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The Origin of the Potential Differences Responsible for Anomalous Osmosis

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THE ORIGIN OF THE POTENTIAL DIFFERENCES RESPONSIBLE FOR ANOMALOUS OSMOSIS.

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I. *Potential Differences in Collodion Membranes Coated with Protein.*

In a series of papers[1] the writer has published the results of experiments showing the peculiar influence of electrolytes on the rate of diffusion of water through collodion membranes. When a collodion membrane separates a solution of an electrolyte (of not too high a concentration) from pure warer, the water diffuses from the side of pure water to the solution side, but the initial rate at which this diffusion occurs does not obey van't Hoff's law. The initial rate of diffusion was measured by the rise of level of liquid in a glass tube inserted through a rubber stopper in a closed collodion bag containing the solution, the collodion bag dipping into a beaker filled with pure water. When the membrane was merely a collodion membrane, the following rules expressed the influence of electrolytes on the initial rate of diffusion of water through the membrane from the water side to the side of the solution.

1. Water diffuses into the solution of an electrolyte through a collodion membrane as if the particles of water were positively charged and as if they were attracted by the anion and repelled by the cation of the electrolyte in solution with a force increasing with the valency.

2. In the case of certain electrolytes the "attracting force" of the anion for water increraes at first more rapidly with increasing concentration than the "repelling force" of the cation, until a point is reached where with a further increase in concentration the "repelling force" of the cation increases more rapidly than the "attractive force" of the anion. Finally, a concentration is reached where the

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[1] Loeb, J., *Science*, 1921, liii, 77; *J. Gen. Physiol.*, 1918–19, i, 717; 1919–20, ii, 173, 387, 577.

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"attracting" and "repelling" forces balance each other and from then on the "attractive" force of the solution increases with the concentration, apparently in accordance with van't Hoff's law.

When, however, the collodion membrane has received a coating of a protein, e.g., gelatin, the hydrogen ion concentration influences the
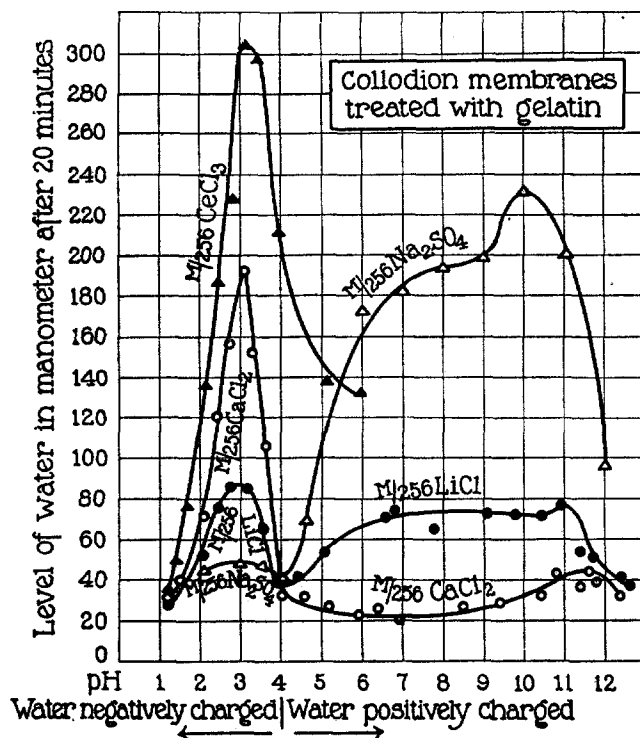

Fig. 1. Influence of hydrogen ion concentration on the initial rate of diffusion of liquid from a solution of HNO₃ or NaOH respectively through a gelatin-coated membrane of collodion into a M/256 solution of LiCl, CaCl₂, CeCl₃, or Na₂SO₄ of the same hydrogen ion concentration. Abscissæ are the initial pH; ordinates, the rise of level of liquid in manometer connected with the salt solution, after 20 minutes.

