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<td>SHINOZAKI, Jutaro</td>
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<td>Citation</td>
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The Permeability of the Sea-Urchin Egg to Water and to Ethylene Glycol

By

Jutaro Shinozaki

(Zoological Institute, Hokkaido University)

(With 4 Text-figures)

In view of the fact that living cells and tissues are bathed in watery medium and the exchange of substances between cells and medium takes place through the plasmic membrane, the phenomena connected with osmosis are very important and yet a fundamental problem. Hence, a number of investigations on the permeability of the plasmic membrane were made by many authors on various types of cells, the most of these, however, were concerned mainly with qualitative characters, and quantitative studies are comparatively few.

Lucké, Hartline and McCutcheon ('31) theoretically treated the permeability in sea-urchin egg and derived from certain simplifying assumptions a differential equation which described the time course of the volume change of the cell. This equation can be integrated. Applying the integrated equation, permeability constant to water was computed from the time course of volume change of the cells placed in hypotonic and hypertonic sea water. Then after, Jacobs ('33) obtained the simultaneous differential equations which expressed the permeability of a cell both to water and to penetrating substance. When sea-urchin eggs are placed in ordinary sea water solution of such a penetrating substance as ethylene glycol, the eggs first shrink and then, as ethylene glycol enters, they return to the original volume. Determining the original and minimum volumes of the egg and the time required for attainment of the minimum volume, the permeability constants to water and to ethylene glycol were read off from a chart which was constructed from numerical integration of these equations by Runge-Kutta’s method. Jacobs adopted the

1) Contribution No. 246 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

one set of units* for ease in integration. In his original treatment the correction for \( b \), the volume which is occupied by osmotically inactive fraction in the cells, was neglected. This correction was first made by Iida (*43). The permeability of the cell both to water and to ethylene glycol may be described by the following equations, applying the Jacobs' units and making the correction for \( b \).

\[
\frac{dS}{dt} = k_1 V^{2/3} \left( 0.510 - \frac{S}{V - b} \right)
\]

and

\[
\frac{dV}{dt} = k_2 V^{2/3} \left( \frac{1 - b + S}{V - b} - 1.510 \right)
\]

where \( dS/dt \) is the rate of entrance of ethylene glycol, \( dV/dt \) the rate of volume change of cell due to movement of water, \( S \) amount of penetrating substance which has entered into cell at time \( t \), \( b \) volume occupied by osmotically inactive fraction, \( V \) volume of cell, \( V^{2/3} \) area of cell surface in Jacobs' unit, \( k_1 \) and \( k_2 \) permeability constants to penetrating substance and to water respectively. In the equation (1), 0.510 is the experimental concentration of ethylene glycol in Jacobs' unit. In the equation (2), 1 in the numerator of the first term within the parenthesis represents the amount of the osmotically active substances contained in the original volume (or unit volume) of the cell, when it is assumed that the cell contains no osmotically inactive substances. And the second term, 1.510 is the sum of the concentrations of ethylene glycol and salts in sea water. If the cells which have shrunk at first and then swelled up to their original volume in sea water containing the penetrating substance, are replaced in ordinary sea water, these first may undergo osmotic swelling followed by shrinking passing through a maximum volume. By making a similar treatment as Jacobs, we can evaluate the permeability constant of the cell to penetrating substance during exosmosis. In this case the external concentration of the penetrating substance is equal to zero, so that the equations (1) and (2) become

\[
\frac{dS}{dt} = -k_1 V^{2/3} \frac{S}{V - b}
\]

and

\[
\frac{dV}{dt} = k_2 V^{2/3} \left( \frac{1 - b + S}{V - b} - 1 \right).
\]

Although the permeability constant of the cell to water was determined both endosmotically and exosmotically, studies on the permeability to penetrating

---

* The one set of units used by Jacobs is defined as follows: unit concentration is osmolar concentration of ordinary sea water, unit volume initial volume of cell, unit surface initial surface of cell and unit amount osmolar equivalent of solutes contained in unit volume of medium which has unit concentration. And one minute was taken as unit time.
substance carried out by Jacobs and by others were confined to the endosmosis. Thence, by using the above equations derived from Jacobs' equations, the author attempted to estimate the permeability constants to ethylene glycol during endosmosis and exosmosis in sea-urchin egg. Further the Lucké, Hartline and McCutcheon's method was also employed and the values were compared with those obtained by Jacobs' method.

