SUMMARY

1. The synthesis of lactose from glucose and glycogen by tissue slices of guinea pig mammary gland has been demonstrated chromatographically, and an attempt to evaluate the synthesis quantitatively has been made by a consideration of the results of two entirely unrelated chemical estimations.

2. No lactose synthesis from glucose or glycogen by tissue slices of cow mammary gland could be demonstrated; a critical assessment of previous claims leads to the conclusion that no *in vitro* demonstration of lactose synthesis by the ruminant gland has yet been made.

3. Maltose (cow and guinea pig glands) and lactate (guinea pig gland) proved ineffective as substrates for lactose synthesis.

We wish to express our thanks to Prof. D. C. Harrison for his interest in the progress of this work, and to Dr Q. H. Gibson for helpful criticism of the manuscript. We are indebted to Messrs Caffrey, Belfast, for regular supplies of brewer's yeast, and to Messrs Gillette Industries Ltd. for a generous gift of special razor blades.

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The Valences of Protein Ions from Electrophoretic and Membrane Potential Measurements

By P. A. CHARLWOOD, *The Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.* 1

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The moving boundary method of electrophoresis, developed by Tiselius (1930, 1937), Philpot (1938), Longsworth (1939), and Svensson (1939, 1940), has many applications, including the small-scale preparative separation of protein mixtures. The purpose of the present studies was ultimately to obtain information likely to be of use in the electrophoretic separation of interesting fractions from pathological human sera.

Convection, due to various causes, is a major difficulty in electrophoresis. The passage of a boundary through the bottom channel of the U-tube due to any cause sets up gravitationally unstable conditions and hence convection, and this is true of the so-called δ-boundary, the origin of which has been discussed by Longsworth & MacInnes (1940).

The separation of pure samples of the components *A* and *B* of a binary protein mixture is illustrated in Fig. 1. If diffusion is neglected, the best possible separation is shown in Fig. 1*a*. If the δ-boundary is eliminated, or reduced to such an extent that its passage through the bottom channel of the U-tube results in negligible convections, the separation theoretically possible is improved to that shown in Fig. 1*b* (Svensson, 1948).

The practical elimination of the δ-boundary may be accomplished either by dilution of the dialysed protein solution (Longsworth & MacInnes, 1940), or by the use of a supernatant buffer solution rather more concentrated than that used for the dialysis (Wiedemann, 1947). There are some objections to these procedures (Pedersen, 1948). Thus, for analy-

tical work it is better to reduce anomalies by suitable choice of protein concentration and buffer (Longsworth, 1942; Svensson, 1946); however, as mentioned by Longsworth (1947), the above modifications may be useful in preparative work.

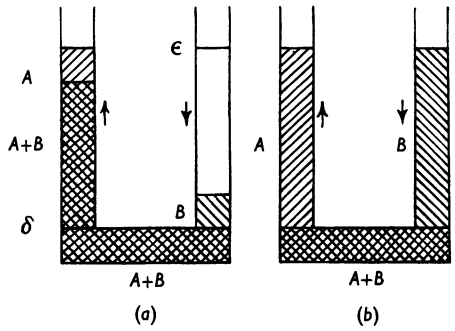


Fig. 1. Theoretical separation of mixture $A+B$ by electrophoresis (*a*) under ordinary conditions, (*b*) when the δ-boundary has been eliminated.

According to the classical theory of Kohlrausch (1897) the development of δ- (and ϵ-) boundaries is due to the different values of the 'beharrliche Funktion', or regulating function, for the dialysed protein solution and the buffer against which it has been dialysed. This buffer will be referred to as the dialysate, in conformity with Adair & Adair (1934). If we know the mobilities of all the ions present in the protein solution and their concentrations in terms of electrochemical equivalents, we can calculate the regulating function, $\Sigma c_1/u_1$ in the notation below, and hence the dilution factor, or the excess concentration of buffer in the supernatant, required to eliminate the δ- and ϵ-boundaries. If all the above information is available, it is probable that the components are already so well characterized as to render of little interest their isolation by the method under discussion. It was therefore decided to work with a comparatively pure protein preparation, and by making arbitrary modifications in the strength of the supernatant buffer, to estimate the least supernatant concentration at which the δ- and ϵ-boundaries were completely eliminated. The regulating functions of the protein solution and the supernatant should then be equal. This information, together with the electroneutrality relation and the equation of the original Donnan equilibrium set up on dialysis, suffices for the estimation of the protein concentration in terms of electrochemical equivalents and hence the valence (Longsworth, 1947).

