J. Membrane Biol. 75, 85-96 (1983)

The Journal of
Membrane Biology

Determination of Permeability Coefficients, Reflection Coefficients, and Hydraulic Conductivity of *Chara corallina* using the Pressure Probe: Effects of Solute Concentrations

Ernst Steudle and Stephen D. Tyerman*

Arbeitsgruppe Membranforschung am Institut für Medizin, Kernforschungsanlage Jülich, D-5170 Jülich, Federal Republic of Germany

Summary. The pressure probe technique which has been used for measurement of water relations parameters of plant cells [hydraulic conductivity (Lp), elastic modulus (ε) and half-time of water flow equilibration for individual cells ($T^w_{1/2}$)] can be used also for measuring reflection and permeability coefficients (σ, P_s) of permeable solutes. In the presence of a permeable osmoticum the pressure/time curves are biphasic, i.e. after a rapid water flow bringing turgor pressure to a minimum value (P_{min}), a second phase occurs in which turgor pressure increases back to the original value (P_o). The second phase ("solute phase") is due to an equilibration of the solute across the cell membrane and can be used to evaluate P_s. The responses are strictly reversible, i.e. when the osmoticum is removed a pressure maximum is quickly reached followed by a slower equilibration of solute. The reflection coefficients for the solutes can be calculated from the change in osmotic pressure of the medium ($\Delta\pi^o_s$) and from the change in turgor at the minimum ($P_o - P_{min}$) after correcting for solute flow. For internodal cells of *Chara corallina*, reflection and permeability coefficients for certain nonelectrolytes (sugars, polyols, monohydroxyalcohols, amides, ketones) are given and compared with data obtained by other methods. For *Chara*, P_s and osmotic values of Lp depended on external stirring, whereas σ corrected for solute flow did not. As expected hydrostatic Lp did not depend on stirring. No polarity of water flow was found for hydrostatic Lp ($Lp_{en}/Lp_{ex} = 1.02 \pm 0.05$, 95% confidence limits) whereas a polarity was observed for osmotic Lp which can be explained in terms of a concentration effect. Using permeable solutes, the concentration dependence of Lp, σ and P_s could be measured over large concentration ranges (up to 1.4 M) at constant cell turgor. P_s was independent of solute concentration for concentrations up to 1.4 M while both σ and Lp decreased with increasing concentration such that there was a linear relationship between $(1-\sigma)$ and $1/Lp$ as predicted by the frictional model for a lipid membrane with pores. The slope of the $(1-\sigma)$ *vs.* $1/Lp$ plot gives a value of P_s and the intercept with the $(1-\sigma)$ axis gives the degree of frictional interaction between solute and water. The frictional term was found to be significantly greater than zero. The values of P_s evaluated from the solute phase were smaller than those obtained from the $(1-\sigma)$ *vs.* $1/Lp$ plots. However, they were of the same order of magnitude and showed the same sequence for the different solutes. The technique for determining P_s and σ is of importance for obtaining quantitative data for the permeation of water-soluble pollutants into plant cells and tissues and for their ecotoxicological significance.

Key Words *Chara corallina* · hydraulic conductivity · pressure probe · reflection coefficient · solute permeability · water relations

Introduction

In a previous paper (Tyerman & Steudle, 1982) it was shown for cells of the isolated epidermis of *Tradescantia virginiana* that the pressure probe (*cf.* Zimmermann & Steudle, 1978, 1980) may be used for measuring transport properties of nonelectrolytes (reflection coefficient σ and permeability coefficient P_s) in addition to the water relation parameters (hydraulic conductivity Lp, volumetric elastic modulus ε). However, for the epidermal cells the proper evaluation of P_s was difficult because of the uncertainty in measuring the effective cell surface area and unstirred layer effects which were difficult to define. For these reasons we have applied the method in this paper to the cylindrical internodes of *Chara corallina* for which the cell geometry can be measured with high accuracy and unstirred layer effects can be better defined.

For *Chara*, values of σ and P_s have been reported for some permeable nonelectrolytes using transcellular osmosis and tracer experiments and the effects of unstirred layers have been examined (Dainty & Ginzburg, 1964*a-d*). Furthermore, Lp depends on cell turgor and concentration (Dainty & Ginzburg, 1964*a*; Steudle & Zimmermann, 1974; Zimmermann & Steudle, 1975) allowing Lp to be varied substantially.

* *Present address:* School of Biological Sciences, The Flinders University of South Australia, Bedford Park, South Australia, 5042.

The coefficients P_s and σ for permeable solutes can easily be obtained by measuring the characteristics of transient changes in pressure/time curves, the theory of which has been known for some time (Philip, 1958; Dainty, 1963; Wilson, 1967). Experiments have been performed in order to develop a rapid method for the determination of quantitative data for the permeation of water-soluble pollutants (ecotoxins) which occur in official lists. Although there are some complications due to unstirred layers, the method may be used to standardize the permeability of the substances in plant tissues and organs which are exposed to them (e.g., in roots and leaves). Experiments with many solutes under different conditions (concentration, turgor, etc.) can be performed yielding σ, P_s and Lp for one cell, thus avoiding complications due to a variation between cells and gaining a better understanding of the interrelations between the coefficients.

Material and Methods

Plant Material

Mature internodal cells of *Chara corallina* were used (length: 40 to 100 mm) which had been grown in artificial pond water (APW) (in mM: 1 NaCl, 0.1 KCl, 0.1 $CaCl_2$, 0.1 $MgCl_2$) in tanks containing a layer of natural pond mud. Temperature was kept at 18 °C and the tanks were continuously illuminated by Osram Fluora lamps.

Methods

A cell was freed from adjacent internodes and branches and placed in a glass tube (2.3 mm diameter) with a node protruding at one end and resting in a slit to make the cell secure. The pressure probe was inserted through the protruding node and solution was pumped through the other end of the glass tube along the cell. The external solution could be stirred vigorously and the APW could be rapidly exchanged by solutions with different osmotica and osmotic pressures. The cells were illuminated during the experiments by a Phillips halogen lamp through glass fiber optics. The temperature was maintained at 20 °C during experiments.

Determinations of ε, $T_{1/2}^w$ *and* Lp. These measurements were carried out as previously described (Steudle & Zimmermann, 1971, 1974; Zimmermann & Steudle, 1975, 1978, 1980). It was ensured that the pressure probe had a low compressibility ($dV_{apparatus}/dP = 4 \times 10^{-3}$ µl bar^{-1}), compared to the extensibility of the cell ($dV/dP = 5$ to 14×10^{-1} µl bar^{-1}) so that little or no correction of the measured ε was required. In addition, a combination of a pressure transducer of high sensitivity (9.2 mV bar^{-1}) and a dc offset allowed pressure relaxations and determinations of ε to be carried out over small pressure intervals (0.4 to 0.6 bar), at high absolute turgor pressure, without any loss of accuracy. The handling of the data was also improved by digitizing the experimental curves and storing the data on "floppy discs" for further analysis. The pressure relaxations were induced either by changing cell turgor pressure with the pressure probe ("hydrostatic experiments") or by varying the osmotic pressure of the medium ("osmotic experiments"). For rapidly permeating substances with low reflection coefficients (*see below*), solute flow could affect the kinetics of water flow and this could be corrected using Eq. (15) of the Appendix.

The effect of external concentration on $T_{1/2}^w$ and Lp was determined in several concentration series using either acetone, ethanol or dimethylformamide as the osmotic solute. The concentration was increased in steps of 200 to 500 mM to reach a final value of 1.2 to 2.0 M and was then lowered again to ensure that the effects were reversible. The solutes equilibrated within about 3 min (acetone) to 16 min (dimethylformamide). Cell turgor was usually greater than 5 bars and could be kept constant during these experiments. Concentration effects on Lp ($T_{1/2}^w$) could be determined from both hydrostatic and osmotic experiments. The high concentrations sometimes reduced the rate of cytoplasmic streaming. Thus the rate of streaming could have been a factor in the reduction of Lp (*see* Results). To investigate this possibility, cells were exposed to cytochalasin B (50 mg liter^{-1}) to stop cyclosis and Lp ($T_{1/2}^w$) was determined again in hydrostatic and osmotic experiments.