II

The materials used in the present work were the unfertilized eggs of two species of sea-urchins, *Strongylocentrotus intermedius* and *S. nudus*. The experiment with the former was carried out at Akkeshi Marine Biological Station, and that with the latter at Oshoro Marine Biological Station. The osmolar concentration of sea water estimated by measuring the freezing point depression is 0.98 M nonelectrolyte in Akkeshi Bay and 0.99 M in Oshoro Bay. We can, therefore, obtain the sea water solution of ethylene glycol, of which concentration is 0.510 in Jacobs' concentration unit, by preparing 0.500 M solution of ethylene glycol with Akkeshi sea water, and 0.505 M solution with Oshoro sea water. The volume of the cell was calculated from its diameter measured directly in each individual. Although no temperature regulation was made during one set of experiment, the range of the temperature change was within 1°C.

The volume occupied by osmotically inactive fraction in the egg was calculated from the volume of the egg in equilibrium with variously diluted sea water, and the data are given in Table I. The value of $b$ is 21 per cent of the volume of the cell in equilibrium with ordinary sea water in *S. intermedius*, and 28 per cent in *S. nudus*.

The permeability constants of the cell to water ($k_w$) and to ethylene glycol ($k_1$) can be read off from the charts which have been previously constructed from the calculation of the equations (1), (2), (3) and (4) by employing the Runge-Kutta's method used by Jacobs ('33). For this calculation we need to obtain the initial values of the variables $t$, $S$ and $V$. When $t=0$, the initial values of $S$ and $V$ in the equations (1) and (2) are 0 and 1.0 respectively, and the initial value of $V$ in the equations (3) and (4) is also 1.0, but the value of $S$ in these equations, which is equal to the final value of $S$ in (1) and (2), can be computed from the (1) and (2) by substituting $V=1$ and $dS/dt=0$ or $dV/dt=0$ in the final (equilibrium) state (when $t=\infty$). It is necessary to convert the permeability constants obtained in this way into the proper units. For this purpose these constants must be multiplied by the conversion factors (see Jacobs, '33, Tab. 1 and p. 435).

Before and after each experiment the fertilizability was examined and it was confirmed that the dilution of sea water as well as ethylene glycol is relatively harmless for the egg.
The results of the equilibrium experiment for evaluation of \( b \). The relative pressure is shown as a fraction of the pressure of ordinary sea water which is taken as unity. The actual volume in cubic micra can be obtained from the multiplication of these values in the table by 100. These data show that \( b \) is 21 and 28 per cent in \( S. \text{intermedius} \) and in \( S. \text{ nudus} \) respectively. Observed volume in the table is the mean of 30 eggs.

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative pressure</th>
<th>Volume observed</th>
<th>( P ) ( V )</th>
<th>( P ) ( V - b )</th>
<th>Volume calculated from ( V_r = \frac{P_0}{P_e} )</th>
<th>( V_r = \frac{P_0(V_a - b)}{P_e} + b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. \text{ intermedius} )</td>
<td>1.0</td>
<td>3,972</td>
<td>3,972</td>
<td>3,134</td>
<td>3,972</td>
<td>3,972</td>
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<tr>
<td></td>
<td>0.8</td>
<td>4,760</td>
<td>3,808</td>
<td>3,138</td>
<td>4,965</td>
<td>4,756</td>
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<td>0.6</td>
<td>6,061</td>
<td>3,637</td>
<td>3,134</td>
<td>6,162</td>
<td>6,061</td>
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<tr>
<td>( S. \text{ nudus} )</td>
<td>1.0</td>
<td>8,386</td>
<td>8,386</td>
<td>6,037</td>
<td>8,386</td>
<td>8,386</td>
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<tr>
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<td>6,082</td>
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<td></td>
<td>0.5</td>
<td>14,491</td>
<td>7,246</td>
<td>6,071</td>
<td>16,772</td>
<td>14,423</td>
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</table>

