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Turgor Pressure and Cell Volume Relaxation in *Halicystis parvula*

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Summary. The elastic modulus, ε, for the cell wall of *Halicystis parvula* $\left(\text{defined by } \varepsilon = V\frac{dP}{dV}\right)$ was determined by two different ways:
1) By measuring the stationary pressure-volume curve and by calculating the long-term elastic coefficient, ε_s, from the slope of the curve at a given volume and
2) By measuring differential changes in cell turgor pressure and cell volume using the pressure probe technique and by calculating the short-term elastic coefficient, ε, according to the definition equation.
The values of the elastic coefficients differ considerably and show different dependences on cell turgor pressure. ε_s is about 0.5 to 2 bar, and is therefore in agreement with measurements of Graves and Gutknecht (Graves, J., Gutknecht, J. (1976) *J. Gen. Physiol.* **67**:579) on perfused cells of *H. parvula*. ε_s is almost pressure independent within the pressure range of 0.05 to 0.9 bar.

On the other hand, ε assumes values of about 1 to 2 bar at a low pressure (about 0.05 to 0.15 bar) and increases to about 16 bar at 0.9 bar turgor pressure. Evidence is presented that the short-term elastic coefficient, ε, determined from differential changes in cell turgor pressure, dP, and cell volume, dV, reflects the true elastic properties of the cell wall, whereas the long-term elastic coefficient, ε_s, also includes other mechanical properties of the cell wall, which could not be identified up to now. The hydraulic conductivity, L_p, of the cell membrane of *H. parvula* was determined by directly measuring both the turgor pressure relaxation process (pressure probe) and the volume changes (microscope) in response to osmotic stress. L_p was calculated from the slope of volume-time curves without knowledge of the elastic modulus. It has a value of about 0.8 to 2×10^{-6} cm sec^{-1} bar^{-1}. The calculation of L_p from the turgor pressure relaxation process leads to identical results when using the short-term elastic coefficient. Under these conditions L_p assumes values of about 1.5 to 2.5×10^{-6} cm sec^{-1} bar^{-1}. L_p increases as the plasmolytic point is approached.

The result demonstrates that the short-term elastic coefficient determined by the pressure probe technique controls the instantaneous water transport between the cell interior and the external medium. The high extensibility of the cell wall (resulting from the low elastic coefficient) is the reason why *H. parvula* exhibits a change in cell volume rather than a change in cell turgor. The results are discussed in relation to pressure measurements in individual cells of higher plant tissues.

The analysis of the swelling and shrinking kinetics of a plant cell in response to external osmotic stress on the basis of the thermodynamics of irreversible processes [11] shows that the rate of uptake or loss of water is not only controlled by the hydraulic conductivity of the cell membrane, but also and equally by a cell wall parameter related to the cell wall elasticity [3, 4, 13, 18, 22]. The cell wall elasticity can be described by the so-called volumetric elastic modulus of the cell wall, ε. ε correlates differential changes in cell turgor pressure, dP, with the corresponding fractional changes in cell volume, dV/V:

$$dP = \frac{\varepsilon}{V}\,dV. \tag{1}$$

Most of our information concerning the absolute value of ε and its dependence on cell turgor, volume, and shape for various giant algal cells and single tissue cells of higher plants is derived from measurements using the pressure probe [18, 22]. The pressure probe technique allows for accurate measurements of

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small cell volume and turgor pressure changes instantaneously as required by Eq. (1). On the other hand, the elastic coefficient has been determined indirectly for some species from the slope of the stationary pressure-volume curve of the plant cell [5]. The latter procedure presupposes that the cell wall is perfectly elastic. However, it is well accepted that the cell wall of most plant cells exhibits mechanical properties which will contribute to long-term (i.e., stationary) deformations [2, 22, 23]. In this communication we report on ε-measurements on cells of *Halicystis parvula* (*Derbesia tenuissima*) by comparing ε-values obtained from both short-term and long-term changes in cell turgor pressure and cell volume.

The high extensibility of the cell wall of *H. parvula* allows for both measurements of the stationary pressure-volume curve over a pressure interval of about 1 bar using the light microscope [5] and of small pressure and volume changes using the nonminiaturized pressure probe. It is shown that as a result of the mechanical properties of the cell wall, which are not known at the present, both the absolute value of the elastic modulus obtained and its dependence on cell turgor pressure are dependent on the method of measurement. The values obtained differ by almost one order of magnitude.

In addition, due to the high extensibility of the cell wall of *H. parvula* the hydraulic conductivity, L_p, can be directly calculated from changes in cell volume (measured under the light microscope) in response to external osmotic stress as a function of time without knowledge of the elastic modulus of the cell wall. On the other hand, determination of L_p from the measured turgor pressure relaxation process (induced by changing the external osmolarity and measured continuously with the pressure probe) leads only to comparable values for L_p when the ε-values determined by short-term deformations are used in the calculation. The result indicates that only the pressure probe technique provides the values of ε that control the instantaneous water flow into and out of plant cells.

Materials and Methods

Halicystis parvula (*Derbesia tenuissima*) was obtained by courtesy of Dr. J. Gutknecht, Duke University Marine Laboratory, Beaufort, North Carolina. The cells were maintained and cultivated at 17 °C in water from the North Sea supplied by the company Biomaris. A detailed description of the life cycle of *H. parvula* (*D. tenuissima*) may be found elsewhere [1, 5–8, 12]. Cells of the gametophyte stage were used for the investigations reported here. The cells were shaped like rotational ellipsoids with a cell volume between 15 and 40 mm^3.

