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Hydraulic Conductivity of *Nitella* Cells Using the Intracellular Perfusion Technique

C.S. Spyropoulos
Department of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Illinois 60637

Summary. The hydraulic conductivity of the intracellularly-perfused internodal cell of *Nitella flexilis* was measured by establishing and maintaining osmotic and hydrostatic pressure gradients between the inside and the outside of the cell. The osmotic filtration coefficient (L_{PD}) determined at zero hydrostatic pressure difference varied between 1.55 and 2.32×10^{-5} cm/sec/atm. Under internal perfusion conditions no polarity between endosmotic and exosmotic flow was observed. The overall hydrostatic filtration coefficient (L_P) was determined with a step change in hydrostatic pressure up to 0.2 atm, while the osmotic pressure difference was maintained at zero. L_P was considerably greater than the L_{PD}, i.e., 14.1 to 19.2×10^{-5} cm/sec/atm. The *overall* L_P of such internodes, which showed protoplasmic streaming and action potentials was the same as that of the isolated cell walls, the latter being 13.2 to 19.9×10^{-5} cm/sec/atm. Some of these results are consistent with previous results on *Nitella* using different techniques. The situation in *Nitella* where at abnormally *low* internal pressure the barrier to hydrostatic pressure-driven water flow does not reside in the plasmalemma but in an in-series structure is comparable to that in the squid axon where the normal internal pressure is close to zero. An interpretation is offered for the finding in the alga that at *high* internal pressures the plasmalemma becomes the rate-limiting structure for hydrostatic pressure-driven water flow. It is suggested that the internal pressure pushes a large fraction of the plasmalemma against skeletal nonporous regions of the cell wall. This suggestion entailing a pressure-dependent cell wall-plasmalemma juxtaposition was also deployed in interpreting previous observations in plant cells on water flow polarity (i.e., observations showing that exosmotic rates are less than endosmotic).

Key Words water · osmosis · pressure · *Nitella* · filtration · permeability

Introduction

Water flow has been measured as a function of osmotic ($\Delta\pi$) and hydrostatic (ΔP) pressure gradients between the bulk inside and the bulk outside fluid phases in the internally-perfused squid axon (Vargas, 1968; Spyropoulos, 1977*a*). In this preparation water flow is measured across a composite structure that includes plasmalemma, residual cytoplasm, and encapsulating materials (i.e., Schwann layer and connective tissue). In the squid axon it was shown that the structure limiting the rate of hydrostatic pressure-driven and diffusional water flow is not the plasmalemma but an in-series surface layer, maybe the axonal sheath (Spyropoulos, 1977*a*, *b*, 1979). The main evidence for this was the finding that the hydrostatic filtration coefficient and the rate of efflux of tritiated water were not affected on destroying the plasmalemma by prolonged (from a few hours to one year) exposure to 3% glutaraldehyde or isosmotic KF. If the rate of hydrostatic pressure-driven water flow were to be limited by a structure in-series with the structure limiting solute flux (i.e., the plasmalemma) then one could expect that the water flow due to an osmotic gradient might be less than that due to a hydrostatic gradient even though the solutes involved are impermeable. Consistent with this expectation is the finding in the squid axon (Vargas, 1968; Spyropoulos, 1977*a*, 1979) that the hydrostatically-driven water flow was two orders of magnitude larger than the osmotically-driven one.

Kedem and Katachalsky (1958, 1963) analyzed the permeability coefficients of membranes composed of different elements arranged in a series array. They pointed out that in such composite structures accumulation or depletion of solutes may occur between the elements and simple additivity rules do not generally hold. Primarily on account of this *theoretical* work and the structural complexity of the real envelope of cells a *quantitative* analysis of the relation between hydrostatic and osmotic filtration coefficients and the extent to which the plasmalemma is the rate-limiting structure for water flow is difficult.

Most people would regard the finding that in the axon the plasmalemma is not the water flow barrier as a mere exception to the rule that it is. The main purpose of the present investigation was to show that the axon in this regard is not so unique. I chose the internode of *Nitella* to extend

these findings. This preparation, in common with the axonal preparation, involves a composite membrane (i.e., cytoplasm, plasmalemma, and encapsulating materials). The reason I chose *Nitella* was that it can be perfused internally and because of reports by Steudle and Zimmermann (1974) and Zimmermann and Steudle (1978), which indicated to me that *Nitella* may behave like the squid axon.

In the work of Steudle and Zimmermann filtration coefficients were calculated from the volumetric elastic modulus of the cell wall and the exponential time course of the internal pressure following a change in hydrostatic or osmotic pressure. These investigators punctured *Nitella* internodes with a micropipette (serving as the probe of a pressure transducer system) and found that after 10 hr the turgor of the cell was reduced to below 2 atm. The reason for this drop in turgor is not made clear. They claim to have excluded leakage (fluid loss), around the point of insertion of the 60-μm diameter pressure probe and instead have invoked a salt loss. The hydrostatic filtration coefficient determined at low turgor pressure (less than 1 atm) was at least twice that determined at turgors exceeding 2 atm. Between 2 and 7 atm turgor, the hydrostatic filtration coefficient (approximately 2.5×10^{-5} cm/sec/atm) did not vary. In this pressure range the endosmotic hydrostatic filtration coefficient was around 10% greater than the exosmotic. At turgors above 2 atm the hydrostatically-determined filtration coefficients were almost equivalent to the osmotically-determined; however, at turgors near the plasmolytic point the hydrostatic filtration coefficients were 2.5–3.0 times the osmotic. Finally these investigators noted that the hydrostatic filtration coefficient at low turgor was close to that of the isolated cell wall. These experiments of these investigators have been questioned by Palta and Stadelman (1977, 1978).

