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DETERMINATION OF THE HYDRAULIC CONDUCTIVITY AND OF REFLECTION COEFFICIENTS IN *NITELLA FLEXILIS* BY MEANS OF DIRECT CELL-TURGOR PRESSURE MEASUREMENTS

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SUMMARY

From direct and continuous measurements of the internal hydrostatic pressure (P) in the internodes of *Nitella flexilis*, the reflection coefficients (σ_s) of some non-electrolytes were determined, using a zero-flow method, and were compared with those found previously on *Valonia utricularis* and with those obtained by Dainty and Ginzburg on other Characean internodes from transcellular osmosis experiments. The hydraulic conductivities (Lp) of the cell membranes were determined by two independent methods, that is, using hydrostatically or osmotically induced flows. From the exponential time course of P in such experiments and from the volumetric elastic modulus (ε) of the cell wall, Lp was calculated. The effect of unstirred layers in the methods described was negligibly small.

In osmotic experiments with different non-plasmolysing external sucrose concentrations (20–200 mM) the exosmotic hydraulic conductivity (Lp_{ex}) decreases markedly with increasing concentration, while the endosmotic hydraulic conductivity (Lp_{en}) shows only a weak dependence. In the hydrostatic experiments the hydraulic conductivities for single cells were constant in the pressure range for P from 2 to 7 atm. In this pressure range Lp_{en} and Lp_{ex} varied for different cells from $2.2 \cdot 10^{-5}$ to $2.8 \cdot 10^{-5}$ and from $1.8 \cdot 10^{-5}$ to $2.5 \cdot 10^{-5}$ $cm \cdot s^{-1} \cdot atm^{-1}$, respectively, with an average ratio Lp_{en} to Lp_{ex} of 1.1, which indicates a polarity in water movement.

These values were the same as those obtained in the osmotic experiments from extrapolation to zero sucrose concentration. At internal pressures below 2 atm the Lp-values markedly increase on approaching the plasmolytic point.

The results are discussed in terms of a dehydration of the membranes (or the cytoplasm) at increased solute concentrations. In addition, the strong dependence of Lp at low internal hydrostatic pressures points to a direct influence of P on the water permeability of the membranes.

INTRODUCTION

The reflection coefficients for some solutes and the hydraulic conductivities during endosmosis and exosmosis were determined by Dainty and Ginzburg [1,2] on *Nitella translucens* and *Chara australis* and by Kamiya and Tazawa

[3,4] on *Nitella flexilis* by means of transcellular osmosis. From the theoretical and experimental point of view this phenomenon is rather complicated. The detailed theory developed by Dainty and Ginzburg [2] shows that the volume flow during transcellular osmosis is caused both by cell shrinkage and true transcellular osmosis. Cell shrinkage occurring during the initial phase of transcellular osmosis is almost over within 5 s and is controlled by the exosmotic hydraulic conductivity. The water flow observed after this time is due to true transcellular osmosis. The rate of water flow during the period is determined both by the exosmotic and endosmotic hydraulic conductivities, Lp_{ex} and Lp_{en}, respectively. Therefore, difficulties can arise in the determination of the two hydraulic conductivities if there is a polar water movement through the membrane, i.e. if $Lp_{ex} \neq Lp_{en}$.

Dainty and Ginzburg [1,2] measured the hydraulic conductivity under exosmotic and endosmotic conditions during true transcellular osmosis over a period of 30–60 s. Tazawa and Kamiya [3,4] at the initial phase of true transcellular osmosis, i.e. in the time interval between 5 and 10 s assuming that the change of turgor and cell volume had been almost completed. From these experiments Dainty and Ginzburg [1,2] and Tazawa and Kamiya [3,4] put forward good reasons for supposing that the demonstrated polarity of the hydraulic conductivities in their dependence on the direction of water movement is an intrinsic characteristic of the membrane because the results could not be explained solely by a dilution effect introduced into the discussion by Dainty [5] as the so-called sweeping-away effect. The sweeping-away effect is correlated with the existence of unstirred layers being built up during the time course of measurement. The calculation of these unstirred layers at the cell-membrane surface is difficult and the shift in the absolute values of the phenomenological coefficients because of unstirred layers can be very remarkable.

For example, Dainty and Ginzburg [1,6] demonstrated theoretically that the reflection coefficients of very permeable substances measured by the method of transcellular osmosis could be underestimated by more than 50% if unstirred layers were not taken into account. Therefore, we feel that it would be useful to establish the theory by investigating the experimental arrangement of transcellular osmosis and the apparent polarity of the hydraulic conductivity. For this purpose, we will use a method which permits a rapid, continuous and direct measurement of the hydrostatic pressure inside the cell during the first phase of shrinking or swelling. Under these conditions errors due to unstirred layers should be negligibly small.