Determination of σ *and* P_s. The reflection coefficient (σ) could be determined from the change in turgor pressure in response to changes in the external concentration of several test solutes. The necessary corrections for the change in cell volume and solute flow (in the case of rapidly permeating solutes) were carried out as previously described (Tyerman & Steudle, 1982; *see* Appendix).

For the rapidly permeating substances, the permeability coefficient (P_s) could be determined from the biphasic kinetics of the change in turgor pressure after the external concentration of test solute was changed (*see* Fig. 1). The second phase, in which turgor pressure returns to the initial value, can be attributed to the equilibration of the solute across the cell membranes. A measurement of the rate constant for this phase and cell geometry allows P_s to be determined (Tyerman & Steudle, 1982; *see* Appendix).

In order to investigate the effect of external unstirred layers, Lp, σ and P_s were determined at different flow rates along the glass tube. The flow rate along the cell could be varied between 0 and 44 cm sec^{-1} (cell length 4 to 10 cm) and thus very effective stirring could be implemented.

Results

Lp Measurements

Figure 1 shows for one cell, pressure/time curves of hydrostatic and osmotic experiments using different solutes. For the osmotic experiments the time course of turgor pressure was biphasic for the permeable solutes (ethanol, methanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, acetone and dimethylformamide) and was completely reversible. When the test solution was added (or exchanged for APW),

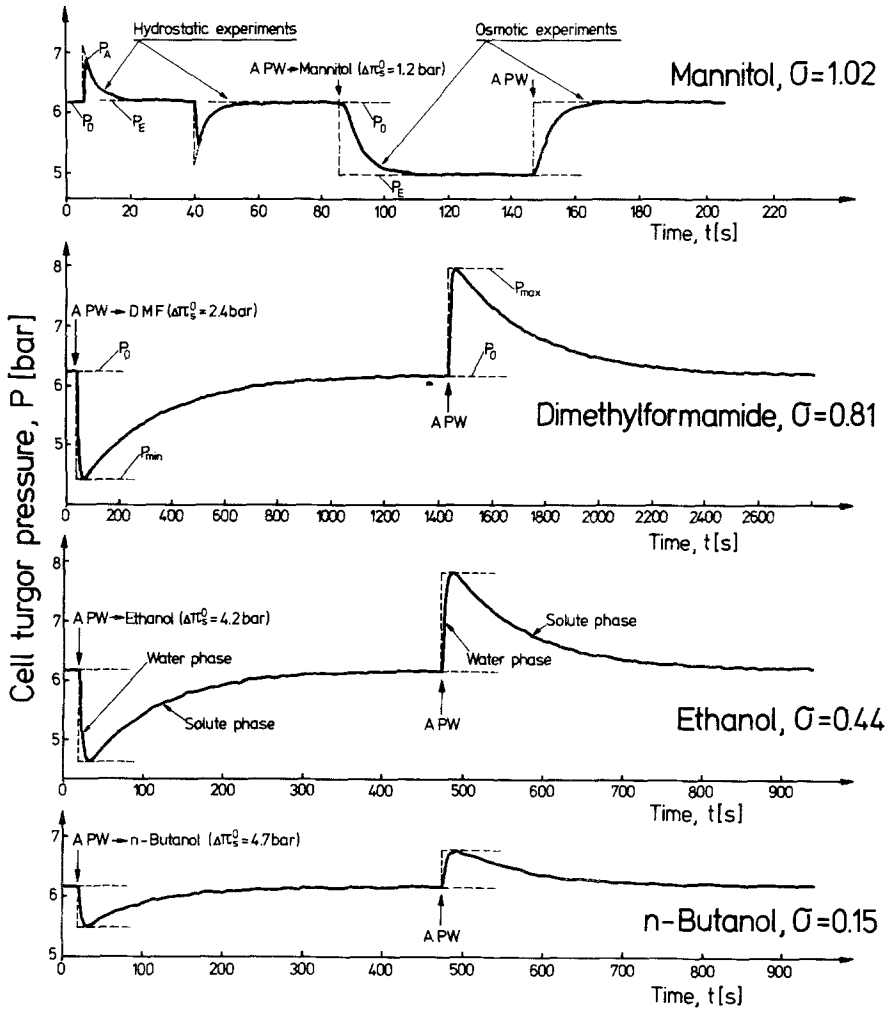

Fig. 1. Responses in cell turgor of an internodal cell of *Chara corallina* to different osmotic solutes. For an impermeable solute (mannitol) the responses after a change from APW to APW +mannitol and back to APW (top) are monophasic, whereas for dimethylformamide, ethanol and *n*-butanol biphasic responses were obtained and the original turgor was re-established. The half-times for the water phases ($T_{1/2}^w$) in the osmotic experiments were a few seconds and similar to those obtained in hydrostatic experiments (top of Figure). For the solute phases half-times of 59 sec (*n*-butanol), 65 sec (ethanol) and 210 sec (dimethylformamide) were measured from which values of P_s could be obtained. From the change in turgor pressure at the minimum (maximum) ($P_o - P_{min}$) and the change in external osmotic pressure ($\Delta\pi_s^0$), values of σ were calculated according to Eq. (13a)

turgor pressure changed rapidly to a minimum (or maximum) followed by a much slower change back to the original turgor pressure which is attributed to solute flow across the cell membrane. The half-times of the water flow equilibration ("water phase") were $T_{1/2}^w = 1.3$ to 7.5 sec and for the equilibration of solutes ("solute phase") $T_{1/2}^s = 31$ to 241 sec depending on the solute used. From the half-times of the water and solute phases the hydraulic conductivity (Lp) and the permeability coefficient (P_s) of the test solute could be calculated (Eqs. 11 and 12 in the Appendix). The hydrostatic experiments (Fig. 1) also yield Lp which can be compared with the values from osmotic experiments.

In osmotic experiments, Lp was dependent on the external flow rate (Fig. 2). For low flow rates Lp increased with increasing flow and tended to reach a saturation value at flow rates greater than about 25 cm sec^{-1}. This effect seems to reflect the fact that the time for the osmotic solute to diffuse through external unstirred layers to the membrane is significant as compared to $T_{1/2}^w$ (*see* Discussion). Using ethanol as the osmotic solute (Fig. 2) the saturation value of Lp was similar to the hydrostatic Lp in APW. In contrast, the saturation value for mannitol was always less than the hydrostatic Lp (e.g. Cell *B*, Fig. 2) and the osmotic Lp for ethanol. This may be due to differences in the diffusion coefficients ($D_{\text{mannitol}} = 0.56 \times 10^{-5}$ cm^2 sec^{-1} and $D_{\text{ethanol}} = 1.26 \times 10^{-5}$ cm^2 sec^{-1}) and the fact that an unstirred layer always remains within the cell wall. An effect of stirring on osmotic Lp has not been reported previously for Characean cells using the probe, since earlier experiments were performed only at one (high) stirring rate (*cf.* Steudle & Zimmermann, 1974; Zimmermann & Steudle, 1975). The range in values for hydrostatic Lp (0.51×10^{-5} to 1.98×10^{-5} cm sec^{-1} bar^{-1}, 18 cells) and osmotic Lp (0.21×10^{-5} to 1.82×10^{-5} cm sec^{-1} bar^{-1}, 9 cells, for concentrations between 50 and