initial rate of diffusion of water from pure water into the solution in a still more complicated way (Fig. 1). The procedure in these experiments was as follows: Both the outside solution as well as the inside solution had the same hydrogen ion concentration,

containing the same concentration of HNO₃ or NaOH respectively.
In addition the inside solution (*i.e.*, the solution inside the collodion
bag which was connected with the manometer) contained a M/256
solution of one of the following four salts: CeCl₃, CaCl₂, LiCl,
and Na₂SO₄. The "attraction" of these M/256 salt solutions
for water was therefore tested at different hydrogen ion concentra-
tions. It is obvious from Fig. 1 that the "attractive" force of one
and the same salt solution for water (measured by the initial rate of
diffusion of water from the outside into the salt solution) varied con-
siderably with the hydrogen ion concentration of the solution. (The
hydrogen ion concentration is expressed by Sörensen's logarithmic
symbol pH; *i.e.*, the log of the concentration with the minus sign
omitted). The abscissæ in the curve are the initial pH of the
solutions, while the ordinates are the rise in level of the watery liquid
in the glass tubes after 20 minutes at 24°C.

Fig. 1 shows that somewhere between pH 4.0 and 5.0 a reversal of
the sign of charge of the water particles occurs. At pH below 4.0 the
water is negatively charged, at pH above 5.0, it is positively charged.
This change coincides with a change in the nature of the charge of
the gelatin ion. At a pH of 4.0 or below the gelatin forms gelatin
nitrate (in the presence of HNO₃) and hence the gelatin ion is a cation.
The water, being negative, has the opposite sign of charge as the gela-
tin ion. At a pH of 5.0 or above the gelatin forms metal gelatinate, Na
gelatinate, Ca gelatinate, etc., and the gelatin ion is negatively charged.
The water, being positively charged, has again the opposite charge as
the gelatin ion. The exact turning point, the isoelectric point of
gelatin, is at a pH 4.7. In a preceding paper[2] the writer has been
able to show that if other proteins than gelatin are used to give the
collodion membrane a coating of a protein, the reversal of the sign
of charge of the water varies with the isoelectric point of the protein
used.

If we return to Fig. 1 we notice that where the water is negatively
charged it is "attracted" by the cation of the salt used and "repelled"
by the anion with a force increasing with the valency of the ion. The
attractive force increases from Li to Ca to Ce, and diminishes from

[2] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 577.

Cl to SO₄. Where the water is positively charged the reverse order of efficiency exists.

The fact that the same concentration of the salts, namely, m/256, was used, has no essential influence on the results. Similar results were obtained when the salt solutions had the osmotic pressure of a m/64 solution of cane sugar.

Those who have studied phenomena of abnormal osmosis through membranes, Girard,[3] Bernstein,[4] Bartell and his collaborators,[5] and Freundlich[6] have reached the conclusion that potential differences on the opposite sides of the membrane are responsible for these anomalies in diffusion. The fact that the sign of charge varies in these experiments with the sign of charge of the protein ion gives a favorable point of attack for the investigation of the origin of the potential differences. In order to simplify the experiments they were confined to pH from 4.6 to pH 1.9; i.e., to that region where the water diffuses as if it were negatively electrified. The method of procedure was as follows. m/256 solutions of one of the four salts mentioned were prepared in different concentrations of HNO₃ and put into collodion bags of about 50 cc. volume, which were lined by a film of gelatin on the inside. These bags were closed with a perforated rubber stopper through which was pushed a glass tube with a diameter of about 2 mm. to indicate the rise of liquid. The collodion bags were put into beakers containing 350 cc. of the same concentration of HNO₃ as that inside the bag. Fig. 2 gives the rise of the level of liquid in the manometer in one set of experiments after 1 hour at 24°C. The abscissæ are the initial pH of the liquid which was the same inside and outside, the ordinates are the rise of the level of liquid after 1 hour. It is obvious in this case that the curves have a minimum near the isoelectric point of gelatin, that the rate of diffusion of water into the solution rises

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

[4] Bernstein, J., Elektrobiologie, Braunschweig, 1912.

[5] 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.