A method which complied with these requirements was developed by us [7] and used for the determination of reflection coefficients [8] and the dependence of the hydraulic conductivities on the direction of water movement [9] on *Valonia utricularis*. It is noteworthy to mention that a polarity of water movement could not be detected in this alga. Using this method it is also possible to measure the volumetric elastic modulus of living cells and to induce water flows both by osmotic and hydrostatic pressure gradients. Therefore, the dependence of the exosmotic and endosmotic hydraulic conductivity on hydrostatic pressure inside the cells and on the osmotic pressure can be determined separately. Such experiments have not been performed before. Furthermore, the method permits (if polarity really exists) a study of this phenomenon over a large pressure range. From these data more insight into the mechanism of polar water movement should be obtained.

MATERIALS AND METHODS

(a) *Material*

N. flexilis was collected from a pond near Jülich and kept at 22–25 °C in daylight for several months in artificial pond water containing 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM $CaCl_2$ and 0.1 mM $MgCl_2$ (pH about 5.6). This was also the normal solution in which the experiments were carried out. The cells actually used were in some cases not those collected, but cells secondarily grown from original plants in a laboratory culture of artificial pond water and pond mud.

(b) *Cell-turgor pressure and volume-flow measurement*

The cells selected for the experiments were 2–5 cm in length and about 0.5 mm in diameter. The cells were freed from neighbouring internodes and leaves and mounted on the slit of the device, which is schematically shown in Fig. 1. In order to avoid large unstirred layers the cells were irrigated with artificial pond water and with the testing solutions, respectively, during the course of the experiments. By means of a two-way stop cock in this device artificial pond water could be rapidly exchanged for media with altered osmolarity and again replaced by artificial pond water.

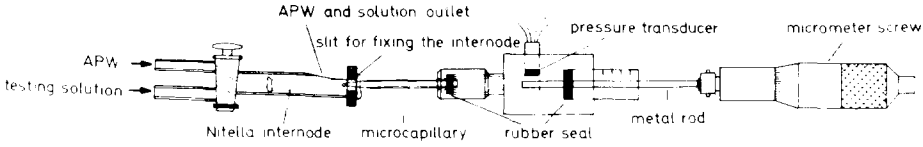

Fig. 1. Experimental assembly for continuous pressure and volume-flow measurements in *Nitella* internodes (diagrammatic representation).

A detailed description of the simultaneous measurement of the cell turgor and of volume flows in giant alga cells had been already given elsewhere [7,9].

For a better understanding of the results presented in this paper the measuring equipment is illustrated in Fig. 1.

A microcapillary tube with a tip diameter of about 60 μm was filled with silicon oil up to the tip and inserted across the nodal cells into the vacuole of the internodal cell with the aid of a micromanipulator viewing the cell under a binocular microscope. The microcapillary was tightly connected to a small pressure chamber, which was also filled with silicon oil. It contained a small silicon pressure transducer (CQS 125–200 Kulite Semiconductor Products, Ridgefield, N. J.) transforming the applied pressure into a proportional voltage, which was monitored by a Keithly 602 electrometer and recorded by a Servogor recorder. Since capillary forces in the tip of the microcapillary were negligibly small, the pressure in the chamber was equal to the pressure in the cell. The boundary between oil and cell sap was usually at the tip of the capillary. It was chosen as the reference point for pressure measurement during volume flow and was regulated by a movable metal rod which was introduced into the pressure chamber and sealed off with a rubber disc. Therefore, errors resulting from leaks in the pressure system could be excluded.

The accuracy of the pressure measurement was about $2 \cdot 10^{-2}$–$3 \cdot 10^{-2}$ atm.

Volume flows and, therefore, changes of the turgor, were induced in two different ways. Outward water movement, i.e. exosmosis, was produced by an osmotic gradient by rapidly replacing the artificial pond water with media containing non-plasmolysing concentrations of a non-electrolyte (20–200 mM). Inward water movement, i.e. endosmosis, was measured by transferring the alga into the original artificial pond water after reaching water flux equilibrium in the exosmotic experiment. These experiments will henceforth be referred to as "osmotic experiments".

In another way of determining the hydraulic conductivities from the time course of the volume flow and of the turgor, water flow was induced by different hydrostatic pressure gradients.

In these experiments, referred to as "hydrostatic experiments" in this paper, an artificial pressure was applied to the vacuole by moving the micrometer screw attached to the metal rod. The response to the shift of the boundary oil/cell sap in a direction towards the vacuole was an expansion of the cell volume and an increase of the turgor. Under these conditions an outward water flow, i.e. exosmosis or, more correctly reverse osmosis, was observed. After reaching the steady state for water fluxes, endosmosis was induced by turning the micrometer screw backwards. During the experiments the boundary oil/cell sap was fixed at the tip of the capillary or an oil droplet with a constant diameter was formed at the tip as the reference point for pressure measurement. Errors due to leakages around the inserted micro-capillary could be easily detected by the hydrostatic experiments. From the exponential decrease or increase of the turgor inside the cell under osmotic and hydrostatic conditions the exosmotic and endosmotic hydraulic conductivities can be calculated as outlined below if the volumetric elastic modulus can be measured. The value of the volumetric elastic modulus can be easily obtained from the hydrostatic experiments. Provided that the time involved for cell expansion and increase of the turgor is short in comparison to the water flow, the change in cell volume can be calculated from the feed and the diameter of the rod (500 μm), since the measuring system is incompressible to a very good approximation.

