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DIFFUSION AND PERMEATION OF WATER IN THE FROG EGG: THE EFFECT OF TENSION AND TONICITY

BY KJELL HANSSON MILD*† AND SØREN LØVTRUP†

**Department of Theoretical Physics and †Department of Zoophysiology, University of Umeå S-901 87 Umeå, Sweden*

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SUMMARY

1. The isotopic water permeability coefficient, P, of the plasma membrane of frog body cavity eggs has been determined as a function of the incubation time in media of different tonicity.

2. It was found that the permeability decreases with the incubation time in hypotonic solutions. The observed changes may be correlated with an increase of the tension in the vitelline membrane.

3. In the evaluation of the experimental results the diffusion of water in the external medium ('unstirred layers') is taken into account.

INTRODUCTION

In most animal cells the net flow of water occurring in a hyperosmotic medium is more rapid than the one taking place under hyposmotic conditions (Heilbrunn, 1952). This observation, which obviously limits the applicability of osmometric methods in permeability studies, may be correlated with the isotope exchange experiments by Berntsson, Haglund & Løvtrup (1964), showing that the permeability coefficient of water is significantly lower in hypotonic, than in isotonic, solutions.

In the quoted note it was not established whether it is the concentration of the bathing medium proper or the tension of the cell membrane (which must differ in shrinking and swelling cells) that is responsible for the observed changes in the permeability coefficient. This question is the subject of the present study.

MATERIAL AND METHODS

The experiments were carried out with body cavity eggs of *Rana temporaria*. The frogs were purchased from commercial dealers in Western Germany and were kept under moist conditions at 5 °C until used. The ovulation was induced by the method described by Rugh (1962). The eggs were surgically removed from the frog, and were kept in amphibian Ringer solution at pH = 7·8 at the experimental temperature, which was either 18 or 25 °C.

Prior to each experiment the radius of the eggs was measured with an optical screw micrometer, a method which has a standard deviation of 1 %.

The exchange of water was followed by determination of the changes in the reduced weight (RW) of an egg placed in Ringer solution containing 20 % D_2O (Løvtrup &

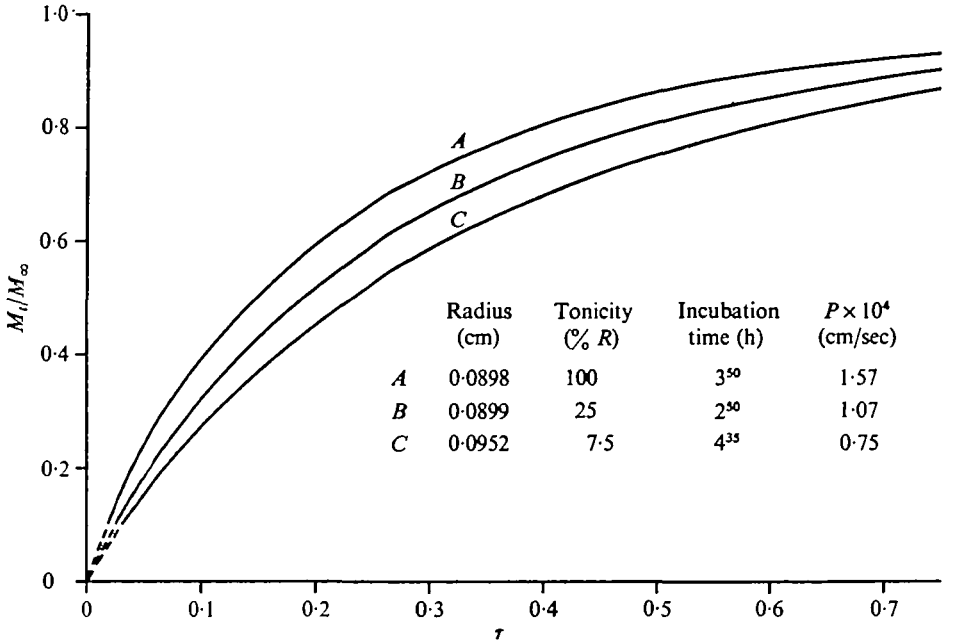

Fig. 1. The curves show the changes in normalized reduced weight,

$$M_t/M_\infty = (RW_t - RW_0)/(RW_\infty - RW_0)$$

as a function of the dimensionless time variable $\tau = D_1 \times t/R^2$, obtained under three different experimental conditions. Curve *A* is recorded under isotonic conditions, curve *B* and *C* are from experiments performed in 25 % *R* and 7·5 % *R*, respectively. The radius of the eggs at the time of the experiment, the incubation time and the computed value of the permeability coefficient are given in the Table inserted in the figure.

