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ELECTRICAL CHARGES OF COLLOIDAL PARTICLES AND ANOMALOUS OSMOSIS.

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I. The Transport Curves on the Acid Side of the Isoelectric Point of the Membrane.

The experiments described in a preceding paper¹ leave no doubt that the Donnan equilibrium is the main source of the potential differences between solid gelatin particles and the surrounding liquid. On the other hand, experiments on the influence of salts on electrical endosmose, cataphoresis, anomalous osmosis, and Quincke's current potentials suggest in certain cases at least a second source which is generally designated as adsorption potentials. The difference between the two kinds of potentials should be that while the potential differences due to the Donnan equilibrium depend on the ionization of the protein, the adsorption potentials should occur regardless of whether or not the solid colloid is ionized. Adsorption potentials should, therefore, be found just as well in the case of isoelectric protein where the protein is practically non-ionized as in the case of metal proteinates or protein-acid salts, while the P.D. due to the Donnan equilibrium should be restricted to the latter two forms of protein.²

It is intended to investigate on the basis of this idea whether or not there exist at the surface of solid gelatin adsorption potentials in addition to potentials due to the Donnan equilibrium. It will be necessary to use for this purpose either electrical endosmose or anomalous osmosis or Quincke's current potentials or cataphoresis. We shall select in this paper anomalous osmosis. By anomalous osmosis

¹ Loeb, J., *J. Gen. Physiol.*, 1921-22, iv, 351.

² Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 667; 1921-22, iv, 351.

is meant the superposition of electrical forces over the purely osmotic forces in the transport of water through a membrane separating pure water from a solution of an electrolyte (or separating two different solutions of electrolytes). When both water and electrolytes are capable of diffusing through the membrane the difference in the mobility of the oppositely charged ions will cause diffusion potentials acting across the membrane. In this case the solution assumes the opposite sign of charge as the water. These potentials we will call E .

There may be a second P.D. inside the pores of the membrane between the solid wall of the pore and the liquid inside the pore. This potential we will call ϵ . If as the consequence of ϵ the liquid inside the pore assumes a negative charge, while as a consequence of E the solution in the collodion bag assumes a positive charge, the liquid cylinder inside the pore will be dragged into the solution by these electrical forces and thus an electrical transport of water will be added to the transport of water by osmotic forces. If the solution, however, has the same sign of charge as the liquid inside the pore, the electrical force will act in an opposite sense from the osmotic force, and the flow of water from the water side of the membrane into the solution will be less than is to be expected on the basis of van't Hoff's law.

This theory of anomalous osmosis was first suggested by Girard³ and has later been supported by Bartell⁴ and others.

In the experiments to be described salt solutions of a definite pH but of different concentrations were put into collodion flasks of about 50 cc. volume, which had received a coating of gelatin as described in previous papers. The collodion bags were dipped into 350 cc. of water of the same pH as that of the salt solution, but containing no salt. The collodion bags were closed with a rubber stopper perforated by a glass tube serving as manometer. The temperature was 24°C. and the rise of the liquid in the manometer was read 20 minutes after the commencement of each experiment. In the close regulation of

³ Girard, P., *Compt. rend. Acad.*, 1908, cxlvi, 927; 1909, cxlviii, 1047, 1186; 1910, cl, 1446; 1911, cliii, 401. Girard, P., *La pression osmotique et le mécanisme de l'osmose*, Publications de la Société de Chimie-physique, Paris, 1912.

⁴ Bartell, F. E., *J. Am. Chem. Soc.*, 1914, xxxvi, 646. Bartell, F. E., and Hocker, C. D., *J. Am. Chem. Soc.*, 1916, xxxviii, 1029, 1036. Bartell, F. E., and Madison, O. E., *J. Physical Chem.*, 1920, xxiv, 593.

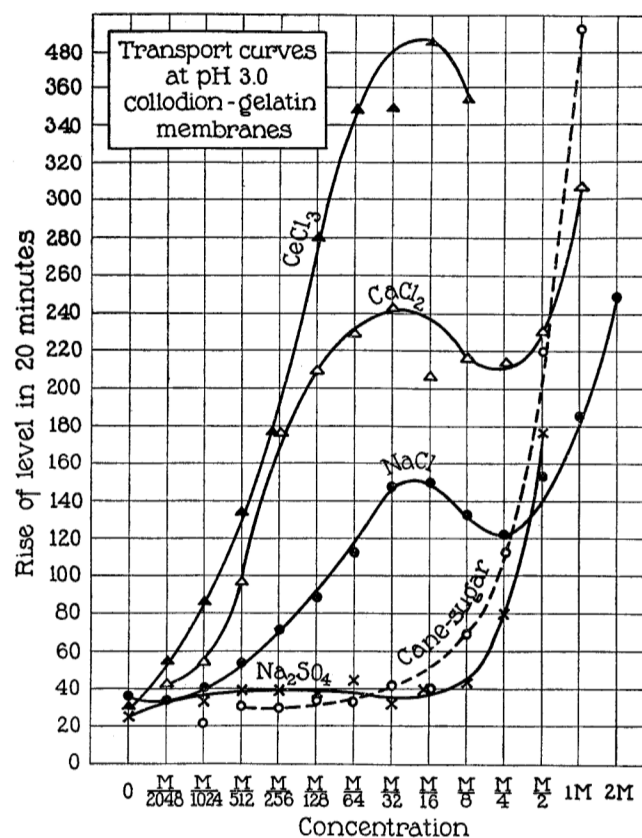


FIG. 1. Transport curves of liquid from the side of water to the side of salt solution through collodion-gelatin membranes of pH 3.0. Abscissæ are the concentration of salt, ordinates the rise in mm. of level in manometer in solution after 20 minutes. Notice the difference between the transport curve for cane-sugar and those for NaCl , CaCl_2 , and CeCl_3 .

the hydrogen ion concentration the experiments to be given in this paper differ from those of previous workers.