The volumetric elastic modulus is calculated from the change in the cell volume and the corresponding change of the turgor using the Philip equation (see below).

The reflection coefficients of several non-electrolytes were determined by measuring the final stationary internal hydrostatic pressure in response to changes of the non-electrolyte concentration in the external medium in exosmotic and endosmotic experiments.

Calculation of the hydraulic conductivity and reflection coefficients

Consider a non-ideal semipermeable membrane separating two non-electrolyte solutions with different solute concentrations. There are in general two fluxes, viz. the volume flow J_v($cm^3 \cdot cm^{-2} \cdot s^{-1}$) and the solute flow J_s ($M \cdot cm^{-2} \cdot s^{-1}$) driven by the hydrostatic pressure difference P (atm) and the osmotic pressure difference $\Delta\pi_s$ (atm), respectively. The interaction between these fluxes can be described by the practical phenomenological equations for coupled flows [10]:

$$J_v = Lp(P - \sigma_s \cdot \Delta\pi_s) \tag{1}$$

$$J_s = (1 - \sigma_s) \cdot \bar{c}_s \cdot J_v + \omega \cdot \Delta\pi_s \tag{2}$$

where the symbols denote: Lp, hydraulic conductivity ($cm \cdot s^{-1} \cdot atm^{-1}$); σ_s, reflection coefficient; ω, solute permeability ($M \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$); and $\bar{c}_s$, average concentration of the non-electrolyte ($M \cdot cm^{-3}$).

In the presence of impermeable solutes Eqn 1 becomes:

$$J_v = Lp(P - \Delta\pi_i - \sigma_s \cdot \Delta\pi_s) \tag{3}$$

where $\Delta\pi_i$ is the osmotic pressure difference due to the impermeable solutes. The volume flow is equal to the water flow, J_w, to a very good approximation.

In order to calculate the hydraulic conductivity from the water flow observed during osmosis we assume that the non-electrolyte is only in the external pond water and that the electrolytes in the cell and in the artificial pond water are impermeable to a first approximation [11]. The last assumption should also be valid for the calculation of the hydraulic conductivity from hydrostatic experiments. Furthermore, we choose the volume of the external medium large in comparison to the cell volume. This means that the osmotic pressure of the external medium, $\pi_i^o + \pi_s^o$, is constant during the hydrostatic and osmotic experiments, only P and the internal osmotic pressure π_i^i are functions of time. Eqn 3 can, therefore, be written:

$$J_w = \frac{1}{A} \frac{dV_w(t)}{dt} = Lp[P(t) - \pi_i^i(t) + \pi_i^o + \sigma_s \cdot \pi_s^o] \tag{4}$$

where $V_w(t)$ denotes the change in cell volume at time t ($V_w = V_0 - V$). V_0 is the cell volume at the original pressure P_0; V is the cell volume at pressure P; A is the cell surface area. Considering the cell as a classical osmometer the following Eqns are valid for $\pi_i^i(t)$ and $P(t)$ [9]:

$$\pi_i^i(t) = \pi_{io}^i \left(1 + \frac{V_w(t)}{V_o}\right) \tag{5}$$

$$P_o - P(t) = \varepsilon \left(1 - \frac{V(t)}{V_o}\right) = \frac{\varepsilon \cdot V_w(t)}{V_o} \tag{6}$$

with π_{i0}^i is the internal osmotic pressure at the original pressure P_0 and ε is the volumetric elastic modules of the cell wall. Eqn 6 is the so-called Philip equation [12]. The elastic modulus, ε, can be determined according to this equation by measuring the change in the cell volume in response to an applied pressure.

It can be shown from Eqns 4–6 that the internal hydrostatic pressure depends exponentially on time provided the only variables are $V_w(t)$, $\pi_i^i(t)$ and $P(t)$. We have found this to be true experimentally. Thus we can write:

$$P - P_E = (P_o - P_E) \cdot e^{-kt} \tag{7}$$

$$P - P_E = (P_A - P_E) \cdot e^{-kt} \tag{8}$$

for osmotic (Eqn 7) and hydrostatic experiments (Eqn 8). P_E denotes the final hydrostatic pressure in such experiments, while P_A is the pressure artificially applied to the cell in the hydrostatic experiment, to produce a volume flow. k is the rate constant for pressure change.

