Biochimica et Biophysica Acta, 373 (1974) 383–396

BBA 76814

DIFFUSION AND PERMEATION OF WATER IN THE FROG EGG
THE EFFECT OF TEMPERATURE

KJELL HANSSON MILD[a,b] and SØREN LØVTRUP[b]

[a]*Department of Theoretical Physics and* [b]*Department of Zoophysiology, University of Umeå, S-90187 Umeå (Sweden)*

(Received February 22nd, 1974)
(Revised manuscript received August 26th, 1974)

SUMMARY

1. The isotopic water permeability (P) of the plasma membrane of body cavity eggs of *Rana temporaria* and *Rana pipiens* has been determined as a function of temperature.

2. It was found that when the temperature is raised above a certain limit the permeability increased towards infinity, and in the low temperature range P was significantly reduced. The activation energy for the intermediate temperatures was found to be 14 kcal/mole.

3. The self-diffusion coefficient of water in the cytoplasm is required for the evaluations of P and this parameter has been measured in ovarian eggs from the ranid species studied. An anomalous temperature dependence of the diffusion coefficient was found. The values increase with increasing temperature until at 16 °C a local maximum is reached. Further increase of the temperature gives first a slight decrease of the coefficient followed by a measurable increase.

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

INTRODUCTION

In all early studies on the water transfer between living cells and their surroundings it has been assumed that the passage through the cell membrane is so slow that the inner and outer compartments can be regarded as being well-stirred compartments.

This approach was criticized by Dick [1] and Løvtrup [2], who showed that the diffusion of water in cytoplasm is slow enough to affect the rate of water permeation, and that the former factor consequently must be taken into account when the value of the permeability coefficient P is calculated from experimental data.

Dainty [3] has claimed that when such studies are carried out with methods involving the exchange of isotopic water, the value of P is grossly underestimated because no allowance is made for the diffusion in the unstirred layer of water, adjacent

to the external face of the cell membrane, a layer that may be as wide as 500 μm.

A justification of this criticism was noted in a consistent discrepancy between the experimental observations on isotope exchanges curves from frog eggs as followed by the automatic diver balance technique and the theoretical curves as given by Løvtrup [2]. Recently, a solution of the diffusion equation with the pertinent boundary conditions for this experimental situation was presented [4]. This new approach takes into account the diffusion in the cytoplasm as well as in the external medium. The first results thus obtained clearly substantiate Dainty's contention, for the P values are significantly higher than those obtained with the old theory.

Comparing the osmotic and the isotopic water permeability coefficients of the frog eggs and oocytes, Prescott and Zeuthen [5, 6] found the former to be larger than the latter and this result was interpreted to demonstrate the existence of water-filled pores in the membrane. However, before any reliance can be placed on this observation it is necessary to re-investigate the isotopic water permeability of these types of cells and evaluate the results on the basis of the theoretical approach given by Hansson Mild [4].

The present paper reports the results of such a study of the isotopic water permeability of ovarian and body cavity eggs of *Rana temporaria* and *Rana pipiens*. In order to gain some insight into the physical nature of the phases in which water exchange occurs, the temperature dependence of the permeation and diffusion processes have also been studied.

MATERIAL AND METHOD

Biological material

The experiments were carried out with ovarian and body cavity eggs of *R. temporaria* and *R. pipiens*. The former were purchased from commercial dealers in Western Germany, the latter from North Carolina, U.S.A. The European frogs were kept at moist conditions at 5 °C, the American ones were in aquaria with a small amount of pond water at 24 °C, and fed with live flies. The ovulation was induced by the method described by Rugh [7]. The eggs were surgically removed from the frog. In all cases normal, uncytolyzed eggs were randomly chosen for experimentation. The eggs were kept in amphibian Ringer solution at pH 7.8 (Table I). The follicle membrane was removed mechanically from the ovarian eggs. Special attention was paid to avoid damaging the plasma membrane.

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

TABLE I

ISOTONIC RINGER's SOLUTION

	mM	mosM
NaCl	112.93	225.86
KCl	2.01	4.02
$CaCl_2$	1.02	3.06
Total	115.96	232.95

Isotope exchange method

The exchange of water was followed by determinations of the changes in the reduced weight of an egg placed in isotonic Ringer solution containing 20 % ^2H_2O [8], by means of the automatic diver balance technique [9–11]. The exchange curve was recorded in two ways, partly on an X–Y recorder where the reduced weight is plotted as a function of time, partly on a paper tape with data points taken at intervals of ten seconds. The latter expedient improves the accuracy of the methods, since optical reading of the curves is avoided. In all cases the exchange process was followed until the curve leveled off, i.e. for about 20–40 min depending on the temperature.