Measurements were made on the δ-, rather than on the ϵ-boundary, as the former is larger and measurable more accurately. Although a single observation of the ϵ-boundary would suffice for deducing the regulating function of the protein solution (Longsworth, 1947), it was anticipated that the error might be less if a series of measurements was made, as in this work.

A completely homogeneous protein preparation would have been desirable for these studies, but such a material is difficult to prepare (Pirie, 1940; Alberty, Anderson & Williams, 1948). An albumin was selected because the relatively high mobilities can be measured accurately, and there is a good separation from the δ-boundary. The albumin used was horse serum albumin A (Kekwick, 1938), provided by Dr R. A. Kekwick.

Valences determined by the above method were compared with those calculated from the electrophoretic mobilities observed under similar conditions. In those cases where the effects were measurable within reasonable limits, membrane potentials were determined, yielding a third independent set of values for the valences. Comparative studies of protein-ion valences obtained by different methods have been relatively few in number (Tiselius & Svensson, 1940; Adair & Adair, 1940; Longsworth, 1941; Abramson, Moyer & Gorin, 1942; Creeth & Jordan, 1949), but great interest attaches to the results in view of the assumptions and approximations made in the different methods.

THEORETICAL

(*a*) *Moving boundary method.* If c = concentration in electrochemical equiv./l., taken with the appropriate sign, suffixes A, R, Pr refer respectively to buffer cation, buffer anion, and protein ion, suffixes 1, 2, 3 refer respectively to conditions inside the dialysis bag, in the dialysate and in the supernatant buffer when the δ- and ϵ-boundaries are eliminated, then the electroneutrality condition gives

$$c_{A,1}+c_{R,1}+c_{Pr,1}=0. \quad (1)$$

If activity coefficients for the same ion on both sides of the dialysis membrane may be considered equal, from the Donnan equilibrium,

$$\left(\frac{c_{A,1}}{c_{A,2}}\right)^{1/z_A}=\left(\frac{c_{R,1}}{c_{R,2}}\right)^{1/z_R}, \quad (2)$$

where z_R, z_A are the valences of the anion and cation respectively, taken with the appropriate sign. The Kohlrausch (1897) regulating function gives

$$\frac{c_{A,1}}{u_A}+\frac{c_{R,1}}{u_R}+\frac{c_{Pr,1}}{u_{Pr}}=c_{A,3}\left(\frac{1}{u_A}-\frac{1}{u_R}\right), \quad (3)$$

where u refers to mobility, including the requisite sign. For the Na acetate solutions $z_A=1$, $z_R=-1$ and for the Na phosphate $z_A=1$, $z_R=-2$. In these cases the best method of solution is by elimination of $c_{R,1}$ and $c_{Pr,1}$ from equations (1) to (3), yielding respectively

$$c_{A,1}^2\left(\frac{1}{u_A}-\frac{1}{u_{Pr}}\right)+c_{A,1}c_{A,3}\left(\frac{1}{u_R}-\frac{1}{u_A}\right)+c_{A,2}c_{R,2}\left(\frac{1}{u_R}-\frac{1}{u_{Pr}}\right)=0, \quad (4)$$

$$c_{A,1}^3\left(\frac{1}{u_A}-\frac{1}{u_{Pr}}\right)+c_{A,1}^2c_{A,3}\left(\frac{1}{u_R}-\frac{1}{u_A}\right)+c_{A,2}^2c_{R,2}\left(\frac{1}{u_R}-\frac{1}{u_{Pr}}\right)=0. \quad (5)$$

These equations contain the single unknown quantity $c_{A,1}$, which may be evaluated by standard algebraical procedures since the value of $c_{A,3}$ is obtained experimentally, as described below. The method of Newton or Horner enables the cubic equation to be solved to any degree of approximation. Substitution of the value of $c_{A,1}$ in the appropriate equation (2) gives the corresponding value of $c_{R,1}$. Then, $c_{Pr,1}$ follows easily from equation (1). It has been found that solution in this way is preferable to the elimination of $c_{A,1}$ and $c_{R,1}$ from equations (1) to (3), which results in clumsy expressions for the quadratic and cubic equations in $c_{Pr,1}$. The protein ion valence, z_{Pr}, can be deduced from $c_{Pr,1}$, provided the stoicheiometric concentration of protein is measured and the molecular weight known.

