[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Interpretation of Simple Electrophoretic Patterns

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Introduction.—An electrophoresis experiment with a single protein may be represented diagrammatically as follows. Initially boundaries are formed at the level a′-a, of Fig. 1, in the two sides of the cell, between the protein solution P and the buffer solution B. After displacement of the boundaries from behind the horizontal glass plates of the cell, passage of a current causes one of the boundaries, a, to descend through a volume V_d to a new position d, as in Fig. 2, and the other,

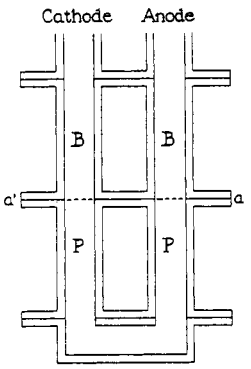

Fig. 1.

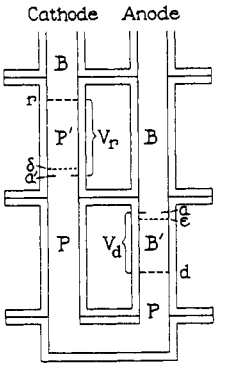

Fig. 2.

Figs. 1 and 2.—Diagrams of the electrophoresis cell illustrating the formation and relative position of the boundaries.

a′, to rise through a volume V_r to the position r of the same figure. Under ideal conditions, approximated with a dilute solution of a protein in a buffer of sufficient capacity, the volumes V_d and V_r are equal and a knowledge of their value permits a precise computation of the mobility of the protein. In general, however, the volume V_r is greater than V_d and an analysis of the concentration changes resulting from the electrolysis is necessary in order to obtain mobilities from the experimental results. It is the purpose of this paper to describe electrophoretic experiments on ovalbumin, at 0°, in which these concentration changes have been determined, and to outline an analysis of the data for the purpose of obtaining accurate mobilities.

Methods.—The recent development of methods for studying and recording refractive index gradients, initiated by Tiselius,[1] has made possible the precise study of electrophoretic boundaries. From "electrophoretic patterns" thus obtained concentration changes during electrophoresis may be computed. The available procedures are the scale method of Lamm,[2] the diagonal schlieren method of Philpot[3] and Svensson[4] and the schlieren scanning method developed in this Laboratory[5,6] and used in the present research.

In outline the schlieren scanning method is illustrated in Fig. 3. An image at P of the illuminated slit S-S is formed by the schlieren lens D. The camera lens O is focused on the electrophoresis cell E and forms an image (full sized in our apparatus) on the screen at G-G. Now if the fluid in the cell is homogeneous this image will be uniformly illuminated. On the other hand, if there is a boundary, B, between, for instance, a protein-bearing solution and a buffer, there will be a region in which the refractive index varies with the height in the cell, and light which would normally pass to P is deflected downward, since the solution with the greater refractive index is on the bottom. If the opaque schlieren diaphragm Q is raised to a point p where it intercepts the most deflected light, a dark band will

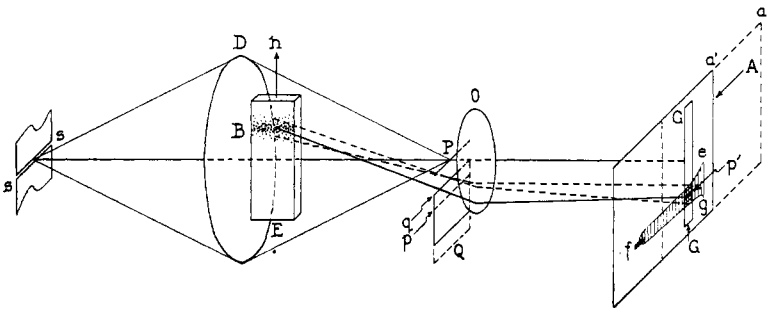

Fig. 3.—Diagram of the schlieren scanning method for the photographic recording of gradients of refractive index.

