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The Identity of Filtration and Diffusion Permeability Coefficients in Frog Egg Membrane

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Summary. The filtration permeability coefficient P_f for water has been determined on ovarian eggs of *Rana temporaria* as a function of the temperature. A local maximum was found at 16 °C and a minimum at 19 °C. This temperature dependence has previously been shown to characterize the cytoplasmic water diffusion coefficient and, since isotope studies have demonstrated that there is no measurable barrier to permeation of water at the surface of ovarian eggs, it is concluded that the previously reported values of P_f for this material are fictitious. Determinations of P_f on body cavity eggs, in which a measurable resistance to water permeation prevails, have given values that are not significantly different from the diffusion permeability coefficients P_d obtained in isotope exchange experiments. With reference to these results, and similar ones reported for other types of membrane, it is concluded that the justification is lacking for the claim $P_f > P_d$, on which many speculations on the mechanism of water passage through biological membranes have heretofore been based.

The movement of water across cell membranes can be studied in two ways, either osmometrically by measuring the rate of swelling or shrinkage of the cell under a gradient of osmotic pressure, or by adding tracer water to the water inside the cell or to the surrounding medium and measuring the rate of transfer out of or into the cell. The latter method gives the diffusion permeability coefficient P_d; the former, the osmotic or the filtration permeability coefficient $P_f = L_p RT/\bar{V}_w$, where L_p is the hydraulic conductivity and $\bar{V}_w$ the partial molar volume of water.

Comparing the values of P_f and P_d, as obtained for frog skin, Hevesy, Hofer and Krogh (1935) found the ratio between the coefficients to be about 4 and a similar result was reported by Koefoed-Johnsen and Ussing (1953). Prescott and Zeuthen (1953) made the comparison between P_f and

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P_d for frog eggs and found, for *Rana temporaria* body cavity and ovarian eggs, the ratios 1.7 and 70, respectively. These results have been interpretated to imply that the water transfer takes place through small pores in the membrane. From these ratios the radius of these pores have been calculated (Stein, 1967), according to a theory put forward by Solomon (1960).

As we shall have the opportunity to substantiate in the Discussion, criticism of these results, as well as of their interpretation, has not been lacking. In particular, it has turned out that the methods originally used for the determination of permeability coefficients are subject to serious sources of error.

We have recently (Hansson Mild & Løvtrup, 1974) measured values of P_d for the frog egg which, so far as we can judge, have been properly corrected, thus giving a correct measure of the resistance towards passage of water residing in the plasma membrane.

Under these circumstances we found it desirable to measure even P_f on this material, trying to minimize the various sources of error. With the acquisition of these sets of data it will be possible to evaluate the justification of the previously reported differences between the two permeability coefficients.

Materials and Methods

Biological Material

The experiments were carried out with oocytes and body cavity eggs, i.e. mature 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 the follicle membrane from the oocytes. In all cases normal, uncytolyzed eggs were randomly chosen for experimentation. The eggs were kept in amphibian Ringer's solution.

Osmometric Method

Osmometric determinations were carried out by following the rate of increase in volume after transfer to hypotonic Ringer's solutions. Diameter measurements were made with microscopes with ocular micrometers.

The vessel for measuring the diameter of a swelling egg is shown in Fig. 1*a*. A hole at the top of a turned nylon block is filled with 1% agar solution. As soon as the agar is solidified, a heated glass-rod with a spherical tip is made to touch the agar to melt a shallow pit. The nylon block is put into a cuvette, which is then filled to the rim with hypotonic Ringer's solution. After the egg is placed on the agar tip, a coverslip is immediately put on the cuvette, and the measurements started.

As shown in Fig. 1*b*, the lower part of the egg is seen through an inclined surface of agar. As the refraction index between agar and the surrounding medium is as low as 1.0016, the error thus introduced in the estimation of the vertical diameter can be neglected. Seven different diameters were measured (Fig. 1*c* and *d*). The average of these values