The nonminiaturized pressure probe is described in detail in a number of publications [14, 15, 18, 20, 22]. In the literature cited the interested reader will also find details of both the ε-determination from differential turgor pressure changes and of the corresponding cell volume changes and of the L_p-calculation from the cell turgor pressure relaxation process. The pressure inside was changed by injection of cell sap using the pressure probe.

Results

Stationary Cell Turgor Pressure-Volume Characteristic

Graves and Gutknecht [5] determined cell turgor pressure-volume curves in perfused cells of *H. parvula* by applying hydrostatic pressure to the cell interior. The final stationary volume established at a given internal hydrostatic pressure was measured under the light microscope. In our experiments the cell turgor pressure was recorded by inserting the microcapillary of the pressure probe (tip diameter of about 50 µm) into the intact cell. The turgor pressure was increased by addition of distilled water and decreased by addition of a 3 mol/liter NaCl or sucrose solution. The resulting external osmotic pressure was measured cryoscopically. The outside osmolarity was varied within a range of 750 to 1500 mosmol/liter, i.e., the osmotic pressure in the bathing solution was changed between 19 and 37 bar. The cell volume was determined by measuring the cell dimensions under the light microscope or by taking photographs of the cell and measuring the cell dimensions from the photographs. A typical pressure-volume characteristic is shown in Fig. 1. It is evident that there is a linear relationship between the cell turgor pressure and the corresponding cell volume in the stationary state over a pressure range of 0.05 to 0.9 bar (correlation coefficient $r = 0.99$) [5]. Different symbols are used to indicate measurements performed either at increasing or decreasing turgor pressures. Within the limits of accuracy of volume measurements of about $\pm 5\%$ it was not possible to detect any hysteresis effects in the function $V = f(P)$ for either rising or falling cell turgor pressure. The value of the long-term (stationary) elastic coefficient denoted by ε_s can be calculated from the slope dP/dV (*see* Eq. (1)), taking into account the cell volume at a given cell turgor pressure. The subscript s is used to indicate that this coefficient has been calculated from measurements in the stationary state.

In Fig. 2 (curve *a*) ε_s is plotted as a function of cell turgor pressure (using the data from Fig. 1). The slight increase in the ε_s-values with increasing turgor pressure is due to the fact that the cell volume changes by about 75% in the pressure range between 0.05 to 0.9 bar. In more than 6 independent experiments ε_s was found to have an average value of about 0.5 to 2 bar.

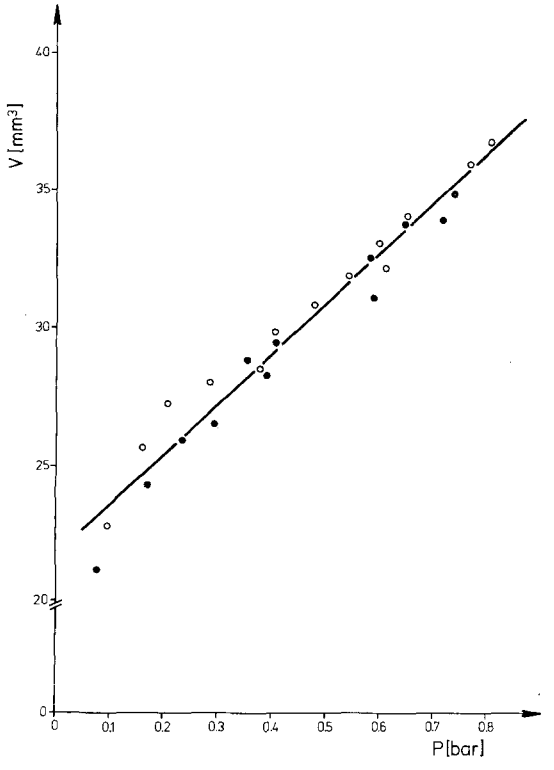

Fig. 1. The stationary cell turgor pressure-volume characteristic of a *Halicystis parvula* cell. The cell turgor was lowered by addition either of NaCl or sucrose to the seawater bathing the cell (●) or it was raised by the addition of distilled water (○). Each time a new stationary state had been established the cell volume was determined by measuring the cell dimensions under the light microscope. The cell turgor pressure was measured with the pressure probe. Within the limits of accuracy of ±5% for the volume determination there was no evidence of hysteresis for rising or declining cell turgor pressure. The correlation coefficient of the straight line is $r=0.99$.

