SODIUM CHLORIDE AND SELECTIVE DIFFUSION IN LIVING ORGANISMS.

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(Received for publication, September 5, 1922.)

1. Proof that NaCl Acts Similarly to CaCl₂ and LaCl₃ on the Rate of Diffusion of Acid into the Egg of Fundulus.

1. Each organism and each cell is in dynamic equilibrium with its surroundings, which means that the chemical changes which occur in the cells are, within certain limits, automatically reversed. Although an understanding of the mechanism of this regulation is one of the fundamental problems of physiology, large gaps exist in our knowledge of these regulatory processes. Why is it, for instance, that the blood of bony fishes can maintain a concentration in NaCl far below that of the ocean and far above that of the fresh water in which they may be living? Why is it that the red blood corpuscles or the cells of muscles (and probably cells in general) remain rich in potassium and entirely or almost free from sodium, while the blood is rich in sodium and poor in potassium? Why is it that the waste products, which are formed in one part of the body, diffuse into the blood but do not, as a rule, diffuse into the other cells of the body?

It is the intention of this paper to show that the salts which surround a cell modify the properties of the cell wall in such a way as to accelerate the rate of diffusion of certain ions and retard the rate of diffusion of other ions, and that aside from calcium chloride, sodium chloride is a very important factor in causing such selective diffusion.

Over 20 years ago the writer made the observation that the newly fertilized eggs of the marine fish Fundulus which normally develop in sea water, are killed when put into a pure NaCl solution of that

concentration in which NaCl exists in the sea water, while they can live indefinitely in such a solution when a small but definite quantity of a salt with bivalent cation (especially CaCl₂) or a still smaller concentration of a salt with a trivalent cation is added. When the eggs are older, they can live for a number of days in a 2.5 M solution of NaCl when the proper amount of CaCl₂ is added, while in a pure NaCl solution of that concentration they are killed in a few hours. Since these eggs can develop as well in distilled water as in sea water, it was obvious that the Ca or the other plurivalent cations were not required for any nutritive purpose. In 1905 the writer suggested as an explanation that a pure NaCl solution, if its concentration exceeded a certain limit, made the membrane of the egg more permeable, so that NaCl could diffuse into the egg, killing the embryo; while this increase in permeability was prevented by the presence of a low concentration of Ca. The writer's further experiments on Fundulus left little doubt concerning the correctness of this suggestion and it was proven beyond doubt and shown to be a general phenomenon by the beautiful experiments of Osterhout and his collaborators on Laminaria and Nitella.

While these experiments were correct as far as the rôle of Ca (and other bivalent cations) was concerned, they were incomplete. In these experiments CaCl₂ appeared as an antagonist to NaCl, which it was under the conditions of the experiments in which an excess of NaCl was used. The experiments, however, were incomplete inasmuch as they failed to show another equally important fact, namely, that in moderate concentrations NaCl acts exactly like CaCl₂ or LaCl₃, and that sodium chloride is, perhaps, as important in determining selective diffusion of ions through the cell wall as is CaCl₂.

In 1910 Wasteneys and Loeb observed that the marine fish Fundulus, which can also live in distilled water, died in 3/10,000 M HNO₃ in less than an hour, while the fish lived indefinitely when the same

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3 Loeb, J., Arch. ges. Physiol., 1905, cvi, 252.
4 Loeb, J., Science, 1912, xxxvi, 637; 1916, xlv, 574; Biochem. Z., 1912, xlvi, 127.
HNO₃ solution was made up in N/10 NaCl. CaCl₂ was even more effective as an antagonist to acid than NaCl. Later the inhibiting effect of salt on the toxic action of acids was observed in the egg of the same fish.

We will show in this paper that NaCl, CaCl₂, and LaCl₃ act all alike, each one retarding the rate of diffusion of acid through the membrane of Fundulus and thereby preventing or retarding the toxic action of the acid on the embryo, with this difference only—that the minimal concentration of the salt in which this inhibiting effect occurs diminishes rapidly with the valency of the cation of the salt.

Two different sets of experiments were made. In one set a certain number of eggs were put into a definite quantity of an acid solution of a definite hydrogen ion concentration and the rate at which acid diffused into the egg was determined by measuring the pH of the solution colorimetrically at different intervals. Since the quantity of acid present in the solution was known in the beginning, it was possible to calculate from the change in pH how much acid had diffused into the egg. In this way the influence of salts on the rate of diffusion of acid into the egg could be measured by following the change of pH of the solution colorimetrically.

