A NOTE ON THE OSMOTIC EFFECT OF VARIOUS STRENGTHS OF DIFFERENT SALTS UPON CELL VOLUME

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Recently, in a paper by Roaf and Whitley,¹ it was pointed out that tadpoles, when placed in solutions of neutral salts, shrank markedly in size but remained active in spite of this great diminution in bulk. As the shrinkage occurred in solutions which were below the isosmotic equivalent for the blood of full grown frogs, it was surmised that the tadpoles maintain a difference of osmotic pressure between their internal fluids and the surrounding medium. Thus the normal concentration of salts is maintained whilst the organism lives in a solution, such as a fresh water pond, of much lower concentration but if it is placed in a stronger solution of salt, it shrinks until there is a fresh equilibrium established between the two and as the isosmotic strength is approached the degree of shrinkage rapidly increases.

The following experiments were performed to obtain more detailed information regarding these changes. The tadpoles were placed in the solutions in flat earthenware dishes capable of holding about 400 c.c. After being laid on blotting paper, to drain off the superfluous water of the stock tank, they were transferred to a tared watch glass and weighed, they were then put in the solution to be tested. At the end of the experiment they were treated in an exactly similar manner and any loss of weight was calculated as a percentage loss of the original weight.

In several of the experiments the weight of the dried solids was obtained, after rinsing in distilled water and drying at 120° C., then the residue was incinerated and the weight of the ash obtained. This was dissolved and titrated with silver nitrate after Mohr's method.

1. *Bio-Chemical Journal*, Vol. I, p. 88, 1906.

EXPERIMENT I

Twenty tadpoles (*Rana temporaria*) were placed in 300 c.c. of each of the following solutions and left for 48 hours.

Solution	Original Weight of Tadpoles	Final Weight of Tadpoles	Loss in Weight of Tadpoles	Percentage of Loss to Original Weigh
Distilled water	1'646 g	1'363 g	0'283 g	17'19
Tap water	1'590 g	1'314 g	0'276 g	17'36
Tap water + $\frac{M}{100}$ NaCl	1'593 g	1'362 g	0'231 g	14'49
Tap water + $\frac{M}{50}$ NaCl	1'722 g	1'613 g	0'109 g	6'33
Tap water + $\frac{M}{40}$ NaCl	1'740 g	1'659 g	0'081 g	4'59
Tap water + $\frac{M}{20}$ NaCl	1'637 g	1'558 g	0'079 g	4'83
Tap water + $\frac{M}{10}$ NaCl	1'731 g	1'561 g	0'170 g	9'82
Tap water + $\frac{M}{8}$ NaCl	1'826 g	1'318 g	0'508 g	27'82

In $\frac{M}{10}$ NaCl, one tadpole was found dead, whilst in $\frac{M}{8}$ NaCl five were found dead, and it is interesting to note the great increase in percentage loss of weight occurring in the strength which kills the tadpoles.

EXPERIMENT II

Eight tadpoles were placed in 300 c.c. of each of the following solutions and left for 24 hours.

Solution	Original Weight of Tadpoles	Final Weight of Tadpoles	Loss in Weight of Tadpoles	Percentage of Loss to Original Weight
Distilled water	2.382	1.945	0.337	14.1
Tap water	1.952	1.738	0.214	10.9
Tap water + $\frac{M}{100}$ NaCl	2.505	2.374	0.133	5.38
Tap water + $\frac{M}{20}$ NaCl	2.142	2.033	0.109	5.09
Tap water + $\frac{M}{10}$ NaCl	2.116	1.788	0.328	15.5
Tap water + $\frac{M}{8}$ NaCl	1.944	1.669	0.275	14.2 ¹
Tap water + $\frac{M}{30}$ CaCl ₂	2.137	1.734	0.403	18.9
Tap water + $\frac{M}{15}$ CaCl ₂	2.186	1.596	0.596	27.3 ²
Tap water + $\frac{M}{11.5}$ CaCl ₂	2.445	1.849	0.596	25.2 ²
Tap water + $\frac{M}{100}$ NaHCO ₃	2.106	1.839	0.267	12.7 ³
Tap water + $\frac{M}{20}$ NaHCO ₃	2.293	1.864	0.429	18.7 ²
Tap water + $\frac{M}{10}$ NaHCO ₃	1.851	1.452	0.399	21.5 ⁴
Tap water + $\frac{M}{100}$ CH ₃ COONa	1.946	1.834	0.102	5.24
Tap water + $\frac{M}{20}$ CH ₃ COONa	2.052	1.812	0.240	11.7
Tap water + $\frac{M}{10}$ CH ₃ COONa	2.197	1.887	0.310	14.1

