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The Pressure-Dependence of the Hydraulic Conductivity, the Membrane Resistance and Membrane Potential During Turgor Pressure Regulation in *Valonia utricularis*

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Summary. The pressure-dependence of the hydraulic conductivity and of electrical membrane parameters in *Valonia utricularis* are determined over a turgor pressure range of 4 to 4.5 atm by means of a direct measurement of the hydrostatic pressure inside the cell. The dependence of the hydraulic conductivity on pressure is calculated from the volume flows and subsequent changes in the turgor induced by both osmotic and hydrostatic gradients. L_p is independent of pressure above 1 atm and equal under osmotic and hydrostatic conditions. Polarity of water movement is not observed. At pressures below 1 atm, L_p increases up to 10 times on approaching the point of plasmolysis. This increase is discussed in terms of membrane folding and of the stretching of pores within the membrane. In contrast to this finding the membrane resistance (normally 300 to 500 Ω cm^2) increases markedly in response to higher pressures and reaches a maximum value of 1500 Ω cm^2 at about 2 atm. Further increase of the hydrostatic pressure reduces the membrane resistance again until the original value is reached at 3 to 4 atm. In the range of the maximum resistance the membrane potential (0 to +15 mV at 1 atm) drops by 10 to 40 mV. At low pressures the membrane potential drops in the dark, while at high pressures and reduced membrane potential an increase of the p.d. is observed. From these results it is suggested that the electrogenic potassium pump postulated by Gutknecht is pressure-dependent and causes the observed changes in membrane resistance. According to this hypothesis, with increasing pressure the potassium influx would be reduced and the potassium efflux accelerated. This means that the electrogenic pump reverses its direction at high pressures.

A characteristic feature of many algae is their capacity to regulate the internal water and ionic content and the cell turgor pressure in response to changes in the osmolarity and ionic composition of the environment. Three mechanisms of regulation are found in nature: regulation via water transport [10], via synthesis of osmotically active substances [4, 16, 21] and via ion transport [18, 24, 29]. The last mechanism has been observed in cells of *Chaetomorpha linum*, a littoral alga, which can adapt to large variations of the external osmotic pressure. As outlined in a previous paper [29], we

demonstrated that the regulation is controlled phenomenologically by the external potassium concentration and by the osmotic pressure of the external medium. To get more insight into the molecular mechanism of the turgor regulation which is coupled with the active transport of ions, we must know the dependence of several transport and membrane parameters, i.e. of the hydraulic conductivity L_p, of the membrane potential ψ_0, of the membrane resistance ρ_0, and of the ion fluxes on the hydrostatic pressure in the cell, P. A method of direct and continuous measurement of changes in the hydrostatic pressure inside the cells has been described for *Valonia utricularis* by us elsewhere [23, 27, 28]. However, when applying this method to *C. linum*, difficulties arise in inserting the microcapillaries, for measuring P and the electrical properties of the membrane, into the cells because of the rigidity of the cell wall and because of the high hydrostatic pressure inside the cells – about 25 atm.

Therefore, we first study in this paper, on *Valonia utricularis*, the pressure-dependence of the hydraulic conductivity and of the membrane potential and the membrane resistance, which is an expression of the salt permeability of the cell membranes. In comparison with other vacuolated cells an unusual feature of *Valonia* is the electropositive potential of the vacuole.

According to Gutknecht [11], *Valonia ventricosa* regulates its vacuolar ionic content by means of an active uptake of K and Na at the tonoplast and by an active extrusion of Na and probably an active uptake of K at the plasmalemma. For our studies we feel that *Valonia* is a suitable object for several reasons. The cells are large enough to insert more than two microcapillaries which are necessary for the measurement of the relation between pressure and the electric membrane parameters. Secondly, *Valonia* cells show the phenomenon of turgor regulation as does *Chaetomorpha linum* but, as will be shown, only in a limited pressure range. Furthermore, Gutknecht [14] found a dependence of the potassium fluxes on the hydrostatic pressure in the pressure range from 0 to 1 atm. Since a part of the short-circuit current measured by Gutknecht [12] could be accounted for by the potassium flux, a strong pressure-dependence of the electrical properties of the cell membranes could be predicted under the conditions of turgor-regulation.

Materials and Methods

Material and Culture Conditions

Cells of *Valonia utricularis* were used for turgor pressure regulation experiments and for measuring the dependence of the hydraulic conductivity, the membrane p.d., and resistance on the hydrostatic pressure of the cell interior. The algae were grown from

cells originally obtained from Naples (Italy). The culture medium was natural seawater from the North Sea enriched by sea salt to raise the osmolarity to that of the Mediterranean "seawater" (main contents in mM/liter 560 Na; 55 Mg; 12 K; 11 Ca; 665 Cl; 30 SO_4). The cultures were illuminated continuously with 25 Watt Osram-Fluora lamps. The temperature of the seawater was 15 to 18 °C.

Turgor Pressure Regulation. Cells of comparable size and age (about 0.5 cm diameter) were preincubated in artificial isotonic seawater for four days (illumination intensity 10,000 Lux), and were transferred into media with a basic composition of (in mM/liter): 545 NaCl; 55 $MgCl_2$; 11 $CaCl_2$; 2.5 $NaHCO_3$; 1 Na_2HPO_4; 1 $NaNO_3$. The osmotic pressure of this medium was changed by varying the sodium chloride concentration (from 415 to 650 mM/liter) to induce turgor pressure changes. At different osmotic pressures the external potassium concentration was varied (0.6 to 50 mM/liter) maintaining the osmolarity of the medium constant by corresponding variation of the sodium chloride concentration. The volume of the external medium was chosen large in comparison to the volume of *Valonia* cells (50 g of *Valonia* cells to 50 to 100 liters of seawater) to avoid changes in the composition of the seawater during turgor pressure regulation. The sodium and potassium concentration and the osmolarity of the external medium were checked flame-photometrically (PMQ II flame photometer with grating monochromator from Carl Zeiss, Oberkochen) and cryoscopically (Cryoscope from Knauer, Berlin) throughout the experiments. At different time intervals cell sap was obtained by a microliter syringe and the sodium, potassium and total osmolar concentrations were determined.

Cell-Turgor Pressure and Volume Flow Measurements

Methods have been developed for monitoring simultaneously the volume flow, the cell-turgor pressure, and the changes of the membrane potential during an applied current. A detailed description of the equipment for measuring the turgor (or the internal hydrostatic pressure) has already been given and illustrated [23, 27]. For a better understanding of the results presented in this paper a description of the measuring principle and a diagrammatic representation of the apparatus (Fig. 1) are given in the following.

