THE EFFECT OF CERTAIN ELECTROLYTES AND NON-ELECTROLYTES ON PERMEABILITY OF LIVING CELLS TO WATER.

By MORTON McCUTCHEON and BALDUIN LUCKE.

(From the Laboratory of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole.)

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Although permeability to water must be regarded as one of the most fundamental properties of living cells, the degree of permeability may be surprisingly low (1), even temporarily nil (2) and varies with the physiological state of the cell (3). It is also thought to depend on the chemical composition of the medium, but direct evidence on this point is scanty. Experiments were therefore undertaken to show quantitatively whether cell permeability to water is regulated by electrolytes in the medium.

A satisfactory method for attacking this problem is to place the cell in anisotonic solutions, so causing water to enter or leave the cell under the driving force of osmotic pressure. The unfertilized egg of the sea urchin, *Arbacia punctulata*, is an excellent natural osmometer for this purpose; the amount of swelling or shrinking occurring in anisotonic solutions, and hence the volume of water entering or leaving the cell, can readily be measured under the microscope.

The effect of temperature and of the salt concentration of the medium on the rate of this process has been previously reported (4–6). In the present experiments, these factors have been held constant, and only the chemical composition of the medium varied. Under these conditions, change in rate of osmosis may be interpreted as change in permeability of the cell to water.

By permeability is understood the quantity of material (in this case, water) passing through unit area and unit thickness[1] of membrane in unit time under unit pressure. In order that results may be expressed

[1] We have assumed that the thickness of the membrane is constant.

in dimensions of permeability, the empirical equation[2] employed in previous papers has not been used here, although the velocity constant

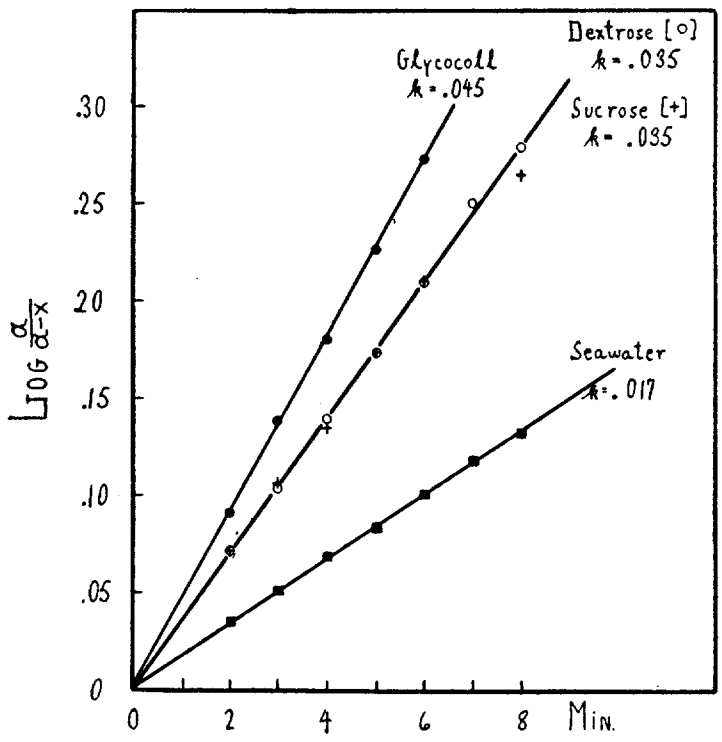

FIG. 1. The effect of non-electrolytes on rate of swelling in hypotonic solutions. Data are taken from Table I. When $\log \frac{a}{a-x}$ (in which a is total increase in cell volume, and $a - x$ is volume increase up to time t) is plotted against times, the graph is a straight line, showing that the process follows the equation $kt = ln \frac{a}{a-x}$, in which k is the velocity constant.

of that equation is approximately proportional to the permeability, provided that, as here, temperature and osmotic pressure of the

[2] $\frac{dx}{dt} = k(a - x)$, where a is the total volume of water that will cross the membrane before equilibrium is established, x the amount that has already crossed at time t and k is the velocity constant.

medium are not varied. The empirical equation fits the present data, as shown in Fig. 1.

In this paper, results are calculated from the equation, permeability $= \frac{dV}{dt} \Big/ SP$ in which $\frac{dV}{dt}$ is cubic micra of water per minute, S is square micra of surface and P is atmospheres.