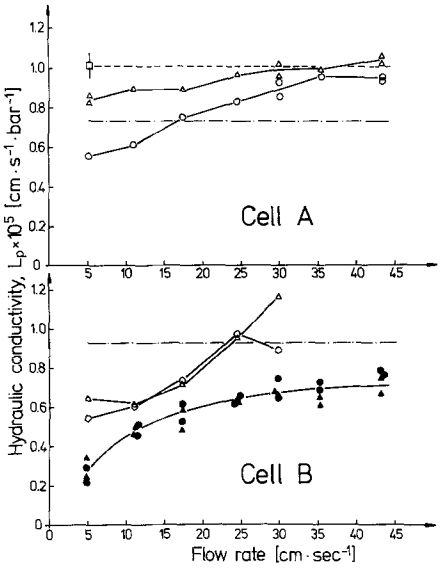

Fig. 2. Effect of stirring on the osmotic *Lp* for two cells (*A*,*B*) of *Chara corallina* and for two solutes (200 mM ethanol, open symbols; 50 mM mannitol, closed symbols; △, exosmotic *Lp*; ○, endosmotic *Lp*). Osmotic *Lp* increased with increasing stirring and tended to reach the hydrostatic *Lp* in APW at high rates. Hydrostatic *Lp* as a function of stirring was also investigated and the regression lines (- · - · -) (slopes not significantly different from zero) are indicated (cell *A* in 200 mM ethanol only, cell *B* in APW only). The hydrostatic *Lp* for cell *A* in APW (- - -) is indicated from determinations at the slowest rate of stirring only (□; ±SD)

200 mM) obtained in this paper are similar to those reported in the past.

External flow rate (stirring) had no influence on *Lp* obtained from hydrostatic experiments at high external concentrations and this indicates that the bulk flow (J_V) induced does not significantly change the concentration gradient across the membrane by sweeping away solutes (Dainty, 1963; *see* Discussion).

With rapidly permeating solutes the effect of concentration on *Lp* could be measured over a large concentration range (up to 1.4 M) at the same turgor pressure. Therefore, the concentration effect on *Lp* could be separated from the effect of turgor pressure also reported in the literature (Steudle & Zimmermann, 1974; Zimmermann & Steudle, 1975; Zimmermann & Hüsken, 1979). Figure 3*A* shows for two permeable substances [ethanol and dimethylformamide (DMF)] the effect of concentration on hydrostatic *Lp*. It was found that the relative reduction in *Lp* with increasing concentration was similar for the solutes (5 experiments on 5 cells), though the values in APW (i.e. at $C_s^o = 0$) were different. As the examples in Fig. 3 indicate, the effect of concentration on *Lp* was reversible, since for increasing and decreasing concentration stepwise the

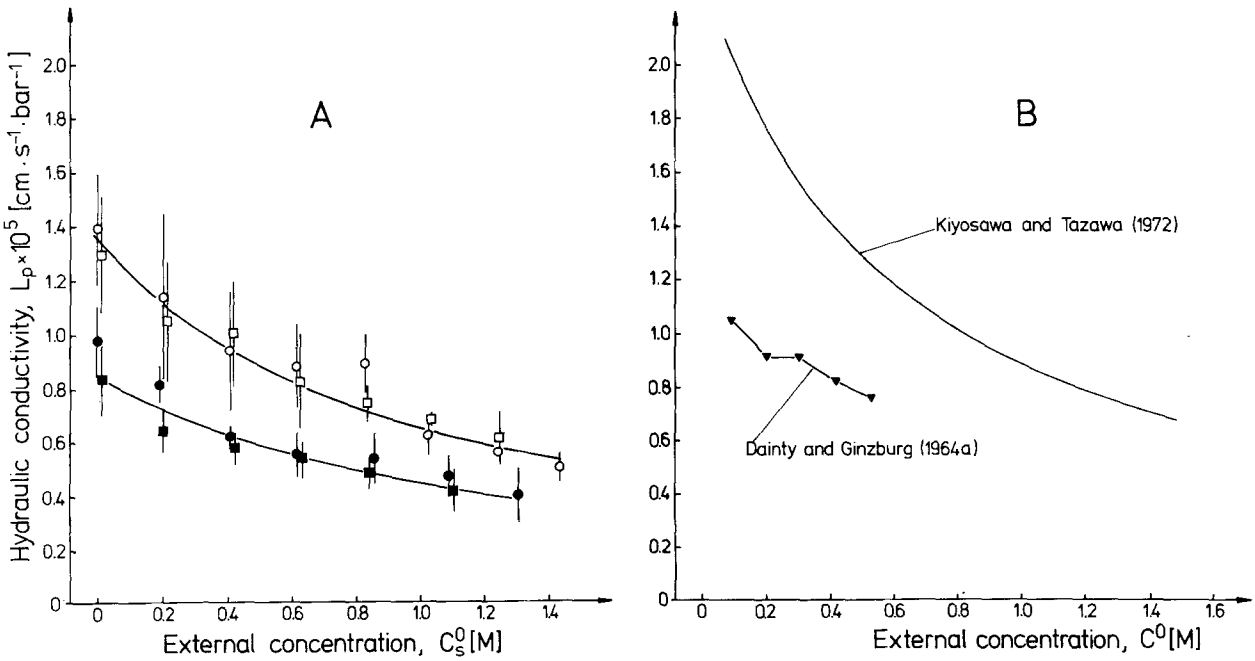

Fig. 3. Effect of concentration of two solutes (ethanol, open symbols; dimethylformamide, filled symbols) on hydrostatic *Lp* for two cells of *Chara corallina*. *Lp* decreases in the presence of the permeable solutes with increasing solute concentration. For lowering (circles) or increasing (squares) concentration stepwise, the same results were obtained. Vertical bars denote standard deviations ($n = 4$ to 10) at a certain concentration. For comparison, results of Dainty and Ginzburg (1964*a*) and Kiyosawa and Tazawa (1972) (Lp_{ex} only) are given in Fig. 3*B* which show the same trend. These results were obtained using transcellular osmosis and sucrose as the external solute

same Lp values were obtained. Furthermore, the high concentrations had no apparent long-term effects which were harmful to the cells as judged by the following two criteria: (1) The reduction in the rate of cyclosis with increasing concentration was reversible upon returning to APW. Since cyclosis is a good indicator of ATP concentration in *Chara* (Reid, 1980), this indicates that ATP production was either reversibly inhibited or not inhibited. (2) Turgor pressure remained at the original value indicating no major changes in permeability of the membrane to the internal solutes.

It should be noted that the reduction in the rate of cyclosis with increasing concentration was not responsible for the reduction in Lp, since stopping cyclosis with cytochalasin B at low C_s^o did not affect Lp (nor σ and P_s, *see below*). In comparison to the data shown in Fig. 3*A*, Fig. 3*B* shows Lp as a function of sucrose concentration as found by Kiyosawa and Tazawa (1972) (Lp for exosmosis only) and Dainty and Ginzburg (1964*a*) using the transcellular osmosis technique. The same trend in Lp as a function of concentration was observed. The results indicate that the effect of concentration on Lp is independent of the chemical nature of the solute.

The values of osmotic Lp for *impermeable solutes* (mannitol, sucrose, etc.) showed a polarity which was expected from previous findings, i.e. Lp for endosmosis (Lp_{en}) was greater than Lp for exosmosis (Lp_{ex}) (t-test, $P<0.05$) with the ratio of Lp_{en} to Lp_{ex} increasing with increasing $\Delta\pi_s^o$ (Dainty & Ginzburg, 1964*a*; Steudle & Zimmermann, 1974). This effect can easily be explained by the concentration effect mentioned above. However, in this investigation it was found that for the *permeable solutes* (at high flow rates) there was a polarity such that $Lp_{ex}>Lp_{en}$ (t-test, $P<0.05$). For example, when APW was exchanged for an ethanol solution, Lp was larger than when the cell was returned to APW after equilibration with the ethanol solution. This atypical polarity may also be explained in terms of a concentration effect provided that the effect of an internal solute on the water permeability of the plasmamembrane is larger than that of an external solute (Kiyosawa & Tazawa, 1972; *see* Discussion).