Fig. 1 represents the transport curves for different concentrations of CeCl_3 , CaCl_2 , NaCl , Na_2SO_4 , and cane-sugar, all of pH 3.0 (HCl having been added). The outside solution was an HCl solution also of pH 3.0 (approximately $N/1,000$ HCl), but containing no salt. The abscissæ are the concentrations, the ordinates the level in mm. to which the liquid had risen in the manometer after 20 minutes. The results of these experiments corroborate similar experiments already published.⁵

The curves for the first three salts, CeCl_3 , CaCl_2 , and NaCl , rise at first until the concentration is about $M/32$, then fall and then rise again at a concentration of about $M/4$. The curves for cane-sugar and Na_2SO_4 commence to rise at a concentration of about $M/32$ or $M/8$ respectively. The transport of water therefore increases with the valency of the cation and inversely with the valency of the anion and the question arises how to account for these curves.

According to Helmholtz's formula modified by Perrin, the transport of liquid through a capillary under the influence of a direct current is

$$v = \frac{q \cdot \epsilon \cdot E \cdot D}{4 \pi \cdot \eta \cdot L}$$

where v is the quantity of liquid carried electroosmotically, q the cross section of the capillary, ϵ is the potential difference between the two strata of the double layer inside the capillary, E the external electromotive force (acting at right angles to the electrical double layer in the capillary), D the dielectric constant of the medium, η the coefficient of internal friction, and L the distance of the external electrodes.

In the experiments on anomalous osmosis the driving force, E , is not furnished by an external P.D. but by a P.D. across the membrane, which has its origin in the difference between the solutions on the opposite sides of the membrane and which acts only through the extremely small distance of the thickness of the membrane. ϵ is the P.D. between the liquid inside the pores and the wall of the gelatin film. At pH 3.0, gelatin exists in the form of gelatin-acid salts, *e.g.* gelatin

⁵ Loeb, J., *J. Gen. Physiol.*, 1919-20, ii, 387, 563, 577, 659, 673.

chloride, when the acid is HCl. The water inside the pores of a film of gelatin chloride is negatively charged while the gelatin is positively charged. This is a consequence of the theory of Donnan's membrane equilibrium.¹

In these experiments the gelatin with which the membrane was coated was originally isoelectric, but the film of gelatin was brought into equilibrium with water of pH 3.0 by putting the collodion bags for several hours into HCl of pH 3.0 before the beginning of the experiment. Since the addition of salts influences both the p.d. across the membrane (*i.e.* the value of E) as well as the p.d. between the liquid inside the pores and the wall of the pore (*i.e.* the value of ϵ) it is necessary to measure the influence of salts on these two p.d. separately and then try to use the results for the analysis of the curves in Fig. 1. The measurements of the p.d. across the membrane were made with the aid of a Compton electrometer and the two electrodes used were calomel electrodes with saturated KCl solution. Measurements of the p.d. across the membrane were made at the beginning of the experiment (Table I) and at the end; *i.e.*, after 20 minutes (Table II). The figures for the p.d. in Table II are lower than in Table I for the reason that during the experiment part of the salt diffused into the outside solution so that the concentration of the salt solution inside the bag diminished while that in the outside increased; hence the value of E diminished. In addition the hydrogen ion concentration which was the same inside and outside the salt solution at the beginning changed and this added a complication which has been discussed in a previous paper.⁶ Table I shows that the p.d. across the membrane (E) increases with the valency of the cation and inversely with the valency of the anion, E being a maximum for CeCl_3 , being lower for CaCl_2 , and still lower for NaCl ; for Na_2SO_4 it becomes about zero or even slightly negative (Table II). It is, therefore, obvious that in a semiquantitative way the results of Tables I and II (*i.e.* the values of E) explain the difference in the ascending branches of the curves in Fig. 1 up to a concentration of about $M/32$.

It can be shown that this p.d. is at least partly due to diffusion potentials. In measuring the diffusion potentials the principle of a

⁶ Loeb, J., *J. Gen. Physiol.*, 1921-22, iv, 213.

TABLE I.
Influence of Concentration of Salt on the Value of E.

P.D. in millivolts across a collodion-gelatin membrane between different concentrations of salts of pH 3.0 against H₂O of pH 3.0 (acid used, HCl), at beginning of experiment. Sign of charge of solution always positive unless minus sign is added.

Concentration.	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M	2 M
	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
CeCl ₃	1.0	9.0	15.0	21.0	32.0	41.0	45.0	41.0	41.0	41.5	42.5	47.0	49.0	
CaCl ₂	0.0	8.0	16.0	16.0	23.0	25.0	35.0	34.0	33.0	39.0	42.5	47.0	49.0	
NaCl.....	1.5	5.5	8.0	12.0	13.0	18.0	22.0	20.0	23.0	23.0	24.0	25.0	28.0	28.0
Na ₂ SO ₄	0.5	5.0	6.0	5.0	8.5	7.5	5.0	-1.5	-2.0	-8.0	-12.0	-17.5		
Cane-sugar.....			2.0	2.0	0	0	0	-1.0	0	0	-0.5	1.0	0	

TABLE II.
Influence of Concentration of Salt on the Value of E.

P.D. in millivolts across a collodion-gelatin membrane between different concentrations of salts of pH 3.0 against H₂O of pH 3.0 (acid used, HCl), after 20 minutes from beginning of experiment. Sign of charge of solution always positive unless minus sign is added.

Concentration.	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M	2 M
	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
CeCl ₃	-0.5	10.0	16.0	22.5	28.0	35.0	41.0	33.0	32.0	29.0	29.0	23.0	20.0	
CaCl ₂	-1.0	6.5	11.5	17.0	22.0	23.0	30.0	27.5	25.0	25.0	24.0	23.0	20.0	
NaCl.....	1.5	4.0	7.5	12.0	10.0	13.0	16.5	16.0	16.0	15.0	11.0	12.0	10.5	8.0
Na ₂ SO ₄	0	3.5	1.0	1.0	1.0	-0.5	-2.5	-6.0	-6.0	-10.0	-10.0	-13.0		
Cane-sugar.....			3.0	6.0	2.0	2.0	3.0	4.0	0	1.0	3.0	5.0	1.0	

flowing junction of Lamb and Larson⁷ was used in a simplified and also less accurate form, which, however, gave results of sufficient accuracy for our purpose. Table III gives some of the results showing that the order of efficiency of the various salts and the influence of concentration are the same in diffusion potentials as in the p.d. across the membrane. The diffusion potentials are, however, considerably lower than the p.d. across the membrane (Tables I and II). The diffusion potentials depend on the difference in the relative velocity of the oppositely charged ions of a salt. A comparison of the values in Table I and Table III suggests that the source of the p.d. across the membrane is the same as that of the diffusion potential if we assume that at pH 3.0 the cations experience a greater retardation in the diffusion through protein films than anions.

