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PERMEABILITY OF VALONIA TO WATER AND SOLUTES: APPARENT ABSENCE OF AQUEOUS MEMBRANE PORES

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SUMMARY

The permeability of the marine alga *Valonia ventricosa* to water and small solutes was studied in cells perfused internally by two micropipets inserted into the vacuole. The protoplast of Valonia was highly permeable to alcohols but relatively impermeable to water and small hydrophilic solutes. For methanol the permeability coefficient of the protoplast was $3.1 \cdot 10^{-4}$ cm·sec^{-1}, and the reflection coefficient was highly negative, about —1.3. The permeability coefficient for urea, on the other hand, was about 10^{-6} cm·sec^{-1}, and the reflection coefficient was unity. No apparent solvent–solute interactions occurred for methanol, urea and water crossing the protoplast, as indicated either by a simple test for "solvent drag" or by a test based on irreversible thermodynamics. The osmotic and diffusional permeability coefficients for water were similar, about $2.4 \cdot 10^{-4}$ cm·sec^{-1}. These results suggest that movements of water, urea and methanol across the protoplast of Valonia are rate limited by a non-porous plasma membrane.

INTRODUCTION

Biological membranes have long been thought to contain aqueous pores which facilitate the penetration of water and small hydrophilic solutes[1–3]. Two main lines of evidence for the existence of these water-filled pores in biological membranes are, first, a discrepancy between the osmotic and diffusional permeabilities of a membrane to water and, second, apparent interactions between water and solutes crossing the membrane. This report deals mainly with the second criterion for water-filled pores, that is, the solvent–solute interaction which may occur if both species cross the membrane *via* aqueous channels. In amphibian skin, for example, an inward osmotic flow increases the flux ratio (influx/efflux) for small hydrophilic solutes such as acetamide and thiourea[4]. Several other tests for solvent–solute interactions in membranes are based on irreversible thermodynamics[5–7]. For example, DAINTY AND GINZBURG[5] have shown that if there are water-filled pores in a lipid membrane then the reflection coefficient, σ, for a particular solute will be given by

$$\sigma = 1 - \omega\bar{v}_s/L_p - a$$

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where ω is a solute permeability coefficient, $\bar{v}_s$ is the partial molar volume of solute, L_p is the hydraulic conductivity, and α expresses a direct interaction between water and solute within the membrane. Thus if the measured reflection coefficient, is less than $1-\omega\bar{v}_s/L_p$, the presence of aqueous pores is suggested.

The giant-celled marine alga Valonia has long been a popular organism for studies of electrolyte transport and bioelectrical phenomena[8–12]. This paper describes the permeability of *Valonia ventricosa* to water and to some small non-electrolytes, with special reference to the possible existence of water-filled pores in the membranes of this cell. I have compared the osmotic and diffusional permeabilities of Valonia to water, and I have tested for solvent–solute interactions by a simple test for "solvent drag" and by a test based on irreversible thermodynamics. All the results suggest an absence of aqueous pores in the membranes of Valonia.

METHODS

General

Cells of *Valonia ventricosa* were shipped by air from either Jamaica or Miami and maintained in natural seawater *plus* enough NaCl and KCl to raise the salinity from about 3.5 to 3.7 %, similar to the water in which Valonia grows naturally. Small amounts of phosphate, nitrate and soil extract were added as prescribed by KECK[13]. The approximate concentrations of major ions in the final seawater medium were Na^+, 505; K^+, 11; and Cl^-, 600 mM. The cells were exposed to room illumination and the temperature was $24° \pm 2°$. Cells were kept under these conditions for up to 2 months without apparent deterioration. During experiments the cells were illuminated continuously at about 3500 lux, and the temperature was $25° \pm 1°$.

Intracellular perfusion

The method of perfusing the vacuole of Valonia is described elsewhere[14]. Briefly, a spherical cell about 1 cm diameter is held with waterproof putty in a dish of seawater on the stage of a dissecting microscope. Two micropipets (0.1–0.3-mm tip diameter) are then forced through the cell wall (7–13 μ thick) and underlying protoplasm[15] (7–12 μ thick) into the vacuole. After a recovery period of 1–2 h the vacuole is perfused with artificial sap at a rate of 100–250 μl/min, depending on the cell volume. The artificial sap contains 1 part seawater and 9 parts 0.68 M KCl, giving approximate final concentrations of Na^+, 50; K^+, 615; and Cl^-, 660 mM, similar to the natural ion concentrations in the sap of *Valonia ventricosa*[16,17]. The inflow and outflow micropipets are connected to the inflow and outflow reservoirs by 1-mm plastic tubing. Perfusion is maintained by gravity with the inflow reservoir 5–10 cm above the outflow reservoir.

