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The Diffusion of Electrolytes Through the Membranes of Living Cells. V. The Additive Effect of Salt and Base and the Antagonistic Effect of Salt and Acid

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THE DIFFUSION OF ELECTROLYTES THROUGH THE MEMBRANES OF LIVING CELLS.

V. THE ADDITIVE EFFECT OF SALT AND BASE AND THE ANTAGONISTIC EFFECT OF SALT AND ACID.

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I. *Introductory Remarks.*

In former papers[1] it has been shown that the washed membranes of the egg of *Fundulus* are impermeable for potassium salts, that the addition of a moderate amount of a second salt accelerates the diffusion, and that an addition of a greater quantity of a second salt inhibits the diffusion of potassium salts into the egg. When alone in solution potassium salt cannot diffuse through the membrane of washed eggs until the potassium salt itself has supplied the "salt effect." It was pointed out that these effects of salt on diffusion were analogous to the effects of salts on globulins, which are insoluble in pure water, soluble in a moderate concentration, and insoluble again in a very high concentration of salt.[2] This would make it appear as if the diffusion of potassium salts depended on the solution of a certain membrane constituent, with qualities resembling those of a globulin. Potassium was used in these experiments since it causes cessation of the heart beat of the embryo as soon as a certain quantity has diffused into the egg. The cessation of the heart beat was, therefore, a convenient indicator for the diffusion of a certain quantity of potassium into the egg.

It seemed desirable to test whether the analogy between the behavior of globulins and the diffusion of electrolytes through

[1] Loeb, J., *J. Biol. Chem.*, 1916, xxvii, 339, 353, 363; 1916-17, xxviii, 175.

[2] Loeb, *J. Biol. Chem.*, 1917, xxxi, 343.

the membrane could be carried further, and for this purpose the effect of neutral salts on the diffusion of acids and alkali was examined. Hardy states that there exists an antagonism between the solvent actions of salts and acids while the solvent actions of salt and base are additive. From this difference Hardy has drawn interesting inferences on the manner of combination between globulins, salts, acids, and alkali.

"There is however one feature of fundamental importance which is never obscured, and that is the antagonism between the solvent actions of salts and acids, and the additive nature of the combined solvent action of salts and alkali. This feature arises I believe from the fact that acid globulin is insoluble by salt. Salts will combine with globulin or with alkali globulin to form soluble compounds; they will not so combine with acid globulin.

If this be true, then, when acid globulin is precipitated and redissolved by salt, the acid must be displaced and the globulin redissolved, not as salt-acid globulin but as salt globulin. The displacement of the acid can readily be followed by methyl orange. When HCl globulin is precipitated by a salt this indicator shows that free acid is liberated. But I failed completely to detect the liberation of free alkali when salt is added to a solution of alkali globulin. This suggests that precipitation of acid globulin is partly a definite chemical replacement of acid by salt,

$$GHS + BS = GBS + HS.$$

The fact that alkalies slightly assist solution in salts while acids very generally depress it suggests an interesting possibility, namely, that in the compounds GHS and GBS the acid HS and the salt BS are united to the molecule of G in the same way, so that they compete with one another, while in GB and GHS the base and salt are united to different parts of the molecule."[3]

It was, therefore, expected that if the analogy between the action of electrolytes upon the solution of globulin on the one hand, and the diffusion through the membrane on the other, would hold, that the addition of a salt should increase the rate of diffusion of alkali into the egg and diminish the rate of the diffusion of acid into the egg. This should show itself in this way, that a neutral salt would increase the toxicity of a base and diminish the toxicity of an acid. This is actually the case, as will be shown in this paper. It was necessary to make sure that the salts used were neutral or at least not alkaline, since

[3] Hardy, W. B., *J. Physiol.*, 1905–06, xxxiii, 323–24.

if they were alkaline the additions of salt to acid would make the acid weaker and when added to alkali increase the alkalinity. This would indeed result in an apparent antagonism of salt to acid and an apparent additive effect of salt to base. Hence to prove a real antagonism between acid and salt it was necessary to make sure that the salt solutions were not alkaline. Tests were made with phenolphthalein, neutral red, and congo red. The neutral red tests showed that the salt solutions used (with the exception, of course, of sodium citrate) were all slightly on the acid side of neutrality (all being as red as or more than distilled water). A trace of alkali sufficed to bring them to neutrality. Thus, one drop (0.05 cc.) of N/100 NaOH sufficed to turn 50 cc. of M/4 NaCl or M/32 $CaCl_2$ or M/32 $SrCl_2$ from red to yellow (with neutral red as an indicator). Na_2SO_4 was more decidedly acid, 0.5 cc. N/100 NaOH being required to turn 50 cc. M/32 Na_2SO_4 yellow. We are, therefore, dealing in the following experiments with an effect of the neutral salt.