The turgor was measured by a silicon miniature pressure transducer (CQS 125–200 Kulite Semiconductor Products, Ridgefield, New Jersey) transforming an applied pressure into a proportional voltage. The pressure transducer was mounted into a closed plexiglass chamber, into which a metal rod was introduced and sealed off by a rubber disc. To change the volume of the chamber the rod could be moved by a micrometer screw. The plexiglass chamber was attached to the holder of a micromanipulator. On the opposite side of the chamber a glass capillary with a tip-diameter of about 60 µm was connected with the pressure chamber by a sealing disc. The chamber and the capillary

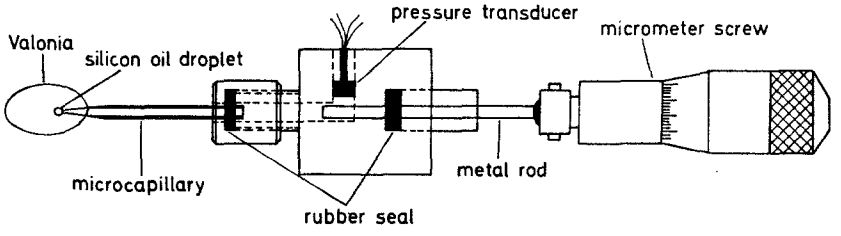

Fig. 1. Pressure and volume flow measurement in a single cell of *V. utricularis* (diagrammatic representation). For further explanation *see* text

were completely filled with silicone oil of low viscosity. The cells were held in a plexiglass chamber filled with natural seawater. The water was well-stirred and rapidly replaced by fresh seawater in order to avoid unstirred layers. The glass capillary was inserted into the cell by a micromanipulator under a binocular microscope. Due to the damage of the cell membrane and the resultant loss of cell sap the hydrostatic pressure dropped first, but returned to its original value after approximately 10 min.

The pressure built up in the chamber of the pressure device was equal to the hydrostatic pressure in the vacuole since the whole device was rigid and the oil was incompressible to a good approximation. Capillary forces in the tip of the capillary were negligibly small. After the steady state had been reached, the cell could be subjected to different volume flows by varying the concentration of an external solute ("osmotic experiments") or by applying artificial pressure inside the cell ("hydrostatic experiments").

Volume flows induced by pressure were realized by slightly increasing the pressure in the chamber with the aid of the measuring rod, so that an oil droplet formed at the tip of the capillary. The size of this oil droplet which adhered to the tip until it reached a diameter of about 100 μm depended on the feed of the rod into the chamber. The change of cell volume could be calculated from the feed and from the diameter of the rod (= 500 μm).

In *Valonia* the change of cell volume is complete after a few seconds, that is, prior to the beginning of the volume flow. To measure the time course of the pressure under "osmotic" and "hydrostatic" conditions the size of the oil droplet or the boundary between oil and cell sap was taken as the reference point. Therefore, any shift of the boundary or a change of the diameter of the droplet due to leakages in the measuring device could be readjusted by the micrometer screw. The volumetric elastic modulus ε of the cells was determined by measuring the response of the hydrostatic pressure to changes of cell volume in small pressure ranges (up to 0.5 atm), where the Philip equation [Eq. (8) in the Appendix] is valid.

In the "osmotic" and "hydrostatic" experiments an exponential time course ($T_{1/2}$=2 to 8 min) of pressure was found in accordance with previous measurements [23, 28]. A new stationary value of the hydrostatic pressure was reached after 30 to 40 min. Due to the linear relationship between pressure and cell volume as expressed by the Philip equation, the rate constant k for the decline of the volume flow during the experiments is the same as for the pressure change. The hydraulic conductivity was calculated from the measured k-values, from the stationary and applied pressures, and from the cell surface area and the cell volume according to Eqs. (17) and (18) in the Appendix.

A possible source of error in the determination of L_p may result from unstirred layer effects. Changes of the local concentration near the cell membranes due to the permeation of the osmotic solute (glucose) could be excluded in the osmotic experiments, since glucose permeates very slowly. Changes of the concentration at the membrane surfaces due to a sweeping away effect are negligibly small as can be calculated from the equation given by Dainty (*cf.* [23]).

Electrical Measurements

Glass microelectrodes of the conventional type (tip diameter about 15 μm) were used for membrane potential and resistance measurements: they had resistances of 1 to 8 MΩ and tip potentials of less than 4 mV. The microelectrodes were inserted across the cell wall and the cytoplasmic layer (thickness of each about 10 μ) into the center of the vacuole; the external electrodes were placed near the cell wall. Both electrodes were

connected to Ag/AgCl electrodes in small glass tubes filled with 3 M KCl/Agar solution. The connection between the microelectrodes and the Ag/AgCl electrodes was very tight to prevent any leakage even in the presence of hydrostatic pressures up to 5 atm.

The membrane p.d. was measured by an amplifier (model P 16 from Grass Instruments) having an input resistance of $10^{11}\ \Omega$, and recorded.

A Keithley 225 was used as a constant current source. The membrane resistance was calculated from the change of the membrane p.d. in response to constant current pulses of a duration of 20 sec at different stationary values of the hydrostatic pressure. The slope of the voltage-current curve was approximately linear over the range of applied current densities up to $\pm 10\ \mu A/cm^2$.

The hydrostatic pressure in the cells was increased stepwise by adding small amounts of distilled water to the external seawater and decreased by stepwise addition of small amounts of NaCl or glucose to the external medium.

After each change of the osmolarity, the measurement of membrane resistance was made after water flux equilibrium had been reached. Leaks occurring around the micropipettes were detected by the drop of the membrane p.d. or, more sensitively, by a drop of the hydrostatic pressure in the hydrostatic experiments. Since the cell behaves like an ideal osmometer, in those experiments the stationary final pressure P_E could be calculated from the initial volume change of the cell and from the original hydrostatic pressure P_0 and could be compared to the measured one. In tight cells, P_E was greater than P_0 because the osmotic pressure was slightly increased by the displacement of water from the cell. In leaky cells, P_E was smaller than P_0 because of the loss of solutes, and therefore the decrease in osmotic pressure. These cells could be easily excluded.

Results and Discussion

Turgor Pressure Regulation

As outlined above, the measurements on *V. utricularis* were performed bearing in mind the interesting phenomenon of turgor pressure regulation demonstrated on *C. linum*. Therefore, we asked whether this phenomenon can also be observed in *Valonia* and how far changes of the intracellular ion content have to be taken into account for the calculation of the hydraulic conductivity during exosmosis and endomosis and for the interpretation of the pressure-dependence of the membrane p.d. and resistance. In Fig. 2 the time course of the intracellular potassium and sodium concentrations is plotted for cells transferred to hypotonic seawaters of different osmolarity and potassium concentrations varying between 0.6 and 50 mM/liter. Curves I denote ion content regulation induced by a change of the external osmotic pressure of about -3.8 atm, whereas curves II indicate the decrease of potassium and the increase of sodium inside the cells in response to a change of the external osmotic pressure of about -13.5 atm. The osmotic stress in experiment II was only tolerated by the cells after preincubation in hypertonic seawater. This indicates that the osmolarity range, in which turgor pressure regulation takes place, is much smaller for *Valonia* than for *Chaetomorpha* [29].