### III

A. Determination of Permeability to Water and to Ethylene Glycol by Jacobs' Method

The permeability constants to water and to ethylene glycol were determined by two experiments: 1st experiment — The measurement of time course of volume change of the cells in ordinary sea water containing a definite amount of ethylene glycol; and 2nd experiment — The measurement of volume change of the cells, which have equilibrated with the sea water solution of ethylene glycol, in ordinary sea water.

The data are shown in Figure 1 and Table 2 for \( S. \text{ intermedius} \) and in Figure 2 and Table 2 for \( S. \text{ nudus} \). The initial volume was obtained by extrapolation. The theoretical curves in these figures are based on the calculated values by equations (1) and (2) in the 1st experiment, and by (3) and (4) in the 2nd experiment. The open circles represent the observed values of volume in per cent of original volume. It will be noted that the theoretical curves give a relatively good agreement with the observed values until the volume of the cell arrives at minimum or maximum; but when passed through this point, the observed values become markedly tending to deviate from the theoretical curves. The deviations in these cases will be discussed later. The permeability constants to water \( (k_a) \) and to ethylene glycol \( (k_b) \) obtained by these experiments and the values converted to proper units are given in Figs. 1 and 2.
In the case of *S. intermedius* the permeability constants to water during endosmosis (2nd exp.) and during exosmosis (1st exp.) are approximately equal to each other; but in the case of *S. nudus* that constant is greater in endosmosis than in exosmosis. This relation obtained in *S. nudus* is contrary to the results obtained by many authors. Such a higher value of permeability constant of *S. nudus* egg to water in endosmosis is very suspicious, because the fertilizability of eggs decreased from 90 per cent before the 1st experiment to 56 per cent after the experiment and finally became zero per cent after 2nd experiment. But also in the eggs of *S. nudus* it may be conceivable that there is no difference between the permeability constants to water during endosmosis and exosmosis.

The permeability constant to ethylene glycol is clearly greater in endosmosis (1st exp.) than in exosmosis (2nd exp.).

![Graphs showing volume change over time](image)

Fig. 1. The time course of the volume change in two experiments (1st and 2nd experiment, see text) with eggs of *Strongylocentrotus intermedius*. The upper curve is based on the theoretical values computed from equation (3) and (4), substituting \( b = 0.21 \). The original volume is obtained by extrapolation and is \( 3,486 \times 10^2 \mu^3 \). Open circles represent the mean values of the observed volumes of 8 eggs in per cent of original one. The values of \( h_1 \) and \( h_2 \) are 0.10 and 0.43 in Jacobs' unit respectively and are \( 1.5 \times 10^{-15} \text{ M} \cdot \text{min}^{-1} \cdot \mu^3 \cdot \text{mol per liter} \) and \( 0.27 \times 10^{-3} \text{ atm}^{-1} \cdot \mu^3 \cdot \text{min}^{-1} \cdot \mu^2 \cdot \text{atm} \) in the proper unit. Temp.: ca. 22°C.

The lower curve is the theoretical one computed from equations (1) and (2). The original volume is \( 3,448 \times 10^2 \mu^3 \). The volume in per cent of original volume is...
the mean of 8 eggs. $k_1$ and $k_2$ are 0.25 and 0.41 in Jacobs' unit and are 3.6 and 0.25 in proper unit. Temp.: ca 19°C.