The addition of a neutral salt to an acid with different anion has no effect on the hydrogen ion concentration. Dr. Haas was kind enough to compare with the aid of his indicators the H ion concentration of M/500 acetic acid made up in H_2O and M/32 Na_2SO_4, and found it identical, namely 3.6×10^{-5}.

II. The Antagonistic Character of the Combined Action of Salts and Acid.

Loeb and Wasteneys[4] have published two papers showing that the adult fish of *Fundulus* can resist acid much better if some neutral salt (NaCl or $CaCl_2$) is added to the acid. Acid killed these fish by altering the surface of their gills whereby respiration became impossible. The effect of the acid on the external surface of the fish was directly visible inasmuch as the outer lining of the body became white and pealed off. The presence of an adequate amount of salt prevented this effect of the acid when the latter was not too concentrated.

2 years ago the writer published similar experiments on the effect of neutral salt on the rate of diffusion of acid into the egg

[4] Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1911, xxxiii, 489; 1912, xxxix, 167.

of *Fundulus*.[5] The acid had to diffuse through the membrane in order to reach the embryo. As soon as the acid penetrates in sufficient quantity into the egg the embryo begins to coagulate and become white, the thinnest part, the tail, showing this effect earlier than the rest. The heart is protected by the pericardium and therefore the heart beats may still be going on at a time when the tail is already coagulated by the acid. As soon as the heart stops beating recovery is no longer possible, since by this time the whole body is coagulated and for this reason the cessation of the heart beat could in this case be used as the criterion for death.

It was found that neutral salts inhibit the rate of diffusion of acid through the membrane. This could be proved by the fact that rhodanates and tartrates prevented the toxic action of acid upon the embryo *inside* the egg, while these salts had no such action upon the fish when outside the egg, the salts themselves being too toxic to be of any use for the fish itself. They were, however, of use in acting upon the external surface of the membrane of the egg, thereby retarding the diffusion of acid through the membrane.

The inhibiting action of salts on the rate of diffusion of acid through the membrane is a function of the anions as well as of the cations. Organic sodium salts antagonize acid better than the inorganic ones and the bivalent anions better than the monovalent ones. Likewise Ca and Sr antagonize better than the univalent cations and also better than Mg and Ba.

The relative efficiency of cations in antagonizing acid is shown in the following table, giving the *minimal* concentration of different neutral salts which permits 50 per cent of the embryos to survive in M/500 acetic acid after 18 hours.

LiCl	Die in all concentrations.
NaCl	" " " "
RbCl	" " " "
CsCl	" " " "
$MgCl_2$	" " " "
$CaCl_2$	M/2048
$SrCl_2$	M/512
$BaCl_2$	M/32

[5] Loeb, *J. Biol. Chem.*, 1915, xxiii, 139.

The superior antagonistic effect of the bivalent over the univalent cations is beyond doubt, as is also the difference between Ca and Sr on the one hand and Mg and Ba on the other.

As far as the anions are concerned, the difference in the antagonistic action between Cl and SO_4 is marked and independent of the nature of the cation. Table I gives the percentage of surviving embryos in M/500 acetic acid in M/8 chlorides and sulfates of different cations.

TABLE I.

After.	Percentage of surviving embryos in M/500 acetic acid made up in chlorides and sulfates of different cations.		
	Cation.	Cl	SO
hrs.			
24	Li	0	80
24	Na	0	100
8	NH_4	0	80
24	Mg	0	100

The sulfates are so much more powerful in their antagonism to acetic acid than the chlorides that at the time when the embryos had all been killed in the chloride solution they were still all active and alive in the sulfate solution.