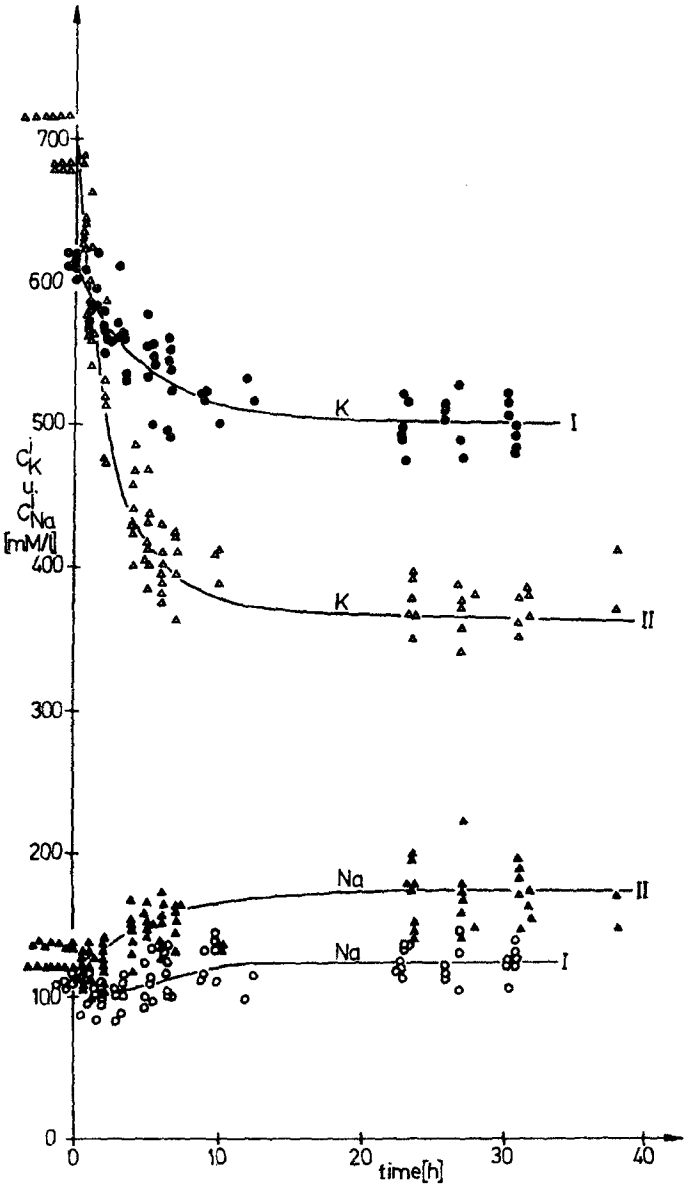

Fig. 2. Time course of the intracellular potassium and sodium concentrations (c_K^i and c_{Na}^i) in *V. utricularis* after transferring the algae into seawaters with reduced osmolarity. In experiment I the external osmotic pressure was reduced by 3.8 atm and in experiment II by 13.5 atm. In experiment II the algae were preincubated in hypertonic seawater (osmolarity: 1.6 M/liter). The external potassium concentration was varied from 0.6 to 50 mM/liter

As indicated in Fig. 2, the new steady state is reached after 8 to 10 hr. Therefore, changes of the ion content in the vacuole during the first phase of rapid water flow (20 min) can be neglected for the calculation of L_p.

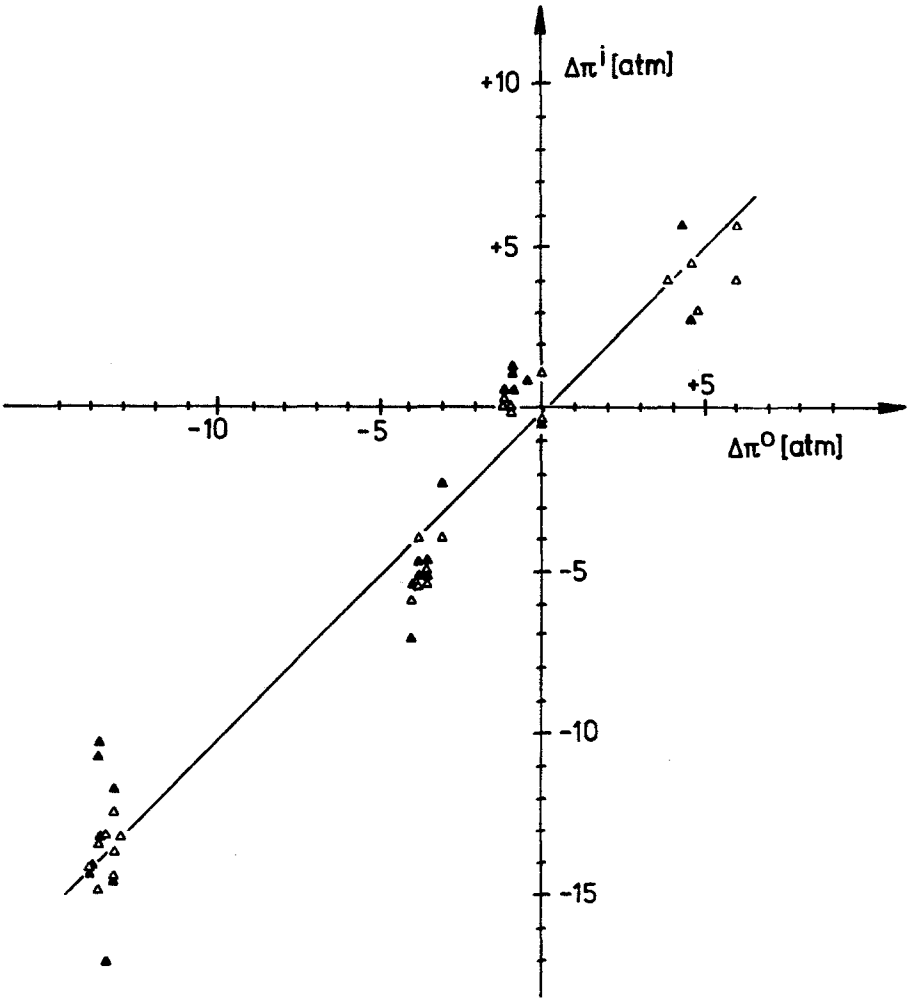

Fig. 3. Change in the osmotic pressure of the cell sap, $\Delta\pi^i$, in response to changes in the external osmotic pressure, $\Delta\pi^o$, after completion of turgor pressure regulation in hypotonic (*see* Fig. 2), isotonic, and hypertonic seawater. The external potassium concentration of the isotonic and hypertonic seawater was varied between 1 and 50, and 5 and 50 mM/liter. $\Delta\pi^i$ was determined cryoscopically (-▲-) or calculated from the internal potassium and sodium concentrations (-△-)

In contrast to the finding on *C. linum* the decrease of the intracellular potassium concentration and the slight increase of the sodium concentration are independent of the external potassium concentration; the intracellular potassium concentration is only a function of the external osmotic pressure.

The same is true for the regulation of the ion content inside the vacuole for cells in isotonic and hypertonic solutions at external potassium concentrations above 1 and 5 mM/liter, respectively.

Plotting the changes of the internal osmolarity after reaching a new steady state against the changes of the external osmolarity the slope of the resulting line is 45° (Fig. 3). This means that $\Delta\pi^i = \Delta\pi^o$ is valid over the whole investigated osmolarity range. This result which is expected for an exact turgor pressure regulation as defined by Kesseler [18] indicates therefore that only the number of the ions in the external medium (i.e., the colligative properties) play a role during turgor pressure regulation. This is important to note for the measurement of the pressure dependence of the membrane p.d. and resistance because the external potassium concentration and the external osmolarity are changed during this experiment (*see below*).