From the data in the accompanying tables, cell volumes were plotted against times, and a smooth curve drawn through the points. The rate of endosmosis was determined in each instance at the *second minute*, since earlier observations were frequently impossible on account of the time required for cells to settle. The rate at the second minute may be determined by drawing the tangent to the curve at this point, but since it is difficult to draw tangents accurately, it was found more satisfactory to determine the times required, beginning at the second minute, for the cells to increase 20,000 cubic micra in volume. The assumption made, that this portion of the curve is a straight line, while not strictly correct, introduces no serious error.

From the figure so obtained, increase in volume per minute was calculated. Practically identical rates were found by drawing tangents.

Surface and pressure were calculated for a point midway between the volume at 2 minutes and a volume greater by 20,000 cubic micra, *i.e.* volume at 2 minutes plus 10,000 cubic micra. Surface was calculated directly from the volume read from the curve at this point. Pressure inside the cell was calculated from the equation $P_t = P_o V_o / V_t$ in which P_o and P_t are osmotic pressures inside the cell at the first instant and at time t respectively, and V_o and V_t are the corresponding cell volumes. P_o was taken as 22 atmospheres, V_o was taken as the mean volume of 30 eggs of the same animal measured in sea water, V_t was read from the curve and P_t was calculated. The osmotic driving force is the difference between P_t and the pressure of the solution -8.8 atmospheres for 40 per cent sea water. The values so obtained were substituted in the expression for permeability.

The technic of measuring volume changes in *Arbacia* eggs by means of a filar micrometer eyepiece has been previously described (4). In the experiments here reported, the cells were first washed in isotonic concentration of the solution to be employed, to remove electrolytes.

A drop of cell suspension was thoroughly mixed with about 20 cc. of this solution, and the cells were then allowed to settle. Temperature was maintained by a water jacket at 12 ± 0.5°C.[3]

Effect of Non-Electrolytes.

The first step, preparatory to studying the effect of electrolytes, was to eliminate them from the medium and to learn whether the rate with

TABLE I.

The effect of non-electrolytes, contrasted with that of sea water, on the permeability of unfertilized *Arbacia* eggs to water. Solutions are isosmotic with 40 per cent sea water. Cell volumes are given in cubic micra $\times 10^2$. Each number represents the mean volume of 6 cells. In the bottom row is given the permeability, which is the number of cubic micra of water entering the cell per minute, per square micron of surface, per atmosphere of pressure. The temperature was 12°C. (data are graphed in Fig. 1).

It is seen that permeability in non-electrolyte solutions is much higher than in sea water.

The mean volume of 30 control cells in 100 per cent sea water was 2060×10^2.

Time	Glycocoll	Dextrose	Saccharose	Sea water
min.				
2	2475	2325	2410	2265
3	2710	2490	2595	2355
4	2900	2675	2725	2450
5	3075	2830	2900	2525
6	3250	2980	3050	2620
7		3145		2700
8		3245		2760
Permeability	0.142	0.097	0.103	0.050

which water entered the cells from hypotonic solutions was thereby affected. Accordingly cells were placed in hypotonic aqueous solutions of non-electrolytes—dextrose and saccharose, and also of glycocoll, which, on account of its slight electrolytic dissociation may be expected to behave toward cells like a non-electrolyte.[4]

[3] Low temperature was necessary to prevent excessively rapid swelling in some of the solutions.

[4] In most experiments solutions were made isotonic with 40 per cent sea water (sea water 40 parts, distilled water 60 parts). At Woods Hole this solution has,

The effect of these non-electrolytes is illustrated in Table I. It is seen that permeability to water is much greater in these solutions than in sea water of the same osmotic pressure (in most experiments it was about twice as great). Other experiments showed the differences in permeability in dextrose, saccharose and glycocoll to be probably not significant.[5]

A possible objection to the significance of these results is that the hydrogen ion concentration was not the same in the several solutions, dextrose, saccharose and glycocoll being on the acid side of neutrality, sea water on the alkaline. This objection is believed not to be valid, as preliminary experiments showed that increase in hydrogen ion concentration obtained by adding HCl to dextrose solution tends to decrease permeability.

Therefore, the rapid osmosis observed in non-electrolyte solutions is not due to increased hydrogen ion concentration, but either to the effect of non-electrolytes or to the absence of electrolytes.

Effect of Sodium and Potassium.

Supposing that the effect of non-electrolyte solutions were due to absence of electrolytes, it seemed possible that addition of electrolytes, especially of those occurring in sea water, to sugar solutions might lower permeability to that observed in sea water. All electrolytes might have this effect or only certain ones. Accordingly NaCl, which is the most abundant electrolyte in sea water, was added to hypotonic dextrose solution; the concentration of NaCl was 0.01 molar.[6]

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according to Garrey (7), a freezing point of 0.73°C. Calculations based on data in the Landolt-Börnstein Tabellen show that 0.38 molar dextrose or 0.39 molar saccharose should be isotonic with this solution. 0.4 molar was assumed as approximately the isotonic concentration of glycocoll.

