

## THE KINETICS OF OSMOTIC SWELLING IN LIVING CELLS.

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The present paper is the first of a series of studies on volume changes in living and dead cells. The simplest case of such volume change, and therefore the easiest and most satisfactory to investigate quantitatively, is the swelling of living cells in hypotonic solutions.

This study is concerned with the *rate* of swelling, and with the way in which the rate is affected by temperature and by the osmotic pressure of the outside solution. The material selected was the unfertilized egg of the sea urchin, *Arbacia*.

This cell is ordinarily in osmotic equilibrium with sea water, which is its natural medium. If sea water is diluted with distilled water, there occurs diffusion of water into the cell which swells, until it again reaches osmotic equilibrium with the outside solution.

For the study of osmosis, the sea urchin egg is particularly favorable material. When the egg is placed in a hypotonic solution and observed under the microscope, the diameter of the egg increases in a very regular way. And since the egg is spherical, the change in volume may easily be calculated from the change in diameter. If measurements are made at minute intervals with an ocular micrometer, and plotted against time, a curve is obtained such as is shown in Fig. 1.

Thus we have an admirable means of studying the *velocity* of osmotic swelling. Most studies of osmosis and of osmotic pressure have been concerned only with the state of equilibrium, or with the amount of swelling or of plasmolysis at equilibrium. A few studies have been made on the initial rate of flow. But in the sea urchin egg the process can be followed practically from beginning to end.

*Method.*

Eggs from a single animal were used in each of the 7 groups of experiments. Their average size was found by measuring 10 to 30 cells. For this purpose a screw ocular micrometer and a 10 mm. objective (immersed in the sea water) were used, the system giving a magnification of 240 diameters.

The eggs were concentrated by slight centrifugation, and a few were then placed in about 50 cc. of hypotonic sea water at known temperature. This solution was contained in a glass dish; its temperature was regulated by a surrounding jacket of water within a larger glass vessel which rested on the stage of a microscope. A thermometer was placed so that the bulb was immersed in the solution containing eggs and in immediate proximity to them. With this arrangement variations in temperature of the solution did not usually exceed  $\pm 0.2^{\circ}\text{C}$ .

Except for a very small percentage of slightly ovate eggs, which were never used for measurement in these experiments, the eggs of *Arbacia* are spherical.

This fact was repeatedly demonstrated by rolling them over with a fine glass needle, a procedure which readily demonstrates any flattening of the egg, such as occurs when they are placed in boiling water. No such flattening was found before or during osmotic swelling. The spherical shape is preserved unless cytolysis occurs; after this event measurement is impossible. But until cytolysis occurred, the eggs were in general uninjured by the experimental procedure, since, when they were returned to ordinary sea water and sperm added, cleavage usually resulted.

*The Rate of Swelling.*

Since the process of osmotic swelling is primarily one of diffusion it is to be expected that when the cell is far from osmotic equilibrium with its surroundings it will swell rapidly, but that the rate of swelling will steadily decrease as equilibrium is approached. The same course is followed by unimolecular chemical reactions. The general equation for such a process is  $\frac{dx}{dt} = k(a-x)$ , where  $\frac{dx}{dt}$  is the rate of transforma-

tion at time  $t$ ,  $a$  is the original amount of substance,  $x$  is the amount of substance converted up to a time  $t$ , and  $k$  the velocity constant.

This is a familiar equation since it expresses a variety of processes besides chemical reactions of the first order, such as rate of diffusion, rate of cooling of bodies, and rate with which vibrations of a spring decrease in amplitude. The mistake must not be made of regarding all processes which follow this equation as being unimolecular reactions.