[6] Freundlich, H., *Kolloid. Z.*, 1916, xviii, 11.

with the increasing hydrogen ion concentration reaching a maximum at pH 3.6 or 3.0 respectively, and that the rate of diffusion falls again with a further increase in the hydrogen ion concentration. $M/256$ Na_2SO_4 shows little "attraction" for water, and $M/128$ cane sugar practically none.
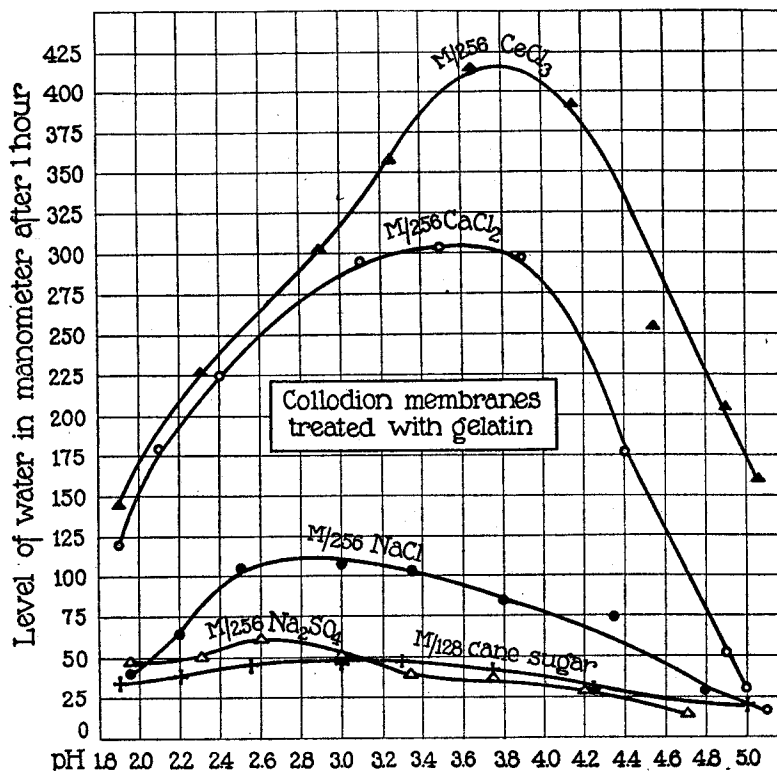

FIG. 2. Influence of hydrogen ion concentration on initial rate of diffusion of liquid from a solution of HNO_3 through a gelatin-coated collodion membrane into a $M/128$ solution of cane sugar, $M/256$ NaCl, $CaCl_2$, $CeCl_3$, or Na_2SO_4 of the same hydrogen ion concentration. Abscissæ are the pH; ordinates, rise of level of liquid in manometer connected with the salt solution, after 1 hour. Water negatively charged.

The writer has recently measured the potential differences between the inside salt solution and the outside solution at the end of an hour with the aid of a Compton electrometer (calomel electrodes with

saturated KCl solution) and obtained a set of curves presented in Fig. 3. At this time some of the salt had diffused from the inside into the outside solution. The P.D. curves in Fig. 3 resemble the osmotic curves in Fig. 2 sufficiently to suspect a connection
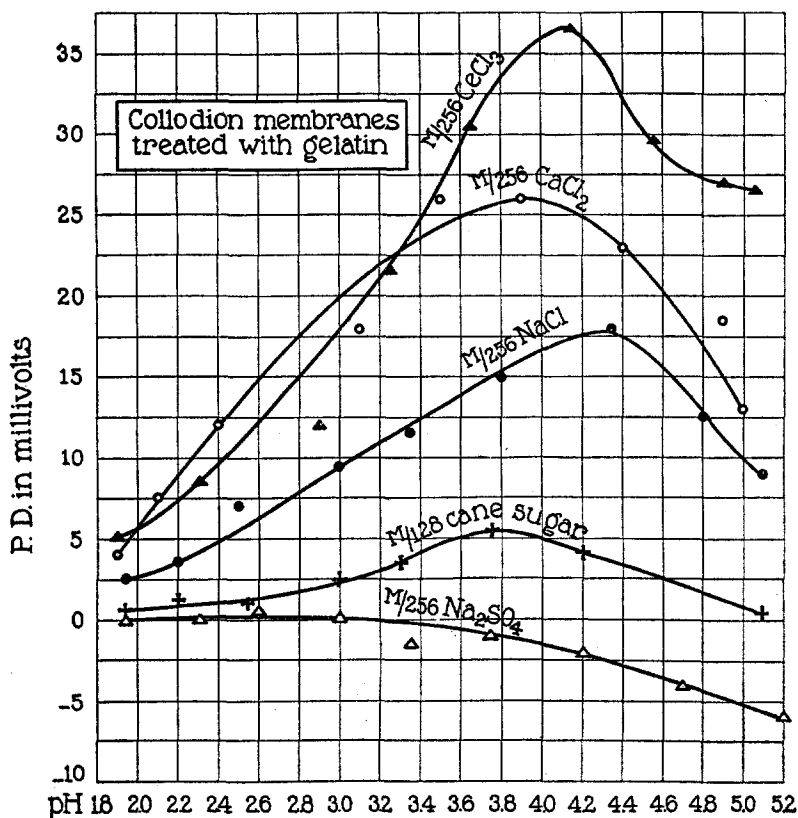

