OSMOTIC PROPERTIES OF THE EGG CELLS OF THE OYSTER
(OSTREA VIRGINICA)

BY BALDUIN LUCKÉ AND R. A. RICCA
(From the Laboratory of Pathology, School of Medicine, University of Pennsylvania,
Philadelphia, Pennsylvania, and the Marine Biological Laboratory,
Woods Hole, Massachusetts)

(Received for publication, July 8, 1941)

There is abundant evidence that living cells function as osmotic systems (1). Quantitative studies, however, on osmotic equilibria and on the kinetics of osmosis with animal cells have been hindered by lack of suitable material. To serve as osmometers for experimental purposes, cells should meet the following requirements: 1. They should be obtainable as isolated cells of a single type. Cell aggregates, such as tissues or organs are unsatisfactory, since they contain not only cells of more than one type, but also blood vessels, lymphatics, tissue spaces, and intercellular substances—all having different properties. 2. The cells should be free to undergo changes of volume, since osmotic phenomena involve such changes. In tissues, free changes of cell volume are interfered with by mutual pressure of component parts; even certain isolated cells are unsuitable, because of the rigidity of their surface membranes (2). The size and shape of the cells should be such as to permit accurate measurement. Few types of animal cells are known that fulfill these requirements; wherefore quantitative investigations of osmotic phenomena, and of the related property of permeability, have been confined almost entirely to two types of cells: mammalian erythrocytes and echinoderm egg cells. In the course of search for new material, the eggs of two other forms of marine invertebrates which belong to different phyla—the annelid Chaetopterus pergamentaceus and the mollusc Cumingia telenoides—have also proved well adapted for osmotic studies (2). Unfortunately, neither of these forms were readily available, nor was the number of eggs from any single specimen of Cumingia sufficient for some experiments.

Further attempts to find suitable cells disclosed that the unfertilized egg of the oyster is excellent material. This animal is widely distributed, its eggs are very abundant, and are obtainable without difficulty. To stimulate shedding of eggs, oysters are placed in bowls of sea water, the temperature of which is suddenly raised to 32–35°C., after which the water is allowed to cool.¹ When

¹ Eggs thus obtained are entirely uninjured and may be fertilized. The mechanism of the spawning of oysters has been extensively studied by Dr. Paul S. Galtsoff, of

The Journal of General Physiology
OSMOTIC PROPERTIES OF OYSTER EGG CELLS

first shed, the eggs are usually ovoid or pear-shaped, but after repeated washing in sea water they become, in a high percentage of samples, uniformly spherical (Fig. 1); the diameter was found to range (in samples from 10 animals) from 50.8 to 54.4μ, with 52.2μ the average. Compared to other marine eggs, the egg of the oyster is relatively small.²

For measuring the eggs a diffraction method was used, the details and advantages of which have been described in previous communications (2, 4). Each measurement of the diffraction pattern gives a statistical average of many thousands of individual cells. Accurate measurements can be made at intervals of 5 seconds (and under favorable conditions in less time); this is a particular advantage when, as in oyster eggs, osmotic volume changes are rapid.

![Fig. 1. Egg cells of Ostrea. The photograph shows the eggs as isolated cells of spherical shape and of uniform size.](image)

The present experiments deal with the question "How good a natural osmometer is the unfertilized egg of the oyster?" To answer this question we have studied: the equilibria the cells attain in several different concentrations of sea water, the reversibility of the volume changes, and the course of osmotic swelling and shrinking. From the data obtained, the permeability of the cells to water and to two solutes, diethylene glycol and glycerol has been computed. The results of these experiments will now be taken up in order.

² For example, *Chaetopterus*, 100μ; *Arbacia* 75μ, *Cumingia* 65μ, approximately.