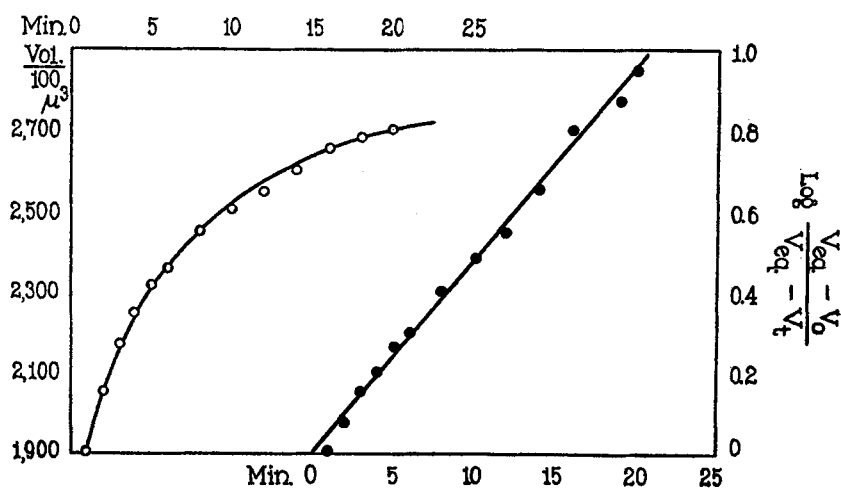


FIG. 1. The rate of swelling of unfertilized sea urchin eggs in 60 per cent sea water at 24.8°. On the left side, volumes are plotted against times. On the right side  $\log \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  is plotted against time.

$V_{\text{eq.}} = 2707 \times 10^2 \mu^3$ ,  $V_0 = 1909 \times 10^2 \mu^3$ , and the values of  $V_t$  are the same as used in the curve on the left. The points fall along a straight line, the slope of which gives the value of  $k$  ( $= 0.048$ ). This graph shows the process follows the equation  $kt = \ln \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$ .

Integrating, we obtain  $kt = \ln \frac{a}{a-x}$ . A modification of this suggested by R. S. Lillie (1) as convenient for calculation of volume changes in cells, is,  $kt = \ln \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  where  $V_{\text{eq.}}$  is volume at equilibrium.  $V_0$  is volume at the first instant, and  $V_t$  is volume at time  $t$ .

It was found by Lillie that this equation correctly represents the rate of swelling of fertilized and unfertilized *Arbacia* eggs in 40 per cent sea water. That it also fits our data may be seen from Fig. 1. When  $\log \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  is plotted against time, a straight line should be obtained, and the slope of this line gives the value of  $k$ , the velocity constant. It is observed that the points do actually fall on a straight line, within the limits of experimental error. In Fig. 2, it is seen that the equation holds over a wide range of temperatures, and, in Fig. 4, over a wide range of osmotic pressures.

It can therefore be safely concluded that the equation  $kt = \ln \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  does correctly express the swelling of cells in hypotonic sea water, as would be expected in a diffusion process.

#### *The Effect of Temperature.*

We now consider evidence that other factors in addition to diffusion are involved in the rate of swelling. We will consider first the effect of temperature.

A pure diffusion process is only moderately accelerated by increasing the temperature. For a rise of  $10^\circ$ , the rate of such a process would ordinarily be increased about 20 to 30 per cent; in other words the temperature coefficient,  $Q_{10}$ , would equal about 1.2 to 1.3. But in our experiments, the effect of temperature was much greater than this.

A typical experiment (one of seven) is represented in Table I. In the first column are placed the times in minutes, in the second, the volumes at  $11^\circ\text{C.}$ , in the third, at  $13.1^\circ$ , and so on for a series of temperatures. Each volume represents the mean of 3 to 6 cells. The original volume was determined by averaging 10 or 20 cells at the beginning of the experiment, and the volume at equilibrium was observed or calculated.<sup>1</sup> The volume at equilibrium was apparently

$$\frac{V_0}{V_{\text{eq.}}} = \frac{\text{intracellular osmolar concentration at equilibrium}}{\text{intracellular osmolar concentration at first instant}}$$

Thus, for experiments in 40 per cent sea water the calculation would be  $\frac{100}{100 + x}$   
 $= \frac{40}{100}$ . Equilibrium volumes were measured directly in several experiments by

keeping the cells at low temperature until no further increase in volume occurred. In 80 and 60 per cent sea water the observed volume at equilibrium was 3 per cent less than the calculated; in 40 per cent sea water, 10 per cent less.

independent of the temperature. In the bottom row are given the velocity constants for the different temperatures.

It is seen that the value of  $k$  increases rapidly with the temperature, from 0.011 at 11° to 0.024 at 20.5°. The effect of temperature is shown graphically in Fig. 2, where  $\log \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  is plotted against time. The slope of the lines gives values of  $k$  at various temperatures.

TABLE I.

