)'s are the metal ion activities at the fixed and scanning electrodes, respectively. If the scanning electrode is in contact with a protein-free area of the paper, then

\[
(a_{M^{n+}})_{\text{scanning}} = (a_{M^{n+}})_{\text{fixed}}
\]

and \( E_{\text{cell}} = 0 \). This is the baseline of the voltage-distance record. When the scanning electrode is in contact with a protein-bearing area, and if the protein binds \( M^{n+} \) thereby lowering the activity of the free metal ion, then

\[
(a_{M^{n+}})_{\text{scanning}} < (a_{M^{n+}})_{\text{fixed}}
\]

and \( E_{\text{cell}} < 0 \), corresponding to the spontaneous transfer of metal ion from the fixed electrode (high activity) to the scanning electrode (low activity). With BSA and Cu\(^{++}\) ion as an example, it is observed that the electromotive force is zero and independent of position along the paper as long as the scanning electrode does not contact any protein, and is negative when the scanning electrode is in contact with the protein-containing region. The observed negative electromotive force implies that the protein has bound some cupric ion and lowered the activity of the free ion. This is the expected behavior for this system.

Since \( (a_{M^{n+}})_{\text{fixed}} \) remains constant as the scanning electrode moves, the value of \( E_{\text{cell}} \) will depend on \( (a_{M^{n+}})_{\text{scanning}} \) in the presence of the protein according to Equation 1. Therefore, in order to compute \( E_{\text{cell}} \) as a function of the total protein concentration, \( P \), it is necessary to relate \( P \) to \( (a_{M^{n+}})_{\text{scanning}} \). For this purpose we must have information about the binding of \( M^{n+} \) to the heat-denatured protein at the given pH. For example, if the protein is assumed to have \( m \) equivalent sites per mole available for binding \( M^{n+} \), each with an intrinsic binding constant \( k \), then the number of occupied sites, \( r \), per mole of protein, will be related to the concentration, \( A \), of free \( M^{n+} \) ions, by the following equation (9), which holds in the absence of electrostatic interactions.

\[
\frac{1}{r} = \frac{1}{mk} A + \frac{1}{m}
\]

Letting \( B \) represent the total concentration of \( M^{n+} \) (i.e. bound plus free ions), then

\[
r = \frac{B - A}{P}
\]

Combining Equations 4 and 5, we obtain

\[
\frac{P}{B} = \frac{1}{m} \left[ \frac{1}{kA} + 1 \right]
\]

Under the conditions of our experiments (i.e. \( E_{\text{cell}} \) as much as \(-300 \) mv), the value of \( (a_{M^{n+}})_{\text{scanning}} \) must be very small (by a factor of \( 10^{10} \) in this example) compared to \( (a_{M^{n+}})_{\text{scanning}} \), according to Equation 1. Therefore, \( A \) must be very small compared to \( B \) in the presence of protein.\(^6\) With this approximation Equation 6 becomes

\[
\frac{P}{B} = \frac{1}{m} \left[ \frac{1}{kA} + 1 \right]
\]

If \( k \) is not too large, say no greater than \( 10^3 \), then \( 1/kA \) will be much greater than unity because \( A \) is very small. With this further approximation, Equation 7 becomes

\[
\frac{P}{B} = \frac{1}{mkA}
\]

Approximating \( (a_{M^{n+}})_{\text{fixed}} \) by \( B \) and \( (a_{M^{n+}})_{\text{scanning}} \) by \( A \), Equation 1 becomes

\[
E_{\text{cell}} = -\frac{0.059}{n} \log \frac{B}{A}
\]

Substituting Equation 8 in Equation 9, we obtain

\[
E_{\text{cell}} = -\frac{0.059}{n} \log P - \frac{0.059}{n} \log mk
\]

In reality, the protein is distributed in an unknown way (in the direction of scanning) through the stripe. Therefore, it is