Using Eqns 4–7 and considering the different boundary conditions, the following equations for Lp_{ex} and Lp_{en} can be easily obtained from osmotic experiments [9, 13]:

$$Lp_{ex} = \frac{k \cdot V_o(P_o - P_E)}{\varepsilon \cdot A \cdot \sigma_s \cdot \pi_s^o} \tag{9}$$

$$Lp_{en} = \frac{k \cdot V_o(P_E - P_o)}{\varepsilon \cdot A \cdot \sigma_s \cdot \pi_s^o} \tag{10}$$

In the same way, an analogous equation for the hydraulic conductivity (Lp_{ex} and Lp_{en}) is derived for the hydrostatic experiments from Eqns 4–6 and 8:

$$Lp = \frac{k \cdot V_o(P_A - P_E)}{\varepsilon \cdot A(P_A - P_o)} \tag{11}$$

The calculation of the reflection coefficients is based on a zero-flow method. With the condition of zero flow, ($J_w = 0$), it follows from Eqn 4 that the measured difference between the initial pressure, P_0, and the final, stationary pressure P_E in an exosmotic experiment is obtained as follows:

$$P_o - P_E = \pi_{io}{}^i - \pi_{iE}{}^i + \sigma_s \cdot \pi_s^o \tag{12}$$

where $\pi_{iE}{}^i$ denotes the final internal osmotic pressure which is reached in the cell at the end of the experiment. From the Eqns 5 and 6 it follows:

$$\pi_{iE}{}^i - \pi_{io}{}^i = \frac{\pi_{io}{}^i}{\varepsilon}(P_o - P_E) \tag{13}$$

Combining the Eqns 12 and 13 one obtains for the reflection coefficient:

$$\sigma_s = \frac{P_o - P_E}{\pi_s^o} \cdot \frac{\varepsilon + \pi_{io}{}^i}{\varepsilon} \tag{14}$$

For the endosmotic experiment an analogous equation can be derived:

$$\sigma_s = \frac{P_E - P_o}{\pi_s^o} \cdot \frac{\varepsilon + \pi_{iE}{}^i}{\varepsilon} \tag{15}$$

In these equations the second factor on the right-hand side corrects the σ_s-values for the shrinkage or swelling of the cell. $\pi_{io}{}^i$ and $\pi_{iE}{}^i$ can be directly calculated from P_0 and P_E respectively, as they differ from these values only by the amount of π_i^o. Combining Eqns 9 and 10 with Eqns 14 and 15, the half-time for the shrinkage or swelling of the cell can be calculated:

$$T_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{\ln 2 \cdot V_o}{Lp \cdot A \cdot (\pi_{io(E)}{}^i + \varepsilon)} \tag{16}$$

This equation differs only from the equation given by Dainty [5] in the choice of the frame of reference of the cell volume, V_0.

RESULTS AND DISCUSSION

Volumetric elastic modulus

The volumetric elastic modulus, ε, must be known for the calculation of the hydraulic conductivity and reflection coefficients. ε is, however, only constant over a limited pressure range. It was clearly demonstrated for *V. utricularis* [13] that as the internal pressure increases by more than 1 atm, the cell wall becomes more resistant to expansion. In the lower pressure range of the cell, the change in volume in response to the pressure becomes larger than predicted by the Philip equation. In Fig. 2 we show the dependence of the volumetric elastic modulus on the hydrostatic pressure inside the cell. The pressure range in which the volumetric elastic modulus was measured is indicated by a line parallel to the abscissa. As shown in Fig. 2, in some cells a linear relationship between volumetric elastic modulus and pressure was found in the pressure range between 1 and 7 atm, whereas in other cells ε was constant. However, in each cell investigated the volumetric elastic modulus decreased in the pressure range below 1 atm as expected from the *Valonia* experiments [13]. The reason for the different dependence of the volume elastic modulus at higher pressures is unknown. Since the calculation of the hydraulic conductivity is based on the validity of the Philip equation (Eqn 6) the *Lp*-values were always measured for only a small pressure interval in which the Philip equation was valid.

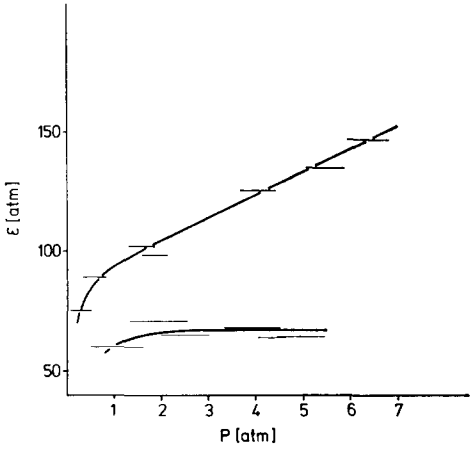

Fig. 2. Dependence of the volumetric elastic modulus of two *Nitella* internodes, ε, on the hydrostatic pressure in the cell (P). The lines parallel to the pressure axis indicate the pressure ranges, for which the ε-values were determined according to the Philip equation (Eqn 6).

