

THE EFFECT OF VALENCE OF IONS ON CELLULAR PERMEABILITY TO WATER.

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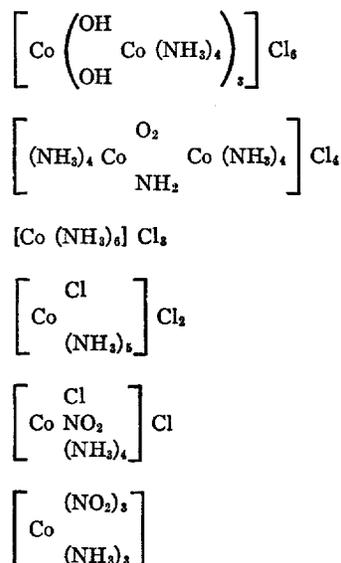
When a cell, such as the egg of the sea urchin, is placed in a solution hypotonic with respect to its natural medium, water enters under the driving force of osmotic pressure, and the cell swells. In the case of the spherical sea urchin egg the osmotic swelling proceeds relatively slowly and permits accurate measurements of the diameter, from which volume and surface of the cell can be calculated. The rate of entrance of water, per unit of time, per unit of surface and per unit of pressure may be defined as the permeability of the cell to water, and depends on a number of factors such as temperature and composition of the medium (1, 2).

While studying the effect of electrolytes on permeability of *Arbacia* eggs to water, striking differences were found in the effects of the chlorides of Ca and Mg on the one hand, and those of Na, K, Li and NH_4 on the other (2).^{*} In quite low concentration, chlorides of the bivalent cations decreased permeability to water while those of the univalent cations had the opposite effect. Since we were unable to find constant differences in the effects of cations of the same valence, it seemed likely that we were dealing with conditions in which effect of valence predominated over other ionic effects.

If this were the case, cations of valence greater than 2 should prove still more effective in reducing permeability to water. In fact preliminary experiments with LaCl_3 and ThCl_4 gave results pointing in this direction, but solutions of these salts were so acid that we regarded them as unsuitable and looked for a valence series of cations the salts of which would form neutral solutions.

^{*} Experiments dealing with Li and NH_4 are unpublished.

This requirement was met by a series of cobaltamine chlorides having the following structure:¹



This series has the advantages of containing cations of widely different valence ranging from 6 to 0; the compounds form approximately neutral solutions, and have been reported to undergo complete electrolytic dissociation as determined by conductivity measurements and other methods (3)*. The action of this cation series on permeability to water was studied by the following method.

Method.

Unfertilized eggs of *Arbacia punctulata* were washed in a solution of dextrose isotonic with sea water to eliminate electrolytes from the medium. The cells were then placed in a dish containing about 20 cc.

¹ The cobaltamine chlorides were kindly prepared for us by Professor Thomas P. McCutcheon, of the University of Pennsylvania. These compounds are representative of a larger number used in the experiments.

* When *Arbacia* eggs were exposed in isotonic dextrose to concentrations of these salts such as were used in the experiments most of them remained capable of fertilization and development.

of 0.38 molal dextrose solution in which had been dissolved the salt to be tested. This solution is isotonic with 40 per cent sea water (40 parts of sea water and 60 parts of distilled water). The course of swelling at constant temperature ($12 \pm 0.5^\circ\text{C}$.) was then observed through the microscope for 4 to 6 minutes and the mean volumes of 5 to 10 cells were plotted against time. The rate of entrance of water in cubic micra per minute is given by the rate of increase in cell volume, $\frac{dV}{dt}$; it is obtained from the slope of the curve at a given time, t . The permeability at this time is then $\frac{dV}{dt}/SP$, where S is the surface of the cell, and P is the difference in osmotic pressure between the interior of the cell at time t , and the medium.²

Effect of Valence of the Cation.

In the first group of experiments permeability was determined with a series of cobaltamine chlorides in 0.0001 molar concentration dissolved in hypotonic dextrose solution; the range of valence of the cation was from 1 to 6. It was found that salts with bivalent cations produced lower permeability than did those with univalent cations. But by this method, no significant difference in effect was found between the bivalent salts and those of higher valence.