\(^6\) The condition that \( A \) be small, deduced from the observed range of values of \( E_{\text{cell}} \), no doubt holds only for sufficiently large \( P \). If \( P \) becomes too small, this condition would no longer be expected to hold.
impossible to specify what fraction of the protein in the stripe is contributing to $E_{cel}$ at any instantaneous position of the scanning electrode. For simplicity, we may imagine the total amount of protein in a stripe to be distributed in the direction of scanning as a step function, i.e. zero outside of the stripe and constant inside the stripe. In passing through the stripe, the scanning electrode may be assumed to pass through $j$ concentration cells, each with a protein concentration $P/j$. If each cell has an electromotive force equal to $E_j$, given by Equation 10, then the area under the curve of the recorded peak will be

$$\text{Area} = \sum E_j = -\frac{0.059j}{n} \log \frac{P}{j} = \frac{0.059j}{n} \log \frac{mk}{j} (11)$$

Thus, a plot of the area under the peak should be a linear function of $\log P$. No significance can be attached to the slope or intercept because of the unknown nature of the quantity $j$ and the approximation of the distribution of $P$ in the stripe by a step-function. In the next section it will be shown that, in the stripping experiments, Equation 12 is obeyed, at least at sufficiently large $k'$. Thus, the origin of the observed phenomenon is explained in terms of the behavior of electrolyte concentration cells.

It remains to justify the approximations made in the above treatment. Although no data are available for the binding of Cu$^{2+}$ ions to heat denatured BSA, we shall resort to data of Klotz and Curme (7) which apply to the native protein at pH 4.8, which is also the pH of our treatment CuSO$_4$ solution. Of course, it is unreasonable to suppose that the binding data for native and denatured BSA will be the same, especially since no doubt there is a significant difference in the internal structure of the protein. Nevertheless, since we are seeking only order of magnitude data to justify our approximations, this procedure is probably not as outrageous as it appears. From the data of Klotz and Curme we obtain values of about $m = 16$ and $k = 800$. Thus $k$ is not too large, as assumed, in transforming Equation 12 into Equation 8. Further, according to Equation 12, $Pmk/j$ must be greater than unity for the area to be negative as observed. Taking $mk$ as $10^4$, $P/k$ must be greater than $10^{-4}$ moles per liter. If the stripe were prepared from 10 $\mu$l of a 1% protein solution, there would be 100 $\mu$g of protein in the stripe. The volume of the stripe was calculated to be about 40 $\mu$l or less, so that the concentration of protein would be between 2.5 and 10 g per liter. If a molecular weight of 69,000 is assumed for the protein, the value of $P/j$ would then be $3 - 15 \times 10^{-4}$ moles per liter, which is approximately equal to the minimal value required for the validity of the approximation under discussion. The large value of $Pmk/j$ will assure that $A$ is small, as assumed, unless $B$ is excessively large. If $B$ becomes large, then the amount of $M^{2+}$ removed by the protein does not lower $B$ significantly, i.e. $B$ and $A$ are comparable. Under these conditions, the approximation that $B \gg A$, used in obtaining Equation 7, would not hold. Also, under conditions where $B$ is comparable to $A$, the value of $E_{cel}$ would become too small to measure accurately. Since we obtained significantly large values of $E_{cel}$ we can be assured that $B \gg A$ and $P$ was sufficiently large (at least in the higher range of concentrations) for these approximations to hold. It may be noted here that the ionic strength on the paper must be sufficiently high to avoid high resistance in the wet strip.

The concentrations of our treatment solutions were high enough to maintain good conductivity. Finally, it may be mentioned that, although we required the validity of all these approximations to test the theory in the stripping experiments, these approximations need not hold rigorously in the electrophoresis experiments, i.e. even if the area cannot be related to the protein concentration by a simple algebraic equation we can still use the method to analyze protein mixtures if we obtain an empirical calibration curve between area and protein concentration for the given metal ion with every protein component of the mixture. Further, even without such calibration curves, it will be possible to compare the electrophoretic patterns of normal and pathological blood plasmas.

**RESULTS AND DISCUSSION**

**Striping Experiments**—The initial studies were designed to obtain an experimental verification of the applicability of Equation 12. In order to maintain the appropriate conditions for the validity of the approximations discussed in the previous section, and also to avoid the additional errors inherent in electrophoresis, the initial experiments were carried out with the striping technique already described. Since BSA was readily available in relatively pure form, this protein was chosen as a typical one; cupric ion was selected as a representative metal-ion because it was known to bind readily to BSA. Measurements were made of electromotive force (i.e. area) of the Cu, Cu$^{2+}$ system at several different protein concentrations on the same strip. A typical...
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result is shown in Fig. 1. Although there is some scatter among the points, it seems that the best relationship between electromotive force (represented by area under the peaks on the pattern) and protein concentration was that predicted from the theory.