In order to get a quantitative expression of the relative efficiency of univalent and bivalent anions the embryos were put into M/500 acetic acid solution to which various concentrations of different sodium salts were added. That concentration of these salts was ascertained which allowed 50 per cent of the eggs to be alive after 10 hours. This concentration was found to be for

NaCl..M/4
NaBr..M/4
$NaNO_3$..Slightly > M/4
Na_2SO_4..M/32

When we add increasing quantities of salt to an acid very soon a concentration is reached where the further addition of salt accelerates the action of the acid; this action takes place at a concentration of the salt where the salt alone is not yet very harmful.

TABLE II.

	Percentage of surviving embryos after 6 hrs. in M/500 acetic acid made up in													
	M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/4096	H_2O
NaCl	0	40	100	75	35	5	5	0	5	0	0	0	5	0
NaBr	50	90	95	50	10	0	0	15	5	0	10	5	0	0
$NaNO_3$	15	95	95	40	20	15	5	0	0	10	0	0	5	0
Na_2SO_4		90	100	100	100	100	80	80	10	5	5	10	10	10

TABLE III.

	Percentage of surviving embryos after 10 hrs. in M/500 acetic acid made up in												
	M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	H_2O
NaCl	0	10	60	30	0	0	0	0	0	0	0	0	0
NaBr	15	85	70	0	0	0	0	0	0	0	0	0	0
$NaNO_3$	0	80	40	5	0	0	0	0	0	0	0	0	0
Na_2SO_4		50	100	100	100	90	10	0	0	0	0	0	0

Especially after 10 hours it is clear that the antagonistic effect of M/1 solutions of NaCl, NaBr, and $NaNO_3$ is less than that of M/2 or M/4 solutions. When the salt concentration exceeds a certain limit its antagonistic action to acid diminishes again.

In a previous paper it was pointed out that when eggs are treated with a low concentration of salt and are *afterwards* exposed to acid the diffusion of acid may be accelerated. Such an effect was never observed when acid and salt were simultaneously in solution no matter how low the concentration of the salt.

III. The Additive Character of the Combined Action of Alkali and Salt.

In working with alkali we have to remember that the CO_2 of the air as well as that produced by the living organism steadily diminishes the concentration of the alkali. This makes it necessary to carry on these experiments in small closed Erlenmeyer flasks in which the volume of solution is large compared with the air space left and the volume of the eggs (20 in each flask). In addition it is necessary to restrict the experiment to short periods. At given intervals the eggs were taken out and examined and the percentage of embryos whose heart was still beating was determined. It was found that the salt always increased the efficiency of the alkali and in no case antagonized it. The following experiments with M/200 NaOH and M/200 tetraethylammoniumhydroxide may serve as an example. The solutions were made up in mixtures of NaCl, KCl, and $CaCl_2$ in the proportion in which these three salts exist in the sea water; namely, 100 molecules of NaCl to 2.2 molecules of KCl to 1.75 molecules of $CaCl_2$ (Table IV).

TABLE IV.

Solution.	Percentage of eggs surviving after 5 hrs. in various alkali solutions made up in NaCl + KCl + $CaCl_2$.										
	M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	H_2O
M/200 NaOH	0	0	0	10	30	45	80	100	100	100	100
M/200 tetraethylammoniumhydroxide	0	0	0	0	0	10	10	45	90	100	100

Even as low a salt concentration as M/32 has an accelerating effect upon the toxic action of alkali. It may be well to point out that these eggs can live for days in neutral solutions of NaCl + KCl + $CaCl_2$ as concentrated as 2.5 M.

In order to ascertain whether at least a trace of an antagonistic effect of salts upon alkali can be discovered, higher concentrations of the latter were used. The writer did not observe any indication of an antagonistic effect between a strong base and salt while the additive effect was always marked.

The next task consisted in ascertaining the influence of valency and nature of anions and cations of the salt on the additive effect. It was found that the same ions which had the greater antagonistic effects upon acid had the greater additive effect upon bases; *i.e.*, it required less of sulfate than of a chloride to accelerate the toxic effect of a base and less Ca than Na. In order to test this M/400 solutions of ethylamine were used. In a pure M/400 ethylamine solution the embryos live at least for a number of days. If we add, however, a neutral salt the same concentration becomes extremely toxic. If different sodium salts are added the toxicity of the solution increases with the valency of the anion, approximately in harmony with Hardy's exponential law. Table V gives the percentage of surviving embryos in M/400 ethylamine when made up in solutions of NaCl, Na_2SO_4, Na_2 tartarte, Na_3 citrate, $CaCl_2$, and $SrCl_2$ after about 22 hours.

