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THE MECHANISM OF DIFFUSION OF ELECTROLYTES  
THROUGH ANIMAL MEMBRANES

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1. In a former paper Loeb and Cattell<sup>1</sup> have shown that embryos of the marine fish *Fundulus* poisoned in a KCl solution until all the hearts stop beating, cannot recover their heart beat when put into H<sub>2</sub>O or into a solution of cane sugar, although they will live in such a medium indefinitely; but that they recover in less than a day when put into a salt solution. Eggs which after being poisoned with KCl were kept in H<sub>2</sub>O for twelve days without recovering, recovered when put into the solution of an electrolyte.

Conversely it could be shown that when eggs of *Fundulus* are kept for twenty-four hours or longer in distilled water they are poisoned by a solution of potassium salts much more slowly than when the eggs are put into KCl solutions of the same concentration directly from sea water. The washing in distilled water frees the surface layer of the egg membrane from salts and this retards the diffusion of KCl into the egg.

These facts suggested to me the idea that it was impossible for KCl to diffuse through a membrane free from salts and that for such a diffusion two conditions are required: first, a sufficiently high osmotic pressure of the KCl solution, and second, a certain modification of the membrane by an electrolyte, which we will call the 'salt effect.' When the concentration of the KCl solution was sufficiently high, e.g.,  $3/8m$  or  $m/2$ , the KCl itself might be able to supply this 'salt effect' upon the membranes of washed eggs; but when the solution of KCl was  $m/8$  or below, the solution might not be sufficiently concentrated to supply the 'salt effect' required to allow the diffusion of KCl. This idea was found to be correct.

An  $m/2$  KCl solution caused cessation of the heartbeat of unwashed eggs in less than two hours, and of the washed eggs in about nine hours. An  $m/8$  KCl solution required six days to cause the cessation of the heartbeat in 70% of the eggs previously washed for twenty-four hours in distilled water, while the same solution had the same effect upon unwashed eggs in less than five hours. This difference was due, according to the theory mentioned above, to the presence of the salts of the sea water in the superficial layers of the membrane of the egg. On this assumption it was to be expected that by adding enough sea water to an  $m/8$  KCl solution to bring this solution to a total salt concentration of  $3/8m$  or  $m/2$  the washed eggs should be poisoned much more rapidly. This was found to be the case. While 70% of 'washed' eggs were poisoned by  $m/8$  KCl in  $H_2O$  in about six days, the same effect was usually produced in less than one day when the  $m/8$  KCl solution was prepared in  $m/4$  sea water. The same effect was produced by the addition of any salt of Li, Na, or  $NH_4$ , and others, to the  $m/8$  KCl solution instead of sea water. Since it might be argued that the addition of a single salt like NaCl might act by merely injuring the membrane and thus increasing its permeability, emphasis should be laid on the fact that these experiments succeeded as well when the 'salt effect' was supplied by balanced solutions like sea water or  $NaCl + CaCl_2$  as by non-balanced solutions.

When any of these salts were added to the  $m/8$  KCl solution in a slightly higher concentration than the moderate concentration required to produce the 'salt effect' upon the membrane, the opposite result was noticed: the diffusion of KCl into the egg was again retarded or rendered impossible (antagonistic salt action). Thus the hearts of *Fundulus* embryos were still practically all beating in  $m/8$  KCl made up in  $m/1$  ( $NaCl + CaCl_2 + MgCl_2$ ) after eighteen days (when the experiment was discontinued), while they had all stopped beating in less than three days and more than 50% in less than one day in  $m/8$  KCl made up in  $m/8$  ( $NaCl + CaCl_2 + MgCl_2$ ). This disposes of the possibility that the general 'salt effect' consists in an increase of the 'permeability' of the membrane through injury by the salt.

The most important fact is that only salts act in this way, namely to accelerate the diffusion of KCl through the membrane when added in moderate concentrations and to retard it again when added in higher concentration. Non-electrolytes, like sugars, glycerin, ethyl or methyl alcohol, and urea, do not show this behavior.

