References and Notes

1. R. Chevallier, *J. Phys. Radium* **12**, 172 (1951).
2. D. W. Strangway, R. M. Honea, B. E. McMahon, E. E. Larson, *Earth Planetary Sci. Letters* **2**, 367 (1967).
3. L. Neel, *J. Phys. Soc. Japan* **17** (suppl. B-1), 676 (1962); J. Cohen, K. M. Creer, R. Pauthenet, K. G. Srivastava, *ibid.*, p. 685.
4. R. W. Smith and M. Fuller, *Science* **156**, 1130 (1967).
5. K. M. Creer, *Geophys. J.* **5**, 16 (1961).
6. A. Z. Hyrnkiewicz, D. S. Kulgowczuk, K. Tomala, *Phys. Letters* **17**, 93 (1965).
7. K. M. Creer, *J. Geomag. Geoelec.* **13**, 86 (1962).
8. M. H. Francombe and H. P. Rooksby, *Clay Minerals Bull.* **21**, 1 (1959); J. Kulp and A. F. Trites, *Amer. Mineralogist* **36**, 23 (1951).

9. We thank NSF for support (project GP-5341). D. Enggren assisted in the laboratory.

14 September 1967

Membranes of Valonia ventricosa: Apparent Absence of Water-Filled Pores

Abstract. *Osmotic and diffusional permeabilities to water have been measured in internally perfused cells of* Valonia ventricosa. *The osmotic and diffusional permeability coefficients for the protoplast are identical, 2.4 × 10^{-4} centimeter per second. Thus, both osmotic and diffusional flows can occur by the same mechanism, that is, by diffusion; and there is no need to postulate the existence of water-filled pores in the membranes of this cell. Supporting evidence for this conclusion is the absence of solvent-solute interactions, that is, "solvent drag," for water, urea, and methanol crossing the protoplast of* Valonia.

A popular hypothesis concerning the structure of cell membranes holds that pores or channels through the membrane facilitate the penetration of water and small hydrophilic solutes. Two main lines of evidence for the presence of these water-filled pores in a membrane are (i) a discrepancy between the osmotic and diffusional permeabilities to water and (ii) frictional interaction between water and solutes, that is, "solvent drag." The evidence for and against the existence of water-filled pores in biological membranes is reviewed by Dainty and House (*1*, *2*) and Dick (*3*).

My report deals mainly with the first criterion for the presence of water-filled pores—a comparison of osmotic and diffusional permeabilities to water. If osmotic flow of water across a membrane occurs by diffusion, then it can be easily shown (*4*) that $L_pRT/\overline{V}_w = P_d$, where P_d (centimeters per second) is the diffusional permeability coefficient obtained from a self-diffusion tracer experiment, L_p (centimeters per second per atmosphere) is the hydraulic conductivity, R is the gas constant, T is the absolute temperature, and $\overline{V}_w$ (cubic centimeters per mole) is the partial molar volume of water. The expression $L_pRT/\overline{V}_w$ is often called the osmotic permeability coefficient, P_{os}, for water. If $L_pRT/\overline{V}_w$ is greater than P_d, a bulk flow of water through continuous channels in the membrane is indicated, and the equivalent radius of these channels can be estimated from the size of the discrepancy (*3*, pp. 83–126).

Both P_{os} and P_d have been measured in many biological membranes, and P_{os} is always greater than P_d, which suggests the presence of water-filled pores. A similar discrepancy appears in artificial phospholipid membranes (*5*). Dainty and House (*1*, *2*) have questioned the reported values for P_d, however, pointing out that P_d was usually underestimated because the effects of the unstirred layers of solution adjacent to the membrane were not taken into account. Thus, in most cells and tissues, the presence of aqueous pores, based on this criterion, is still in doubt.

The marine alga *Valonia* (*8*), in contrast to erythrocytes (*6*) and the freshwater algae *Nitella* and *Chara* (*7*), is relatively resistant to water flow under an osmotic-pressure gradient. The high resistance to osmotic flow suggested to me that *Valonia* might be particularly suitable for a study of water permeability, because the effects of the unstirred layers on the apparent permeability should be relatively small. Another advantage of these giant cells is that they can be perfused internally, which facilitates permeability measurements with rapidly penetrating substances. A third advantage is that the important correction for the diffusional resistance of the cell wall and unstirred layers can be made on each cell after the protoplast has formed aplanospores (*9*).

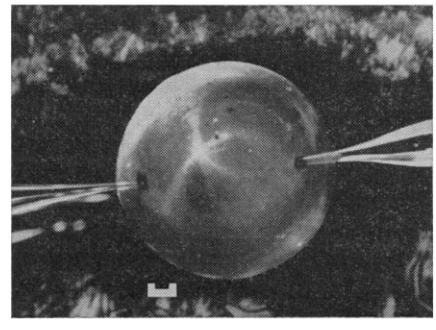

Fig. 1. A single cell of the marine alga *Valonia ventricosa* perfused internally by means of two micropipets inserted into the vacuole. Scale is 1 mm.