The mathematical treatment of the exchange process is based on the assumption that the diffusion takes place isotropically from a sphere. Due to the low internal pressure in the egg in an isotonic solution, the egg, when placed on the diver, will deform slightly under the influence of the gravity force and establish a small contact area with the diver. This part of the egg cannot participate in the diffusion process and a deviation from the assumption of isotropy thus occurs. In order to reduce this source of error, the top part of the diver, approximately twice the diameter of the egg, is made of a 1 % agar. Diffusion of water is only slightly retarded in this medium [12], and this modification, therefore, implies an approach towards the assumption of a spherically symmetrical diffusion process.

Dainty [3] has submitted that a possible source of error in the diver balance method may arise through changes in the density of the medium when exchange with ^2H_2O takes place. The formal solution of this problem is very complicated, but it can be seen from the following reasoning that the effect probably is negligible.

The reduced weight (RW) of an egg is given by:

$$RW = V(\rho_e - \rho_m)g \tag{1}$$

where V is the volume of the egg, ρ_e is the density of the egg, ρ_m the density of the external medium and g the gravity. When the egg is placed in a solution containing ^2H_2O an exchange of water molecules take place. This will alter ρ_e and also RW. Since the diffusion coefficient of the surrounding medium is not infinitely large, and no mechanical stirring is applied, there will be a change in the density of the medium in the immediate surrounding of the egg when water molecules leave the egg. A consequent change in the buoyancy force might be anticipated. However, a change in ρ_m would lead to an alteration of the hydrostatic pressure around the egg and the system thus would no longer be in mechanical equilibrium. Since the egg and the diver are placed in a cuvette with a volume approximately 1000 times larger than the egg, the cuvette can be regarded as a pressure reservoir acting to keep the hydrostatic pressure constant at each horizontal level. The pressure equilibration thus ensured is much faster than the diffusion process.

For an exact solution of this problem the hydrodynamic equations would have to be solved for fluctuations in pressure, density and temperature, taking into consideration physical properties of the liquid such as heat capacity, viscocity, velocity of sound, compressibility, etc.. Such an analysis presumably would show that for our purposes we can regard the system as being in mechanical equilibrium, and that, therefore, no change in buoyancy force occurs during the experiment (see further de Groot [13] and Hanley [14]).

The fluctuations in the density of the medium would be largest at the very

beginning of the exchange process where the concentration gradients of the different water isotopes are largest. For reasons given later in the text, it is impossible to start the readings during the first 60 s of the experiment, and this further strengthens the assumption of mechanical equilibrium.

Mathematical treatment

The theory of the tracer exchange, as recorded in a diver balance experiment, has been outlined by Løvtrup [2] and by Hansson Mild [4, 15]. The main principles are as follows,
the exchange is described by the diffusion equation:

$$D_i \nabla^2 c_i = \frac{\partial c_i}{\partial t}, \qquad i = 1, 2 \tag{2}$$

where D_1 is the cytoplasmic diffusion coefficient, D_2 the diffusion coefficient in the external medium and c_i is the concentration of heavy water in the two compartments. The proper boundary conditions to be used are:

$$-D_1 \left(\frac{\partial c_1}{\partial r}\right)_{r=R} = -D_2 \left(\frac{\partial c_2}{\partial r}\right)_{r=R} = P(c_1 - c_2)_{r=R} \tag{3}$$

where P is the permeability coefficient, and R the radius, of the egg. Allowing for the effect of diffusion in the external medium, Eqn 3 implies that the so-called unstirred layer [3] is taken into account. The solution of the equations given by Eqn 2 and the boundary conditions according to Eqn 3 cannot be obtained in an analytical form and, therefore, numerical methods are used. In this case Laplace transformation in the dimensionless time variable $\tau = D_1 \times t/R^2$ is employed. The transformed function for the reduced weight (RW) is:

$$\overline{\left(\frac{M_t}{M_\infty}\right)} = \frac{3L}{p^2}\left(\frac{\sqrt{p}\coth\sqrt{p}-1}{L-1+\sqrt{p}\coth\sqrt{p}+L\dfrac{\sqrt{p}\coth\sqrt{p}-1}{A(1+\sqrt{p}/A)}}\right) \tag{4}$$

where p is the Laplace variable, $L = RP/D_1$, $A = D_2/D_1$. M_t stands for the amount of substance exchanged after time t (i.e. τ) and expressed in terms of RW

$$\frac{M_t}{M_\infty} = \frac{RW_t - RW_0}{RW_\infty - RW_0}. \tag{5}$$

The numerical inversion method of Nordén [16] is applied to Eqn 4 to obtain the desired formal solution. This method has recently been thoroughly tested and compared with other methods for numerical inversion of Laplace transforms [17] and it was concluded that it is one of the most efficient methods for this type of function, with respect both to the amount of computation work that has to be done and to the accuracy of the result.