TABLE V.

Solution.	Percentage of surviving embryos after 22 hrs. in M/400 ethylamine made up in various concentrations of salt solutions.										
	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/4096	M/8192	H_2O
NaCl	30	50	75	70	75	70	95	90			95
Na_2SO_4	25	30	20	65	80	80	95	95			95
Na_2 tartrate	40	20	25	50	65	80	75				95
Na_3 citrate	0	0	0	10	5	30	70	80			90
$CaCl_2$	5	5	0	5	20	30	50	60	85	100	95
$SrCl_2$	25	30	30	35	55	100	85	95	85	90	95

Comparing the concentration of these salts which permits 50 per cent of the embryos to remain alive we find this to be for

NaCl	M/32
Na_2SO_4	M/128
Na_2 tartrate	M/128
Na_3 citrate	M/1024

The ratio of efficiency of monovalent to bivalent to trivalent anion is, therefore, as 1:4:32, while Hardy's rule demands in this case 1:4:16. The exception in citrate is probably due to the fact that citrate is in itself alkaline. In experiments of shorter duration the ratio between tartrate, sulfate, and citrate was M/128: M/128: M/512, which shows citrate to be four times as efficient as sulfate. In the writer's previous papers it was pointed out that Hardy's valency rule holds also for the influence of salts on the rate of diffusion of KCl into the egg.

As far as the cations are concerned, it is obvious that the addition of a slight amount of $CaCl_2$ accelerates the action of the base considerably more than NaCl, M/1024 $CaCl_2$ being as efficient as M/32 NaCl. $SrCl_2$ is slightly less efficient than $CaCl_2$.

It is of interest that the addition of salts to NH_4OH gave no clear results; there was neither a clear additive effect nor an antagonistic effect between salt and NH_4OH.

IV. Proof That the Salt Action Described in This Paper Is Due to an Influence upon the Rate of Diffusion by Alkali and Acid Through the Membrane.

As stated in previous papers the experiments on the *Fundulus* egg have the striking advantage of demonstrating directly whether a salt action is due to an influence upon the rate of diffusion through a membrane or whether it is due to an action on the living protoplasm. The embryo lives inside the membrane of the egg from which it is separated by a watery liquid. By comparing the effect of a solute on the embryo while *in* the egg, and on the embryo *immediately after hatching,* we can make sure whether or not the effect observed on the egg is merely due to an influence upon the rate of diffusion through the membrane or to an effect upon the embryo inside the egg. When the difference in the two cases is merely one of degree a doubt might still be entertained; but when it becomes one of kind, as is the case in these experiments, all doubt vanishes.

The *Fundulus* embryo inside the egg remains alive indefinitely when the eggs are put into M/400 ethylamine but they all die in less than 24 hours when the M/400 ethylamine is made up in M/64 $CaCl_2$ or in some other salt. The salt accelerates death. When we make the same experiment on the newly hatched embryo we find that the latter dies in about 20 minutes in M/400 ethylamine and the addition of $CaCl_2$ does not accelerate the action but retards it markedly, the embryo living from 40 to 60 minutes in M/400 ethylamine made up in M/32 $CaCl_2$. The same is true for other salts, *e.g.*, Na_2SO_4 or $SrCl_2$; instead of accelerating the action of alkali on the hatched embryo they retard it. This effect of the salt then is exactly the reverse from the effect of the salt upon the embryo while *inside* the egg. This does not permit of any other interpretation than that the combined effect of salt and alkali upon the egg is due to an action on the membrane surrounding the egg and not upon the embryo inside the egg. We must therefore conclude that the addition of a neutral salt to the alkali allows the latter to diffuse more rapidly into the egg. The embryo which is killed in 20 minutes in M/400 ethylamine, when brought directly into this solution, remains alive in this solution, as long as it is surrounded by the egg membrane, and the reason can only be that this solution never reaches the embryo as long as it is surrounded by the membrane. If, however, a salt of the proper concentration is added to the M/400 ethylamine solution the latter can diffuse into the egg. In a stronger ethylamine solution, *e.g.*, M/200, the embryos are all killed inside of 24 hours when inside the egg. In this case the higher concentration of the alkali itself supplies the "salt effect" upon the diffusion of base into the egg.