The reason for this appears to be that, at a given temperature, permeability can be reduced only to a certain value. This numerical value is obtained in sea water (presumably on account of the presence of Ca and Mg) and also in dextrose solution containing cobaltamines of bivalent or higher valence in sufficient concentration.

It occurred to us that differences in effect of cobalts of higher valence might be demonstrated if we added some substance which increased permeability, and then determined the amount of cobalt necessary to reduce permeability to that obtained in sea water. This proved to be the case. It had been found that small amounts of K_2SO_4 and especially of K_3 citrate greatly increased permeability. When one of these

² For details of calculation see (2). In the present experiments slopes of curves were obtained by drawing tangents. Permeability varies but little during the course of an experiment; as a matter of convenience permeability was calculated at the 3rd minute.

TABLE I.

The effect of cobaltamine chlorides, having cations of different valence, on permeability to water. These salts in the concentrations shown are dissolved in 0.38 molal dextrose solution containing 0.01 M K_3 citrate. Solutions are isosmotic with 40 per cent sea water. Cell volumes are given in cubic micra $\times 10^3$. Each number represents the mean volume of 5 to 10 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 $12 \pm 0.5^\circ C$.

It is seen that the effectiveness of the cobaltamine chlorides in lowering permeability increases with the valence of the cation.

The mean volume of 20 control cells in sea water was 2000×10^3 cubic micra.

Molar concentration	6 valent		4 valent			3 valent			2 valent
	1×10^{-4}	2×10^{-4}	2×10^{-4}	4×10^{-4}	8×10^{-4}	4×10^{-4}	8×10^{-4}	1.6×10^{-3}	1.6×10^{-3}
<i>min.</i>									
1	2156	2139	2105	2205	2200	2187	2197	2139	2191
2	2308	2253	2280	2344	2313	2363	2313	2285	2423
3	2447	2326	2447	2442	2381	2636	2466	2377	2616
4	2631	2404	2611	2591	2452	2893	2626	2490	2834
5		2533		2721	2572		2778	2601	
Permeability	0.089	0.041	0.097	0.072	0.048	0.159	0.088	0.063	0.131

TABLE II.

The effect of valence in a cation series of cobaltamine chlorides. The salts in the concentrations shown are dissolved in 0.38 molal dextrose solution containing 0.005 M K_3 citrate. Numbers represent permeability to water.

The effectiveness of the cation in reducing permeability to water increases with the valence; for example, 5×10^{-5} of the 6 valent salt are required to reduce permeability to the value usually obtained in sea water, while 64 times this concentration is required in the case of the 2 valent cobalt (data are graphed in Fig. 2).

Molar concentration	2.5×10^{-5}	5×10^{-5}	1×10^{-4}	2×10^{-4}	4×10^{-4}	8×10^{-4}	1.6×10^{-3}	3.2×10^{-3}
6 valent	0.194	0.048	0.042					
4 valent		0.172	0.058	0.038				
3 valent			0.180	0.068	0.061			
2 valent					0.171	0.113	0.157	0.043
1 valent								0.058

salts in proper concentration was added to the solution it was found that much less of 6 valent cobalt was required to reduce permeability than of the 4 valent, and so on down the series. Thus in Table I it is seen that when 0.01 M K_3 citrate was used, 0.0002 M 6 valent cobalt was sufficient to reduce permeability to the value obtained in sea water,* 0.0008 M of the 4 valent was required, while twice this amount of the 3 valent and 2 valent salts were incompletely effective.

When a lower concentration of K_3 citrate (0.005 M) was used, smaller amounts of the cobalts were necessary to reduce permeability. In Table II it is seen that 0.00005 M of the 6 valent salt was sufficient to reduce permeability, more than twice as much of the 4 valent cobalt was required, more than 8 times as much 3 valent and 64 times as much of the 2 valent salt, while this amount of the 1 valent salt was incompletely effective.

