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 electrolyte concentration cells, the variable voltage arising from gradients in the concentration of hydrogen and buffer ions. However, because of instrumental difficulties (e.g. poor signal to noise ratio, inability to obtain a well defined electrode reaction because of oxide coating on the platinum electrode, etc.) quantitative results could not be obtained to test the validity of the method in order to develop a scanning procedure.

Since proteins bind various metal ions it was suggested that the combination of a metal ion to the protein on the paper strip, followed by scanning with an electrode of the same metal, might provide a well defined reversible electrode and might also overcome the problem of a poor signal to noise ratio. Sharpsteen carried out such experiments using silver and blood plasma in a procedure involving photochemical reduction of silver ion to metallic silver, before scanning, and obtained a significant improvement in the appearance of the scanning records. Having been apprised of this initial success, we proceeded with a systematic investigation of the problem along the following lines: (a) developed a simple scanning procedure based on metal-protein interaction, (b) showed that the origin of the voltage is indeed in the 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), (c) related the observed electromotive force to the concentrations of the various protein components, and (d) 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. P67508. Bovine fibrinogen was prepared from plasma Fraction I (Armour Lot No. 82094) by a modification of the method of Laki, as described by Sturtevant et al. 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 Hg⁺⁺⁺ and Hg⁺⁺ ions prepared by dissolving 0.025 mole each of mercurous 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 recording and obtaining 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 minimum 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 strip, 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. Since the Spinco sample strip 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 strip 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++-Fe+++ mixture was used instead of a Fe-Fe+++ electrode because the latter is not easily reversible. The instability of ferric 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 0.03 M mercurous-mercuric solution for 10 seconds and then scanned with a movable silver electrode and a fixed platinum electrode.

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.
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 $M^{+}$ and then withdrawn, the activity of the metal ion will be uniform throughout the paper (and equal to that of the treatment solution if interference of the protein according to Equation 1. Therefore, in order to compute $E_{cell}$ as a function of the total protein concentration, $P$, it is necessary to relate $P$ to $(a_{M^{+}})_{total}$. For this purpose we must have information about the binding of $M^{+}$ 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 $M^{+}$, 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 $M^{+}$ ions, by the following equation (9), which holds in the absence of electrostatic interactions.

$$\frac{1}{r} = \frac{1}{mkA} + \frac{1}{m}$$  \hspace{1cm} (4)

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

$$r = \frac{B}{A}$$  \hspace{1cm} (5)

Combining Equations 4 and 5, we obtain

$$\frac{P}{B - A} = \frac{1}{mkA} + \frac{1}{m}$$  \hspace{1cm} (6)

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

$$\frac{P}{B} = \frac{1}{m} \left[ \frac{1}{kA} + 1 \right]$$  \hspace{1cm} (7)

If $k$ is not too large, say not greater than $10^{6}$, then $1/kA$ will be much greater than unity because $A$ is very small. With this further approximation, Equation 7 becomes

$$\frac{P}{B} = \frac{1}{mkA}$$  \hspace{1cm} (8)

Approximating $(a_{M^{+}})_{fixed}$ by $B$ and $(a_{M^{+}})_{total}$ by $A$, Equation 1 becomes

$$E_{cell} = -\frac{0.059}{n} \log \frac{B}{A}$$  \hspace{1cm} (9)

Substituting Equation 8 in Equation 9, we obtain

$$E_{cell} = -\frac{0.059}{n} \log P - \frac{0.059}{n} \log mk$$  \hspace{1cm} (10)

In reality, the protein is distributed in an unknown way (in the direction of scanning) through the stripe. Therefore, it is 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 $(a_{M^{+}})_{fixed}$ remains constant as the scanning electrode moves, the value of $E_{cell}$ will depend on $(a_{M^{+}})_{total}$ in the presence of the protein according to Equation 1. Therefore, in order to compute $E_{cell}$ as a function of the total protein concentration, $P$, it is necessary to relate $P$ to $(a_{M^{+}})_{total}$. For this purpose we must have information about the binding of $M^{+}$ 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 $M^{+}$, 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 $M^{+}$ ions, by the following equation (9), which holds in the absence of electrostatic interactions.
impossible to specify what fraction of the protein in the stripe is contributing to $E_{cell}$ 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 $f$ concentration cells, each with a protein concentration $P_i$. If each cell has an electromotive force equal to $E_i$, given by Equation 10, then the area under the curve of the recorded peak will be

$$\text{Area} = \sum E_i = -\frac{0.059j}{n} \log \frac{P}{j} = -\frac{0.059}{n} \log \frac{mk}{j}$$