1. One dead. 2. All dead. 3. Three dead, several others lethargic. 4. Six dead, one lethargic.

As in the preceding table it is interesting to note the great increase in the percentage loss of weight occurring in solutions which kill the tadpoles.

EXPERIMENT III

Twelve tadpoles were placed in 300 c.c. of each of the following solutions and left for *four* hours.

Solution	Original Weight of Tadpoles	Final Weight of Tadpoles	Loss in Weight of Tadpoles	Percentage of Loss to Original Weight
Distilled water	1.241	1.142	0.099	8.67
Tap water	1.480	1.387	0.093	6.29
Tap water + $\frac{M}{20}$ NaCl	1.236	1.117	0.119	9.63
Tap water + $\frac{M}{10}$ NaCl	1.175	1.013	0.162	13.79
Tap water + $\frac{M}{8}$ NaCl	1.204	1.003	0.201	16.69
Tap water + $\frac{M}{60}$ CaCl ₂	1.220	1.105	0.115	9.43
Tap water + $\frac{M}{30}$ CaCl ₂	1.048	0.938	0.110	10.51
Tap water + $\frac{M}{20}$ CaCl ₂	1.067	0.940	0.127	11.90
Tap water + $\frac{M}{100}$ NaHCO ₃	1.193	1.133	0.060	5.03
Tap water + $\frac{M}{20}$ NaHCO ₃	1.200	1.110	0.090	7.50
Tap water + $\frac{M}{10}$ NaHCO ₃	1.231	1.086	0.145	10.97
Tap water + $\frac{M}{100}$ CH ₃ COONa	1.098	1.011	0.087	7.92
Tap water + $\frac{M}{20}$ CH ₃ COONa	1.380	1.262	0.118	8.55
Tap water + $\frac{M}{10}$ CH ₃ COONa	1.233	1.059	0.174	14.11

EXPERIMENT IV

Twelve tadpoles were placed in 300 c.c. of each of the following solutions and left for *four* hours.

Solution	Original Weight of Tadpoles	Final Weight of Tadpoles	Loss in Weight of Tadpoles	Percentage of Loss to Original Weight
Distilled water	1.195	1.103	0.092	7.70
Tap water	1.166	1.115	0.051	4.37
Tap water + $\frac{M}{100}$ NaCl	1.114	1.072	0.042	3.77
Tap water + $\frac{M}{20}$ NaCl	1.051	0.992	0.059	5.61
Tap water + $\frac{M}{10}$ NaCl	1.250	1.153	0.097	7.76
Tap water + $\frac{M}{8}$ NaCl	1.193	1.056	0.137	11.48
Tap water + $\frac{M}{60}$ CaCl ₂	1.136	1.087	0.049	4.31
Tap water + $\frac{M}{30}$ CaCl ₂	1.309	1.239	0.070	5.35
Tap water + $\frac{M}{20}$ CaCl ₂	0.983	0.913	0.070	7.12
Tap water + $\frac{M}{100}$ NaHCO ₃	1.142	1.118	0.024	2.10
Tap water + $\frac{M}{20}$ NaHCO ₃	1.012	0.929	0.083	8.20
Tap water + $\frac{M}{10}$ NaHCO ₃	0.980	0.863	0.117	11.94
Tap water + $\frac{M}{100}$ CH ₃ COONa	1.090	1.062	0.028	2.57
Tap water + $\frac{M}{20}$ CH ₃ COONa	0.993	0.935	0.058	5.84
Tap water + $\frac{M}{10}$ CH ₃ COONa	1.082	0.975	0.107	9.89
Tap water (kept running)	1.168	1.116	0.052	4.45