A polarity for hydrostatic Lp which has been reported for *Nitella flexilis* (i.e. $Lp_{en}>Lp_{ex}$; Steudle & Zimmermann, 1974) could not be found for *Chara corallina*. The ratio Lp_{en}/Lp_{ex} was 1.02 (mean) for 17 cells with upper and lower 95% confidence limits of 1.07 and 0.97, respectively. Finally, it should be mentioned that in our experiments which were carried out mostly at turgor pressures above 5 bar, the relaxation curves were well fitted by a single exponential down to pressure differences ($P-P_E$ in Eq. 11a) of about 0.02 bar ($r^2>0.98$, number of points, $n>50$).

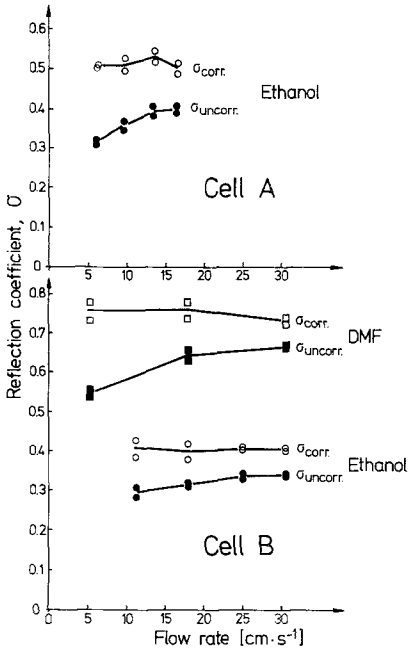

Fig. 4. Reflection coefficients of two solutes as a function of external stirring (flow rate in the chamber). Values of σ corrected for solute flow (σ_{corr}, *see* Eq. 13a) and uncorrected (σ_{uncorr}) are given for two cells (*A*, *B*) and ethanol and dimethylformamide. σ_{corr} is independent of stirring, whereas σ_{uncorr} increases with stirring

Reflection Coefficient Measurements

From Fig. 1 it can be seen that the osmotic responses to different solutes are quite different, i.e. the ratios $(P_o-P_{min})/\Delta\pi_s^o$ [or $(P_o-P_{max})/\Delta\Pi_s^o$] are different. It is shown in the Appendix (Eq. 13a) that these ratios can be used to evaluate the reflection coefficients of the solutes. In Eq. (13a) the term $\exp\left(-\frac{P_s\cdot A_o}{V_o}t_{min}\right)$ corrects for the solute flow which tends to decrease the $(P_o-P_{min})/\Delta\pi_s^o$ ratio.

The reflection coefficients uncorrected for solute flow (σ_{uncorr}) were found to depend on external stirring, whereas the corrected values (σ_{corr}) were practically independent of stirring (Fig. 4). This is plausible since the correction term ($\exp(-P_s A_o t_{min}/V_o)$) becomes larger with

Table 1. Reflection coefficients (σ) for different nonelectrolytes of internodes of *Chara corallina* at low solute concentrations[a]

Solute	Reflection coefficients of:					
	Chara corallina		*Chara corallina*[b]	*Nitella translucens*[b]	*Nitella flexilis*[c]	*Tradescantia virginiana*, isolated epidermis[d]
	σ	$1-\frac{P_s \cdot \bar{V}_s}{L_p \cdot RT}$				
Sucrose	0.95	–	–	–	0.97±0.01(5)	1.04±0.06(4)
Mannitol	1.02±0.04(8)	–	–	–	–	1.06±0.05(5)
Formamide	0.99	–	1	–	0.79±0.04(3)	0.99±0.06(4)
Dimethylformamide	0.76±0.06(3)	0.98	–	–	–	–
Methanol	0.38±0.02(4)	0.95	0.30	0.25 (0.50)	0.31±0.04(2)	0.15±0.29(4)
Ethanol	0.40±0.06(4)	0.95	0.27	0.29 (0.44)	0.34±0.02(2)	0.25±0.31(4)
n-Propanol	0.24±0.05(4)	0.93	0.22	0.16	0.17±0.06(2)	−0.58±0.28(3)
iso-Propanol	0.45±0.06(4)	0.95	–	0.27 (0.40)	0.35±0.05(2)	0.26±0.33(4)
n-Butanol	0.14±0.03(4)	0.91	–	–	–	–
Iso-Butanol (2-Methylpropanol-1)	0.21±0.01(4)	0.91	–	–	–	–
Acetone	0.17±0.03(3)	0.92	–	–	–	–

[a] For comparison, the values of $1-\bar{V}_s \cdot P_s/L_p \cdot RT$ (*see* Eq. 1) and literature data for Characean cells and *Tradescantia virginiana* are given. Values for *N. flexilis* were obtained from pressure probe measurements without correcting for solute flow. Values for *N. translucens* and *C. corallina* were obtained by transcellular osmosis. For *N. translucens* σ values corrected for unstirred layers are given in brackets. Mean values are given ±SD; in brackets the number of cells investigated.

[b] Data taken from Dainty and Ginzburg (1964*d*).

[c] Data taken from Steudle and Zimmermann (1974).

[d] Data taken from Tyerman and Steudle (1982).

Table 2. Permeability coefficients (P_s) for different nonelectrolytes of internodal cells of *Chara corallina*.[a]

Solute	Permeability coefficients, $P_s \times 10^4$ (cm s^{-1}) of:			
	Chara corallina	*Chara corallina*[b]	*Nitella translucens*[b]	*Nitella mucronata*[c]
Dimethylformamide	0.81±0.14 (3)	–	–	0.71
Methanol	3.29±1.33 (4)	4.0	4.8	5.7
Ethanol	2.36×0.28 (15)	2.8	4.3	5.5
n-Propanol	2.63±0.33 (4)	–	–	7.2
iso-Propanol	1.86±0.48 (4)	2.0	2.1	3.8
n-Butanol	2.51±0.69 (4)	–	–	–
iso-Butanol (2-Methylpropanol-1)	2.30±0.60 (4)	–	–	–
Acetone	3.36±0.14 (4)	–	–	–

[a] The data obtained by the pressure probe are similar to those obtained by Dainty and Ginzburg (1964*c*) using ^{14}C-labelled tracers and correcting for unstirred layers; with the exception of dimethylformamide they are significantly smaller than those given by Collander (1954) for *Nitella mucronata*.

[b] Data taken from Dainty and Ginzburg (1964*c*).

[c] Data taken from Collander (1954).

slow stirring due to the dependence of Lp on stirring (Eq. 14 and Fig. 2).

Values of σ for the different solutes investigated are given in Table 1, which also comprises data from the literature. σ ranged between 0.1 and 1.0, and for some of the alcohols there is good agreement between literature values for Characean cells and the values reported here.

Permeability Coefficients

The values of P_s for the different solutes examined in this paper are listed in Table 2 and compared with the data of Collander (1954) and of Dainty and Ginzburg (1964*c*). Dainty and Ginzburg determined the permeability coefficients of methanol, ethanol and isopropanol by measuring the initial uptake of ^{14}C-labeled alcohols and corrected for unstirred layers. Despite the probable underestimation of our values due to not correcting for unstirred layers, a comparison with the corrected data of Dainty and Ginzburg (1964*c*) shows that they are very similar. Taking this result by itself may indicate that the effect of unstirred layers in our experiments was small (but *see below*).

