

BIOPHYSICAL ASPECTS OF PERMEATION AND
DIFFUSION OF WATER IN FROG EGGS

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"It is water that, on taking different forms, constitute this earth, this atmosphere, this sky, these mountains, gods and men, beast and birds, grass and trees and animals, down to worms, flies and ants. All of these are but different forms of water. Meditate on water."

Chāndogya Upanishad ca. 1000 B.C. quoted in Ann.N.Y.Acad.Sci. (1973) 204 , p. 134 .

This dissertation is based on the following papers:

- I. Modifications in the automatic diver balance technique.
J. Exp. Biol. 53 (1970) 187-193. Together with T. Bergfors
and S. Løvtrup.
- II. The kinetics of diffusion between a spherical cell and a surrounding
medium with different diffusion properties.
Bull. Math. Biophys. 33 (1971) 19-26.
- III. Diffusion exchange between a membrane-bounded sphere and its surrounding
Bull. Math. Biophys. 34 (1972) 93-102.
- IV. On the mechanical properties of the vitelline membrane of the frog egg.
J. Exp. Biol. (in press). Together with S. Løvtrup and T. Bergfors.
- V. Diffusion and permeation of water in the frog egg. I. The effect temperature.
Submitted for publication. Together with S. Løvtrup.
- VI. Diffusion and permeation of water in the frog egg. II. The effect of tension
and tonicity.
Submitted for publication. Together with S. Løvtrup.

These papers will be referred to by their Roman numerals.

1. Introduction

All animal cells are surrounded by a membraneous envelope, called the cell membrane or 'plasma' membrane. This structure constitutes a barrier separating the intracellular from the extracellular fluid. Numerous cellular properties have been attributed to the plasma membrane, but it is not always evident whether all of these actually reside there.

Since Overton's (1899) work, one of the main sources of information about the structure and function of the cell membrane has been permeability studies involving measurements of the rate at which dissolved molecules can enter or leave the cell.

Water is essential to the functioning of the cell and it is therefore of importance to understand the means by which water passes into and out of cells. The first students who studied water permeability employed the so-called 'osmometric' method, in which a cell is exposed to hyper- or hyposmotic conditions and the resulting changes in volume measured as a function of time. The use of isotopic water in permeability studies was first introduced by von Hevesy et al. (1935), who studied the movement of labelled water across frog skin. By the combination of the isotope exchange method and the Cartesian diver balance (Pigon and Løvtrup, 1951), it became possible to follow the exchange of water in an individual cell. This method was further improved by Larsson and Løvtrup (1966)

through the introduction of the automatic electromagnetic diver balance.

The present thesis comprises studies on the diver balance technique, outlined in § 2, and the theory of water exchange between a cell and its surroundings, a brief summary is given in § 3. The results obtained have been applied to experiments on ovarian and body cavity eggs of frogs and are summarized in §§ 4 and 5.

2. The automatic diver balance technique

The principle of the automatic diver balance is that a submerged body, the diver, with density less than the surrounding medium, is prevented from rising to the surface by an electromagnetic force acting on a small piece of iron placed in the diver. The early versions of the balance displayed certain imperfections which hampered experimental progress. The primary causes of these disturbances have been traced to temperature oscillations in the flotation medium, to details in the electronic design and to the magnetic material used in the core and the diver. A brief outline of the theory and the modifications introduced is given in (I).

3. The mathematics of diffusion in spherical cells

In all early permeability studies it has been assumed that the cell membrane is the only barrier limiting the rate of water transfer between the living cell

and the surrounding medium. This assumption was shown to be erroneous (Dick, 1959; Løvtrup, 1963). The diffusion of water in the cytoplasm has to be taken into account in order to obtain the proper permeability coefficient, P , of the membrane. The equation to be used for the evaluation of isotope exchange curves was given by Løvtrup (1963). However, Dainty (1963) criticized the studies of isotopic water exchange performed on cells and artificial membranes and claimed that the values of P were grossly underestimated due to disregard of the diffusion in the unstirred layers of water adjacent to the membrane, layers that may be as large as 500 μ .

A justification of this criticism was noted in a consistent discrepancy between the experimental observations and the theoretical curves calculated according to the equation given by Løvtrup (1963). In (II) is presented the first attempt to attack this problem. The diffusion equation is solved for a homogenous sphere located in media with different diffusion properties. The method of Laplace transformation is used to obtain the formal solution, however, no inversion can be found for all times and an expansion is performed valid for small times, i.e. $\tau \ll 1$ where $\tau = Dxt/R^2$ and D is the diffusion coefficient, t the time and R the radius. This new theory for the evaluation of isotopic exchange curves was applied to experiments performed on frog ovarian eggs. The eggs had been treated with chemicals in order to remove the diffusion barrier at the surface. The D values

found (Løvtrup, Hansson Mild and Berglund, 1971) were significantly higher than those obtained with the old theory. It has later been shown (Hansson Mild, James and Gillen, 1972), however, that the chemical treatment profoundly alters the properties of the cytoplasm and that the values found are too high to represent the diffusion coefficient in untreated ovarian eggs.