The figures in Table I, II, or III do, however, not explain the drop in the curves of Fig. 1 which occurs when the concentration of the salt reaches $m/32$. The cause for this depression lies probably in the influence of the concentration of the salt on the value of ϵ ; *i.e.*, p.d. between gelatin chloride and water inside the pores of the membrane. It has been shown in previous experiments that salts depress the p.d. between gelatin particles and surrounding liquid and that the reason for this depression is furnished by the Donnan theory of membrane equilibria.¹ The method of these experiments was briefly as follows:

1 gm. of fine particles of powdered gelatin rendered first isoelectric and of an equal size of grain was put into 200 cc. of various concentrations of a salt (NaCl, CaCl₂, etc.) in water and containing 8 cc. of 0.1 N HCl per 100 cc. The gelatin remained in this solution for 2 hours at 20°C. under frequent stirring. The suspension was then put on a filter and the gelatin freed from the supernatant liquid. The gelatin was then melted by heating to 45° and cooled rapidly to cause solidification and the p.d. between the gelatin and the supernatant liquid was then measured with the aid of a Compton electrometer. Table IV gives the result. The reader will notice that in no case do any of the salts cause a rise in the p.d. between gelatin and liquid. The observed p.d. could, however, be calculated with a fair degree of accuracy from Donnan's equilibrium equation.

⁷ Lamb, A. B., and Larson, A. T., *J. Am. Chem. Soc.*, 1920, xlii, 229.

TABLE III.

Diffusion potentials of different concentrations of salts of pH 3.0 against H₂O of pH 3.0 (acid used, HCl). Sign of charge of salt solution always positive unless minus sign is added.

Concentration.	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
CeCl ₃		2.0	4.0	7.0	10.0	16.0	19.0	24.0	31.0	36.0			
CaCl ₂		2.0	3.0	6.0	10.0	13.0	17.0	22.0	26.0	30.0	35.0	43.0	47.0
NaCl.....	0	0	0.5	1.5	3.0	5.0	7.0	9.0	12.0	15.0	15.0	17.0	18.0
Na ₂ SO ₄		-1.0	-1.0	-1.5	-2.0	-3.0	-5.0	-7.0	-9.0	-12.0	-15.0	-22.0	
Cane-sugar.....	0	0	0	0	0	0	-0.5	-0.5	-0.5	-1.0	-1.5	-3.0	-3.0

TABLE IV.

Influence of Different Concentrations of Salts on the Value of ϵ .
r.D. in millivolts between solid gelatin particles and HCl solution. pH of gelatin particles about 2.8 (in absence of salt).

Concentration.	0	M/8192	M/4096	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8
	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
NaCl.....	25.5	23.0	25.0	25.0	23.0	21.5	18.5	14.0	10.5	7.0	5.5	2.5
CaCl ₂	25.5	25.5	23.0	24.0	21.0	19.0	15.5	11.5	7.5	5.0	3.0	2.5
BaCl ₂	0.92	25.0	24.0	23.0	22.0	18.5	15.0	11.0	7.5	5.5	2.5	2.0
CeCl ₃		25.0	22.0	21.5	0.61	16.0	11.5	8.0	5.0	2.5	2.5	
Na ₂ SO ₄	25.0	22.0	22.5	21.0	18.1	16.0	11.5	8.5	6.5	4.0	3.0	1.5

The effect of the salt on ϵ was depressing and no increase in ϵ is noticeable in the low concentrations of CeCl_3 or CaCl_2 . It is therefore obvious that the effect of the salts on ϵ exhibited by our method of experimenting can account for the drop in the curves in Fig. 1 but apparently not for the initial rise or the augmenting effect of low concentrations of CeCl_3 or CaCl_2 . If Helmholtz's formula holds for these experiments curves representing the product of the values $E \times \epsilon$ should therefore show an initial rise, followed by a drop; moreover, they should show the relative order of the rise as exhibited in the transport curves in Fig. 1.

In Fig. 2 the curves for $E \times \epsilon$ are plotted with the concentration of the salt as abscissæ and the value of $E \times \epsilon$ as ordinates. The values of ϵ are taken from Table IV and those for E from Table II. The general order of the four curves in Fig. 2 is sufficiently similar to that in Fig. 1 to indicate that our interpretation of the curves in Fig. 1 is approximately correct. Thus the transport curve for Na_2SO_4 in Fig. 1 is flat, and so is the $E \times \epsilon$ curve for Na_2SO_4 in Fig. 2. The curves for NaCl , CaCl_2 , and CeCl_3 rise in both figures in the order named and in both curves the rise is followed by a drop. The second rise of the curves in Fig. 1 after a concentration of $m/4$ is due to the osmotic effect and has no connection with the electrical effect, as is shown by the fact that this rise occurs also in the cane-sugar curve. Hence, as far as the electrical effect in Fig. 1 is concerned, the character of the curves resembles that of the curves in Fig. 2, as is proven by the fact that if we superpose the curves for $E \times \epsilon$ in Fig. 2 over the transport curve for cane-sugar in Fig. 1 we get the curves of the type of Fig. 1. This is still more approximately the case if we substitute for the purely osmotic transport effect not the curve for cane-sugar but the curves for NaCl and CaCl_2 at the isoelectric point of gelatin (Fig. 4). Since the curve for NaCl at pH 4.7 (Fig. 4) rises more slowly than the corresponding curve for CaCl_2 , the lowest point in the NaCl curve in Fig. 1 must be lower than that for CaCl_2 .