Electrical measurements

The potential difference between vacuole and seawater was measured with a high-impedance voltameter, which made electrical contact with the inflow reservoir and external seawater *via* KCl–agar bridges and calomel electrodes. The vacuole potential was monitored throughout each experiment and provided a sensitive indicator of the condition of the cell. Serious leaks which occasionally develop caused an immediate drop in the potential difference to near zero. The average vacuole potential

of all the cells used in this study was 16 mV (range, 10–28 mV), vacuole positive to external seawater, which is the normal value for *V. ventricosa*[16].

Fluid movements

The volume flow across the protoplast was measured by closing the inflow tube and observing the rate of fluid movement in a calibrated capillary (0.10 μl/mm) attached to the outflow tube. Because the cell volume was large (about 1 cm^3) an osmotic water flow of about $4 \cdot 10^{-7}$ $cm \cdot sec^{-1}$ could be measured during a period of at least 10 min without an appreciable change in the internal solute concentrations. Fluid movements were measured with artificial sap inside the cell and with either seawater or seawater *plus* various non-electrolytes, usually mannitol or methanol, outside. The osmotic pressures of the mannitol and methanol solutions were checked by the freezing-point depression, and the values agreed within 5 % of the osmotic pressures predicted by the Van 't Hoff relation: $\pi = RTc$ where π is the excess osmotic pressure due to the added non-electrolyte and c is the concentration of non-electrolyte.

Isotope fluxes

Unidirectional movements of water and solutes across the protoplast were measured with radioisotopes and standard detectors. Tritiated water (^3HHO) and [^{14}C]methanol were measured by liquid scintillation, and [^{14}C]urea was measured in a gas-flow counter. To measure the permeability of the protoplast to a labeled substance, the tracer was added to the inflow reservoir and about 40 min was allowed for the tracer efflux to reach a constant value. Then 30 ml of non-labeled seawater were placed outside, and 5–6 0.5-ml samples of this solution were withdrawn at 2–5-min intervals. The total counts in the external solution were plotted against time (see Fig. 1), and the gradient of this curve, $V_1(dc_1^*/dt)$, was determined. The permeability coefficient, P ($cm \cdot sec^{-1}$), was calculated by the equation

$$P = \frac{V_1(dc_1^*/dt)}{Ac_2^*} \qquad (1)$$

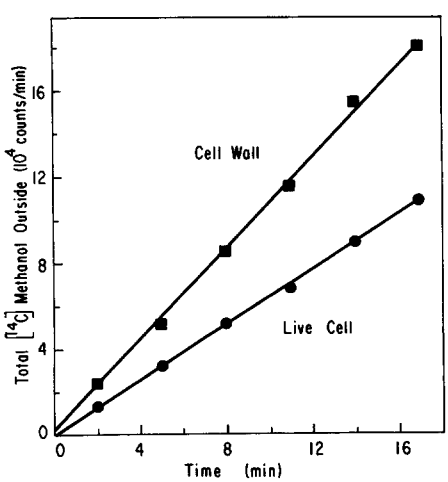

Fig. 1. Efflux of labeled methanol from an internally perfused cell of *Valonia ventricosa*. Efflux of methanol was measured first from a live cell and then, after the formation of aplanospores, methanol efflux was measured from the remaining cell wall.

where c_1* and c_2* are the concentrations of labeled substance (counts·min^{-1}·cm^{-3}) outside and inside, respectively, A is the surface area (cm^2) of the spherical cell, V_1 is the volume (cm^3) of the external solution, and t is the time (sec). The influx of tracer was neglected because c_1* was never greater than 4 % of c_2*. During the flux measurements, the external solution was stirred gently by means of a Pasteur pipet. Vigorous stirring was impossible because of the fragility of the cytoplasmic seal around the micropipets.