Fig. 2. The time course of volume change in the 1st and 2nd experiments with the egg of *S. nudus*.

The upper curve is a theoretical one calculated from the equations (3) and (4), substituting $b = 0.28$. The initial volume is estimated by extrapolation and its value is $7,555 \times 10^2 \mu^3$. Open circles represent the mean values of observed volume of 8 eggs in per cent of original one. The values of $k_1$ and $k_2$ are 0.09 and 0.57 in Jacobs' unit and are 1.7 and 0.45 in proper unit. Temp.: ca 22°C.

The lower curve is theoretically drawn by using equations (1) and (2). The initial volume is 7,555. The mean of 10 eggs. Temp.: ca 22°C. The values of $k_1$ and $k_2$ are 0.14 and 0.48 in Jacobs' unit and are 2.7 and 0.38 in proper unit. The concentration of ethylene glycol employed in 1st experiment with *S. nudus* egg is exceptionally 0.526 in Jacobs' concentration unit.

Table 2. The volume change in the 1st and 2nd experiment. The initial volume was estimated from extrapolation. The concentration of ethylene glycol used in each experiment is 0.510 in Jacobs' concentration unit.

*Strongylocentrotus intermedius*

The volume change in 1st experiment. Each figure in the table represents the mean of 8 measurements. The value of $b$ has been taken as 21 per cent of the cell volume in ordinary sea water. Temp.: ca 22°C.

<table>
<thead>
<tr>
<th>Time in minute</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. in $10^2 \mu^3$</td>
<td>3,448</td>
<td>3,037</td>
<td>2,929</td>
<td>2,986</td>
<td>3,037</td>
<td>3,170</td>
<td>3,261</td>
<td>3,284</td>
<td></td>
</tr>
<tr>
<td>Vol. in % of original one</td>
<td>100.0</td>
<td>88.0</td>
<td>85.0</td>
<td>86.8</td>
<td>88.0</td>
<td>92.0</td>
<td>93.1</td>
<td>94.6</td>
<td>95.2</td>
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<table>
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<th>11</th>
<th>12</th>
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<tr>
<td>Vol. in $10^2 \mu^3$</td>
<td>3,307</td>
<td>3,330</td>
<td>3,354</td>
<td>3,377</td>
</tr>
<tr>
<td>Vol. in % of original one</td>
<td>98.0</td>
<td>98.5</td>
<td>97.2</td>
<td>97.9</td>
</tr>
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</table>

The volume change in 2nd experiment. The mean of 8 measurements. Temp.: ca 19°C.

<table>
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<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Vol. in $10^2 \mu^3$</td>
<td>3,496</td>
<td>3,946</td>
<td>4,104</td>
<td>4,299</td>
<td>4,266</td>
<td>4,377</td>
<td>4,405</td>
<td>4,377</td>
<td>4,294</td>
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<tr>
<td>Vol. in % of original one</td>
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<td>117.9</td>
<td>117.4</td>
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<td>122.0</td>
<td>125.2</td>
<td>126.0</td>
<td>125.2</td>
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<table>
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<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. in $10^2 \mu^3$</td>
<td>4,239</td>
<td>4,185</td>
<td>4,104</td>
<td>4,024</td>
<td>3,946</td>
<td>3,894</td>
<td>3,817</td>
<td>3,817</td>
</tr>
<tr>
<td>Vol. in % of original one</td>
<td>121.3</td>
<td>119.7</td>
<td>117.4</td>
<td>115.1</td>
<td>112.9</td>
<td>111.4</td>
<td>109.1</td>
<td>109.1</td>
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</table>

*S. nudus*

The volume change in 1st experiment. Each figure is the mean of 10 measurements. The concentration of ethylene glycol in this experiment is exceptionally 0.526 in Jacobs' concentration unit. Temp.: ca 22°C. The $b$ has been taken as 28 per cent of the cell volume in ordinary sea water.
Permeability of the Sea-Urchin Egg

The volume change in 2nd experiment. The concentration of ethylene glycol used in this experiment is 0.510. The mean of 8 measurements. Temp. ca 22°C.