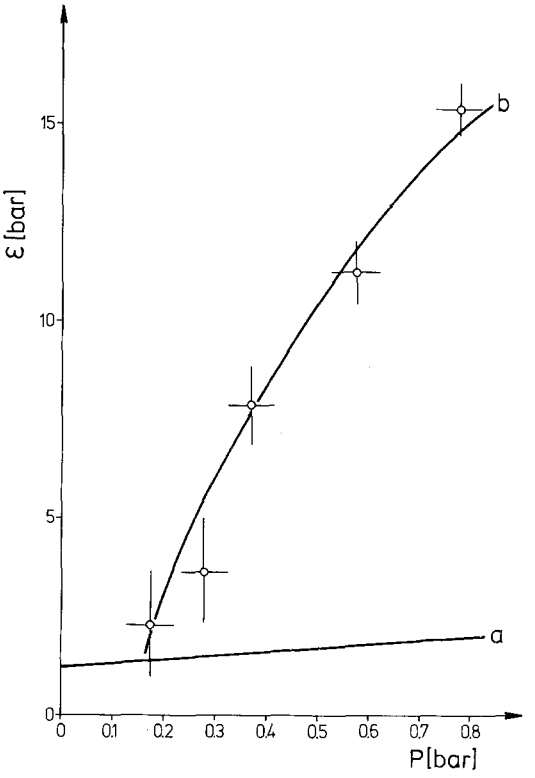

Fig. 2. Dependence of the long-term and short-term elastic coefficient of *Halicystis parvula* on cell turgor pressure. (Curve *a*): According to Eq. (1) the long-term (stationary) elastic coefficient, ε_s, can be calculated from the slope of the function $V=f(P)$ as shown in Fig. 1 at a given volume, V. Data from Fig. 1 were taken for the calculation of the function $\varepsilon_s=f(P)$. Note that ε_s is almost constant over the entire pressure range. (Curve *b*): Dependence of the short-term elastic coefficient, ε, on the cell turgor pressure determined by measuring the changes in turgor pressure, ΔP_I, and the corresponding changes in cell volume, ΔV, using the pressure probe technique. At various cell turgor pressure values ε was determined over a pressure interval denoted by horizontal bars. The measurements shown were performed on the same cell shown in Fig. 1. The curve indicates that the short-term elastic coefficient, ε, is strongly dependent on cell turgor pressure, as also observed for other giant algal cells [22]. The vertical bars represent the SD of the ε-measurement.

Determination of ε by Short-Term Changes in Cell Turgor Pressure and Volume

The volumetric elastic modulus, ε (short-term elastic coefficient), as defined by Eq. (1) was determined from differential pressure and volume changes induced and recorded directly with the pressure probe. The changes in cell volume were executed within 2 to 4 sec. Thus, the changes in cell volume were so rapid in comparison to the half-time of water exchange (*see* below) that we can assume that the induced volume changes are not affected by the onset of water flow. Cell volume changes generated by the pressure probe technique were between 0.1 to 5% of the total cell volume, both in shrinking and in swelling experiments. The corresponding instantaneous pressure changes were of the order of 30 to 300 mbar. Larger pressure changes lead to irreversible destruction of the cell. Measurements of the short-term elastic coefficient, ε, as a function of cell turgor pressure are shown in Fig. 2 (curve *b*). The experiments were performed on the same cell as in Fig. 1. At very low pressure values (about 100 mbar) both the short-term ε-value and the long-term (stationary) ε_s-value assume the same value of about 1 to 2 bar, whereas towards higher pressure values the short-term elastic coefficient, ε, increases much more rapidly than the long-term elastic coefficient, ε_s. This statement is in agreement with similar findings in other giant algal and higher plant cells [9, 16, 18, 21–23]. ε assumes a value of up to 15 bar at a cell turgor pressure of 0.8 bar. Similar results were obtained from eight other

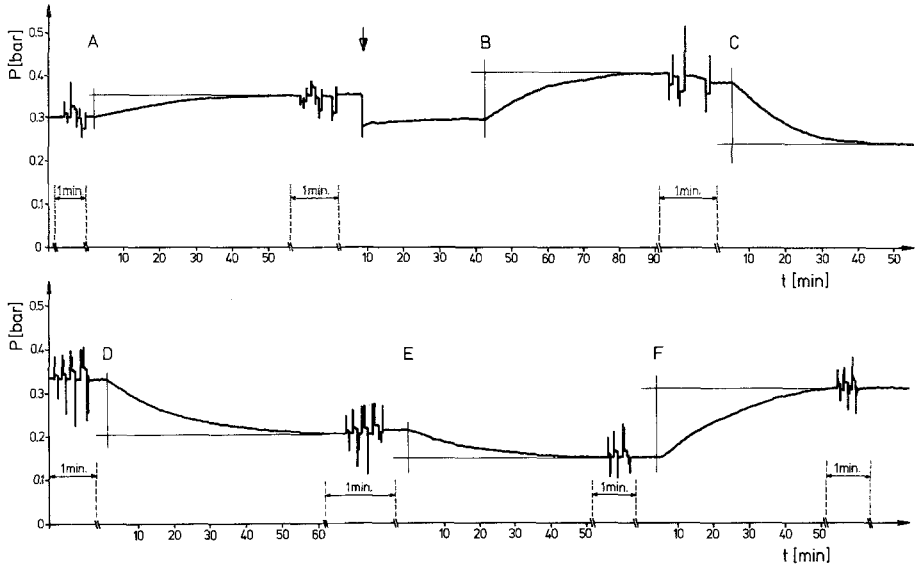

Fig. 3. Time courses of cell turgor pressure changes in *Halicystis parvula* following changes in the osmolarity of the external solution. The turgor pressure relaxation processes were induced either by the addition of various amounts of distilled water (curve *A*, *B*) or sucrose (curve *C*, *D*, *E*) to the seawater or by replacing these solutions by seawater (curve *F*). Measurements of the volumetric elastic modulus were interspersed between the turgor pressure relaxation processes, as indicated in the figure. The spikes on the top of the pressure pulse injected into the cell for ε-measurement result from shock-wave pressures. This effect is of no consequence for the ΔP_I-determination since the ΔP_I-values are calculated from the plateau values. The arrow indicates an experiment in which a negative pressure pulse was injected into the cell. Under these conditions no significant turgor pressure relaxation is observed due to the high extensibility of the cell wall. The measurements presented were all performed on the same cell.