The perfusion technique employed in the present work offers certain advantages over previous methods. For example, the volumetric elastic modulus of the cell wall does not enter into the calculations of the filtration coefficients and the osmotic pressure of the interior can be controlled and maintained constant, which is not the case in the previous work. The disadvantage is that the operational pressure range is limited.

A short description of the work in this paper was presented elsewhere (Spyropoulos, 1980).

Materials and Methods

Nitella flexilis purchased from, and identified as such, by Everglades Aquatic Nursery (Tampa, Fla.) was used. It was kept and grown at room temperature (20–23 °C) with artificial light and pond water for 3–6 months before using. The artificial pond water, APW, contained 0.8 mM NaCl and 0.1 mM each of KCl, $CaCl_2$ and $MgCl_2$. Some pond mud was included in the tank. The pH was around 5.5 ± 0.1. The internodes selected were mature (i.e., at least the third posterior to the growing region).

The internode of *Nitella* has been perfused previously (Tazawa, 1964; Strunk, 1970; Tazawa, Kishimoto & Kikuyama, 1974; Tazawa, Kikuyama & Nakakawa, 1975; Kikuyama & Tazawa, 1976*a, b*; Shimmen, Kikuyama & Tazawa, 1976; Kiyosawa & Tazawa, 1977; Shimmen & Tazawa, 1977). Some features of these previous perfusion techniques were incorporated in the perfusion technique I used: (i) Mg^{++} and EGTA were included in the perfusate to preserve excitability and to "destroy" the tonoplast. (ii) Following cannulation and before pressure was applied to initiate perfusion, a slight negative pressure was applied to the middle internode. My technique differed in that my double cannulation method used in the axon (*cf.* Spyropoulos, 1977*a, b*, 1979 and previous work referred to therein) was adapted for use in *Nitella* (*cf.* Fig. 1) and in that the cell under study was not cut; instead the glass cannulae were fixed to the cell wall of the neighboring internodes (*cf.* Fig. 1). The cannula was attached to the cell wall by washing the latter with distilled water and acetone and allowing it to dry on the glass. The dried portion was covered with dental sticky wax. The space between the dried portion of the cannulated neighboring internode and the partitioning node was filled with a mixture of Vaseline® and mineral oil. My previous work on the axon (e.g., Spyropoulos, 1977) should be referred to for testing leakage of the cannular region and for being assured that the flow measured was through the internode under study and not through adjacent regions[1]. Transmembrane water flow induced by either osmotic or hydrostatic pressure gradients did not affect intracellular solute concentrations during flow readings. Except when water flow readings were made, the internal perfusate was flushed out into a large resevoir interposed between the cannulated region and the pressure head. The contents of the reservoir were stirred with an externally operated magnet. The extracellular fluid was stirred constantly by vibration with a probe attached to a "massage" vibrator. At high magnifications it can be seen microscopically that this vibration was transmitted to the interior of the cell and stirred it as well. In two experiments inclusion of carbon dust in the internal perfusate demonstrated very clearly the internal mixing.

The perfusion fluid contained 5 mM EGTA, 6 mM $MgCl_2$, 5 mM tris-maleate buffer (adjusted to pH 7.0 with KOH), 1 mM ATP and 180 mM sucrose instead of sorbitol (used in previous perfusion experiments). Sucrose was preferred as an osmoticum instead of a combination of salts approaching that of the normal internal milieu, for I felt that there would be more assur-

[1] Briefly, the fluid bathing the entire *Nitella* preparation was divided into three pools by means of partitions applied exactly on the nodes. The partitions were made of prophylactic rubber. Holes were punched in the rubber. The diameter of the holes was $^2/_3$ to $^3/_4$ of the diameter of the node. The internode was threaded through the holes so that the nodes were gripped snugly. The nodes are quite sturdy and can easily withstand the forces involved. At the end of the experiment sucrose C-14 was introduced into the internode by perfusion, and after effluxes were measured at $\Delta P = 0$ and at $\Delta P = 0.1$ atm, 2–3.4 atm pressure was applied for 1–2 hr. The lack of appreciable radioactivity in the lateral pools, even at 2–3.5 atm internal pressure, indicated that the cannulation system was leak-proof. At $\Delta P = 0$ and $\Delta P = 0.1$ atm (where the plasmalemma is still intact) the efflux of sucrose C-14 in the middle pool was barely detectable.