In (III) the solution of the diffusion equation is extended to include a membrane-bounded sphere situated in media with different diffusion properties. In order to obtain the formal solution, Laplace transformation in the time variable is employed. It is not possible to find a closed-form solution in terms of known analytical functions, and a numerical inversion technique is applied to obtain the final solution valid for all times.

Applying the equation of Løvtrup (1963) to exchange curves obtained on body cavity eggs show that the permeability coefficient varies with the time range of the curve employed for the calculation. The highest value was obtained working with the first part of the curve and the lowest when the calculations were performed on the later part of the curves. When the whole time range of the curves were used an average value was obtained. This source of error is eliminated by the theory presented in (III). As expected, from the work of Dainty (1963), the P values are somewhat higher than with the old theory. These results thus show the importance of taking into account the diffusion in the cytoplasm as well as

in the external medium when P is to be determined.

4. The influence of temperature on the permeability coefficient

Temperature is one of the most important variables of the physico-chemical environment of the living material. Most organisms are able to live only within a very narrow temperature range, usually of the order of 20° to 30°C . In (V) the results of the studies on the temperature dependence of the cytoplasmic diffusion coefficient and the permeability coefficient of the plasma membrane of frog eggs are presented. It is shown that the permeability barrier breaks down ($P \rightarrow \infty$) when the temperature is raised above a certain limit and P is significantly reduced in the low temperature range. The temperatures where these drastical changes occur coincide with the limits for normal embryonic development of the ranid species studied (Rana temporaria and Rana pipiens). The experimental results are interpreted to reveal the existence of a broad thermal phase transition of the lipids in the membrane going from a rigid crystalline gel to a liquid crystalline state at high temperatures (order \rightarrow disorder), and the observed correlation with biological observation suggest that the normal functioning of the plasma membrane requires that it prevails in this transition phase.

The self-diffusion coefficient of water in cytoplasm, D_1 , is required for the calculation of P , and this parameter has been measured in ovarian eggs. An anomalous temperature dependence of D_1 was found. The values increase with in-

creasing temperatures until at 16°C a local maximum is found. Further increase of the temperature gives at first a slight decrease of D_1 followed by a measurable increase. The D_1 value at 25°C is still lower than the peak value at 16°C.

Drost-Hansen (1971, 1973) has convincingly argued that the properties of water near interfaces, including biological ones, are notably different from those of bulk water, and that higher order phase transitions may result when the temperature is changed. These transitions frequently occurs around the temperatures 13-16°, 29-32°, 44-45° and 60-62°C. The results on the temperature dependence of D_1 thus indicate that most of the water in the ovarian egg of frog cannot be regarded as ordinary water.

5. The influence of tension in the vitelline membrane on the permeability coefficient.

In most animal cells the exit of water which occurs in a hyperosmotic medium is more rapid than the entrance of water taking place under hyposmotic condition. This fact, which obviously limits the applicability of osmometric methods in permeability studies, was confirmed in isotope exchange experiments (Berntsson et al. 1964) showing that P is significantly lower in hypotonic than in isotonic solutions. It could not be established whether it is the concentration of the bathing medium proper, or rather the tension of the cell membrane, that is responsible for the observed changes in P . This question is the subject of the study presented in (VI).

The main result, obtained from experiments with body cavity eggs of Rana temporaria, is that the permeability decreases with the incubation time in hypotonic solution. The observed changes may be correlated with an increase of the stress resultant (i.e. tension) in the vitelline membrane.

This correlation of P with the tension was made possible through the results reported in (IV) in which the increase of the internal pressure in the eggs was measured as a function of the incubation time in different hypotonic solutions and at different temperatures. It was found that the pressure increases from 10°C to a maximum at 16°C , reaches a minimum at 19°C , which is followed by a further increase. The most likely reason for this anomalous behaviour is that it is a reflection of the temperature dependence of D_1 recorded in (V), where a mechanism to explain the observations is put forward.

6. Conclusions

In many biological systems the permeability coefficient measured osmometrically, L_p , is larger than the corresponding isotopic permeability. This has been interpreted to indicate that the permeation of water takes place through water-filled cylindrical pores across the membrane. The measurements of Prescott and Zeuthen (1953) gave the ratio of L_p/P to 1.61 and 70 for body cavity and ovarian eggs (R. temporaria), respectively. Stein (1967) used these ratios to calculate the pore radius to 2.8 and 30 Å for these eggs. In view of the

results of the present study it is seen that the ratio for body cavity eggs probably is less than unity and in ovarian eggs it tends to zero due to the infinitely large isotopic permeability. This would give a complex value for the radius according to the formula used to calculate the pore radius.

These findings suggest that in order to compare the two permeability coefficients, L_p and P , new and more accurate values of the former must be obtained.

The results of the temperature dependence of the self-diffusion coefficient of water in the cytoplasm of the ovarian eggs and of the pressure in body cavity eggs show that the state of the intracellular water is significantly different from bulk water.

The temperature dependence of P was interpreted as a reflection of a broad thermal phase transition of the lipids in the membrane and this point must be further investigated by a different technique, for instance differential scanning calorimetry.

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