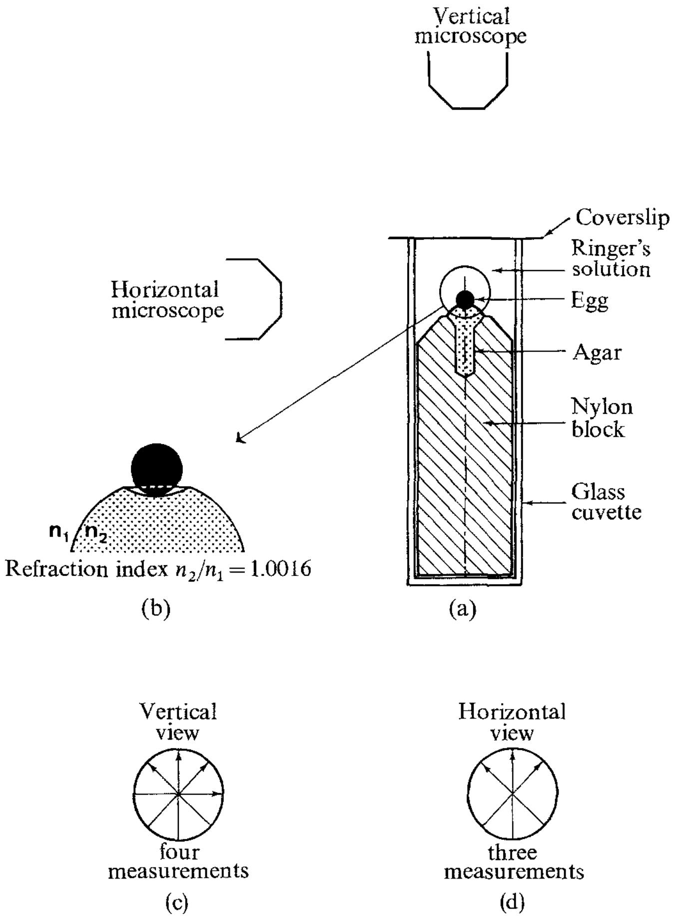

Fig. 1. Experimental apparatus for measuring the diameter of an egg swelling in hypotonic solution

was used to calculate the volume of a sphere representing by the egg. Depending on the rate of swelling, the readings were made at different time intervals. Working with oocytes the measurements were done with 1- to 2-min intervals for the first 10 min; thereafter readings were taken at approximately 5-min intervals. For the body cavity eggs the time intervals were about 3 to 5 min at the beginning and 20 min in the later stages of the swelling process.

The set-up shown in Fig. 1 was used for measurements at room temperature. When the influence of temperature was investigated, the cuvette was inserted in a plexiglass container through which water from a thermostat was circulated.

The permeability coefficient was calculated from the initial slope of the curve obtained by plotting the radius against time (*see* Dick, Dick & Bradbury, 1970). On the assumption that the factors limiting the rate of the passage of water reside exclusively in the surface of the egg we have, according to Katchalsky and Curran (1965),

$$J_v = L_p(\Delta p - \sigma \Delta \Pi) \tag{1}$$

where J_v is the volume flow per unit area, Δp is the difference in hydrostatic pressure across the membrane, σ the reflexion coefficient and $\Delta\Pi$ the osmotic pressure difference.

It has been shown that Δp is negligible compared to $\Delta\Pi$ for the concentrations used here (Hansson Mild, Løvtrup & Bergfors, 1974), namely, 7.5, 25 and 50% Ringer's solution.

The osmotic pressure difference can be expressed as

$$\Delta\Pi = RT\Delta C_s \tag{2}$$

where ΔC_s is the concentration difference across the membrane with respect to dissolved salts (Kotyk & Janáček, 1970). In the evaluation of ΔC_s it is assumed that the cell cytoplasm is isosmotic with 100% Ringer's.

Dealing with spherical cells the volume flow can be expressed as the negative time derivative of the radius; introducing this together with the expression for $\Delta\Pi$ into Eq. (1) the equation usually employed in osmotic swelling experiments is obtained:

$$\frac{dr}{dt} = \sigma P_f \bar{V}_w \Delta C_s \tag{3}$$

where P_f is defined as above. This gives the osmometric permeability coefficient as:

$$P_f = \frac{\left(\frac{dr}{dt}\right)_{t=0}}{\sigma \bar{V}_w \Delta C_s}. \tag{4}$$

Eq. (4) is valid only for $t = 0$, since for $t > 0$, the elastic properties of the membrane may influence the passage of water and, furthermore, the concentration difference across the membrane is very difficult to evaluate due to the non-negligible effect of diffusion in the cytoplasm (Dick *et al.*, 1970). No attempts were made in this study to obtain a theoretical expression for the whole swelling curve.

Some experiments were made to estimate the reflection coefficient for Ringer's solutions of different concentration *vis-à-vis* the membrane of the mature frog egg, principally according to the method of Goldstein and Solomon (1960). Because of the very small volume changes occurring when the eggs are placed in a nearly isotonic solution, it is impossible to make accurate measurements; all we can conclude from these observations is that σ is close to 1.0. In our calculations of P_f the latter value was assumed.

Results

The influence of temperature on P_f was studied on ovarian eggs in 50% Ringer's. The results obtained are shown in Fig. 2. It is seen that a maximum obtains at 16 °C, and a minimum at 19 °C. The evaluation of these results will be made in the discussion.