(1) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).
(2) Lamm, *Nova Acta Reg. Soc. Sci. Upsaliensis*, Series IV, **10**, No. 6 (1937).
(3) Philpot, *Nature*, **141**, 283 (1938).
(4) Svensson, *Kolloid-Z.*, **87**, 181 (1939).
(5) Longsworth, THIS JOURNAL, **61**, 529 (1939).
(6) Longsworth, Shedlovsky and MacInnes, *J. Exptl. Med.*, **70**, 399 (1939).

appear on the screen, conjugate to the region of steepest gradient in the boundary B. Such a band appears in the image G-G at p′.

However, a boundary between two solutions does not consist of a single geometric plane, but of a region in which the composition varies from that of one solution to that of the other. The refractive index, n, in such a region changes continuously with the height, h, of the liquid in the cell. The gradient, dn/dh, of refractive index, for each boundary, will thus, theoretically at least, vary from zero to a maximum, and back to zero. In Fig. 3 the variation of the gradient, dn/dh, of the boundary B in the cell E, is represented by the density of the shading. The pencil

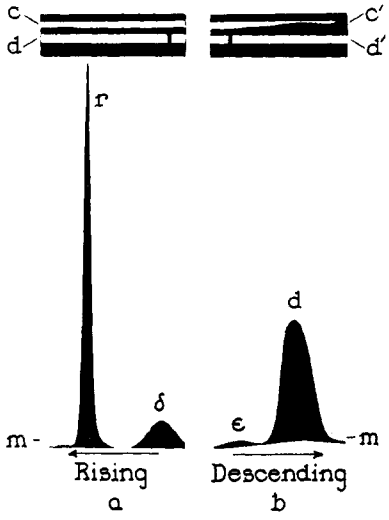

Fig. 4.—Electrophoretic patterns of a 1.36% solution of ovalbumin in a 0.1 N sodium acetate buffer at pH 3.92 and 0°. Scanning exposure made after electrolysis for 13,000 seconds at 5.39 volts per cm.

of light passing through the layer having the maximum value of the gradient will be most bent from the normal path and will be the first to be intercepted when the schlieren diaphragm Q is raised. On lifting this diaphragm still further, *i. e.*, to the level q, less refracted pencils of light will also be intercepted and the schlieren band in the image G-G will widen. This obviously can be continued until the whole field has been covered. In the schlieren scanning method this process is made continuous and is recorded photographically. The image of the cell at G-G is masked by a narrow vertical slit and a photographic plate A is moved in the direction of the arrow at a constant rate across this slit. Actuated by the same mechanism, the schlieren diaphragm Q is given a steady movement upward. The resulting (positive) photographic record for a typical single boundary is indicated by the area e-f-g. The displacement of the diaphragm Q from the position P is proportional to the gradient at levels in the cell E conjugate to the edges of the schlieren bands. Thus the contour of the area e-f-g indicates both the position and the magnitude, of the refractive index gradients existing in the boundary. Since the photographic plate A was in position a at the time the schlieren diaphragm was at p, a section of the band p′ appears at f when the plate has been moved to a′. It will be seen that the usual schlieren bands are narrow sections through the area e-f-g. By a system of gears the ratio of the rates of motion of the schlieren diaphragm Q to that of the plate A is given a constant value, such as one to three, and this ratio can be varied by changing the gears. For establishing the base line of the schlieren patterns the position of the diaphragm Q may be read accurately with a micrometer.