In the second set of experiments, twenty eggs were always put into 50 cc. of an acid solution of a definite pH with or without salts, and after a certain number of hours, the percentage of eggs in which the hearts were no longer beating was determined. It may be assumed that the heart of the embryo stops beating when a certain quantity of acid has diffused into the egg, and that this quantity is approximately the same for each heart at the same age of the embryo. There are, however, individual variations in the membranes as a consequence of which the rate of diffusion of acid is not the same for different eggs. Assuming that these individual variations are distributed equally in each group consisting of a larger number of eggs, we may further assume that the percentage of hearts which have stopped beating in each group after a certain time is the expression of the average quantity of diffusion of acid during this time. In this way we gain

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7 Loeb, J., J. Biol. Chem., 1915, xxiii, 139; 1917, xxxii, 147.
also a measure of the influence of salts on the rate of diffusion of acid through the membrane with the aid of mortality curves.

The eggs used were about 5 days old or older, the heart beat and circulation of the embryos being well developed. The eggs were kept for at least 2 days previous to the experiment in distilled water to free the outside of the membrane from salts. This is absolutely necessary for the study of the influence of salts on the rate of diffusion of acid into the eggs. Twenty eggs which had previously been examined to make sure that their hearts were beating normally were put into an Erlenmeyer flask containing 50 cc. of an acid solution, generally (unless the contrary is stated) HCl of pH 3.7. These acid solutions were made up in either H₂O or salt solution and they were brought to the desired pH with the aid of the proper indicators. It was necessary to compare only such experiments with each other as were made simultaneously to eliminate the influence of temperature. After a certain time the eggs were examined microscopically to determine the percentage of eggs in which the hearts had stopped beating. The pH of the supernatant acid solution was then determined colorimetrically to check the results. It may be said that the colorimetric check was always parallel to the percentage of hearts which had stopped beating. We will first give the results of the influence of salts on the rate of diffusion of acid into the egg by the method of mortality curves.

2. In Fig. 1 are found the mortality curves for HCl of pH 3.7 in the presence of different concentrations of NaCl, CaCl₂, and LaCl₃. The abscissae are the concentrations of the salts, the ordinates the percentage of eggs killed by the acid (the stopping of the heart beat by acid becomes rapidly irreversible after a certain time). When all were killed, the value of the ordinate in these mortality curves is 100. The duration of the experiment given in Fig. 1 was about 17 hours. In 17 hours 90 per cent of the eggs were killed by the acid when no salt was present; when salt was present the mortality was less, but the retarding influence of the three different chlorides NaCl, CaCl₂, and LaCl₃ on the diffusion of acid into the egg increased rapidly with the increasing valency of the cation. Thus to reduce the mortality of eggs in 50 cc. of HCl of pH 3.7 from 90 per cent to 40 per cent, M/500,000 LaCl₃ (or CeCl₃ or AlCl₃) was sufficient, while a con-
centration of between \( \frac{m}{8,192} \) and \( \frac{m}{4,096} \) \( \text{CaCl}_2 \), and of \( \frac{m}{64} \) \( \text{NaCl} \) was required. In order to overcome completely the toxic effect of \( \text{HCl} \) of \( \text{pH} \) 3.7 (i.e. to prevent completely the diffusion of acid into the egg) about \( \frac{m}{32,000} \) to \( \frac{m}{16,000} \) \( \text{LaCl}_3 \) (or \( \text{CeCl}_3 \) or \( \text{AlCl}_3 \)), about \( \frac{m}{1,024} \) to \( \frac{m}{512} \) \( \text{CaCl}_2 \), and, as we shall see presently, about \( \frac{m}{8} \) to \( \frac{m}{4} \) \( \text{NaCl} \) were required. The influence of the valency of the
cation on the rate of diffusion of \( \text{HCl} \) of \( \text{pH} \) 3.7 into the egg is therefore enormous, \( \text{Ca} \) being about 100 times as effective as \( \text{Na} \), and \( \text{La} \) being at least 40 times as effective as \( \text{Ca} \). What interests us here is the fact that \( \text{NaCl} \) acts qualitatively like \( \text{CaCl}_2 \) and \( \text{LaCl}_3 \) and that only the concentration of salt required differs. The concentration of maximal efficiency of \( \text{NaCl} \), namely \( \frac{m}{8} \) to \( \frac{m}{4} \), is that in which it exists in the blood of mammals and many cold blooded vertebrates.