The curve-fitting procedure

In order to obtain the values for P and D_1 from the experimental curves, these have to be compared with the theoretical expression for the exchange process by means of a curve-fitting procedure.

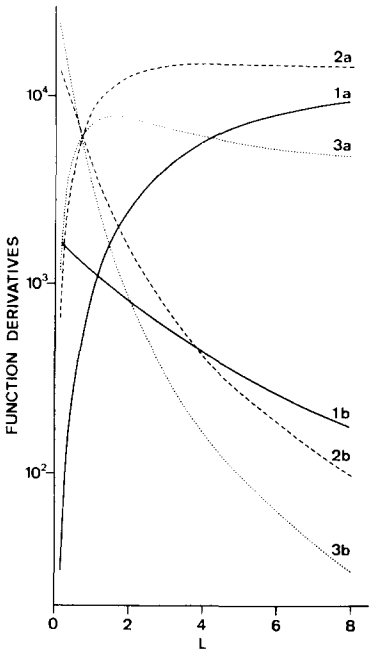


Fig. 1. The derivatives of the theoretical function of the exchange process with respect to D_1 (a) and P (b), respectively. The following parameter values were employed: $R = 0.1$ cm, $D_2 = 1.8 \cdot 10^{-5}$ cm^2/s, $D_1 = 6.0 \cdot 10^{-6}$ cm^2/s. The two sets of curves corresponds to different values of the dimensionless time variable τ, namely 0.036 (1), 0.36 (2) and 0.72 (3).

In almost all efficient methods of this kind the derivatives of the theoretical function with respect to the parameters are required if rapid convergence is desired. Since the function is known only numerically, the derivatives also have to be calculated numerically. In order to obtain as good accuracy as possible in these calculations the derivatives were first derived in the Laplace transform of the function; subsequently the numerical inversion method [16] was applied to obtain the final values. The derivatives of $[M_t/M_\infty]$ with respect to D_1 and P for a particular case are shown in Fig. 1. It can be seen that for large values of L, the dimensionless permeability coefficient, the function becomes rather insensitive to changes in P, the process thus being mainly controlled by diffusion. The reverse situation is obtained when $L \ll 1$, where the derivate with respect to D_1 is very small and the process is controlled largely by permeation.

To obtain optimum efficiency in the curve-fitting when both the diffusion and the permeability coefficient are to be obtained simultaneously from the exchange curve, the derivatives should be of the same order of magnitude, i.e. the value of L should be about 1.

When the experimental curves are to be compared with the numerical inversion of Eqn 4, both RW_0 and RW_∞, must be known. This requires two further parameters besides D_1 and P. With the diver balance technique it is impossible to obtain the weight at the beginning of an exchange experiment since, in order to reduce the error in the time scale, the egg is dropped down onto the diver. This causes a mechanical

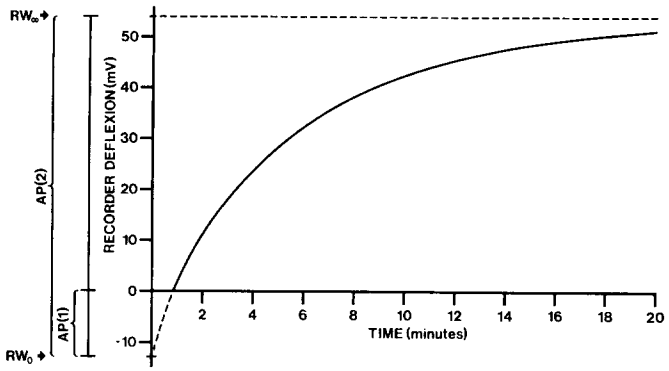


Fig. 2. Reproduction of an experimental curve from a body cavity egg of *R. temporaria* at 18 °C in 100 % Ringer solution. The radius of the egg was 0.0834 cm. The relation between the $AP(i)$, $i = 1,2$, and the reduced weight is shown in the figure.

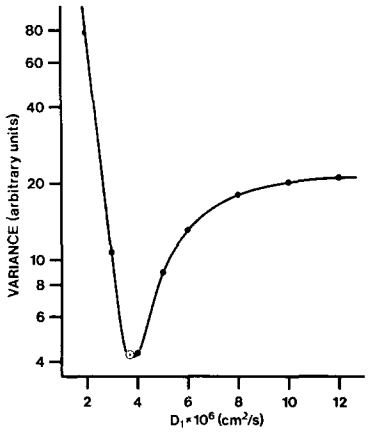
disturbance of the system, and it takes approximately 30 s before the diver is stabilized enough to give reliable readings. It is for this reason that our measurements start at $t = 60$ s. In principle, RW_∞ could be estimated experimentally, but this would require a very careful calibration of the diver balance before each separate experiment. Furthermore, a very long time must be employed in order for RW to approach the equilibrium value. To circumvent these difficulties the function employed for the curve-fitting procedure is:

$$F = -AP(1)+AP(2)\times f(P, D_1) \tag{6}$$

where $AP(i)$, $i = 1,2$ are two new parameters related to RW_0 and RW_∞, see Fig. 2.