(*b*) *Mobility method.* The method of Abramson, Gorin & Moyer (1939) and Abramson *et al.* (1942) was used to calculate valences from the relation

$$z=\frac{6\pi\eta r}{f(\kappa r)}\frac{(1+\kappa r+\kappa r_i)}{(1+\kappa r_i)}\,u\times\frac{300}{4{\cdot}80\times10^{-10}},$$

where r=radius of protein ion (assumed spherical); r_i=average radius of ions of ionic atmosphere; κ=the Debye-Hückel ionic strength function; $f(\kappa r)$=Henry's function; η=viscosity of solvent.

In this relation, a modification of the formula of Henry (1931), derived on the basis of the theory of Debye & Hückel (1923), several assumptions and limitations are implicit; these are discussed in detail by Abramson *et al.* (1939, 1942). One such limitation is that the equation can be applied only in the case of a spherical ion, but the authors of the method evolved a means of applying the relation to cylindrical particles, by expressing the mobilities of such particles in terms of those of spheres of the same molecular volume.

(*c*) *Membrane potentials.* The measured potential difference between the protein solution and the dialysate is given by the relation

$$E=-\frac{RT}{z_AF}\ln\frac{c_{A,1}}{c_{A,2}}=-\frac{RT}{z_RF}\ln\frac{c_{R,1}}{c_{R,2}} \qquad (6)$$

where R, T, F have their usual significance. This equation has the same implications regarding activity coefficients as has equation (2).

A very full discussion of the errors inherent in the membrane potential method has been given by Adair & Adair (1934). Probably the chief sources of error are the liquid junction potentials, but these can be considerably reduced by use of saturated KCl solution in the electrode elements. Adair & Adair (1934) have shown that, under the conditions of these experiments, the remarks above about activity coefficients are sufficiently close to the truth. The possibility of alterations in the solutions during equilibration has been minimized by working near 0° in stoppered Pyrex vessels, and by avoiding the use of excessively dilute buffers. Owing to the low protein concentrations used the volume correction of Adair & Adair (1934) has been dispensed with.

The protein-ion valence can be calculated with the help of equations (1) and (6).

EXPERIMENTAL

Electrophoresis experiments. These were carried out in the Tiselius (1937) apparatus at +0·5°. The protein solutions were dialysed in cellophan bags for 4–5 days against the appropriate buffer solutions, the dialysates being changed twice daily. Three sets of measurements were carried out at different protein concentrations (0·5 – 1·5 %) after dialysis against Na acetate-acetic acid buffer, ionic strength 0·02, pH 3·82. Five other sets were carried out at approximately 1·4 % protein concentration dialysed against disodium phosphate solutions of various ionic strengths. The more dilute phosphate solutions differed somewhat in pH from the more concentrated (Table 1), indicating the presence of small amounts of monovalent phosphate ion. The latter was ignored in the calculations. Potential gradients used in the U-tube ranged from 3·0 to 9·5 V./cm. according to the buffer. Measurements of the area of the δ-boundary were carried out by the Svensson (1939, 1940, 1946) technique, as this gives more consistent results with small refractive index differences measured at large inclinations of the diagonal slit to the vertical, when the base line may deviate appreciably from linear (Svensson, 1946). Mobility measurements were made in solutions with a fairly low protein to buffer ratio (Longsworth, 1942; Svensson, 1946), approximately 1 % protein being used in buffer of ionic strength 0·2, and proportionally less protein at lower ionic strengths. Even so the ascending boundary moved about 2 % faster than the descending. Consequently, mobilities were measured in the descending limb, the conductivity of the protein solution being used for computation (Svensson, 1946). Corrections for volume changes at the electrodes were considered to be negligible in these experiments (Longsworth, 1942, 1943). The positions of the boundaries were identified with those of the median ordinates (Longsworth, 1941, 1943), estimated from patterns traced on millimetre graph paper with an enlargement factor of five. From the mercury vapour lamp light source monochromatic light (λ=546 mμ.) was isolated by means of a filter. The patterns were recorded on Ilford thin film half-tone panchromatic plates. Conductivity measurements were made in a Washburn-type conductivity cell immersed in the electrophoresis bath at +0·5°.