It has been suggested [5] that mechanical deformation of membrane structure in response to pressure might alter the permeability of water and ions. In the following, therefore, we investigated how far changes of L_p induced by the high pressure observed during turgor pressure regulation on *V. utricularis* and *C. linum* must be taken into consideration for the discussion of the mechanism.

The Pressure-Dependence of L_p

The equations (17) and (18) (Appendix) for calculation of L_p from osmotic and hydrostatic volume flows have been derived on the assumption that both the area and the volumetric elastic modulus ε are pressure-independent. This may be true for the area of *Valonia* cells, but not for ε. As demonstrated in a previous paper [23], ε is only constant over a limited pressure range. At larger changes of the hydrostatic pressure, deviations from the Philip equation [Eq. (8) in the Appendix] occur. To calculate correct L_p values over larger pressure ranges the complete function $\varepsilon = f(P)$ must be known. Therefore, we measured ε stepwise for small pressure ranges, in which the Philip equation is valid. The result given in Fig. 4 confirms a very strong dependence of ε on the hydrostatic pressure. The curve resembles that found on *N. flexilis* [25]. The values of ε for both algae are of the same order and are lower than that calculated by Barry [1] for *N. translucens* making the assumption that the relative volume change was approximately four times the relative change in length.

In Fig. 5 the L_p values calculated from Eq. (18) are plotted versus hydrostatic pressure. The lines parallel to the pressure axis indicate the pressure ranges in which L_p has been determined at a constant ε. A polarity of water movement could not be observed. The values of L_p for exosmosis and endosmosis are equal.

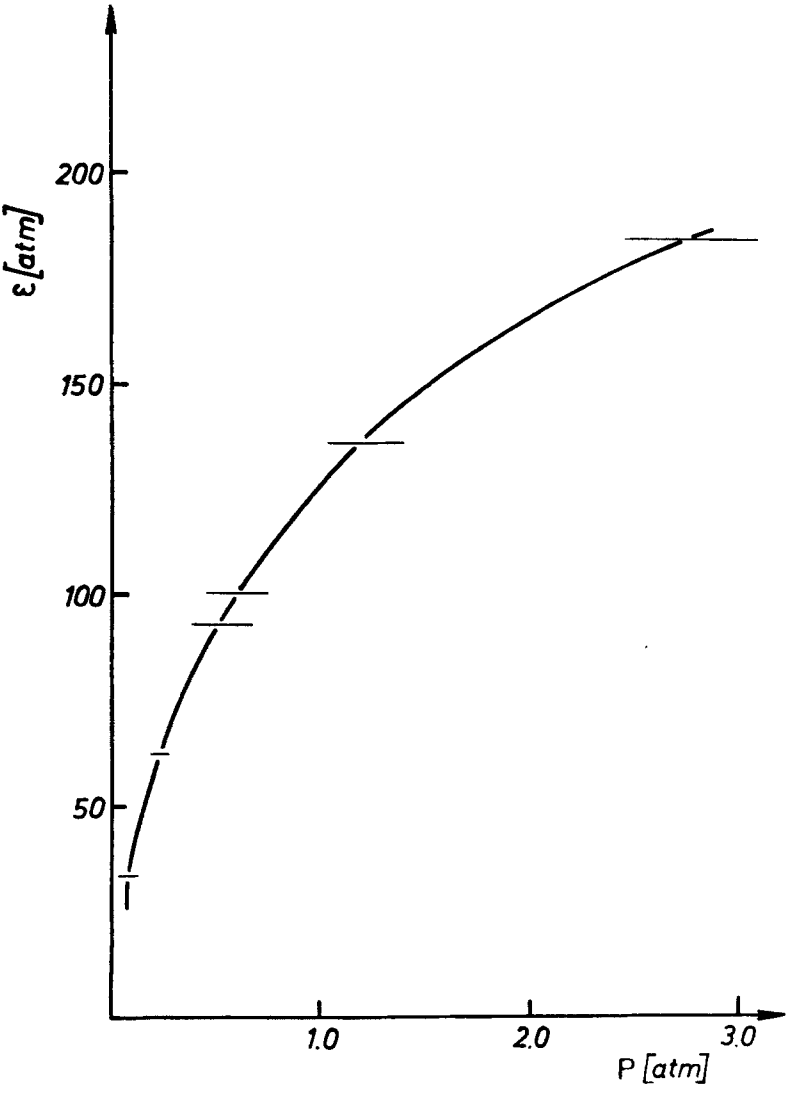

Fig. 4. Dependence of the volumetric elastic modulus ε of a cell of *V. utricularis* on the cell-turgor pressure P. The lines parallel to the pressure axis indicate the pressure ranges for which the ε-values were determined according to the Philip equation (Eq. 8)

As the hydrostatic pressure tends toward zero, L_p increases strongly; this is similar to the results obtained on *N. flexilis* [25]. In some *Valonia* cells the value of L_p in the pressure range below 1 atm increased to 10 times its value at normal pressure (about 1.5 atm). The same trend was found in osmotic experiments using glucose as the osmotic solute (Fig. 6). For hydrostatic pressure above 1 atm, L_p is pressure-independent and, therefore,