Pigon, 1951; Bergfors, Hansson Mild & Løvtrup, 1970; Hansson Mild & Løvtrup, 1974). The tonicity of the isotopic medium was the same as that of the incubation medium.

The water exchange parameters are calculated from the recorded changes in RW (Hansson Mild, 1972) on a CDC 3300 computer at the data processing central of Umeå University (UMDAC). A discussion of the isotope exchange method and the curve-fitting program has been published recently by Hansson Mild & Løvtrup (1974).

RESULTS

The experiments carried out in isotonic (100 % R) solution at 18 °C were first evaluated with a computer program designed for the simultaneous determination of both the cytoplasmic diffusion coefficient for water, D_1, and the permeability coefficient, *P*. The mean value of D_1 from 12 experiments was found to be $(5{\cdot}0 \pm 0{\cdot}8) \times 10^{-6}$ cm²/sec. The difference between the mean value of *P* when D_1 is kept constant and when it is evaluated from the individual experiment is less than 5 %. The recorded value of D_1 has been used throughout our experiments for the calculation of *P*, an expedient that is justified by the fact that we have been unable to register any effect of the medium upon D_1.

The isotope exchange was also followed in eggs which had been incubated f

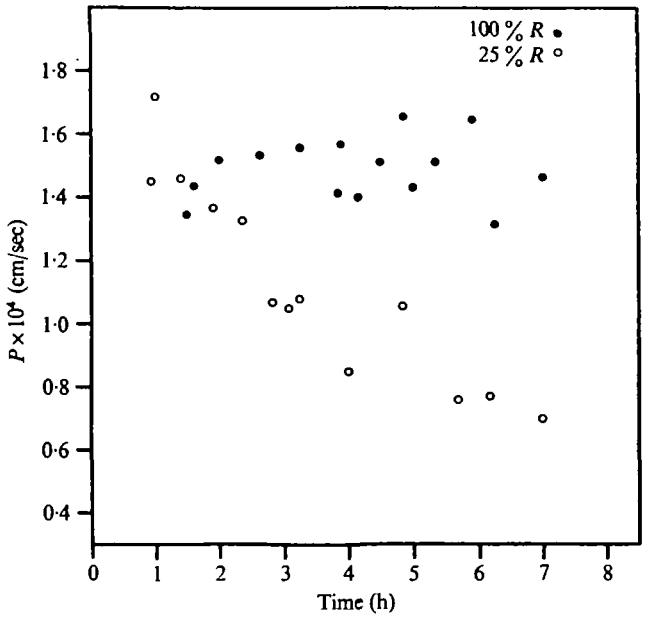

Fig. 2. The permeability coefficient as a function of incubation time in 100% (●) and 25% Ringer (○) solutions. The same D_1 value, $5{\cdot}0 \times 10^{-4}$ cm²/sec, is used for the evaluation of P of all points.

various times in different hypotonic media. In Fig. 1 are shown examples of curves obtained under three different experimental conditions. In order to make the comparison between the curves easier they are plotted as the normalized changes in reduced weight versus the dimensionless time variable $\tau = D_1 \times t/R^2$, where t is the time in seconds and R the radius of the egg (see further Hansson Mild, 1972). Since D_1 is constant, the difference between the curves are due solely to changes in the membrane permeability coefficient, P. The corresponding computer-fitted theoretical curves are not shown in the plot since the deviations from the experimental curves occur in the third or fourth digit – an effect too small to be seen in our diagram. For reasons outlined earlier (Hansson Mild & Løvtrup, 1974), the first minute of the exchange curve cannot be used in the curve fitting procedure and this part of the curves is therefore indicated with a broken line in Fig. 1.