This explains the empirical rule at which the writer had arrived in his previous papers⁵ on anomalous osmosis; namely, first, that the transport of water through the collodion-gelatin membrane from the side of the water to the side of solution is increased by that ion of the salt which has the same sign of charge as the membrane, and dimin-

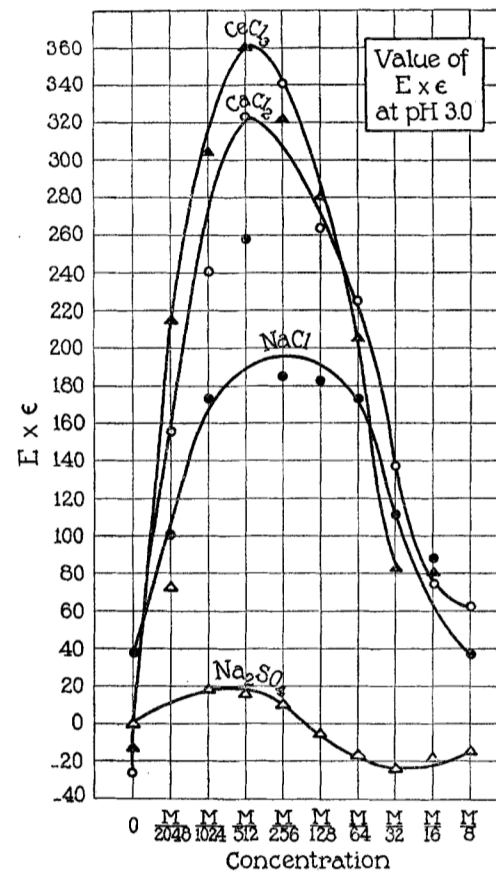


FIG. 2. Value of product of P.D. across the membrane (E) and P.D. inside the pores of the membrane (e) at pH 3.0. Abscissæ are the concentration of salt solution, ordinates $E \times e$. Notice similarity of curves for $E \times e$ with the curves in Fig. 1 at lower concentrations of salt.

ished by the oppositely charged ion with a force increasing with the valency of the ion; and second, that the relative effect of the oppositely charged ions is not the same at different concentrations, but that at low concentrations the augmenting effect of the ion with the same sign of charge as the membrane increases more rapidly than the depressing effect of the oppositely charged ion, while at high concentrations the reverse is true. The augmenting effect of the ion with the same sign of charge as that of the membrane on the transport of liquid is due chiefly, if not exclusively, to the effect of the salt on the P.D. across the membrane (E), which depends upon that ion which gives the salt solution the opposite charge from that of the liquid inside the pores. This ion is in this case the cation and hence the transport increases with the valency of the cation (Tables I and II). The depressing effect of the oppositely charged ion on the transport of liquid is due to the effect on ϵ . If ϵ is determined by the Donnan equilibrium between the solid gelatin salt and the bounding solution, it must, according to the theory, be depressed by that ion which has the opposite sign of charge as the protein ion.¹ The oppositely charged ions of a salt act, therefore, each on a different type of P.D., the one on diffusion potentials, the other on a P.D. due to an equilibrium condition.

There are three discrepancies between the curves in Figs. 1 and 2 which need further explanation. First, the location of the maximum of the curves in Figs. 1 and 2 is not identical, being located at $m/32$ in Fig. 1 and at $m/256$ or $m/512$ in Fig. 2. This may be partly or entirely due to the fact that the concentration of the liquid was lower inside the pores than in the salt solution since water was flowing constantly from the side of water into the solution, thus causing a considerable dilution inside the pores.

Second, the curves in Fig. 1 do not come down to zero while those in Fig. 2 come down to nearly zero at a nominal concentration of $m/8$. For this we may have two reasons, first, that when the concentration exceeds $m/4$ the transport due to osmotic forces becomes so great that a drop of the transport curves to zero is no longer possible; or it may mean that after the concentration exceeds $m/4$ a new source of electrification of the gelatin inside the pores not accounted for by the ionization of the protein commences. We shall return to this

possibility later and show that there is no adequate support for this second assumption, though it cannot be absolutely excluded.

Third, the difference between the curves for CeCl_3 and CaCl_2 is smaller in Fig. 2 than in Fig. 1. It is possible that Ce increases the value of ϵ beyond that accounted for by the ionization of gelatin chloride.

Aside from these discrepancies we can say that Helmholtz's formula explains the curves for anomalous osmosis given in Fig. 1 when the values for ϵ used are those to be expected on the basis of the Donnan equilibrium. It may, therefore, be stated that the Donnan theory is able to explain the phenomena of anomalous osmosis more completely than any other theory thus far offered.

II. The Transport Curves on the Alkaline Side of the Isoelectric Point.

In these experiments the salt solutions were rendered alkaline by adding enough KOH to bring the salt solution to a pH of 11.0. The outside solution was a pure KOH solution also of pH 11.0 but free from salt. Fig. 3 gives the curves for the transport of liquid in the solution during the first 20 minutes. The curves show a rise—until the concentration of the salt is about $\text{m}/64$ —followed by a drop, and then a second rise follows at a concentration of about $\text{m}/8$. The general character of the curves in Fig. 3 is about the same as that in Fig. 1 but the relative efficiency of the cations and anions is reversed. In solutions whose pH is on the alkaline side of the isoelectric point of gelatin, the "attraction" of the solution for water increases with the valency of the anion but inversely with the valency of the cation; while on the acid side the relative efficiency of the two oppositely charged ions is the reverse. Thus in Fig. 1 the curve for Na_2SO_4 is flat while that for CaCl_2 rises; in Fig. 3 the curve for Na_2SO_4 rises while that for CaCl_2 is flat. The reason for this reversal is the fact that the sign of charge between the liquid inside the pores of the gelatin film and the gelatin wall of the pore is reversed on the opposite sides of the isoelectric point. At pH 3.0 the gelatin is positively charged and the liquid inside the pores is negatively charged; while at pH 11.0 the gelatin is negatively charged and the liquid inside the pores is positively charged. The sign of charge of the solution in the p.d. across the membrane, *i.e.* of E , remains, however, the same in alkali and acid solutions (Table V).

Table V gives the p.d. across the membrane at the beginning of the experiments represented in Fig. 3 and Table VI gives the diffusion potentials between the same salt solutions of pH 11.0 against KOH of pH 11.0 without salts and with no membrane between them.