**Fig. 1.** Inhibiting influence of salts on the toxic effects of \( \text{HCl} \) solution of \( \text{pH} \) 3.7. Abscissae are the concentrations of salts; ordinates, percentage of eggs which died in 18 hours. \( \text{LaCl}_3 \), \( \text{CeCl}_3 \), \( \text{AlCl}_3 \), \( \text{CaCl}_2 \), and \( \text{NaCl} \) inhibit the toxic effects of acid, but the concentration of salt required diminishes rapidly with the valency of the cation.
This striking valency effect of the cations suggested a number of additional experiments; namely, first, a comparison of the effect of Na₂SO₄ with that of NaCl. In this case the experiments lasted 3 days. The results are found in Fig. 2. It is obvious that the inhibiting action of Na₂SO₄ on the diffusion of acid is greater than that of NaCl and that therefore the SO₄ ion cannot be said to have an effect opposite to that of the cation. Fig. 2 gives also a comparison of the inhibiting effect of NaCl alone and of NaCl + CaCl₂ (in the proportion of 100 molecules of NaCl to 1.5 molecules of CaCl₂). It is obvious that the inhibitory effect of NaCl + CaCl₂ on acid is greater than that of NaCl alone, and the effects of NaCl and of CaCl₂ seem to be additive. Complete inhibition of the toxic effect of acid (or rather complete inhibition of the diffusion of acid into the egg)
during the 3 days was caused by LaCl₃ at a concentration below \( \frac{m}{4.096} \), by CaCl₂ at a concentration between \( \frac{m}{256} \) and \( \frac{m}{16} \), by NaCl at \( \frac{m}{4} \), by \( \text{Na}_2\text{SO}_4 \) at \( \frac{m}{8} \), and by a mixture of NaCl + CaCl₂ (balanced solution) at \( \frac{m}{16} \).

When the concentration of salts exceeds the concentrations mentioned, they cease to inhibit the diffusion of acid into the egg (Fig. 2). LaCl₃ becomes entirely ineffective at a concentration of \( \frac{m}{8} \), NaCl at a concentration of \( 1 \text{ m} \), CaCl₂ at a concentration of \( \frac{m}{4} \), \( \text{Na}_2\text{SO}_4 \) at \( \frac{m}{4} \), while the balanced solution NaCl + CaCl₂ remains efficient even at a concentration of \( 2 \text{ m} \). It is impossible to connect the concentrations at which the salts no longer prevent the diffusion of acid with any purely electrostatic action of ions, and the increase in permeability caused by higher concentrations of salts must be ascribed to an alteration of the membrane due to other than mere electrostatic effects.

This conclusion is corroborated by experiments on the influence of electrolytes other than those thus far mentioned on the rate of diffusion of acid into the egg (Fig. 3). It is obvious that according to the mortality curves in Fig. 3 the inhibiting effect of MgCl₂ on the diffusion of acid is very much less than that of CaCl₂ and almost approaches that of LiCl or NaCl; and the inhibiting effect of BaCl₂ is only slightly greater than that of MgCl₂. On the other hand, Mn acts almost as well as Ca. Sr also acts almost like Ca.

These latter facts are, however, only of secondary importance for our purpose which is to show that in the proper concentration NaCl acts exactly like CaCl₂ and like LaCl₃ on the rate of diffusion of acid through the membrane of the Fundulus egg (and, perhaps, through the membranes of cells in general).

3. We will now give the results of the second method of following the diffusion of acid into the eggs colorimetrically. HCl solutions were made up in water or salt solutions to a pH 3.8, and ten eggs, about 5 or 6 days old (with beating hearts) were put into 10 cc. of these HCl solutions of pH 3.8 in a test-tube. 1 drop of methyl orange was added to each solution and the test-tubes were closed with rubber stoppers. From time to time (about every 10 minutes) the test-tubes were slightly agitated to change the solution in immediate contact with the eggs. The color of the indicator made it possible to follow the rate of diffusion of the acid into the egg. It was found
that when salt was present in proper concentration, the color of the solution changed very little or not at all during 20 hours at about 22°C., while when no salt was present the color changed, betraying a gradual diminution of the acid in the solution. By comparing the color of the solution with standardized indicator solutions, the change in pH could be ascertained. Fig. 4 contains some of the results. The ordinates are the pH of the acid solutions while the abscissae are the times.