Hydraulic conductivity, permeability coefficients and reflection coefficients

In a normal, non-perfused Valonia cell, the osmotic pressure of the sap exceeds the osmotic pressure of the external seawater by about 1 atm (ref. 18). Osmotic equilibrium is maintained by the turgor or wall pressure, which is equal to the difference between the osmotic pressures of the external seawater and vacuolar sap. The cytoplasm is assumed to be in osmotic equilibrium with the sap. In a perfused Valonia cell the turgor pressure is virtually abolished, for the hydrostatic pressure of the perfusion system (10–20 cm of water) is only 2–3 % of the normal turgor pressure. An osmotic pressure difference still exists, however, and thus there is an inward osmotic flow, J_v, of about $3 \cdot 10^{-7}$ cm·sec^{-1}. Not all of this volume flow is due to simple osmosis, however, because even when identical solutions bathe both sides of the protoplast there is still an inward volume flow of almost $1 \cdot 10^{-7}$ cm·sec^{-1} which appears to be coupled to inward salt transport[14]. I will designate this "non-osmotic" volume flow $J_{v(n)}$ and will assume $J_{v(n)}$ to be constant throughout these experiments. This assumption is justified because salt transport in Valonia is insensitive to small changes in external osmotic pressure, *i.e.*, over a range of 26–30 atm (ref. 14 and unpublished data). The total inward volume flow, J_v (cm·sec^{-1}) in a perfused cell is thus given by

$$J_v^1 = L_p(\pi_i^2 - \pi_i^1) + J_{v(n)} \tag{2}$$

where L_p is the hydraulic conductivity (cm·sec^{-1}·atm^{-1}) and π_i^1 and π_i^2 are the osmotic pressures (atm) due to the impermeant ions in seawater and sap, respectively. If a small amount of impermeant non-electrolyte is added to the external seawater, a second volume flow, J_v^2, is observed, which is given by

$$J_v^2 = L_p(\pi_i^2 - \pi_i^1) + J_{v(n)} - L_pRTc_i^1 \tag{3}$$

where c_i^1 is the concentration (mole·cm^{-3}) of added non-electrolyte. Subtracting Eqn. 3 from Eqn. 2 gives

$$J_v^1 - J_v^2 = L_pRTc_i^1 \tag{4}$$

from which the hydraulic conductivity of the protoplast can be easily calculated. To estimate the permeability of Valonia to a relatively impermeant solute such as mannitol, we may neglect the diffusional resistance of the cell wall and unstirred layers and assume that the cell wall is relatively permeable to small solutes (see, *e.g.*, ref. 19), so that the permeability coefficient of the wall *plus* protoplast will be equal to the permeability coefficient of the protoplast alone. To estimate the permeability of the protoplast to permeant substances, however, we must know the concentration difference across the protoplast, $c^4 - c^3$, which is not the same as the concentration difference between the bulk solutions, $c^2 - c^1$. Fortunately, the permeability

of the isolated cell wall and unstirred layers can be measured after the entire protoplast of Valonia forms aplanospores[15,20], a reproductive process triggered by perfusing the vacuole with seawater. Thus we can first measure the permeability of an intact cell (P_{tot}), then measure the permeability of the cell wall and associated unstirred layers (P_{wall}), and then calculate the permeability coefficient of the protoplast (P_{pro}) by the equation

$$P_{pro}^{-1} = P_{tot}^{-1} - P_{wall}^{-1} \tag{5}$$

which is accurate if P_{wall} is considerably greater than P_{tot} (ref. 8).

To estimate the reflection coefficient, σ, of the protoplast for a permeant solute, the diffusional resistance of the wall and unstirred layers must again be taken into account. First the inward volume flow is measured with seawater outside, as given by Eqn. 2. Then a small amount of permeant solute is added to the external seawater and the volume flow, J_v^3, is measured.

$$J_v^3 = L_p(\pi_i^2 - \pi_i^1) + J_{v(n)} + L_p\sigma RT(c_p^4 - c_p^3) \tag{6}$$

where $(c_p^4 - c_p^3)$ is the difference in the concentration of permeant solute across the protoplast. Subtracting Eqn. 6 from Eqn. 2 gives

$$J_v^2 - J_v^3 = -L_p\sigma RT(c_p^4 - c_p^3) \tag{7}$$

which contains two unknowns, σ and $(c_p^4 - c_p^3)$. To estimate $(c_p^4 - c_p^3)$ we measure isotopically the permeability coefficients for the permeant solute in both the intact cell (P_{tot}) and in the isolated cell wall (P_{wall}), as described above, then calculate the permeability coefficient of the protoplast (P_{pro}) by Eqn. 5. The concentration differences, $(c_p^1 - c_p^2)$ and $(c_p^3 - c_p^4)$, are related to the permeability coefficients by the equation

$$P_{tot}(c_p^1 - c_p^2) = P_{pro}(c_p^3 - c_p^4) \tag{8}$$

Solving for $(c_p^3 - c_p^4)$, we now calculate the reflection coefficient by Eqn. 7.