Experimental Results and Discussion.—The type of phenomenon to be discussed in this paper is illustrated in Fig. 4, which represents electrophoretic patterns of a 1.36% solution of ovalbumin at pH 3.92 in a 0.1 normal sodium acetate buffer.[7]

In order to interpret these patterns in terms of concentrations and mobilities, information must be available about (a) the base line of the diagram and (b) the starting position of the boundaries. The base line is obtained as shown at c-c′ of Fig. 4 by a scanning exposure of the cell immediately after the formation of the boundaries but before the latter have been shifted into view from behind the horizontal glass plates. The procedure consists in setting the schlieren diaphragm, Q of Fig. 3, just below P, the exact position of the diaphragm being given by the reading, m of a micrometer, and then scanning until the diaphragm is somewhat above P. The lower edges of the strips c and c′ of Fig. 4 are thus the schlieren patterns of the windows of the cell and thermostat, deviations of these edges from the horizontal indicating imperfections in the windows. The upper edges of the strips c and c′

(7) The ovalbumin used in this research was prepared from fresh hen's eggs. Although three times recrystallized, it was not electrophoretically homogeneous. At most pH values the main component is accompanied by a second component migrating somewhat more slowly, as shown in Fig. 2 of reference (5) and in Fig. 6 of this paper. Similar patterns have been obtained with samples of ovalbumin from four different laboratories. A study of egg white now in progress indicates that the slow component is present in the starting material. Ovalbumin appears homogeneous in the patterns reproduced in Fig. 4, because the pH-mobility curves of the two components apparently intersect in the neighborhood of the isoelectric point. At pH 3.92 the protein may be considered electrically homogeneous, thereby simplifying the interpretation of the patterns.

correspond to a reading, m, of the micrometer. The equivalent position on the patterns a and b, Fig. 4, is at m-m. Consequently when the upper edges of the strips c-c′ are brought into coincidence with the line m-m the lower edges of these strips constitute the base line for the patterns. In order to obtain the position of the freshly formed boundaries after they have been shifted into view, the schlieren bands shown at d-d′ in Fig. 4 are recorded on the photographic plate. As Guggenheim[8] has suggested, the diffusion process smooths out the slight irregularities in the distribution of concentrations resulting from the formation and shifting of the boundaries. As illustrated by the finite width of the bands in the strip d-d′, the boundaries are not infinitely sharp. However, the positions of the mid-points of these bands may be taken as the positions at zero time from which mobilities can be computed.

It will be seen in Fig. 4 that two peaks or maxima are evident in the patterns of both the rising and descending boundaries but that the patterns are far from being mirror images of each other. This may, possibly, be an extreme case but is all the more useful in illustrating deviations from ideal behavior and in testing the validity of the interpretation to be given below.

The deviations, or so-called "boundary anomalies," illustrated by these patterns are as follows. (1) The rising boundary r is much sharper than that, d, descending into the protein solution. (2) The boundary r has swept through a larger volume V_r, than that, V_d, of the boundary d. (3) The total shaded area of Fig. 4a is equal to that of Fig. 4b, but the partial area A_r under the rising boundary r is less than that, A_d, for the descending boundary d. Since these areas, as will be shown, are proportional to the concentration changes at the boundaries it is apparent that the change is less at the rising boundary than at the descending one. Also (4), the concentration distribution in a boundary is not, in general, symmetrical about the maximum as is shown by the shapes of the peaks d and r.

These deviations were explained qualitatively by the authors[9] as follows. In practice the protein solution is prepared by dialysis against a buffer solution. A difference, due to the Donnan equilibrium, of salt composition exists between the two solutions when dialysis is complete. Moreover, the protein solution contains conducting constituents, the protein ions, that are not present in the buffer solution. When passage of a current causes a boundary between two such solutions to move from a to d of Fig. 2, for example, there is found in the intervening volume V_d a buffer solution of composition [B′]. This composition has been adjusted, in general, to a value different from that of B.[10] The boundary ϵ, which moves very slowly, thus forms between two solutions of the same buffer, but at different concentrations,[11] and is quite evident in Fig. 4. With the boundary r moving upward into the buffer solution, there is a similar but more complicated adjustment of the composition of the protein solution which replaces the buffer as the boundary rises. The resulting concentration boundary δ, between the solutions P and P′, Fig. 2, also moves very slowly under the influence of the current. The δ boundary, also shown in Fig. 4, is much more visible than the ϵ boundary because the former involves a gradient of protein concentration whereas the latter does not.