Membrane potentials were measured at +0·5° by a technique closely similar to that of Adair & Adair (1934). Two modifications were made. Good quality cellophan tubing was tied at one end and supported by sliding it over the end of a short test tube in which several large holes had been blown. The upper end of the cellophan was held tightly to the tube by a stout rubber band. The levels of both protein solution and dialysate were kept below the level of the rubber band. No necessity was felt to work at the equilibrium osmotic pressures of the solutions in view of the conclusions of Northrop & Kunitz (1926) and Adair & Adair (1934). Dialysis was able to proceed freely under the above conditions, and the membranes were protected against damage from the electrode capillary tubes during measurements. Instead of calomel electrodes Ag-AgCl in saturated KCl were used. These were prepared according to MacInnes & Beattie (1920). Polarization was negligible.

Boundaries were formed in the electrode capillary U-tubes by the method of Maclagan (1929), and readings taken 5–10 min. later. If contamination of the solutions by KCl occurred at this stage, dialysis had to be repeated. The potential difference between two electrodes was measured, then the potential when they were immersed in protein solution and dialysate respectively, and finally the electrodes were checked against each other. This procedure was repeated, but the electrode previously dipped into the dialysate was this time used in the protein solution. The membrane potentials, corrected for the electrode differences, were then averaged. Owing to liquid-junction potentials it was difficult to obtain reproducibility better than 0·1 mV. At the

protein concentrations used only measurements in ionic strengths of 0·02 and below were sufficiently large to yield results of any quantitative significance.

Nitrogen estimations were carried out by the method of Ma & Zuazaga (1942), but Na selenate replaced selenium in the digestion catalyst, and digestion was for at least 4 hr. The conversion factor of 6·97 was used for this protein (Kekwick, 1938).

pH *measurements* were made at 20° by means of a Cambridge pH meter with glass electrode. 0·05 M-Potassium hydrogen phthalate (pH 3·97) and 0·05 M-borax (pH 9·18) were taken as standards.

RESULTS

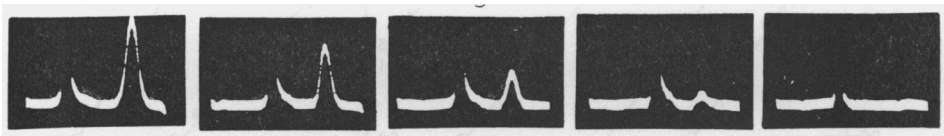
Patterns obtained by electrophoresis of samples of the horse serum albumin A are shown in Fig. 2. There are indications of the presence of a small amount of globulin impurity; furthermore, the albumin peak in Fig. 2 (*a*) and (*c*) shows evidence of being composite in accordance with the observations of Luetscher (1939).

series are not necessarily directly comparable. The results of experiments in acetate buffer are shown in Fig. 5.

(*a*) (*b*) (*c*)

Fig. 2. Electrophoretic patterns given by preparation of horse serum albumin A. Migration is from left to right. (*a*) Sodium acetate-acetic acid, $I=0{\cdot}2$, pH 3·8. Protein 1·2%. After 369 min. at 3·5 V./cm. (*b*) Sodium phosphate, $I=0{\cdot}05$; sodium chloride, $I=0{\cdot}15$; pH 8·0. Protein 0·9%. After 349 min. at 2·3 V./cm. (*c*) Sodium phosphate, $I=0{\cdot}2$, pH 8·8. Protein 0·9%. After 216 min. at 2·8 V./cm.

From the intercept on the abscissae of the curves in Figs. 4 and 5 the value of $c_{A,3}$ can be obtained directly for substitution in equations (4) and (5).



(*a*) (*b*) (*c*) (*d*) (*e*)

Fig. 3. Philpot-Svensson curves showing the reduction in size of the δ-boundary as the excess concentration of buffer in the supernatant is increased from (*a*) 0%, through (*b*) 15%, (*c*) 25%, (*d*) 35%, to (*e*) 40%. Results obtained with a solution of 1·5% horse serum albumin A; dialysate disodium phosphate, $I=0{\cdot}02$, pH 8·2.