independent experiments. The ε-value at 0.9 bar in these experiments varied between 6 and 16 bar. It should be noted that the pressure measurements at very low pressure values are subjected to an error of about ± 15 mbar because of the limits of resolution imposed by the pressure transducer. This error is small when compared with the absolute values of 1 to 2 bar (at 0.05 bar cell turgor pressure) and 6 to 16 bar (at a cell turgor pressure of 0.9 bar). The possibility of artifacts caused by impaling the cell with the microcapillary can definitely be excluded, because the ratio of the intact membrane area to the area injured by insertion of the microcapillary was more than 10,000. Recent experiments on ligatured internodes of *Chara corallina* with various cell volumes using different tip diameters of the capillary have shown that this ratio is too large to have any disruptive influence on the measurement of the elastic coefficient and the hydraulic conductivity [19]. A further argument against the presence of leakages, particularly in the low pressure range, was provided by the experimental evidence that the pressure in the cell can be lowered to 0.05 bar and returned to higher values by addition of distilled water. Any leakages that did occur occasionally were reflected in an instantaneous pronounced drop in pressure.

As we have already pointed out elsewhere [20], the pressure probe is very sensitive to leakages in comparison with methods using the electrical potential as an indicator for irreversible changes in membrane structure.

Determination of the Hydraulic Conductivity from the Turgor Pressure Relaxation

In order to induce water flows across the cell membrane of *H. parvula* cells the external osmolarity was varied by the addition of NaCl, sucrose, or distilled water and by subsequent replacement of the solution of a higher or lower osmolarity by seawater. The time course of the cell turgor pressure relaxation process was monitored continuously with the pressure probe. Figure 3 shows typical turgor pressure relaxation curves recorded from a cell in response to alternating external osmotic conditions. Each time a stationary condition was established measurements of the volumetric elastic modulus (short-term elastic coefficient), ε, were performed, as illustrated in Fig. 3. Further for each stationary state the cell volume was determined by measuring the cell dimensions with the microscope. From the measured volume and turgor pressure differences, ΔV and ΔP, respectively, between two stationary states, the long-term (stationary) elastic coefficient, ε_s, can be calculated (*see* above). The values are in agreement with those obtained from the stationary pressure-volume characteristics.

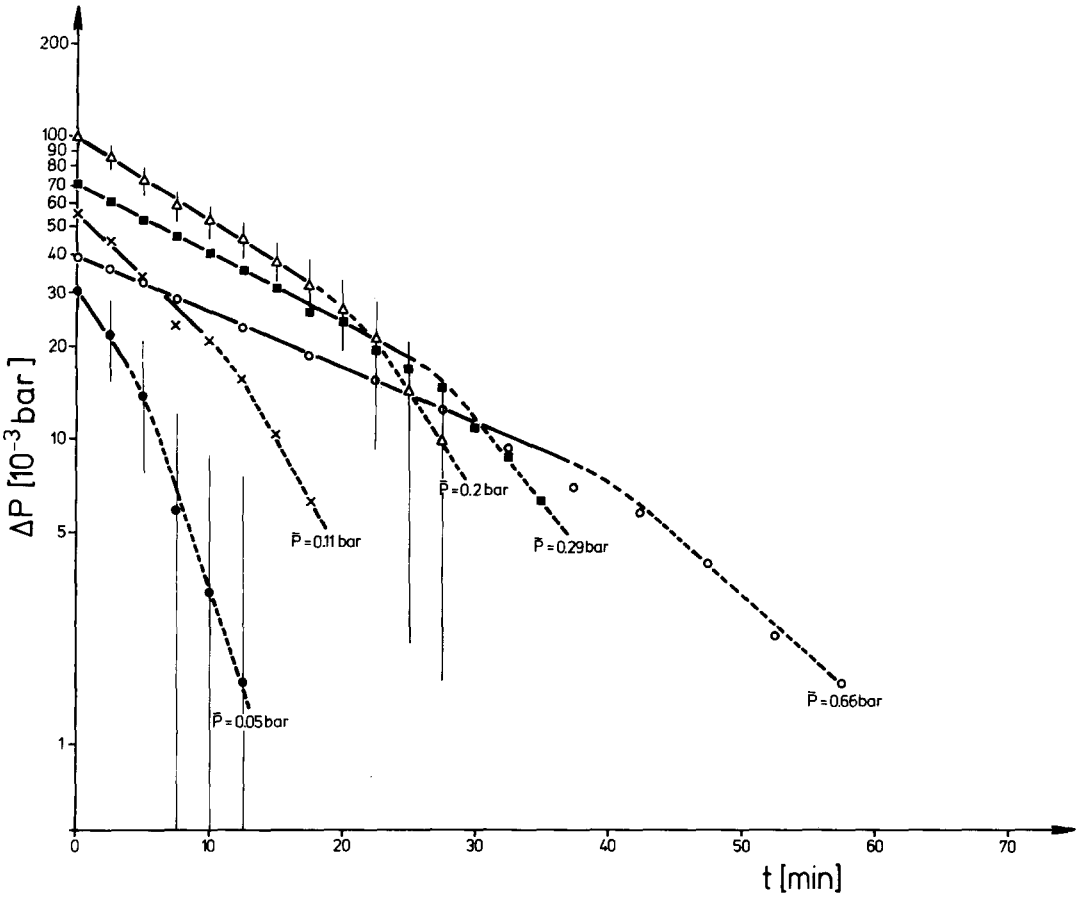

Fig. 4. Semilogarithmic plot. It is evident that the turgor pressure relaxation processes cannot be represented by a single exponential function over the whole time range. This is true regardless of the pressure range in which the experiment was performed. The straight lines representing the turgor changes after the onset of turgor relaxation were calculated with the method of least squares. The half-time for the initial phase is of the order of 5 to 15 min, depending on the turgor pressure range. The corresponding "half-time" for the subsequent water transport phase (denoted by dotted lines) is in the range of 3 to 8 min. The vertical bars drawn only for two curves indicate the error in the pressure determination.