*A Typical Experiment on Swelling of Unfertilized Arbacia Eggs in 40 Per Cent Sea Water. Volumes  $\times 10^{-2}$  Are Given in Cubic Micra. Each Number Represents the Mean Volume of 3 Cells. In the Bottom Row Is Given the Velocity Constant for Each Temperature.*

Time.	11.0°C.	13.1°C.	14.8°C.	17.6°C.	20.5°C.	22.8°C.	24.8°C.
<i>min.</i>							
1	2310	2330	2180	2437	2310	2340	2365
2	2388	2355	2330	2581	2460	2515	2580
3	2482	2438	2400	2665	2640	2717	2730
4	2518	2615	2496	2767	2782	2835	2975
5	2580	2645	2570	2852	2905	2978	
6	2645	2745	2665	2980	2980	3195	3240
8	2768	2850	2770	3120	3145	3465	3510
10	2875	3010	2880	3370	3390	3736	3815
12	2993	3105	3004	3540	3615	3940	4105
14	3105	3290	3120	3758	3817	4140	4210
16	3215	3370	3215	3836	3920		
18	3271	3540	3346		4091		
20		3585					
$k =$	0.011	0.014	0.012	0.021	0.024	0.033	0.036

Over the range studied the value of the temperature coefficient is fairly uniform at about 2.4, that is, the velocity constant would be increased 2.4 times by a rise in temperature of 10°. In other experiments  $Q_{10}$  varied between 2 and 3, but was always fairly uniform throughout the temperature range in any given experiment (see Table II).

This magnitude of the temperature coefficient is a common one in chemical reactions. But in pure diffusion processes, the temperature coefficient is usually low, about 1.2 or 1.3.

In the last 2 years another measure for the effect of temperature on biological processes has been strongly advocated by Crozier and others (2). It was found by Arrhenius that in certain chemical reactions the velocity is proportional to the reciprocal of the absolute temperature, or,  $\ln \frac{k_1}{k_2} = \frac{q}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right)$  where  $k_1$  and  $k_2$  are velocity constants at different temperatures,  $q$  is heat of activation,  $R$  is the gas constant, and  $T$  the absolute temperature.

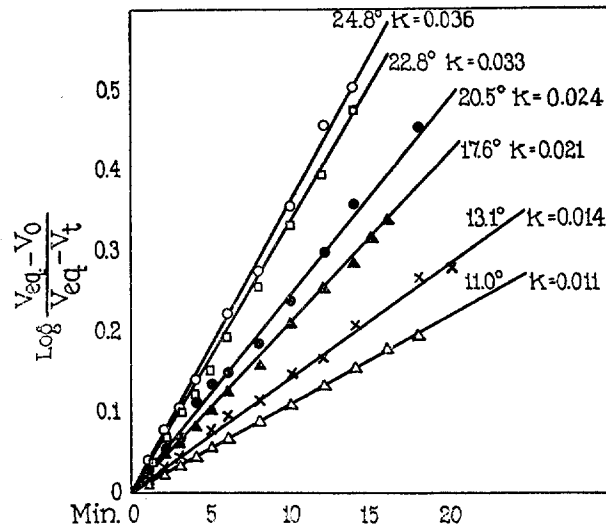


FIG. 2. The effect of temperature on the rate of swelling of unfertilized *Arbacia* eggs in 40 per cent sea water.  $\text{Log} \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t}$  is plotted against time. The resulting graph is for each temperature a straight line, the slope of which gives the value of  $k$ , the velocity constant. The experiment at  $14.8^\circ$  has been omitted to avoid confusion. Data are taken from Table I.

For chemical reactions the value of  $q$  usually lies between 10,000 and 30,000 calories, while, if calculated for diffusion processes, its value would be about 7,000.

In Fig. 3, the logarithms of the velocity constants have been plotted against the reciprocals of the absolute temperature. This graph is a composite of three experiments in 40 per cent sea water. The value of  $q$  or  $\mu$ , as it is often called, is about 16,000—corresponding to that of a chemical reaction.