A typical series of patterns of the δ-boundaries, obtained in 0·02 ionic strength phosphate buffer, is shown in Fig. 3. The results of all the experiments in phosphate buffers are shown in Fig. 4. Although the diagonal slit was kept at a set inclination throughout a particular series, the areas obtained in different

Convection is very liable to occur in solutions where the protein to buffer ratio is too high, whether or not the ordinary dialysate is employed as supernatant. This is especially true of the region just below the descending albumin peak. In confirmation of the warning of Pedersen (1948), there was an in-

Table 1. *Horse serum albumin valences calculated from moving boundary studies in phosphate buffers*

Dialysate: Ionic strength, $I=\frac{3}{2}c_{A,2}$	Dialysate: pH	Excess buffer concentration to eliminate δ-boundary (%) $=100\,(c_{A,3}/c_{A,2}-1)$	Total N (mg./ml.)	Protein concentration (electrochemical equiv./l.) $=c_{Pr,1}$	Valence $=z_{Pr}$
0·2	8·83	8·0	1·99	−0·00655	−33·2
0·1	8·81	12·4	1·99	−0·00618	−31·3
0·05	8·63	19·5	1·95	−0·00556	−28·8
0·02	8·22	38·4	2·15	−0·00508	−23·9
0·01	8·05	59·6	1·68	−0·00337	−20·3

Table 2. *Horse serum albumin valences calculated from moving boundary studies in acetate buffers*

Dialysate: Ionic strength, $I=c_{A,2}$	Dialysate: pH	Excess buffer concentration to eliminate δ-boundary (%) $=100\,(c_{A,3}/c_{A,2}-1)$	Total N (mg./ml.)	Protein concentration (electrochemical equiv./l.) $=c_{Pr,1}$	Valence $=z_{Pr}$
0·02	3·82	20·2	0·774	0·00267	+34·8
0·02	3·82	32·7	1·39	0·00429	+31·1
0·02	3·82	76·5	2·69	0·00982	+36·8

creased tendency to convection just below the δ-boundary as the limiting concentration of buffer was reached in the supernatant. It is, of course, obvious that convections are inevitable in this region should this limiting concentration be exceeded. The conditions employed in the mobility determinations ensured that the solutions were free from detectable convection.

$$u_{Na}^{+} = 23{\cdot}5 \times 10^{-5} \text{ cm.}^2 \text{V.}^{-1}\text{sec.}^{-1}$$
$$u_{HPO_4}^{--} = -21{\cdot}9 \times 10^{-5} \text{ cm.}^2 \text{V.}^{-1}\text{sec.}^{-1}$$

after Hoch (1948) and

$$u_{CH_3COO^-} = -18{\cdot}5 \times 10^{-5} \text{ cm.}^2 \text{V.}^{-1}\text{sec.}^{-1},$$

based on the relative mobility of acetate ion given by Longsworth (1947). Protein-ion mobilities used were the measured values shown in Table 3.

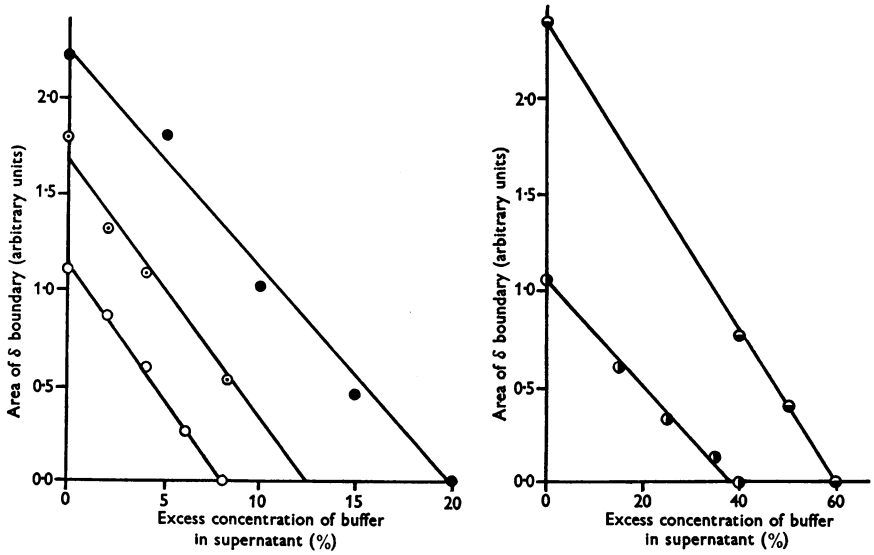


Fig. 4. Elimination of the δ-boundary in the phosphate buffers of Tables 1 and 3. Points correspond to ionic strengths as follows: ○, 0·2; ⊙, 0·1; ●, 0·05; ◑, 0·02; ◒, 0·01.