Definite though less striking differences in effectiveness were found between several bivalent and univalent cobaltammines, the former decreasing permeability to water, whereas univalent cobaltammines, as well as the undissociated compound, did not decisively either increase or decrease permeability.³

From these and similar experiments it is concluded that there are distinct differences in the effectiveness of cobaltammines of different valence in reducing permeability and that this effectiveness increases rapidly with the valence of the cation.

Effect of Valence of the Anion.

The results obtained with the cobalt cation series suggested the question, do anions act in the opposite direction? Do they tend to increase permeability, and is their effectiveness related to valence?

Preliminary experiments indicated that this is the case. When 0.0001 M KCl , K_2SO_4 , K_3 citrate and $K_4Fe(CN)_6$ were added to dextrose solution, sulfate gave higher permeability than chloride, while citrate and ferrocyanide gave higher permeability than did sulfate.

* In 40 per cent sea water, or in 0.38 molal dextrose solution containing 0.0005 M $CaCl_2$, the value for permeability at 12°C. is approximately 0.05, but varies somewhat in different lots of cells.

³ When cobaltamine appears as a univalent anion, $\left[\begin{array}{c} Co \\ (NO_2)_4 \\ (NH_3)_2 \end{array} \right] Na$. definite increase in permeability was demonstrable.

However with such solutions, tendency to cytolysis was a disturbing factor; this was overcome by addition of CaCl_2 in 0.0005 molar concentration. It was now possible to determine the minimal concentration of the potassium salt which would increase permeability above the magnitude obtained in sea water or in CaCl_2 solution. Two series of potassium salts were used, one with inorganic, one with organic anions.

TABLE III.

The effect of valence in an anion series of inorganic potassium salts. The salts in the concentrations shown are dissolved in 0.38 molal dextrose solution containing 0.0005 M CaCl_2 . Cell volumes measured at minute intervals are given in cubic micra $\times 10^2$. Each number represents the mean volume of 6 to 8 cells.

In the bottom row is given the permeability in the units stated in Table I. The temperature was $12 \pm 0.5^\circ\text{C}$.

It is seen that the effectiveness of the salts in raising permeability above the value of the CaCl_2 control increases with the valence of the anion.

The mean volume of 20 control cells in sea water was 2236×10^2 cubic micra.

Molar concentration	$\text{K}_4\text{Fe}(\text{CN})_6$	$\text{K}_2\text{Fe}(\text{CN})_6$			K_2SO_4		KCl		CaCl_2
	1×10^{-3}	1×10^{-3}	2×10^{-3}	4×10^{-3}	2×10^{-3}	4×10^{-3}	4×10^{-3}	8×10^{-3}	
<i>min.</i>									
1	2428	2423	2418	2405	2405	2433	2414	2368	2428
2	2616	2523		2626	2499	2676	2494	2509	2567
3	2861	2656	2676	2824	2616	2882	2567	2641	2671
4	3059	2757	2840	3032	2742	3154	2676	2793	2742
5	3290	2871	3026		2876		2783	2935	2824
6	3478	3005	3199		3005		2887	3059	2924
Permeability	0.118	0.057	0.081	0.115	0.062	0.133	0.046	0.073	0.048

With the former, Table III illustrating a typical experiment, shows that ferrocyanide was more effective in increasing permeability than 8 times the amount of chloride and had approximately the same effect as 4 times the amount of sulfate, while sulfate was more than twice as effective as the chloride.

The question might be raised, however, whether this result could be due to differences in concentration of potassium ions rather than to valence of the anion. That this is probably not the correct or at least the complete explanation is evident from the ratios of the effective concentrations as given in the preceding paragraph.

Table IV brings out the effect of valence still more clearly. In this experiment 0.001 M ferrocyanide was required definitely to increase permeability above the value of the control (CaCl_2 solution), twice as much ferricyanide, 4 times as much sulfate and 8 times as much chloride.

TABLE IV.

The effect of valence in an anion series of inorganic potassium salts. The salts in the concentrations shown are dissolved in 0.38 molal dextrose solution containing 0.0005 M CaCl_2 . Numbers represent permeability to water.