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 $P$. 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$^{+}$ 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 $x = 800$. Thus $k$ is not too large, as assumed, in transforming Equation 7 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$^4$, $P/j$ 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 $M^{+}$ 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 $E_{cell}$ would become too small to measure accurately. Since we obtained significantly large values of $E_{cell}$ 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$^{+}$ system at several different protein concentrations on the same strip. A typical
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 \( \gamma \)-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, \( \gamma \)-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
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. β-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” plasmas, 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

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**Fig. 4.** Relation between area and concentration of BSA after electrophoresis of mixtures of BSA and fibrinogen, using the copper system. A typical Versascribe pattern, this one having a BSA concentration of 2%, is shown at the top. The short vertical line indicates the location of the origin, with the arrow indicating the direction of migration. The data for the areas of the albumin peaks in the 8 runs in this figure were all obtained from a single experiment and are plotted on a logarithmic scale.

**Fig. 5.** Solutions of three purified plasma components, 4% BSA (labeled A), 0.8% bovine fibrinogen (labeled φ) and 1.6% human γ-globulin (labeled γ), were subjected to electrophoresis and then scanned, using the copper, silver, iron, and mercury systems, respectively. The vertical line in each case indicates the point at which the protein was applied to the filter paper. Migration of albumin and fibrinogen was toward the right, γ-globulin toward the left. The electromotive force scales are not the same for all systems.
The concentration of albumin 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 migration of most of the components toward the right. The pattern labeled BPB was obtained by scanning a strip bearing the normal plasma with the Spinco Analytrol, after staining with bromphenol blue in the conventional manner. The concentration of albumin was too high for this apparatus to measure accurately at the sensitivity used.

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 demonstration 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 γ-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 pattern, 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, as previously mentioned, calibrations are required with each metal-protein combination in order to convert areas to protein concentrations. 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 comparing the patterns of normal plasma with those of abnormal serum. Referring first to the silver patterns, the absence of both γ-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 β-globulin and the α2-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 γ-globulin present in the abnormal serum, in low concentration, but that its mobility is lower than that of the normal individual. Or perhaps only the fast-moving γ-globulins are completely absent, while the slow-moving components continue to be present.

It has been somewhat disappointing that the β(metal-binding)-globulin has not been selectively brought out by the iron treatment, 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 values. 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 appearance of a peak in the β-globulin region in one of the abnormal plasmas analyzed (in which the concentration of β-globulin is abnormally high), as is shown in Fig. 7. Coincidentally, the doubly spiked γ-globulin pattern was also obtained with this particular plasma. The pattern obtained with the copper system, shown for purposes of comparison, indicates that the β-globulin content is indeed considerably higher than usual, and that there are also some slow γ-globulins present, causing an unusually skewed peak.

These results give some indication of the possible wide application 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

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**Fig. 6.** A comparison of patterns obtained with four different metal systems on normal plasma and agammaglobulinemia serum, after electrophoresis. The vertical lines indicate the point at which the samples were applied to the filter paper, with the migration of most of the components toward the right. The pattern labeled BPB was obtained by scanning a strip bearing the normal plasma with the Spinco Analytrol, after staining with bromphenol blue in the conventional manner. The concentration of albumin was too high for this apparatus to measure accurately at the sensitivity used.

**Fig. 7.** An abnormal plasma (diagnosis not known), subjected to electrophoresis. At the top is a pattern obtained upon treatment with the iron system, showing the doubly spiked γ-globulin peak and the β-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 γ-globulin content, as well as that of the β-globulin, is unusually high in this plasma.

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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, vari-
a tions 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 im-
proved 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 forma-
tion 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 evi-
dence is presented which tends to corroborate the theory; furth-
more, 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 differ-
ent 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 selec-
tively.

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