Note to Tables.—There is a large percentage loss of weight which occurs in the tadpoles kept in tap water. This must be due to some condition of experiment, as it is inconceivable that they could continue losing weight all the time that they were kept in the laboratory. In the last experiment a second control was kept in running water to test if that would make any difference, but the loss was the same as with those kept in still water.

The results of these experiments show that there is a certain strength of solution in which the tadpoles are best suited to maintain an equilibrium between their body fluids and the surrounding medium. A decrease in the concentration of the salt causes an increased loss of weight, which reaches a maximum in distilled water. Similarly, increase in the concentration of the salt causes a progressive increase in the loss of weight which becomes more marked as the strength at which the organisms are killed is approached. The great loss in weight which occurs in distilled water is probably due to loss of salts and water from the organism by means of dialysis, aided by an effort on the part of the organism to keep its body fluids of constant concentration. The first effect would be a loss of salt by dialysis and this would be followed by excretion of water in an attempt to keep a constant ratio of salts to water in the animal's body fluids. Addition of a salt to tap water lessens the loss by dialysis and consequently less water would be excreted to keep a balance between the concentration of the fluids of the body and the surrounding medium. At a certain strength of solution the point is reached at which equilibrium is nearly attained between the two solutions but as there is only a single salt in solution the true equilibrium is not found in these experiments. If a mixture of salts were used it is conceivable that at a definite concentration the only loss of weight would be that due to the excretions of the organism during the time of the experiment.¹

Beyond this optimum strength the loss in strength increases due to loss of water by the increase of osmotic pressure in the surrounding medium. Whether any appreciable exchange of salts occurs could not be determined as the total amount of salt contained in the organism is too small to show any relative change with accuracy but there was, as a rule, a progressive increase in the chlorides in the case where chlorides were present in the external medium and a decrease of chlorides in the case of solutions of sodium bicarbonate and sodium acetate. The dried solids and the ash showed a progressive

1. Cf. Ostwald Wolfgang, *Arch. f. d. ges. Physiol.*, Vol. CVI, p. 568, 1905.

increase as the strength of solution increased, no matter what was the nature of the salt used. It is probably this change in the salts which causes death to occur in solutions of a single salt in distilled water,¹ and when single salts are increased markedly above the other salts (as in using high concentrations of salts in tap water). This latter case has been discussed in a previous paper where the relationship between the proteids and electrolytes has been pointed out.²

In view of these facts the current view, that when plasmolysis occurs the solution used is isosmotic with the cell contents, needs to be carefully revised. If the cells used are accustomed to live in fresh water it is extremely probable that they habitually maintain a higher concentration of salt than is present in their surroundings. Any increase in the osmotic strength of the surrounding medium would tend to abstract water but it is only when this pressure is sufficient to overcome the adhesion of the protoplasm to the cell wall that plasmolysis becomes visible.

It is evident from the tables that long before the isosmotic strength is reached there is a progressive loss of weight, as the strength of solution increases; hence showing that it is not equality of pressure but a difference which is maintained by the cell.³

In conclusion, I wish to thank Professor Benjamin Moore for his assistance and advice during the course of this research.

1. Loeb and Gies, *Arch. f. d. ges. Physiol.*, Vol. XCIII, p. 246, 1903; also Loeb, *Arch. f. d. ges. Physiol.*, Vol. CVII, p. 252, 1905.

2. Roaf and Whitley, *loc. cit.*, pp. 97-101.

3. Cf. Macallum, *Journ. Physiol.*, Vol. XXIX, p. 213, 1903.