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## THE INFLUENCE OF SALTS ON THE RATE OF DIFFUSION OF ACID THROUGH COLLODION MEMBRANES.

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(From the Laboratories of The Rockefeller Institute for Medical Research.)

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When a solution of a salt, *e.g.*,  $\text{CeCl}_3$  or  $\text{CaCl}_2$  or  $\text{NaCl}$ , in  $\text{HCl}$ , with a pH of 3.0, is separated by a collodion membrane from a pure solution of  $\text{HCl}$  of the same pH, salt will diffuse out until the solution on both sides of the membrane is identical. Since the concentration of  $\text{HCl}$  measured by the hydrogen electrode is the same on opposite sides of the membrane at the beginning of the experiment, one would expect that no change in the pH should occur during the experiment. The writer found, however, that at first acid is rapidly driven by the salt to the other side of the membrane, so that at first the concentration of the acid is diminished on the side of the salt solution and increased on that side of the membrane where the solution was originally free from salt.<sup>1</sup> This occurs, of course, only during the first hours of the experiment. When the concentration of the salt has become the same on the opposite sides of the membrane, the pH also becomes the same again on both sides of the membrane.

The following experiments may serve as an illustration. Collodion bags, treated with gelatin, of about 50 cc. contents, were filled with a  $\text{M}/256$  solution of a salt made up to pH 3.0 through the addition of  $\text{HCl}$ . These bags dipped into beakers filled with 350 cc. of  $\text{HCl}$  of pH 3.0 (about  $\text{N}/1,000$ ) but without salt. After different intervals the experiment was terminated and the pH inside and outside was determined. It was found that after an hour the pH inside had increased considerably (*i.e.* the concentration of hydrogen ions in the salt solution had diminished), while outside the change of pH was small on account of the fact that the volume of solution was seven times as great as inside the bag. Table I gives some of the results. In Experiments 1 to 4 the collodion membrane was coated with gelatin, while in Experi-

<sup>1</sup> Loeb, J., *J. Gen. Physiol.*, 1921-22, iv, 213.

ment 5 the membrane was not treated. The phenomenon is observed in both cases. The initial concentration of the salt inside the bag was always  $M/256$ , but, of course, diminished during the experiment.

TABLE I.

*Change of Hydrogen Ion Concentration on Opposite Sides of the Membrane during Period of Diffusion of Salts.*

Time, hrs.		0	1	2	3	5	6	8	22
1. $M/256$ $CeCl_3$ .	pH inside.....	3.01	3.29	3.29	3.32	3.17	3.20	3.16	3.04
	pH outside.....	2.97	2.97	2.95	2.95	2.97	2.98	2.97	2.99
	Difference.....	0.04	0.32	0.34	0.37	0.20	0.22	0.19	0.05
2. $M/256$ $CaCl_2$ .	pH inside.....	2.99	3.26	3.26	3.21	3.20	3.21	3.10	3.02
	pH outside.....	2.97	2.97	2.97	2.96	2.98	2.98	2.98	2.99
	Difference.....	0.02	0.29	0.29	0.25	0.22	0.23	0.12	0.03
3. $M/256$ $NaCl$ .	pH inside.....	2.98	3.13	3.12	3.09	3.05	3.05	3.00	
	pH outside.....	2.98	2.98	2.99	2.98	2.99	3.00	2.99	
	Difference.....	0	0.15	0.13	0.11	0.06	0.05	0.01	
4. $M/256$ $Na_2SO_4$ .	pH inside.....	3.06	3.08	3.01	3.01	3.01	3.01	3.05	
	pH outside.....	2.98	3.01	3.00	3.01	3.00	3.02	3.03	
	Difference.....	0.08	0.07	0.01	0	0.01	0.01	0.02	
5. $M/256$ $CaCl_2$ . (Membrane not coated with gelatin.)	pH inside.....	2.98	3.11	3.11	3.08	3.04	3.04	3.02	
	pH outside.....	2.98	2.95	2.97	2.99	2.97	2.97	2.99	
	Difference.....	0	0.16	0.14	0.09	0.07	0.07	0.03	

These experiments show, first, that the expulsion of acid from the salt solution into the solution free from salt during the first hours of the experiment is in the case of chlorides the more considerable the higher the valency of the cation; in other words, it increases with the concentration of Cl ions in the solution. Thus after 3 hours the difference in pH inside and outside is 0.37 for  $CeCl_3$ , 0.25 for  $CaCl_2$ , and 0.11 for  $NaCl$ . This expulsion of acid occurs regardless of the fact whether or not the membrane is coated with gelatin, as a comparison of Experiment 2 with Experiment 5 shows. After 3 hours the difference in acid on the opposite sides of the membrane becomes less and finally disappears. In the case of  $Na_2SO_4$  this effect is not noticeable.