Since the specific conductance, κ_P, of the protein solution P has been found experimentally to be less than that, κ_B', of the adjusted buffer solution, the electric field is greater in the protein solution than in the buffer and variations of this field thus exist at the descending boundary d. The protein ions in the dilute uppermost layers of the boundary d are therefore in weaker fields than are those in the concentrated layers and thus tend to lag behind, causing the boundary to become diffuse, as is shown in Fig. 4b. In the case of the boundary r rising into the buffer solution $\kappa_P' < \kappa_B$ so that the dilute, slowly moving protein ions tend to be overtaken by the faster concentrated ones, with the result that this boundary tends to remain sharp, as is illustrated by Fig. 4a. Due to these field gradients at the boundaries, and in some instances to *p*H gradients also, the distribution through a boundary may be unsymmetrical about the ordinate passing through the maximum of the refractive index gradient curve. This lack of symmetry, shown clearly by the boundary d of Fig. 4b, has been studied by Tiselius and Horsfall.[12] Assuming a proportionality between refractive index and protein concentration it is apparent that in such cases the position of the maximum gradient is not identical with that of the ordinate which divides the area under the

(8) Guggenheim, THIS JOURNAL, **52**, 1315 (1930).

(9) Longsworth and MacInnes, *Chem. Rev.*, **24**, 271 (1939).

(10) This adjustment is understood for some simple cases (Kohlrausch, *Ann. Physik*, **62**, 209 (1897); Weber, "Die partiellen Differential-Gleichungen der mathematischen Physik," 5te Aufl., Braunschweig, 1910, **1**, 195–203, 503–527; Longsworth, THIS JOURNAL, **52**, 1897 (1930); Henry and Brittain, *Trans. Faraday Soc.*, **29**, 798 (1933)) but the theory for the more complicated systems frequently encountered in practice has not been developed.

(11) See MacInnes and Longsworth, *Chem. Rev.*, **11**, 199 (1932).

(12) Tiselius and Horsfall, Jr., *J. Exptl. Med.*, **69**, 83 (1939).

curve into two equal parts. The position of the latter ordinate is the better value to use in mobility computations since it is approximately the position the boundary would have if it retained its original sharpness. The precise location of an unsymmetrical boundary requires an integration of the concentration–distance curve. Computations for the cases considered in this paper show that the position of the ordinate dividing the gradient curve into equal parts differs from the true value by at most 1%, whereas the position of the maximum ordinate may be in error by 19%.

It is important in this connection to be clear as to what is meant by the mobility, u, of a protein. It is the distance moved per second in a unit electric field by an average particle in the body of the protein solution. The movement of the particle through this distance corresponds to the transport of uA [P] grams of protein through a plane (to be discussed below) in the body of the protein solution, A being the cross-sectional area of the channel and [P] the concentration of protein in grams per milliliter. If the electric field, F, differs from unity and the transport continues for t seconds the total quantity, p, of protein transported through a reference plane is $p = uA[P]Ft$, from which $u = p/FAt[P]$. Since the field strength, F, in the body of the protein solution is $i/\kappa_P A$ this expression becomes

$$u = p\kappa_P/[P]it \qquad (1)$$

in which i is the current. The quantity p is determined analytically in the Hittorf or "transport" method, whereas in the moving boundary method it is determined by following the motion of a boundary between the solution and the buffer. If the passage of electricity causes a boundary to descend from a to d, of Fig. 2, it sweeps through a volume $A(a - d) = V_d$. Thus the amount of protein passing any plane in the unmodified solution is $A(a - d)[P] = V_d[P]$. Equation 1 thus becomes

$$u_d = \frac{A(a-d)[P]}{[P]}\frac{\kappa_P}{it} = \frac{V_d\kappa_P}{it} \qquad (2)$$

in which u_d indicates the mobility computed from data on the descending boundary.