<table>
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<tr>
<th>Time in minute</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. in % of original one</td>
<td>100.0</td>
<td>86.4</td>
<td>82.7</td>
<td>83.9</td>
<td>86.3</td>
<td>88.7</td>
<td>90.4</td>
<td>92.0</td>
<td>93.2</td>
</tr>
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</table>

Time in minute 9 10 11 12
Vol. in \(10^2 \mu^3\) 7.142 7.218 7.294 7.339
Vol. in % of original one 94.5 95.5 96.5 97.1

B. Determination of Permeability to Water By Lucké, Hartline and McCutcheon's Method

The data obtained by this method are given in Figure 3 for the eggs of *S. intermedius* and in Figure 4 for those of *S. nudus*. The volume at time zero was obtained by extrapolation, and the volume of the egg in equilibrium with experimental solutions was evaluated from the equation, \(V_c = \frac{P_0(V_o - b)}{P_e} + b\). The value of \(b\) had been taken as 21 per cent of the cell volume in ordinary sea water for *S. intermedius* and as 28 per cent for *S. nudus*. The theoretical curves in these figures were drawn from the values calculated by the integrated form of Lucké’s equation. As is obvious from these figures, the theoretical curves give satisfactory agreement with the experimental ones with the exception in 20 per cent. The permeability constant \((k)\) of the cells to water for endosmosis is about 0.2 \((\mu^3\) min. \(/\mu^3\) atm.) at 20°C for *S. intermedius* and is about 0.25 at 24°C for *S. nudus*. The determination for the permeability constant in exosmosis of water was made only with *S. nudus*. It is about 0.38 at 23°C. In the case of *S. nudus* the permeability constant to water is greater in exosmosis than in endosmosis as previously has been ascertained by many authors.

The permeability constant to water in unfertilized eggs of *Arbacia* is 0.1–0.2 at ca. 20°C (Lucké, Hartline and McCutcheon, '31; Stewart and Jacobs, '36; Lucké, Hartline and Ricca, '39, and others), the value obtained in the present work, however, is somewhat greater than that in *Arbacia* egg.
Fig. 3. Volume-time curves of swelling of *S. intermedius* eggs in hypotonic sea water. The open circles, the solid circles and the crosses indicate the observed volumes in 60%, in 40% and in 20% sea water respectively. The curves are drawn from the values theoretically calculated by the integrated form of Lucké's equation.

Fig. 4. Curves of swelling and shrinking of *S. nudus* eggs. The crosses indicate the observed volumes in 80% sea water and the squares and the open circles in 60%.
The solid circles give the volumes, when the cells, previously equilibrated with 60% sea water, are replaced in 100%. The curves are the theoretical ones.

**IV**

In the eggs of *S. intermedius* the permeability constants to water for endosmosis and for exosmosis, which were obtained by Jacobs' method, are approximately equal to each other and this value is in the same order of magnitude as the value obtained by the method of Lucké et al. The well-known relation that the permeability constant to water is greater during exosmosis than during endosmosis, was also recognized in the results with the eggs of *S. nudus* obtained by the method of Lucké et al. On the contrary in the eggs of *S. nudus* the permeability constant to water estimated by Jacobs' method is inversely less in exosmosis than in endosmosis, and further, the absolute values are considerably greater than the ones obtained by the method of Lucké et al. But this experiment must be re-examined, as the physiological conditions of eggs used were not suitable for experiment.