Of the different methods tested for the parameter estimation the best results were obtained with a modification of the Marquardt method [18–20]. The function to be minimized is the sum of squares of the differences between the experimental points and the theoretical curve. The search direction in the Marquardt method interpolates between the gradient direction and directions within $\pm$ 90° from the latter. The angle between the gradient and the search direction has been monitored and combined with a line search method. If a given search direction fails to give a decrease in the sum of squares, a new direction closer to the gradient is chosen. The process usually terminates after 20 iterations and the difference between experimental point and the theoretical value is then mostly to be found in the fourth digit for the whole time range of the curve. All the computational work was done at the data processing centre of Umeå University (UMDAC) at a CDC 3300 computer. An example of the performance of the computer program is shown in Fig. 3, where the curve-fitting is done with different fixed D_1 values and compared with the result obtained from a computer run with no constraints on D_1. It is seen that the D_1 value in the latter case lies at the minimum of the variance. In Fig. 4 the corresponding values of P for this curve are shown as a function of D_1.

Although much effort has been laid down on increasing the efficiency of the computer program, some difficulties are not yet overcome. Thus, working with body cavity eggs it was found very difficult to obtain reliable values for D_1 and P simultaneously. In some cases D_1 assumed very large values, sometimes even larger than the



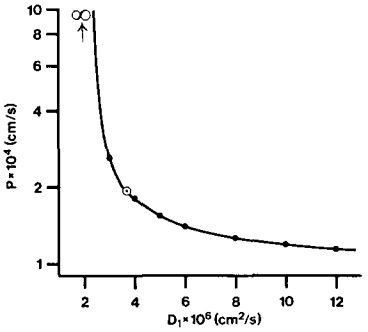


Fig. 3. (●) The variance (sum of squares of the deviation between the experimental and the theoretical curve) as a function of D_1, when the latter is fixed at the values shown. ○, the result obtained from a simultaneous determination of D_1 and P. The experimental curve is the same as shown in Fig. 2.

Fig. 4. The value of P as a function of different fixed values of D_1. ○, the result obtained from a simultaneous determination of D_1 and P.

self-diffusion coefficient of water in water. The cause of this failure is probably to be found in the high values of L, around 5 and higher, where the derivatives of the function with respect to D_1 and P are beginning to differ by an order of magnitude.

A further complication arises for the simultaneous determination of D_1 and P for this type of exchange curve, because it is very difficult to distinguish between an exchange curve with is rate limited by the membrane (L finite) and one which is diffusion controlled (L infinitely large) when both $AP(1)$ and D_1 are variables. From studies of the theoretical curves it appears that by a suitable choice of values for the two sets of parameters the difference between the function values can be made very small for times $t \geqslant 60$ s, but various attempts to place constraints on the parameters have been unsuccessful. It was decided to perform the curve fitting for body cavity eggs with a fixed value of D_1 corresponding to that found for ovarian eggs. This expedient causes an error in the individual permeability determinations, since there is a small variation in the diffusion coefficient from egg to egg. However, the reported values for P are mean values of several experiments, and this will reduce the error. This conclusion is corroborated by results from earlier experiments with body cavity eggs in which the simultaneous determination of D_1 and P was possible. The difference between the mean values of P obtained in this way and those obtained from determinations with fixed D_1 values was less than 5 %.

RESULTS

In the ovarian eggs of both species studied no measurable permeability barrier towards water movement can be detected. At all temperatures the values of P are larger than 10^{-1} cm/s. The difference between an exchange curve with P of this order and one where $P \rightarrow \infty$ lies in the fourth or fifth digit of the theoretical function and is,

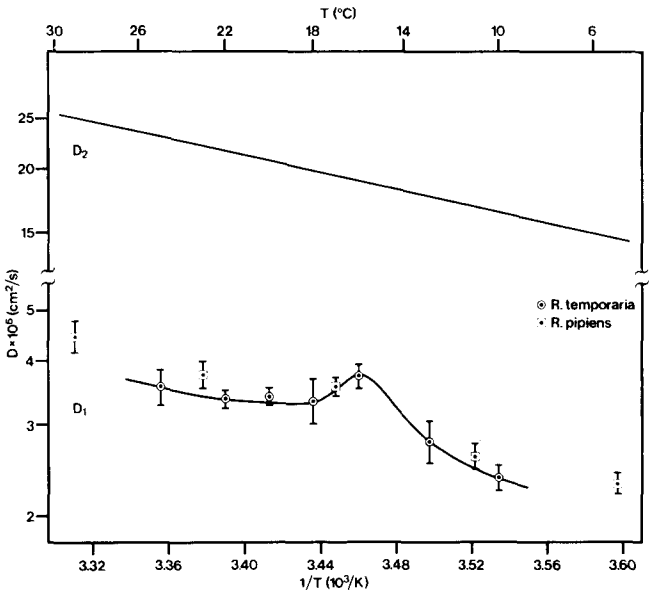


Fig. 5. The self-diffusion coefficient D_1 of water in the cytoplasm of ovarian eggs of *R. temporaria* (○) and *R. pipiens* (⊡) as a function of $1/T$. The vertical lines indicate the standard deviation of the mean. (n ≈ 6). For comparison the self-diffusion coefficient for bulk water, D_2, is also shown [21].

