THE REVERSAL OF THE SIGN OF THE CHARGE OF COLLODION MEMBRANES BY TRIVALENT CATIONS.

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I.

The reversal of the sign of charge of collodion membranes (treated previously with a protein) by hydrogen ions has been discussed in a preceding paper\(^1\) and in this paper the influence of trivalent cations on the reversal will be considered. We shall omit the discussion of the action of tetravalent cations (e.g. ThCl\(_4\)) since their solutions have so high a concentration of hydrogen ions that this alone suffices to bring about a reversal in the sign of charge.

We will prove first that collodion membranes which have previously been treated with a protein give the reversal in the sign of charge in the presence of trivalent cations, while collodion membranes not so treated do not show the reversal. When a solution of CaCl\(_2\) of not too high a concentration (e.g. below m/8) is separated from pure water by a collodion membrane, which is negatively electrified, the solution shows no attraction for water, while it attracts water powerfully when the membrane is charged positively. The Ca ion acts as if it repelled positively charged water and as if it attracted negatively charged water. To find out whether or not trivalent cations reverse the sign of charge of the membrane we have to add a low concentration of a "neutral" salt with trivalent cation to the solution of CaCl\(_2\). Weak solutions of CeCl\(_3\) and LaCl\(_3\) satisfy this condition. The solutions of CaCl\(_2\) are for this purpose made up in m/1,024 solutions of CeCl\(_3\) or LaCl\(_3\) instead of in distilled water; and the distilled water surrounding the collodion bag is also replaced by

m/1,024 CeCl₃, so that in regard to CeCl₃ or LaCl₃ the liquid is the same on both sides of the membrane. Any osmotic effect can therefore only be due to the CaCl₂. This allows us to investigate the question of the reversal of the sign of charge of the membrane by the Ce ions. Fig. 1 shows that the CeCl₃ can only reverse the sign of charge of the membrane if the latter has previously been treated with gelatin. The lower curve gives the initial rate of diffusion of water (after 20 minutes) into CaCl₂ solutions (of different concentration) through collodion membranes not treated with gelatin. The membrane is negatively charged since no rise of the level of water in the solution occurs as long as the concentration of CaCl₂ is below m/8. The rise of the curve at a higher concentration has a different cause which need not be considered in this connection. The upper curve gives the influence of the same CaCl₂ solutions on the rate of diffusion of water from pure water into solution when the membrane has previously received a gelatin treatment. In this case the level of liquid rises in the solution and the more so the higher

![Graph showing the initial rate of diffusion of water through collodion membranes treated and not treated with gelatin.](image-url)
the concentration of the CaCl₂ solution. The CeCl₃ has therefore caused a reversal in the sign of charge of the membrane making the latter positive. This made it possible for the CaCl₂ solution to increase the rate of diffusion of water through the membrane into the solution. The pH of the solutions varied between 5.1 (in the lowest concentration of CaCl₂) and 5.9 (for the highest concentration); i.e., the solutions were all on the alkaline side of the isoelectric point of gelatin.

Experiments with electrical endosmose confirmed the conclusion that in the presence of CeCl₃ the membrane assumes a positive charge when the membrane had previously been treated with gelatin, but that the membrane remains negatively charged when it has not been treated with a protein. In these experiments with electrical endosmose the solutions inside and outside the collodion bag were solutions of CeCl₃ of identical concentration.

II.

We have shown in another paper⁵ in this number of the *Journal* that in the case of collodion membranes treated with gelatin and rendered positive by acid the rate of diffusion of water from the side of pure water through the membrane to the solution is raised by cations in the order K < Na < Li < divalent cations < trivalent cations. Since the Ce ions render the gelatin film of the collodion membrane positive the influence of different cations on the attraction of water should increase in the same order as when the membrane is rendered positive by acid; and the attraction of water by the solution should be a minimum in the case of K. Fig. 2 shows that this is true. In these experiments different concentrations of the four salts, KCl, NaCl, LiCl, and CaCl₂, from ½/2,048 to 1M were made in ½/1,024 CeCl₃. The H₂O surrounding the collodion bags containing the solutions was replaced by ½/1,024 CeCl₃ to eliminate the osmotic effect of CeCl₃ in the experiments. The experiments show that the attraction of water by the four salts follows the order we should expect if Ce caused the membrane to assume a positive charge; i.e., K < Na < Li < Ca. The pH in these experiments varied from

5.2 to 6.0, thereby proving that the Ce reversed the sign of the charge of the gelatin film on the inside of the membrane without raising the hydrogen ion concentration to that point where an acid reversal occurs. In Fig. 3 m/1,024 AlCl₃ solutions are used for rendering the membrane positive. In this case the pH was about 4.1 in all solutions.