In contrast to analogous experiments on cells of other giant algal cells (20–22), it is not possible to induce a turgor pressure relaxation process by shifting the cell sap/oil boundary in the tip of the capillary thus changing the cell turgor pressure directly. The arrow in Fig. 3 indicates an experiment in which a negative pressure pulse was injected into the cell by means of the pressure probe. It is obvious that due to the high extensibility of the cell wall of *H. parvula* only a very slight turgor pressure change can be induced under these experimental conditions. It is not possible to calculate the half-time of water transport from such small pressure changes. Thus, the only way to induce a measurable water flow, i.e., a turgor pressure relaxation of cells of *H. parvula*, is to change the external osmolarity. Figure 4 shows semilogarithmic plots of pressure relaxation curves performed in different pressure intervals. It is immediately obvious that the turgor pressure relaxation processes cannot be described by a single exponential curve over the entire regulatory range. Figure 4 shows that the time taken for water transport seems to decrease with time.

For the first 10 to 40 min after onset of water flow, the pressure relaxation curve can be approximated by an exponential curve; the half-time is about 5 to 15 min, depending on the turgor pressure range. After this initial phase the slope changes continuously until after 40 to 50 min the turgor pressure relaxation process proceeds almost exponentially with a half-time of about 3 to 8 min. However, as indicated by the error bars (which consider the resolution of the pressure probe which tends to decline, particularly towards the end of relaxation when the new stationary state is reached) it seems to be very likely that this "second exponential phase" does not reflect the true time course of pressure relaxation in this time interval due to the limitation in the resolution of the pressure probe.

Assuming that the first exponential curve of the

turgor pressure relaxation process reflects water transport and that salt transport during this phase does not occur [18, 22] the hydraulic conductivity, L_p, can be calculated from the rate constant or from the half-time of the water exchange, $T_{1/2}$, according to the following equation [3, 4, 18, 22]:

$$L_p = \frac{V}{A}\frac{\ln 2}{T_{1/2}}\frac{1}{\varepsilon + \pi_0^i} \tag{2}$$

where V = cell volume, A = cell surface area, and π_0^i = internal osmotic pressure determined by assuming that the cryoscopically measured osmotic pressure in the bathing solution is nearly identical to the internal one due to the low turgor pressure values of *H. parvula.*

The volume, V, and the membrane area, A, are functions of cell turgor pressure because of the large volume changes undergone by the cells of *H. parvula.* On the other hand, the ratio V/A can easily be shown to vary by only 15–20% over the whole turgor pressure range of 0.05 to 0.9 bar. In a pressure interval of about 0.1 bar in which the half-time of water transport is usually determined, we thus can assume V/A to be constant.

The knowledge of the internal osmotic pressure is not required when calculating the L_p-value from the instantaneous water flow and turgor pressure change dP/dt, respectively, after the onset of the relaxation process, using the following equation [3, 22]:

$$L_p = \frac{V}{A}\frac{1}{\varepsilon}\frac{1}{\sigma \Delta\pi}\left(\frac{\Delta P}{\Delta t}\right) \quad (t=0) \tag{3}$$

where $\Delta\pi$ is the induced change in the external osmolarity and σ is the reflection coefficient of the solute used to change external osmolarity.

Measurements with ^{14}C-surcose showed that the membrane of *H. parvula* is virtually impermeable to sucrose, i.e., that $\sigma_{\text{sucrose}} \approx 1$. Since the changes in osmolarity brought about by the addition of NaCl lead to the same pressure changes as the addition of the equivalent amount of sucrose, we may conclude that σ_{NaCl} is also 1.

Calculation of L_p-values from the semilogarithmic plots shown in Fig. 4 using both Eqs. (2) and (3) leads to comparable results. L_p is calculated to be about $1.5\text{–}2.5 \times 10^{-6}$ cm sec^{-1} bar^{-1} in the pressure range of 0.3 to 0.6 bar both for water efflux or influx when using the short-term elastic coefficient ε. A polarity in water flow is not observed within the limits of accuracy. The values are in the range reported by Gutknecht et al. [8]. Towards very low pressures (close to the plasmolytic point) L_p increases (*see* Fig. 5) as it is known to do for other giant algal cells

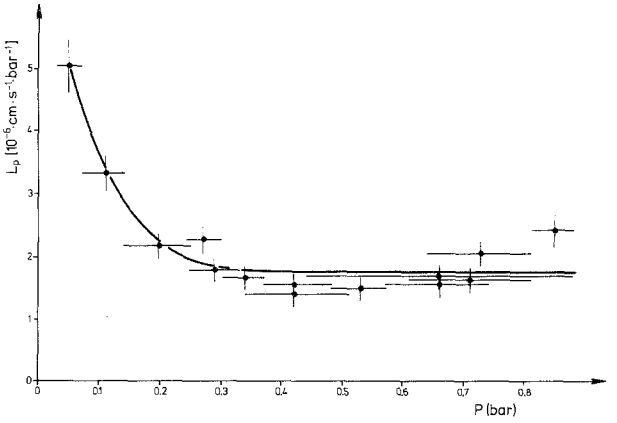

Fig. 5. The dependence of the hydraulic conductivity, L_p, of the cell membrane of *Halicystis parvula* on the turgor pressure, P. The individual values for the hydraulic conductivity were calculated from the initial exponential phase of the turgor relaxation process, using Eqs. (2) and (3). For further details, *see* text. The L_p-values increase as the plasmolytic point is approached. This is also found to be the case in some other algal species [18, 22].