It is important to point to the following possible source of error in the determination of the volumetric elastic modulus. The time required for applying a pressure by turning the micrometer screw and for measuring the change of the pressure inside the cell must be very short in order to avoid an error due to volume flow. The half-time of water flow in *Nitella* is of the order of 3 s. This means that the determination of the volumetric elastic modulus has to be performed in less than 1 s. According to the experiments of Kamiya et al. [14] on the cell wall tube of

Nitella the change in cell volume in response to pressure includes two componenst: an instantaneous change in a fraction of a second, followed by a very slow expansion which ceased after 20 min. The change of volume during the latter phase amounts to at most 10% of the total volume change. Since the hydraulic conductivity was calculated, however, from the volume flow during the first few seconds, only the first component of the cell volume change had to be taken into consideration.

Reflection coefficients

The results for the reflection coefficients of some solutes are listed in Table I. For comparison the reflection coefficients of the same solutes measured on *V. utricularis* [8] and on *N. translucens* and on *C. australis* [1] are also given.

TABLE I

REFLECTION COEFFICIENTS

Reflection coefficients, σ_s, of some non-electrolytes for the internode of *N. flexilis* (with standard deviations, the number of the cells is given in brackets). The non-electrolyte concentration in the testing solution was $c_s = 160$ mM. For comparison the data for *N. translucens*, *C. australis* and *V. utricularis* are given.

Solute	Molecular radius (A^0)	Reflection coefficients of			
		N. flexilis	*V. utricularis*[a]	*N. translucens*[b]	*C. australis*[b]
Sucrose	5.3[c]	0.97 ± 0.01 (5)	1		
Glucose	4.4[c]	0.96 ± 0.07 (2)	0.95		
Glycerol	2.74[d]	0.80 ± 0.04 (2)	0.81		
Acetamide	2.27[d]	0.91 ± 0.02 (2)	0.79		
Urea	2.03[d]	0.91 ± 0.01 (2)	0.76	1	1
Formamide		0.79 ± 0.04 (3)		1	1
Ethylene glycol		0.94 ± 0.02 (3)		1	1
Isopropanol		0.35 ± 0.05 (2)		0.27 (0.40)[f]	
n-Propanol		0.17 ± 0.06 (2)		0.16	0.22
Ethanol	2.13[e]	0.34 ± 0.02 (2)		0.29 (0.44)[f]	0.27
Methanol	1.83[e]	0.31 ± 0.04 (2)		0.25 (0.50)[f]	0.30

[a] Data taken from Zimmermann and Steudle [8].
[b] Data taken from Dainty and Ginzburg [1].
[c] Data taken from Durbin [15].
[d] Data taken from Goldstein and Solomon [16].
[e] Data taken from Villegas and Barnola [17].
[f] Data corrected for unstirred layers [6].

The reflection coefficients of the membranes of *N. flexilis* for sucrose, glucose and glycerol are in good agreement with the values found for *V. utricularis*. Differences exist between the reflection coefficients for urea and acetamide. It should be noted that the reflection coefficients given in Table I are values for the whole barrier including two membranes, i.e. tonoplast and plasmalemma. Therefore, it is necessary to discuss the differences in the values of the reflection coefficients of the single membranes; however, at present these are still lacking. For *N. translucens* Dainty and Ginzburg [6] found that the permeability of the plasmalemma for urea was 30 times higher than that of the tonoplast. In this case the high value of the

reflection coefficient of *N. flexilis*, *C. australis* and *N. translucens* for urea may reflect the properties of the tonoplast. In contrast the lower value of *V. utricularis* for this substance will point to a higher permeability of the tonoplast. Large differences between the values of the reflection coefficients measured by us on *N. flexilis* and by Dainty and Ginzburg on *N. translucens* seem to exist for methanol, ethanol, *n*-propanol and isopropanol although the ratio of the reflection coefficients of *n*-propanol and isopropanol is roughly the same for both algae.

If the correction for unstirred layers is taken into account as done by Dainty and Ginzburg [6] for the values of the reflection coefficients on *N. translucens* these values will be found higher than those measured by us on *N. flexilis*. Although we determined the reflection coefficients using a zero-flow method, a discussion of the unstirred layers must be undertaken.

A rough correction for the unstirred layers can be performed using the ratio $P \cdot \delta / D$ where P denotes the permeability coefficient of the solute, D its diffusion coefficient and δ the thickness of the unstirred layer. Dainty (personal communication) estimates the thickness of the unstirred layers built up under our experimental conditions to about 50 μm. Using this value, the permeability coefficients for methanol, ethanol and isopropanol given by Dainty and Ginzburg [6], and their diffusion coefficients [18] the ratio $P \cdot \delta / D$ is calculated to be 0.1–0.2 for these solutes. This means, that the solute concentration at the membrane surface is about 10–20% smaller than in the bulk solution, and that the reflection coefficients of the rapidly permeating alcohols are underestimated by the same amount. The corrected values of the reflection coefficients measured on *N. flexilis* would then be in better agreement with those given by Dainty and Ginzburg [6].