RESULTS

Osmotic and diffusional permeabilities to water

The osmotic and diffusional permeabilities of Valonia to water are discussed in more detail elsewhere[21]. Briefly, the hydraulic conductivity of *V. ventricosa* was $1.85 \pm 0.27 \cdot 10^{-7}$ cm·sec^{-1}·atm^{-1} (12 cells). (Results are expressed in the sequence: mean ± S.E. and, in parentheses, the number of measurements.) The diffusional permeability (P_d) of the protoplast, corrected for the diffusional resistance of the cell wall and unstirred layers, was $2.36 \pm 0.17 \cdot 10^{-4}$ cm·sec^{-1} (5 cells). The hydraulic conductivity can be compared to the diffusional permeability coefficient by the equation, $P_{os} = L_p RT/\bar{v}_w$, where P_{os} is the osmotic permeability coefficient and $\bar{v}_w$ is the partial molar volume of water. This conversion gives a value for P_{os} of $2.51 \pm 0.37 \cdot 10^{-4}$ cm·sec^{-1}, which is similar to the value of P_d. The similarity between P_{os} and P_d suggests that osmotic and diffusional flows can occur by the same mechanism, *i.e.*, by diffusion, and there is no need to postulate a bulk-flow component of the osmotic flow across the protoplast of Valonia.

Permeabilities to methanol and urea

The permeability coefficient of intact cells to labeled methanol was 1.37 $\pm$ 0.11·10^{-4} cm·sec^{-1} (7 cells), and the permeability coefficient for the cell walls of these cells was 2.57 $\pm$ 0.23·10^{-4} cm·sec^{-1}. These values were obtained from data such as those shown in Fig. 1. The calculated permeability coefficient for the protoplast was 3.11 $\pm$ 0.36·10^{-4} cm·sec^{-1}, which is slightly larger than the permeability to water. The permeability coefficient for urea was 9.8 $\pm$ 4.8·10^{-7} cm·sec^{-1} (5 cells). This value is not corrected for the diffusional resistance of the cell wall and unstirred layers.

Possible solvent–solute interactions, as indicated by "solvent drag", were tested by measuring the permeability coefficients for urea and methanol during first an inward and then an outward osmotic flow, produced by adding mannitol to the external solution. The osmotic flows were measured immediately following each tracer flux measurement. The results, shown in Table I, indicate that the presence or direction of a small osmotic flow does not affect the apparent permeability of the protoplast to these solutes. The apparent absence of solvent drag on urea and methanol suggests that these solutes cross the protoplast independently of water.

TABLE I

EFFECT OF AN OSMOTIC FLOW ON THE APPARENT PERMEABILITY OF VALONIA TO UREA AND METHANOL

Solute	*Permeability coefficient** ($cm \cdot sec^{-1}$)	*Volume flow*** ($cm^3 \cdot cm^{-2} \cdot sec^{-1}$)
Urea	1.61 $\pm$ 0.04·10^{-6} (4)	3.5·10^{-7} (inward)
	1.62 $\pm$ 0.04·10^{-6} (4)	−3.7·10^{-7} (outward)
Methanol	1.25 $\pm$ 0.07·10^{-4} (4)	3.4·10^{-7} (inward)
	1.15 $\pm$ 0.30·10^{-4} (4)	−4.9·10^{-7} (outward)

* Mean, S.E., and number of measurements in parentheses. All of the measurements for each solute were obtained from one cell. These permeability coefficients are not corrected for the diffusional resistance of the cell wall and unstirred layers.

** The outward osmotic flows were created by adding mannitol to the external seawater.

Possible solute–solute interaction in the membranes of Valonia was tested by measuring tracer efflux in the presence and absence of an opposing net influx. If solute molecules cross the membrane *via* narrow channels, then molecules moving against the net flux may be retarded. This "long-pore" effect[22] describes the movements of labeled water and solutes across some cell membranes. To test for a long-pore effect the permeability coefficient for urea efflux was measured with 0.1 mM urea inside and no urea outside. Then the external urea concentration was raised to 10 mM and the permeability coefficient for tracer efflux was measured again. The two permeability coefficients were similar, 2.90 $\pm$ 0.15·10^{-6} (7) and 3.06 $\pm$ 0.12·10^{-6} (4) cm·sec^{-1}, respectively. Thus no long-pore effect is apparent. Similar results were obtained with methanol; the permeability coefficient for methanol efflux was independent of the external methanol concentration over a range of 0.1–200 mM.