If cells were perfect osmometers, their volume should increase in direct proportion as the osmotic pressure of the medium with which they are in equilibrium decreases; stated alternatively, the product of volume and pressure should be constant. It has been shown (1) for several types of living cells (plant cells, erythrocytes, marine eggs) that this relation holds provided that cell volume is corrected for osmotically inactive cell content. The significant volume is not the volume of the cell as measured, but is this volume diminished by a quantity which represents the space occupied by osmotically inactive material. Boyle's law as applied to cells becomes:

$$P (V - b) = K \text{ constant}$$

where \(P\) is the osmotic pressure of the dissolved substances in the cell (this pressure at equilibrium is identical with the known osmotic pressure of the

![Graph showing applicability of Boyle's law to oyster eggs. Results of six equilibrium experiments. Volumes of cells are plotted against reciprocals of corresponding concentrations of sea water with which they are in equilibrium (concentration of ordinary sea water = 1.0). The graph drawn weighs all the observed points and is linear; it represents the equation \(P (V - b) = \text{constant}\). The volume of osmotically inactive cell contents, \(b\), is obtained by extrapolation of the graph to \(\frac{1}{C} = 0\) (this extrapolation is not shown in the figure). The mean value of \(b\) is 44 per cent of the initial cell volume.]
medium), \( V \) is the measured cell volume, and \( b \) the space within the cell occupied by osmotically inactive material (this term includes bound water if any exist).

The applicability of Boyle's law was tested in duplicate or triplicate experiments with eggs from six oysters. The cells were measured in ordinary sea water, and in several dilutions of sea water with which they had been brought to equilibrium. The results are shown in Fig. 2, in which the observed volumes are plotted against the reciprocals of the corresponding concentrations of sea water; a straight line may be fitted to the points, which is in accordance with equation 1. Thus it is evident that the egg cells of the oyster closely obey the law of Boyle-van't Hoff, provided that the cell volume is corrected for osmotically inactive contents.

The correction factor, \( b \), may be computed from the observed volumes arithmetically, or, more simply by extrapolating the linear graph of Fig. 2; the intercept with the axis \( \frac{1}{C} = 0 \) gives directly the mean volume of osmotically inactive cell content. The value of \( b \) computed in this way\(^3\) is equal to 44 per cent of the cell volume in ordinary sea water. The amount of \( b \) for the oyster egg is much greater than \( b \) for the *Arbacia* egg which has only 12 per cent of dead space \((4, 5)\); on the other hand, the amount for the oyster egg is approximately the same as \( b \) for mammalian erythrocytes. In different plant cells, even greater variations of \( b \) values are found; for certain yeasts the correction factor is 64 per cent of vell volume; on the other hand, for certain other cells which have large sap vacuoles and an extremely thin layer of protoplasm, \( b \) approaches zero \((1)\).

2. **Reversibility of Osmotic Swelling; Preservation of Semipermeability**

In the experiments discussed in the preceding section, the relation of cell volume to osmotic pressure was investigated over a wide range of concentration of sea water—from 100 to 40 per cent, corresponding to pressures of 22 to 8.8 atmospheres. At all concentrations, the volume of the cells reached a steady state, which for a number of hours remained unaltered. This fact made it unlikely that swelling in the hypotonic solutions had led to injury and consequently to loss of semipermeability.

Reversibility of the volume changes was now tested by returning the swollen cells to ordinary sea water, and measuring them after they had again come into equilibrium. The results of a representative experiment, with duplicate meas-

\(^3\) A graphic method for evaluating \( b \) is perhaps preferable to arithmetic computation, for such computations are greatly affected by slight accidental errors of measurement, whereas a graphic method at once weighs all the points. The values of \( b \) obtained arithmetically ranged from 31 to 56 per cent, with two-thirds of the values being between 40 and 48 per cent.
measurements for each concentration, are shown in Table I. It is seen that the original and final volumes are in good agreement. It may consequently be concluded that osmotic volume changes of oyster eggs (within the range stated) are reversible, and that the semipermeability of these cells remains intact.