The differences of the values existing for both species can be explained in terms of the different properties of the membranes, since *N. translucens* is less permeable for water-soluble solutes than *N. flexilis* as can be seen by comparing the reflection coefficients for formamide, ethylene glycol and urea. The assumption of different properties of the membranes of *N. flexilis* and *N. translucens* is also supported by the different values of the hydraulic conductivity being about 2 times greater for *N. flexilis* than for *N. translucens* (see below) [2].

It is important to point out that the corrected σ_s-values of Dainty and Ginzburg [6] should be used because the uncorrected values of these authors have been mistakenly quoted in reviews [19].

Concentration and pressure dependence of the hydraulic conductivity

In Fig. 3 the hydraulic conductivity is plotted for endosmotic and exosmotic water flow against increasing external concentrations of sucrose. The hydraulic conductivity of exosmosis decreases with the increase of the sucrose concentration. The endosmotic hydraulic conductivity was measured at each concentration of sucrose by replacing the artificial pond water+sucrose by artificial pond water alone. In some experiments the endosmotic hydraulic conductivity was approximately constant over the whole concentration range investigated, in most experiments the endosmotic hydraulic conductivity decreases slightly above 50 mM sucrose as indicated in Table II.

Extrapolation of the values for the endosmotic and exosmotic hydraulic conductivity to zero concentration of sucrose yields a ratio of the endosmotic to

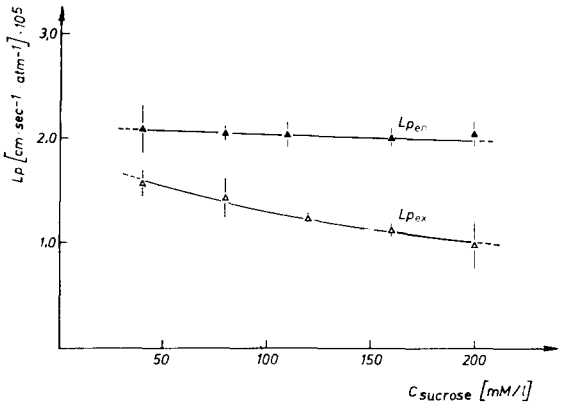

Fig. 3. Concentration dependence of the exosmotic and the endosmotic hydraulic conductivities (Lp_{ex} and Lp_{en}) from osmotic experiments with a single *Nitella* internode (Cell Nr. 2 from Table II) for different non-plasmolysing sucrose concentrations. The vertical lines to each value denote the standard deviation. From the extrapolation to $c_{sucrose}=0$ one obtains for $Lp_{ex}=1.82\cdot10^{-5}$ and for $Lp_{en}=2.12\cdot10^{-5}$ cm·s^{-1}·atm^{-1} ($Lp_{en}/Lp_{ex}=1.16$).

the exosmotic hydraulic conductivity of about 1.1. This value for the polarity of water movement in *Nitella* under natural and stationary conditions is in good agreement with the value of 1.2–1.7 given by Dainty and Ginzburg[2] and Tazawa and Kamiya [4] indicating that the assumptions of the theory of transcellular osmosis are valid. However, the polarity of water movement increases at higher concentrations up to values of about 2.1 (Table II).

Dainty and Ginzburg [2] concluded from the similar trend of the hydraulic conductivity with an increasing concentration of the external solute that it is the osmolarity of the solution and not the cell-turgor pressure, which determines the change in the hydraulic conductivity. Since we were able to directly change the hydrostatic pressure inside the cell we studied the dependence of the hydraulic conductivity on the turgor pressure in cells of *N. flexilis* to test the assumption of Dainty and Ginzburg [2]. In order to use this method at a low internal hydrostatic pressure it was necessary to decrease the intracellular electrolyte concentration. This can be easily done by incubating the punctured cells in artificial pond water for 10 h or more. During this time the turgor drops to 0.5–1 atm in response to the decreasing electrolyte concentration in the vacuole. This slow decrease can be accelerated by inducing exosmotic and endosmotic water flows alternatively. This effect was not fully understood. It might be explained by a slow loss of solute across the plasmadesmata of the internodes. Considering the short time of measurement of the hydraulic conductivities the change in the electrolyte concentration of the vacuole during such a measurement is negligible. In any case, the cells containing a low internal electrolyte concentration are not leaky since they react in hydrostatic experiments as classical osmometers. In Fig. 4 the values of the endosmotic and exosmotic hydraulic conductivities in a typical hydrostatic measurement are plotted against the hydrostatic pressure. In Table III the values for both coefficients and their ratio are listed for several cells. In the pressure range between 2 and 7 atm a polarity of water movement can be observed; the average ratio of the endosmotic

TABLE II

INFLUENCE OF EXTERNAL SUCROSE CONCENTRATION ON HYDRAULIC CONDUCTIVITY

Concentration dependence of the hydraulic conductivities (Lp_{ex} and Lp_{en} in 10^{-5} cm·s^{-1}·atm^{-1}) and of the ratio Lp_{en}/Lp_{ex} from osmotic experiments on different non-plasmolysing concentrations of sucrose. For all values the standard deviations and the number of experiments (in brackets) are given.