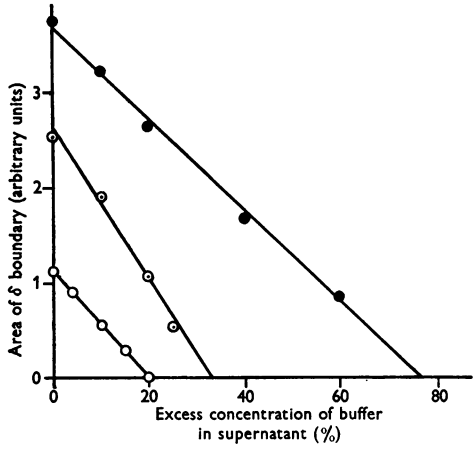


Fig. 5. Elimination of the δ-boundary. Dialysate acetate buffer, $I = 0{\cdot}02$, pH 3·8. Points correspond to albumin concentrations as follows: ○, 0·5%; ⊙, 1·0%; ●, 1·8%.

Tables 1 and 2 show the values of the valences calculated from the moving-boundary studies by the method discussed above. In these calculations the following mobility values were used:

Tables 3 and 4 show, respectively, the valences calculated from the mobility data and from the membrane potentials. In the calculations from mobility data the physical constants of the protein were assumed equal to those used by Abramson *et al.* (1942) for horse serum albumin B, on the basis of the sedimentation and diffusion measurements of Kekwick (1938). Two sets of valences are recorded, based on the extreme molecular models of a hydrated sphere and an unhydrated cylinder of axial ratio 5/1. A value of 2·5 A. was retained for r_i in phosphate buffers. This was considered justified on the grounds that: (*a*) sodium ions will predominate in the ionic atmosphere close to the negatively charged protein ion; (*b*) the phosphate ion will probably not differ greatly in size from this value; and (*c*) this factor affects the results relatively little except at the higher ionic strengths.

DISCUSSION

The most direct method of determining protein-ion valences would appear to be from observations of electrophoretic mobility, but considerable difficulties arise in the theory (Abramson *et al.* 1942). In

Table 3. *Horse serum albumin valences calculated from mobility measurements*

Dialysate			Mobility, u_{Pr} ($cm.^2 V.^{-1} sec.^{-1}$ $\times 10^{-5}$)	Valence (for hydrated sphere)	Valence (for an unhydrated rod of axial ratio 5/1)
Buffer	Ionic strength I	pH		z_{Pr}	z_{Pr}
Phosphate	0·2	8·83	− 6·3	−18·5	−20·6
	0·1	8·81	− 7·5	−18·5	−20·1
	0·05	8·63	− 8·5	−17·8	−19·1
	0·02	8·22	− 9·9	−16·5	−17·6
	0·01	8·05	−11·8	−16·7	−17·5
Acetate	0·02	3·82	+ 7·2	+12·0	+12·8

Table 4. *Horse serum albumin valences calculated from membrane potential measurements*

Dialysate			Membrane potential (mV.)	Total N (mg./ml.)	Protein concentration (electrochemical equiv./l.) $c_{Pr,1}$	Valence, z_{Pr}
Buffer	Ionic strength (I)	pH				
Phosphate	0·02	8·22	−2·31	1·92	−0·00374	−19·5
	0·01	8·05	−3·70	1·79	−0·00293	−16·4
Acetate	0·02	3·86	+2·55	3·30	+0·00433	+13·2
	0·02	3·85	+1·89	2·70	+0·00320	+12·0
	0·02	3·86	+1·58	2·13	+0·00264	+12·5
	0·02	3·85	+1·22	1·52	+0·00206	+13·7

addition, there are specific buffer-ion effects (Svensson, 1946). A new theoretical approach has recently been made by Booth (1948). Other methods depend mainly on the estimation of inorganic ions and application of the electroneutrality equation. Chemical analysis, either directly (Svensson, 1946) or by isotope dilution (Velick, 1949), gives the total of free and combined material. Titration data may be lacking in accuracy, unless suitable corrections are made for the combination of the protein ions with ions other than hydrogen or hydroxyl, or with undissociated molecules (Tiselius & Svensson, 1940; Abramson *et al.* 1942). Membrane potential measurements may involve the errors already discussed. The first comparative studies (Tiselius & Svensson, 1940; Adair & Adair, 1940) did not take into account several factors, so that the apparent agreement between mobility and membrane potential values depended to some extent on compensating errors (Longsworth, 1941; Abramson *et al.* 1942). The recent results of Creeth & Jordan (1949) show excellent agreement at low ionic strengths in the case of the highly asymmetric particles of nucleic acids.