Initially, we presumed that the permeability depends on the external osmolarity, C_e. However, when the P values were plotted against C_e, a considerable scatter was observed, much greater than warranted by the accuracy of the method. This variation was found to be the outcome of incubating the eggs in hypotonic solutions, as can be seen from Figs. 2 and 3, in which P is plotted as a function of the incubation time. From Fig. 2 it appears that, in an isotonic solution, P remains constant at a mean value of $1{\cdot}5 \pm 0{\cdot}1 \times 10^{-4}$ cm/sec. When the eggs are kept in hypotonic solutions there is a marked decrease in P with time. Fig. 2 shows that after 3 h in 25% Ringer P is reduced by 30% and after 6 h it is only half of the initial value. In Fig. 3 the corresponding results for 50% Ringer and 7·5% Ringer are shown.

In parallel experiments the internal pressure in the eggs was determined as a function of temperature and incubation time in different media (Hansson Mild, Løvtrup &

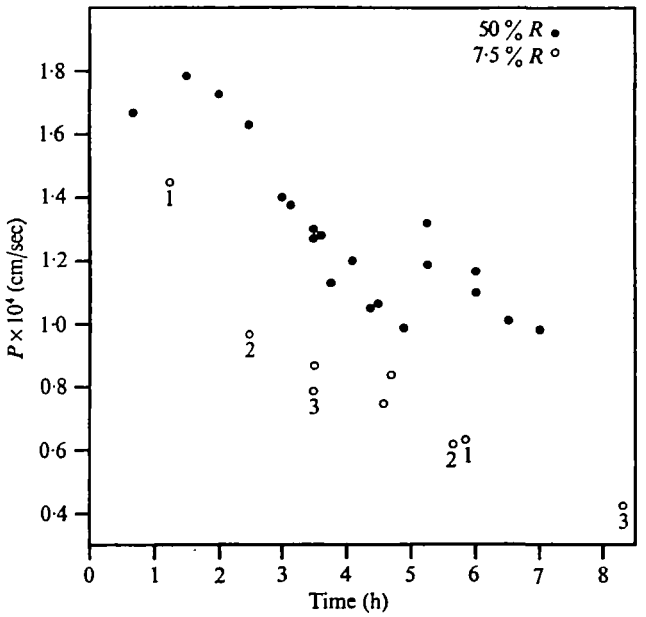

Fig. 3. The permeability coefficient as a function of incubation time in 50% (●) and 7·5% Ringer (○) solutions. The same D_1 value, $5{\cdot}0 \times 10^{-6}$ cm²/sec, is used for the evaluation of P of all points. The numbers on the points are explained in the text.

Bergfors, 1974*a*). When the pressure is known, the stress resultant (i.e. the tension) in the vitelline membrane (which is the structure responsible for the tension) can be calculated from

$$N = \frac{pR}{2}, \tag{1}$$

where p is the pressure and R the radius of the egg. In Fig. 4 the permeability is shown as a function of N for each of the experiments in Figs. 2 and 3, showing an evident correlation between permeability and tension, whereas the tonicity *per se* appears to be without influence.

In some cases P was measured a second time on the same egg and the results of these experiments have been marked in Figs. 3 and 4. The same number on two points indicate that they refer to the same egg.

Some experiments were also carried out at 25 °C. It was found that at this temperature the P value of body cavity eggs of *R. temporaria* is infinitely large. When the eggs are incubated in hypotonic solution the permeability becomes finite. There is a considerable scatter in the results, but when the exchange of heavy water is repeated several times in the same egg P is seen to decrease similarly to that observed in the experiments at 18 °C. The special importance of these experiments is the finding that the effect of high temperatures on P can be counteracted by an increase in the membrane tension.