3. Kinetics of Osmosis

The rates at which the oyster eggs attain osmotic equilibria were now studied as follows. After measuring their initial size, the cells were mixed with diluted sea water in such proportion as to give a suspension of suitable density of cells in a medium of known concentration. This suspension was quickly introduced into the chamber of the apparatus and the changes in

<table>
<thead>
<tr>
<th>Initial size in 100 per cent sea water</th>
<th>Size in equilibrium with</th>
<th>Final volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 → 40</td>
<td>100 → 50</td>
</tr>
<tr>
<td>72,780µ² (51.8)µ</td>
<td>134,100</td>
<td>110,300</td>
</tr>
<tr>
<td>73,620µ² (52.0)µ</td>
<td>132,200</td>
<td>110,300</td>
</tr>
</tbody>
</table>

size of the cells were followed by making readings of the diffraction pattern until a steady state was reached.

The course of swelling and of shrinking during endosmosis and exosmosis, respectively, is illustrated in Figs. 3 and 4. It is seen that either swelling or shrinking is completed in considerably less than 5 minutes. Osmotic changes of oyster eggs are rapid when compared to similar processes in *Arbacia* eggs, which require approximately 30 minutes to reach equilibrium.

With data obtained from the measurements of oyster eggs made during swelling or shrinking in anisotonic solutions we can test the applicability of equations (based upon theoretical grounds) which for other types of living cells have satisfactorily described the course of osmotic volume changes. It has been shown (4,6) that rate of change of cell volume is, at any instant, proportional to the area of the surface and to the difference in osmotic pressure existing between the cell and the surrounding medium; i.e.,

\[
\frac{dV}{dt} = K \cdot S \cdot (P - P_e)
\]
OSMOTIC PROPERTIES OF OYSTER EGG CELLS

Fig. 3. Course of swelling of cells in two concentrations (40 and 50 per cent) of hypotonic sea water. Permeability constants ($K$) are in $\mu^3$/min./$\mu^2$/atmosphere.

Fig. 4. Course of shrinking of cells upon transfer from 40 per cent sea water (with which they had previously been brought to equilibrium) to ordinary sea water.

where $\frac{dV}{dt}$ is rate of change of cell volume due to passage of water, either inward or outward; $S$ is the area of the cell surface, $P$ the osmotic pressure of the in-
terior of the cell, $P_s$ that of the surrounding medium, and $K$ is a factor of proportionality. Correcting cell volume for $b$ (equation 1), and making necessary substitutions before integrating, equation 2 when integrated becomes:

$$k(36\pi)^4 P_0 (V_0 - b)t = (V_s - b) \left[ \left(1 - \frac{b}{V_s}\right) V_s^4 \left(\frac{1}{V^4} \ln \frac{V_0 - V}{(V_s^4 - V^4)^{1/2}} + \frac{2V^4}{\sqrt{3} V_s^4} \tan^{-1} \frac{2V^4}{\sqrt{3} V_s^4}\right) - 3V^4 \right] V_{t=0}$$

In the application of this equation to the experimental data it will be convenient to designate the entire right hand side of equation 3 as $f(V, V_s)$. Using the data of two representative swelling experiments and plotting $\frac{1}{(36\pi)^4 \cdot P_0 (V_0 - b) \cdot f(V, V_s)}$ against time, the fit obtained is linear (Fig. 5). This is in harmony with the demands of the equation, which may therefore be regarded as describing satisfactorily the course of osmotic volume changes of the oyster egg.

4. Permeability of the Oyster Egg to Water during Endosmosis and Exosmosis

The permeability of a cell to water, $K$ of equations 2 and 3, is defined as the amount of water that enters or leaves the cell in unit time, through unit of cell surface, as a result of a unit of pressure. In convenient units, permeability is expressed as the number of cubic micra of water that pass per minute through each square micron of cell surface, per atmosphere of difference in osmotic pressure between the cell interior and the medium. This quantity may be obtained directly from the slopes of linear graphs such as are shown in Fig. 5.