Cell No.		$c_{sucrose}$ (mM)					
		20	40	80	120	160	200
1	Lp_{ex}	2.03 ± 0.11 (5.4%) (5)	1.91 ± 0.08 (4.2%) (3)	1.67 ± 0.02 (1.2%) (3)	1.72 ± 0.01 (0.6%) (4)	—	—
	Lp_{en}	2.41 ± 0.10 (4.1%) (5)	2.39 ± 0.11 (4.6%) (4)	2.21 ± 0.06 (2.7%) (5)	2.09 ± 0.09 (4.3%) (4)	—	—
	Lp_{en}/Lp_{ex}	1.19	1.25	1.32	1.22	—	—
2	Lp_{ex}	—	1.57 ± 0.13 (8.3%) (2)	1.43 ± 0.18 (12.6%) (2)	1.23 ± 0.04 (3.3%) (2)	1.13 ± 0.06 (5.3%) (2)	0.98 ± 0.22 (22.4%) (2)
	Lp_{en}	—	2.09 ± 0.23 (11.0%) (3)	2.05 ± 0.07 (3.4%) (3)	2.04 ± 0.12 (5.9%) (3)	2.01 ± 0.09 (4.5%) (4)	2.04 ± 0.12 (5.9%) (3)
	Lp_{en}/Lp_{ex}	—	1.33	1.43	1.66	1.78	2.08
3	Lp_{ex}	—	—	2.12 (1)	1.64 (1)	1.58 (1)	—
	Lp_{en}	—	2.56 ± 0.09 (3.5%) (3)	2.43 ± 0.30 (12.3%) (2)	2.10 ± 0.21 (10.0%) (2)	1.82 ± 0.05 (2.7%) (2)	—
	Lp_{en}/Lp_{ex}	—	—	1.15	1.28	1.15	—
4	Lp_{ex}	—	1.14 ± 0.09 (7.9%) (3)	0.79 ± 0.10 (12.7%) (6)	0.77 ± 0.07 (9.1%) (3)	0.70 ± 0.00 (0.0%) (2)	—
	Lp_{en}	—	1.25 ± 0.06 (4.8%) (3)	1.24 ± 0.08 (6.5%) (5)	1.15 ± 0.04 (3.5%) (3)	1.04 ± 0.04 (3.8%) (3)	—
	Lp_{en}/Lp_{ex}	—	1.10	1.57	1.49	1.49	—

TABLE III

INFLUENCE INTERNAL HYDROSTATIC PRESSURE ON HYDRAULIC CONDUCTIVITY

Exosmotic and endosmotic hydraulic conductivities and the ratio Lp_{en}/Lp_{ex} from hydrostatic experiments in ranges of the internal hydrostatic pressure, where Lp is independent of the cell turgor. For all values the standard deviation and the number of the determinations are given.

Cell No.	Pressure range	$Lp_{ex} \times 10^5$ (cm·s^{-1}·atm^{-1})	$Lp_{en} \times 10^5$ (cm·s^{-1}·atm^{-1})	Lp_{en}/Lp_{ex}
1	2.0–7.3	2.21 ± 0.14 (6.3%) (18)	2.69 ± 0.14 (5.2%) (17)	1.22
2	2.0–7.4	2.49 ± 0.12 (4.8%) (26)	2.72 ± 0.14 (5.1%) (19)	1.09
3	2.1–6.4	2.42 ± 0.18 (7.4%) (14)	2.80 ± 0.15 (5.4%) (14)	1.16
4	1.8–7.4	2.01 ± 0.19 (9.5%) (20)	2.18 ± 0.18 (8.4%) (17)	1.08
5	2.1–5.8	1.84 ± 0.15 (8.4%) (24)	2.15 ± 0.20 (9.3%) (17)	1.17