[5] Samples of dextrose from three manufacturers gave similar results. It is assumed that no significant penetration of these substances occurred during the short time that experiments lasted.

These non-electrolytes were selected because they are known not to penetrate cells readily. The choice of non-electrolytes is important because if substances were used which rapidly enter the cell, its osmotic pressure would increase and endosmosis would be accelerated.

[6] Dextrose was chosen as the most convenient non-electrolyte with which to work.

Electrolytes were dissolved in distilled water in 1 molar concentration, the

The effect of NaCl and of KCl is illustrated in Table II. It is seen that the permeability values were even greater than in solutions of dextrose alone. Repeated experiments showed that there is probably no significant difference in the effect of Na and K, nor did these ions

TABLE II.

The effect of sodium, potassium, calcium and magnesium on permeability to water. The chloride of the several cations is added to dextrose in amounts sufficient to give 0.004 molar concentration of the electrolytes. Solutions are isosmotic with 40 per cent sea water. Each number represents the mean volume of 6 to 9 cells.

It is seen that in NaCl and KCl, permeability to water is of the same order of magnitude as in dextrose alone, while in $CaCl_2$ and $MgCl_2$ it is approximately the same as in sea water.

The mean volume of 30 control cells in 100 per cent sea water was 2035×10^2.

Time	Dextrose	NaCl in dextrose	KCl in dextrose	$CaCl_2$ in dextrose	$MgCl_2$ in dextrose	Sea water
min.						
1						2210
1.5		2205				
2	2180	2310	2290	2245	2240	2285
2.5		2435				
3	2305	2560	2470	2365	2365	2370
3.5		2655				
4	2480		2580	2460	2435	2465
4.5		2900				
5	2655		2750	2535	2520	2545
6	2840		2855	2610	2595	2630
7	2955			2685	2635	2685
8	3070			2745		2740
Permeability	0.093	0.129	0.096	0.054	0.050	0.048

invariably increase the rate of osmosis above that occurring in solution of dextrose alone. Lower concentrations of NaCl and KCl produced similar though less definite results; higher concentrations led to rapid cytolysis.

amounts of these solutions added to dextrose being so small as not to alter its osmotic pressure significantly.

Effect of Calcium and Magnesium.

There remained the possibility that only certain of the electrolytes of sea water have a restraining action on osmosis. Since Na and K tended to increase permeability to water, it seemed possible that Ca and Mg would have the opposite effect. This proved to be the case. In the same Table (II), the value in hypotonic sea water is 0.048, in dextrose solution of the same osmotic pressure, 0.093. On adding $CaCl_2$ to this dextrose solution in amount sufficient to obtain 0.004 molar concentration, the permeability was reduced to 0.054, approximately the value in sea water. This result was regularly reproducible. A similar effect was produced by $MgCl_2$.

Remarkably small amounts of Ca were found effective in slowing osmosis in dextrose, though a concentration of 0.00005 molar proved to be too low. Thus in one experiment in which the concentration of $CaCl_2$ was varied, the following results were obtained: 0.001 M, 0.044; 0.0005 M, 0.042; 0.0001 M, 0.049; 0.00005 M, 0.077.

These experiments indicate that the rapid osmosis observed in dextrose solutions is not due to the effect of non-electrolytes, but to absence of Ca and Mg. Also, the conclusion appears justified, that the relatively slow osmosis observed in sea water is due, at least in part, to the presence of these two bivalent cations.

It has been seen that the chlorides of Ca and Mg in dextrose solutions decrease permeability to the value found in sea water, whereas NaCl and KCl have no such effect. Numerous experiments invariably gave similar results.

Antagonism of NaCl and KCl with $CaCl_2$ and $MgCl_2$.

As stated above, Na and K in dextrose solution did not always increase permeability more than did dextrose alone. In order to determine the effect of these cations more definitely the principle of salt antagonism was used. Having found a concentration of $CaCl_2$ in dextrose solution which was just sufficient to lower permeability to the value obtained in sea water, varying amounts of NaCl were added. In appropriate concentrations, NaCl antagonised the slowing effect of $CaCl_2$, the degree of permeability being intermediate in value between that of Ca in dextrose and that in dextrose alone. K was just as effective as Na in this respect. A typical experiment is represented in

Table III. Ca could be replaced by Mg and similar antagonism with the univalent cations demonstrated. Such experiments show definitely that NaCl and KCl tend to increase permeability to water.