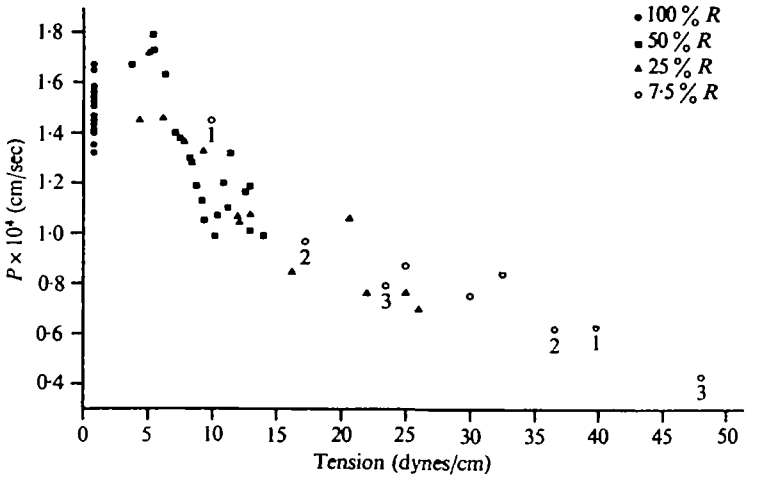

Fig. 4. The permeability coefficient as a function of the stress resultant (i.e. the tension) of the vitalline membrane. The numbers on the points are explained in the text.

DISCUSSION

In the boundary condition used for the solution of the diffusion equation it is assumed that no volume flow occurs. This is an approximation when hypotonic solutions are employed. The pertinent equations in this case have been given by Kedem & Katchalsky (1958). In the presence of a non-zero volume flow, J_v, the solute flow is

$$J_s = \bar{C}_s J_v + P\Delta C_s, \tag{2}$$

where $\bar{C}_s$ is to be regarded as the average concentration of D_2O in the membrane, ΔC_s is the concentration difference across the membrane and $P = \omega RT$ in the notation of Kedem & Katchalsky (1958).

From this equation it can be seen that the flow of D_2O will be different depending on whether the bulk flow enters or leaves the cell. Prescott & Zeuthen (1953) have interpreted their observations on frog eggs to show this effect.

That this 'solvent drag' does not measurably affect the RW can be seen from the following reasoning. Working with the diver balance technique it is the outflow of water from the object that is actually measured (Løvtrup & Pigon, 1951; Løvtrup, 1963) and since we work only with hypotonic solutions, it follows that the volume flow and the measurable isotope flux occur in opposite directions. The bulk flow is greatest at the beginning of the swelling process when the osmotic concentration difference across the membrane is largest. This is exemplified by Fig. 5, showing the changes of the radius as a function of time for an egg incubated in 7·5% Ringer solution at 19 °C (for details of the experimental arrangement, see Hansson Mild, Carlson & Løvtrup (1974*b*)). After 7 h of incubation the volume flow is reduced, compared to the initial stage, by a factor of 5. If the solvent drag hypothesis is valid for our experimental conditions, the retardation of the isotope flux should be greatest initially, diminishing with the incubation time. Consequently we should observe a gradual rise in the value of P, whereas the actual observations show the opposite

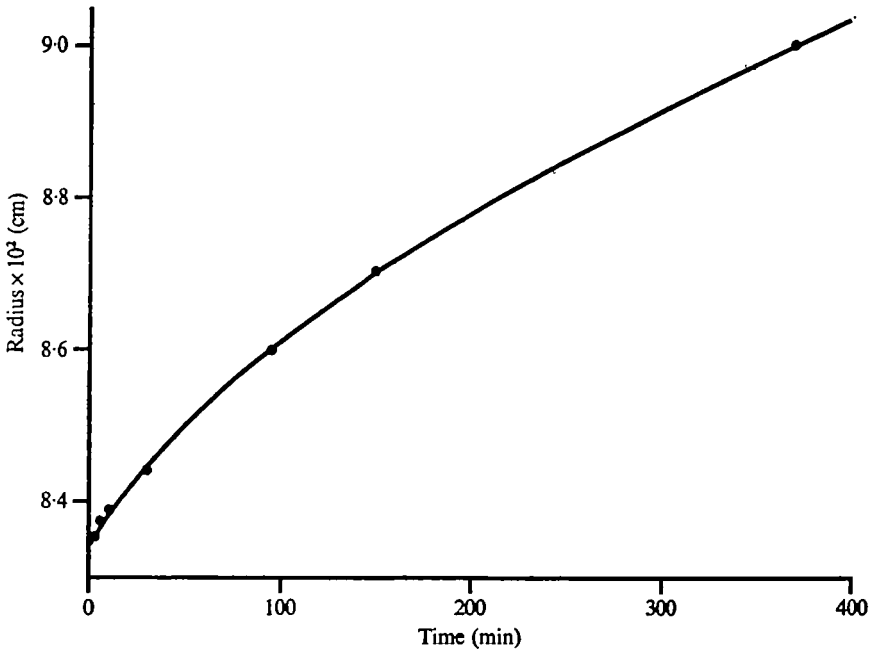

Fig. 5. The change of the radius as a function of time for an egg incubated in 7·5% Ringer solution at 19 °C.