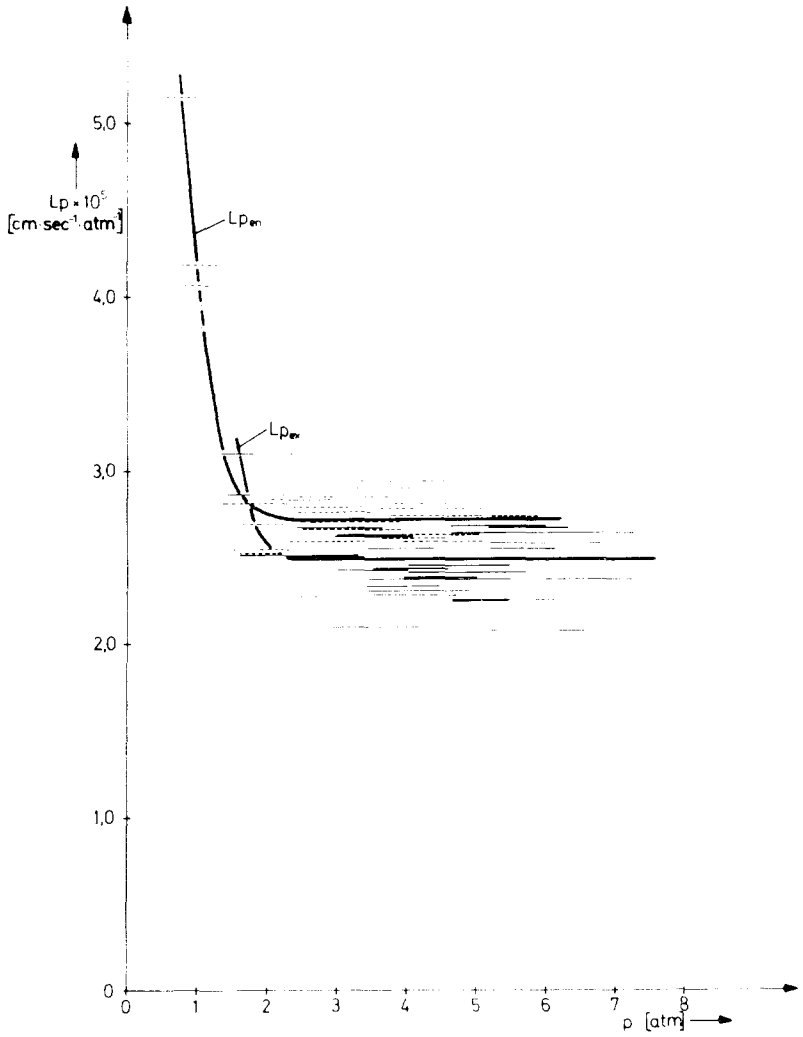

Fig. 4. Dependence of the endosmotic (- - -) and of the exosmotic (——) hydraulic conductivities on the hydrostatic pressure in a *Nitella* internode (Cell Nr. 2 in Table III). The broken and the full lines parallel to the pressure axis indicate the pressure ranges, for which the *Lp*-values were determined.

to the exosmotic hydraulic conductivity is about 1.1 as found by extrapolation in the osmotic experiments and is constant between 2 and 7 atm. This means that the turgor has no effect on hydraulic conductivity in this pressure range. As the hydrostatic pressure decreases the ratio decreases and the absolute values of the hydraulic conductivities increase markedly (see Fig. 4).

At first sight this result appears puzzling for it is the reverse trend to the exosmotic hydraulic conductivity in the osmotic experiments. The apparent discrepancy between the results of the hydrostatic and osmotic experiments can be interpreted

in terms of the dehydration theory [2,4,20,21], and in terms of a direct influence of the hydrostatic pressure on the membrane permeability.

Considering the osmotic experiment the decrease of the chemical water potential in the vacuolar and external phases due to the decrease of the hydrostatic pressure and to the increase of the sucrose concentration reduces the water content in the membranes or cytoplasm. The membranes will be dehydrated and, therefore, shrink and become less permeable. The dehydration theory also seems to explain satisfactorily the weak dependence of the endosmotic hydraulic conductivity on the osmolarity assuming that the dehydration and hydration of the membrane components are rapid processes. If this is true one should expect the endosmotic hydraulic conductivity to be independent of the osmolarity of the external medium since this parameter was measured in each case under the same conditions, i.e. after reaching water flux equilibrium at different sucrose concentrations and replacing the external medium by artificial pond water.

The observed influence of the sucrose at higher concentrations in some experiments on the Lp_{en}-values (Table II) points to a longer time necessary to rehydrate the strongly dehydrated membranes.

The independence of the hydraulic conductivity on the hydrostatic pressure in the pressure range between 2 and 7 atm is also in agreement with the dehydration theory, since the chemical water potential of both phases is constant over the whole pressure range. Therefore, the increase of the hydraulic conductivity in the low pressure range in the hydrostatic experiments must be related to a direct influence of the hydrostatic pressure on the membrane permeability with subsequent increase of the hydraulic conductivity and disappearance of polarity. Surely, one can argue that this increase of the hydraulic conductivity results from the decrease of the electrolyte concentration in the vacuole assuming an influence of the electrolyte on the membrane water permeability. However, the assumption that the turgor is responsible for the change in the water permeability is supported by similar results observed on *V. utricularis* [13]. With this alga the hydraulic conductivity also increased strongly when approaching the plasmolytic point. In contrast to the experiments on *Nitella* the internal electrolyte concentration was practically constant, and, therefore, an influence of the vacuolar electrolyte concentration on the hydration of the membranes could be excluded.

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