There was an effect of external flow rate on P_s (an increase of about 20% for flows from 5 to

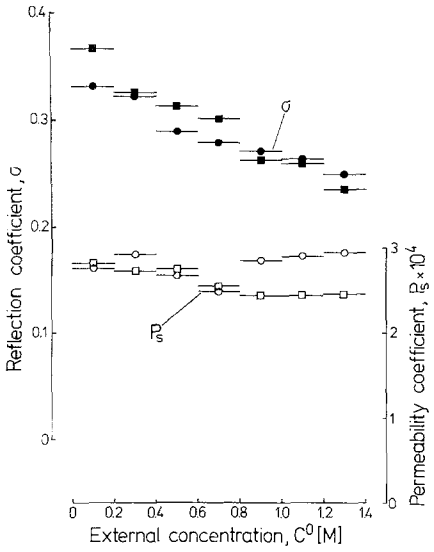

Fig. 5. Effect of external solute concentration (ethanol) on the reflection (σ) and permeability coefficient (P_s) of a *Chara corallina* internode. σ decreases with increasing external concentration, whereas P_s remains practically constant. As for Lp (*see* Fig. 3) no differences were found when the concentration was decreased (squares) or increased (circles) stepwise. Note that all experiments were performed at the same constant turgor

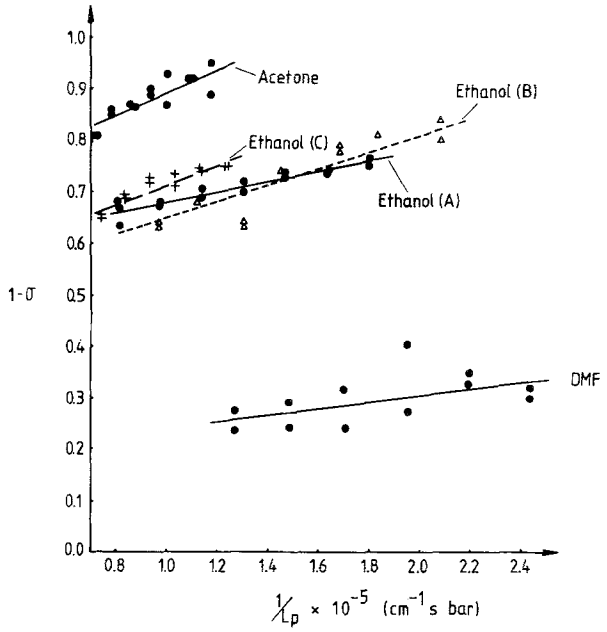

Fig. 6. Plot of $1-\sigma$ *vs.* $1/Lp$ according to Eq. (1) for three solutes (acetone, ethanol and dimethylformamide) and five cells. Lp was varied for each cell by changing the external concentration of the solute (*see* Fig. 3). As postulated by Eq. (1) the plots yield straight lines to a rather good approximation. From the slopes of the lines permeability coefficients can be estimated (P_s') (Eq. 1) by a procedure which is independent of the determination of P_s from the rate constant of the solute phase. The results of the regressions are listed in Table 3

44 cm sec^{-1}) indicating that external unstirred layers may result in a small underestimation of P_s. An underestimation would be expected since for an unstirred layer of 30 μm in thickness (3 × cell wall thickness) and diffusion coefficients for the solutes of around 10^{-5} cm^2 sec^{-1}, the membrane P_s would be 10 to 20% larger than the measured value.

The effect of concentration on both P_s and σ was also investigated using ethanol, acetone or DMF as the solute and in the same concentration experiments described above for Lp (*see* Fig. 3*A*). There was no effect of concentration on P_s (ethanol, acetone and DMF) but σ decreased significantly with increasing concentration (Fig. 5 for ethanol). The change in σ with concentration was completely reversible for each solute and, as for Lp, it may be concluded that the concentration effect is independent of the chemical nature of the solute. That P_s did not change also indicates that the rather high solute concentrations used did not affect the membrane integrity.

The reduction in σ with increasing concentration would be expected when Lp decreases since the contribution of solute flow to the volume flow across the membrane should increase. This can be seen from the simple frictional model for a lipid-pore membrane (Kedem & Katchalsky, 1961; Dainty & Ginzburg, 1963), where σ is given by:

$$\sigma = 1 - \frac{\bar{V}_s \cdot P_s}{Lp \cdot RT} - \frac{K_s^c \cdot f_{sw}^c}{f_{sw}^c + f_{sm}^c}. \quad (1)$$

$\bar{V}_s$ is the partial molar volume of the solute, K_s^c is the partition coefficient between the membrane pores (phase c) and the membrane; f_{sw}^c represents the frictional interaction between solute and water in the pores and f_{sm}^c the interaction between solutes and the wall of the pores. Since P_s is independent of concentration and provided the frictional (last) term in Eq. (1) is constant, a plot of $1-\sigma$ *vs.* $1/Lp$ should yield a straight line. The slope of the line would give an independent estimate of the membrane permeability coefficient (denoted by P_s' in contrast to the permeability P_s measured from the solute phase), while the intercept with the $1-\sigma$ axis would give an estimate of the frictional term.

Figure 6 shows plots of $1-\sigma$ *vs.* $1/Lp$ for acetone, ethanol and DMF. Although there is

Table 3. Values of the permeability coefficient (P_s') obtained from the slope of $1-\sigma$ *versus* $1/L_p$ (*see* Fig. 6).[a]

Solute	$P_s' \times 10^4$ (cm sec^{-1})	Intercept	Correlation coefficient
Acetone	7.43±2.68	0.67±0.08	0.87
Ethanol (A)	4.34±0.91	0.57±0.03	0.95
Ethanol (B)	6.68±2.49	0.49±0.09	0.88
Ethanol (C)	7.71±2.25	0.53±0.05	0.92
Dimethyl-formamide	2.09±2.18	0.17±0.13	0.56

[a] The intercept with the $1-\sigma$ axis is also given and according to Eq. (1) this should correspond to the frictional term. Values of P_s' and the intercept were calculated from a linear regression for which 95% confidence limits and correlation coefficients are given.

some scatter in the data, there is no evidence to suggest that the relationships are not linear. This indicates that the assumption of the frictional term being constant is probably correct. Table 3 gives the results of the analysis of the plots in Fig. 6. The intercepts with the $1-\sigma$ axis range from 0.17 for DMF to 0.67 for acetone and in each case the values are significantly different from zero. For ethanol and acetone reasonable correlation coefficients were obtained (*see* Table 3) and the 95% confidence limits for P_s' obtained from the slopes are fair at ±20 to 40%. For DMF the slope was not significantly different from zero. This could be expected since DMF has a low P_s compared to the other solutes but σ and Lp would have errors similar in magnitude to those for acetone and ethanol. It should be noted that P_s and P_s' show the same sequence for the different solutes, i.e. $P_s(\text{DMF}) < P_s(\text{ethanol}) < P_s(\text{acetone})$ which indicates that the application of Eq. (1) is basically correct. However, it was found in each case that P_s' was by a factor of about 2 larger than P_s. This may reflect the fact that the P_s measured from the solute phase is almost certainly underestimated as a result of unstirred layers.

Discussion

The absolute values of Lp were similar to those determined by Dainty and Hope (1959) and Dainty and Ginzburg (1964*a*) for *Chara corallina* but somewhat larger than those given by Dainty, Vinters and Tyree (1974). They are identical with those reported by Zimmermann and Hüsken (1979) and Wendler and Zimmermann (1982) for the high pressure range ($Lp = 1.2$ to 1.8×10^{-5} cm sec^{-1} bar^{-1} for $P > 1$ to 2 bar). The data are similar to those found for other Characean internodes in osmotic and hydrostatic experiments (*see* reviews: Dainty, 1976; Zimmermann & Steudle, 1978, 1980). However, it has been found for low stirring rates, that in osmotic experiments Lp becomes dependent on stirring and this is most likely due to an effect of solute diffusion to the membrane surface (after a change in the osmotic pressure of the medium) rather than a change in the osmotic driving force across the membrane due to solute flow into the cell. If we assume that (for lower stirring rates) an unstirred layer of 50 to 100 µm may be formed, a few seconds would be required for a solute molecule to diffuse from the bulk solution to the membrane surface. This is of the same order as the half-time for water-flow equilibration. On the other hand, the effect of solute flow across the membrane on the kinetics of water flow during the water phase is relatively small as long as $T_{1/2}^w \ll T_{1/2}^s$. The data given for osmotic Lp in this paper are corrected for solute flow using Eq. (15) of the Appendix.