The valences deduced from δ-boundary measurements assume that the charge operative in membrane equilibria is identical with that on the ion migrating in an electric field. Evidence is accumulating in support of this (Svensson, 1946). Thus valences calculated from membrane potentials and from mobility measurements are in reasonably good agreement. In addition to the above assumption of identity of charge, there is the requirement of the Kohlrausch (1897) theory that the mobility of a given ion should be independent of its position in the U-tube. In practice there may be appreciable alterations of pH across the boundaries (Longsworth, 1947), and the variation of protein ion mobility with pH is usually quite large. Under some conditions in the present experiments the buffering capacity of the salts present is not as great as would be desirable. In some cases this is due to the endeavours to obtain measurements under the same conditions as used for membrane potentials. The disodium phosphate solutions of pH 8·8 also have poor buffering capacity; these solutions were selected to simplify the algebra involved. In calculation no account has been taken of possible contributions of hydrogen ions, etc., to the conductivity, or various equilibria. In order partly to suppress changes of pH at boundaries, the acetic acid/sodium acetate ratio in the supernatant was kept constant, in contrast to the procedure of Longsworth (1947), who diluted his protein solution with acetic acid solution.

The present work shows quite good correspondence between the valences determined from membrane potentials and those from mobilities, particularly where the latter are calculated on the basis of a cylindrical model which approximates to the most probable form of the horse serum albumin molecule (Oncley, 1941). It should be noted that the protein solutions used for mobility measurements were at practically the same pH values as the corresponding dialysates, whereas the relatively high protein concentrations necessary in the membrane potential measurements involved a change of pH across the membrane amounting to approximately 0·07 unit (calc.) in some cases.

The valences calculated from mobility measurements in phosphate solutions are subject to specific influences (Longsworth, 1941); but it was desired to work under conditions approximating to those liable to be encountered in the course of electrophoretic separations and phosphate buffers are commonly used for such work, particularly with serum proteins. The agreement with the membrane potential results is perhaps slightly better than might therefore be expected.

The valences calculated from the δ-boundary measurements are considerably larger than those deduced by the other methods. The best agreement is in phosphate of low ionic strength, where the anomalies might be expected to be larger. The apparent variation of charge with ionic strength in phosphate buffers is partly due to the differences in pH. The only data available for comparison are those of Longsworth (1947), who obtained from ε-boundary measurements a value of 10·5 for the valence of ovalbumin in acetate buffer of pH 3·92 and ionic strength 0·1. Although this corresponds well with titration data, Abramson *et al.* (1942) have shown that correction of the titration figures reduces the value to approximately 5·0, in accordance with mobility calculations. Thus the results of Longsworth also are rather higher than would be expected. Since neither Longsworth's ovalbumin, nor the present horse serum albumin, is a homogeneous material the deviations could be due partly to this cause; but, no doubt, must be attributed mainly to the inadequacy of the Kohlrausch (1897) theory for the present systems. Recent theoretical advances (Dole, 1945; Svensson, 1946) are not easy to apply in a general case, although Hoch (1948) has obtained promising results based on the Svensson (1946) theory, when certain assumptions are made regarding protein interactions. Further improvements in correlating results of the various methods of determining the valences of protein ions may be expected when the new theories are applied in studies conducted on the relatively pure proteins which are becoming available through the researches into fractionation methods such as those of Cohn, Hughes & Weare (1947).

The conclusion to be drawn from the results of the δ-boundary experiments is that, at present, it is not possible in eliminating the δ-boundary for preparative work to improve on the following semi-empirical method. From a preliminary run and measurement of the δ- or ε-boundary the buffer concentration needed in the supernatant is calculated. A second run using this strength buffer will generally still result in δ- and ε-boundaries, smaller in size. A second, and if necessary further, approximation results in the required elimination. Under these circumstances the ideal separation illustrated in Fig. 1 (*b*) may be almost attained in the ascending limb, where there is a greater sharpening effect than usual, whilst in the descending limb the modified conditions favour more diffuse boundaries whereby the increased separation is partly, or even wholly, offset.