![Graph showing the relative influence of different cations upon the attraction of water](image)

**Fig. 2.** The relative influence of different cations upon the attraction of water is in the order Ca > Li > Na > K, proving that the membrane is positively charged in the presence of Ce.

The order of efficiency of cations remains the same as when CeCl₃ is used, but the effect is quantitatively larger in Fig. 3 than in Fig. 2. All these experiments and many others show that trivalent cations cause the membrane previously treated with gelatin to be charged positively on the alkaline side of the isoelectric point of gelatin.
The next question was as to the minimal concentration of a trivalent cation required to render the membrane positive. For this purpose various concentrations of CeCl₃ were prepared, beginning with m/65,536 CeCl₃. To each of these solutions so much CaCl₂ was added that the concentration was m/256 in regard to CaCl₂. These
solutions were put into collodion bags previously treated with gelatin. The bags were put into beakers containing the same solutions of CeCl₃ as that inside the bag but free from CaCl₂. Hence the Ce acted only on the sign of the charge of the membrane but not on the attraction of water. This latter was done by the CaCl₂. Whenever the level of the solution of CaCl₂ rises we know the membrane must be positively charged. The curve in Fig. 4 shows that the membrane already assumes a positive charge in M/32,768 CeCl₃ and probably in lower concentrations of CeCl₃.

When the CeCl₃ was replaced by AlCl₃ the membrane assumed a positive charge when the concentration of AlCl₃ was less than M/65,536. The pH of this latter solution of AlCl₃ was 5.0, so that the reversal was due to the Al ion and not to the hydrogen ion concentration. The fact that Al is more efficient than the Ce (or La) ion may be connected with the fact that the ionic radius of Al is considerably smaller than that of Ce.
smaller than that of either Ce or La. The drop in the curves when the concentration of the CeCl₃ or AlCl₃ solution exceeds M/1,024 is due to the concentration of the anion, as is shown by the fact that the drop is greater in the case of Al₂(SO₄)₃ than in the case of AlCl₃. This latter phenomenon has been discussed in previous papers. The pH of the Al₂(SO₄)₃ solutions almost coincided with that of the AlCl₃ solutions of the same concentration.

IV.

It is therefore certain that the trivalent ions bring about a positive electrification in the collodion membrane in contact with water when the membrane has been treated with a protein, but that they cannot reverse the sign of the charge of collodion membranes not so treated. This indicates that the reversal is at least partly due to an action of the trivalent cations on the protein. A chemical combination can only occur between Ce or Al and gelatin on the alkaline side of the isoelectric point where the gelatin is capable of combining with metals. In the experiments described thus far the pH of the CeCl₃ solutions was with one exception always > 4.7. The question arises, how will a CeCl₃ solution act on the acid side of the isoelectric point of the protein forming the inner lining of the collodion bag where the membrane is already rendered positive by the acid?

M/256 LaCl₃ solutions were prepared at different pH, from 7.0 to 2.6, by adding KOH or HNO₃ to the distilled water used for the solutions as required for the pH. These solutions were put into collodion bags and the latter were dipped into H₂O of exactly the same pH as that of the solutions inside the bag. The initial rise of water (in the first 20 minutes) inside the bags was then observed. Similar experiments were made with CeCl₃. The collodion membranes had previously been treated with gelatin. Fig. 5 gives the results. It is plain that the curves consist of two distinct parts and that the dividing line seems to lie near the isoelectric point of gelatin. On the alkaline side of the point, i.e. for pH 4.7 or above, the initial rate of diffusion is near 140 mm.; and it varies very little with a change of pH. As soon, however, as the pH falls to 4.7 or below, the rate of diffusion rises steeply to 300 and 340 mm. for the two salts.
To understand this result we must discriminate between the effects of the trivalent cations on the reversal of the sign of charge and on the increase of the rate of diffusion of water into the solution, which seems to be due to an increase in the density of charge of a membrane already positive. On the alkaline side of the isoelectric point, although m/256 solutions of La and Ce charge the membrane positively, their attraction for water rises very steeply on the acid side of the isoelectric point of gelatin.
point of gelatin the trivalent cation makes the membrane positive by combining with gelatin and forming a salt; e.g., La gelatinate. But, in addition, the salts of trivalent cations attract the water, and the more the higher their concentration until a certain maximum is reached, as shown in Fig. 4. On the acid side from the isoelectric point the protein film cannot react chemically with the La or Ce and the membrane is charged positively through the influence of the

![Graph showing the relationship between pH and initial rate of diffusion of water](https://via.placeholder.com/150)

**Fig. 6.** Showing that the critical point for this steep rise varies with the isoelectric point of the protein with which the membrane has been treated.

acid. In this case the trivalent cation acts apparently by increasing the density of charge of the positive membrane considerably, as was to be expected.