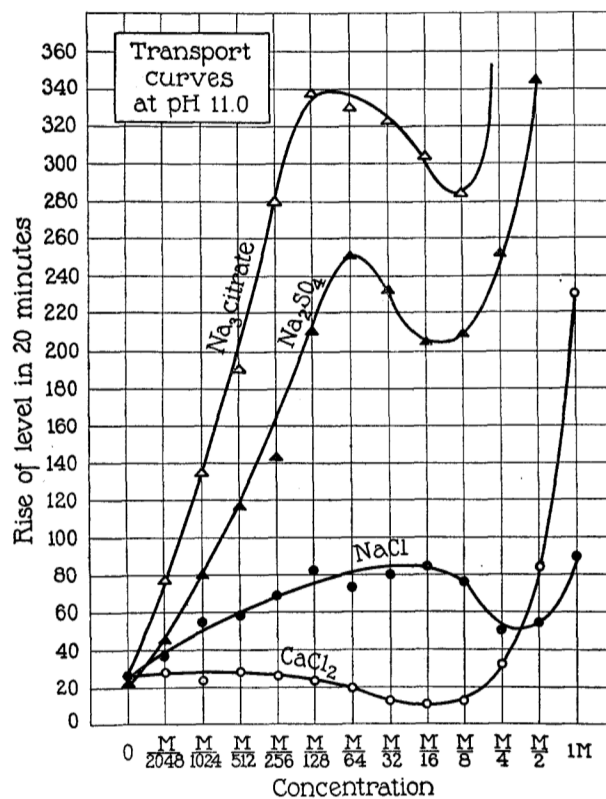


FIG. 3. Transport curves at pH 11.0. Notice reversal of relative efficiency of anions and cations between the curves in Figs. 1 and 3, due to the fact that the sign of charge of liquid inside the pores is positive at pH 3.0 and negative at pH 11.0.

TABLE V.

P.D. across a collodion-gelatin membrane between different concentrations of salts of pH 11.0 against H₂O of pH 11.0 (alkali used, KOH) at beginning of diffusion. Sign of charge of salt solution always positive unless minus sign is added.

Concentration.	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
Na ₃ citrate.....	millivolts -20.0	millivolts -27.0	millivolts -38.0	millivolts -34.0	millivolts -37.0	millivolts -39.0	millivolts -33.5	millivolts -37.0	millivolts -44.0	millivolts -46.0	millivolts -52.0	millivolts -56.0
Na ₂ SO ₄	millivolts -15.0	millivolts -23.0	millivolts -25.0	millivolts -28.0	millivolts -29.0	millivolts -25.0	millivolts -23.0	millivolts -21.0	millivolts -23.0	millivolts -26.5	millivolts -30.0	millivolts -30.0
NaCl.....	millivolts -5.0	millivolts -8.0	millivolts -13.0	millivolts -7.0	millivolts -10.0	millivolts 1.0	millivolts 4.5	millivolts 9.5	millivolts 13.5	millivolts 19.0	millivolts 22.0	millivolts 24.0
CaCl ₂	millivolts -4.0	millivolts -2.5	millivolts 0	millivolts 6.5	millivolts 14.0	millivolts 20.0	millivolts 26.5	millivolts 33.0	millivolts 38.0	millivolts 42.5	millivolts 49.0	millivolts 50.0

TABLE VI.

Diffusion potentials in millivolts of different concentrations of salts of pH 11.0 against H₂O of pH 11.0 (alkali used, KOH.) Sign of charge of salt solution always positive unless minus sign is added.

Concentration.	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2
Na ₃ citrate.....	millivolts 0	millivolts -4.0	millivolts -6.0	millivolts -9.0	millivolts -12.0	millivolts -15.5	millivolts -20.0	millivolts -24.0	millivolts -28.5	millivolts -33.5	millivolts -38.0	millivolts -43.0
Na ₂ SO ₄	millivolts 0	millivolts -2.5	millivolts -3.0	millivolts -4.0	millivolts -5.0	millivolts -6.5	millivolts -8.5	millivolts -11.0	millivolts -14.0	millivolts -17.0	millivolts -19.5	millivolts -22.0
NaCl.....	millivolts 0	millivolts 1.0	millivolts 1.5	millivolts 3.0	millivolts 5.0	millivolts 8.0	millivolts 10.0	millivolts 12.5	millivolts 15.0	millivolts 18.0	millivolts 20.5	millivolts 23.5
CaCl ₂	millivolts 0	millivolts 4.5	millivolts 7.0	millivolts 10.5	millivolts 15.0	millivolts 19.5	millivolts 23.5	millivolts 28.5	millivolts 34.0	millivolts 39.0	millivolts 43.0	millivolts 48.0

We can say that the sign and order of the values of the potentials are the same in Tables V and VI indicating that the p.d. across the membrane is essentially of the nature of a diffusion potential. The negative p.d. across the membrane in Table V is, however, always greater than the corresponding p.d. across the membrane in Table VI.

The drop in the curves in Fig. 3 beyond a concentration of $m/64$ is due again to the diminution of ϵ through the increase in the concentration of salts.

III. The Transport Curves at the Isoelectric Point.

The main purpose of this paper is the investigation of the transport curves at the isoelectric point. At this point the gelatin is not ionized and salts cannot cause a charge of the particles unless they alter the pH or cause the formation of complex protein salts which undergo an electrolytic dissociation. This latter seems to occur when salts with trivalent (or tetravalent?) cations or salts with tetravalent anions are added to isoelectric gelatin, since the addition of this kind of salts has a similar effect as the addition of acid or alkali respectively to isoelectric gelatin. No such effect seems, however, noticeable in the case of salts of the type of NaCl, CaCl_2 , or Na_2SO_4 . These latter salts influence the transport curves at the isoelectric point in approximately the same way as does cane-sugar or grape sugar; *i.e.*, only osmotically. The transport curves for these latter salts show no electrical effect at the isoelectric point but only the osmotic effect (Fig. 4). In these experiments at the isoelectric point special care was taken that the gelatin film of the membrane was at the isoelectric point at the beginning of the experiment; *i.e.*, that the pH was 4.7. The gelatin used for the film formation was isoelectric and in addition the collodion-gelatin bags were kept in water which had been brought to pH 4.7, by adding acetic acid. The salt solutions also were carefully brought to pH 4.7.

Table VII shows that the p.d. across the membrane is very high at pH 4.7.⁸ If, therefore, at this pH there exists only a small p.d.

⁸ If we compare the p.d. across the membrane for the NaCl and CaCl_2 and CeCl_3 solutions with the diffusion potentials (which are not given in this paper), it is seen that the p.d. across the membrane is greater than the diffusion potentials for the same solutions, as if the membrane retarded the motion of the cations. The same was found when the pH was 3.0. It is, therefore, obvious that this cation retention is not caused by the charge of the gelatin.

TABLE VII.