As far as the direct action of alkali on the embryo is concerned it is visibly due to the solution of the surface elements of the embryo. In a M/400 ethylamine solution the surface of the head is dissolved very quickly, leaving the eyes protruding.

We have already mentioned the fact that tartrates and rhodanates can be used to antagonize the action of acid on the egg, while they cannot be used to counteract the injurious effect of acids on the fish when outside the egg.[5] This shows that the antagonistic action of salt to acid, as long as the embryo is inside the egg, is due to an effect on the membrane of the egg and not on the embryo itself.

V. Theoretical Remarks.

We have pointed out in this and in a previous paper the analogy which exists between the conditions for the diffusion of electrolytes through the membrane of the *Fundulus* egg and the condition for the solution of globulins. It seems on the whole that those conditions which tend to make globulins soluble also permit or accelerate the diffusion of certain electrolytes through the membrane of the egg of *Fundulus;* while those which render globulins less soluble also inhibit or retard the diffusion of certain electrolytes. Thus it was shown in a former paper that potassium salts diffuse through the membrane of *Fundulus* when the membrane has had a chance to combine with a certain moderate quantity of salt; while either a membrane free from salt or one having an excess of salt is more or less impermeable for the same potassium salt. This corresponds to the fact that globulins are soluble in a moderate amount of salt but insoluble in distilled water or in an excess of salt. It was also pointed out that the valency rule found by Hardy for the precipitation of globulins holds also for the action of salt on the diffusion of potassium through the membrane of the *Fundulus* egg. It was pointed out, however, that there exists merely an analogy and not an identity in the diffusion of salts through the membrane and the solubility of globulins; since the ratio for the solvent and precipitating effect of a neutral salt on globulins differs widely from the ratio for the accelerating and inhibiting effect of the same salt for the diffusion through the membrane.

In this paper the analogy is carried further. Salts and acids have an antagonistic and salt and bases an additive effect upon the solution of globulins. It could be shown that the relation holds for the diffusion of acid and bases through the membrane. Salts accelerate the diffusion of bases and retard the diffusion of acid through the membrane. The analogy apparently breaks down again through the fact that the increasing valency of both cations and anions of salts increases the antagonistic action to the diffusion of acid and increases the accelerating action on the diffusion of alkali.

Salts, therefore, increase the rate of diffusion of certain electrolytes through the membrane of the egg of *Fundulus* when they are also able to dissolve globulins; and they retard or prevent the diffusion when they are likely to prevent the solution of globulins. The facts, however, do not permit us to say that the substance in the membrane upon which the diffusion of electrolytes depends *is* a globulin. A difference in the action of salts on acids and bases was also observed by the writer in his old experiments on the swelling of muscle, where he found an antagonistic action of neutral salts to acid but not to base.[6]

Some authors assume that the proof of an increase in permeability of a membrane to *one* substance indicates an increase of permeability to *all* substances. The fact that salts increase

[6] Loeb, *Arch. ges. Physiol.*, 1899, lxxv, 303.

the permeability of the *Fundulus* membrane to alkali while they have the opposite effect on the permeability to acids should warn us against such generalizations.

SUMMARY OF RESULTS.

1. When *Fundulus* eggs are put into solutions of bases not sufficiently concentrated to injure the embryo the bases become injurious when neutral salts in low concentration are added. This additive effect between salts and bases was observed also at higher concentrations of the base.

2. When *Fundulus* eggs are put into an acid which kills the embryo rapidly the addition of a neutral salt has an antagonistic effect. The same antagonism between acid and salt was observed also for lower concentrations of acids.

3. The minimal quantity of a salt required for these effects diminishes with an increase in the valency of both anion and cation of the salt. In the case of the anion the quantity diminishes according to Hardy's valency rule. In addition the chemical nature of the salt is of importance.

4. It can be shown that the additive effects of salt to base and the antagonistic effect of salt to acid are due in the case of the egg of *Fundulus* to an influence of the salt upon the rate of diffusion of alkali and acid through the membrane of the egg; this influence being accelerating for the diffusion of alkali and retarding for the diffusion of acid.

5. The partial similarity of the action of neutral salts in these cases with the action of salt on the solution of globulins in the presence of acid and alkali is pointed out.