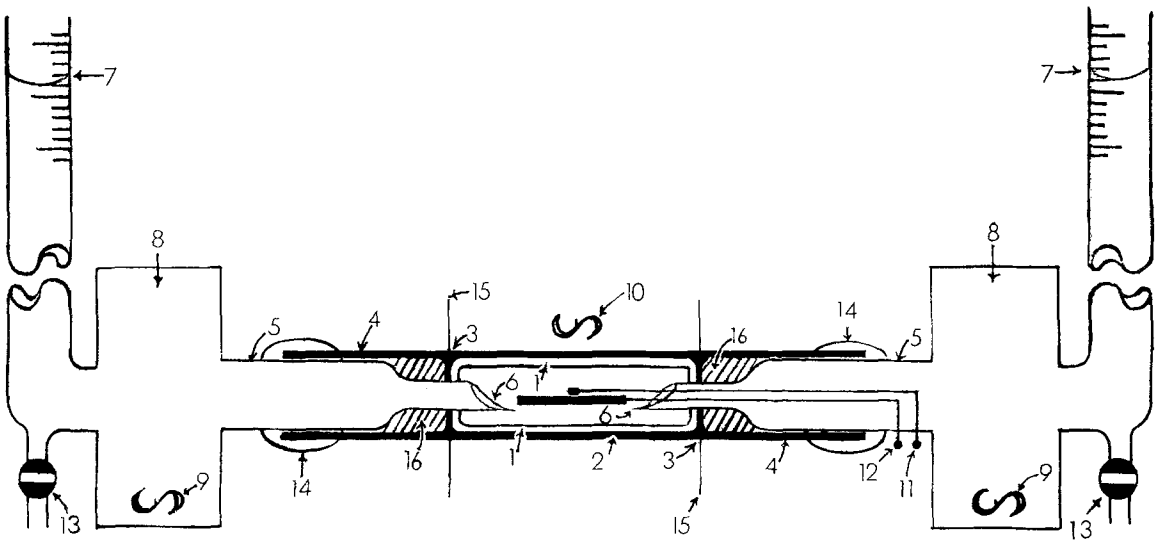

Fig. 1. Method of filtration coefficient measurement using the internal perfusion technique. *1* Protoplasmic layers; *2* internode under study; *3* node; *4* cell wall of adjacent internode used for cannulation; *5* cannula; *6* beveled tip of cannula penetrated through node; *7* pressure head; *8* reservoir for mixing of intracellular fluid altered by water flow; *9* stirrer for intracellular compartment; *10* stirrer for extracellular compartment; *11* potential wire electrode; *12* current wire electrode; *13* combination of valves for changing perfusate; *14* dental sticky wax for reinforcement of cannulated region of cell wall of neighboring internode; *15* thin rubber partition dividing external fluid into three pools; *16* mixture of Vaseline® and mineral oil to reinforce sealing at point of entrance of cannula into internode under study

ance that with sucrose the internal solutes were impermeable. For hydrostatic filtration coefficient measurements the external fluid was APW made isosmotic to the internal perfusate (*see* below) by addition of sucrose. For osmotic filtration coefficient measurements $\Delta\Pi$ was controlled by varying the sucrose either in the external or the internal phase. Before cannulating the cell the concentration of sucrose for incipient plasmolysis was determined. It varied between 198 and 212 mM[2]. This is somewhat disturbing since published values on *Nitella flexilis* are higher. I also noticed that the cell wall thickness was smaller than that published for adult cells. After perfusing the cell and before filtration coefficients were measured, the external concentration of sucrose was adjusted for zero net water flow at zero hydrostatic pressure difference. In two cells I switched from sucrose as the main osmoticum in the perfusate to sorbitol. I noticed a slowing down in the streaming rate when sorbitol was used but no change in excitability or the osmotic filtration coefficient.

Since at higher hydrostatic pressures the cell became irreversibly damaged, I did not apply more than 0.23–0.25 atm to the interior of the cell; consequently I could not study the hydrostatic pressure dependence of the filtration coefficients. The "damage" at pressures above 0.20–0.25 atm entailed a spotty irreversible dislodging of the protoplasm from the cell wall. These spots were concentrated near the cannula.

Pressures for filtration coefficient measurements on cell walls were attained with water and mercury columns or (for very high pressures) using compressed nitrogen cylinders.

Cells walls were isolated as follows: After filtration coefficient measurements of the "intact" *Nitella* were completed, the hydrostatic pressure difference was zero and the cell bathed in a hypertonic (~300 mM sucrose) solution. Since the internal solute concentration was not kept constant (i.e., low) by perfusion, plasmolysis was induced. When plasmolysis was clearly visible, a pressure of ~100 cm H_2O was applied to one cannula only. Thus the protoplasm was flushed out through the other cannula. Filtration coefficient measurements on cell walls were then carried out with distilled water inside and outside the internode. Cell wall thickness was measured in the light microscope by making wet cross-sections. My accuracy was only around 0.35 μm.

Electrical responses were recorded while the internode was immersed in APW. The internal solution was 5 mM EGTA, 6 mM $MgCl_2$, 5 mM tris-maleate buffer (pH 7.0), 1 mM ATP and 180 mM sucrose. Before recording, the intracellular compartment was made into a closed system by closing a valve (not shown in figure) interposed between the cannula and the reservoir and then changing the isosmotic external medium to APW. When electrical responses were absent the results were discarded. As noted previously (e.g., Tazawa, Kikuyama & Shimmen, 1976), the action potentials in internodes perfused with low ionic strength solutions were prolonged and rectangular. Excitability was tested by inserting two wires (twisted about each other) into the internode through the cannula. One served as the current electrode and the other as the potential electrode. The current electrode was a 25–30 μm platinum wire that was insulated except for a 2-cm length. The potential wire was silver, 10 μm in diameter and insulated except for 2 mm at its end that was located in the middle of, but not in contact with, the uninsulated region of the current wire.

