THE REVERSAL OF THE SIGN OF THE CHARGE OF MEMBRANES BY HYDROGEN IONS.

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I. INTRODUCTION.

The similarity between the influence of ions on free and on electrical endosmose through collodion membranes leads to the conclusion that this influence is due to an alteration of the density of the electrical charge of the membrane by the ions. This conclusion rests on the assumption that the Helmholtzian theory of the double layer and the formula connecting the volume of transport of liquid with the density of charge is correct. We have seen that on this assumption the observations lead to the conclusion that the ions with the same sign of charge as the membrane increase and the ions with the opposite sign of charge diminish the density of charge of the membrane. Whether, therefore, a given ion increases or diminishes the density of charge of the membrane depends upon the sign of the charge of the membrane, and, hence, the sign of the charge precedes the influence of ions on the density of charge. One of the problems in the theory of the double layer at the boundary of membrane and watery solution is therefore the origin of the sign of the charge of membranes in contact with water.

Chemists consider the double layer at the boundary of membrane and water as ionic in character and as due to the preferential adsorption of one kind of ions by the membrane, while the watery phase of the double layer is formed by the ions with the opposite charge from those adsorbed. This view has been developed with remarkable lucidity and consistency by Perrin who assumes that the H and OH

ions are especially influential in determining the sign of charge of the membrane. In alkaline solutions the OH ions are said to be adsorbed by the membrane, while the mobile, watery stratum of the double layer is formed by the cations; in acid solutions the H ions are thought to be adsorbed while the anions form the mobile stratum of the double layer. When the OH ions are adsorbed by the membrane the latter is negatively charged, when the H ions are adsorbed the membrane is positively charged. This view meets a difficulty in the fact that as a general rule membranes are negatively charged when in contact with neutral water. The adsorption hypothesis meets this difficulty with an additional assumption; namely, that in a neutral solution the OH ions have a greater tendency to be adsorbed by a membrane than the H ions. We should, however, be forced to assume that the preferential adsorption of OH ions occurs also in some cases in acid solutions, since we have seen that collodion membranes (not treated with a protein) are negatively charged even in strong acid solutions.\(^8\) Another difficulty was pointed out by Perrin himself, namely that no other monovalent ion except the H and OH ions were able to reverse the sign of charge of a membrane, and he intimated that this might be due to the fact that the velocities of H and OH ions are greater than those of any other ion. But if the velocity determines the relative degree of adsorption of ions then the H ions should be more readily adsorbed by a membrane in neutral solutions than the OH ions.

A second view, which is held chiefly by physicists, considers the formation of an electrical double layer at the boundary of membrane and water as a case of contact electricity, which may be influenced but which need not be caused by ions. Lenard⁴ has shown that when very minute particles are torn off from the free surface of water the minute particles are negatively charged while the water assumes a positive charge. He concludes from this that at the surface of the water there exists an electrical double layer the external stratum of which is negatively charged, while the internal stratum is positively charged. Since he was able to show that such a double layer exists at the surface of water even in a vacuum, this double layer cannot

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have its origin in an adsorption of ions. He assumes that the double layer at the boundary of water and membrane is also entirely inside the watery phase. This seems, however, to be disproved by an experiment of Coehn and Franken. A sphere of paraffin dipped into water possesses a high negative charge which can be demonstrated electrometrically when the paraffin is lifted out of the water. If, however, a film of water is caused to adhere temporarily to the surface of the paraffin when it is lifted out of the water, the electrometer indicates no charge as long as this film exists, but indicates a charge the moment the film has disappeared. This leaves no doubt that when a solid is bounded by water the one charge is in the membrane and the opposite charge in the watery phase. The fact that paraffin and solid substances in general are negatively charged when in contact with water is ascribed by Coehn to the difference in the dielectric constant of the two phases. Coehn has found that substances of a higher dielectric constant assume a positive charge when in contact with a substance of a lower dielectric constant. This would explain why membranes in general assume a negative charge when in contact with water since the dielectric constant of the latter is relatively high.