![Mortality curves. HCl of pH 3.7 in different salt solutions](image)

**Fig. 3.** Proof that the inhibiting effect of MgCl₂ and BaCl₂ on the rate of diffusion of acid into the egg is less than that of CaCl₂.

It is seen from Curve I in Fig. 4 that without salt the pH of the solution rose gradually in 7 hours from 3.8 to pH 4.2. Since there were 10 cc. of solution of HCl present the amount of acid which diffused into the ten eggs can be calculated from the curve. We may omit the calculation, since it is of no special interest in this connection. After 20 hours the pH was over 5.0; i.e., practically all the acid had diffused from the solution into the eggs, and all the embryos were dead.
after 20 hours. Curve II in Fig. 4 shows that in m/8, m/4, and m/2 NaCl the pH of the solution did not change during the 20 hours of the experiment; i.e., practically no acid diffused into the eggs and the embryos were all alive after that time. Curve III shows that in

![Graph showing pH vs. time for different salt concentrations](image)

**Fig. 4.** Influence of salts on rate at which acid diffuses into the egg. Abscissa are the time in hours, ordinates the pH of the acid solution. The original pH of the acid solution was 3.8.

2 m NaCl the acid diffused even more rapidly into the eggs than without salt and all the embryos were killed rapidly. In 1 m NaCl (Curve IV) the acid diffused first rapidly and later more slowly than without salt; seven of the ten eggs were killed after 20 hours. In m/1,024 LaCl₃ and m/64 CaCl₂ (Curve V) no color change occurred in the solution
and no acid diffused into the eggs in 24 hours or more and all the embryos remained alive.

In all experiments with mortality curves the pH of the supernatant solution was measured at the end of the experiments. Thus Fig. 5 shows that the curves for the change in pH for NaCl and NaCl + CaCl₂ respectively at the end of the experiment corresponded to the mortality curves for these two solutions in Fig. 2. Where the mortality was lower, the pH curve was also lower, and since the mortality was lower in NaCl + CaCl₂ than in NaCl (Fig. 2), we had a right to expect that the same would be true for the pH curves of the two mixtures, which was the case as Fig. 5 shows.
We therefore reach the conclusion that NaCl acts like CaCl$_2$ or LaCl$_3$ in preventing the diffusion of strong acids through the membrane of the egg of *Fundulus*. The only difference is that the concentration in which this salt effect is complete is considerably higher in the case of NaCl than in the case of CaCl$_2$ or LaCl$_3$. The greatest efficiency of NaCl is between $\mu/8$ and $\mu/2$; i.e., that concentration in which NaCl exists in the sea water and the blood of the majority of animals.

II.

*Proof of the Lack of Inhibiting Action of Salts on the Diffusion of Non-Dissociated Weak Acids.*

According to Hantzsch, fatty acids, like acetic acid, occur in two tautomeric forms, true acids (which always undergo complete electrolytic dissociation), and pseudo-acids which are not dissociable. There exists an equilibrium between the two forms which upon the addition of a salt like Na acetate shifts in favor of the non-dissociable pseudo-acid form.

As long as the concentration of acid is not too high, so that there is not too high a concentration of pseudo-acid, acetic acid acts much like HCl in the egg of *Fundulus* and the rate of diffusion of acetic acid into the egg of *Fundulus* is retarded by the presence of salt. If, however, much pseudo-acid is present, the egg is no longer protected by salts.

Fig. 6 shows the mortality curve of *Fundulus* eggs in acetic acid, HCl, $H_2$SO$_4$, and $H_2$PO$_4$, plotted over the pH as abscissa with the percentage of mortality (in 14 hours) as ordinates. It is obvious that acetic acid of the same pH is a little more toxic than HCl and this difference may be ascribed to undisassociated acid. The fact that fatty acids are more efficient physiologically than HCl of the same hydrogen ion concentration has been known for a long time. Richards$^8$ observed it as far as the minimal concentration of acid for sour taste is concerned, the writer observed it in regard to the effect of the velocity of swelling of muscle in acid,$^9$ and later in a most striking


way in his experiments on membrane formation in sea urchin eggs in which CO₂ and fatty acids were very efficient and strong mineral acids inefficient. He explained the phenomenon on the assumption of the greater diffusibility of the non-dissociated acid molecule. As soon as the non-dissociated acid molecule gets through the cell wall, it will be transformed from the non-dissociable pseudo-acid form into the dissociable-acid form, producing the acid effect, e.g., sour taste, swelling of muscle (due to formation of a dissociable pro-

