Theoretical and Experimental Exclusion of Errors in the Determination of the Elasticity and Water Transport Parameters of Plant Cells by the Pressure Probe Technique\textsuperscript{1, 2}

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ABSTRACT

The volumetric elastic modulus of the cell wall and the hydraulic conductivity of the cell membranes were measured on ligatured compartments of different sizes of Chara corallina internodes using the pressure probe technique. The ratio between intact cell surface area and the area of puncture in the cell wall and membrane introduced by the microcapillary of the pressure probe was varied over a large range by inserting microcapillaries of widely varying diameters in different sized compartments. The relationship of the elastic modulus and the hydraulic conductivity to turgor pressure was independent of the ratio of intact cell surface area to the area of injury. The increase in the hydraulic conductivity below 2 bar turgor pressure and the volume dependence of the elastic modulus were shown to be the same as those observed in intact nonligatured cells. Theoretical considerations of the possible influence of injury of the cell wall and cell membrane around the inserted microcapillary on the measurement of the water transport and cell wall parameters do not explain the experimental findings. Thus, mechanical artifacts, if at all present, are too small to account for the observed dependence of the hydraulic conductivity and the elastic modulus on turgor pressure. The pressure probe technique thus represents an accurate method for measuring water transport parameters in both giant algal cells and in tissue cells of higher plants.

The analysis of the kinetics of swelling and shrinking of plasmolyzed protoplasts is one of the classical methods for the measurement of the hydraulic conductivity of the membranes of higher plant cells (1, 11). Zimmermann and Steudle (13, 19, 21) cast doubt on the accuracy of this method when they were able to show, by way of a direct measurement of the turgor relaxation process, that the hydraulic conductivity of membranes of some turgid giant algal cells increases markedly in the low pressure range, on approaching the plasmolytic point. In Characean cells, this increase was observed at pressures below 2 bar (normal turgor, 4.5–6 bar), that is in a pressure range in which the cell membrane does not normally become detached from the cell wall (19, 21). These results suggest that the $L_p$ values of a plasmolyzed cell may differ from those of a turgid plant cell. However, in the bladder cell of Mesembryanthemum crystallinum, $L_p$ is pressure-independent over the whole pressure range (12). Palta and Stadelmann (8) also recently reported that the $L_p$ value was constant over a wide range of pressures in Allium cepa epidermis cells, using the plasmolytic method, and expressed doubts about the measurements obtained with the pressure probe technique developed by Zimmermann and Steudle (18, 22). They argued that mechanical injury to the cell membrane and cell wall could arise from the introduction of the microcapillary of the pressure probe or from the application of a pressure pulse in pressure ranges close to the plasmolytic point. In order to investigate this issue, we carried out a closer theoretical and experimental examination of the possible interferences caused by elastic changes of the cell wall and leakage of the microcapillary on the determination of both the half-time for water exchange and $\epsilon$ values from turgor relaxation processes. In this communication we report $L_p$ measurements on different sized ligatured internodes of Chara corallina in which microcapillaries of varying tip diameters were inserted. The ratio of intact cell surface to the area of a possible injury was varied over a wide range.

Both experiments upon and theoretical analysis of the possible influence of mechanical injury of the cell wall and cell membrane on the determination of the water transport parameters revealed that such mechanical artifacts, if at all present, are so small that they are not measurable. Mechanical artifacts, therefore, cannot be held in any way responsible either for the observed increase in the values of the hydraulic conductivity at low pressures or the pressure dependence of the volumetric elastic modulus of the cell wall in this species. Thus, the half-time for water exchange measured with the pressure probe technique reflects the true values for water transport between cell and environment.

MATERIALS AND METHODS

C. corallina cells originally obtained from Sydney, Australia, were cultivated in artificial pond water for several months. The cells were freed from neighboring internodes and then kept in artificial pond water containing 0.1 mm KCl, 1 mm NaCl, 0.1 mm CaCl$_2$, and 0.1 mm MgCl$_2$ (pH about 5.6) (13) for at least 24 hours. Internodes approximately 6 to 8 cm long were ligatured with a silk thread into different sized compartments. The volume and surface of the two ligatured compartments on a given cell were both varied over a wide range. A pressure probe was inserted into each of the compartments. The pressure probe technique has been described and reviewed in detail elsewhere (16, 17, 22).