When the writer first observed these phenomena he was under the impression that they were due to the action of the membrane, and he expressed this belief in a paper published a year ago.<sup>1</sup> Recent experiments to be mentioned presently have convinced him that the membrane has little if anything to do with this phenomenon and that it finds its explanation in the influence of salts on the diffusion constant of acids discovered by Arrhenius and described by him as follows:

“The theoretical study of the phenomenon of diffusion has led to a conclusion which seems very paradoxical. If hydrochloric acid diffuses in water, its diffusion constant is found to be 2.09 at 12°C., which also agrees very well with the theory of Nernst. If instead of using pure water for the diffusion, I take a solution of sodium chloride, I might expect that the molecules of HCl moved, *i.e.*, diffused more slowly in that medium than in water because of the increase of the viscosity on addition of NaCl. But instead of that an increase of the constant of diffusion is observed. For instance into a cylindrical vessel was poured a layer of 1 cm. height of 1.04 *n* HCl and over it was placed pure water to a height of 3 cm. The diffusion constant was found to be 2.09 at 12°C. In another experiment 0.1 *n* NaCl was used instead of water, so that at the bottom of the cylindrical vessel was a 1 cm. high layer 1.04 *n* in regard to HCl and 0.1 *n* in regard to NaCl and over it were placed 3 cm. of 0.1 *n* NaCl.

“The diffusion constant was now 2.50. According to Nernst's theory I calculated 2.43. In 0.67 *n* NaCl the constant was still higher 3.51, calculated 3.47. Many analogous experiments with results agreeing with the dissociation theory were performed with nitric and hydrochloric acid, caustic potash and soda.

“The explanation is that when the H-ions (*i.e.*, the acid) diffuse in pure water they must drag the (about 5 times) more immobile Cl-ions with them in equivalent number. If now Na-ions are distributed in the same fluid, these ions are carried back in the opposite direction of the diffusing H-ions because of the electric forces which hold back the H-ions and pull on the Cl-ions in the direction of diffusion (upwards in the experiments). The driving back of the Na-ions partly neutralizes these electric forces, so that therefore the H-ions are not so strongly held back nor the Cl-ions pushed up as in pure water. Therefore the H-ions diffuse more rapidly and that in a so much higher degree as the Na-ions are more numerous relatively to the H-ions. The maximal velocity which the H-ions may reach at 12° is that corresponding to no hindrance from electrical forces and gives a diffusion constant 6.17. With 0.52 *n* HCl and 3.43 *n* NH<sub>4</sub>Cl I reached a value of 4.67 instead of theoretically calculated 5.72.—It must be borne in mind that at these high concentrations the degree of dissociation of the HCl is diminished, which is not taken into consideration, and therefore the observed dissociation constant is smaller than the calculated one determined with the supposition that the electrolytic dissociation is complete.

“This phenomenon is a so-called salt action, which can not be explained if we suppose the molecules of the diffusing acids or bases and the added salts not to be dissociated into electrically charged ions. It is a real proof of the electrolytic dissociation hypothesis.”<sup>2</sup>

On the basis of Arrhenius' statement, the phenomenon observed by the writer might find its explanation in the following way. When a solution of, *e.g.*,  $M/1,000$   $CeCl_3$  is made up in  $HCl$  so that the  $pH$  is 3.0, and if this solution is put into a collodion bag of 50 cc. volume, which is immersed in a beaker containing pure  $HCl$  (without salt) also of  $pH$  3.0, the osmotic pressure of  $Cl$  ions is about four times greater in the salt solution than on the opposite side of the membrane. Hence four times as many  $Cl$  ions will diffuse through the membrane from the salt solution into the water as will diffuse simultaneously in the opposite direction.  $H$  and  $Ce$  ions will be dragged along electrostatically with the moving  $Cl$  ions, but on account of their greater mobility  $H$  ions will follow the  $Cl$  ions more readily than will the slow  $Ce$  ions. As a consequence the hydrogen ion concentration will rise on the water side of the membrane and diminish on the side of the salt solution; but on account of the fact that the volume of water was seven times as great as that of the salt solution inside the collodion bag, the change in  $pH$  was more marked in the salt solution inside the bag than outside. If this explanation is correct, the membrane itself has nothing to do with the phenomenon except to make it easier to demonstrate the salt effect on the rate of diffusion of the acid. If this conclusion is correct, it should be possible to prove that the rate of diffusion of acid into water through a membrane is accelerated through the addition of salt and the more the higher the concentration of the salt.