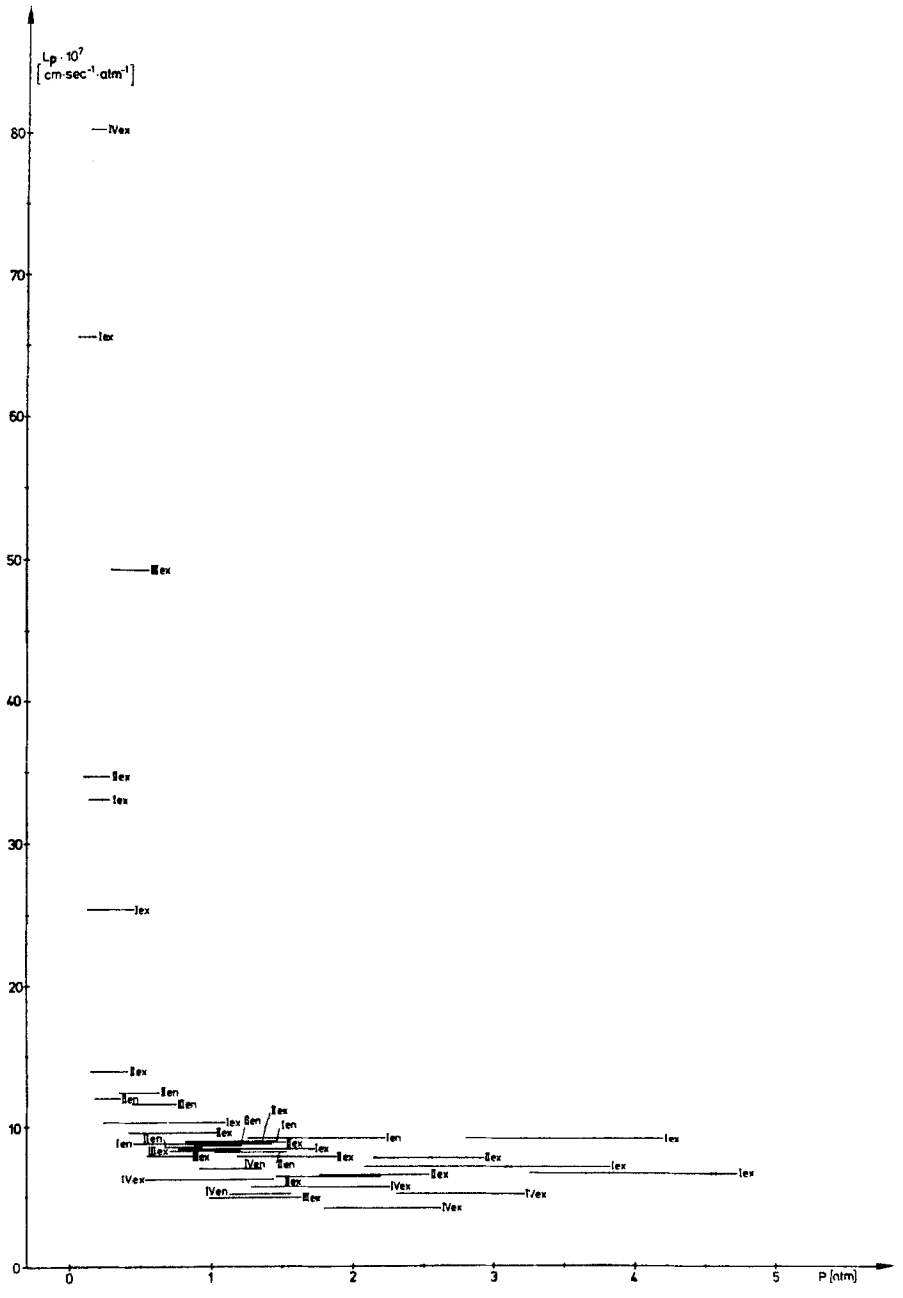

Fig. 5. Dependence of the hydraulic conductivity L_p on the hydrostatic pressure P in *V. utricularis* cells. L_p was determined from endosmotic and exosmotic volume flows induced by hydrostatic pressure gradients according to Eq. (18). The direction of the volume flow and the number of the cell are given for each experiment

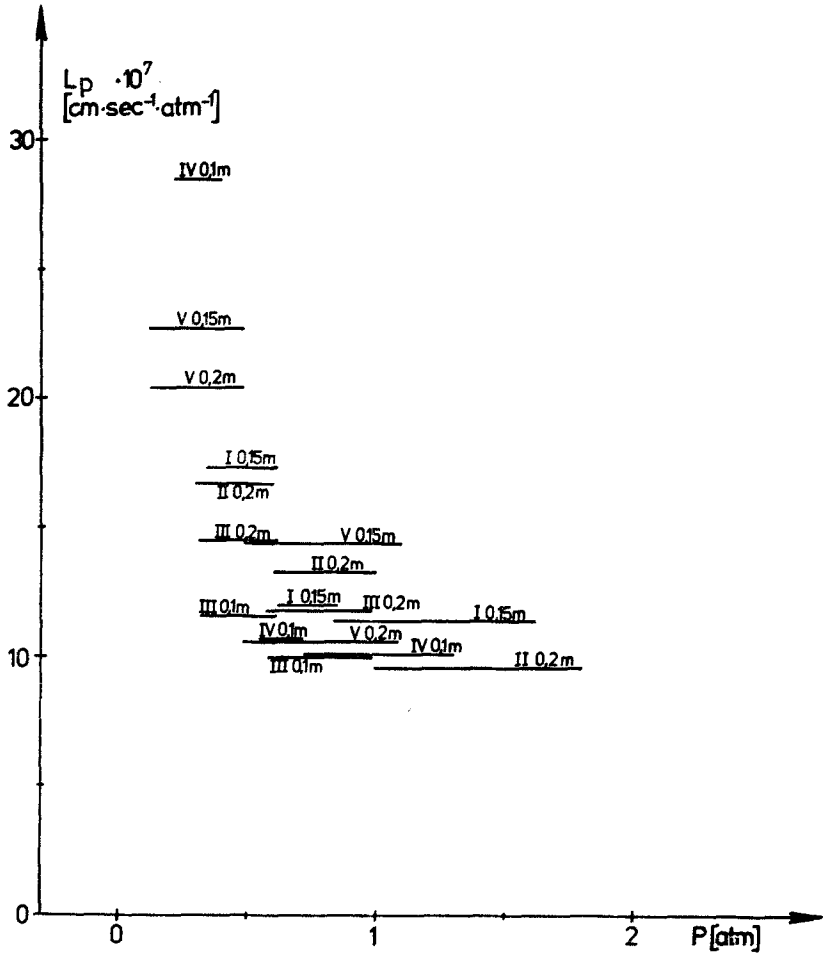

Fig. 6. Dependence of the hydraulic conductivity L_p on the hydrostatic pressure P in *V. utricularis* cells. L_p was determined from exosmotic volume flows induced by osmotic gradients (glucose) according to Eq. (17). The glucose concentration and the number of the cell are given for each experiment

changes of L_p during turgor pressure regulation have to be taken into account only in hypertonic solutions. The results suggest that it is the decrease of the hydrostatic pressure and not a dehydration or hydration effect of the electrolytes and the osmotic solute on the membrane as discussed in the case of *N. flexilis* [25] which is responsible for the increase of the water permeability, on approaching the point of plasmolysis. In contrast to our experiments on *Nitella* this conclusion can be drawn easily since the internal electrolyte concentration remained practically constant during volume flow induced by an applied pressure. Therefore, a possible influence

of different vacuolar electrolyte concentrations on the hydration of the membranes of protoplasma can be excluded.

The increase of L_p of the two membranes may be more dramatic than indicated in Fig. 5, since the value of the hydraulic conductivity of the cell wall must be taken into consideration, when measuring overall values in the order of 10^{-5} cm/sec atm. The value of L_p for the cell wall of *Valonia* was not measured up to the present. However, using the value of 6×10^{-5} which was determined for the cell wall of *Nitella* (*unpublished data*) one can postulate that the cell wall becomes the restricting barrier for the water flow on approaching the plasmolytic point.

A model which can explain the increase of L_p on approaching the plasmolytic point was introduced into literature by Richardson, Ličko, and Bartoli [20]. Performing a theoretical analysis of flows through folded and planar membranes the authors demonstrated that a preferential increase of water flow is created by folded membranes in the neighborhood of the isotonic state. The calculations of Richardson *et al.* [20] show that the increase of water flow results from salt concentration gradients established within the membrane folds giving a continuous and nonlinear distribution of thermodynamic driving forces across the membrane. Since a folding of the membrane on approaching the plasmolytic point seems plausible, this effect could play a role, although it is difficult to prove this hypothesis experimentally.

Another model – highly speculative, but interesting – is the following. From the standpoint of the "pore model" the dependence of L_p on pressure is unexpected. The "pore model" would suggest an increase in the size or number of pores, and, therefore, of L_p in response to a uniform stretch of the cell membrane induced by an applied pressure. However, we measured the largest value of L_p in an approximately unstretched state of the cell membrane. Burton [5] demonstrated experimentally an increase of L_p to a maximum for about 20% linear stretch of the frog muscle, but a decrease for further stretch. His theory of one-way stretch would predict this maximum of L_p in dependence on a linear stretch. According to this theory the maximum of L_p will occur at a smaller degree of stretch if the pores of the membranes are already elliptical in the unstretched state.