The formation of a double layer at the boundary of two phases is thus, according to Coehn, a phenomenon of contact (or frictional) electricity. Lenard and more recently Frenkel have offered suggestions concerning the origin of the double layer which make it dependent on the Rutherford model of the atom. Lenard points out that the atoms at the surface of a body are generally oriented in such a way that the electrons are at the surface and the more massive part (the positive nucleus) is towards the interior. This idea has been elaborated by Frenkel into a theory of surface electric double layers of solid and liquid bodies. According to this theory “double layers must exist on the surface of all liquid and amorphous solid bodies, whatever their chemical constitution. The latter will determine but the magnitude and distribution of electric charges on both sides of the surface.”

6 Frenkel, J., Phil. Mag., 1917, xxxiii, 297.
On the basis of this theory it seems natural that membranes should as a rule assume a negative charge when in contact with water. The fact which requires a further explanation is the possibility of a reversal of this sign of charge.

Perrin has shown that in certain cases acids are able to cause a membrane in contact with water to be positively charged. The fact that addition of alkali to an acid solution restores the original negative charge to such a membrane was explained by Perrin on the assumption that in an alkaline solution the OH ions are adsorbed. This latter assumption seems unnecessary since the addition of alkali to an acid may merely serve to lower the hydrogen ion concentration below the level required to make the membrane positive. It is, therefore, only necessary to explain why hydrogen ions in sufficient concentration impress a positive charge on so many membranes and the following is a contribution to the solution of this problem.

We have seen in the preceding papers of this series that a collodion membrane not treated with a protein always assumes a negative charge when in contact with a watery solution, even if this solution is acid. This was demonstrated in two ways, first by electrical endosmose, and, second, by common osmosis. The experiments with electrical endosmose do not lend themselves so well to the exact determination of that hydrogen ion concentration at which the membrane becomes positive on account of electrolysis and the secondary chemical reactions at the electrodes. The method of common osmosis is free from this source of error. When we separate a watery solution from pure water by a collodion membrane the pure water diffuses into the solution at a rate which is not only a function of the gas pressure of the solute but also of the electrostatic forces of the ions in solution. The rate increases with increasing valency of the anion and diminishes with increasing valency of the cation. When, however, the collodion membrane has been treated for some time with a protein it assumes a positive charge when the hydrogen ion concentration exceeds a certain value; and in this case the watery phase of the double layer assumes a negative charge. This is proved by the fact that when we separate a watery solution with a sufficiently high hydrogen ion concentration from pure water by a collodion membrane
treated with a protein, the water will diffuse into the solution at a rate increasing with increasing valency of the cation and diminishing with increasing valency of the anion. It is not difficult to find out by this method the critical hydrogen ion concentration at which the reversal in the sign of charge of the membrane occurs. The experiments to be described in this paper have led to the result that this critical hydrogen ion concentration is connected but not identical with the isoelectric point of the protein with which the collodion membrane has been treated.

Proteins are amphoteric electrolytes which can form salts with metals (metal proteinates, e.g. Na proteinate) as well as with the anions of acids (protein-acid salts, e.g. protein chloride). Whether they do the one or the other depends upon the hydrogen ion concentration of the solution. Below a certain hydrogen ion concentration the proteins form salts of the type of metal proteinates, e.g. Na gelatinate; above this critical hydrogen ion concentration they form salts of the type of protein-acid salts; e.g., gelatin chloride. Between the two concentrations proteins form salts with neither cation nor anion, and this is the so called isoelectric point. For a number of proteins (gelatin included) the isoelectric point lies in the neighborhood of a hydrogen ion concentration of $2 \times 10^{-9}$ N; for oxyhemoglobin it lies at a very different hydrogen ion concentration; namely, $1.8 \times 10^{-7}$ N. We will show in this paper that the hydrogen ion concentration required to produce a positive charge in a collodion membrane previously treated with a protein varies in the same sense as the isoelectric point of the protein used.