This proof was furnished in the following way. 350 cc. of different concentrations of  $NaCl$ ,  $CaCl_2$ ,  $LaCl_3$ , or  $Na_2SO_4$  were made up in  $HCl$  so that each solution was  $N/2,000$  in regard to the acid. Into these mixtures of  $N/2,000$   $HCl$  and salt were dipped collodion bags with a volume of about 50 cc. filled with water of  $pH$  4.7. In this case the salt solution was outside the bag and the water free from salt was

<sup>2</sup>Arrhenius, S., *Theories of solutions*, New Haven, London, Oxford, 1912, 163-165.

inside the collodion bag. Both salt and acid diffused into the bag. After 20 minutes the collodion bags were taken out, the volume of liquid measured, and 10 cc. of the solution were titrated with  $N/400$  NaOH to pH 5.4, using methyl red as an indicator.

In these experiments the concentration of the acid outside did not change materially since the volume of the outside solution was seven times as great as the inside solution, and the volume of the liquid inside the bag also remained practically the same except at the higher concentrations of the salt where the osmotic effect became noticeable. We may therefore use the titration value for 10 cc. of the content of the bag as a measure for the rate with which acid diffuses from the outside solution into the collodion bag. These titration values are plotted in Fig. 1 as ordinates over the concentrations of the salts as abscissæ. 0.25 cc. of  $N/400$  NaOH were required to bring the acid contained in 10 cc. of the contents of the collodion bag to pH 5.4 when no salt was present. If we call this rate for the diffusion of acid through the membrane 1, then it is seen that the rate of diffusion of HCl is doubled when the concentration of NaCl is between  $M/64$  and  $M/128$ , when the concentration of  $CaCl_2$  is between  $M/128$  and  $M/256$ , and when the concentration of  $LaCl_3$  is about  $M/512$  or perhaps a little below. It is obvious that the effect of these three salts on the rate of diffusion of HCl through the membrane increases with the concentration of the Cl ions and that all three salts accelerate the rate of diffusion of HCl through the membrane.  $H_2SO_4$  diffuses more slowly through the membrane than HCl, since the titration value for acid without salt is 0.20. This rate of diffusion is doubled when the concentration of  $Na_2SO_4$  is between  $M/256$  and  $M/128$ . At higher concentrations both  $Na_2SO_4$  and  $CaCl_2$  show anomalies which need not be discussed in this connection.

These experiments were open to the objection that the pH of the  $N/2,000$  HCl solution was different in  $LaCl_3$ ,  $CaCl_2$ , or NaCl and that this caused the difference in the influence of the three salts on the rate of diffusion. Measurements of the pH showed that it was practically the same in all three solutions and had therefore nothing to do with the result. This was, however, not true in the case of mixtures of  $H_2SO_4$  and  $Na_2SO_4$  (as had already been found by Thomas and Baldwin<sup>3</sup>), and for high concentrations of  $CaCl_2$ . These interesting phenomena can, however, not be discussed in this connection.