For all experiments the temperature was 20–23 °C.

Results

In my treatments (1977*a*, *b*, 1979, and the present paper) L_p denotes merely water flow in cc/sec/cm^2

[2] This determination of plasmolysis in the intact internode prompted by choosing 180 mM sucrose in my standard near-isosmotic perfusate.

surface area/atmosphere applied hydrostatic pressure between the internal perfusion channel and the outside bulk fluid phase, while $\Delta\pi$ is maintained at zero, L_{PD} denotes water flow in cc/sec/cm^2 surface area/atmosphere applied osmotic pressure difference, while ΔP is maintained at zero and J_v denotes water flow in cc/sec/cm^2 surface area induced hydrostatically or osmotically. It should be emphasized that in this usage, L_P, L_{PD} and J_v involve the *composite* surface of the cell not the plasmalemma alone [*cf.* Kedem & Katchalsky (1963) for a theoretical discussion of this problem of composite membranes].

In each internode an attempt was made to measure successively (i) L_{PD}, (ii) the overall L_P of the composite surface of the cell (L_P), and (iii) the L_P of the isolated cell wall (L_{PW}). L_{PD} measurements were carried out first since occasionally during L_P measurements the protoplasmic layer inexplicably sloughed off. L_{PW} measurements were carried out last since they entailed flushing out the rest of the interior of the internode. The cell number in the Table and the curve number in the figures help identify these multiple experiments. In the determination of J_v allowance was not made for the slight increase (2% at 5 atm) in surface area. The data from three cells showing $\frac{L_P}{L_{PD}}=3$–6 were not-included in the table or the figures. In the middle of the experiment the cells had become inexcitable.

Osmotic Filtration Coefficient (L_{PD})

L_{PD} determinations were carried out at $\Delta P=0$. Six experiments on different internodes were undertaken. The results are given in the Table and Fig. 2. At the beginning of the experiment the interior of the internode contained 180 mM sucrose, 5 mM EGTA, 6 mM $MgCl_2$ and 5 mM tris-maleate buffer (adjusted to pH 7.0 with KOH). The external fluid was APW made isosmotic to the internal fluid by adding sufficient sucrose to obtain zero net water flow. The $\Delta\Pi$ for exosmotic flow was then controlled by adding variable amounts of sucrose to the initially isosmotic medium bathing the perfused internode. The $\Delta\Pi$ for endosmotic flow was controlled by increasing the sucrose concentration in the initially isosmotic internal perfusate. The $J_v-\Delta\Pi$ relation for exosmotic flow was slightly nonlinear. Endosmotic flows (cells No. 6 and 4) were undertaken in an attempt to test a hypothesis on the nature of the polarity in water movement seen in some plant cells. No polarity was observed; however, endosmotic experiments were limited in that the cell was damaged at $\Delta\Pi=2.65$ atm; i.e., it showed no streaming or action potentials and the L_{PD} steadily declined. Although L_{PD} experiments were limited both in number and in the $\Delta\Pi$ range explored, they were reproducible. In cell No. 4 I alternated between endosmotic and exosmotic flow three times and in cell No. 6, four times. Invariably endosmotic flow was the same as exosmotic ± 2 to 4.5%. This is within the limits of my accuracy.

Hydrostatic Filtration Coefficients on Living Cells (L_P)

L_P was determined at $\Delta\pi=0$. Of the five experiments attempted on different cells, four were com-

Table. Filtration coefficients in *Nitella*

Cell No.	Cell Dimensions[a]			Filtration Coefficients (cm/sec/atm) $\times 10^5$					
	Diameter (μm)	Length (mm)	Cell wall thickness (μm)	L_P	L_{PW}	L_{PD}			
						Endosmotic $\Delta\Pi$ range	Exosmotic $\Delta\Pi$ range		
						1.35 atm	1.35 atm	2.65 atm	3.95 atm
1	545	38.5	5.7	14.1	13.2		1.55	1.61	1.79
2	502	39.3	5.2	16.3	15.5		1.70	1.76	1.92
3	478	31.6	4.5	17.7	18.5		1.74	1.84	2.09
4	417	20.5	4.0		20.4	1.95	1.91	2.01	2.25
5	390	18.2	3.7	19.2	21.6		2.06	2.11	2.32
6	462	23.5	4.8		17.5	1.60	1.63		
7	407	25.8	4.3		19.0				
8	405	21.7	4.0		19.9				

[a] Accuracy: Diameter ± 5 μm due to variations along the length. Length ± 1.0 mm due to obscurity of nodal region. Cell wall thickness ± 0.35 μm.

pleted. One internode (internode No. 4 in Fig. 2) was lost after L_{PD} determinations. The results of these four successful experiments are presented in the Table and Fig. 3. These experiments, as mentioned in Materials and Methods, are limited in that it was not possible to attain high intracellular pressures without cell damage. In the range of pressure investigated, L_P varied from 14.1 to 19.2×10^{-5} cm/sec/atm.