FIG. 3. Potential difference between salt solutions and external solution in preceding experiment after 1 hour of diffusion. Abscissæ are the pH at beginning of experiment; ordinates, P.D. at the end of the experiment. Attention is called to a certain similarity of the curves in Figs. 2 and 3.

between the two, so that we may assume that the P.D. between the opposite sides of the membrane is the main driving force which (for low concentrations of electrolytes) moves the water from the water side to the solution side of the membrane.

The next question was: What is the origin of the p.d.? It occurred to the writer that a measurement of the hydrogen ion concentration inside and outside after 1 hour might possibly throw a light on this problem. The results of these measurements are plotted in Fig. 4. The reader will remember that at the beginning the hydrogen ion concentration was the same inside and outside. Yet after 1 hour
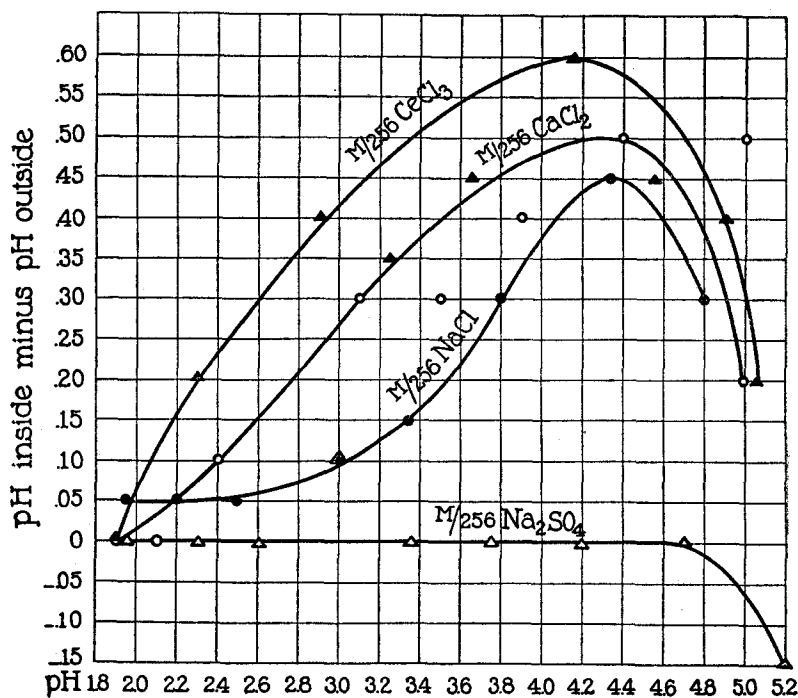

FIG. 4. Abscissæ are the initial pH; ordinates, the values (pH inside minus pH outside) in preceding experiment. Attention is called to the similarity of the curves in Figs. 3 and 4.