Reflection coefficients for non-electrolytes

The reflection coefficients for some non-electrolytes were measured to provide a further test for aqueous pores in the membranes of Valonia. The reflection coefficient, σ, for a lipid membrane containing water-filled pores is given by

$$\sigma = 1 - \omega\bar{v}_s/L_p - \alpha$$

where $\bar{v}_s$ is the partial molar volume of the solute, α reflects a direct frictional interaction between solvent and solute in the membrane, and ω is a solute permeability coefficient (mole·cm^{-2}·sec^{-1}·atm^{-1}), related to the conventional permeability coefficient by $\omega = P/RT$. If $\sigma < 1 - \omega\bar{v}_s/L_p$, then the frictional term is non-zero, and the presence of water-filled pores is suggested[5].

To provide the best possible comparison between the measured reflection coefficient and the quantity $1 - \omega\bar{v}_s/L_p$, I measured all three membrane parameters —σ, ω, and L_p—for methanol in each of 5 cells. First a cell was perfused with a tracer quantity of labeled methanol and the permeability coefficient for methanol efflux was measured at approximately zero-volume flow (seawater *plus* 80 mM mannitol outside). Then the inflow tube was closed and the hydraulic conductivity was measured as described above (Eqns. 2–4). Then methanol (200 mM) was added to the external seawater and J_v[3] was measured (Eqn. 6). After the formation of aplanospores, the permeability coefficient of the cell wall was measured, and the permeability coefficient of the protoplast was calculated by Eqn. 5. The concentration difference across the protoplast was then calculated by Eqn. 8, and the reflection coefficient was calculated by Eqn. 7.

Table II compares the measured reflection coefficient for methanol with the quantity $1 - \omega\bar{v}_s/L_p$. The mean value of $\sigma - (1 - \omega\bar{v}_s/L_p)$ does not differ significantly from zero, which means that the frictional term, α, is near zero. These results are consistent with the results in Table I which also suggest that water and methanol molecules cross the protoplast independently. It would be informative to measure σ, ω, and L_p for a hydrophilic solute whose reflection coefficient was intermediate between 1 and —1.3. However, the other solutes studied—urea, formamide, acetamide, and ethylene glycol—all gave reflection coefficients of near unity. That is, they were indistinguishable (within 4 %) from mannitol in their ability to induce an osmotic flow across the protoplast. The reflection coefficient for ethanol was roughly

TABLE II

HYDRAULIC CONDUCTIVITY (L_p), REFLECTION COEFFICIENT (σ) AND PERMEABILITY COEFFICIENT (ω) FOR METHANOL CROSSING THE PROTOPLAST OF VALONIA

Cell	L_p (*cm·sec^{-1}·atm^{-1}*)	ω (*mole·cm^{-2}·sec^{-1}·atm^{-1}*)	σ	$1 - \omega\bar{v}_s/L_p$	$\sigma - (1 - \omega\bar{v}_s/L_p$
July 1	$2.70 \cdot 10^{-7}$	$2.09 \cdot 10^{-8}$	—1.66	—1.94	0.28
July 9	$1.93 \cdot 10^{-7}$	$1.25 \cdot 10^{-8}$	—1.59	—1.46	—0.13
July 12	$3.65 \cdot 10^{-7}$	$0.78 \cdot 10^{-8}$	—0.18	0.18	—0.36
July 21	$1.62 \cdot 10^{-7}$	$1.11 \cdot 10^{-8}$	—1.28	—1.62	0.34
July 28	$1.91 \cdot 10^{-7}$	$1.25 \cdot 10^{-8}$	—1.88	—1.49	—0.39
				Mean ± S.E.	0.05 ± 0.16

similar to that of methanol, *i.e.*, highly negative. Ethyl acetate caused a rapid change in the membrane potential; therefore this compound was not studied further.

DISCUSSION

All the results presented above indicate either that the membranes of Valonia are not porous or that the number or size of the pores is too small to provide an important pathway for water, urea and methanol movements. The osmotic and diffusional permeabilities of the protoplast to water are identical, provided that the diffusional resistance of the cell wall and unstirred layers is taken into account. Furthermore, there is no evidence so far for solvent–solute interactions in the membranes of Valonia, as indicated either by a test for "solvent drag" or by a test based on irreversible thermodynamics. Finally, there is no indication of solute–solute interactions, *i.e.*, a "long-pore" effect, for urea or methanol crossing the protoplast.