Cells of *Valonia ventricosa* about 1 cm in diameter were shipped by air from Jamaica and maintained in laboratory cultures as described previously (*10*). The method of perfusing the vacuole of *Valonia* is also described elsewhere (*8*). Briefly, a spherical cell is held with waterproof putty in a dish of seawater (*11*) on the stage of a dissecting microscope. Two micropipets are inserted through the cell wall and underlying protoplasm into the vacuole (Fig. 1). After a 1- to 2-hour recovery period the vacuole is perfused with artificial sap (*11*) at a rate of 100 to 300 μl/min. The outer solution is stirred gently with a Pasteur pipet. Rapid stirring is impossible because of the fragility of the cytoplasmic seal around the micropipets.

The potential difference between vacuole and seawater is monitored continuously and provides a sensitive indicator of the condition of the cell. Serious leaks which occasionally develop cause an immediate drop in the potential difference to near zero. The vacuole potential of these cells was +16±1 mv (13 cells) (*12*), which is the normal value for *V. ventricosa* bathed in seawater (*10*).

I measured the diffusional permeability to water by adding water labeled with tritium (THO) to the inflow reservoir and allowing about 40 minutes for the THO efflux to attain a steady value. Then 30 ml of seawater was placed outside the cell, and five 0.5-ml samples of this solution were removed at 2-minute intervals and counted by liquid scintillation. The total counts in the outside solution were plotted against time, and the gradient of this curve, $V_o \cdot dC_o^*/dt$, was determined. The permeability coefficient, P_d (centimeters per second), is equal to $V_o \cdot dC_o^*/dt \cdot A \cdot C_i^*$, where C_i^* and C_o^* are the THO concentrations inside and outside (counts per minute per cubic centimeter), A is the surface area of the spherical cell (square centimeters), V_0 is the volume

Table 1. Osmotic and diffusional permeability coefficients (P_{os} and P_d) for water movement across the protoplast of *Valonia ventricosa*. Values of P_d are corrected for the effects of the cell wall and unstirred layers. The mean P_{os} and P_d (± standard errors) for all cells and, in parentheses, the number of cells used are given in the first line. In cells 12 and 13, P_{os} and P_d were both measured in the same cell.

Cell	P_{os} (cm sec^{-1} × 10^4)	P_d (cm sec^{-1} × 10^4)
All cells	2.40 ± 0.29 (8)	2.36 ± 0.17 (5)
No. 12	1.89	1.92
No. 13	2.53	2.56

of the external solution (cubic centimeters), and t is the time (seconds). The influx of THO was neglected because C_o* was never greater than 4 percent of C_i*. The C_i* in the perfusate leaving the vacuole was 80 to 90 percent of C_i* entering the vacuole, and the mean of these two values was used in calculating P_d. The THO was assumed to be an ideal tracer for water (*3, 5*). The value obtained for P_d was $1.22 \pm 0.03 \times 10^{-4}$ cm sec^{-1} (five cells).

After measurement of the diffusional permeability coefficient in a living cell, the vacuole was perfused with seawater, which caused the entire protoplast to form multinucleate aplanospores (*9*). The diffusional permeability of the remaining cell wall and associated unstirred layers was then measured in a manner identical to the previous measurement on the living cell. The P_d for the cell wall and unstirred layers was $2.52 \pm 0.10 \times 10^{-4}$ cm sec^{-1} (five cells).

The cell wall and unstirred layers have a considerable effect on the diffusional permeability of *Valonia* to water, and this must be taken into account when we estimate the P_d of the protoplast alone. The wall and unstirred layers are, in effect, diffusional barriers in series with the protoplast, and thus the permeability coefficients are analogous to conductances in series. The diffusional permeability of the protoplast (P_p) may thus be estimated from the equation

$$P_p^{-1} = P_t^{-1} - P_w^{-1}$$

where P_t is total permeability and P_w is the permeability of the wall and unstirred layers (*1*, pp. 300–307). The calculated P_d for the protoplast was $2.36 \pm 0.17 \times 10^{-4}$ cm sec^{-1} (five cells).

I measured the hydraulic conductivity (L_p) of *Valonia* by closing the inflow tube and measuring the rate of fluid movement in a calibrated capillary attached to the outflow pipet. Cells bathed in seawater showed an inward volume flow of about 3×10^{-7} cm sec^{-1}, whereas cells bathed in seawater plus 100 mM mannitol showed an outward volume flow of about 1×10^{-7} cm sec^{-1}. The L_p was calculated by the equation

$$L_p = \Delta J_v / (\sigma RT \Delta C)$$

where ΔJ_v centimeters per second is the difference between the two osmotic flows, ΔC is the concentration of mannitol (usually 10^{-4} mole cm^{-3}) added to produce ΔJ_v, and σ is the reflection coefficient of the membrane for mannitol (*13*). The reflection coefficient for mannitol was assumed to be unity (*14*). The osmotic pressures of the seawater and mannitol solutions were checked by measuring the freezing-point depression. The hydraulic conductivity was not corrected for the effects of the cell wall and unstirred layers, because the L_p of these was about 500 times the L_p of a living cell (*1*, p. 304). The hydraulic conductivity of *Valonia* was $1.75 \pm 0.21 \times 10^{-7}$ cm sec^{-1} atm^{-1} (eight cells).