P.D. in millivolts across a collodion-gelatin membrane between different concentrations of salts of pH 4.7 (isoelectric point of gelatin) against H_2O of pH 4.7, at beginning of experiment. Sign of charge of solution always positive unless minus sign is added.

Concentration.	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M	2 M
KCl.....	millivolts	1.0	0.5	-1.0	3.5	-2.5	-1.0	-1.0	-0.5	0	0	0	0	0
NaCl.....	4.0	9.0	10.0	13.0	15.0	19.0	22.0	25.0	28.0	27.0	28.0	33.0	34.0	0
LiCl.....	0	13.0	14.0	18.0	26.0	32.0	38.0	36.0	39.0	49.0	51.0	57.0	55.0	59.0
MgCl ₂	4.0	20.0	25.0	35.0	81.0	48.0	52.0	55.0	62.5	64.0	74.0	72.5	75.0	
CaCl ₂	5.0	27.0	31.5	33.0	39.0	45.0	44.0	52.0	54.0	62.5	68.0	70.0	71.0	
BaCl ₂	3.0	14.0	23.0	25.0	27.5	33.0	43.0	48.0	52.0	57.0	57.0	61.0	64.0	
MgSO ₄	1.5	1.0	4.0	6.0	8.0	10.0	11.0	10.0	9.0	10.0	9.5	9.0	5.0	
Ce(NO ₃) ₃	0	17.0	28.0	41.0	44.0	49.0	50.0	54.0	55.0					
Na ₂ SO ₄	4.0	-4.5	-7.0	-8.0	-11.0	-14.0	-16.0	-18.5	-20.0	-25.0	-28.0	-32.5		
Na ₂ Fe(CN) ₆		-28.0	-30.0	-30.0	-31.0	-33.0	-35.0	-37.5	-45.0	-50.0	-60.0			
Cane-sugar.....	3.0	2.5	5.0	2.5	4.0	3.0	3.5	3.0	2.5	2.5	3.0	-1.0	-1.5	

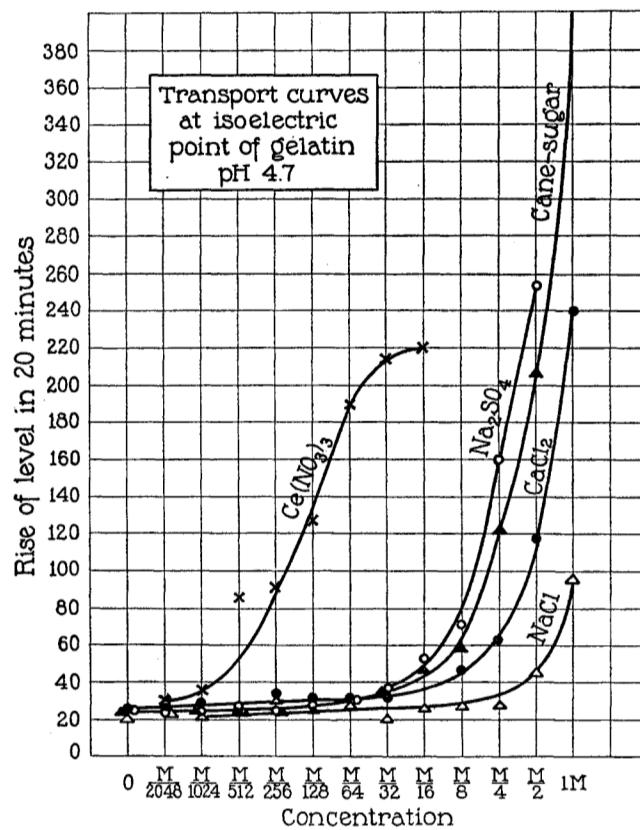


FIG. 4. Transport curves at the isoelectric point of gelatin. The curves for NaCl , CaCl_2 , and Na_2SO_4 resemble the transport curve for cane-sugar showing that osmotic forces suffice to explain these curves. The curve for $\text{Ca}(\text{NO}_3)_2$ suggests that this salt confers a positive charge to the gelatin.

inside the pores of the membrane a considerable transport of water by electrical forces must occur. If no such transport is noticeable, it means that ϵ is zero (as the Donnan theory demands, since gelatin

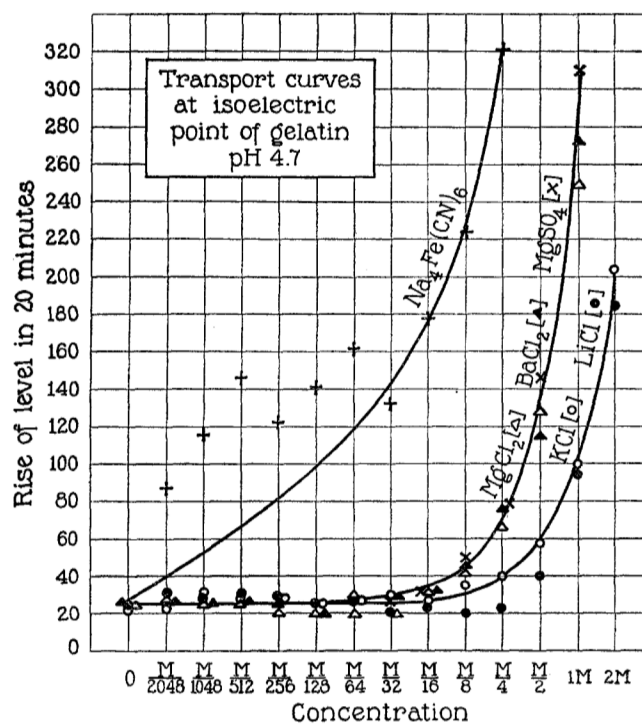


FIG. 5. Transport curves at the isoelectric point. Those for KCl, LiCl, MgCl_2 , BaCl_2 , and MgSO_4 resemble the transport curves for cane-sugar while $\text{Na}_4\text{Fe}(\text{CN})_6$ seems to transfer a negative charge to the isoelectric gelatin.

is not ionized at its isoelectric point and does not combine with salt), and that there is no additional source of P.D. which might be ascribed to adsorption. Figs. 4 and 5 give the transport curves for cane-sugar, NaCl, KCl, LiCl, MgCl_2 , MgSO_4 , BaCl_2 , $\text{Ce}(\text{NO}_3)_3$, and $\text{Na}_4\text{Fe}(\text{CN})_6$ at pH 4.7; *i.e.*, when gelatin is non-ionized. There cannot be the least doubt that the curves for all these salts (with the exception of $\text{Ce}(\text{NO}_3)_3$ and $\text{Na}_4\text{Fe}(\text{CN})_6$) are of the nature of the cane-sugar curve; *i.e.*, they show only that part of the curve which corresponds to the second rise of the transport curves in Figs. 1 and 3, and which must be ascribed chiefly, if not exclusively, to the osmotic forces. It might be added that the curve for Na_2 oxalate does not commence to rise until the concentration of the salt is M/16. The initial rise and drop of the transport curves in Figs. 1 and 3, which is the expression of the electrical forces, is entirely lacking in all the curves at the isoelectric point of gelatin, Figs. 4 and 5, with the exception of the curves for $\text{Ce}(\text{NO}_3)_3$ and $\text{Na}_4\text{Fe}(\text{CN})_6$, to which we shall now give our attention.