The computation of mobilities from data on the rising boundary is, however, complicated by the change of protein concentration at the δ boundary. Assuming, in essential agreement with experiment, that the latter remains stationary, the quantity, p, of protein migrating upward through a plane in the body of the solution on the passage of it coulombs is $(r - a')A$ [P′] = V_r[P′] in which [P′] is the concentration of protein in the adjusted solution between the δ and r boundaries. Introducing this value of p into equation 1 we obtain

$$u_r = \frac{[P']}{[P]}\frac{V_r\kappa_P}{it} \qquad (3)$$

which differs from equation 2 in that it contains the concentration ratio [P′]/[P]. Since it is one of the main conclusions of this paper that, correctly interpreted, the data on rising and descending boundaries yield the same value of the mobility, *i. e.*, $u_d = u_r$, it is important to establish values of the ratio [P′]/[P]. This requires an investigation, which is given below, of the changes of protein and salt concentrations in the channel of the apparatus during electrolysis.

Strictly, the reference plane from which mobilities are measured should be fixed with respect to the solvent. Since the observed boundary displacements refer to a plane fixed with respect to the apparatus they should be corrected for the displacement the solvent experiences as a result of electrolysis (see reference 11, page 203) otherwise the mobilities found will depend, among other things, upon the nature of the electrode reaction. This computation is greatly facilitated if one electrode chamber is closed since only the volume changes occurring on this side of the protein solution can affect the position of the boundaries. In the experiments reported in this paper the anode chamber was closed and the volume change, ΔV, was equal to

$$\Delta V = f(T_-\varphi_{NaAc} - \varphi_{NaCl} + V_{AgCl} - V_{Ag}) - [P]V_d\bar{\varphi}_P$$

in which V_d is the volume swept through by the descending boundary, for example, on the passage of f Faradays, T_- the anion transference number of sodium acetate, φ and V are the apparent molal and molal volumes, respectively, of the materials indicated by the subscripts, and $\bar{\varphi}_P$ is the apparent specific volume of the protein. On the basis of the available data

$$\Delta V = 19.03f - 0.749[P]V_d$$

A positive value for this volume change represents an expansion of the solution in the closed side and consequently the observed V_d is too large by an amount ΔV. The values of V_d and V_r recorded in Table I have been corrected for this volume change. The corrections for the descending boundary are 2.1, 1.5 and 0.5% at protein concentrations of 0.64, 1.36 and 2.74%,

respectively, and are thus by no means negligible. The validity of the correction is difficult to prove in the case of the electrophoresis of proteins but has been amply demonstrated for strong electrolytes.[11]

We have observed repeatedly, within a small experimental error, that (1) the total area of an electrophoretic pattern is independent of the time during the electrolysis at which a pattern may be obtained, *i. e.*, the area is independent of the mode of variation of the refractive index through the boundaries and (2) the total areas of the patterns for the two sides of the channel are identical. These observations are consistent with the fact that the total area of a pattern is given by the integral $\int_B^P (dn/dh)dh$, in which n is the refractive index at the level h in the cell, the limits of integration being the same for both sides of the channel and independent of the time of electrolysis. Thus the results of planimeter measurements on enlarged images of Figs. 4a and b, in arbitrary units, yielded $A_\epsilon = 18$, $A_d = 354$, $A_\delta = 72$ and $A_r = 302$, from which the sum of the areas for the descending boundaries, $A_\epsilon + A_d$, is 372, whereas the sum, $A_\delta + A_r$, for the rising boundaries has the closely agreeing value of 374.