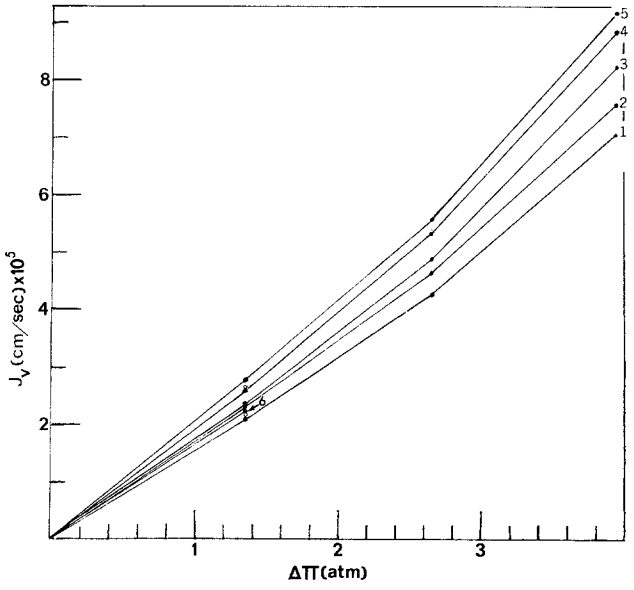

Fig. 2. Water flow (J_v) as function of the difference in somotic pressure ($\Delta\Pi$) between the inside and the outside of the cell. Solid points for exosmotic flow. The two open circles at $\Delta\Pi = 1.35$ for endosmotic flow

Hydrostatic Pressure Filtration Coefficients of Cell Walls (L_{PW})

A total of eight experiments on different cell walls were undertaken. Five of these internodes had been used previously for L_{PD} and L_P measurements. These results are presented in the Table and Fig. 4. L_{PW} values ranged from 13.2 to 19.9×10^{-5} cm/sec/atm. These L_{PW} values are higher than reported previously (*cf.* Discussion); however, this may be explained by the observation that the thickness of my cell walls was less.

In three cell walls the effects on L_{PW} of 100% ethanol, acetone, methanol, and drying were studied. Such treatments (lasting 5 min in the case of ethanol, methanol, and acetone and one day in the case of drying) affects L_{PW} by less than 7%. In two cell walls the cannulae were removed, the walls cut so that the nodes were excluded and then recannulated. The L_{PW} measured did not vary more than 5%.

It is merely a coincidence that the L_{PW} of the

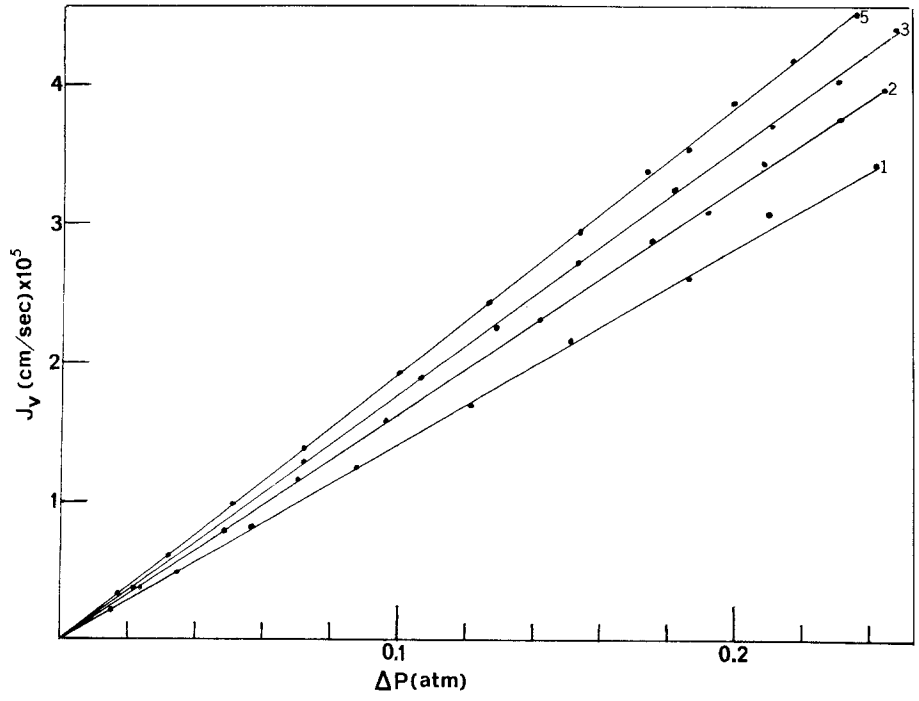

Fig. 3. Water flow (J_v) as a function of the difference in hydrostatic pressure (ΔP) between the inside and the outside of the living cell