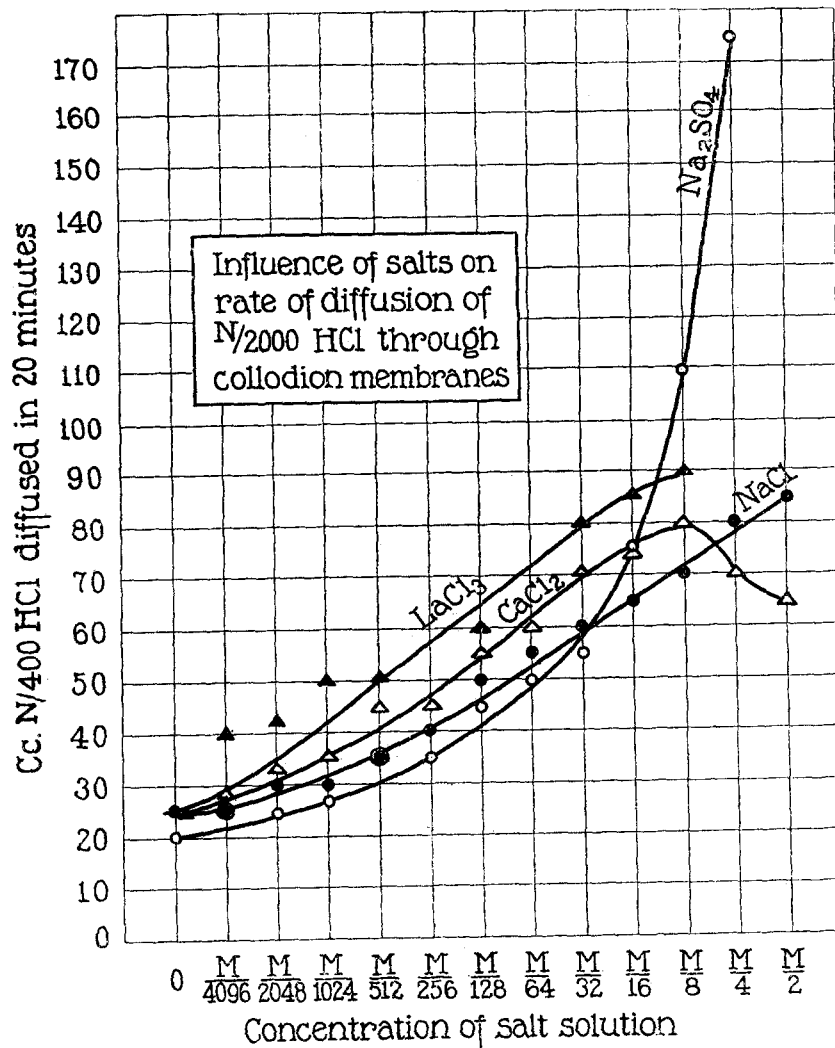


FIG. 1. Influence of salts on rate of diffusion of N/2,000 HCl through collodion membranes. Abscissæ are the concentrations of salts; ordinates, cubic centimeters of N/400 NaOH required to bring 10 cc. of water inside to pH 5.4 after 20 minutes of diffusion.

In a series of papers and a recent book the writer has tried to prove that the influence of electrolytes on the colloidal properties of protein solutions, such as osmotic pressure, viscosity, membrane potentials, is merely the consequence of the Donnan equilibrium. The equilibrium rests on the fact that many membranes which are freely permeable for small crystalloidal ions are impermeable for protein ions. The writer has shown that proteins behave in regard to chemical reactions like crystalloids and the same seems to be true as far as solubility is concerned. This then leads to the conclusion that the only reason for the colloidal behavior of protein solutions in regard to osmotic pressure, viscosity, etc., lies in the non-diffusibility of proteins through membranes and gels which are permeable to crystalloids. If we could produce membranes permeable to Na but not to  $\text{SO}_4$  ions it would be found that a  $\text{Na}_2\text{SO}_4$  solution would show colloidal behavior in regard to osmotic pressure and membrane potentials. This postulate of the theory of colloidal behavior is met in the facts published in this paper. It is not necessary that the membrane should be completely impermeable for one ion; it suffices that the rate of diffusion of one type of ions be considerably slower than that of another kind. The slow ions are the cations of salts, especially the polyvalent cations.

These experiments were undertaken to find out whether the influence of salts on the rate of diffusion of acid through the membranes of living cells was the same as through collodion membranes. This is not the case since salts retard the rate of diffusion of acid through membranes of living cells instead of accelerating it. This retarding effect of salt on the diffusion of acid—the so called antagonistic salt effect—was observed by Loeb and Wasteneys in the case of the marine fish *Fundulus*.<sup>4</sup> Thus the fish died in 3 M/10,000  $\text{HNO}_3$  in less than an hour while they lived indefinitely when the same  $\text{HNO}_3$  solution was made up in M/10 NaCl.  $\text{CaCl}_2$  was much more effective in the prevention of the toxic effects of acid. Later the same inhibiting effect of salt on the toxic effect of acid was observed in the eggs of *Fundulus*.<sup>5</sup>

<sup>3</sup> Thomas, A. W., and Baldwin, M. E., *J. Am. Chem. Soc.*, 1919, xli, 1981.

<sup>4</sup> Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1911, xxxiii, 489; 1912, xxxix, 167.

<sup>5</sup> Loeb, J., *J. Biol. Chem.*, 1915, xxiii, 139; 1917, xxxii, 147.



Since the effect of salts on the diffusion constant of acids must be the same in the case of membranes of living cells as in the case of collodion membranes, the retarding effect of salts on the diffusion of acids in the egg of *Fundulus* can only be ascribed to a modification of the membranes of *Fundulus* by the salts whereby a block is created to the diffusion of acids.

## SUMMARY AND CONCLUSION.

1. The writer had previously published the observation that if a salt solution made up in an acid solution (*e.g.* HCl) of a definite pH (*e.g.* 3.0) is separated by a collodion membrane from pure water containing the same acid of the same pH, acid is at first driven from the salt solution into the water, so that the pH of the latter becomes at first lower than that of the solution.

2. It is shown in this paper that this paradoxical phenomenon is not due to any peculiarity of the membrane but is a consequence of the well known fact that the diffusion constant of an acid is increased by a salt.