From the discussion given above it is evident that the partial area A_ϵ of Fig. 4 is due to a gradient of buffer concentration. Since the solution P retains its original composition, in which the buffer electrolyte concentration differs but little from that of solution B, another variation of salt concentration, opposite in sign to that at the ϵ boundary, must exist in the boundary d. Consequently the area A_d must be increased by an amount A_ϵ in order to measure the change of protein concentration at the boundary d. Conductance measurements of the B′ and P′ solutions, to be described later in this paper, indicate that the δ boundary also contains a gradient of buffer salts. Since the same quantity of electrolyte enters the channel from the electrode vessel on one side as leaves the channel on the other side, it follows that the increment of buffer electrolytes in the B′ solution must be compensated by a corresponding decrement in the P′ solution. The concentrations of salts [B] and protein [P] as functions of the height can thus be represented schematically, as is shown in Fig. 5. The increment of buffer salts in the B′ solution is proportional to $A_\epsilon V_d$ while the corresponding and equal decrement in the P′ solution is proportional to $A'_\epsilon V_r$ in which A'_ϵ is that portion of the area A_δ due to the electrolyte gradient in the δ boundary. Therefore $A'_\epsilon = A_\epsilon V_d/V_r$. Moreover, since an electrolyte gradient, equal in magnitude but opposite in sign to that in the δ boundary, must also exist in the boundary r, the area A_r must be increased by an amount $A_\epsilon V_d/V_r$ in order to measure the change of protein concentration at this boundary. The ratio [P′]/[P] of equation 3 thus takes the value $(A_r + A_\epsilon V_d/V_r)/(A_d + A_\epsilon)$ and equation 3 becomes

$$u_r = \frac{V_r \kappa_P}{it} \times \frac{A_r + A_\epsilon V_d/V_r}{A_d + A_\epsilon} \qquad (3a)$$

A small error may arise from the use of equation 3a due to the fact that account has not been taken of slight *p*H changes at the boundaries.

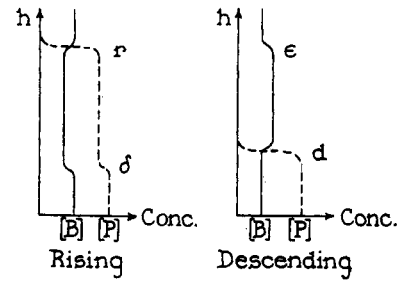

Fig. 5.—Schematic diagram of the changes of buffer electrolyte (———) and protein (– – –) concentrations at each of the electrophoretic boundaries.

The data necessary for the computation, and the resulting mobilities, at three concentrations of ovalbumin are recorded in Table I. The figures in the second column include data obtained from Fig. 4 and at the other concentrations

TABLE I

ELECTROPHORETIC MOBILITIES, u, OF OVALBUMIN IN A 0.1 NORMAL SODIUM ACETATE BUFFER AT *p*H 3.92 AND 0°

1	Protein concn., g. %	0.64	1.36	2.74
2	Conductance, κ_P, at 0°	0.003491	0.003434	0.003326
3	Coulombs, it	143.3	171.7	148.5
4	Volume, V_d, ml.	1.114	1.339	1.15_2
5	Volume, V_r, ml.	1.244	1.609	1.58_0
6	A_d, area	169	354	715
7	A_ϵ, area	7	18	36
8	A_r, area	151	302	533[a]
9	A_δ, area	23	72	218
10	Relative viscosity,[b] η, 0°	1.022	1.047	1.095
11	$u_r^* \times 10^5$	3.03	3.22	3.54
12	$u_r \times 10^5$ (Equation 3a)	2.71	2.74	2.63
13	$u_d \times 10^5$ (Equation 2)	2.71	2.68	2.59
14	$u_d\eta$	2.77	2.81	2.84

[a] By difference; the maximum refractive index gradient of this boundary was too great to be recorded. [b] Interpolated from measurements made in this Laboratory.

from similar electrophoretic patterns. The mobilities u_d given in line 13 have been computed with the aid of equation 2 whereas the values u_r in line 12 were obtained from equation 3a. It will be noted that they agree reasonably closely, as would be expected if our interpretation is correct. Line 11 contains values of u_r^* computed from the relation $\kappa_P V_r/it$, in which the change of protein concentration at the δ boundary, *i. e.*, [P']/[P], has been ignored. The large error which may arise from this procedure may be seen by comparing the figures of lines 11 and 12. The percentage deviations reach a maximum of 37% in our experiments.[13]

It will be observed that the mobilities of ovalbumin, computed as described above and recorded in lines 12 and 13 of Table I, change but slightly with the protein concentration and much of this change disappears if the mobilities are multiplied by the relative viscosity, η (line 10), the product $u_d\eta$ increasing but slightly with the protein concentration. This observation is important since it indicates that mobility measurements may aid in the identification of components in an electrophoretic pattern.