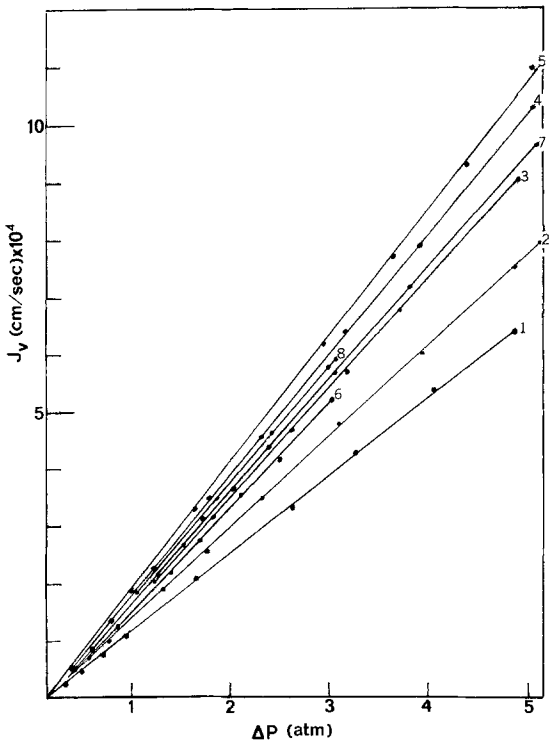

Fig. 4. Water flow (J_v) as a function of the difference in hydrostatic pressure (ΔP) between the inside and the outside of the isolated cell wall

cell wall (or the L_P of the intact *Nitella* at $\Delta P \sim 0$) is the same as that of the squid axon with or without a plasmalemma. Other algae show a different L_{PW} (Zimmermann & Steudle, 1978) and L_{PW} of the *Nitella* cell wall depends upon thickness (Kamiya, Tazawa & Takata, 1962). This dependence is also indicated from the data given in the Table.

Discussion

Previously L_{PD} in algal cells had been measured by a number of different methods: (i) the plasmolytic method [for review, *see* Stadelman (1966) and Dainty (1969)]; (ii) the transcellular osmosis method (Osterhout, 1949; Kamiya & Tazawa, 1956; Dainty & Hope, 1959*a*; Dainty & Ginzburg, 1964; Tazawa & Kamiya, 1965; Kiyosawa & Tazawa, 1972; and in tonoplast free cells by Kiyosawa & Tazawa, 1977); (iii) by measuring the rate of change of length of a cell as a function of $\Delta\pi$ (Kelly, Kohn & Dainty, 1963); (iv) by measuring the time course of change in internal pressure following a step change in external osmotic pressure (Steudle & Zimmermann, 1974; Zimmermann & Steudle, 1974, 1975); (v) by an internal perfusion technique in Valonia (Gutknecht, 1967, 1968); (vi) by the rate of change of the reduced weight of the cell during osmotic water flow (Palva, 1939). With the possible exception of the plasmolytic method the values obtained with the other methods on *Nitella* were similar to the ones obtained in the present work.

L_P at low ΔP was previously determined by measuring the rate of change in internal pressure following a step change in intracellular volume (Steudle & Zimmermann, 1974; Zimmermann & Steudle, 1974, 1975). I could not investigate with my technique the hydrostatic pressure dependence of L_P. They found that below 1–2 atm, internal pressure L_P was increased by a factor of between 2 and 10. At $\Delta P \sim 0$, the L_P observed by me is rather higher than that observed by Zimmermann and Steudle. Comparison with Fig. 2 clearly shows that L_{PD} is almost an order of magnitude smaller than L_P. This is a large difference, though not as striking as it is in the axon where it is two orders of magnitude.

Previously (Kamiya et al., 1962; Tazawa & Kamiya, 1965; Tyree, 1968; Zimmermann & Steudle, 1975) L_{PW} was determined by methods similar to mine. The main difference was that in the previous work the internode under study was cannulated, not the neighboring one as in my work. My L_{PW} values are somewhat larger than previous ones; however, this may be due to my cell walls being also thinner (*cf.* Table). The fact that my cell walls were thinner may also explain why my L_P values at $\Delta P \sim 0$ were higher than previous ones. This would follow if the cell wall is the rate-limiting structure for pressure-driven water flow. The most important finding on L_{PW} measurements was that the values obtained were almost indistinguishable from the L_P values, indicating that the rate-limiting structure for pressure-driven flow is not the plasmalemma but the (in-series) cell wall. Zimmermann and Steudle (1975), using a different technique also noted that $L_{PW} \sim L_P$. The same observation had already been made on the squid axon, namely the L_P observed in the intact axon was not altered by destruction of the plasmalemma. In the axon, in large part, this observation led me to conclude that the reason the L_P is so much larger than the L_{PD} was related to the pressure-driven water flow being limited by in-series structures and not by the plasmalemma itself.

The present study was prompted by a desire to demonstrate that the property of the squid axon surface wherein the barrier to hydrostatic water flow is in-series with the plasmalemma (and the observed overall L_P is not equivalent to the L_{PD}) is not as unique or as paradoxical as thought by

most. It is evident now that when $\Delta P \sim 0$ the hydraulic behavior of the cell surface of some algae is similar to that of the surface of the axon; however, the aforementioned necessary condition that ΔP be low or approach zero although physiological for the axon is not so for the algae cells that normally are at high turgor pressure. Zimmermann and Steudle (1974, 1975, 1978) have found in algae that L_P increases by a factor of at least 2 (in *Nitella*) or 10 (in *Valonia*) when the internal hydrostatic pressure is lowered from a level of a few atmospheres to well below one atmosphere. It would seem then that the analogy advocated between the axon and the algae would be more convincing if there were a straightforward explanation of the pressure dependence of the hydraulic conductivity of the plasmalemma. Zimmermann and Steudle (1975, 1978) offered three interpretations: (i) that the hydraulic conductivity of the plasmalemma is decreased at higher pressures due to stretching and closure of elliptical pores, (ii) that the hydraulic conductivity of the plasmalemma is increased near the plasmolytic point due to an increase in its folding and consequent changes in salt gradients within the folds and (iii) that there is a pressure dependence of the contribution of solute flow to water flow and that this contribution "can arise either from a pressure dependence of the coupling coefficient and/or of the efficiency of the energy transfer between active solute flow and water flow, or from a direct effect of pressure on active solute flow." Apparently these authors currently prefer the third interpretation (Zimmermann & Steudle, 1978).