The equation as here given is a somewhat simplified form of that previously published (6). The expression $\frac{V_s - V}{(V_s^4 - V^4)}$ in the present equation has been obtained by multiplying numerator and denominator of $\frac{V_s^4 + (V_s V)^4 + V^4}{(V_s^4 - V^4)^2}$ in the old form by $V_s^4 - V^4$; this eliminates calculation of the three quantities $V_s^4, V^4$, and $(V_s V)^4$, none of which appears elsewhere in the equation. We are obliged to Dr. T. N. Harris, of the Laboratory of Bacteriology, University of Pennsylvania, for drawing our attention to this simplification.

The symbol $V_s$ indicates volume of the cell at equilibrium, $V_0$ and $P_0$ are volume and pressure, respectively, in ordinary sea water. Suggestions for the construction of conversion charts, by means of which the computation is rendered a relatively easy task, are given on page 409 of a previous publication (6).

More correctly this equation describes the swelling and shrinking process from the beginning to approaching equilibrium. As the cells approach their equilibrium, deviations from equation 3 appear which are as yet unexplained.
In these experiments, the values of $K$ for water entering cells were the same; namely 0.6. In similar experiments with eggs from four other oysters which were caused to swell in either 40 or 50 per cent sea water, permeability ranged from 0.5 to 0.6, with most values close to an average permeability of 0.6 for endosmosis.

For the reverse process, exosmosis, permeability may be computed by two methods. In the first method, equation 3 is used and the value of permeability derived as has been described for endosmosis. In three duplicate experiments, cells previously swollen in 40 or 50 per cent sea water were measured during shrinking after their return to ordinary sea water; in these experiments the values of permeability for exosmosis averaged 0.5.

By a second method, based upon entirely different procedures, values of permeability were slightly, but not significantly, higher. This method, developed by Jacobs (7) makes it possible to evaluate permeability to water and to solutes as well. The cells are measured in ordinary sea water, and then transferred to a medium made by dissolving in sea water 0.5 M of a relatively harmless substance to which the cells are permeable—in the present experi-

![Figure 5. Applicability of equation 3 to swelling of oyster eggs in 50 per cent sea water.](image)
ments, diethylene glycol or glycerol. In this initially hypertonic medium the cells at first shrink, but as the solute penetrates, a minimum volume is attained and then the water lost during shrinking is gradually regained (Fig. 6). From

![Diagram showing volume changes over time for diethylene glycol and glycerol](image)

**Fig. 6.** Two experiments with eggs from different oysters showing the course of shrinkage and subsequent swelling when placed in a solution of 0.5 M diethylene glycol (upper graph) or glycerol (lower graph) in sea water. Volumes correspond to averages of diffraction scale measurements. Permeability constants for solutes are in mols × 10^{-18}/min./μ²/mol per liter.

For details of computation see (7). In the determinations of the permeability constants for water by this method, the volume of osmotically inactive cell contents has not been considered. Probably no serious error is thereby introduced for such correction is of less importance when dealing with volumes which differ only slightly from the initial size; i.e., less than 15 per cent in the present experiments.
OSMOTIC PROPERTIES OF OYSTER EGG CELLS

The significant data of five experiments with diethylene glycol as the penetrating solute are recorded in Table II; in the last column are stated the values of permeability to water; the average is 0.7. In three similar experiments with glycerol, the average is 0.6. These values are but slightly higher than the values computed from the course of shrinking by the first method.

By two entirely different methods, therefore, the permeability of the oyster egg is found to be 0.5–0.7 for exosmosis. For endosmosis, as shown above, the values obtained are exactly the same. This agreement is of considerable interest, since with another type of living cell, the Arbacia egg, water appears to leave the cell more readily than it enters: the calculated value of permeability for exosmosis is at least 25 per cent greater than the value of endosmosis. No such difference is found for the oyster egg, which in this respect may be regarded as a more perfect osmotic system.

5. Permeability to Diethylene Glycol and to Glycerol

The ingenious method of Jacobs permits, as has been stated, simultaneous measurement of cell permeability to water and to dissolved substances. The necessary data for computation of either are the same.