therefore, not measurable with out technique. At each temperature the fluctuations in the permeability coefficient were very large, sometimes several orders of magnitude. No significance can be attached to this, it merely reflects the failure of the method to detect a finite permeability.

The results for the diffusion coefficient D_1 is presented in an Arrhenius plot in Fig. 5. For comparison the temperature dependence of D_2, the self-diffusion coefficient of water [21] is also shown. The latter follows a straight line according to the equation:

$$D = D_0 e^{(-E_a/RT)} \tag{7}$$

where D_0 is a constant, E_a the apparent activation energy and R the gas constant. For diffusion of water in water (D_2) E_a is found to be 4.1 kcal/mole.

From Fig. 5 it appears that the D_1 values for *R. temporaria* do not follow a straight line but show a pronounced peak at 16 °C. Even at 25 °C D_1 is still below the peak value. Eqn 7 is therefore, not applicable in the whole temperature range.

The experiments on *R. pipiens* were carried out at temperatures with intervals of 6 °C and, therefore, no curve is drawn through these points. However, it is seen from Fig. 5 that the D_1 values for this specie are slightly higher than the corresponding values from *R. temporaria*. This difference in D_1 between the species has also been found before [11]. The two species seem to have the same anomalous temperature dependence at 16 °C.

In Fig. 6 the permeability coefficients of the body cavity eggs are plotted as log E versus $1/T$. In some cases it has been possible to obtain D_1 and P simultaneously from the same curve and these values are marked with a filled circle. The D_1 values

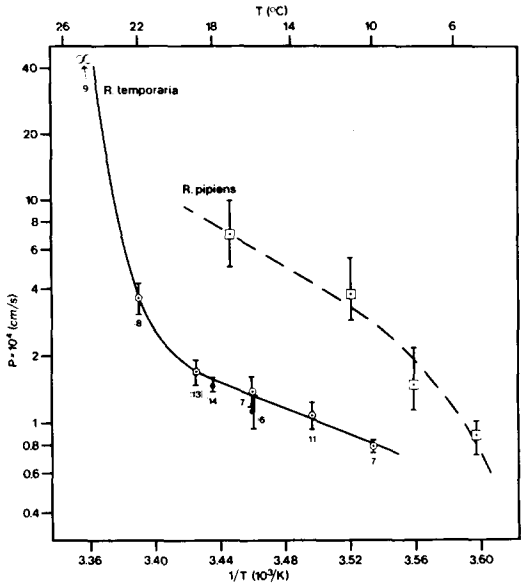


Fig. 6. The permeability coefficient of water in body cavity eggs of *R. temporaria* (○ and ●) and *R. pipiens* (⊡) as a function of 1/T. The points indicated with a filled symbol are from curves where a simultaneous determination of D_1 and P was carried out. The number (n) of experiments at each temperature is indicated in parentheses. The vertical lines indicate the standard deviation of mean. The error limits for *R. pipiens* have been estimated as the maximum error in the extrapolation.

(*R. temporaria*) found in this way are: $(4.0\pm0.3)\cdot10^{-6}$, $(5.0\pm0.8)\cdot10^{-6}$ and $(5.0\pm0.3)\cdot10^{-6}$ cm^2/s for 16, 18 and 25 °C, respectively, thus slightly higher than the corresponding ones presented in Fig. 5. However, since these experiments were carried out before diver with agar top were introduced, too much significance cannot be attached to the discrepancy.

The most remarkable thing about the curve for *R. temporaria* in Fig. 6 is the disappearance of the permeability barrier at 25 °C. In some experiments a finite value of P (not shown in the figure) was found, but in the great majority of cases the values were outside the range of the diagram, i.e. greater than 10^{-1}. The P values at this temperature are not very sensitive to the D_1 value employed in the curve-fitting procedure. In order to obtain P values of the order $3\cdot10^{-4}$ cm/s (corresponding to the extrapolation of the straight line to 25 °C), D_1 has to be taken as high as $1.0\cdot10^{-5}$ cm^2/s.

Application of Eqn 7 in the temperature range 10–19 °C, gives an apparent activation energy of 14 kcal/mole for the permeation process in *R. temporaria*.