I am considering an alternate interpretation that, in common with the three of Zimmermann and Steudle, has no solid experimental support. I prefer it mainly because it appeared to me to bring the behavior of the cell surface of the algae more in line with that of the axon. Briefly, I suggest that since internal pressure in the algae presses the plasmalemma against the porous cell wall, those regions of the plasmalemma in juxtaposition to the interporous (presumably water impermeable) regions of the cell wall may be expected to be less available for water flow than those regions of the plasmalemma facing the pore opening. If at higher internal pressures the hydraulic conductance of the plasmalemma is thus sufficiently reduced then the plasmalemma may replace the cell wall as the rate-limiting layer for pressure-driven water flow and probably the L_{PD} should become more equivalent to L_P.

It may be reasonable to assume that the cellulose microfibril component of the cell wall is in large part the structure that provides the steric hindrance to pressure-driven water flow[3]. For architecture of cell wall, *cf.*, e.g., Preston's review (1974) also Green (1958), and Probine and Preston (1961). Considering the striking in-parallel packing of microfibrils [especially on the surface of the wall facing the plasmalemma (*cf.* Green, 1958) one may speculate that the pathways for water flow tend to be slits between microfibrils not cylindrical pores. Estimates of an equivalent "pore" radius have been made by Dainty and Hope (1959*b*). On the basis of measurements of the free space of the cell wall, they proposed a system of pores more than 100 Å in diameter and normal to the surface and less than 100 Å and parallel to the surface. I have also made some *very rough* measurements (*unpublished*) that may indicate an equivalent "pore" radius 25–45 Å (or a smaller equivalent half "slit" width).[4]

[3] This is somewhat indicated by the observation mentioned in Results that L_{PW} is not affected by treatment of the wall with pure acetone, ethanol, and methanol. It would have been more convincing had I also tried dilute acids or bases that dissolve hemicelluloses, polyuronides, etc.

[4] The value of 25–45 Å was estimated from the ratio P_f/P_d, namely the ratio of the permeability determined by hydrostatic pressure experiments to that by tracer diffusion experiments. In the P_d measurements the isolated cell walls were internally perfused continuously so that the tritiated water in the internal compartment was kept constant and the stagnant layers minimized. External stagnant layers were also minimized by stirring. It should be emphasized that the effects of unstirred layers on P_f/P_d can be very large and very difficult to evaluate. The 25–45 Å range is consistent with the finding that inulin C-14 ($\sim$12 Å radius) included in the internal perfusate readily crosses the wall. In one experiment, the internal perfusate of isolated cell walls contained various concentrations of Ficoll 400 in distilled water. The hydrostatic pressure required to sustain zero net volume flow was determined for each concentration of Ficoll in the internal perfusate. As done previously (Spyropoulos, 1979), periodically brief high pressure heads were required to assure flushing of internal contents. The osmotic pressure values thus obtained corresponded to those obtained previously with dialysis membranes and also those obtained with KF-treated axons whose axoplasm was bored out and whose interior was perfused with isosmotic KF containing various concentrations of Ficoll and subjected to various pressure heads (Spyropoulos, 1979)! Mistakenly, in this previous paper it was stated that Ficoll was outside instead of inside the axon. In the second type of experiment indicating that the cell wall is impermeable to Ficoll intact *Nitella* cells were immersed in a medium containing sucrose at a concentration barely insufficient for plasmolysis. Addition of Ficoll to this medium resulted in a steady-state cytorrhysis, not a plasmolysis. The mol wt of Ficoll is 400,000. A value given previously (Spyropoulos, 1979) for the Stokes' radius of Ficoll 400 was cited inadvertently from the Pharmacia, Inc., literature where it was quite incorrect. According to David A. Weber of Pharmacia, Inc., the correct Stokes' radius is $\sim$100 Å.

On account of the theoretical limitations in analyzing composite membranes (e.g., *cf.* Kedem & Katchalsky, 1963) also on account of the structural complexity of the composite membranes encountered in algae, I can only go so far as to suggest that at high internal pressures one of the elements of the composite surface structure, i.e., the plasmalemma, is pushed against another of its elements, i.e., the cell wall, in such a way that the conductance of the former may become limiting. Attempts at being more quantitative than just that would be precarious.