In order to test Equation 12 more extensively, this experiment was repeated with other proteins and other metals; bovine fibrinogen and human γ-globulin were used for this purpose. Typical results obtained with fibrinogen, using the Cu, Cu++ system, are shown in Fig. 2. It should be noted that this protein may be detected with copper at much lower concentration than is BSA. Also, more consistent results were obtained than with BSA. The reason for these improved features is not immediately apparent, but it is possible that the binding capacity of fibrinogen for copper is much greater than that of BSA, so that a higher electromotive force is obtained with a smaller amount of protein. It is also possible that the more erratic results obtained with BSA are due to side effects arising from the large amount of protein required, making it difficult for Cu++ ions to penetrate the stripe completely. If so, then it would be understandable that more consistent results should be obtained with the fibrinogen.

As a final example, γ-globulin solutions were striped on filter paper strips, and were scanned using the Ag, Ag+ system. A typical Versascribe pattern and graph are shown in Fig. 3. It should be noted that the range of concentrations used here is approximately the same as that of fibrinogen. The points lie fairly close to a straight line, as predicted, particularly at higher concentrations, where the theory would be expected to be more accurate. Presumably, curvature will be evident at lower concentrations where the approximations break down. In summary, the data of Figs. 1 to 3, and other similar data not shown, provide an experimental test of the applicability of Equation 12. No serious problems seem to arise from variation in the microstructure of the filter paper or from evaporation.

Electrophoresis of Fractionated Proteins—Similar experiments were next carried out with fractionated proteins which had been subjected to electrophoresis under the conditions already described. At the same time it was desired to check the applicability of Equation 12 to a given protein component in a synthetically prepared mixture. For this purpose 8 solutions were prepared, each of which contained the same amount of bovine fibrinogen (0.47%) but varying amounts of BSA. These mixtures were each separated by paper electrophoresis, heat-denatured, treated with the copper solution, and scanned with a copper electrode in the manner previously described. A typical Versascribe pattern and set of data are shown in Fig. 4. It should be noted that the presence of a constant amount of fibrinogen does not interfere with the migration of the albumin (i.e. there are no significant albumin-fibrinogen interactions). Further, the area under the albumin peak is a linear function of the logarithm of the albumin concentration as predicted by Equation 12. The deviation from linearity at low protein concentration may again be due to the breakdown of the approximations. These results are especially satisfactory considering that diffusion, and adsorption of protein along the paper, can occur during electrophoresis. Despite these possibilities, a very good baseline was obtained (see Fig. 4).

After we had obtained this further verification of the theory, under conditions of electrophoresis, a variety of metals and protein fractions were investigated in order to improve the

![Fig. 2](image_url)

**Fig. 2.** The relation between area and concentration in stripes of bovine fibrinogen, without electrophoresis, recorded using the copper system. The pattern obtained resembled that shown in Fig. 1, except that much lower protein concentration was required to give equivalent peak size with fibrinogen. Areas under the peaks are plotted against protein concentration on a semi-logarithmic scale.

![Fig. 3](image_url)

**Fig. 3.** The relation between area and concentration in stripes of human γ-globulin, without electrophoresis, recorded using the silver system. A typical Versascribe pattern is shown at the top, with protein concentration in percent indicated. The areas under the peaks are plotted against the protein concentration on a semi-logarithmic scale.
selectivity of the method. The choice of metals was somewhat limited by the requirement that the pH of the system should not be too low, to enhance binding (8), and also that the electrodes should be rapidly reversible. In addition to the silver and copper systems, cobalt, nickel, iron, and mercury were tried. The results obtained with cobalt and nickel were less satisfactory than the others; therefore, it was decided to concentrate on the four most reproducible systems which provide a fair variety of the available types of metals.

