This hypothesis explains why the increase in osmotic pressure, viscosity, and the swelling of gelatin caused by the salt does not become noticeable unless the excess of salt is washed away, since the presence of the salt represses the electrolytic dissociation of the gelatin salt formed.

Some of the data on which these conclusions are based have recently been published.[1]

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Studies on salt action. II. The effect of transfer from stronger to weaker salt solutions upon the viability of bacteria in water.

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In continuation of work previously reported, upon the viability of *B. communis* in salt solutions of various kinds,[2] we have studied the effect of transfer from salt solutions to weaker salt solutions, in order to see if phenomena could be detected analogous to those observed by Loeb[3] and others in the study of the influence of salt solutions upon the swelling of animal membranes and powdered colloids.

B. communis was grown on nutrient agar slants at 37° C. for 16–18 hours. The growth was washed off in pure redistilled water, shaken for five minutes to break up clumps and added in 1 c.c. portions to the electrolyte solutions in which the preliminary treatment was to be accomplished ("primary" solutions), which had been previously warmed to 37° C. The solutions were then shaken for one minute to give a homogeneous suspension, 1 c.c. was withdrawn and agar plates poured. The bottles containing the suspensions were then replaced in the incubator at 37° C. and kept there for 30 minutes (in one case 60 minutes). At the end of this interval plates were again made, 1 c.c. withdrawn and transferred to another bottle containing 99 c.c. of water or salt solution ("secondary" solution). These secondary solution bottles were

[1] Loeb, J., *J. Biol. Chem.*, 1917, XXXI, 343; 1918, XXXIII, 531; XXXIV, 77; 395.

[2] PROC. SOC. EXP. BIOL. AND MED., 1918, Vol. XV, p. 67.

[3] *Jour. Biol. Chem.*, Vol. XXX, p. 343.

then returned to the incubator and kept there throughout the remainder of the experiment. The number of organisms surviving was determined at stated intervals. The counts were made on standard agar after 24 hours incubation at 37° C.

All the reagents used in these experiments were of the same high purity as those which were employed in the experiments reported in our earlier communication. All the glassware had been very carefully cleaned with chromic acid oxidizing mixture, rinsed in tap water and in distilled water.

Table I shows the result of transfer from 0.1 isotonic NaCl solution to 0.001 isotonic NaCl (1 c.c. of the first solution containing the bacteria to 99 c.c. of sterile distilled water), from 0.1 isotonic NaCl to another bottle of the same solution, and from 0.1 isotonic $CaCl_2$ to another bottle of the same solution. Three independent experiments are cited in each group. In each of these three series of experiments the reduction in bacterial numbers was relatively small in 9 hours although in the case of the $CaCl_2$ the normal toxicity of this salt manifested itself to a slight degree. In each case, of course, there is a decrease in actual count in the transfer to about 1 per cent. of the original number present, but this is the result of the dilution and does not imply the death of the bacteria.

The transfer from 0.1 isotonic $CaCl_2$ to 0.001 isotonic $CaCl_2$ as shown in Table II is accompanied on the other hand by a rapid destruction of bacteria so that after 8–9 hours only a few hundred bacteria are left out of hundreds of thousands.

TABLE I.

VIABILITY OF *B. communis* TRANSFERRED FROM A STRONG TO A WEAK SODIUM SOLUTION AND FROM SODIUM AND CALCIUM SOLUTIONS TO SOLUTIONS OF THE SAME STRENGTH.

		Time after Seeding.		Time after Transfer.			
		1 Min.	30 Min.	120 Min.	240 Min.	360 Min.	540 Min.
0.1 isotonic	Bacteria	29,600,000	29,700,000	280,000	270,000	260,000	270,000
NaCl to 0.001	per	35,500,000	32,500,000	285,000	240,000	235,000	210,000
isotonic NaCl	c.c.	33,500,000	30,000,000	215,000	195,000	195,000	180,000
0.1 isotonic	Bacteria	35,500,000	32,500,000	370,000	312,000	310,000	240,000
NaCl to 0.1	per	33,500,000	30,000,000	260,000	245,000	255,000	210,000
isotonic NaCl	c.c.	33,500,000	30,000,000	255,000	230,000	260,000	230,000
0.1 isotonic	Bacteria	26,500,000	26,500,000	300,000	300,000	240,000	170,000
$CaCl_2$ to 0.1	per	29,200,000	28,300,000	200,000	170,000	70,000	40,000
isotonic $CaCl_2$	c.c.	33,500,000	26,500,000	295,000	255,000	210,000	170,000

TABLE II.

VIABILITY OF *B. communis* TRANSFERRED FROM A STRONG TO A WEAK CALCIUM SOLUTION.

		Time after Seeding.			Time after Transfer.											
		1 Min.	30 Min.	60 Min.	15 Min.	30 Min.	60 Min.	105 Min.	120 Min.	240 Min.	280 Min.	310 Min.	360 Min.	460 Min.	490 Min.	540 Min.
0.1 isotonic $CaCl_2$ to .001 isotonic $CaCl_2$	Bacteria per c.c.	86,000,000	81,000,000		500,000	480,000	65,000		30,000			650			110	
		26,500,000	26,500,000						260,000	80,000			32,500			<1,000
		63,000,000		68,000,000	540,000	520,000	360,000	150,000			1,900			150		

In Loeb's colloid studies it was a transfer from NaCl to more dilute NaCl or water which produced a marked increase in swelling. In the case of the viability of the bacteria it is a transfer from $CaCl_2$ to a very dilute solution which causes a profound effect. Whether the death of the bacteria in the latter case is due to the fact that compounds are formed in the cell wall or protoplasm which hinder the elimination of toxic waste products of cell metabolism or whether such compounds favor the ingress of water which causes deadly hydrolyses (which Phelps has suggested as the lethal factor in similar cases), or whether such compounds favor the loss of necessary constituents of the cell to the water outside, we are not prepared to say. Experiments are now being conducted to throw light upon this point. It seems clear in any case from the conditions of the experiment that the effect is not due to any direct toxicity of the salt but to some change which it produces in the rate of exchange between the inside of the cell and its environment.

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Experimental tri-nitro-toluene poisoning.

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In an attempt to produce experimentally in dogs a state of poisoning by tri-nitro-toluene analogous to the condition recently observed among munition workers in England and America, the following methods of administering the poison have been employed: (1) Feeding by mouth (TNT in butter); (2) skin inunction (TNT in lard); (3) subcutaneous injections (TNT in olive oil); (4) intravenous injections (TNT in acetone); (5) intraperitoneal injections (TNT in albolene). Only the first three methods have proven satisfactory. Intravenous injection of any considerable quantity of an acetone solution causes immediate death, probably from a precipitation of the TNT in the blood stream, and consequent pulmonary embolism. The toxic action of the acetone, too, may be a factor. Negative results with intraperitoneal injections of an albolene solution were probably due to faulty absorption.