Among other cells and tissues the strongest evidence for membrane pores comes from the human erythrocyte. Workers in SOLOMON's laboratory[23] recently confirmed their previous value of P_{os}/P_d of about 2.4, from which they estimate that the erythrocyte membrane contains equivalent pores of about 3.5 Å radius. This value is close to their previous pore radius of 4.2 Å, based on the reflection coefficients for small hydrophilic solutes[24]. In Nitella DAINTY AND GINZBURG[25] present tentative evidence for water-filled pores on the basis of an apparent frictional interaction between water and alcohols crossing the protoplast. Their measured reflection coefficient for methanol, for example, is about 0.5, whereas their estimated value of $1-\omega\bar{v}_s/L_p$ is about 0.9. In amphibian skin a large body of data can be explained by the presence of membrane pores, but DAINTY AND HOUSE[2] believe that the evidence for pores is still inconclusive. The evidence for membrane pores in some other cells and tissues is reviewed by DAINTY[1] and DICK[3].

In artificial phospholipid membranes a discrepancy between the osmotic and diffusional permeabilities to water appeared in early work by HANAI, HAYDON AND TAYLOR[26] and THOMPSON AND HUANG[27,28]. This discrepancy has now been abolished by correcting for the effect of the unstirred layers[29,30]. CASS AND FINKELSTEIN[29] recently found no evidence for aqueous pores in several types of phospholipid membranes. Especially notable is their finding that the presence of a protein which greatly increases the permeability to ions has no effect on the permeability of their membrane to water. The electrical resistance of their phospholipid membrane with protein is roughly equal to the resistance of the Valonia protoplast, *i.e.*, $10^4\ \Omega\cdot cm^2$ (ref. 14).

Two other notable features of Valonia are the low osmotic permeability to water and the highly negative reflection coefficient for methanol. The osmotic permeability of Valonia is about 50 times less than the hydraulic conductivity of the characean algae[31–33] and mammalian erythrocytes[23,24], both of which are especially permeable to water. However, Valonia and the characeans are fairly similar in their permeabilities to methanol and other small non-electrolytes[19,35–37]. Thus the reflection coefficient for methanol is negative in Valonia and positive in Nitella chiefly because Valonia is much less permeable to water than Nitella. Finally, in an artificial phospholipid membrane the reflection coefficient for methanol is also negative (E. J. A. LEA, personal communication).

So far I have treated the protoplast of Valonia as a single membrane, although

it is clearly a complex system containing at least two probable barriers (plasmalemma and tonoplast) to the movement of water and solutes. It is unlikely, however, that the agreement between the osmotic and diffusional permeabilities to water could be a fortuitous result of series-membrane or mosaic-membrane effects. For example, if aqueous pores allowed a bulk flow of water under an osmotic pressure gradient, then, in order to make P_{os} equal to P_d, an equivalent amount of water would have to cross the membranes by exchange diffusion in a tracer experiment. Another possible interpretation of all my results is that the bulk protoplasm rather than a membrane limits the movement of water and solutes. LING, OCHSENFELD AND KARREMAN[38], for example, report that ^{3}HHO diffusion in frog ovarian oöcytes is limited by the bulk phase rather than by the surface membrane and, furthermore, that the self-diffusion coefficient for ^{3}HHO in cytoplasm is only 30–50 % of the normal self-diffusion coefficient for water. If we assume in Valonia that the bulk protoplasm rather than a membrane is rate-limiting for the diffusion of ^{3}HHO, then the self-diffusion coefficient for ^{3}HHO in the protoplasm will be equal to the product of the permeability coefficient ($2.4 \cdot 10^{-4}$ cm·sec^{-1}) and the thickness of the protoplast (7–12 μ). This gives a value of about $2.5 \cdot 10^{-7}$ cm^2·sec^{-1}, roughly one-hundredth the normal self-diffusion coefficient for ^{3}HHO in water, which seems improbably low. A second point is that if the bulk protoplasm limits the rate of movement of water, then the osmotic flow across the protoplast will be governed by the mutual diffusion coefficient of water and cytoplasmic macromolecules[3,39]. The observed similarity between P_{os} and P_d will require, therefore, a fortuitous agreement between the self-diffusion coefficient of ^{3}HHO and the mutual coefficient of water and cytoplasmic colloids, which again seems improbable. Therefore the simplest interpretation of all my results is that the movements of urea, methanol and water across the protoplast of Valonia are rate limited by a non-porous plasma membrane.

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