In previous publications the writer has already called attention to the fact that on the alkaline side of the isoelectric point the presence of salts with a trivalent cation has the effect of reversing the sign of the P.D. between gelatin and water. When the pH is > 4.7 , *i.e.* when gelatin exists in the form of Na gelatinate, the Donnan equilibrium causes the expulsion of NaOH from the gelatin into the bounding liquid with the result that gelatin assumes a negative and the bounding liquid a positive charge. When, however, some CeCl_3 or LaCl_3 is added the liquid assumes a negative and the gelatin a positive charge.⁹ This reversal of the sign of charge by trivalent cations had been discovered by Perrin in his experiments on electrical endosmose.¹⁰ The reversal may either be due to a reaction between $\text{Ce}(\text{NO}_3)_3$ and isoelectric gelatin, in which a compound is formed which dissociates into a complex positively charged gelatin-Ce cation and a negative ion, presumably NO_3 , or the addition of the salt brings the pH to a value below 4.7. In either case the behavior of the curve for $\text{Ce}(\text{NO}_3)_3$ in Fig. 4 becomes clear. When $\text{Ce}(\text{NO}_3)_3$ solutions of pH 4.7 are separated by a collodion-gelatin membrane (of

⁹ Loeb, J., *J. Gen. Physiol.*, 1919-20, ii, 659.

¹⁰ Perrin, J., *J. chim. physique*, 1904, ii, 601; 1905, iii, 50. Notice sur les titres et travaux scientifiques de M. Jean Perrin, Paris, 1918.

pH 4.7) from water of pH 4.7, the $\text{Ce}(\text{NO}_3)_3$ solution assumes a positive charge as is shown in Table VII. If now the Ce causes the liquid cylinders inside the pores to be charged negatively an electrical transport of water into the solution must occur commencing at a low concentration of the salt and in the way characteristic for the electrical transport curves in Figs. 1 and 3. The curve for $\text{Na}_4\text{Fe}(\text{CN})_6$ in Fig. 5 suggests that $\text{Na}_4\text{Fe}(\text{CN})_6$ causes solid isoelectric gelatin to assume a negative charge. The question is, whether this happens only in the case of trivalent (and probably tetravalent) cations and tetravalent anions. It is obvious that all the transport curves for salts with divalent cations, Mg, Ca, and Ba, in Figs. 4 and 5, commence to rise at a slightly lower concentration than the transport curves for the salts with monovalent cation, KCl, NaCl, and LiCl. It might be argued that the salts with bivalent cation transfer also a positive charge to isoelectric gelatin at a concentration above $M/8$ and that the same is true for the salts with monovalent cations, the difference being that the concentration of the salt required for this effect is very high for salts with monovalent cation, slightly lower for salts with bivalent cation, and very low for salts with trivalent cation.

On the other hand it should be pointed out that the transport curve for cane-sugar rises even more rapidly than that for CaCl_2 so that there is in reality no need to assume that the CaCl_2 charges solid isoelectric gelatin positively. The difference between the transport curves for NaCl and CaCl_2 in Fig. 4 is no greater than the difference between the transport curves for cane-sugar and grape sugar. As far as the experiments on anomalous osmosis are concerned, there is no reason to assume that the salts with divalent cations or monovalent cations transfer a positive charge to the isoelectric gelatin or to gelatin of any pH or that the bivalent anions transfer a negative charge, though it is not absolutely disproved that this may not happen at high concentrations of the salts.

It was thus far left undecided whether the positive electrification of isoelectric gelatin by $\text{Ce}(\text{NO}_3)_3$ in Fig. 4 was due to a change of the pH so that the gelatin was no longer isoelectric or to the formation of a salt between isoelectric gelatin and $\text{Ce}(\text{NO}_3)_3$ dissociating into a positively charged complex gelatin-Ce ion and negatively charged NO_3 ions. To settle this question experiments were made with

buffer solutions consisting of mixtures of $M/50$ acetic acid and $M/50$ Na acetate in the proper proportions to give a pH 4.7, 5.6, or 3.4. Solutions of different concentrations of $Ce(NO_3)_3$ were made up in these buffer solutions and put into the collodion bags. The outside solutions

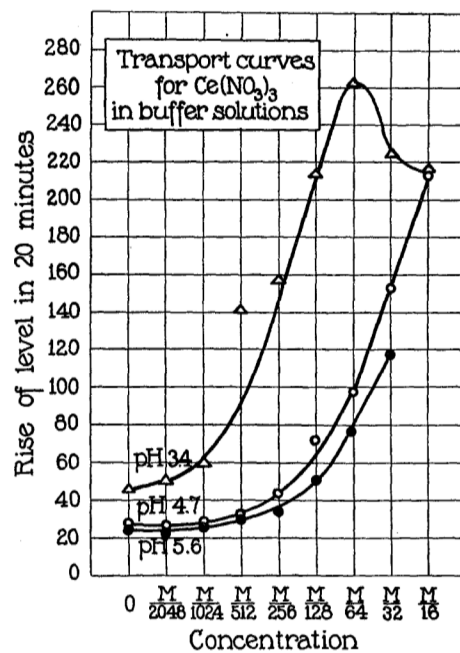


FIG. 6. Influence of $Ce(NO_3)_3$ on the transport curve in the presence of buffer solutions (mixtures of $M/50$ acetic acid and $M/50$ Na acetate).

were the same buffer solutions without the $Ce(NO_3)_3$. The curves in Fig. 6 represent the transport values in 20 minutes. We will first direct our attention to the transport curve at pH 4.7. It is clear that at the isoelectric point the transport curve for $Ce(NO_3)_3$ rises again steeply, thus supporting the idea that the $Ce(NO_3)_3$ reacts with the

protein in the way suggested, thereby giving rise to a double layer in the pores in which the gelatin is positively and the liquid negatively charged. Table VIII shows that at pH 4.7 there is a high p.d. across the membrane in which the $\text{Ce}(\text{NO}_3)_3$ solution assumes a positive charge. If now the liquid inside the pores is negatively charged an electrical transport of water from the water side into the solution must occur.