Since the Fe, Fe++ electrode is not as rapidly reversible as would be desired, and the construction of a pure mercury electrode presents technical difficulties, a somewhat different technique was applied in the use of these metals. In general, a binding site on a protein tends to discriminate between the ions of a metal in various oxidation states so that, for example, ferric ion might be bound very strongly, and ferrous ion might be bound to a very limited extent or very weakly by the same protein (e.g. γ-globulin (6)). Therefore, it is possible to treat a protein-bearing strip with a mixture of ions in two different oxidation states; if one of the ions is bound preferentially, the ratio of activities will be different from that at the reference point, giving rise to an electromotive force in the same manner as at the types of electrode previously considered. This electromotive force may be detected by simply scanning the strip with a platinum electrode. Even though the relative affinities of the two ionic species would depend upon their relative concentrations in the solution, no attempt was made in these experiments to determine these relative concentrations or to obtain quantitative relationships between protein concentration and observed electromotive force. It was considered satisfactory, for our purpose, to show that different types of patterns (including peak size and shape) can be obtained with various metals.

In order to gain some knowledge of the kind of results that could be expected, the fractionated plasma components used for the previous studies were subjected to electrophoresis, and then scanned, using each of the systems described. Typical results are shown in Fig. 5. The most striking feature is the great difference between the patterns obtained with the various metal systems. With silver, for example, the fibrinogen seems to be quite homogeneous, whereas with copper and mercury two components are clearly evident. It was found, in fact, that this particular fibrinogen preparation was clottable only to an extent of 85%, so that there is a distinct possibility that the "active" and "inactive" species might be separated by electrophoresis, but have been heretofore undetected. The peculiar pattern of γ-globulin obtained with iron is also interesting, although it has not as yet been clearly demonstrated whether this is fact or artifact. This doubly spiked pattern seems to appear only when the concentration of γ-globulin is rather high (greater than about 1.5%) and might be a consequence of the fact that both ferrous and ferric ions are bound by the protein, but with different binding constants, rather than actually indicating the presence of several separated components. This type of pattern appeared quite often in "abnormal" plasma, but not in those samples considered to be most likely "normal." Albumin, also, appears to have been fractionated, with several peaks showing up in the pattern obtained with silver.

Electrophoresis of Plasma—Several samples of human blood plasma, collected for routine clinical tests were obtained from the
The concentration of albumin was too high for this apparatus to measure accurately at the sen-
plasma with the Spinco Analytrol, after staining with bromphenol labeled BPB was obtained by scanning a strip bearing the normal
migration of most of the components toward the right. The pattern which the samples were applied to the filter paper, with the mi-
after electrophoresis. The vertical lines indicate the point at
metal systems on normal plasma and agammaglobulinemic serum,
variation was noted from one to another. However, duplicate
patterns leads one to suspect that there is y-globulin present in
component in the abnormal serum, between the beta-globulin and the alpha-globulin, which does not appear in the normal plasma.
Furthermore, careful comparison of either the silver or copper patterns leads one to suspect that there is y-globulin present in
in low concentration, but that its mobility is lower than that of the normal individual. Or perhaps only the fast-moving y-globulins are completely absent, and the slow-
moving components continue to be present.

It has been somewhat disappointing that the beta metal-binding
globulin has not been selectively brought out by the iron treat-
ment, as was expected in view of the fact that ferric ion is strongly
bound to this protein, whereas ferrous ion is hardly bound at all
(6). This might be explained by the fact that it was necessary
to work at very low pH in order to keep the ferric ion in solution,
and the extent of binding decreases rapidly at the lower pH val-
ues. Or the absence of a peak might be due to the small number of
binding sites (two) for this metal, requiring a rather high protein
concentration before B/A becomes significantly small. That the latter might be the explanation is indicated by the ap-
pearance of a peak in the beta-globulin region in one of the abnor-
mal plasmas analyzed (in which the concentration of beta-globulin
is abnormally high), as is shown in Fig. 7. Coincidentally, the
doubly spiked y-globulin pattern was also obtained with this
particular plasma. The pattern obtained with the copper sys-
tem, shown for purposes of comparison, indicates that the beta-
globulin content is indeed considerably higher than usual, and
that there are also some slow y-globulins present, causing an un-
usually skewed peak.