(and in fact much earlier) it is obvious that some of the acid has diffused from the inside into the outside solution so that the hydrogen ion concentration is higher outside than inside the collodion bag containing the salt solution.

This then leads to the result that when we separate identical acid solutions by a collodion membrane coated with a film of gelatin, and put inside the collodion bag a solution of a neutral salt with a mono-

valent anion (*e.g.*, Cl), acid is driven from the side where the salt is added to the side originally free from salt. This difference in the pH inside minus pH outside gives rise to a potential difference which was discussed in a preceding paper.[7] In this paper it was shown that when we separate a gelatin chloride solution from a solution of HCl (without gelatin) both having at the beginning the same hydrogen ion concentration, acid is forced from the gelatin solution into the outside solution. The same happens when solid jelly of gelatin chloride is separated from a HCl solution. In this case there arises a P.D. between the two phases; and the writer has been able to show that this P.D. can be calculated with a good degree of accuracy from the difference of the hydrogen ion concentration inside and outside, on the basis of Nernst's well known formula. By multiplying the value (pH inside minus pH outside) by 58 we get the value for the P.D. actually observed at 18°C. in terms of millivolts.[7]

This unequal distribution of acid inside and outside is due to a peculiar membrane equilibrium the theory of which was developed by Donnan.[8] Our new experiments show that such an equilibrium condition is produced also between a gelatin membrane and a solution of HCl free from gelatin. The coating of gelatin on the collodion membrane behaves like a solid jelly of gelatin, the gelatin being transformed into gelatin chloride when in contact with HCl. The Donnan equilibrium demands that the concentration of free HCl inside the gelatin membrane be less than the concentration of free HCl of the solution bounding the membrane. This gives rise to the P.D. The value of pH inside minus pH outside is diminished when a neutral salt is added—in accordance with Donnan's theory—and the fact that a salt is added in our diffusion experiments on one side of the membrane but not on the other is the cause of the fact that the pH of the acid solution containing the salt solution (inside solution) becomes higher than the pH of the solution containing no or less salt (outside solution). The reader will find the experiments proving this in the writer's paper referred to.[7]

[7] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 667,

[8] Donnan, F. G., *Z. Elektrochem.*, 1911, xvii, 572.   Donnan, F. G., and Harris, A. B., *J. Chem. Soc.*, 1911, xcix, 1554.   Donnan, F. G., and Garner, W. E., *J. Chem. Soc.*, 1919, cxv, 1313.

A comparison of Figs. 3 and 4 shows that the two sets of curves, that for the P.D. (Fig. 3) and for the values pH inside minus pH outside (Fig. 4) when plotted over the initial pH as abscissæ, have certain features in common. Both sets of curves have a minimum at or near the isoelectric point of gelatin (pH 4.7). They both rise to a maximum at or not far from pH 4.2, and then drop again when the initial pH of the solution drops further. This parallelism suggests that this form of the P.D. curve is determined by the differences in the hydrogen ion concentration produced on the opposite sides of the membrane in conformity with Donnan's theory of membrane equilibrium.

II. Potential Differences in Collodion Membranes Free from Protein.

The experiments just described were repeated with collodion membranes which had not been treated with a protein. The difference between the P.D. in this case (Fig. 5) and the P.D. observed in the case of collodion membranes coated with a film of gelatin (Fig. 3) is striking. The method of experimentation was the same in the case of the collodion bags free from gelatin as in the experiments of the preceding chapter. The initial concentration of HNO_3 was always the same inside and outside. The inside solution, however, contained in addition to the acid a solution of $M/256$ of one of the four salts, $CeCl_3$, $CaCl_2$, $NaCl$, and Na_2SO_4. After 1 hour the P.D. inside and outside was measured.

We notice that the P.D. is no longer a minimum at or near pH $=4.0$ and we can therefore be certain that this feature of the curves in Fig. 3 was due to the gelatin. The curves in Figs. 3 and 5 have, however, one feature in common; namely, that the P.D. increases with the increasing valency of the cation and diminishes with the increasing valency of the anion. This feature is, therefore, not a specific function of the film of gelatin.