Our results may be explained by the theory of Burton, if we assume a linear stretch in the membranes of *Nitella* and *Valonia* in response to pressure and very elliptical pores in the membranes which shift the maximum to pressures in the neighborhood of the plasmolytic point being below the pressures at which the experiments were performed. In this case we should expect a decrease of L_p to higher pressures. The assumption of narrow

elliptical pores is consistent with results of Gutknecht [13] because they indicate the absence of water-filled pores in *V. ventricosa*, only at normal pressure (1 atm).

If it were true that the hydraulic conductivity decreases again on reaching the point of plasmolysis, the discrepancy between our results and those obtained and referred by Stadelmann [22] would not be so large. These authors calculated L_p from the time course of swelling and shrinking of plasmolyzed protoplasts and applied this method to a wide range of plant cells. Their values of L_p are in the range of 10^{-8} to 10^{-6} cm sec^{-1} atm^{-1} and, therefore, very low in comparison to those found generally in giant algal cells [6, 25]. Dainty [6] pointed out several objections to this method, probably the most serious one being that the membranes may have quite different properties in the plasmolyzed state than in the turgid. Our results confirm this assumption of Dainty that at normal values of cell turgor, care has to be taken in the use of L_p values determined under plasmolytic conditions.

Considering the phenomenon of turgor pressure regulation it is important to note that a change of L_p does not have to be taken into consideration during regulation in hypotonic seawater, i.e. in the range of high pressure, in contrast to changes of the ion permeabilities as will be shown in the next section.

Pressure-Dependence of the Membrane p.d. and Resistance

To study the pressure-dependence of the membrane potential and resistance the hydrostatic pressure, normally about 1 to 1.5 atm, was increased by adding distilled water in steps to the external medium until a pressure of 3.5 to 4.5 atm was reached, and then again lowered by adding stepwise small amounts of sodium chloride or glucose. After each addition and after reaching a new equilibrium for the water fluxes, the membrane resistance ρ_0, the membrane potential ψ_0, and the hydrostatic pressure P were measured.

In this way, the plot of ψ_0 and ρ_0 against P in Fig. 7 was obtained. To prove a possible influence of the dilution of the external seawater (maximal about 15%) or of the increase of the sodium chloride or glucose concentration (maximal about 5%) on the shape of the curves, the experiments of increasing and decreasing the hydrostatic pressure were performed several times on the same cell. As seen in Fig. 7, ρ_0 increases in the pressure range from 1.5 to 2.0 atm by a factor of 2 to 3. At higher pressures, ρ_0 decreases again and reaches, at about 3 atm, the original value found in the pressure range around 1.0 atm, the original pressure of most of the cells. In the pressure range, in which the maximum of ρ_0 can be observed and which

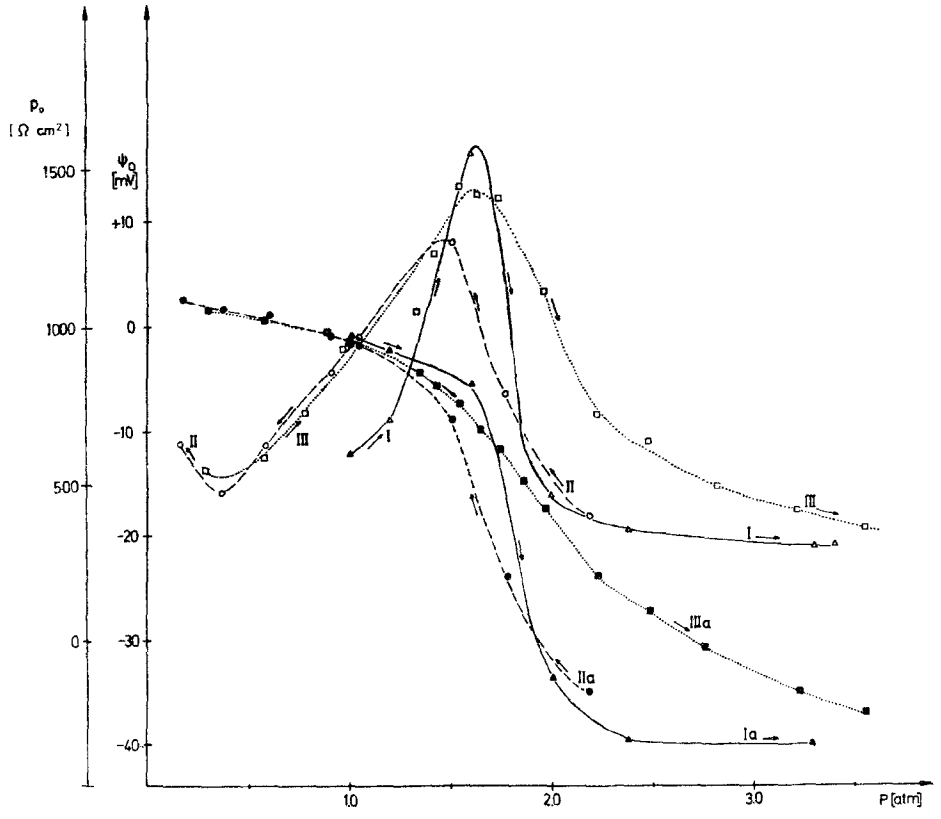

Fig. 7. Pressure-dependence of the membrane resistance ρ_0 (curves I–III), and of the membrane potential ψ_0 (curves Ia–IIIa). The hydrostatic pressure P was increased by stepwise additions of water up to a dilution of 15% (curves I and Ia), then lowered by stepwise additions of sodium chloride (curves II and IIa), and again increased with distilled water (curves III and IIIa). After each addition and after reaching water equilibrium ψ_0 and ρ_0 were measured

differs from cell to cell, ψ_0 becomes more negative by about 10 to 40 mV. The absolute value of ψ_0, reached finally at pressures about 3 atm, depends on the original potential (which varied between 0 and +15 mV).