For hydrostatic Lp, only the sweep away effect could have changed the osmotic driving force at the membrane surface, i.e., a water flow (J_V) induced by a change in cell turgor could have altered the concentration at the membrane surface (C_s^{membrane}) as compared with the bulk solution (C_s^{bulk}) resulting in a reduction of the driving force for water flow. For the steady state, C_s^{membrane} would be given by (Dainty, 1963):

$$C_s^{\text{membrane}} = C_s^{\text{bulk}} \exp\left(-\frac{J_V \delta}{D_s}\right) \tag{2}$$

where δ is the thickness of the unstirred layer and D_s the diffusion coefficient of the solute. However, in our case δ would be very small since for a *Chara* cell ($\varepsilon + \pi = 500$ bar, $V = 40$ µl, $V/A = 2 \times 10^{-2}$ cm) subjected to a change in turgor pressure of 0.5 bar, it can be calculated that the water extruded from the cell forms an unstirred layer of not more than 0.2 µm in thickness. This value is an overestimate since the calculation assumes that the water is extruded instantaneously. Using this value of δ it can be calculated (Eq. 2) that the sweep away effect would result in less than a 0.7% reduction of driving force when $J_V = 5 \times 10^{-6}$ cm sec^{-1}, $C_s^{\text{bulk}} = 1.4$ M, $\sigma = 0.5$, $\Delta P = 0.5$ bar and D_s in the cell wall (cytoplasm) $= 10^{-6}$ cm^2 sec^{-1}. Clearly this could not account for the much larger reduction in Lp with increasing concentration which

is observed (Fig. 3). It is also not surprising that an effect of stirring on Lp (hydrostatic) was not found.

The effect of external concentration on Lp is well known for Characean cells (*see* reviews: Dainty, 1976; Zimmermann & Steudle, 1978, 1980). However, with the rapidly permeating solutes the concentration dependence could be measured over a large concentration range (0 to 1.4 M for ethanol) *at constant turgor pressure.* The results show (at least for the solutes used here) that the effect is independent of the solute used. This was also found for red blood cells (Sha'afi et al., 1970).

The polarity of water movement which has been observed in osmotic experiments using an impermeable solute was due to the concentration effect and has been found previously (Kamiya & Tazawa, 1956; Dainty & Ginzburg, 1964*a*; Steudle & Zimmermann, 1974). However, in contrast to other findings on Characean spp. (Steudle & Zimmermann, 1974), no polarity has been observed in this study with hydrostatic experiments either in APW or in the presence of osmotic solutes. As already mentioned this may be due to very small, or no polarity for *Chara corallina* (i.e. $Lp_{en}/Lp_{ex} < 1.05$). Another possibility is that the extent of polarity depends on the water flow (driving force) produced during the experiments. If this is true, the much smaller pressure steps used to produce a water flow (J_V) in this paper could be the reason for the missing polarity.

Tazawa and Kiyosawa (1973) explain the polarity of water movement in *Chara* in terms of a concentration effect which is bigger when the solute is on the inside rather than on the outside of the plasma membrane. They show evidence for this from measurements in which they varied C^i and C^o by transcellular osmosis. Our results with permeable solutes point in the same direction because it was found that $Lp_{ex} > Lp_{en}$ (i.e. the reverse to the case with impermeable solutes). For exosmotic water flow the cell membrane only "sees" the high concentration at the outer surface and for endosmotic water flow, the same concentration only at the inner surface. If C_s^i reduces Lp more than C_s^o does, a polarity in which $Lp_{ex} > Lp_{en}$ would result. In contrast, in an experiment with an impermeable solute, the solute is always at the outer surface leading to the normally observed polarity mentioned above.

The values of σ reported in this paper are similar to those given previously for Characean cells for some of the solutes (Table 1). The values of σ_{corr} appear to be corrected for unstirred layers (Fig. 4). This occurs because the correction term for solute flow ($\exp(P_s A_o t_{min}/V_o)$) essentially reduces $\Delta\pi_s^o$ in Eq. (13a) (Appendix) to the true value across the membrane and incorporates the effect of unstirred layers on t_{min} and P_s. The true concentration difference across the membrane at t_{min} is obtained from extrapolating the solute phase of the pressure/time curves to t_{min}. The concentration difference between the membrane faces is obtained because in the solute phase (for $Lp(\varepsilon+\pi) \gg P_s$) the measured turgor pressure is developed from differences in osmotic pressure at the two membrane surfaces. The values of σ for *Nitella flexilis* previously measured using the probe (Steudle & Zimmermann, 1974) were not corrected for solute flow and are thus underestimated.

We have calculated $1-\bar{V}_s P_s/RTLp$ (Table 1) to give an indication of the magnitude of the frictional term in Eq. (1). For all the permeable solutes $\sigma \ll 1-\bar{V}_s P_s/RTLp$ and even if P_s were underestimated substantially, say by a factor of 2 or 3 due to unstirred layers, the same inequality will hold. Also from the plots of $1-\sigma$ *vs.* $1/Lp$ the intercepts with the $1-\sigma$ axis were significantly different from zero indicating that the frictional term is significant. That the intercepts with the $1-\sigma$ axis are different for the different solutes could result from the partition coefficients and friction terms being different for the different solutes (*see* Eq. 1). The presence of the frictional term has been used in the past as evidence for the presence of pores in the membrane in which water and solute may interact (thus K_s^c and/or f_{sw}^c in Eq. (1) are large) (Dainty & Ginzburg, 1964*d*; Rich et al., 1967).

The values of P_s found in this paper are underestimates due to the effects of internal and external unstirred layers. It is thus surprising that there is a rather good agreement with Dainty and Ginzburg's (1964*c*) values for the same species which have been corrected for unstirred layers. These authors measured P_s from the initial rate of uptake of ^{14}C-labeled solutes, i.e. for a time interval between 0 and 10 sec. Our values, however, refer to a time interval between 30 and 240 sec after the addition of solute when the influence of unstirred layers could be more pronounced. Therefore, it is astonishing that similar values of P_s are obtained. One possible reason for the agreement is that P_s measured when $J_V \neq 0$ (our experiments) is larger than P_s measured from tracer diffusion (i.e. J_V

$=0$). However, it should be mentioned that similar P_s values are obtained when $J_V=0$ in our experiments by applying the minimum method of Sha'afi et al. (1970) (Tyerman & Steudle, *unpublished results*).

That the underestimation of P_s may be substantial, is suggested from the permeability coefficients evaluated from plots of $1-\sigma$ *vs.* $1/Lp$ (P_s') (Fig. 6 and Table 3), which are by a factor of about 2 larger than the values directly measured. P_s' may be free of unstirred layer effects (since both σ and hydrostatic Lp are) and may be closer to the true membrane permeability. However, it should be emphasized that all the equations used in this paper (including Eq. 1) are for a single membrane only and not for two membranes in series. It is difficult to predict how the presence of the tonoplast influences values of σ used to calculate P_s' according to Eq. (1).

At first sight, the finding that P_s is independent of solute concentration whereas Lp depends on external concentration seems to contradict a high frictional interaction between solute and water flow. However, in terms of the dehydration hypothesis (*see above;* Kamiya & Tazawa, 1956; Dainty & Ginzburg, 1964*a*) it could be possible that a change in water potential affects water rather than solute transport. It could be envisaged that a shrinking of the cell membrane and small changes in pore diameters may cause big changes in the hydraulic conductivity at a level where solute transport through the pores is not affected. On the other hand, if only a small proportion of the solutes entering the cell passes through pores (proportion depending on K_s^c in Eq. 1) then a change in P_s due to changes in pore properties may not be observed when the overall P_s of the membrane (including pores) is measured. In fact, an effect of external concentration on P_s has been observed for urea by Dainty and Ginzburg (1964*b*). These authors eluted *Chara* cells preloaded with ^{14}C-urea in a transcellular osmosis chamber and determined changes of internal and external ^{14}C-urea with time. However, they used high concentrations of an impermeable solute (sucrose) which, although not plasmolyzing the cell (due to the transcellular osmosis set up), may have resulted in large changes in turgor pressure. Thus turgor pressure rather than external concentration may have affected the permeability to urea. This possibility was excluded in this paper by using permeable solutes to increase the concentration.