Since the water is positively charged even in the presence of acid when the membrane consists of collodion free from protein, it is to be expected that only the Na_2SO_4 solution should attract the water, while no such attraction should occur in the case of $M/256$ $CeCl_3$ or $M/256$ $CaCl_2$. Fig. 6 shows that this is true.

The question then arises: How can we account for this valency effect of the ion with the same sign of charge as that of the particle of water? The answer seems to be that we are dealing here with diffusion potentials which are independent of the membrane. This was proved
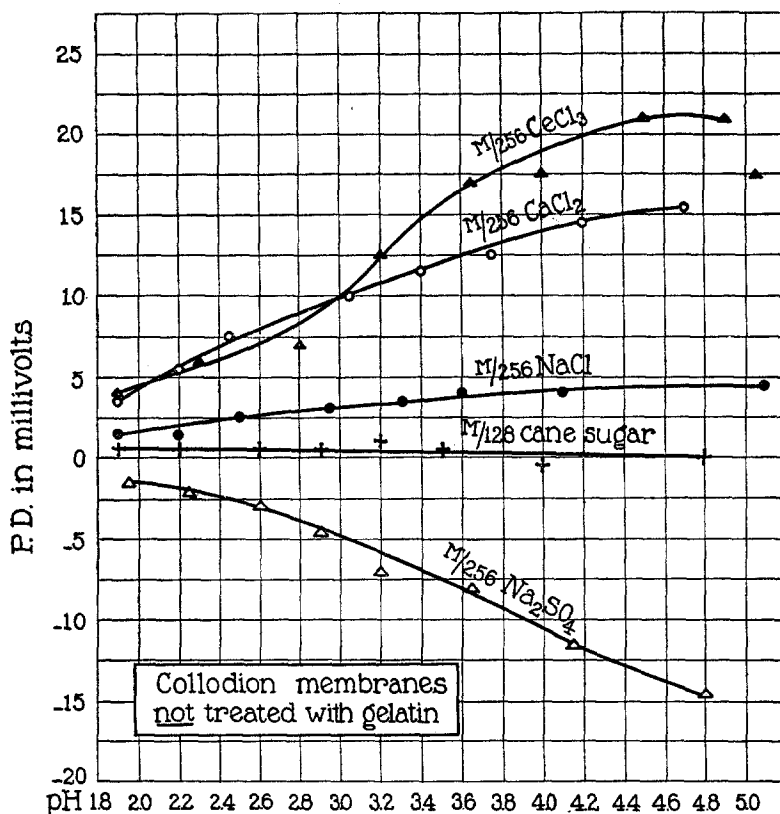

FIG. 5. Influence of neutral salts on the P.D. across collodion membranes not treated with gelatin. Otherwise same experiment as in Fig. 3. Notice difference between curves in Figs. 5 and 3, revealing the share of the protein in the origin of the P.D.

in the following way. Two beakers were filled with identical solutions of HNO_3, the HNO_3 solution in one of the beakers being dissolved in $M/256$ $CeCl_3$ or $CaCl_2$ or $NaCl$ or Na_2SO_4, while the HNO_3 in the other was dissolved in pure water. The pH was identical in

the two solutions. They were connected by a bent glass tube, filled
with a pure HNO₃ solution, of the same pH as that in the
two beakers. Into each beaker was dipped a calomel electrode with
saturated KCl solution and the diffusion potential was ascertained
with the aid of a Compton electrometer. Fig. 7 gives the P.D. A
comparison between Figs. 5 and 7 shows that the curves represent-
ing the P.D. in the two cases are very similar.