The experimental results from body cavity eggs of *R. pipiens* showed that 100 % Ringer solution is not isotonic for these eggs. The P values decrease with time in a manner similar to that found when experiments are carried out on eggs of *R. temporaria* incubated in hypotonic solutions [22]. The results presented in Fig. 6 are extrapolations corresponding to the values to be anticipated under isotonic conditions (see further Hansson Mild and Løvtrup [22]). The extrapolation procedure unfortunately does not entail a satisfactory accuracy particularly at high values of P, and thus of

the temperature. We have, therefore, chosen not to draw the curve for *R. pipiens* in the whole investigated temperature range. However, the values of P at high temperatures, indicate conformance with the results on *R. temporaria*, although the temperature limit where $P \rightarrow \infty$ is shifted to around 30 °C.

In spite of fact that the P values are found through extrapolation there is no doubt that the permeability is significantly reduced when the temperature is lower than 8 °C.

DISCUSSION

The diffusion coefficient for water in cytoplasm

The present studies constitute a correction of the results obtained in earlier work with the diver balance. Haglund and Løvtrup [23] reported values on the temperature dependence of D_1 in ovarian eggs of *R. temporaria* 30–40 % lower than those recorded by us. The reason for this discrepancy is to be found in the fact that they neglected the diffusion in the external medium. It has been shown [15] that this leads to an underestimation of the diffusion coefficient.

In the study by Løvtrup et al. [11] the influence of this factor was considered, but it was believed at that time that ovarian eggs have a permeability barrier towards water, and in order to remove this the eggs were treated with chemicals such as digitonin, ethanol and formaldehyde. According to Hansson Mild et al. [24] this kind of treatment profoundly alters the properties of the cytoplasm, leading to values of D_1 that are too high. By using spin echo techniques they found $D_1 = 6.8 \cdot 10^{-6}$ cm^2/s for ovarian eggs of *R. pipiens* at 22 °C. The values obtained in the present study are somewhat lower, but at present it cannot be decided whether this discrepancy is due to the methods or to the biological material.

The temperature dependence of D_1, particularly the peak at 16 °C, is remarkably different from that of ordinary water (Fig. 5). Drost-Hansen [25–27] has convincingly argued that the properties of water near interfaces, including biological ones, are notably different from those of bulk water and that phase transitions may be expected to occur as the temperature is changed. Transitions are frequently found in the temperature range 13–16 °C. In view of this it is tempting to suggest that a significant part of the cytoplasmic water prevails in one or more stabilized water structures, and that a high-order phase transition between such structures is indicated by the peak at 16 °C.

The observed temperature dependence of the cytoplasmic diffusion coefficient may afford an explanation of the thermal anomaly of the internal pressure in the body cavity eggs of *R. temporaria* found in this laboratory [28]. In these experiments the eggs were incubated in hypotonic Ringer solutions of varying strength and the pressure measured after a specified length of time. It was found that the pressure increases from 10 °C to a maximum at 16 °C, reaches a minimum at 19 °C, which was followed by a further increase.

Under the experimental conditions the eggs swell through uptake of water, the movement of which across the membrane is given by:

$$J_v = L_{pd} \Delta \Pi \tag{8}$$

where J_v is the volume flow per unit area, L_{pd} the phenomenological permeability

coefficient and $\Delta\Pi$ the osmotic pressure difference across the membrane.

When, as observed in the present paper, D_1 is higher at 16 °C than at either 13 or 19 °C, then, after entering the egg, the water will diffuse away from the membrane faster at the intermediate temperature. This, in turn, will lead to an increased effective concentration gradient, and thus $\Delta\Pi$, over the membrane, thereby causing proportionately more water to enter the egg per unit time and area. Since the internal pressure was found to be proportional to the stretching of the vitelline membrane surrounding the egg, we may thus account for the pressure maximum at 16 °C. The anomalous behaviour of D_1 at 16 °C is also reflected in a recent study [29] of the osmotic water permeability coefficient of ovarian eggs of *R. temporaria*.

Unstirred layers

The early studies on the exchange of isotopic water were based on the assumption that the water movement takes place between two well-stirred compartments, the cell membrane thus being the only rate-limiting barrier. It was later shown [1, 2] that this simple model cannot be used because the diffusion in the cytoplasm is so slow that it measurably affects the rate of water exchange, a circumstance leading to an underestimation of P.

However, even this approach is an oversimplification, for the diffusion in the surrounding medium is slow enough to affect the rate of the overall process, and correction for this factor will thus lead to a further increase of the permeability coefficient [4]. As an illustration of this successive correction of P it may be mentioned that Prescott and Zeuthen [5, 6] found the value $0.75 \cdot 10^{-4}$ cm/s for the body cavity egg of *R. temporaria*. When the cytoplasmic diffusion was taken into account a value of $1.6 \cdot 10^{-4}$ cm/s was recorded [30] and in the present work, where even the external diffusion is incorporated into the equations, we find a value of $2.6 \cdot 10^{-4}$ cm/s (22 °C).