II. Membranes Treated with Gelatin.

The isoelectric point of gelatin is, according to Michaelis, at a hydrogen ion concentration of about $2 \times 10^{-9}$ N (or in Sörensen's logarithmic symbol, pH = 4.7). A 1 per cent solution of gelatin was put into a collodion bag over night and was removed the next morning. The bag was rinsed a number of times (ten or twenty times or more) with water to remove all the gelatin except that film which remained apparently attached to the inner side of the col-

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lodion membrane. When such membranes were used for osmotic experiments it could be shown that the membrane had a positive charge as soon as the hydrogen ion concentration exceeded $10^{-4}$ N or a value slightly below $10^{-4}$. The point of reversal was therefore near the isoelectric point of gelatin but on the acid side of that point.

The reader will remember that it had been shown in the preceding papers that when we separate a solution of an electrolyte by a collodion membrane from distilled water the water will be attracted by that ion which has the opposite sign of charge and will be repelled by the ion which has the same sign of charge as the watery phase of the double layer, and that both the attractive and repulsive effects increase generally with the valency of the ion. In the case of a salt like CaCl₂ the repulsive effect of Ca upon positively charged water prevails over the attractive effect of Cl upon such water and no diffusion of such water into the CaCl₂ solution will occur; while when the water is negatively charged the attractive effect of Ca prevails over the repulsive effect of the Cl ion upon the water and water will diffuse rapidly into the solution. We therefore can use a solution of CaCl₂ to find out at which hydrogen ion concentration the reversal of sign in the charge of the watery phase occurs. When we separate a $\frac{m}{256}$ solution of CaCl₂ from a solution of water by a collodion membrane, water will commence to diffuse into the solution as soon as the water in contact with the membrane assumes a negative charge; while otherwise practically no such diffusion will occur. In these experiments the collodion flasks described in the previous experiments were used. They were closed with rubber stoppers perforated by a glass tube with a bore of 2 mm. in diameter serving as a manometer. The collodion bags were dipped into beakers filled with water. The $\frac{m}{256}$ solution of CaCl₂ and the water in the beaker into which the bags were dipped were always given the same hydrogen ion concentration; they were rendered acid by the addition of HNO₃ and alkaline by the addition of KOH. We can in this way ascertain at which hydrogen ion concentration the water will commence to diffuse into the solution of $\frac{m}{256}$ CaCl₂ and this will give us that hydrogen ion concentration where the reversal of the sign of charge on the membrane occurs. Such a curve is given in Fig. 1. The abscissae are the hydrogen ion concentrations (expressed in terms of pH), the ordinates the rise of
level of liquid in the glass tube after 20 minutes. The reader will notice that the curve for \( \frac{m}{256} \text{CaCl}_2 \) is low and flat as long as the pH > 4.0 and that a sharp rise in the curve begins at a pH of about 4.0 or a little less. The curve rises steeply with diminishing pH (i.e., increasing hydrogen ion concentration) until it reaches a maximum at

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**FIG. 1.** Collodion membranes previously treated with 1 per cent gelatin solution. Abscissae, pH (logarithms of hydrogen ion concentration with minus sign omitted). Ordinates, initial rate of diffusion of water from pure water through collodion membranes into solutions of salt indicated. Reversal of sign of charge of membrane at pH = 4.0; i.e., on the acid side of the isoelectric point of gelatin.
about pH = 3.1 and then drops again. This drop is the concentration effect discussed in preceding papers and due to the ions with the opposite sign of charge as that of the membrane, in this case Cl and NO$_3$ (the latter being added with the nitric acid).\textsuperscript{1}