The Delta and Epsilon Boundaries.—Although the interpretation, given in the foregoing pages, of patterns of the type shown in Fig. 4, appears to be satisfactory, two questions may arise in the mind of the reader. Do gradients of buffer electrolyte really exist in the boundaries? And has the possibility that the δ and ϵ boundaries may be due, in part, to a slowly moving protein constituent been completely eliminated?

To answer the first of these questions the procedure was as follows. After the electrophoresis in which the patterns shown in Fig. 4 were obtained, the contents of each section of the cell were isolated and the specific conductances at 0° were measured. The results, before and after electrolysis, are given in Table II. In the lower cathode and upper anode sections of the cell, Fig. 2, no boundaries were present and no significant change of conductance occurred, showing, as would be expected, that the compositions of the solutions B and P are unchanged by the passage of current. In the upper cathode section, however, where the adjusted protein solution P' has partially replaced the buffer solution B originally present, the specific conductance had decreased 5.1%. A corresponding *increase* in the lower anode section accompanied the partial replacement of the protein solution P by the adjusted buffer solution B'. The conductance of the solution P', between the boundaries δ and r, can be readily estimated. The height of the upper cathode section is known, as well as the positions of the r and δ boundaries. In addition data are available, in Table II, on the conductances of the B and P solutions, and of the mixture of these with the P' solution. These, assuming simple proportionality, are sufficient data for computing the conductance of the latter solution, which is given as "P' solution, computed" in the table. A change of equivalent conductance from $\kappa_P = 0.00343$ to $\kappa_{P'} = 0.00322$ is therefore indicated at the δ boundary. Since the conductances of these solutions are due mainly to the buffer ions and are roughly proportional to their concentrations, this indicates that there is a decrease of concentration of buffer electrolyte in passing from solution P to solution P' across the δ boundary in accord with Fig. 5. It is of interest, however, that the ratio $\kappa_{P'}/\kappa_P (= 0.939)$ differs appreciably from that [P']/[P] (= 0.853) computed from refractive index measurements, showing that the P' solution is not obtained from the P solution simply by dilution with water as required by the theory of electrophoretic boundaries discussed by Henry and Brittain.[14] The theory, however, was developed for a ternary ionic system, *i. e.*, protein ions and the anions and cations of a binary buffer salt. At the *p*H of the experiments and with the buffer solution

TABLE II

CONDUCTANCE CHANGES ON ELECTROLYSIS OF A 1.36% OVALBUMIN SOLUTION IN A 0.1 NORMAL SODIUM ACETATE BUFFER, AT *p*H 3.92

Section	κ (before)	κ (after)	Diff. in %
Cathode, upper, B	0.00353	0.00336	−5.1
Cathode, lower, P	.00343	.00344	...
Anode, upper, B	.00353	.00353	...
Anode, lower, P	.00343	.00361	+5.3
P' solution, computed		.00322	

(13) This result indicates that mobility measurements involving observations of rising boundaries, without considering the δ effect, may be in error. Thus Landsteiner, Longsworth and van der Scheer (*Science*, **88**, 83 (1938)) used a mean value, $(u_d + u_r^*)/2$, which is about 2.5% higher, for the data of their Table I for example, than the correct value, u_d. It should be noted, however, that this correction does not affect the order of the mobilities, and consequently the conclusions of that paper remain unchanged. In a recent electrophoretic study of hemoglobin Davis and Cohn (THIS JOURNAL, **61**, 2092 (1939)) observed a mobility increase of 27% on increasing the protein concentration from 0.17 to 1.33%. They computed their results from observations on the rising boundary and it seems probable that most of this apparent mobility variation is due to neglect of the correction for the concentration change at the δ boundary.