The order of magnitude of the error introduced by neglecting the diffusion in the external medium may be estimated from the equation [3]:

$$\frac{1}{P_a} = \frac{1}{P} + \frac{\delta}{D} \tag{9}$$

where P_a is the permeability coefficient obtained without regard to the diffusion in the outer medium, P the correct permeability coefficient, δ the thickness of the unstirred layer and D the diffusion coefficient.

If the values of P given above are inserted in Eqn 9 we get a δ of about 500 μm, in close agreement with the value estimated by Dainty [3], thus supporting the claim that our method of calculation takes into account the unstirred layers.

The permeability coefficient of the ovarian egg

The values reported in the literature for the permeability coefficient of the ovarian egg fall in two groups. In the first are the results of Prescott and Zeuthen [5, 6] who, assuming exchange between two well-stirred compartments, found P to be $1.3 \cdot 10^{-4}$ cm/s in *R. temporaria*. Haglund and Loeffler [30] reported a value of about $3 \cdot 10^{-4}$ cm/s in three different anuran species (22–24 °C).

Løvtrup [2], Haglund and Løvtrup [23] and Ling et al. [31] came to the result that P is infinitely large, i.e. that no permeability barrier to water obtains in the ova-

rian egg. The present observations thus corroborate those in the latter group.

It remains to be seen whether the striking difference between the permeability coefficient of ovarian and body cavity eggs can be explained, as suggested by Dick et al. [32], with reference to the enlargement of surface area due to the microvilli present in the former case. The high P value for the ovarian egg might, of course, suggest that the cell membrane has become damaged through the isolation procedure. This explanation seems unlikely, however, in view of the fact that the extensive modification of the egg surface, involving the breakdown of the microvilli occurs during maturation, when the oocyte is transformed into a body cavity egg.

The permeability coefficient of the body cavity egg

Very few observations have been obtained on the activation energy for permeation of water through biological membranes. For red blood cells a very low value, 5–6 kcal/mole, has been reported [33]. In osmometric studies on the permeability coefficient in sea urchin eggs [34] E_a was observed to lie between 14.7 and 20.3 kcal/mole. Haglund and Løvtrup [23], using the isotope exchange method, found in amphibian eggs E_a to be 14.0–22.7 kcal/mole. It may also be mentioned that in artificial membranes activation energies of 12–15 kcal/mole have been reported [35, 36]. Apart from the results on erythrocytes the published values are seen to be in reasonable agreement with that obtained by us for the temperature range 10–19 °C.

An interesting comparison can be made between the temperature dependence of P at high and low temperature and the temperature range over which normal embryonic development is possible. Comparative studies [37, 38] on different species of frogs show that normal development of embryos is possible only in a temperature range of the order 22–24 °C. Thus, eggs of *R. pipiens* collected in the New York City area have a lower temperature limit of 6 °C and an upper one of 28 °C. The corresponding temperatures for *R. pipiens* living in Florida are 9–33 °C. Hertwig [39] found that eggs of *R. temporaria* cannot develop normally below 0.5–1 °C and above 24 °C. This upper limit is also confirmed in preliminary studies of our own.

From Fig. 6 it can be seen that the upper temperature limit for *R. temporaria* coincides with that where a drastic increase in water permeability occurs. The results on *R. pipiens* at high temperatures are indicative of a similar correlation. At the lower temperature limit of *R. pipiens* a marked decrease of P is found. No attempts were made to establish the lower limit of *R. temporaria* because further extension of the experimental temperature range involves great technical difficulties.

It is known that the fatty acids of the lipids in exothermic animals are adjusted to the environmental temperature [40], and Heilbrunn [41] first pointed out that the difference in sensitivity to heat of various organisms is correlated with differences in the melting point of their lipids. It is, therefore, likely that the observed temperature dependence of P could be interpreted to reflect a broad thermal phase transition of the membrane lipids. At temperatures below the lower limit the lipids should be in a rigid gel state, offering a great resistance towards passage of water, and above the upper limit they might be in a liquid crystalline state with very high permeability properties. However, this interpretation needs verification by further investigations, for instance with techniques such as electron spin resonance or differential scanning calorimetry.

The limiting temperatures are not sharply defined but extend over a few de-

grees. This offers a possible explanation of the finite P found in some experiments on *R. temporaria* at 25 °C. These particular experiments were carried out during the years 1971 and 1972 and thus with a material different from that used in the main part of this study. A small disparity in membrane lipid composition between separate groups of animals might entail a slightly shifted upper temperature limit and a substantially different permeability value.