![Mortality curves for different acids. Acetic acid is more toxic than HCl of the same pH, especially at lower pH where the quantity of undissociated acetic acid becomes greater.](image)

It is possible to retard the diffusion of HCl and of acetic acid of pH 3.8 by NaCl, but this inhibitory effect of NaCl is much less in the

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10 Loeb, J., Artificial parthenogenesis and fertilization, Chicago, 1913.
case of the diffusion of acetic acid than in the case of HCl. This is shown by the mortality curves in Fig. 7. Thus when HCl of pH 3.8 was dissolved in m/32 NaCl, only 20 per cent of the embryos had been killed after 12 hours, while when acetic acid of pH 3.8 was dissolved in m/32 NaCl, 40 per cent were killed. To keep all the eggs alive for 12 hours in acid of pH 3.8, m/16 NaCl was required in the case of HCl and m/4 NaCl in the case of acetic acid. This could be explained on the assumption that the NaCl prevents the diffusion of the dissociated acid in both cases but does not prevent the diffusion of the non-dissociated molecules of acetic acid to the same degree, if at all. HCl solution of pH 3.8 has less than n/8,000 titrable acid, while an acetic acid solution of pH 3.8 has about ten times as much titrable acid, nine-tenths of which is not dissociated.
To test further the idea that salts only inhibit the diffusion of dissociated but not of non-dissociated acid, experiments were made with mixtures of acetic acid of pH 5.0 and Na acetate. Acetic acid of pH 5.0 is harmless for the egg, as Fig. 6 shows. It was ascertained that \( \frac{1}{32} \) to \( \frac{1}{4} \) solutions of Na acetate were also harmless for the egg of *Fundulus* at pH 5.8. Acetic acid of pH 5.0, however, kills all the eggs rapidly if it is made up in Na acetate of \( \frac{1}{16} \) or above. The reason is that to give a mixture of \( \frac{1}{16} \) Na acetate and acetic acid a pH of 5.0, a concentration of between \( \frac{1}{64} \) and \( \frac{1}{32} \) acetic acid is required. The diffusion of non-dissociated acetic acid into the eggs killed the embryos at as low a pH as 5.0, at which strong acids like HCl or \( \text{H}_2\text{SO}_4 \) are quite harmless for the egg of *Fundulus*.

The fact that salts like NaCl can prevent the diffusion of dissociated acid but cannot prevent the diffusion of non-dissociated acid into the egg of *Fundulus*, seemed of such physiological importance as to make further proof more desirable. This was done in the experiments recorded in Fig. 8. The abscissae in this figure are the pH while the ordinates are the percentage of embryos killed by the acid in about 14 hours. Curve II in Fig. 8 shows that when acetic acid is made up in \( \frac{1}{1,024} \) LaCl\(_3\), the eggs are not killed until the pH is below 3.5; while Curve III shows that in acetic acid alone the eggs are already killed when the pH is below 4.3. This difference is considerable and shows that LaCl\(_3\) has a great retarding effect on the diffusion of dissociated acetic acid. This retarding effect of LaCl\(_3\) on acetic acid is, however, less than on HCl, since Curve I shows that LaCl\(_3\) inhibits the effect of the strong acid HCl much more effectively than it does the weak acetic acid. The difference is due to the fact that the LaCl\(_3\) retards the diffusion of dissociated acid, HCl or acetic, but not of the non-dissociated pseudo-acid, CH\(_3\)COOH. This is made certain by Curves IV and V. Curve IV shows that in a mixture of acetic acid and \( \frac{1}{16} \) Na acetate the eggs were killed at a pH of 5.1, and Curve V shows that this mixture is only a little less toxic in the presence of \( \frac{1}{1,024} \) LaCl\(_3\). The slight antagonistic effect revealed by a comparison of Curves IV and V is due to the inhibitory effect of LaCl\(_3\) on the diffusion of the dissociated acid into the egg. The experiment was repeated with \( \frac{1}{64} \) CaCl\(_2\) instead of LaCl\(_3\) and the result was the same.
By mixing acetic acid with M/16 Na acetate the equilibrium is shifted in favor of non-dissociated acetic acid, and M/64 acetic acid is required to bring a solution of M/16 Na acetate to a pH of 5.1. The high concentration of undissociated acetic acid killed the eggs and the experiments all proved that the rate of diffusion of this

non-dissociated acid cannot be inhibited at all or only to a negligible extent by the presence of salt.