(14) Henry and Brittain, *Trans. Faraday Soc.*, **29**, 798 (1933).

used (0.1 N NaAc + 0.5 N HAc) it is probable that the effects of the hydrogen ion constituent and of the undissociated acid cannot be neglected.

In this and other laboratories the δ and ϵ boundaries were first thought to be due to slowly migrating protein or carbohydrate constituents.[15] Attempts to isolate such materials were unsuccessful, however. Although the explanation, outlined in this paper, of the boundaries was soon evident[9] the possibility of a small concentration of electrophoretically inert material was not entirely eliminated. The work of Henry and Brittain, mentioned above, suggested a method by which the δ and ϵ boundaries might be suppressed under certain conditions if no inert material were present. In the case of the electrophoresis of ovalbumin in the neutral range, where the contribution of the hydrogen ion conductance to the system is negligible, the conductance ratio $\kappa_{P'}/\kappa_P$ is in fair agreement with the ratio [P']/[P] determined refractometrically. Thus in the electrophoresis experiment (the patterns for which are shown in Fig. 6) of a 1.36% solution of ovalbumin in a phosphate buffer at 6.80 the conductance, $\kappa_{P'}/\kappa_P$, and protein concentration, [P']/[P], ratios were found to have nearly the same value, *i. e.*, 0.935 and 0.923, respectively. Under these conditions the concentrations of the constituents of the P′ solution are such as would result from dilution of the P solution with water. Thus in this case if the boundaries were initially formed between the buffer and the solution P′, obtained by diluting solution P by the factor 1/0.935, there should be no δ and ϵ boundaries left behind when the protein boundaries migrate away from their original positions. This idea was tested experimentally with the result shown in Fig. 7. It will be seen that the δ and ϵ boundaries, quite evident in Fig. 6, have been eliminated. This would appear to be conclusive evidence of the nature of the δ and ϵ boundaries, as outlined above, and of the absence of inert constituents in the sample of ovalbumin.

(15) Tiselius, *Biochem. J.*, **31**, 1464 (1937).

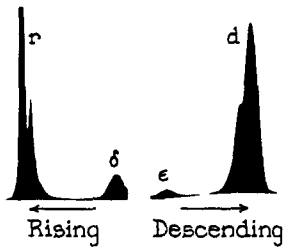

Fig. 6.—Electrophoretic patterns of a 1.36% solution of ovalbumin in a 0.1 μ sodium phosphate buffer at pH 6.80 and 0°. Scanning exposure made after electrolysis for 6000 seconds at 7.23 volts per cm.

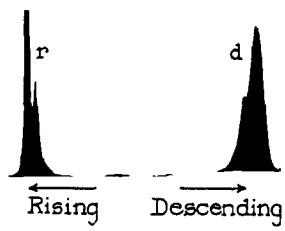

Fig. 7.—Same as for Fig. 6 except that the dialyzed ovalbumin solution was diluted in order to eliminate the δ and ϵ boundaries.

Summary

Electrophoretic studies of ovalbumin, at 0° and at pH 3.92, have been made by the moving boundary method. The research was undertaken in order to establish the conditions under which accurate values of protein mobilities may be found from moving boundary data. This involved investigation of the nature of the δ and ϵ boundaries, and of the concentration distributions in the rising and descending protein boundaries. The results indicate that, although in general more diffuse, the descending boundaries yield correct values of the mobility. The rising boundaries may give results that are greatly in error unless additional data are available from which corrections may be made, in which case the two types of boundary yield the same value of the mobility. Contrary to some recent conclusions, the mobilities have been found to vary but slightly with the protein concentration, if a small viscosity correction is made, within the composition limits studied. There is evidence that gradients of buffer concentration exist, in general, in all the boundaries. The correctness of the interpretation has been indicated by showing that, in certain cases, the δ and ϵ boundaries may be suppressed.

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