The values of P_f for body cavity eggs were determined in two different series of experiments. The first of these was carried out at room temperature (22 to 23 °C) and at different tonicities (7.5, 25 and 50% Ringer's). No

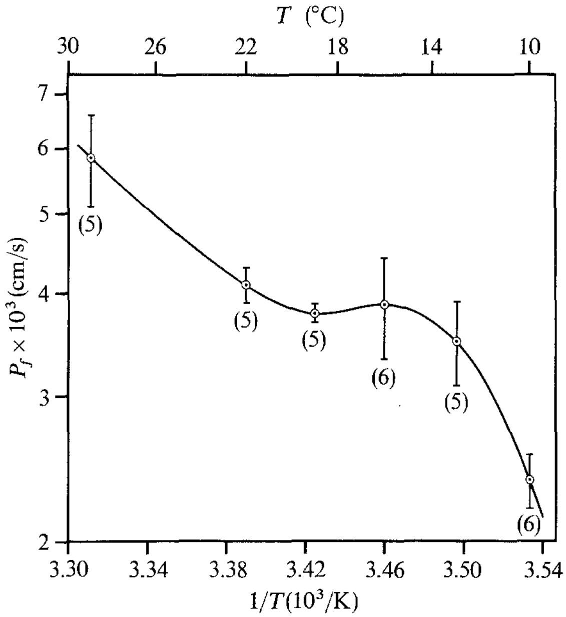

Fig. 2. Logarithmic plot of P_f of oocytes of *R. temporaria* against $1/T$. The number in parentheses indicates the number of experiments. The vertical lines indicate the standard error of the mean

significant effect of the medium could be observed. The mean value of P_f obtained from 10 separate determinations was $2.4 \pm 0.6 \times 10^{-4}$ cm sec^{-1}. In a second series, P_f was measured at 19 °C and 7.5% Ringer's. The result of seven experiments was $1.8 \pm 0.3 \times 10^{-4}$ cm sec^{-1}.

These results should be compared with the corresponding values obtained with the isotope exchange method (Hansson Mild & Løvtrup, 1974), which are: 22 °C – $2.9 \pm 0.5 \times 10^{-4}$ cm sec^{-1}; 19 °C – $1.7 \pm 0.2 \times 10^{-4}$ cm sec^{-1}.

Discussion

Our results on ovarian eggs gave values which, although higher by an order of magnitude than those obtained on mature eggs, are still finite. This outcome should be contrasted with our previous findings (Hansson Mild & Løvtrup, 1974), according to which there is no measurable barrier at the surface of the ovarian egg, i.e. $P_d \to \infty$, that consequently the exchange of water is rate-limited only by the diffusion in the egg and in the surrounding medium.

That we are indeed measuring the former process in our determinations of the 'permeability coefficient' is directly demonstrated by the temperature dependency of the measured values. In the quoted paper we found that the three parameters involved in the isotope exchange, D_2, the diffusion in the external medium, D_1, the diffusion in the egg cytoplasm and P_d, the membrane permeability coefficient, each is affected in a very typical way by changes in the temperature, and the one for D_1 is distinguished by a maximum at 16 °C and a minimum at 19 °C, closely similar to the result shown in Fig. 2.

It is important to notice that these results do not imply that there is no barrier to water transfer at the surface of the ovarian egg, only that the resistance is so low that it cannot be measured with any available method. We therefore feel entitled to conclude that the assumptions made in the osmometric determinations on oocytes are not fulfilled, and that previously published values of P_f for this material do not represent an empirical reality.

In the presence of a membrane the latter will be the one which primarily determines the rate of the net flow of water across the egg surface. But, as shown by Dick (1959) (*see also* Dick *et al.*, 1970), the effect of diffusion is not negligible. Further factors that may affect the net flow are the tension in the egg surface and the loss of solutes from the egg (Sigler & Janáček, 1971). This circumstance implies that it is very complicated to describe mathematically the course of the volume change. All that can be established is the extrapolated rate at $t = 0$.

Nevertheless, in work with body cavity eggs, in which the membrane permeability is rather low, the accuracy of our method is acceptable. As reported above, the result is that, within the experimental errors, the filtration permeability coefficient is equal to that obtained in isotope exchange experiments (Hansson Mild & Løvtrup, 1974). Consequently, as concerns the frog egg, there is no justification for the assumption $P_f > P_d$. The same conclusion has been arrived at for artificial membranes (e.g. Cass & Finkelstein, 1967; Everitt, Redwood & Haydon, 1969; Andreoli, 1970; Haydon, 1970*a*, *b*). Although he did not reach equality between the two coefficients, Hays (1972) has shown that under proper experimental conditions the previously recorded difference in the frog skin can be substantially reduced. This author also arrives at the view advanced earlier (Løvtrup, 1963) that, since the undirectional fluxes across the membrane are very large compared to the net flow, the solvent drag, the basis for the postulated inequality, must be very slight.

Although one biological material, for which $P_f > P_d$ has been postulated, remains to be scrutinized, viz., the erythrocyte, we feel that already there is rather good support for the postulate

$$L_p RT/\bar{V}_w = P,$$

where P stands for the permeability coefficient without regard to the method by which it is estimated.

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