In preliminary experiments, we attempted to measure the permeability of oyster eggs to ethylene glycol, a solute which because of its convenient rate of

---

TABLE II

Permeability of eggs of Ostrea to water, and to diethylene glycol, at 22°C. Permeability values are given in the last two vertical columns. Permeability to water is expressed as number of cubic micra of water which enter the cell, per minute, through each square micron of cell surface, per atmosphere of difference in osmotic pressure. The values for diethylene glycol are the number of mols $\times 10^{-16}$ of this substance which pass per minute through each square micron of cell surface, at a concentration difference of 1 mol per liter.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial volume</th>
<th>Minimum volume</th>
<th>Time to minimum volume</th>
<th>$V_m/V_o$</th>
<th>Permeability to diethylene glycol $\times 10^{-16}$</th>
<th>Permeability to water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68,600</td>
<td>60,100</td>
<td>0.38</td>
<td>0.875</td>
<td>29.6</td>
<td>0.73</td>
</tr>
<tr>
<td>2</td>
<td>78,390</td>
<td>66,240</td>
<td>0.33</td>
<td>0.845</td>
<td>26.6</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>77,950</td>
<td>69,500</td>
<td>0.42</td>
<td>0.892</td>
<td>28.2</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>73,200</td>
<td>61,600</td>
<td>0.38</td>
<td>0.845</td>
<td>24.2</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>70,690</td>
<td>63,770</td>
<td>0.50</td>
<td>0.902</td>
<td>27.3</td>
<td>0.50</td>
</tr>
<tr>
<td>Mean</td>
<td>73,770</td>
<td>64,240</td>
<td>0.40</td>
<td>0.872</td>
<td>27.2</td>
<td>0.70</td>
</tr>
</tbody>
</table>

---

$^7$ For Chaetopterus and Cumingia we do not have as yet sufficient data on exosmosis for computation by equation 3. It is of interest, however, to note that permeability values for endosmosis by this equation agree remarkably well with values computed by Jacobs' method for shrinking.
penetration has been a favorite with other types of cells (2, 8). But oyster eggs proved so highly permeable to ethylene glycol that our measurements were not sufficiently accurate. We therefore used a related substance, diethylene glycol, which, as Stewart and Jacobs had shown with Arbacia eggs (8), penetrates more slowly. This substance was found suitable for quantitative measurements with oyster eggs; it penetrates into these cells rapidly but at a rate readily measurable by the diffraction method. The values of permeability of this substance are shown in the column next to the last of Table II. The figures express the number of mols \(10^{-16}\) of diethylene glycol which enter the cells per minute through each square micron of surface, at a concentration difference of 1 mol per liter; the average value is 27.2.

In other experiments, glycerol was chosen as the solute to be studied, since we had previously observed that this substance penetrates very slowly into one type of cell, the Arbacia egg, whereas it penetrates readily into another, the Chaetopterus egg. The oyster egg proved still more permeable to glycerol; in three experiments its permeability averaged 20.7.

6. Comparison of Permeability Values

It may now be of interest to compare the values of permeability obtained for the oyster egg with values previously obtained by us for three other invertebrate eggs. This comparison is restricted to determinations made under similar experimental conditions, using the same technique of measuring cell volume and the same methods for computing permeability. The values are collected in Table III. Inspection of the table brings out first, that for each of the four types of cells permeability to water computed from the course of swelling (by equation 3) is in good agreement with values obtained from the course of shrinking by Jacobs' method; with the exception of the slightly higher permeability obtained by Jacobs' method for Arbacia, the determinations for the other cells are almost identical. It follows, as a corollary, that permeability to water is not affected by the presence in the medium (sea water) of the non-electrolytes, ethylene glycol, diethylene glycol, or glycerol; moreover, when the object of experiments is solely evaluation of cell permeability to water, it becomes a matter of convenience which of the two methods is employed.

It will next be noted that the oyster egg is considerably more permeable to water and to solutes than are the other three kinds of marine eggs. Thus, for water the ratio is, very nearly: Arbacia, 1; Chaetopterus, 4; Cumingia, 4; Ostrea, 6.

Ethylene glycol penetrates three of the cells in approximately the same ratio as water, but for oyster eggs the ratio is probably much higher. Diethylene glycol has the very high permeability of 27 for Ostrea; this is ten times the value obtained by Steward and Jacobs for Arbacia (8).