Preliminary experiments indicate that this method of studying salt antagonism can also be used quantitatively. Table IV shows that increasing amounts of NaCl added to $CaCl_2$ in dextrose give increasing values for permeability.

TABLE III.

Antagonism of sodium or potassium with calcium. In 0.0005 molar $CaCl_2$ in dextrose, permeability to water has the same low value as is usually obtained in isosmotic (40 per cent) sea water. Upon the further addition of NaCl or KCl (final concentration 0.01 molar), permeability increases to values intermediate between those in calcium-dextrose solution and in dextrose alone.

Each figure represents the mean volume of 5 to 7 cells. Volume of control in 100 per cent sea water, 2030×10^2.

Time	Dextrose	KCl and $CaCl_2$ in dextrose	NaCl and $CaCl_2$ in dextrose	$CaCl_2$ in dextrose
min.				
1		2170	2015	2195
2	2285	2325	2145	2255
3	2470	2435	2295	2355
4	2595	2585	2405	2450
5	2735	2695	2520	2520
6	2890	2820	2645	2580
7	2995	2935	2770	2650
8		3045	2850	2720
Permeability	0.091	0.076	0.065	0.050

DISCUSSION.

These experiments indicate that the cations of sea water are important in regulating the permeability of the cell to water. There is an extensive literature on the effect of ions on permeability of cells and tissues to various substances.[7] In most cases, as in our experiments

[7] The literature on this subject is reviewed by Osterhout, W. J. V., Injury, recovery, and death in relation to conductivity and permeability, Philadelphia and London, 1922. Jacobs, M. H., in Cowdry, E. V., General cytology, Chicago, 1924. Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 6th edition, 1926. von Tschermak, A., Allgemeine Physiologie. I, Berlin, 1924.

given above, Na and K have been found to increase permeability, Ca and Mg to decrease it. Particularly striking is the agreement between our results and those of Osterhout (8), who studied the effect of various electrolytes on the electrical conductivity of plant tissues, using this property as a measure of permeability. For these reasons it is probable that the action of ions stated above has general application.

TABLE IV.

Antagonism of sodium and calcium. Having determined permeability to water in solution of 0.0005 molar $CaCl_2$ in 0.38 molar dextrose, increasing amounts of NaCl are added to this solution. Permeability is seen to increase with the amount of NaCl added. Each figure represents the mean volume of 5 or 6 cells. The mean volume of 30 control cells in 100 per cent sea water was 2010×10^2.

Time	$CaCl_2$ and 0.016 M NaCl	$CaCl_2$ and 0.008 M NaCl	$CaCl_2$ and 0.004M NaCl	$CaCl_2$
min.				
1	2115			
1.5	2180			
2	2270	2245	2170	2230
3		2330	2265	2325
4	2525		2385	2420
5	2675	2520	2430	2480
6	2830	2640	2535	2545
7		2760	2675	2620
8		2890	2765	2710
Permeability	0.080	0.056	0.050	0.047

On the other hand the effect of non-electrolytes seems to vary with the material and method used (9).

The method employed by us permits direct measurement of the rate with which water crosses the cell membrane. The effect of different ions on permeability is striking and reproducible. Since the experiments were carried out at constant temperature and constant osmotic pressure of the medium, the conclusion is justified that the permeability of the cell to water is increased by NaCl and KCl, decreased by $CaCl_2$ and $MgCl_2$.[8]

[8] The *mechanism* of these changes in permeability to water has not been investigated in the present series of experiments. It may be said, however, that injury

SUMMARY.

1. Permeability to water in unfertilized eggs of the sea urchin, *Arbacia punctulata*, is found to be greater in hypotonic solutions of dextrose, saccharose and glycocoll than in sea water of the same osmotic pressure.

2. The addition to dextrose solution of small amounts of $CaCl_2$ or $MgCl_2$ restores the permeability approximately to the value obtained in sea water.

3. This effect of $CaCl_2$ and $MgCl_2$ is antagonized by the further addition of NaCl or KCl.

4. It is concluded that the NaCl and KCl tend to increase the permeability of the cell to water, $CaCl_2$ and $MgCl_2$ to decrease it.

5. The method here employed can be used for quantitative study of salt antagonism.

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to the cell is probably involved. Preliminary experiments of various types—such as fertilization tests on cells previously exposed for a few minutes to isotonic unbalanced solutions—suggest that NaCl and KCl are more toxic than $CaCl_2$ and $MgCl_2$, and that recovery from injury may be possible to a certain degree. But on all these points more data are necessary before definite conclusions are drawn. It would be of considerable importance if it could be shown that under certain conditions, increase in permeability of the cell to water could be taken as a measure of injury.