In the giant cells of *Loligo vulgaris, Dosidicas gigas, Nitella flexilis, Valonia ultricularis, Chara intermedia,* and *fragilis* and *Nitelopsis obtusa,* where *both* L_P and L_{PD} have been measured (Spyropoulos, 1977*a*, 1979, 1980; Steudle & Zimmermann, 1974, 1978; Vargas, 1968; Zimmermann & Steudle, 1974, 1975, 1978). L_P was found under certain conditions to be larger than L_{PD} even though impermeable solutes were involved. In all other cells, both plant and animal, water flow has been studied only as a function of $\Delta\pi$ and not of ΔP; nevertheless, even when impermeable solutes are involved it is assumed that the flow due to ΔP is the same as that due to $\Delta\pi$. This is so even in cells that are closely related to the ones listed above, namely, the giant axon of *Dorytheuthis plei* (Villegas & Villegas, 1960) and the giant cell of *Valonia ventricularis* (Gutknecht, 1967, 1968). In both these cells it is assumed that water flow due to $\Delta\Pi$ was equivalent to that due to ΔP and in both[5] $\Delta P \sim 0$ but in both only water flow due to $\Delta\Pi$ not ΔP was measured. In both of these studies the ratio of the permeability determined by osmotic experiments, P_f, to that determined by diffusion (of labeled water) experiments, P_d, was used to ascertain the mode of passage of water through the plasmalemma, namely, whether it was viscous or diffusive and, if viscous the equivalent pore radius. It is implicit in the use of this ratio for such purposes that unless the flow due to $\Delta\Pi$ is equivalent to that due to ΔP, it is the P_f that is measured by hydrostatic experiments, not by osmotic, that is more meaningful. In the giant axon of *Loligo vulgaris* both diffusional and hydrostatic pressure-driven water flow are limited by structures in series with the plasmalemma. So unless this is shown not to be the case in *Dorytheuthis plei* one cannot draw any conclusions about the pore sizes in the plasmalemma. Gutknecht (1967, 1968) in *Valonia ventricularis* found that the P_d of the wall was of the same order as the P_d of the plasmalemma, but claimed that the P_f of the wall (determined by L_{PD} experiments after the plasmalemma was destroyed) was 500 times the P_f of the plasmalemma. In the related species *Valonia ultricularis* Zimmermann and Steudle (1974) using a direct method found that, at $\Delta P \sim 0$, L_P is increased by a factor of 10, indicating according to our picture that the wall may have become more rate limiting for pressure-driven flow.

A number of investigators (Kamiya & Tazawa, 1956; Dainty & Hope, 1959*a*; Dainty & Ginzburg, 1964; Tazawa & Kamiya, 1965, 1966; Kiyosawa & Tazawa, 1973, 1977; Tazawa & Kiyosawa, 1973; Steudle & Zimmermann, 1974, 1978) have made quite an issue of a vexing polarity, a kind of rectification effect in the water flow (i.e., wherein exosmotic flow is less than endosmotic).

I think that the polarity may be approached in the same way as the pressure dependence of L_P. In Steudle and Zimmermann's (1974, 1978) experiments using the pressure sensor method, the internal compartment of the cell is a *closed* system so that upon inducing exoplasmic flow by osmotic means the internal hydrostatic pressure was lowered. Conversely, upon inducing endosmotic flow the internal hydrostatic pressure is increased. One would expect that upon lowering the internal pressure, the plasmalemma would become less contiguous to the cell wall and less rate limiting since its hydraulic conductance would be increased. On the other hand, an increase in the internal pressure would make the plasmalemma more forcefully in contact with the cell wall, thereby its conductance would decrease. Consistent with this interpretation is the experiment mentioned in my Results where no polarity was observed in the perfused cell in which, on account of the internal compartment being an open system, osmotic water flows were measured at nearly zero hydrostatic pressure difference. This interpretation is also consistent with the experiment of Steudle and Zimmermann (1974) who noticed polarity in *Nitella,* when the interior was a *closed* system at high *but not* at very low internal hydrostatic pressure. My interpretation of the polarity in water flow is not consistent with many trancellular osmosis experiments (*cf.* above for references) where the phenomenon was discovered. For this, as yet, I have no *clear* explanation. In Steudle and Zimmermann's internal sensor and my internal perfusion technique the internal compartment is more easily defined as an open or a closed system. On the other hand I have difficulty in defining the interior of the cell in the transcellular osmosis experiments. The main reason for this difficulty is that the interior in the transcellular osmosis experiments is divided.

[5] The experiments on *Valonia ventricularis* were carried out under internal perfusion conditions where $\Delta P = 0$.

It appears that in the squid axon and under some conditions in some giant algae that a surface structure in-series with the plasmalemma and not the plasmalemma *per se* is the rate-limiting structure for pressure-driven water flow. This has been demonstrated using the techniques of intracellular perfusion by myself and introduction of a pressure sensor into the cell interior by Steudle and Zimmermann. Such methods that are *required* to ascertain whether or not the plasmalemma is the water flow barrier have been applied to a *limited* number of cells. The reason is that with these techniques the cell must be giant. However, most cells are not naked, i.e., completely devoid of surface material (cellular or acellular) closely associated with the external surface of the plasmalemma. This extra-plasmalemmal material cannot be ignored so, unless it is proven directly by inventing some method sort of equivalent to those used in the giant alga or axon, the plasmalemma need not be regarded in a given cell as the barrier to pressure-driven water flow.

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