If we now return to a discussion of the curves in Figs. 1 and 2, repre-
senting the influence of the pH on the attraction of a m/256 solution
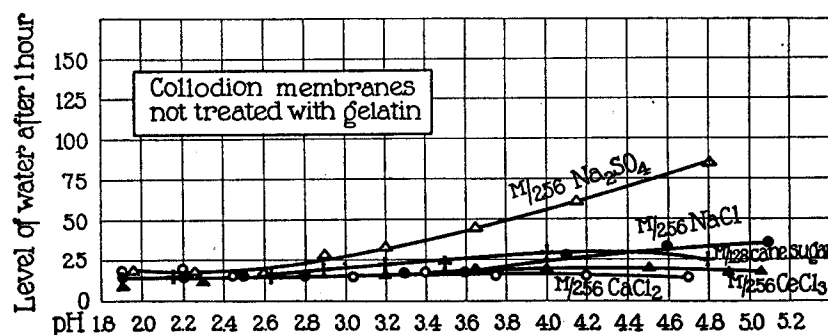of a neutral salt for water through a collodion membrane impregnated

FIG. 6. Influence of m/128 cane sugar, m/256 NaCl, CaCl₂, CeCl₃, and Na₂SO₄
on the initial rate of diffusion of water through collodion membranes not treated
with gelatin. Otherwise the experiment is the same as in Fig. 2. Abscissæ are
the pH; ordinates, rise of level of water in salt solution after 1 hour. Only
Na₂SO₄ attracts water to a noticeable amount since the latter is positively charged
when diffusing through the membrane.

with gelatin, we can say that these curves resemble the curves in
Fig. 3 for the P.D. on the opposite sides of the membrane.

Figs. 4 and 7 show that the P.D. curves in Fig. 3 have a double
source. One is connected with the influence of the gelatin layer on
the unequal distribution of the acid on the opposite sides of the mem-
brane, which finds its explanation probably in the Donnan effect.
The other source of the P.D. seems to be the diffusion potentials as
shown in Fig. 7.

These diffusion potentials which exist regardless of the presence or
absence of a membrane and regardless of the nature of the membrane
seem to be responsible for the fact that the ions with opposite sign

of charge as that of the water "attract" the water with a force increas-
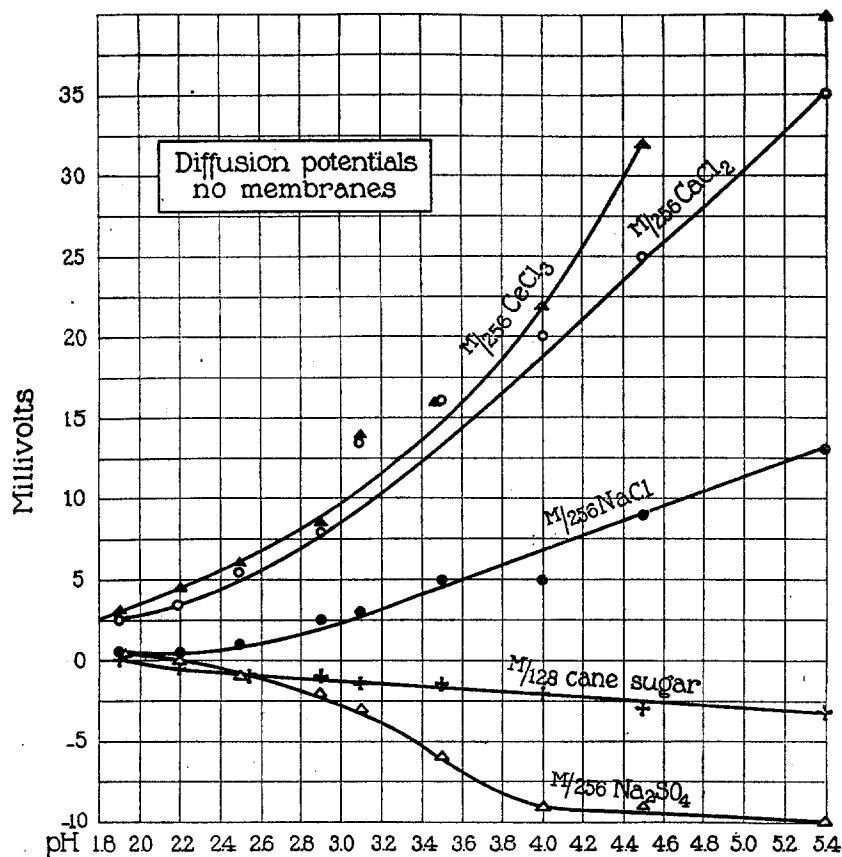ing with the valency of the ion, while the Donnan effect seems to be