Instead of using CaCl$_2$ we can also use a m/256 solution of Na$_2$SO$_4$ as a test for the sign of the electrification of water. We put m/256 Na$_2$SO$_4$ into the collodion flask and dip the latter into H$_2$O. The outside water as well as the m/256 solution of Na$_2$SO$_4$ is brought to the same pH by adding HNO$_3$ (or KOH). The SO$_4$ ion attracts positively charged water and the Na ion repels it, but the attractive effect of the SO$_4$ ion is greater than the repelling effect of the Na ion. Water will commence to be attracted by m/256 Na$_2$SO$_4$ as soon as the water is positively charged; when water is negatively charged it will be repelled more powerfully by SO$_4$ than it will be attracted by Na. Hence the hydrogen ion concentration at which the water commences to be positively charged will be indicated by a rise in the level of liquid in the glass tube serving as a manometer. Fig. 1 shows that water commences to be positively charged at a pH of about 4.0 or slightly above where the turning point was also found when we used m/256 CaCl$_2$ solution as a test.

We can finally use solutions with monovalent anion and monovalent cation as a test solution; e.g., m/256 LiCl. In this case we get an attraction for water both in alkaline and in acid solutions. In the acid solution the negatively charged water is attracted by the Li ion and in the alkaline solution the positively charged water is attracted by the Cl ion. Between the two effects there should be a point where the water is neither positive nor negative and hence is not attracted by either ion. Fig. 1 shows that this point lies again at a pH near 4.0.

Fig. 2 gives the point of reversal in the presence of m/256 solutions of NaH$_2$PO$_4$, Na$_2$ oxalate, and NaCl. The point of reversal lies between 4.4 and 4.0, but the steep rise of the curve commences at pH 4.0.

Experiments with a number of other salts and other acids were made, all giving the same result; namely, that the membrane commences to be distinctly positively charged as soon as the pH is 4.0 or slightly above, but below 4.7. At the isoelectric point, pH = 4.7, no acid combines with the gelatin and no change of the sign of charge.
can be expected at this point. When the hydrogen ion concentration
of the solution becomes higher than this, an increasing amount of
gelatin is transformed into gelatin-acid salt. With an increase in the
concentration of the gelatin-acid salt an increasing proportion of the
surface of the membrane assumes a positive charge. Hence the steep

Collodion membrane treated with gelatin

![Graph showing the initial rate of diffusion of water vs pH]

**Fig. 2.** Collodion membranes treated with gelatin. Reversal of sign of charge of membrane on acid side of the isoelectric point of gelatin.

rise of the curves in which CaCl₂ or MgCl₂ are used as test solutions
must lie near the isoelectric point but on the acid side from the
latter.

It may be pointed out that the influence of acids on the reversal of
the sign of charge is not an additive effect of the oppositely charged
ions, as was the case in regard to the influence of ions on the density of charge of the membrane. If this were the case, SO₄ should shift the steep rise of the curve more to the acid side and Ca more in the opposite direction. The curves given in this paper (and many other curves not published) show that this is not the case. This supports the view that the mechanism for the influence of ions on the density of charge and for the influence of the hydrogen ions on the reversal of the sign of charge is not the same.

The curves in Fig. 1 show that there are three relative minima of the charge of the membrane. One lies at about pH = 4.0 or slightly above, when the membrane is neither positively nor negatively charged. The drop leading to this minimum is not an additive function of the oppositely charged ions and differs in this respect from the drop leading to the other two minima, the latter being due to the diminution of the density of charge on the membrane caused by that ion which has the opposite sign of charge as the membrane. We have shown in the preceding experiments¹ that this depression occurs when the concentration of the electrolyte exceeds a certain value. One of these minima lies on the acid side, namely at a pH less than 2, owing to the fact that the concentration of the HNO₃ added is not 1/100 or more. This minimum is due to the depressing effect of the anions (NO₃ and SO₄) of the solution upon the density of the positive charge of the membrane. The third minimum lies at a pH of about 12 or above and is caused by the depressing effect of the cation of the solution, Na, K, etc., upon the density of the negative charge of the membrane. If we had continued to increase the concentration of acid there would have been another rise of the curve due to the gas pressure effect of the acid solution; and the same might have happened if we had been able to increase the concentrations of the KOH added; strong alkali solutions, however, dissolve the membrane.