We therefore see that while the proper concentrations of salts like LaCl₃, CaCl₂, and NaCl prevent the diffusion of electrolytically dissociated acid through the membranes of Fundulus, they will not prevent the diffusion of non-dissociated acetic acid—the so called pseudo-acid CH₃COOH. It is needless to say that this difference in the
action of salts on dissociated and non-dissociated (or pseudo-) acids must be of importance for the selective diffusion of acids through the kidneys.

In the following table is shown the concentration of acetic acid required to bring a m/16 solution of Na acetate to different pH. The pH was practically the same when m/1,024 LaCl₃ was in the solution.

<table>
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<th>Concentration of acetic acid</th>
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<th>m/4</th>
<th>m/8</th>
<th>m/16</th>
<th>m/32</th>
<th>m/64</th>
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<td>4.0</td>
<td>4.2</td>
<td>4.5</td>
<td>4.8</td>
<td>5.1</td>
<td>5.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>

III.

The Acceleration of the Diffusion of Alkali into the Fundulus Egg by Salts.

The writer had already published the fact that either NaOH or ethylamine kills the embryo of Fundulus more rapidly when a salt is added. It was intended to find out whether CaCl₂ and NaCl acted alike in this case. Fig. 9 gives the mortality curves for Fundulus eggs after 15 hours in n/250 NaOH in different concentrations of CaCl₂ or NaCl. It is obvious that the eggs can live in this concentration of NaOH when little or no salt is present, but that they are killed in 15 hours when the concentration of salt exceeds a certain low limit. The lowest concentration of CaCl₂ in which the eggs can live more than 15 hours at 24°C. is m/1,024, while the corresponding minimal concentration of NaCl is m/256.

Fig. 10 was intended to compare the effect of Na₂SO₄ and NaCl for a n/300 solution of NaOH. There is little difference between NaCl and Na₂SO₄, and less between NaCl and BaCl₂ than between NaCl and CaCl₂ in Fig. 9. In all cases there is an acceleration of the diffusion of alkali into the cell due to salt.

What interests us is the fact that NaCl and CaCl₂ act alike on the rate of diffusion of alkali into the egg with this difference only that CaCl₂ is more effective than NaCl in accelerating the rate of diffusion of NaOH through the membrane of the egg of Fundulus.

Fig. 9. CaCl₂ and NaCl accelerate the rate of diffusion of NaOH into the egg, CaCl₂ being more effective than NaCl.

Fig. 10. Accelerating influence of different salts on the diffusion of NaOH into the egg.
IV.

The Influence of NaCl on the Diffusion of KCl through Membranes.

While NaCl and CaCl₂ behave alike in their influence on the diffusion of acid and alkali, they differ strikingly in their influence on the diffusion of KCl through the cell wall. The writer is inclined to believe that this fact may have an effect in maintaining the equilibrium of KCl inside the cells. Cells are rich in KCl and poor in NaCl, while the reverse is true for the blood serum and the tissue fluids. In 1911 Wasteneys and the writer made the observation that Fundulus could live indefinitely in a low concentration of KCl, while when a slight amount of NaCl was added the fish died rapidly; when more NaCl was added the fish again lived indefinitely. A slight amount of NaCl therefore accelerated the diffusion of KCl into the egg while a higher concentration of NaCl had the opposite effect, retarding the rate of diffusion of KCl into the fish.¹³

Similar results were observed by Cattell and the writer on the egg of Fundulus.¹⁴ A concentration of KCl which alone was not able to cause the heart to stop beating was rendered efficient when a little NaCl was added, while with the addition of more NaCl the diffusion of KCl into the egg was blocked again.

When embryos whose hearts had been caused to stop beating in a KCl solution were put into a NaCl solution (or solutions of certain other salts), the heart beat was resumed again after some time; but when the eggs poisoned by KCl were put into distilled water or a solution of a non-electrolyte, such as cane-sugar or glucose or glycerol or urea, the hearts would not recover. These embryos have the peculiarity that they can survive for as long as a week or more without heart beat. It was found that such eggs which had failed to recover from the KCl in distilled water or sugar solutions for several days would recover promptly when put into the solution of the proper salts. All these experiments proved that the diffusion of KCl through the membrane of the egg was only possible when the egg was in a solution of a sodium salt or when the membrane contained some such