TABLE II.  
*Summary of Seven Experiments. The Table Shows That the Velocity Constant,  $k$ , Varies Directly with the Temperature, and Inversely with the Concentration of the Sea Water. In the Bottom Line Are Given the Values of  $\mu$  for the Several Experiments.*

Sea water Distilled water 40		Sea water Distilled water 60		Sea water Distilled water 80		Sea water Distilled water 20	
Temp- erature.	$k$	Temp- erature.	$k$	Temp- erature.	$k$	Temp- erature.	$k$
°C.		°C.		°C.		°C.	
9.9	0.016	11.8	0.037	11.0	0.011	11.7	0.015
12.3	0.017	14.6	0.071	13.1	0.014	12.9	0.013
13.2	0.020	16.8	0.047	14.8	0.012	15.2	0.015
15.2	0.023	21.3	0.089	17.6	0.021	17.7	0.020
16.2	0.027	22.3	0.085	20.5	0.024	20.9	0.034
18.5	0.029	24.8	0.124	22.8	0.033	24.3	0.044
18.8	0.024	26.8	0.101	24.8	0.036	25.9	0.057
19.6	0.041	29.8	0.157				
21.8	0.043						
26.3	0.069						
28.3	0.061						
$\mu \dots$	14,500		14,800		15,600		19,000
					16,500		13,400

Similar results were obtained in three experiments in 60 per cent sea water and in one experiment in 20 per cent sea water. The value of  $\mu$  in these several experiments ranged from 13,000 to 19,000, but was fairly uniform throughout the temperature range in any one experiment.

It may be concluded from these data that the temperature coefficient of osmotic swelling in living cells is regulated by factors other than diffusion. That the effect of temperature lies in alteration in the permeability of the cell membrane to water seems probable, but at present cannot be proved.

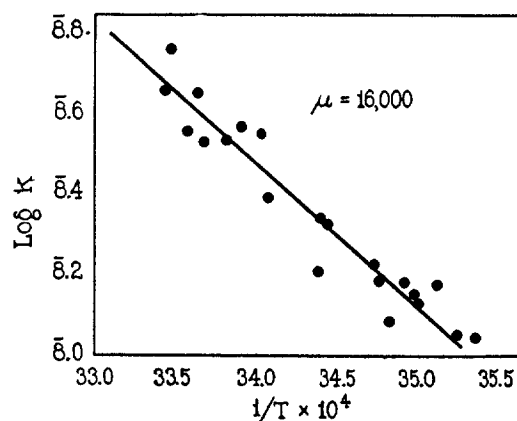


FIG. 3. Swelling of unfertilized *Arbacia* eggs in 40 per cent sea water. The logarithms of the velocity constants of three experiments are plotted against the reciprocals of the absolute temperature. The slope of the line gives the value of  $\mu$  (= 16,000).

The rate of water absorption in its relation to temperature has hitherto been studied chiefly with plant tissue, either by the method of plasmolysis or by weighing the material. High temperature coefficients have been reported by Krabbe (3), van Rysselberghe (4), Brown (5), and Delf (6). With animal material the most significant work is that of Bialaszewicz (7), who found that unfertilized frog eggs took up water five times more rapidly at 20° than at 10°. But, as Kanitz (8) points out, his method is open to the objection that he compared the amounts of water taken up in equal times instead of the times required to produce a certain fraction of the total amount of swelling.



The literature on the effect of temperature on absorption of water has been reviewed by Kanitz (8) and Stiles (9). Przibram has lately collected in tabular form all available data of biologic interest on temperature coefficients (10).

While in the light of present knowledge it seems likely that the effect of temperature is exerted chiefly on permeability of the cell membrane, yet it is possible that the effect is a more complicated one. Thus it might be suggested that changes in viscosity possibly play some part in establishing a high temperature coefficient. Available evidence on this point, though not conclusive is against such a supposition. Heilbrunn (11) has studied the effect of temperature on the viscosity of several marine eggs. With *Cumingia* eggs he found that the curve of viscosity passes through a maximum at about 15°. As the temperature rises above 15° or falls below it, the protoplasm becomes more and more fluid until suddenly it undergoes a sharp increase in viscosity, at about 4° and 30° respectively. In the *Arbacia* egg the general relation of viscosity to temperature is similar.

If the temperature coefficient of osmotic swelling were largely influenced by viscosity changes in the cell, we should expect different values of  $Q_{10}$  in different parts of the temperature range. This, however, is not the case for, as has been stated above, the value of the temperature coefficient remains practically constant over the range studied, from 10° to 30°. It therefore seems unlikely that viscosity greatly influences the temperature coefficient of osmotic swelling in living cells.