Table 1 compares P_{os} (which equals $L_pRT/\overline{V}_w$) and P_d in *V. ventricosa*. The similarity between P_{os} and P_d indicates that osmotic and diffusional flows can occur by the same mechanism, that is, diffusion. Water molecules apparently move independently under an osmotic-pressure gradient, and there is no need to postulate the presence of water-filled pores in the membranes of *Valonia*. This is the first time that a value for $L_pRT/\overline{V}_wP_d$ of unity has been found in a biological membrane system.

The protoplast of *Valonia* is, of course, not a single membrane but a series membrane system containing at least two probable barriers (the plasmalemma and tonoplast) to the movement of water and solutes. That the agreement between P_{os} and P_d might be a fortuitous consequence of series-membrane or mosaic-membrane effects is unlikely, however. Another possible explanation for these results is that the protoplasm rather than a membrane is rate-limiting for the movement of water (*15*). This would require, however, that the self-diffusion coefficient for water in the protoplasm (about 12 μ thick) be about 100 times less than the normal self-diffusion coefficient for water.

Additional evidence for the absence of water-filled pores in *Valonia* membranes comes from measurements of C^{14}-methanol and C^{14}-urea fluxes. Using methods similar to those described above, I find that P_{meth} is about 10^{-4} cm sec^{-1} and P_{urea} is about 10^{-6} cm sec^{-1}. The permeability coefficients for methanol and urea are independent of the direction of osmotic water flow; that is, there is no apparent solvent drag. Thus in terms of the two criteria for water-filled pores stated above, I find no discrepancy between the osmotic and diffusional permeabilities to water and no evidence so far for solvent-solute interactions in the membranes of *Valonia*.

JOHN GUTKNECHT*
School of Biological Sciences,
University of East Anglia,
Norwich, England

References and Notes

1. J. Dainty, in *Advances in Botanical Research*, R. D. Preston, Ed. (Academic Press, New York, 1963), vol. 1, p. 279.
2. J. Dainty and C. R. House, *J. Physiol.* **185**, 172 (1966).
3. D. A. T. Dick, *Cell Water* (Butterworths, Washington, 1966).
4. J. F. Thain, *Principles of Osmotic Phenomena* (Royal Institute of Chemistry, London, 1967), pp. 48–51.
5. T. Hanai, D. A. Haydon, W. R. Redwood, *Ann. N. Y. Acad. Sci.* **137**, 731 (1966); T. E. Thompson and C. Huang, *ibid.*, p. 740.
6. R. I. Sha'afi, G. T. Rich, V. W. Sidel, W. Bossert, A. K. Solomon, *J. Gen. Physiol.* **50**, 1377 (1967).
7. N. Kamiya and M. Tazawa, *Protoplasma* **46**, 394 (1956); J. Dainty and B. Z. Ginzburg, *Biochim. Biophys. Acta* **79**, 102 (1964).
8. J. Gutknecht, *J. Gen. Physiol.* **50**, 1821 (1967).
9. The formation of aplanospores in *V. ventricosa* is described in detail by W. L. Doyle, *Carnegie Inst. Wash., Pap. Tortugas Lab.* **32**, 144 (1940).
10. J. Gutknecht, *Biol. Bull.* **130**, 331 (1966).
11. The approximate concentrations (millimoles per liter) of major ions in the seawater were Na, 505; K, 11; and Cl, 600. The major ion concentrations in the artificial sap were Na, 50; K, 615; and Cl, 650. The artificial sap contains one part seawater and nine parts 0.67M KCl. Natural seawater was used, but supplementary NaCl and KCl were added to raise salinity from about 3.5 to 3.7 percent, similar to that of water in which *Valonia* grows naturally. The temperature of the experimental solutions was 25° ± 1°C.
12. All results are quoted in the sequence: mean ± standard error and (in parentheses) number of measurements.
13. A. J. Staverman, *Rec. Trav. Chim.* **70**, 344 (1951); O. Kedem and A. Katchalsky, *Biochim. Biophys. Acta* **27**, 229 (1958).
14. Acetamide, formamide, ethylene glycol, and urea were indistinguishable from mannitol in their ability to induce an osmotic flow across the protoplast of *Valonia*. Thus the reflection coefficients for all these small solutes appears to be unity.
15. G. N. Ling, M. M. Ochsenfeld, G. Karreman, *J. Gen. Physiol.* **50**, 1807 (1967).
16. I thank Prof. J. Dainty and Drs. N. A. Walker, J. F. Thain, and E. J. A. Lea for constructive criticisms of this work. I thank Dr. R. Harden-Jones for the use of his osmometer, Prof. A. D. Skelding for supplying the *Valonia*, and Mrs. Lyn Barber for technical assistance. Supported in part by a PHS postdoctoral fellowship (GM-13,804).
* Present address: Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27706.

5 September 1967