[18, 19, 21, 22]. The increase in L_p may be caused by a coupling to solute flow [22].

The large volume changes of *H. parvula* allow, in contrast to other giant algal cells, the direct measurement under the light-microscope of the rate of volume change (dV/dt) as a function of the change in the external osmotic pressure. Therefore, it is possible to calculate the value of the hydraulic conductivity independently of measurements of the turgor pressure relaxation process. This is achieved by following the initial rate of swelling or shrinking of the cell volume under the microscope in response to a change in the outside osmolarity, $\Delta\pi$, described by the following equation [3, 15]:

$$L_p = \frac{1}{A}\frac{1}{\sigma \Delta\pi}\left(\frac{\Delta V}{\Delta t}\right) \quad (t=0) \tag{4}$$

which is identical with Eq. (3), but contains $\Delta V/\Delta t$ instead of $\Delta P/\Delta t$. In the calculation of the hydraulic conductivity of the cell membrane using Eq. (4) it is not required to know the value of the volumetric elastic modulus of the cell wall.

Combined measurements of both the volume change, ΔV, and the cell turgor pressure change, ΔP, as a function of the time on a given cell after the onset of osmoregulation in the pressure range of about 0.3 to 0.8 bar are shown in Fig. 6. The semilogarithmic plot of the function $\Delta V = f(t)$ (*see* inset) demonstrates that the change in cell volume with time is exponential over the whole time range (within the limits of accuracy) indicating that the "second exponential phase" of turgor pressure relaxation (*see* Figs. 4 and 6) can be traced back to the inaccuracy of

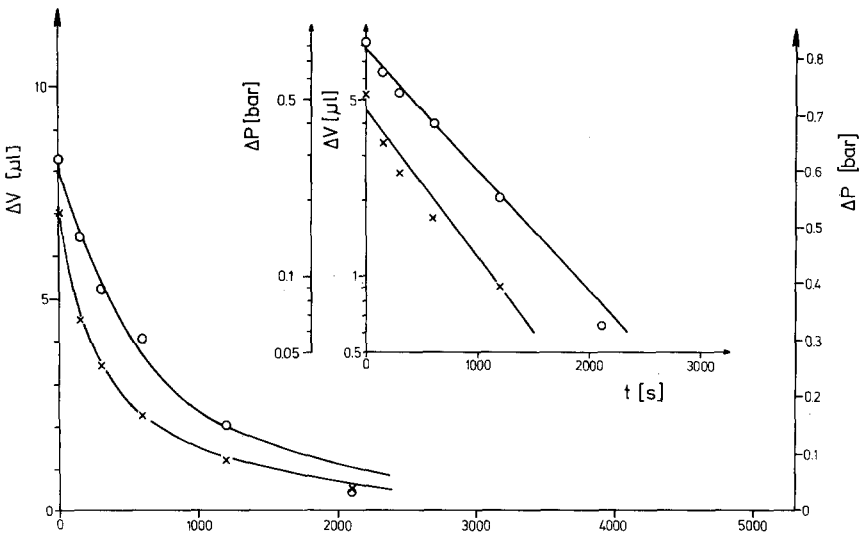

Fig. 6. Combined measurement of turgor pressure and cell volume relaxation of a cell of *Halicystis parvula* (cell volume 36.6 µl, surface area 0.62 cm^2) induced by a change of about 13 bar in the external osmolarity by addition of a 3 mol/liter NaCl solution to seawater. Semilogarithmic plot of the cell volume and turgor pressure change with time yields a straight line within the limits of accuracy (*see* inset and text). The turgor pressure values are denoted by crosses, the cell volume values by circles. From the initial rate of volume change $dV/dt = 1.05 \times 10^{-5}$ cm^3 sec^{-1}, the hydraulic conductivity, L_p, is calculated to be 1.25×10^{-6} cm sec^{-1} bar^{-1} using Eq. (4). From the initial change in turgor pressure $dP/dt = 1.7 \times 10^{-3}$ bar sec^{-1} the same L_p-value is calculated according to Eq. (3) when the short-term elastic coefficient, $\varepsilon = 6$ bar, is used.

the pressure measurement at these small pressure changes, as mentioned above. The hydraulic conductivity calculated from the measured initial water flow (i.e., volume change) using Eq. (4) assumes a value of 1.26×10^{-6} cm s^{-1} bar^{-1}, which is only identical to that value calculated from the turgor pressure relaxation when using the short-term elastic coefficient, $\varepsilon = 6$ bar. On the average, the L_p-values calculated for the initial volume change were found to be between 0.8 to 2×10^{-6} cm sec^{-1} bar^{-1}.