#### *Effect of the Osmotic Pressure of the Solution.*

If one egg is placed in a mixture of sea water and distilled water containing 60 per cent sea water, and another egg is placed in 40 per cent sea water, it is evident that the latter egg will take up water more rapidly and swell to a greater volume than the former. But it is not evident which egg will reach equilibrium first (or, more correctly, swell half way to equilibrium first).

The answer to this question can be obtained from the data already given (Table II). As seen in Fig. 4, it took much longer for cells to swell in 20 per cent sea water than in 40, and longer in 40 per cent than in 60 per cent. The velocity constants become correspondingly lower

as the concentration of sea water is decreased. In the typical example given in the chart, at  $15 \pm 0.2^\circ$  for 80 per cent sea water,  $k = 0.072$ , for 60 per cent sea water,  $k = 0.024$ , for 40 per cent, 0.012, and for 20 per cent, 0.006.

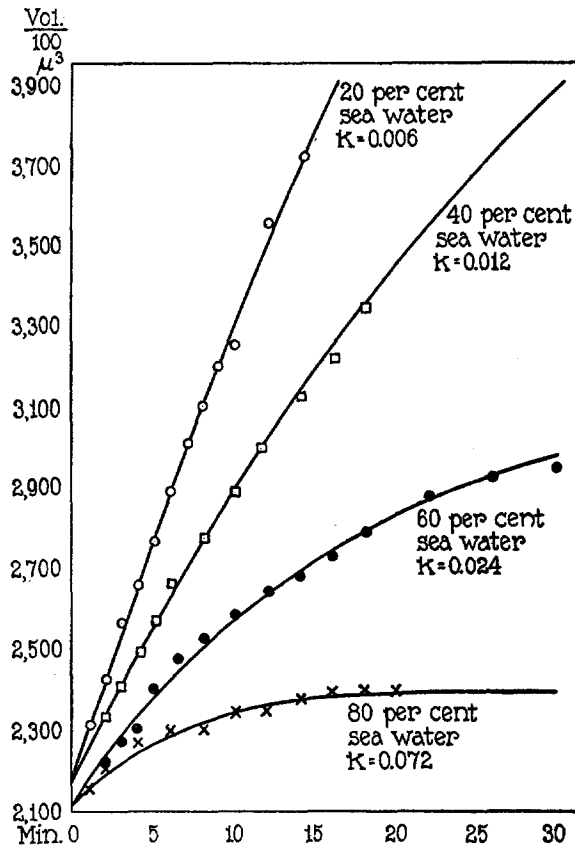


FIG. 4. The effect of osmotic pressure of the solution on the rate of swelling of unfertilized *Arbacia* eggs, at  $15 \pm 0.2^\circ\text{C}$ . Volumes are plotted against times. Points represent *observed* volumes; curves represent *calculated* volumes  $\left( kt = \ln \frac{V_{\text{eq.}} - V_0}{V_{\text{eq.}} - V_t} \right)$ .

Our data are not at present adequate for working out the law that connects concentration with rate of swelling, but the general principle stands out clearly, that the more dilute the sea water is made, the longer it takes the cell to swell.

## SUMMARY.

The rate of swelling of unfertilized sea urchin eggs in hypotonic sea water was investigated. Analysis of curves leads to the following conclusions.

1. The rate of swelling follows the equation,  $kt = \ln \frac{V_{eq.} - V_0}{V_{eq.} - V_t}$  where  $V_{eq.}$ ,  $V_0$ , and  $V_t$  stand for volume at equilibrium, at first instant, and at time  $t$ , respectively, the other symbols having their usual significance. This equation is found to hold over a wide range of temperatures and osmotic pressures. This relation is the one expected in a diffusion process.

2. The rate of swelling is found to have a high temperature coefficient ( $Q_{10} = 2$  to 3, or  $\mu = 13,000$  to 19,000). This deviation from the usual effect of temperature on diffusion processes is thought to be associated with changes in cell permeability to water. The possible influence of changes in viscosity is discussed.

3. The lower the osmotic pressure of the solution, the longer it takes for swelling of the cell. Thus at 15° in 80 per cent sea water, the velocity constant has a value of 0.072, in 20 per cent sea water, of 0.006.

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