In order to test this idea further, membranes treated with oxyhemoglobin instead of with gelatin were tried. The isoelectric point of oxyhemoglobin is at pH = 6.8. In this case the steep rise in the curve with LaCl₃ should be nearer this value than 4.7. The curves
of Fig. 6 show that this is the case. It may be mentioned incidentally that the initial rate of diffusion of water into solutions was greater when the membranes had been treated with oxyhemoglobin than when they had been treated with gelatin. We shall return to this phenomenon in another connection.

![Graph showing concentration of electrolytes](Fig. 7. Showing that the attraction of salts with trivalent cations for water is considerably greater than the attraction of acid (HCl) for water, although acids are very efficient in making the membrane positive.)
The experiments represented in Figs. 5 and 6 seem to show that the efficiency of ions in causing the reversal of the sign of charge is as great in the case of hydrogen ions as in the case of trivalent cations; while the influence on the rate of diffusion of water is considerably greater in the case of trivalent cations than in the case of hydrogen ions. Fig. 7 permits a comparison of the attraction of water by solutions of equal concentration of HCl, LaCl₃, Al₂Cl₆, and ThCl₄ through membranes treated with gelatin. On one side of the membrane was the solution, on the other pure water. If we consider only concentrations up to m/32 this attraction is very slight in the case of HCl as compared with LaCl₃ or Al₂Cl₆. (It should be stated that in the experiments with HCl the level of the water was considerably higher at the beginning of the experiment than in the other solutions.)

Theoretical Remarks.

The experiments have shown that a reversal of the sign of charge of a collodion membrane can be brought about by acids and by "neutral" salts with trivalent cation after the inside of the membrane has been in contact for an hour or more with a sufficiently strong solution of a protein whereby apparently a protein film is formed on the inside of the membrane. Collodion membranes not treated are always negatively charged in contact with water, no matter whether or not hydrogen ions and trivalent cations are present in sufficient concentration. Collodion membranes when treated with a protein are also negatively charged when the reaction of the solution is on the alkaline side of the isoelectric point of the protein and when the solution is free from trivalent or tetravalent cations. In this case the protein exists in the form of a metal proteinate dissociating into a positive metal ion, the protein adhering to the inside of the collodion membrane. This might suggest that the membrane becomes negative in contact with water on account of these metal ions dissolving in water, while the solid film of protein anions adhering to the membrane is negatively charged. When the hydrogen ion concentration is raised sufficiently to cause the transformation of the protein
film into a protein-acid salt the reversal in the sign of charge of the membrane would be easily intelligible since the protein-acid salt dissociates into a positive protein cation forming the surface film of the membrane, and an anion which goes into solution.

A difficulty arises, however, if we try to explain the reversal of the sign of charge of the membrane by trivalent cations on the alkaline side of the isoelectric point of the protein. In this case the protein film consists of La or Ce proteinates which are practically insoluble. Practically no dissociation into a negative protein anion and a trivalent cation would be supposed to occur and it seems not possible to state why an insoluble La proteinate should assume a positive charge when in contact with water. As long as this fact is not explained it remains doubtful whether the tentative explanation just given for the acid reversal is correct or complete. It also remains to be explained why the collodion membrane not treated with a protein always assumes a positive charge regardless of the hydrogen ion concentration or the concentration of trivalent cations. It might be argued that the collodion membrane differs from proteins in not being an amphoteric electrolyte and in not being able to combine with trivalent cations. Another possibility must, however, be considered; namely, that the hydrogen ions and trivalent cations influence the surface electrical potential of the solution and that the combined effects of these ions on the surface electrical potential of the membrane and of the liquid determine the phenomena described in our papers.

SUMMARY.

1. Trivalent cations cause a collodion membrane covered with a protein film to be charged positively while they do not produce such an effect on collodion membranes not possessing a protein film. The same had been found for the reversal of the sign of charge of the membrane by acid.

2. This reversal in the sign of charge of the membrane by trivalent cations occurs on the alkaline side of the isoelectric point of the protein used; while the reversal by acid occurs on the acid side of the isoelectric point.
3. The reversal seems to be due to or to be accompanied in both cases by a chemical change in the protein. The chemical change which occurs when the hydrogen ions reverse the sign of charge of the protein film consists in the formation of a protein-acid salt whereby the H ion becomes part of a complex protein cation; while the chemical change which occurs when trivalent cations reverse the sign of charge of the protein film consists in the formation of an insoluble and therefore sparingly or non-ionizable metal proteinate.