SUMMARY

1. Measurements have been made in acetate and phosphate buffers of the variations in size of the δ-boundary formed during the electrophoresis of horse serum albumin A with modified concentrations of buffer salts in the supernatant fluid.

2. The valences of the protein ions calculated from these observations have been compared with those deduced from mobility and membrane potential measurements.

3. The last two methods compare very favourably, but the first one gives rather higher values. The reasons for this are discussed.

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The Selective Hydrolysis of Amylopectin and Amylose by β-Amylase

By R. H. HOPKINS and B. K. JHA
Department of Brewing and Industrial Fermentation, University of Birmingham

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The common starches are now generally recognized as consisting of a branched component (amylopectin) and a linear one (amylose), hydrolysable by β-amylase which attacks the α-glucosidic chains at their non-reducing ends to yield about 50 and 100% maltose respectively. Potato amylopectin has one such chain end for every 24–26 glucosidic groups present, whereas potato amylose has one to every 200–500 or more.

An investigation has been undertaken of the relative rates of disappearance of these two components of starch during hydrolysis by β-amylase. The proportion of amylose and amylopectin remaining in a partly hydrolysed starch paste can be calculated from the absorption spectra of its iodine colour under standard conditions. If amylopectin is preferentially hydrolysed amylose will accumulate and the iodine colour will be relatively bluer.

MATERIALS AND METHODS

Substrates. Three commercial samples of potato starch, one each of wheat, rice, a soluble starch (A.R.) and a Lintner soluble starch prepared in our laboratory from potato starch were used. Amylose was prepared from potato starch by the thymol precipitation method of Bourne, Donnison, Haworth & Peat (1948), and amylopectin by *cyclo*hexanol and thymol precipitation as described by Hopkins & Jelinek (1948). In some cases the amylopectin was preserved in solution, sterile.

Starch and amylopectin were made into a cream with cold water, poured into boiling water and boiled with continuous mechanical stirring for 15 min. With native starches, 0·05 g. of NaCl/100 ml. was added 10 min. before the end of boiling. Amylose was preserved under butanol and dissolved in hot water freshly for use, the butanol being driven off at about 80–90°.

Preparation of amylopectin. The purest preparation of amylopectin made in these laboratories was obtained by precipitation of potato starch paste with *cyclo*hexanol followed 3 days later by thymol (Hopkins & Jelinek, 1948). It had been stored for 2 years and was difficult to get into solution unless finely ground, and all the precautions required in the preparation of starch paste were employed. A white flocculum was thrown out, apparently retrograded amylose, which was separated and the solution centrifuged clear. There were indications that unless it was well dispersed it hydrolysed only half as rapidly as other specimens of amylopectin. The data on which Fig. 1 was constructed were obtained with this sample.

Preparation of amylose and amylopectin for determination of Michaelis constants. Potato starch was stirred with water at 63° for 15 min., the clear portion separated and the amylose prepared from it by thymol precipitation (amylose I, yield 4·6–6·2%). The starch residue was dispersed by boiling for 30 min., centrifuged to remove small amounts of residues, and the residual amylose (II) precipitated by thymol, leaving amylopectin II. The amylose preparations were purified and stored as described by Hopkins & Jelinek (1948). The amylopectins were kept in sterile solution, amylopectin II for 3 months and amylopectin III (prepared in the same way as II) for 6 days. (Amylopectin I derived from the 63° infusion (yield 0·8–1·1%), will be referred to later.)

Amylose I corresponds to the β-amylose of Baldwin (1930) and the amylose of Meyer, Bernfeld & Wolff (1940), but, having been precipitated with thymol, it should be free from amylopectin. In Fig. 2 it is described as 'Baldwin amylose'.

Enzyme preparations. Both β-soya and barley amylase were prepared as described by Hopkins, Jelinek & Harrison (1948).

Reaction mixtures. Conditions of pH, temperature, etc., are given in the appropriate tables or in the text. In view of the effects of salts on spectrometric measurements of iodine coloration, the concentration of buffer solutions was kept down to a 1% addition of 0·1 M-acetate (for pH 4·6) and citrate-phosphate for pH 5·8 and 7.