Before drawing conclusions from these results we must discuss several possible sources of error. Considering the procedure for the measurement of the relationships presented in Fig. 7 there was no steady state for the ion fluxes during the increase and decrease of the hydrostatic pressure. According to Figs. 1 and 2 the internal potassium concentration is independent of the external potassium concentration over a wide range, but decreases or increases in response to changes of the external osmolarity. We feel that this objection can be eliminated by three observations. As indicated in Fig. 7

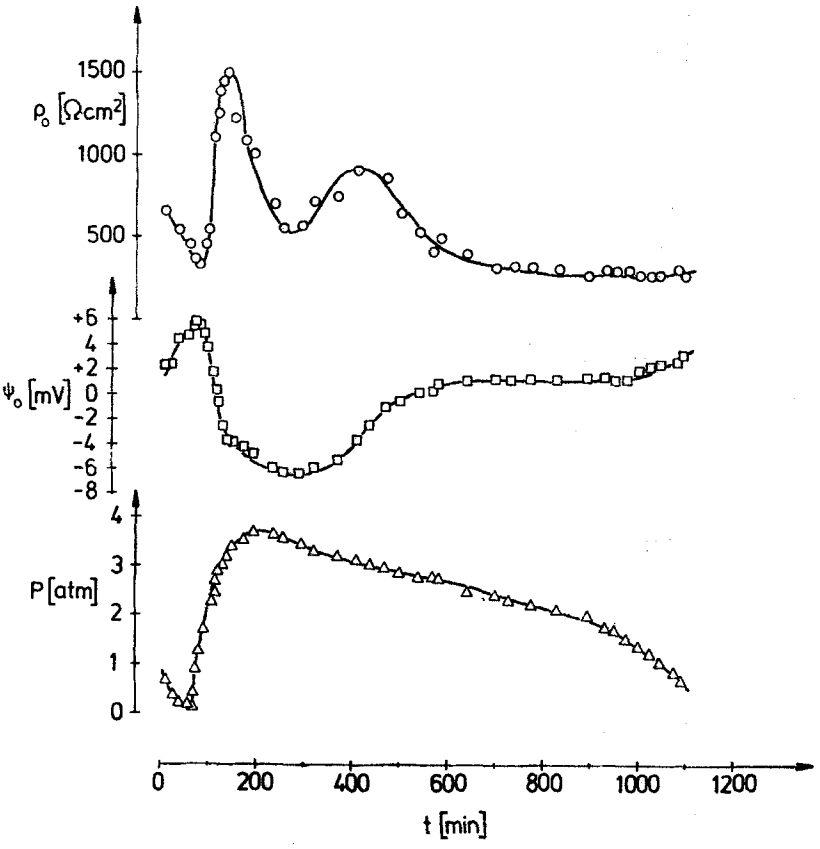

Fig. 8. Time course of the membrane potential ψ_0 and resistance ρ_0 during turgor pressure regulation in *V. utricularis*. The original pressure in the cell was lowered to 0.1 atm by stepwise addition of sodium chloride, and then increased by the addition of water in one step (15% dilution)

the changes of ψ_0 and ρ_0 are reproducible and independent of the way in which the pressure was altered. The slight shift of the maximum membrane resistance may be explained by long time of measurement or by a hysteresis phenomenon (*see below*). Furthermore, this objection was also invalidated by changing the time course of the applied pressure. In Fig. 8 a typical experiment is shown in which a turgor pressure regulation was simulated. The hydrostatic pressure was lowered to 0.1 atm and then rapidly increased to 3.5 atm by adding distilled water to the external medium in one step. During the increase of the hydrostatic pressure the usual course of ψ_0 and ρ_0 was observed. Subsequently the hydrostatic pressure decreases again slowly and reaches a value of about 1 atm after about 20 hr. During this decline similar changes of ψ_0 and ρ_0 occur. Sometimes a shift of the maximal

resistance to higher pressures (to about 3 atm) and changes of the height of the maximal resistance (Fig. 8) could be observed during the slow decrease of the hydrostatic pressure. These effects were not fully understood, but seemed to depend on the duration of applying high pressures to the cell and, therefore, can probably be explained by a hysteresis phenomenon.

In some experiments, such as those described in Fig. 8, we increased the hydrostatic pressure again after reaching the original value of 1 atm in a period of 20 hr. A similar change of the two parameters in their dependence on pressure was measured although the intracellular potassium concentration was lowered (*see* Figs. 1 and 2). Errors due to the decrease of the external potassium concentration from the dilution of the external medium (up to 15%) could also be excluded, since the membrane p.d. and resistance showed no significant change in response to a reduction of the external potassium concentration from the normal value of 12 mM/liter to about 6 mM/liter. In addition, the same results were obtained, if the external medium was diluted with 12 mM KCl-solution instead of distilled water. Another source of error may result from electrokinetic phenomena such as electroosmosis, transport number effects, etc. [1, 2, 7, 8]. Although an influence of these electrokinetic phenomena is excluded partly by the experiments represented in Fig. 8, we checked the influence of these phenomena by inducing an endosmotic volume flow in pressure ranges below the pressure at which the increase of the resistance was usually observed. This was done by applying a pressure to the cell with the aid of the apparatus for continuous cell turgor pressure measurement as described above. The volume flows were of the order of 1.5×10^{-6} cm^3/cm^2 sec and, therefore, one magnitude greater than the maximum volume flows produced during the other experiments. Under these conditions a slight change of ρ_0 occurred, which cannot, however, explain the large changes of ρ_0 in Fig. 7 and Fig. 8. Furthermore, the potential remained constant. In another set of experiments we applied high currents of the order of 100 μA/cm^2 to the membrane and simultaneously measured the pressure. No significant volume flow caused by electroosmosis could be detected. This result is in agreement with the assumption of Gutknecht mentioned above that there are no water-filled pores in the membranes of *Valonia* at normal pressure, and also with our finding that L_p is constant at pressures above 1 atm.

We also measured the dependence of ψ_0 on the hydrostatic pressure without inserting the current-microelectrode into the cell to exclude any influence of this electrode. The same voltage drop in the pressure range in which the increase of ρ_0 was usually found could be observed. Therefore, it is proved, that the changes of ψ_0 and ρ_0 are directly affected by the pressure.

The results appear to point to the existence of pressure-dependent ion fluxes in *V. utricularis*. Considering the finding of Gutknecht we can suppose that the increase of the resistance is due to the decrease of potassium influx in response to increasing pressure. To explain the decrease of ρ_0 at higher pressures we must further postulate that, on the effect of pressure on the potassium influx, is superposed a pressure-dependent potassium efflux which is increased by increasing pressure. There is some evidence from the light-dependence of the membrane p.d. that it is the electrogenic pump in the tonoplast which is pressure-dependent. In the dark the potential drops by an amount of 5 mV at normal pressure in accordance with Gutknecht [12]. In contrast, at pressures of about 3 to 4 atm and lowered p.d., an increase (up to 10 mV) of the stable membrane potential is obtained in the dark. This result can be explained by the assumption of a change of the direction of the electrogenic potassium pump in response to pressure. At low pressure the pump transports positive charge into the vacuole; at higher pressures, at which the influx is inhibited and the direction of the pump has been changed, positive charge is pumped into the cytoplasm. In this latter case the vacuolar potential would become more negative in the light than in the dark. The reversal of the electrogenic potassium pump is a reasonable hypothesis, but at the present stage of information, a pressure-dependence of other ion fluxes cannot be excluded.

In any case, it seems possible that the pressure-dependence of the ion fluxes is coupled with primary changes of the fixed charge of the two membranes [26] or with structural changes of the membranes in response to pressure. For example, configurational transitions of the α-helix of polypeptides and of the triple helix of collagen in dependence on load are known [9]. P. Zahler *(personal communication)* found a selective discrimination between uptake of hydrophilic and hydrophobic proteins in bilayers dependent on tension. Therefore, such phenomena could play a role in our system. However, configurational transitions should be restricted only to ion transport since L_p and, therefore, the water transport was not affected in this pressure range.