2. The difference in the behavior of electrolytes and non-electrolytes, and the influence of the chemical nature of the salt in this general 'salt

effect' upon the membrane can best be studied in experiments on the recovery of eggs previously poisoned with KCl. Eggs were put directly from sea water into m/2 KCl for twelve hours. These eggs must have contained an excess of KCl, since in this solution all the hearts of unwashed eggs stop beating in less than two hours. The heartbeat was resumed when enough KCl had left the eggs after they had been transferred into the proper solutions. The resumption of the heartbeat became, therefore, an indicator for the rapidity of diffusion of the KCl from the heart into the membrane or the surrounding solution. Since the concentration of KCl was approximately equal in each egg at the beginning of the experiment, the differences in time required for recovery were due to the influence of the outside solution upon the rapidity of the diffusion of KCl from the egg into the membrane. Table I shows the percentage of hearts which recovered from the effects of KCl in various salts of Na, NH<sub>4</sub>, and Mg in twenty-four hours. It is obvious that beginning with a limiting lower concentration all the hearts were beating again at that time in all the solutions, although most of the solutions are so poisonous that they would have killed the embryo in a short time had they been able to diffuse in more than traces into the egg. They acted by bringing about a certain modification of the membrane rendering possible the diffusion of the potassium salts.

TABLE I

	PERCENTAGE OF HEARTS RECOVERING FROM POTASSIUM STANDSTILL IN TWENTY-FOURS IN													H <sub>2</sub> O
	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	
Sea water.....		100	100	91	60	50	26	15	45	15	10	25	35	5
NaCl + CaCl <sub>2</sub> .....	100	95	100	90	55	55	25	25	5	5				10
NaCl.....	100	100	100	100	100	90	40	25	15	10				5
NaBr.....	100	100	100	100	90	75	66	50	35	30	40			5
NaNO <sub>3</sub> .....				100	100	70	35	15	10	15	10	0	10	5
Na <sub>2</sub> acetate.....		100	100	100	100	100	85	35	15	0	5	5	0	5
Na <sub>2</sub> SO <sub>4</sub> .....		100	100	100	100	90	93	90	81	38	14	5		
NaHCO <sub>3</sub> .....				100	100	100	100	75	35	10	5			
Na <sub>2</sub> CO <sub>3</sub> .....									35	30	20	20	5	5
Na <sub>2</sub> HPO <sub>4</sub> .....					95	100	90	55	50	25	10	14	5	0
Na <sub>3</sub> citrate.....									95	60	80	30	20	
NH <sub>4</sub> Cl.....		38	72	89	83	77	41	16	15	15				11
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....			45	75	70	75	65	65	50	31	15	10	30	10
(NH <sub>4</sub> ) <sub>3</sub> citrate.....									80	68	55	25	14	
MgCl <sub>2</sub> .....			100	85	95	75	55	25	25	25	15	10	0	5
MgSO <sub>4</sub> .....		100	95	70	95	90	80	45	70	30	10	10	15	

The limiting lower concentration to bring about the recovery of 70% or more eggs in twenty-four hours depends in a definite way upon the nature of the anion of all these salts, being for Cl: SO<sub>4</sub>: citrate in the ratio of approximately 1: 4: 16, as stated in a previous publication.<sup>1</sup>

In time recovery took also place in the solutions below the limiting concentration. In the recovery experiments no antagonistic salt action is noticeable, probably on account of the fact that the decisive boundary is the inner surface of the membrane which receives only traces of the salt from the outside solution, enough to accelerate the diffusion of KCl but not enough to inhibit this process.

Table II shows the behavior of the eggs in twenty-four hours when put into solutions of non-electrolytes. The characteristic influence of increasing concentrations of the solution upon recovery which is so striking in Table I is entirely lacking in this case, m/2 solutions of non-electrolytes acting no better than weaker ones. What little recovery takes place in the solutions of non-electrolytes is 'accidental' and where the figures for recovery are 20% or more, it is probably due to the secondary formation of some acid or alkali through microorganisms or in the embryo. The main fact is that the number of hearts which recovered did *not* increase when the eggs were left for a number of days in the solution of non-electrolytes. When the eggs were transferred from the solution of non-electrolytes to a solution of the proper electrolyte the 'salt effect' and hence the recovery took place.

TABLE II

	PERCENTAGE OF HEARTS WHICH RECOVERED FROM KCl POISONING IN											
	m/1	m/2	m/4	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	H <sub>2</sub> O
Glucose.....	0	15	10	5	0	0	0	5	0	0	0	15
Cane sugar.....	14	10	14	20	14	15	15	37	21	32	10	5
Glycerin.....		20	20	15	11	11	0	15	5	15		10
Methyl alcohol.....	20	45	25	15	20	10	10	10	20	10	15	10
Ethyl alcohol.....		5	25	25	35	0	15	5	5	15		10
Urea.....	5	40	15	10	5	30	10	25	10	20	15	10

Table III illustrates the effect of the cation suggesting an influence of the periodic law. Thus recovery occurs neither in RbCl and CsCl nor in the salts of the alkali earth metals occupying the corresponding position in the next group of the periodic series; namely, Sr and Ba. These cations act by preventing the diffusion of the KCl from the egg for if we transfer the eggs from such a solution to one with an efficient

cation, like Na, they will recover. In experiments on the diffusion of KCl into the egg these salts acted somewhat differently, Mg and especially Ca inhibiting the diffusion.