III. Membranes Treated with Casein and Egg Albumin.

The isoelectric point of casein is, according to Michaelis,² identical with that for gelatin, since it lies at a pH of about 4.7. When we treat collodion membranes over night with a 1 per cent casein solution in-

instead of with gelatin solution, and make the same experiments as described for gelatin, we should expect to find the hydrogen ion concentration at which the collodion membrane treated with casein is rendered positive to be identical with that found for membranes treated

![Collodion membrane treated with casein](image)

**Fig. 3.** Collodion membranes treated with casein. Reversal of sign of charge of membrane at pH below 4.7, on the acid side of the isoelectric point of casein.

with gelatin. Fig. 3 shows that this is correct. The curves for membranes treated with casein show a steep rise at a pH of 4.0 or slightly above, but below 4.7. The same is true for membranes treated with egg albumin (Fig. 4). The curves show a steep rise
Collodion membrane treated with egg albumin

Membrane charged positively
Membrane charged negatively

Fig. 4. Collodion membranes treated with egg albumin. Reversal of sign of charge of membrane at pH near 4.0, on the acid side of the isoelectric point of egg albumin.
at a pH of about 4.0. The isoelectric point of egg albumin is, according to Sörensen, at $1.5 \times 10^{-5}$ N or pH about 4.8.

IV. Membranes Treated with Oxyhemoglobin.

Defibrinated ox blood was diluted repeatedly with isotonic solutions of NaCl and centrifuged to remove practically all the proteins.

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Fig. 5. Collodion membranes treated with oxyhemoglobin. Reversal of sign of charge of membrane at pH of about 6.0, on the acid side of the isoelectric point of oxyhemoglobin.

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of the blood except those contained in the red corpuscles. After repeating this process five or more times the corpuscles were laked by adding four times their volume of distilled water. Newly prepared Collodion membrane treated with oxyhemoglobin

![Graph showing the reversal of sign of charge at pH of about 6.0, on the acid side of the isoelectric point of oxyhemoglobin.]

Fig. 6. Collodion membranes treated with oxyhemoglobin. Reversal of sign of charge at pH of about 6.0, on the acid side of the isoelectric point of oxyhemoglobin.
collodion bags were filled over night with this crude oxyhemoglobin solution and the bags were used the next day for similar experiments to those described for membranes treated with gelatin. Fig. 5 shows the results of such experiments. The MgCl₂ curve begins to rise at a pH of 6.0 while the curve for Na₂SO₄ rises at a pH of about 7.0 or 8.0. Hence when the membrane has been treated with oxyhemoglobin a much lower hydrogen ion concentration is required to induce a positive charge on the membrane than when the membrane has been treated with gelatin. This was to be expected if the reversal of the sign of charge of a membrane treated with a protein depends on the isoelectric point of the protein. According to Michaelis the isoelectric point of oxyhemoglobin is pH = 6.7 to 6.8, and we notice accordingly that the membrane treated with oxyhemoglobin assumes a positive charge when the pH is a little less than 6.7; e.g., 6.0. In Fig. 6 the curve for NaH₂PO₄ shows a distinct rise at a pH of 6.0 and that for CaCl₂ at about 6.0 or a little less. In judging the curves in Figs. 5 and 6 the reader must bear in mind that the CO₂ of the air lowers the pH during the experiments and although the experiments lasted only 20 minutes the error so caused was noticeable in the neighborhood of the point of neutrality. Thus the beginning of the rise in the curve for Na₂SO₄ was in reality not at pH = 8, but at a lower pH, either 7.0 or probably less.