In any case, the results presented in this paper suggest that mechano-electrical processes are involved in turgor pressure regulation. Further experiments in this field measuring the pressure-dependence of the electrical parameters of the two membranes separately and using other algae such as *Nitella* or *Chaetomorpha* in which the hydrostatic pressure can be changed over a large pressure range should give more insight into these phenomena which could also be interesting for better understanding of the molecular mechanism of mechano-receptors.

Appendix

If two compartments are separated by a membrane which is permeable to solutes and to water, the solvent flow through the membrane can be described by the principles of irreversible thermodynamics according to Kedem and Katchalsky [17] and Katchalsky and Curran [15]. These authors have given the following relation to express the solvent (volume) flow, J_v (cm^3/cm^2 sec), in terms of the driving forces of the hydrostatic and osmotic pressure difference between two well-stirred bulk solutions separated by the membrane.

$$J_v = L_p(P - \Delta\pi_i) + L_{pD} \cdot \Delta\pi_s \tag{1}$$

or

$$J_v = L_p(P - \Delta\pi_i - \sigma_s \cdot \Delta\pi_s) \tag{2}$$

in which P is the hydrostatic pressure difference, and $\Delta\pi_i = \pi_i^i - \pi_i^o$ and $\Delta\pi_s = \pi_s^i - \pi_s^o$ are the osmotic pressure differences due to the impermeable (subscript i) and the permeable (subscript s) solutes. (The solutions bathing the external and the internal surface of the cell membrane are denoted by the superscripts o and i, respectively.) L_p is the hydraulic conductivity, L_{pD} the osmotic coefficient and σ_s the reflection coefficient defined as $\sigma_s \equiv -\frac{L_{pD}}{L_p}$. To a good approximation the volume flow is equal to the water flow. As demonstrated experimentally elsewhere [23], the change in the hydrostatic pressure in *Valonia* cells depends exponentially on time during exosmotic and endosmotic volume flow, induced both by osmotic (Eq. (3)) and hydrostatic (Eq. (4)) pressure gradients.

$$P - P_E = (P_0 - P_E)\, e^{-k \cdot t} \tag{3}$$

$$P - P_E = (P_A - P_E)\, e^{-k \cdot t}. \tag{4}$$

In these equations P is the hydrostatic pressure at time t, P_0 is the initial, P_E the final pressure, and P_A the pressure initially applied to the cell in hydrostatic experiments; k is the rate constant for pressure changes. Using Eqs. (3) and (4) it is possible to evaluate the phenomenological coefficients of Eq. (1). In the following, L_p will be derived from the change in the hydrostatic pressure observed due to concentration changes of a permeable solute (e.g., glucose) in the external solution. The calculation of L_p from water flow induced by hydrostatic gradients was given elsewhere [23].

We assume that only the testing solute (s) is permeable in the system and that all other solutes are impermeable ($\sigma = 1$) [3]. Due to the experi-

mental conditions the permeable solute is only in the external solution and the volume of the external solution is large in comparison to the cell volume, so that π_s^o and π_i^o are constant during volume flow. Under these conditions the water flow J_v, can be written:

$$J_v=\frac{1}{A}\cdot\frac{dV_w(t)}{dt}=L_p[P(t)-\pi_i^i(t)+\pi_i^o]-L_{pD}\cdot\pi_s^o. \tag{5}$$

$\pi_i^i(t)$ is given by

$$\pi_i^i(t)=\frac{n_iRT}{V_0-V_w}=\frac{\pi_{io}^i}{1-V_w/V_0} \tag{6}$$

in which V_0 and π_{io}^i are the cell volume and the internal osmotic pressure of the impermeable solute, respectively, at the initial pressure P_0, and V_w is the change in the cell volume at time t. Since $V_w \ll V_0$ in the case of small deviations from the steady state, Eq. (6) can be written:

$$\pi_i^i(t)=\pi_{io}^i\left(1+\frac{V_w(t)}{V_0}\right). \tag{7}$$

Eqs. (6) and (7) are valid if one assumes the cell to be a classical osmometer. In this case, the cell wall is considered to be elastic having a volumetric elastic modulus ε defined by the modified equation of Philip [19]

$$P-P_0=\varepsilon(V/V_0-1) \tag{8}$$

in which V is the cell volume at time t during water flow. As $V(t)=V_0-V_w(t)$, Eq. (8) becomes

$$P-P_0=-\frac{V_w}{V_0}\varepsilon. \tag{9}$$

Substituting Eqs. (7) and (9) into Eq. (5) and rearranging yields

$$\frac{dV_w}{dt}+\frac{L_pA(\pi_{io}^i+\varepsilon)}{V_0}V_w=L_pA(P_0-\pi_{io}^i+\pi_i^o)-L_{pD}\cdot A\cdot\pi_s^o. \tag{10}$$

Furthermore, according to Eq. (5), if $J_v=0$

$$P_0=\pi_{io}^i-\pi_i^o+\frac{L_{pD}}{L_p}\pi_{s(J_v=0)}^o. \tag{11}$$

Introducing this expression for P_0 into Eq. (10), it follows that

$$\frac{dV_w}{dt}+\frac{L_pA(\pi_{io}^i+\varepsilon)}{V_0}V_w=-L_{pD}A(\pi_s^o-\pi_{s(J_v=0)}^o). \tag{12}$$

The initial rate of water flow, i.e. the initial rate of shrinkage or swelling of the cell, is given by

$$\left(\frac{dV_w}{dt}\right)_{t=0} = -L_{pD} A(\pi_s^o - \pi_{s(J_v=0)}^o). \tag{13}$$

On the other hand, by substituting Eq. (9) into Eq. (3), an equation for the experimental determination of V_w is obtained:

$$V_w = \frac{V_0}{\varepsilon}(P_0 - P_E)(1 - e^{-k \cdot t}) \tag{14}$$

or for the initial rate of water flow at time $t = 0$

$$\left(\frac{dV_w}{dt}\right)_{t=0} = k \frac{V_0}{\varepsilon}(P_0 - P_E). \tag{15}$$

Comparing Eqs. (13) and (15) it follows that

$$L_{pD} = -\frac{kV_0(P_0 - P_E)}{\varepsilon A(\pi_s^o - \pi_{s(J_v=0)}^o)} \tag{16}$$

or

$$L_p = \frac{kV_0(P_0 - P_E)}{\varepsilon A \sigma_s(\pi_s^o - \pi_{s(J_v=0)}^o)}. \tag{17}$$

In Eq. (17) $\pi_s^o - \pi_{s(J_v=0)}^o$ denotes the change of the external osmolarity inducing the water flow. Under the experimental conditions of this paper ($\pi_{s(J_v=0}^o = 0$) the change of the external osmolarity is π_s^o during exosmosis and $-\pi_s^o$ during endomosis.

If a volume flow is induced by an artificial change of the internal hydrostatic pressure [*see* Eq. (4)], a similar expression for L_p is found as outlined in a previous paper [23]:

$$L_p = \frac{kV_0(P_A - P_E)}{\varepsilon A(P_A - P_0)}. \tag{18}$$

L_p and L_{pD} can be calculated from Eqs. (16) and (17) if the volumetric elastic modulus is known since the other parameters can be measured easily. According to the Philip equation, ε can be determined by measuring the change in cell volume in response to an applied pressure.

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