A comparison of the transport curve for $\text{Ce}(\text{NO}_3)_3$ of pH 4.7 in Fig. 6 with that in Fig. 4 shows that the latter is higher. This finds its explanation in the fact (evident by a comparison of Tables VIII and VII) that the p.d. across the membrane, *i.e.* the value E , is less in the experiment with buffer salt in Fig. 6 than without it in Fig. 4.

TABLE VIII.

p.d. in millivolts across the collodion-gelatin membrane between solutions of $\text{Ce}(\text{NO}_3)_3$ made up in buffer solution, and buffer solution free from $\text{Ce}(\text{NO}_3)_3$. The $\text{Ce}(\text{NO}_3)_3$ solution is always positively charged.

Concentration of $\text{Ce}(\text{NO}_3)_3$	0	M/2048	M/1024	M/512	M/256	M/128	M/64	M/32	M/16
pH	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts	millivolts
4.7	0	2.0	4.0	7.0	11.5	16.0	21.0	27.0	34.0
5.6	0	1.0	2.5	4.5	7.5	11.5	17.0	23.0	28.0
3.4	2.0	10.5	17.5	26.0	29.0	32.0	35.0	38.0	44.0

The transport curve at pH 3.4 is higher than at pH 4.7 and the transport curve at pH 5.6 is lower than at pH 4.7 (Fig. 6). Table VIII shows that this finds its explanation in the fact that the p.d. across the membrane varies correspondingly with the pH.

As a consequence it seems possible that the $\text{Ce}(\text{NO}_3)_3$ causes the positive charge of gelatin by forming a dissociable salt with gelatin in which the positive ion is a complex gelatin-Ce ion. In other words, the salt reacts with isoelectric gelatin in a similar way as acid does. If this assumption is correct the p.d. between gelatin and bounding $\text{Ce}(\text{NO}_3)_3$ solution must be ascribed to a Donnan equilibrium, in which the $\text{Ce}(\text{NO}_3)_3$ plays a similar rôle as the HCl.

The difference between gelatin chloride and the hypothetical gelatin- $\text{Ce}(\text{NO}_3)_3$ salt is this that it is much easier to remove by washing the $\text{Ce}(\text{NO}_3)_3$ from powdered gelatin than it is to remove the HCl.

In an analogous way we must assume that isoelectric gelatin can combine loosely with $\text{Na}_4\text{Fe}(\text{CN})_6$ whereby negatively charged complex gelatin- $\text{Fe}(\text{CN})_6$ ions and positively charged Na ions are formed.

SUMMARY AND CONCLUSIONS.

1. It has been shown in previous publications that when solutions of different concentrations of salts are separated by collodion-gelatin membranes from water, electrical forces participate in addition to osmotic forces in the transport of water from the side of the water to that of the solution. When the hydrogen ion concentration of the salt solution and of the water on the other side of the membrane is the same and if both are on the acid side of the isoelectric point of gelatin (*e.g.* pH 3.0), the electrical transport of water increases with the valency of the cation and inversely with the valency of the anion of the salt in solution. Moreover, the electrical transport of water increases at first with increasing concentration of the solution until a maximum is reached at a concentration of about $M/32$, when upon further increase of the concentration of the salt solution the transport diminishes until a concentration of about $M/4$ is reached, when a second rise begins, which is exclusively or pre-eminently the expression of osmotic forces and therefore needs no further discussion.

2. It is shown that the increase in the height of the transport curves with increase in the valency of the cation and inversely with the increase in the valency of the anion is due to the influence of the salt on the p.d. (E) across the membrane, the positive charge of the solution increasing in the same way with the valency of the ions mentioned. This effect on the p.d. increases with increasing concentration of the solution and is partly, if not essentially, the result of diffusion potentials.

3. The drop in the transport curves is, however, due to the influence of the salts on the p.d. (ϵ) between the liquid inside the pores of the gelatin membrane and the gelatin walls of the pores. According to the Donnan equilibrium the liquid inside the pores must be negatively charged at pH 3.0 and this charge is diminished the higher the concentration of the salt. Since the electrical transport is in proportion to the product of $E \times \epsilon$ and since the augmenting action of

the salt on E begins at lower concentrations than the depressing action on ϵ , it follows that the electrical transport of water must at first rise with increasing concentration of the salt and then drop.

4. If the Donnan equilibrium is the sole cause for the p.d. (ϵ) between solid gelatin and watery solution the transport of water through collodion-gelatin membranes from water to salt solution should be determined purely by osmotic forces when water, gelatin, and salt solution have the hydrogen ion concentration of the isoelectric point of gelatin ($\text{pH} = 4.7$). It is shown that this is practically the case when solutions of LiCl , NaCl , KCl , MgCl_2 , CaCl_2 , BaCl_2 , Na_2SO_4 , MgSO_4 are separated by collodion-gelatin membranes from water; that, however, when the salt has a trivalent (or tetravalent?) cation or a tetravalent anion a p.d. between solid isoelectric gelatin and water is produced in which the wall assumes the sign of charge of the polyvalent ion.

5. It is suggested that the salts with trivalent cation, *e.g.* $\text{Ce}(\text{NO}_3)_3$, form loose compounds with isoelectric gelatin which dissociate electrolytically into positively charged complex gelatin-Ce ions and negatively charged NO_3 ions, and that the salts of $\text{Na}_4\text{Fe}(\text{CN})_6$ form loose compounds with isoelectric gelatin which dissociate electrolytically into negatively charged complex gelatin- $\text{Fe}(\text{CN})_6$ ions and positively charged Na ions. The Donnan equilibrium resulting from this ionization would in that case be the cause of the charge of the membrane.