V. Collodion Membranes not Treated with a Protein.

When we use membranes not treated with gelatin and repeat the experiments described in Fig. 1, we get altogether different results (Fig. 7). There is no minimum or drop at pH of about 4.0 or 6.0 since the sign of charge of collodion membranes not treated with proteins is not reversed by acid.³ Collodion membranes not treated with a protein are always negatively (and the watery phase positively) charged within the range of our experiments. Hence a m/256 solution of CaCl₂ cannot attract pure water through such a membrane even in acid solution and the curve for the effect of m/256 CaCl₂ is flat for the whole range of hydrogen ion concentrations (up to 10⁻² N). The curve for m/256 Na₂SO₄ does not reach a minimum at pH 4.0 but drops slowly reaching a minimum at pH 2.0 or below, this drop being due to the depressing effect of the high concentration of the cat-
ions Na and H of the solution on the negative charge of the membrane. The drop of the curve at the alkaline end (pH = 10 to 12) is due to the high concentration of the cations Na and K, the latter introduced with the KOH added.

Collodion membrane not treated with protein

![Graph](image)

**Fig. 7.** Collodion membranes not treated with protein. No reversal of sign of charge of membrane.

**VI. Theoretical Remarks.**

Collodion membranes treated with oxyhemoglobin in the way mentioned remain red after they are washed, showing that the treatment of the membrane with a protein solution results in the formation of a durable protein film at the surface of the collodion membrane and probably also in the interstices of the membrane, since traces of oxyhemoglobin or at least of red pigment diffuse out of the membrane. Applying Werner's idea concerning the combination between acid and NH₃ to proteins we should expect that acids are added to an amino-acid group of a protein in a way similar to that in which they are added to NH₃; namely (if the acid added is HNO₃), by forming a salt of the type $R - N\underset{H_2}{\overset{HNO_3}{\text{H}}}^-$ which dissociates into a positive $R - NH_3$ ion.
and a negative $\text{NO}_3^-$ ion. On this assumption, the double layer at the boundary of the protein film and a solution would appear to be ionic in character, the positive stratum of the double layer being formed by the protein cations of the surface films of the membrane, while the mobile, watery stratum of the double layer contains the anions of the protein-acid salt. On this assumption it is clear why the hydrogen ion concentration required to make the membrane positive varies in the same sense as the isoelectric point of the protein used and why it is always higher than that of the isoelectric point, since a certain part of the surface of the protein film must be transformed into protein-acid salt before the membrane assumes a positive charge. At a hydrogen ion concentration below that of the isoelectric point the protein film is negatively charged since the protein exists here in the form of metal proteinate dissociating into a protein anion and a metal ion, the latter forming the positive watery stratum of the double layer, while the membrane owes its negative charge to the protein anion.

It does not follow, however, that this transformation of protein into protein-acid salt is the only, or even the essential, condition for the reversal of the sign of charge of the double layer by acid. Since acid can bring about a reversal in the electrical double layer of water bounded by air (or possibly even in a vacuum), it is obvious that the modification of the surface layer of the water which is in contact with the membrane may also play a rôle in the reversal of the sign of charge of the protein film. This possibility has to be considered also in view of the fact that trivalent or tetravalent cations can bring about a reversal in the sign of charge of a membrane treated with protein, even when the hydrogen ion concentration excludes a salt formation between protein and trivalent cation. These facts will be discussed in a subsequent paper.

**SUMMARY.**

1. It had been shown in previous papers that when a collodion membrane has been treated with a protein the membrane assumes a positive charge when the hydrogen ion concentration of the solution
with which it is in contact exceeds a certain limit. It is pointed out in
this paper that by treating the collodion membrane with a protein
(e.g. oxyhemoglobin) a thin film of protein adheres to the membrane
and that the positive charge of the membrane must therefore be
localized in this protein film.

2. It is further shown in this paper that the hydrogen ion concen-
tration, at which the reversal in the sign of the charge of a collodion
membrane treated with a protein occurs, varies in the same sense as
the isoelectric point of the protein, with which the membrane has
been treated, and is always slightly higher than that of the isoelectric
point of the protein used.

3. The critical hydrogen ion concentration required for the reversal
seems to be, therefore, that concentration where enough of the pro-
tein lining of the membrane is converted into a protein-acid salt (e.g.
gelatin nitrate) capable of ionizing into a positive protein ion (e.g.
gelatin) and the anion of the acid used (e.g. NO₃).