¹³ Loeb J., and Wasteneys, H., Biochem. Z., 1911, xxxi, 450; xxxii, 155.
salts. This salt effect required for the diffusion of KCl through the membrane increased for sodium salts with the valency of the anion. The reader is referred for further details to the previous publications. In these older experiments one question was left undecided; namely, whether or not CaCl₂ acts like NaCl on the rate of diffusion of KCl through the membrane of the egg. In order to obtain a definite answer a special method must be used. Eggs of Fundulus in which the circulation was developed were taken from sea water and put for 48 hours into a 1/2 KCl solution of pH about 5.8 at a temperature of about 24°C. The purpose was to fill the eggs with an excessive dose of KCl so that the recovery should not be too quick. This precaution had been neglected in the writer's previous experiments and as a consequence recoveries occurred for which the outside solution was not always responsible. It was assumed that the recovery, i.e. the resumption of the heart beat, occurred when the excess of KCl had diffused out of the egg. The eggs thus poisoned with KCl were then transferred into distilled water for 2 days to remove all salts (adhering to the outer surface of the egg membrane). None of the hearts recovered in the distilled water. The eggs were then put into different concentrations of different salts all of a pH of 5.8 (i.e. the pH of the distilled water) and examined after certain intervals. Twenty eggs were always put into 50 cc. of the salt solution. Fig. 11 gives the curves for the recovery of eggs in 3 days in solutions of NaCl, CaCl₂, and LaCl₃. The abscissæ are the concentrations of the salt solutions, the ordinates the percentage of eggs that had recovered. It is obvious that recovery occurred rapidly in NaCl, while the recovery was very slow in CaCl₂ and while practically no recovery occurred in LaCl₃. It was not due to any injurious action of CaCl₂ that the eggs did not recover since it was found in control experiments that eggs which had failed to recover after 2 or 3 days in, e.g., a 1/64 solution of CaCl₂, recovered in less than a day when put into a solution of 1/64 Na₄Fe(CN)₆ or some other sodium salt.

Since in sea water and in blood NaCl is always accompanied by CaCl₂ in the proportion of approximately 100 molecules of NaCl to 1.5 molecules of CaCl₂, it seemed of importance to determine the influence of different concentrations of such mixtures of NaCl + CaCl₂ on the rate of diffusion of KCl through the membrane. These experiments
yielded the interesting results that the balanced solution of NaCl + CaCl₂ accelerated the diffusion most at a concentration of \( \frac{m}{4} \) and \( \frac{m}{2} \) while at higher concentrations the influence became less (Curve I, Fig. 12). This was not due to any injury to the egg but corresponded to the earlier results that when the concentration of NaCl exceeds a certain limit it retards the diffusion of KCl through the membrane. That the higher concentration of NaCl + CaCl₂ only retarded the rate of diffusion of KCl without injuring the egg follows from the fact that 24 hours later more eggs had recovered in these higher concentrations than had after 20 hours (Curve II, Fig. 12).

It had already been noticed in the older experiments that the Na salts caused the recovery of the eggs the more promptly the higher
the valency of the anion of the salt. Since in these experiments no attention had been given to the pH of the solution, it was necessary to repeat the experiments. In the new experiments the salt solutions always were made up for the same pH; namely, 5.8. It is obvious that the recovery is the most rapid in $\text{Na}_4\text{Fe(CN)}_6$, next in $\text{Na}_2\text{SO}_4$, and next in $\text{NaCl}$, while it is slowest in $\text{MgCl}_2$ (Fig. 13).

![Curves of recovery from KCl in NaCl + CaCl₂](image)

**Fig. 12.** Influence of concentration of NaCl + CaCl₂ (balanced solution) on recovery of eggs from KCl poisoning, showing that when the concentration of salt increases from $\text{m}/128$ to $\text{m}/2$ the rate of recovery increases, while after that the rate of recovery diminishes with further increase in concentration.

It may incidentally be stated that the recovery as a rule was a little quicker in MgCl₂ or BaCl₂ than in CaCl₂, while MnCl₂ and SrCl₂ acted very much like CaCl₂. In LiCl and NH₄Cl the recovery was decidedly slower than in NaCl (Fig. 14).

Entirely similar results as those described for the rate of diffusion of KCl were obtained in regard to the influence of these salts on the rate of diffusion of Rb through the membrane of the egg of *Fundulus*. 
Fig. 13. Influence of different salts on the rate of recovery. The rate increases for Na salts with the increase in valency of the anion. pH 5.8.