These results give some indication of the possible wide applica-
tion of this general technique. Further research will undoubtedly
develop other metal-ion systems which can impart even better
selectivity than those studied thus far. However, aside from
the promise of improved selectivity, the procedure described here
also makes it possible to obtain results in a much shorter time
than is required by the dye-binding techniques. Within perhaps
half an hour after the protein-bearing strips are removed from
laboratory of Tompkins County Memorial Hospital,10 subjected
to electrophoresis, and scanned, using each of the four metal
systems described above. Since these samples were obtained
from hospitalized patients, it was not surprising that considerable
variation was noted from one to another. However, duplicate
strips showed good reproducibility in most cases. In order to
obtain a standard of comparison, i.e. a presumably normal
plasma, the blood of one of the authors was drawn; clotting was
prevented by the addition of heparin, and patterns were made
with each of the metal systems, as well as in the conventional
manner with the Spinco Analytrol, after dyeing some strips with
bromphenol blue. In Fig. 6 the normal plasma is compared
with the serum of a patient having agammaglobulinemia.

It is, first of all, interesting to compare the patterns obtained
by this metal-ion scanning technique with that obtained by the
conventional method. Although there is no clear-cut demon-
stration of additional components by use of the metal-ion binding
technique, the various fractions seem to be more clearly defined
and, upon close examination of the different patterns, it may be
inferred that several sub-fractions are discernible. The clear
resolution of the y-globulin fraction of the normal plasma is
especially evident, compared to the pattern obtained with the
Analytrol. It should also be noted that, in the Analytrol pat-
tern, the highly concentrated albumin peak is not obtained at
the sensitivity required to give reasonable peak heights to the
other components. On the other hand, by proper choice of
metal, it is possible to control the albumin peak height so that
all components are easily detected in a single scan. Of course,
previously mentioned, calibrations are required with each metal-
protein combination in order to convert areas to protein concen-
trations. At the same time, this is one of the great advantages
of the method, i.e. by appropriate choice of metal a given peak
can be surpressed or enhanced, providing selectivity in the
analysis of complex mixtures.

This feature of selectivity is demonstrated in Fig. 6, by com-
paring the patterns of normal plasma with those of abnormal
serum. Referring first to the silver patterns, the absence of both
y-globulin and fibrinogen in the abnormal serum is immediately
apparent. In the same patterns, there seems to be an additional
component in the abnormal serum, between the beta-globulin and the alpha-globulin, which does not appear in the normal plasma.

Fig. 6. A comparison of patterns obtained with four different
metal systems on normal plasma and agammaglobulinemic serum,
after electrophoresis. The vertical lines indicate the point at
which the samples were applied to the filter paper, with the mi-

Fig. 7. An abnormal plasma (diagnosis not known), subjected
to electrophoresis. At the top is a pattern obtained upon treat-
ment with the iron system, showing the doubly spiked y-globulin
peak and the beta-globulin peak which is generally absent in patterns obtained with this system. Below, for purposes of comparison,
is a pattern obtained by treatment with the copper system. It
is noted that the y-globulin content, as well as that of the beta-globu-
in, is unusually high in this plasma.

10 We are indebted to Dr. W. L. Lanyon for these samples.
the drying oven following electrophoresis, it is possible to have the analytical results. This is to be compared to the 7 hours or so, required in the dye-binding method. Furthermore, variations of this technique should make it possible to scan other media, such as starch gel, agar, glass fiber, etc., thereby taking advantage of the improved resolution obtained with some of these other supports, and still have the added advantage of improved selectivity of the metal-ion binding.

SUMMARY

It has been found by Sharpsteen that if a wet protein-bearing filter paper strip is contacted at one end by an electrode, and a second electrode is drawn along the strip, an electromotive force is produced when the moving electrode encounters regions of the paper which bear the protein. This effect can be enhanced by binding various metal ions to the protein and scanning the paper strip with an electrode of the corresponding metal. It is shown here that this electromotive force arises as a result of the formation of a concentration cell due to binding of metal ions to the protein, and a theory is presented which relates this electromotive force to the concentration of protein present. Experimental evidence is presented which tends to corroborate the theory; furthermore, it is shown that an electromotive force is obtained with each of several different plasma proteins, e.g. bovine serum albumin, bovine fibrinogen, and human γ-globulin, and with several different metals, viz. Cu, Ag, Fe, and Hg. Finally, it is demonstrated that strikingly different patterns are obtained by using different metal systems with the same electrophoretically separated plasma, and it is suggested that this might make it possible to determine certain components of protein mixtures more selectively.

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