trend, i.e. very little change during the first hour of incubation and a substantial decrease subsequently.

With this source of error eliminated we are thus entitled to conclude that the permeability of the plasma membrane of the frog egg varies with the tension in the vitelline membrane surrounding it. Since the tension in the latter is much higher than in the surface of the naked egg (Berntsson *et al.* 1964), the results in Fig. 4 can be interpreted in two different ways. Either the tension measured in the vitelline membrane is transmitted to the subjacent plasma membrane, in which case the figure is valid as it stands, or else the swelling causes proportionate changes in the two membranes, and if this is true the measured tension values are only indices of substantially lower values in the plasma membrane proper.

Our results show that data obtained in osmometric experiments must be interpreted with great discretion. Evaluation of the permeability coefficient is usually made by fitting the experimental curve to a theoretical curve derived on the assumption that diffusion occurs between two well-stirred compartments and that P is constant during the experiment. It has been found (Lucké, 1940; Rich *et al.* 1968) that it is not possible to account for the whole experimental curve on these premises, and our results suggest that this may be due to the erroneous assumption of a constant permeability coefficient.

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REFERENCES

BERGFORS, T., HANSSON MILD, K. & LØVTRUP, S. (1970). Modifications in the automatic diver balance technique. *J. exp. Biol.* **53**, 187–93.

BERNTSSON, K.-E., HAGLUND, B. & LØVTRUP, S. (1964). Water permeation at different tonicities in the amphibian egg. *J. exp. Zool.* **155**, 317–24.

HANSSON MILD, K. (1972). Diffusion exchange between a membrane-bounded sphere and its surrounding. *Bull. math. Biophysics* **34**, 93–102.

HANSSON MILD, K. & LØVTRUP, S. (1974). Diffusion and permeation of water in the frog egg: The effect of temperature. *Biochim. Biophys. Acta* (in the Press).

HANSSON MILD, K., LØVTRUP, S. & BERGFORS, T. (1974*a*). On the mechanical properties of the vitelline membrane of the frog egg. *J. exp. Biol.* **60**, 807–20.

HANSSON MILD, K., CARLSON, L. & LØVTRUP, S. (1974*b*). The identity of filtration and diffusion permeability coefficients in frog egg membrane. *J. Membrane Biol.* (in the Press).

HEILBRUNN, L. V. (1952). *An Outline of General Physiology*. Philadelphia: W. B. Saunders.

KEDEM, D. & KATCHALSKY, A. (1958). Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biophys. Acta* **27**, 229–46.

LØVTRUP, S. (1963). On the rate of water exchange across the surface of animal cells. *J. theor. Biol.* **5**, 341–59.

LØVTRUP, S. & PIGON, A. (1951). Diffusion and active transport of water in the amoeba *Chaos chaos* L. *C. R. Trav. Lab. Carlsberg* (Ser. Chim.), **28**, 1–36.

LUCKÉ, B. (1940). The living cells as an osmotic system and its permeability to water. *Cold Spring Harbor Symp. quant. Biol.* **8**, 123–32.

PRESCOTT, D. M. & ZEUTHEN, E. (1953). Comparison of water diffusion and water filtration across cell surfaces. *Acta physiol. scand.* **28**, 77–94.

RICH, G. T., SHA'AFI, R. I., ROMUALDEZ, A. & SOLOMON, A. K. (1968). Effect of osmolarity on the hydraulic permeability coefficient of red cells. *J. gen. Physiol.* **52**, 941–54.

RUGH, R. (1962). *Experimental Embryology*, 3rd ed. Minnesota: Burgess Publishing Company.