Discussion

The results reported here indicate that the value of about 0.6 bar for the long-term (stationary) elastic coefficients of cells of *H. parvula* reported by Graves and Gutknecht [5] is of the right order of magnitude. However, there is evidence that this parameter does not reflect the pure elastic properties of the cell wall but includes also other mechanical properties. On the other hand, according to Eq. (1) the short-term elastic coefficient, ε, determined from differential turgor pressure and volume changes represents the intrinsic elastic coefficient of the cell wall. The two different coefficients have different pressure dependencies. The short-term elastic coefficient, ε, increases with increasing turgor pressure, whereas the long-term coefficient, ε_s, remains virtually constant over the entire experimentally accessible cell turgor pressure range (*see* Fig. 2*a* and *b*). Although the two ε-values hardly differ at a turgor pressure of about 0.1 bar, the short-term elastic coefficient, ε, is larger than the long-term coefficient, ε_s, by about one order of magnitude at about 0.9 bar. At the present, we have no information which mechanical properties influence ε_s. A contribution of visco-elastic properties may be excluded, since no hysteresis was observed when measuring the pressure-volume curve and volume changes were reversible. Recalculation of data reported by Kamiya et al. [10] and Tazawa and Kamiya [17] for isolated, mercury-filled cell walls of Characean cells demonstrates that the absolute values of both coefficients also differ in this species. Figure 7 shows that the short-term elastic coefficient, ε, (determined from rapid pressure and volume changes) increases significantly towards higher pressure values in comparison with the long-term ε_s-value determined from stationary measurements, which remains practically constant over the entire pressure range (0 to 6 bar).

The short-term elastic coefficient, ε, determines, along with other parameters (i.e., hydraulic conductivity, L_p, and cell geometry, V/A), the magnitude of the instantaneous water flow across the membrane-wall barrier. This conclusion can be drawn from the agreement of the values of the hydraulic conductivity, L_p, derived from direct measurements of both the volume and turgor pressure changes with time in response to osmotic stress. Care has to be taken, therefore, if values of the elastic coefficient obtained by different methods are discussed with respect to their relevance to the water exchange of cells with their environment [5, 22]. The short-term elastic coefficient also controls the overall turgor pressure relaxation process provided that the half-time of water exchange is short enough (say in seconds). Under these conditions the visco-elastic properties of the cell wall will not contribute to the turgor pressure-cell volume changes. This is the case for Characean cells [22]. In cells of *Valonia utricularis* on the other hand, which exhibit a

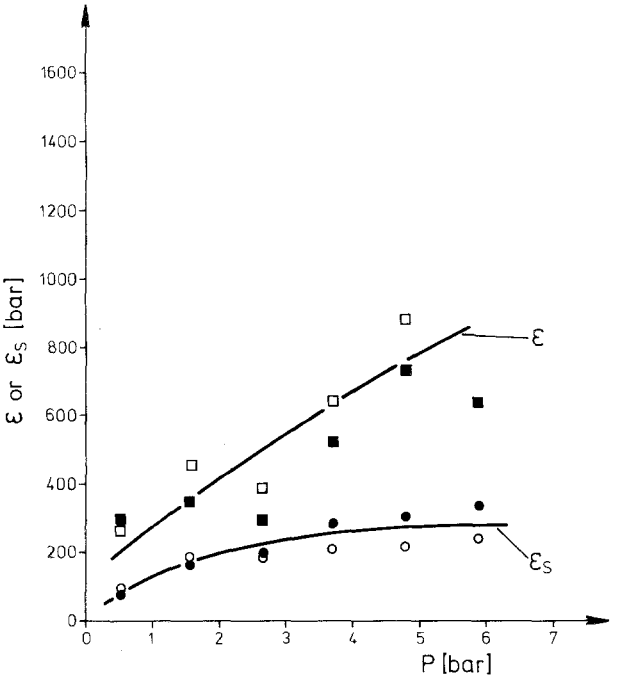

Fig. 7. The function $\varepsilon = f(P)$ (from instantaneous volume changes) and $\varepsilon_s = f(P)$ (from retarded volume changes) for an isolated, mercury-filled cell wall tube of *Nitella flexilis* (length: 22 mm; diameter: 0.51 mm). The values are recalculated from data measured by Kamiya et al. [10]. □ and ■: By analogy with the pressure probe measurements, the volume changes, ΔV, were measured instantaneously after a change in turgor. ε increases with increasing pressure. ○ and ●: Volume changes measured 20–30 min after a pressure change. The ε_s-values for stationary wall extension are almost constant over the whole range of turgor pressure. They are considerably lower than the corresponding ε-values. Turgor pressures were changed stepwise (in steps of ~1 bar) of either increasing (□, ○) or decreasing (■, ●) turgor. ε and ε_s-values are plotted over the width of pressure intervals.

half-time of 3 to 7 min for water exchange comparable with that of *H. parvula*), no differences between the two elastic coefficients were found, indicating that the mechanical properties of the cell wall of this species only comprise elastic ones [20].

The short-term elastic coefficient, ε, measured differentially in cells of *H. parvula* are of the same order of magnitude or even smaller than the corresponding ε-values reported for higher plant cells [9, 22, 23]. In contrast, the short-term ε-values of pond water and marine giant algal cells are considerably higher; they are generally in the range between 100 to 700 bar. The low ε-values imply that cells of *H. parvula* primarily change their volume rather than their turgor pressure in response to osmotic stress. For this reason, and this has been shown to be the case experimentally, a turgor pressure relaxation process in *H. parvula* cannot be induced by means of a direct change in turgor pressure inside the cell by means of the pressure probe (*see* Fig. 3). This is in contrast to other giant algal and higher plant cells [9, 14, 20, 21].

Table 1.

Cell	P_0 [bar]	P_I [bar]	ΔP_I [bar]	π_0^i [bar]	$\varepsilon, \varepsilon_s$ [bar]	P_F [bar]
V. utricularis[a]	2.0	2.2	0.2	25	200	2.02
		4.0	2.0	25	250	2.18
C. corallina[a]	4.0	4.2	0.2	6	400	4.00
		6.0	2.0	6	500	4.02
H. parvula	0.5	0.8	0.3	25	1	0.79

Dependence of the final internal cell turgor pressure, P_F, on the elastic properties of the cell wall after an induced pressure pulse with the pressure probe technique, ΔP_I, into several giant algal cells. The values are calculated using Eq. (7).