The effectiveness of the salts in increasing permeability above the value of the CaCl_2 control increases with valence of the anion.

Molar concentration	2×10^{-4}	8×10^{-4}	1×10^{-3}	2×10^{-3}	4×10^{-3}	8×10^{-3}
KCl				0.066	0.067	0.095
K_2SO_4				0.048	0.152	
$\text{K}_3\text{Fe}(\text{CN})_6$			0.068	0.107		
$\text{K}_4\text{Fe}(\text{CN})_6$	0.056	0.063	0.087	0.192		

($\text{CaCl}_2 = 0.046$)

TABLE V.

The effect of valence in an anion series of organic potassium salts.

The effectiveness of the salts in increasing permeability above the value of the CaCl_2 control increases rapidly with the valence of the anion (data are graphed in Fig. 1).

Molar concentration	2.5×10^{-4}	5×10^{-4}	1×10^{-3}	2×10^{-3}	4×10^{-3}	8×10^{-3}
K acetate			0.044	0.049	0.048	0.116
K_2 tartrate		0.055	0.060	0.090	0.150	
K_3 citrate	0.052	0.086	0.184			

($\text{CaCl}_2 = 0.046$)

Similar results were obtained using a series of organic salts with increasing valence of the anion (Table V): 0.0005 molar citrate was required to increase permeability above the value of the CaCl_2 control; 4 times as much tartrate and 16 times as much acetate were needed.

In both inorganic and organic anion series it was invariably found that the effectiveness of the salts in increasing permeability to water increases rapidly with the valence of the anion.

The relation between permeability and valence in the anion and cation series is graphically shown in Figs. 1 and 2. In the first graph (Fig. 1) permeability to water is plotted against the molar concentration of an anion series. It is seen that with the trivalent anion a relatively slight increase in concentration causes a marked change in

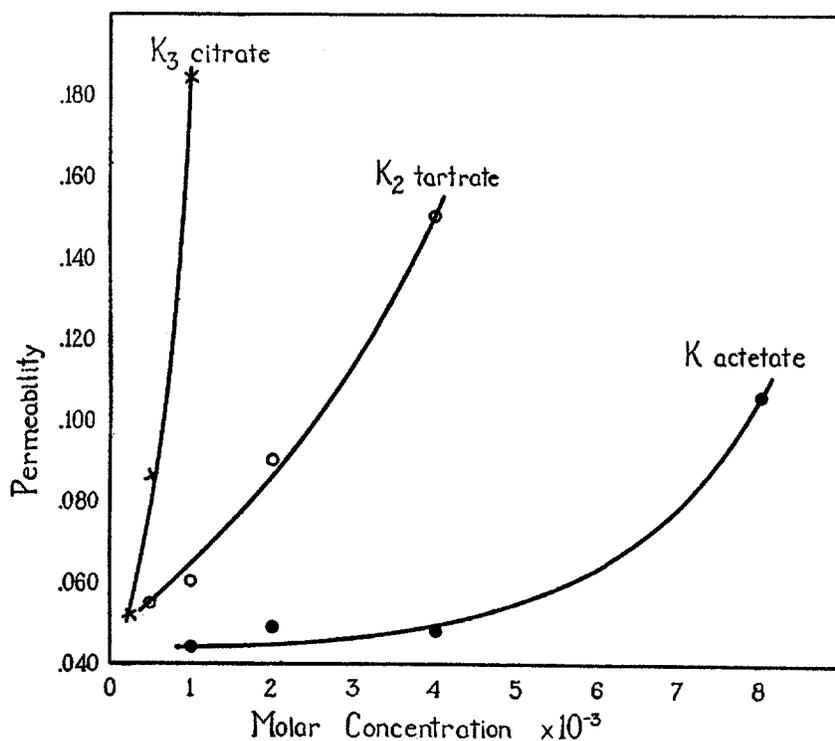


Fig. 1. The relation between permeability and molar concentration of salts having anions of different valence (data are taken from Table V).

permeability; whereas with the univalent anion a considerable change in concentration is required to bring about an increase in permeability; and the bivalent anion occupies an intermediate position.