The results of this paper show that the pressure probe can provide P_s values of plant cells from osmotic experiments. Although unstirred layers are a problem, the values of P_s which can be measured are those which are relevant for the osmotic relations of plant cells, and, therefore, they are of great practical importance. For example, the transport of water-soluble toxic substances could be characterized quantitatively for higher plant tissues using the probe, thereby gaining more information about the uptake and transport of these substances in tissues. Furthermore, plants need to have efficient adaptive mechanisms at the solute transport level to survive under conditions of water stress (e.g. osmotic adjustment). As shown, the pressure probe may be used to work out whether changes in passive solute transport are involved in the adaptation of plants to osmotic stress.

We would like to thank Prof. U. Zimmermann and Dr. S. Wendler, KFA Jülich, for reading and discussing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft, Zi 99-8, and the BMFT (No. 037266).

Appendix

Time Course of Turgor Pressure of a Cell in the Presence of a Permeable Solute

In order to describe the pressure-time course of a cell it is assumed that all other solutes except for the permeable test solute are impermeable (internal concentration prior to the experiment $=C^i_{o\,\mathrm{imp}}$; external concentration $=C^o_{\mathrm{imp}}$) and that the solute is added at $t=0$ to the external medium at a concentration of C_s^o. The volume of the external medium is large compared with that of the cell so that C_s^o is constant during the experiment. The equations given below briefly summarize results given previously (Philip, 1958; Dainty, 1963; Wilson, 1967).

The two membranes (tonoplast and plasmalemma) are considered as one barrier. Treating the cell and the medium as two well-stirred compartments the water (J_V) and solute (J_s) flows are given by:

$$J_V=-\frac{1}{A_o}\frac{dV}{dt}=Lp[P-\sigma RT(C_s^i-C_s^o)-RT(C^i_{\mathrm{imp}}-C^o_{\mathrm{imp}})] \quad (3)$$

and

$$J_s=-\frac{1}{A_o}\frac{dn_s^i}{dt}=P_s(C_s^i-C_s^o)+(1-\sigma)\bar{C}_s J_V \quad (4)$$

where A_o=cell surface area, $V(t)$=cell volume and V_o =cell volume at $t=0$. $C_s^i(t)$=internal concentration of solute; $\bar{C}_s$=average concentration of solute across the cell membrane; $n_s^i(t)$=amount of solute in the cell. Turgor pressure and cell volume are related to each other by the volumetric elastic modules (ε) of the cell:

$$\frac{P-P_o}{\varepsilon}=\frac{V-V_o}{V_o}. \quad (5)$$

Using this relation and the abbreviations:

$$v=V/V_o; \quad \tau=\frac{(RTC^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_o}t$$

$$\alpha=\frac{\sigma RTC^o_s}{RTC^i_{o\,\mathrm{imp}}+\varepsilon}; \quad s=\frac{n^i_s}{V_oC^o_s}, \tag{6}$$

Eq. (3) yields:

$$v\frac{dv}{d\tau}=1-v+\alpha(s-v). \tag{7}$$

Rewriting also Eq. (4) in terms of nondimensional variables we get:

$$v\frac{dv}{d\tau}=b(v-s)+(1-\sigma)\frac{\bar{C}_s}{C^o_s}v\frac{dv}{d\tau} \tag{8}$$

where

$b=P_s/[(RTC^i_{o\,\mathrm{imp}}+\varepsilon)Lp]$.

Equations (7) and (8) are identical with those given by Wilson (1967), except that they introduce turgor pressure and the volumetric elastic modulus (ε) for the application to plant cells. It has been shown by the same author that Eqs. (7) and (8) are solved by:

$$v=1-\frac{\alpha}{1-b}(e^{-b\tau}-e^{-\tau}). \tag{9}$$

For the solution of the differential equations it has been assumed that α is small and that the solvent drag effect (i.e. the second term on the right side of Eq. 4) can be neglected. However, the time course of swelling or shrinking of the cell should be given by Eq. (9) within the order of $(1-v_{\min})^2$ (Wilson, 1967; $v_{\min}$ = relative change in volume at the minimum). Since in our experiments with *Chara*, $\varepsilon=300$ to 500 bar and $P_o-P_{\min}=1$ to 2 bar at the most, $v_{\min}$ would be 0.2 to 0.7% and $(1-v_{\min})^2=0.996$ to 0.987. Thus Eq. (9) should be a rather good estimate of the time course of pressure or volume. It should be noted that Eq. (9) has already been derived and used before by Philip (1958) and Dainty (1963). Writing the equation in terms of observable variables, we obtain:

$$\frac{V-V_o}{V_o}=\frac{P-P_o}{\varepsilon}=\frac{\sigma\Delta\pi^o_sLp}{(RTC^i_{o\,\mathrm{imp}}+\varepsilon)Lp-P_s}\cdot\left[\exp\left(-\frac{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_o}t\right)-\exp\left(-\frac{P_sA_o}{V_o}t\right)\right]. \tag{10}$$

$\Delta\pi^o_s$ = change in the external osmotic pressure; $\pi^i_{o\,\mathrm{imp}}=RTC^i_{o\,\mathrm{imp}}$. It can be seen that the time history of turgor pressure or volume can be described as the sum of two exponentials provided that the influence of solvent drag is small.

For $Lp(\varepsilon+RTC^i_{o\,\mathrm{imp}})\gg P_s$ (i.e. for $T^w_{1/2}\ll T^s_{1/2}$ as for *Chara*) the second term in the brackets on the right side can be neglected for sufficiently short time intervals. In this case we get:

$$\frac{V-V_o}{V_o}=\frac{P-P_o}{\varepsilon}=\frac{\sigma\Delta\pi^o_s}{\pi^i_{o\,\mathrm{imp}}+\varepsilon}\exp\left(-\frac{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_o}t\right). \tag{11}$$

It can be shown that Eq. (11) is identical with the equation used for osmotic pressure relaxation experiments in the presence of impermeable osmotic solute, i.e.:

$$P-P_E=(P_o-P_E)\exp\left(-\frac{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_o}t\right) \tag{11a}$$

where P_E = final pressure reached in an osmotic experiment (*see* Fig. 1). Furthermore, after sufficiently long time intervals, the first term in the brackets should have vanished (for rapid water flows) and then Eq. (11) reduces to ($Lp(\pi^i_{o\,\mathrm{imp}}+\varepsilon)\gg P_s$):

$$\frac{V-V_o}{V_o}=\frac{P-P_o}{\varepsilon}=-\frac{\sigma\Delta\pi^o_s}{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)}\exp\left(-\frac{P_sA_0}{V_o}t\right). \tag{12}$$

Equation (12) is identical with that of solute equilibration between cell and surroundings in a two-compartment system.