Glycerol penetrates into the Arbacia egg at an extremely slow rate; hence
accurate determinations of time required to attain minimum value are difficult, and the present permeability value must be regarded as an approximation. But Chaetopterus is freely permeable to glycerol, more permeable in fact than is Arbacia to the readily penetrating ethylene glycol. Ostrea, by contrast, is only three times as permeable as Chaetopterus. Taking the value for Arbacia as sufficiently accurate for obtaining ratios, we find the following: Arbacia, 1; Chaetopterus, 210, and Ostrea, 690.

From these several comparisons it becomes evident that the relative ease of penetration of water is not paralleled by permeability to solutes. For every solute, as well as for water, the permeability of the four kinds of cells is specific. This finding is in harmony with observations made on other kinds of living cells (9).

### TABLE III

Comparative permeability to water, and to ethylene glycol, diethylene glycol, and glycerol. Values for water are given in $\mu^3$/min./$\mu^3$/atmosphere; they are computed from the course of swelling by equation 3 (1st method), and from the course of shrinking by Jacobs method (2nd method). Permeability constants for the solutes are in mols $\times 10^{-15}$/min./$\mu^3$/mols per liter. All values are based on measurements made by the diffraction method at a temperature of $22^\circ \pm 0.5^\circ$C.; values for Arbacia, Chaetopterus, and Cumingia have been taken from previous publications (2, 4).

<table>
<thead>
<tr>
<th>Kind of egg</th>
<th>Water (1st method)</th>
<th>Water (2nd method)</th>
<th>Ethylene glycol</th>
<th>Diethylene glycol</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbacia</td>
<td>0.10; 0.12</td>
<td>0.17</td>
<td>3.5</td>
<td>14.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Chaetopterus</td>
<td>0.44</td>
<td>0.46</td>
<td>15.6</td>
<td>27.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Cumingia</td>
<td>0.46</td>
<td>0.41</td>
<td>15.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostrea</td>
<td>0.60</td>
<td>0.6; 0.7</td>
<td>(Very rapid penetration)</td>
<td>20.7</td>
<td></td>
</tr>
</tbody>
</table>

**SUMMARY**

Investigations of the osmotic properties of oyster eggs by a diffraction method for measuring volumes have led to the following conclusions:

1. The product of cell volume and osmotic pressure is approximately constant, if allowance is made for osmotically inactive cell contents (law of Boyle-van't Hoff). The space occupied by osmotically inactive averages 44 per cent of cell volume.

2. Volume changes over a wide range of pressures are reversible, indicating that the semipermeability of the cell during such changes remains intact.

3. The kinetics of endosmosis and of exosmosis are described by the equation, \[
\frac{dV}{dt} = K \cdot S \cdot (P - P_s),
\] where $dV$ is rate of volume change; $S$, surface area of cell, $(P - P_s)$, the difference in osmotic pressure between cell interior and medium, and $K$, the permeability of the cell to water.
4. Permeability to water during endosmosis is 0.6μg of water per minute, per square micron of cell surface, per atmosphere of pressure. The value of permeability for exosmosis is closely the same; in this respect the egg cell of the oyster appears to be a more perfect osmometer than the other marine cells which have been studied. Permeability to water computed by the equation given above is in good agreement with computations by the entirely different method devised by Jacobs.

5. Permeability to diethylene glycol averages 27.2, and to glycerol 20.7. These values express the number of mols × 10⁻¹⁵ which enter per minute through each square micron of cell surface at a concentration difference of 1 mol per liter and a temperature of 22.5°C.

6. Values for permeability to water and to the solutes tested are considerably higher for the oyster egg than for other forms of marine eggs previously examined.

7. The oyster egg because of its high degree of permeability is a natural osmometer particularly suitable for the study of the less readily penetrating solutes.

BIBLIOGRAPHY
1. Lucké, B., and McCutcheon, M., Physiol. Rev., 1932, 12, 68.