FIG. 7. Diffusion potentials between solutions of M/128 cane sugar, M/256
NaCl, CaCl₂, CeCl₃, and Na₂SO₄ in various concentrations of HNO₃ and solutions
free from these salts but of the same concentrations of HNO₃.   The salt solutions
and the solutions free from salt had always the same pH.   Abscissæ are the pH;
ordinates, diffusion potentials.   Attention is called to the resemblance between
the curves in Fig. 7 and those in Fig. 5.

responsible for the fact that when the collodion membrane is impreg-
nated with a protein the rate of diffusion of the negatively charged
water is a minimum at the isoelectric point of the protein, increasing

with a diminishing pH until a maximum is reached at a pH of about 4.2, and diminishing with a further diminution of the pH.

These experiments then lead to the result that the anomalous osmosis discussed in this paper is determined by a potential difference between the opposite sides of the membrane, and that this P.D. seems to have a double source; namely, the Donnan equilibrium and diffusion potentials.

In addition to these two sources of P.D. between the opposite sides of the membrane there may be others, as, e.g., the Coehn effect due to the difference in the dielectric constant between liquid and membrane. If such a P.D. plays a rôle in our experiments it can be only of minor importance.

The experiments suggest the possibility that the establishment of a Donnan equilibrium between membrane and solution is one of the factors determining the Helmholtzian electrical double layer, at least in the conditions of our experiments.

SUMMARY AND CONCLUSION.

1. Collodion bags coated with gelatin on the inside were filled with a M/256 solution of neutral salt (e.g., NaCl, CaCl₂, CeCl₃, or Na₂SO₄) made up in various concentrations of HNO₃ (varying from N/50,000 to N/100). Each collodion bag was put into an HNO₃ solution of the same concentration as that inside the bag but containing no salt. In this case water diffuses from the outside solution (containing no salt) into the inside solution (containing the salt) with a relative initial velocity which can be expressed by the following rules: (a) Water diffuses into the salt solution as if the particles of water were negatively charged and as if they were attracted by the cation and repelled by the anion of the salt with a force increasing with the valency of the ion. (b) The initial rate of the diffusion of water is a minimum at the hydrogen ion concentration of about N/50,000 HCl (pH 4.7, which is the point at which gelatin is not ionized), rises with increasing hydrogen ion concentration until it reaches a maximum and then diminishes again with a further rise in the initial hydrogen ion concentration.

2. The potential differences between the salt solution and the outside solution (originally free from salt) were measured after the

diffusion had been going on for 1 hour; and when these values were plotted as ordinates over the original pH as abscissæ, the curves obtained were found to be similar to the osmotic rate curves. This confirms the view expressed by Girard, Bernstein, Bartell, and Freundlich that these cases of anomalous osmosis are in reality cases of electrical endosmose where the driving force is a P.D. between the opposite sides of the membrane.

3. The question arose as to the origin of these P. D. and it was found that the P.D. has apparently a double origin. Certain features of the P.D. curve, such as the rise and fall with varying pH, seem to be the consequence of a Donnan equilibrium which leads to some of the free HNO_3 being forced from the solution containing salt into the outside solution containing no (or less) salt. This difference of the concentration of HNO_3, on the opposite sides of the membrane leads to a P.D. which in conformity with Nernst's theory of concentration cells should be equal to 58 $\times$ (pH inside minus pH outside) millivolts at 18°C. The curves of the values of (pH inside minus pH outside) when plotted as ordinates over the original pH as abscissæ lead to curves resembling those for the P. D. in regard to location of minimum and maximum.

4. A second source of the P.D. seems to be diffusion potentials, which exist even if no membranes are present and which seem to be responsible for the fact that the rate of diffusion of negatively charged water into the salt solution increases with the valency of the cation and diminishes with the valency of the anion of the salt.

5. The experiments suggest the possibility that the establishment of a Donnan equilibrium between membrane and solution is one of the factors determining the Helmholtzian electrical double layer, at least in the conditions of our experiments.