The permeability constant to ethylene glycol without correction for $b$ for the unfertilized egg of *Arbacia* is estimated to be $3.5 \times 10^{-13}$ M/min./$\mu^2$/mol per liter at ca. 20°C (Stewart and Jacobs, '32 and '36; Hunter, '38 and Lucké, Hartline and Ricca, '39). Correcting for $b$, the permeability constant in endosmosis to ethylene
glycol obtained in the present work is 3.6 for *S. intermedius* and 2.7 for *S. nudus* respectively, and the value of the constant for exosmosis gives 1.5 for *S. intermedius* and 1.7 for *S. nudus*. From these facts it seems probable that ethylene glycol passes through the plasmic membrane during endosmosis more easily than during exosmosis, and this relation is contrary to the case of water. This may be partly due to the different diffusibility of ethylene glycol in sea water and in protoplasm.

The theoretical curves based on Jacobs' equation are well fitted for the observed values until the cell volume arrives at its maximum or minimum, but when passed through these points the observed values become to deviate from the theoretical curves. Some factors, which might be the causes for this deviation, may be as follows. 1) The concentration of the medium would not exactly remain constant during the experiment, for the amount of medium in which the eggs are suspended is relatively small and the movement of water or solutes takes place between the medium and the cell interior. 2) When the volume of the cell passes through its maximum or minimum, the direction of movement of water begins to be reversal, so that the permeability constant of the cell to water might be changed. It is assumed in the theoretical treatment that the permeability constant to water remains constant throughout the course of the volume-change. But strictly speaking this is not the case. In addition to these factors described above, the tension at the surface and the mobility (or the diffusibility) of the solute as well as of water through the protoplasm may be also factors in the cell permeability. The former can be neglected, because this value is extremely small (Kamada and Yamamoto, '31; E. N. Harvey, '31 and Cole, '32). However, the latter closely relates to whether the following assumption is valid or not, that is, the resistance to the movement of water and of the solute is confined to the plasmic membrane, in other words, water and solute entered into the cell instantaneously distribute homogeneously throughout the cell interior. Unless this assumption is valid, the osmotic pressure within the cell at the time *t* could not be obtained from the equation, \[ P = \frac{P_0 \sqrt{a-b}}{1 - \frac{t}{T}}. \] In the experiment with the egg of *Ceratocephale* it was found by Kamada ('36) that, as soon as the egg is placed in hypotonic medium, the swelling of the egg as a whole begins, but the swelling of the nucleus is clearly delayed. This phenomenon shows that this factor being concerned with the diffusion should not be neglected in the theoretical treatment of the cell permeability. Further, exact knowledge in regard to the structure of the plasmic membrane will be also significant in the cell permeability. Some of these factors should be considered in the mathematical treatment of the equation of Lucké et al.

The author wishes to thank Prof. K. Aoki for many helpful suggestions concerning this work.

**Summary**

1. Permeability constants to water and to ethylene glycol were estimated
with the unfertilized eggs of two species of sea-urchins: *Strongylocentrotus intermedius* and *S. nudus*.

2. Permeability constant to water during endosmosis is 0.2 \( \mu^3/\text{min.} /\mu^2/\text{atm.} \) at 20°C in *S. intermedius* and 0.25 at 24°C in *S. nudus*, and during exosmosis is obtained only in *S. nudus* and is 0.38 at 23°C.

3. Permeability constants to ethylene glycol were determined both for endosmosis and for exosmosis. The value for endosmosis is 3.6 \( 10^{-15} \text{M/min.} /\mu^2/\text{mol per liter} \) at 22°C in *S. intermedius* and 2.7 at 22°C in *S. nudus*, and that for exosmosis is 1.5 at 19°C in *S. intermedius* and 1.7 at 22°C in *S. nudus*. From these facts it seems probable that ethylene glycol penetrates more easily from the surrounding medium into the cell interior.

4. The theoretical values of the egg volume calculated from the integrated form of Lucké's equation give a good agreement with actually estimated values. The calculated values from the Jacobs' equation satisfactorily fit the observed values until the cell volume arrives at its maximum or minimum, but after passing through this point, the observed values begin to deviate from the theoretical values, and some causes of this deviation were discussed briefly.

**Literature Cited**