[a] Data were taken from Zimmermann et al. [19, 20].

The final turgor pressure in the cells to be expected under these conditions can be estimated with relative ease. If the original turgor pressure, P_0, is changed to an initial pressure, P_I, by the injection of a pressure pulse, ΔP_I, the relationship between the initial stationary osmotic pressure in the cell π_0^i, and the final value, π_F^i, when a new state of equilibrium has been reached, is expressed by [3, 21]:

$$\pi_F^i = \pi_0^i \left(1 + \frac{\Delta V}{V}\right) \tag{5}$$

where $\Delta V = V_0 + \Delta V_I - V_F$, V_0 = original stationary volume, V_F = final stationary volume, and ΔV_I = induced volume change. This equation holds for ΔV_I, $\Delta V \ll V$.

The relative volume change, $\Delta V/V$, is correlated to the cell turgor pressure change, ΔP, by the long-term elastic coefficient, ε_s, in *H. parvula* and by the short-term elastic coefficient, ε, in *Valonia* and Characean cells (*see* above). Therefore Eq. (5) can be written as

$$\pi_F^i = \pi_0^i \left(1 + \frac{\Delta P}{\varepsilon}\right) \tag{6}$$

with $\Delta P = P_0 + \Delta P_I - P_F$ and ΔP, $\Delta P_I \ll \varepsilon$.

With the assumption that the osmotic pressure in the surrounding solution, π_E, is constant, the final cell turgor pressure, P_F, after turgor pressure relaxation has been completed can be calculated using Eq. (6) and the steady-state conditions $P_0 = \pi_0^i - \pi_E$ and $P_F = \pi_F^i - \pi_E$ as a function of the induced pressure change, ΔP_I, providing that the reflection coefficient of the solutes is nearly unity.

$$P_F = P_0 + \frac{\pi_0^i}{\varepsilon + \pi_0^i} \Delta P_I. \tag{7}$$

For giant algal cells such as *V. utricularis* and *C. corallina* which exhibit a high ε-value [22], P_F takes on the values shown in Table 1 for typical pressure pulses of 0.2 to 2 bar. In the calculation it is assumed that for *Valonia* π_0^i was about 25 bar while a π_0^i-value of about 6 bar is assumed for Characean cells. The data demonstrate that the final turgor pressure, P_F, established in the cells after reaching a new stationary state is very close to the original turgor pressure value, P_0, regardless of the amplitude of the pressure pulse.

In contrast, the P_F-value for cells of *H. parvula* are close to the P_I-value, i.e., the initial turgor pressure value established by injection of a pressure pulse using the pressure probe. Calculations were carried out under the assumption that ΔP_I does not exceed the maximum of about 0.3 bar because in this event the cell would burst (*see* above). This result is immediately evident from Eq. (7) because P_F becomes approximately equal to P_I (when the values for the short-term or long-term elastic coefficient are very small). We thus can conclude that the cells of *H. parvula* predominantly exhibit a cell volume relaxation in response to osmotic stress in the water transport phase of osmoregulation [18]. The internal osmotic pressure, in turn, changes so considerably during volume changes that a new quasi-stationary condition between the osmotic pressure difference on the one hand and the cell turgor pressure on the other hand is achieved after a relatively small turgor pressure change. This behavior is similar to that found in cells without walls and in some cells of higher plant tissues [9, 18, 23] and demonstrates that there is a continuous transition from turgor to volume relaxation [18].

Further inspection of Eq. (7) shows that, generally speaking, the changes in cell turgor pressure are independent of the external osmotic pressure, π_E, provided that π_E remains unchanged during the relaxation process. This condition is always fulfilled experimentally with giant algal cells suspended in a large volume of solution. For cells in higher plant tissue, it would appear that this condition might not be fulfilled. However, by means of the nonminiaturized and miniaturized pressure probe techniques [9, 18, 22] it has been possible to demonstrate in a variety of tissue cells, that no pressure change is registered in a neighboring cell when the turgor pressure in a given cell is changed by the injection of a pressure pulse [22]. This finding permits us to conclude that changes in the osmotic pressure in the apoplasmic space are so small during water flow across the cell membrane that in practice the osmotic pressure in the apoplasmic space can be assumed to be constant. Using Eq. (2) derived for isolated cells in a large environmental compartment and assuming that the cells exchange water across their entire surface, the hydraulic conductivity of the cell membrane, L_p, may be estimated to a good approximation from the turgor pressure relaxation process induced in a single cell of a higher plant tissue by the injection of a pressure pulse.

On the other hand, it should be noted that, in the light of the arguments presented above, the hydraulic conductivity of an individual cell in plant tissues cannot be measured in response to osmotic pressure changes in the bathing solution since under these conditions osmotic pressure changes occur both in all tissue cells and in the apoplasmic space. In addition to these difficulties, the effective osmotic pressure near a cell situated deep within the tissue may not be known with accuracy as changes of the osmotic pressure in the bathing medium require time to diffuse through the tissue. This delay may, in some cases, be longer than the half-time of turgor pressure relaxation. On the other hand, comparative measurements of both osmotically and hydrostatically induced turgor pressure relaxation processes measured in a single tissue cell would yield information on the transport pathways in the tissue if the hydraulic conductivity of individual tissue cells were determined initially by injecting pressure pulses.

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