Fig. 14. Influence of NaCl, LiCl, and NH₄Cl on the rate of recovery from KCl. Influence of NaCl is greater than that of LiCl or NH₄Cl.

Curves of recovery from KCl in different salt solutions

Concentration of salt solution

Percentage of recovery

Concentration of salt solution

Percentage of recovery
THEORETICAL REMARKS.

It has been customary to explain phenomena of selective diffusion through membranes as well as differences in the relative concentration of ions between cells and blood by the assumption that the diffusion depends on the lipoids of the cells and that only substances that are soluble in these lipoids can diffuse through the cell walls. Thus K was considered lipoid-soluble, and Na and Ca were not considered lipoid-soluble. These ideas are purely speculative and the best that can be said for them is that they are not contradicted by the facts and that possibly at some future date these guesses may turn out to have been correct. The facts enumerated in this paper point, however, to a definite influence of NaCl and CaCl₂ on selective diffusion and these facts exist and are of importance regardless of the ultimate explanation of the mechanism by which salts influence diffusion through the membrane of cells.

We understand why Na and Ca cannot diffuse into the cells of our body, since such a diffusion is rendered impossible by both the CaCl₂ and the NaCl present in blood, which seem to render the membrane of most kinds of cells impermeable for Na as well as Ca. We see, on the other hand, that the NaCl must favor the diffusion of KCl into the membrane, and this favorable effect is not counteracted by the Ca, as Fig. 12 shows. If it could be shown that NaCl + CaCl₂ favor also the rate of diffusion of Cl ions through the membrane, it would follow that KCl must accumulate in the cells until the molar concentration of Cl ions in the cells equals the Cl ions in the blood or tissue fluid. While this may be the case, the writer does not wish it understood that he considers this influence of NaCl upon the diffusion of KCl through the membrane as the sole cause of the unequal distribution of K and Na in cells and blood. That such a suggestion would be premature is indicated by the work of Loeb, Atchley, and Palmer. It is certain, however, that the effect of NaCl + CaCl₂ upon the rate of diffusion of KCl through the membrane must influence the ease with which this ionic equilibrium between blood and cells is maintained.

The fact that salts accelerate the rate of dissociated alkalies and retard the rate of diffusion of dissociated acids, while they have no retarding influence on the rate of diffusion of non-dissociated acid (and, perhaps, also non-dissociated alkali) has probably some bearing on secretions and certainly an important bearing on the use of drugs and disinfectants.\textsuperscript{14} It is also possible that the lack of inhibitory action of salts on the rate of diffusion of non-dissociated acids may ultimately furnish a clue for the understanding of the mechanism by which salts modify the rate of diffusion of ions through the membrane.

Finally the writer calls attention to the fact that salts accelerate the rate of diffusion of acids through collodion membranes\textsuperscript{17} while they have the opposite effect on the rate of diffusion of acids through the membranes of \textit{Fundulus}. This must be due to the fact that the \textit{Fundulus} membrane or the membranes of cells in general differ from collodion membranes in a chemical or physical way.

It may also be stated that acid diffused more rapidly into eggs which had been killed by keeping them for 5 minutes at a temperature of 80°C., but that the rate of diffusion of acid into such eggs is also retarded by salts.

\textbf{SUMMARY AND CONCLUSIONS.}

1. It is shown that NaCl acts like CaCl\textsubscript{2} or LaCl\textsubscript{3} in preventing the diffusion of strong acids through the membrane of the egg of \textit{Fundulus} with this difference only that a \textmu{}/8 solution of NaCl acts like a \textmu{}/1,000 solution of CaCl\textsubscript{2} and like a \textmu{}/30,000 solution of LaCl\textsubscript{3}.

2. It is shown that these salts inhibit the diffusion of non-dissociated weak acid through the membrane of the \textit{Fundulus} egg but slightly if at all.

3. Both NaCl and CaCl\textsubscript{2} accelerate the diffusion of dissociated strong alkali through the egg membrane of \textit{Fundulus} and CaCl\textsubscript{2} is more efficient in this respect than NaCl.

4. It is shown that in moderate concentrations NaCl accelerates the rate of diffusion of KCl through the membrane of the egg of \textit{Fundulus} while CaCl\textsubscript{2} does not.

\textsuperscript{16} Michaelis, L., and Dernby, K. G., \textit{Z. Immunitätsforsch., Orig.}, 1922, xxxiv, 194.

\textsuperscript{17} Loeb, J., \textit{J. Gen. Physiol.}, 1922-23, v, 255.