Similarly a plot of a cation series (Fig. 2) shows that permeability to water decreases with concentration of the 6 valent cation more rapidly than with cations of lower valence.

DISCUSSION.

The experiments indicate that permeability of cells to water is regulated, at least in part, by the sign and the number of charges on the ions in the medium, in the sense that cations decrease and anions increase permeability to water; and these effects increase rapidly with the valence of the ion.

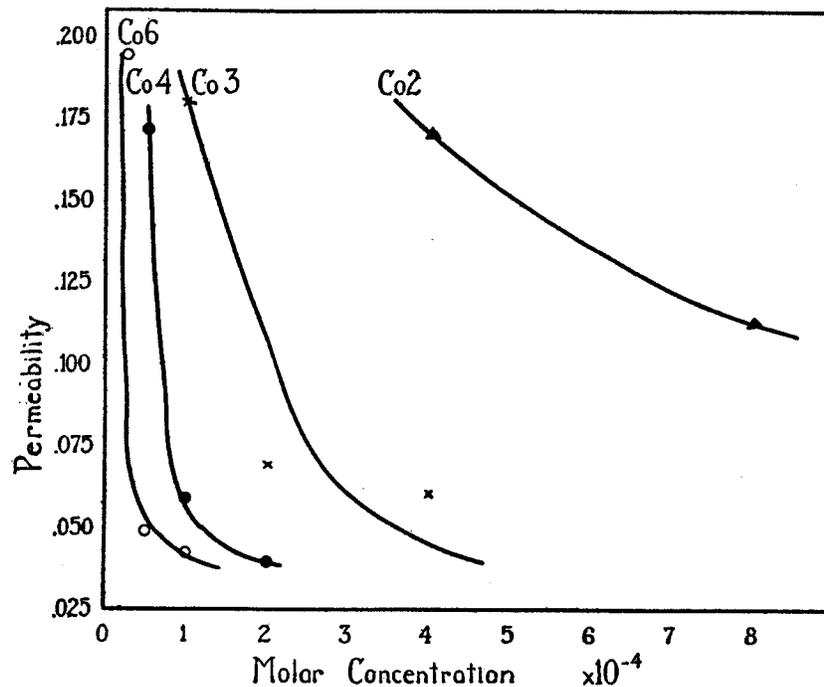


Fig. 2. The relation between permeability and molar concentration of salts having cations of different valence (data are taken from Table II).

It is interesting to note that valence and the sign of the charge were found by Osterhout (4) and Raber (5) to produce changes in conductivity of plant tissues that are closely paralleled in our experiments on permeability to water.

The experiments also show that it is possible to determine the concentration of a cation which will oppose the effect of an anion in its tendency to increase permeability to water, and conversely, the

restraining action of a cation on osmosis may be balanced by the proper concentration of an anion. The method used, therefore permits quantitative study of antagonism between cations and anions in their effects on permeability to water.

SUMMARY.

1. Permeability to water in unfertilized eggs of the sea urchin, *Arbacia punctulata*, was studied by measuring the rate of swelling in hypotonic dextrose solution.

2. Permeability is greatly affected by addition of electrolytes in low concentration.

3. A decrease in permeability to water was found with increasing valence of the cation, using a series of cobaltamine chlorides in which the valence of the cation ranged from 1 to 6.

4. Conversely, an increase in permeability to water was found with increasing valence of the anion, using two series of potassium salts in which the valence of the anion ranged from 1 to 4, and 1 to 3, respectively.

5. It is concluded that the effect of electrolytes on permeability to water depends chiefly on the sign and the number of charges on the ion, in the sense that positive ions decrease permeability to water, while negative ions increase permeability to water; and the effectiveness of the ion is greater the higher its valence.

6. Antagonism has been demonstrated between cations and anions in their effect on permeability, and the method employed permits quantitative study of such antagonism.

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