TABLE III  
INFLUENCE OF CATION UPON THE RECOVERY

	PERCENTAGE OF EMBRYOS POISONED BY KCl RECOVERED AFTER TWENTY-FOUR HOURS IN													
	m/1	m/2	m/4	m/8	m/16	m/32	m/64	m/128	m/250	m/512	m/1024	m/2058	m/4096	H <sub>2</sub> O
LiCl.....		30	45	80	60	80	60	30	5	0	10			5
NaCl.....	90	100	100	100	100	90	40	25	15	10				0
RbCl.....		0	0	0	5	5	0	1	0	2	0			0
CsCl.....		5	5	0	0	5	5	0	0	0	0			0
MgCl <sub>2</sub> .....			100	85	95	75	55	25	25	25	15	10		5
CaCl <sub>2</sub> .....				100	100	75	45	20	20					
SrCl <sub>2</sub> .....				30	15	30	15	20	0	5	25	0	0	10
BaCl <sub>2</sub> .....			5	0	5	10	15	25	10	20	35	10	20	0
NH <sub>4</sub> Cl.....		38	72	89	83	77	41	16	15	15				11
N(C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> Cl.....					100	78	60	23	20	14	5	14	12	10

3. If we wish to formulate a theory of the general 'salt effect' upon the membrane required to make the diffusion of potassium salts possible, we must remember that the conditions for the diffusion of acid into the egg resemble those described for KCl.<sup>2</sup> We know that acids form salts with proteins, which dissociate rather completely into protein ion and the anion of the acid used; while ordinarily the protein molecule (being usually a very weak base and acid) does not dissociate electrolytically to any extent. We can understand the phenomena described in this paper if we assume that the ionization of the proteins of the membrane is the prerequisite for the diffusion of KCl as well as of acid through the membrane. When this ionization of proteins of the membrane is to be brought about by neutral salts, as in the case of the diffusion of KCl, a much higher concentration of the salts is required than when the ionization is caused by acid or base, and the limiting concentration varies with the constitution of the salts. This ionizing effect need not be produced entirely by KCl but, for the 'salt effect,' the KCl can be partly replaced by other neutral salts. When more than a moderate amount of salt is added the ionization of the proteins of the membrane is diminished instead of increased, and the diffusion of KCl may again become as impossible as if a non-electrolyte had been added. A fuller

discussion of the theory will be given in the complete paper. Those cases in which the possibility of diffusion through a membrane was diminished by the addition of too much salt or by a salt with bivalent cation have thus far been singled out for discussion under the heading of antagonistic salt action, but the proof furnished in this paper that the absence of electrolytes acts in a similar but more complete way shows that the case of antagonistic salt action is only a part of the more general problem of the role of electrolytes (or of the ionization of the protein molecules of the membrane) in the diffusion of salts through animal membranes.

*Summary and Conclusions.*—Our experiments show that for the diffusion of certain electrolytes (potassium salts and acid) through animal membranes besides the osmotic pressure of the electrolyte a second effect is required which we call the 'salt effect' upon the membrane. This consists probably in an ionization of the protein molecules of the membrane. KCl and acid cannot diffuse through a membrane free from salts. The condition necessary for the diffusion of acid and KCl is produced when a trace of acid or a moderate amount of salt is added whereby the protein molecules of the membrane become ionized ('salt effect'). The addition of more salt (whereby the dissociation of the protein molecules of the membrane is diminished and probably other changes are brought about) annihilates this condition again. The latter fact is the special case of antagonistic salt action. The influence of the nature of the salt upon the limiting concentration for the 'salt effect' is stated in the paper.

The nature of the forces by which the ionization of the protein molecules of the membrane may bring about the diffusion of acids and potassium salts (and possibly of electrolytes in general) will be discussed in the main paper.

<sup>1</sup> Loeb, J., these PROCEEDINGS, 1, 473 (1915); Loeb, J., and Cattell, McK., *J. Biol. Chem.*, 23, 41 (1915); Loeb, J., and Wasteneys, H., *Biochem. Zs.*, 31, 450 (1911); 32, 155.

<sup>2</sup> Loeb, J., *J. Biol. Chem.*, 23, 139 (1915); *Science*, 34, 653 (1911); *Biochem. Zs.*, 37, 127 (1912); Loeb, J., and Wasteneys, H., *Biochem. Zs.*, 33, 489 (1911); 39, 167 (1912).