At the minimum (maximum) turgor pressure, $dP/dt=0$ and we get from Eq. (10):

$$\exp\left\{\frac{A_o}{V_o}[Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})-P_s]t_{\min}\right\}=\frac{Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})}{P_s} \tag{13}$$

or

$$\frac{P_o-P_{\min}}{\Delta\pi^o_s}=\frac{\varepsilon}{\varepsilon+\pi^i_{o\,\mathrm{imp}}}\sigma\exp\left(-\frac{P_sA_o}{V_o}t_{\min}\right). \tag{13a}$$

Equation (13a) has been used in this paper to evaluate σ from $P_o-P_{\min}/\Delta\pi^o_s$ ratios. The term $\varepsilon/(\varepsilon+\pi^i_{o\,\mathrm{imp}})$ in the equation corrects for the shrinking (or swelling) of the cell and the exponential term for solute flow. The time to reach the minimum (maximum) will be given by (from Eq. 13):

$$t_{\min}=\frac{V_o}{A_o[Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})-P_s]}\ln\frac{Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})}{P_s}. \tag{14}$$

$t_{\min}$ is independent of σ and only determined by the ratio of $Lp(\varepsilon+RTC^i_{o\,\mathrm{imp}})/P_s$ (i.e. $T^w_{1/2}/T^s_{1/2}$). As long as $Lp(\varepsilon+RTC^i_{o\,\mathrm{imp}})\gg P_s$, Lp in osmotic experiments may be calculated from the "water phase" (*see* Fig. 1) according to Eq. (11).

The influence of solute flow on water flow can be corrected for provided that P_s is determined from the solute phase. From Eqs. (10) and (13a) it follows ($Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})\gg P_s$):

$$P-P_{\min}=(P_o-P_{\min})\left[\exp\left(-\frac{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_o}t+\frac{P_sA_o}{V_o}t_{\min}\right)-\exp\left(-\frac{P_sA_o}{V_o}(t-t_{\min})\right)+1\right]. \tag{15}$$

It can be easily verified from Eq. (15) that for impermeable solutes ($P_s=0$), Eq. (15) is identical with Eq. (11a). To correct for solute flow during the water phase ($t<t_{\min}$), $\ln[P-P_o+(P_o-P_{\min})\exp\{P_sA_o(t_{\min}-t)/V_o\}]$ has to be plotted against time to get the rate constant for water flow from the slope of the straight line. For *Chara* the correction for solute flow was up to about 20%. If the condition $Lp(\varepsilon+\pi^i_{o\,\mathrm{imp}})\gg P_s$ does not hold, $(P-P_{\min})/(P_o-P_{\min})$ is given by:

$$\frac{P-P_{\min}}{P_o-P_{\min}}=\frac{Lp(\pi^i_{o\,\mathrm{imp}}+\varepsilon)}{Lp(\pi^i_{o\,\mathrm{imp}}+\varepsilon)-P_s}\cdot\left[\exp\left\{-\frac{(\pi^i_{o\,\mathrm{imp}}+\varepsilon)LpA_o}{V_0}t+\frac{P_sA_0}{V_0}t_{\min}\right\}-\exp\left\{-\frac{P_sA_o}{V_0}(t-t_{\min})\right\}\right]+1. \tag{16}$$

However, under these conditions it would be difficult to determine P_s from the solute phase.

References

Collander, R. 1954. The permeability of *Nitella* cells to non-electrolytes. *Physiol. Plant.* **7**:420–445

Dainty, J. 1963. Water relations of plant cells. *Adv. Bot. Res.* **1**:279–326

Dainty, J. 1976. Water relations of plant cells. *In:* Encyclopedia of Plant Physiology. New Series. U. Lüttge and M.G. Pitman, editors. Vol. 2, Part A, pp. 12–35. Springer-Verlag, Berlin

Dainty, J., Ginzburg, B.Z. 1963. Irreversible thermodynamics and frictional models of membrane processes, with particular reference to the cell membrane. *J. Theor. Biol.* **5**:256–265

Dainty, J., Ginzburg, B.Z. 1964*a*. The measurement of hydraulic conductivity (osmotic permeability to water) of internodal Characean cells by means of transcellular osmosis. *Biochim. Biophys. Acta* **79**:102–111

Dainty, J., Ginzburg, B.Z. 1964*b*. The permeability of the cell membranes of *Nitella translucens* to urea and the effect of high concentrations of sucrose on this permeability. *Biochim. Biophys. Acta* **79**:112–121

Dainty, J., Ginzburg, B.Z. 1964*c*. The permeability of the protoplasts of *Chara australis* and *Nitella translucens* to methanol, ethanol and isopropanol. *Biochim. Biophys. Acta* **79**:122–128

Dainty, J., Ginzburg, B.Z. 1964*d*. The reflection coefficient of plant cell membranes to certain solutes. *Biochim. Biophys. Acta* **79**:129–137

Dainty, J., Hope, A.B. 1959. The water permeability of cells of *Chara australis* R. Br. *Aust. J. Biol. Sci.* **12**:136–145

Dainty, J., Vinters, H., Tyree, M.T. 1974. A study of transcellular osmosis and the kinetics of swelling and shrinking in cells of *Chara corallina*. *In:* Membrane Transport in Plants. U. Zimmermann and J. Dainty, editors. pp. 59–63. Springer-Verlag, Berlin

Kamiya, N., Tazawa, M. 1956. Studies on water permeability of a single plant cell by means of transcellular osmosis. *Protoplasma* **46**:394–422

Kedem, O., Katchalsky, A. 1961. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* **45**:143–179

Kiyosawa, K., Tazawa, M. 1972. Influence of intracellular and extracellular tonicities on water permeability of Characean cells. *Protoplasma* **74**:257–270

Philip, J.R. 1958. The osmotic cell, solute diffusibility, and the plant water economy. *Plant Physiol.* **33**:264–271

Reid, R.J. 1980. A study of adenylate concentrations and chloride active transport in *Chara corallina*. Ph. D. Thesis. University of Sydney, Australia

Rich, G.T., Sha'afi, R.I., Barton, T.C., Solomon, A.K. 1967. Permeability studies on red cell membranes of dog, cat and beef. *J. Gen. Physiol.* **50**:2391–2405

Sha'afi, R.I., Rich, G.T., Mikulecky, D.C., Solomon, A.K. 1970. Determination of urea permeability in red cells by minimum method. A test of the phenomenological equations. *J. Gen. Physiol.* **55**:427–450

Steudle, E., Zimmermann, U. 1971. Hydraulische Leitfähigkeit von *Valonia utricularis*. *Z. Naturforsch.* **266**:1302–1311

Steudle, E., Zimmermann, U. 1974. Determination of the hydraulic conductivity and of reflection coefficients in *Nitella flexilis* by means of direct cell-turgor pressure measurements. *Biochim. Biophys. Acta* **332**:399–412

Tazawa, M., Kiyosawa, K. 1973. Analysis of transcellular water movement in *Nitella*. A new procedure to determine the inward and outward water permeabilities of membranes. *Protoplasma* **78**:349–364

Tyerman, S.D., Steudle, E. 1982. Comparison between osmotic and hydrostatic water flows in a higher plant cell: Determination of hydraulic conductivities and reflection coefficients in isolated epidermis of *Tradescantia virginiana*. *Aust. J. Plant Physiol.* **9**:461–480

Wendler, S., Zimmermann, U. 1982. A new method for the determination of hydraulic conductivity and cell volume of plant cells by pressure clamp. *Plant Physiol.* **69**:998–1003

Wilson, T.A. 1967. Osmotic volume changes induced by a permeable solute. *J. Theor. Biol.* **17**:304–311

Zimmermann, U., Hüsken, D. 1979. Theoretical and experimental exclusion of errors in the determination of the elasticity and water transport parameters of plant cells by the pressure probe technique. *Plant Physiol.* **64**:18–24

Zimmermann, U., Steudle, E. 1975. The hydraulic conductivity and volumetric elastic modulus of cells and isolated cell walls of *Nitella* and *Chara spp.*: Pressure and volume effects. *Aust. J. Plant Physiol.* **2**:1–12

Zimmermann, U., Steudle, E. 1978. Physical aspects of water relations of plant cells. *Adv. Bot. Res.* **6**:45–117

Zimmermann, U., Steudle, E. 1980. Fundamental water relations parameters. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas and J. Dainty, editors. pp. 113–127. Elsevier/North Holland Biomedical

Received 16 November 1982; revised 2 February 1983