ACKNOWLEDGEMENTS

We wish to thank Professor Arne Claesson for many stimulating discussions during the course of this work.

We also gratefully acknowledge the assistance of Mr André Berglund and Mr Ronald Grönlund in various aspects of the present work.

The work was supported by the Swedish Natural Science Research Council.

REFERENCES

1 Dick, D. A. T. (1959) Exp. Cell Res. 17, 5–12
2 Løvtrup, S. (1963) J. Theor. Biol. 5, 341–359
3 Dainty, J. (1963) Adv. Bot. Res. 1, 279–326
4 Hansson Mild, K. (1972) Bull. Math. Biophys. 34, 183–189
5 Prescott, D. M. and Zeuthen, E. (1953) Acta Physiol. Scand. 28, 77–94
6 Prescott, D. M. and Zeuthen, E. (1953) Acta Physiol. Scand. 28, 77–94
7 Rugh, R. (1962) Experimental Embryology (3rd edn), Burgess, Minnesota
8 Løvtrup, S. and Pigon, A. (1951) C. R. Lab. Carlsberg, Sér. Chim. 28, 1–36
9 Larsson, S. and Løvtrup, S. (1966) J. Exp. Biol. 44, 47–58
10 Bergfors, T., Hansson Mild, K. and Løvtrup, S. (1970) J. Exp. Biol. 53, 187–193
11 Løvtrup, S., Hansson, Mild, K. and Berglund, A. (1970) J. Cell. Physiol. 76, 167–174
12 Nakayama, F. S. and Jackson, R. D. (1963) J. Phys. Chem. 67, 932–933
13 de Groot, S. R. (1951) Thermodynamics of Irreversible Processes. North-Holland, Amsterdam
14 Hanley, H. J. M. (1969) Hydrodynamics in Transport Phenomena in Fluids, Dekker, New York
15 Hansson Mild, K. (1971) Bull. Math. Biophys. 33, 19–26
16 Nordén, H. (1961) Acta Acad. Aboensis Math. Phys. 22, 1–31
17 Hennesy, T. R. (1973) New Mathematical Model of Inert Gas Transport through Biological tissue at Hyperbaric Environments. Thesis, University of Cape Town
18 Marquardt, D. W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441
19 Bard, Y. (1970) SIAM J. Numer. Anal. 7, 157–186
20 Stusnick, E. and Hurst, R. P. (1972) J. Theor. Biol. 37, 261–271
21 Wang, J. H., Robinson, C. V. and Edelman, I. S. (1953) J. Am. Chem. Soc. 75, 466–470
22 Hansson Mild, K. and Løvtrup, S. (1974) J. Exp. Biol., in the press
23 Haglund, B. and Løvtrup, S. (1966) J. Cell. Physiol. 67, 355–360
24 Hansson Mild, K., James, T. L. and Gillen, K. T. (1972) J. Cell. Physiol. 80, 155–158
25 Drost-Hansen, W. (1971) Chemistry of the Cell Interface (Brown, H. D., ed.) Vol. B, pp. 1–184, Academic Press, New York
26 Drost-Hansen, W. (1972) Water and biological interfaces/structural and functional aspects. Paper presented at XV Solvay conference, Brussels
27 Drost-Hansen, W. (1973) Ann. N.Y. Acad. Sci. 204, 100–108
28 Hansson Mild, K., Løvtrup, S. and Bergfors, T. (1974) J. Exp. Biol. 60, 807–820
29 Hansson Mild, K. Carlson, L. and Løvtrup, S. (1974) J. Membrane Biol., in the press
30 Haglund, B. and Loeffler, C. A. (1969) J. Cell. Physiol. 73, 69–80
31 Ling, G. N., Ochensfield, M. M. and Karreman, G. (1967) J. Gen. Physiol. 50, 1807–1820
32 Dick, E. G., Dick, D. A. T. and Bradbury, S. (1970) J. Cell. Sci. 6, 451–476
33 Vieira, F. L., Sha'afi, R. I. and Solomon, A. K. (1970) J. Gen. Physiol. 55, 451–466

34 McCutcheon, M. and Lucké, B. (1932) J. Cell. Comp. Physiol. 2, 11–26
35 Price, H. D. and Thompson, T. E. (1968) Biophys. Soc. Meeting 12th, Abstr. M.C. 5
36 Redwood, W. R. and Haydon, D. A. (1969) J. Theor. Biol. 22, 1–21
37 Moore, J. A. (1939) Ecology 20, 459–478
38 Moore, J. A. (1949) Evolution 3, 1–24
39 Hertwig, D. (1898) Arch. Entw. Mech. 130, 266–339
40 Barańska, J. and Wlodawer, P. (1969) Comp. Biochem. Physiol. 28, 553–570
41 Heilbrunn, L. V. (1924) Am. J. Physiol. 69, 190–199