The hydraulic conductivity is determined from the half-time, $\tau$, of the turgor relaxation process initiated either osmotically or most commonly hydrostatically by means of the pressure probe. The half-time is given by equation 1:

$$\tau = \frac{\ln 2 \cdot V_c}{A_c \cdot L_p \cdot (E + \Pi t)}$$

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where \( V_c \) = volume of the cell, \( A_c \) = area of the cell surface, \( \Pi \) = internal osmotic pressure, and \( \epsilon \) = volumetric elastic modulus of the cell wall.

The volumetric elastic modulus describes the elasticity of the cell wall and is defined by the following equation introduced by Philips (9):

\[
\epsilon = V_c \frac{d \Pi}{dV}
\]

The elastic modulus was determined in independent experiments by injecting pressure pulses into the cell interior by means of the pressure probe. The pressure changes and the corresponding volume changes were monitored (19).

Interference by the elasticity (compressibility) of the measuring set-up has to be taken into account in order to calculate the accurate value for the volumetric elastic modulus of the cell wall. Taking the compressibility of the measuring set-up into consideration, the following equation holds (6):

\[
\Delta V_M = \Delta V_C - \Delta V_P
\]

The negative sign in this equation expresses that (due to compression) \( \Delta V_P \) decreases with increasing pressure, whereas \( \Delta V_M \) and \( \Delta V_V \) increase. Dividing this equation by \( \Delta \Pi \) and \( V_c \) and using the Philips equation (equation 2) yields after rearrangement:

\[
\epsilon_M = f \cdot \epsilon
\]

where the correction factor, \( f \), is defined by:

\[
f = \frac{\epsilon_P + V_P/V_c \cdot \epsilon}{\epsilon_P}
\]

\( \epsilon_M \) is the measured volumetric elastic modulus of the whole system and is defined by \( \epsilon_M = (V_c/\Delta V_M) \Delta F. \) \( \epsilon_M \) is of the order of 50 to 600 bar in the pressure range between 0 and 6 bar. \( \epsilon_P \) is the elastic modulus of the pressure probe which is defined by \( \epsilon_P = -(V_P/\Delta V_P) \Delta \Pi \) and determined in independent experiments to be about 6,500 bar; \( V_P \) is the volume of the pressure probe (about 10 \( \mu l \)), and \( V_c \) is the cell volume (about 3–40 \( \mu l \)). \( \epsilon \) is the volumetric elastic modulus of the cell wall (equation 2), and \( \Delta V_M \) is the measured volume change.

The error in the \( \epsilon \) determination was calculated to be about 10% by propagation of errors. The range of the data covers experiments both on intact and ligatured *C. corallina* internodes, and the correction factor, \( f \), was calculated to be from 0.76 to 0.99.

Whereas the values of the measured elastic modulus \( \epsilon_M \) have to be corrected to obtain the true \( \epsilon \) value of the cell wall, it should be noted that for the calculation of \( L_p \) the uncorrected values for the volumetric elastic modulus, \( \epsilon_M \), must be used (6), because the relaxation processes are controlled by the over-all system parameters including both cell-specific and apparatus parameters; thus the measured \( \epsilon_M \) value determines the rate of water exchange.

In order to achieve different extremes in the ratio of intact cell surface area to the area of possible injury resulting from the puncture by the microcapillary of the pressure probe, microcapillaries with very small tip diameters of about 5 to 10 \( \mu m \) were used for pressure manipulation in the larger part of the ligatured internodal cell, whereas microcapillaries introduced into the smaller compartment had tip diameters of about 150 to 200 \( \mu m \).

Two different methods were employed to test whether the ligature between the two internodal compartments was pressure-tight. One was by applying pressure pulses of differing magnitudes (up to 2 bar) to one of the internodal compartments with the pressure probe, and the other by injecting 5 mm EGTA \(^3\) dissolved in artificial isotonic cell sap containing 80 mm KCl, 30 mm NaCl, 5 mm CaCl\(_2\), and 5 mm MgCl\(_2\) (Sanders and Zimmermann, unpublished results) into one of the internodal cylinders with the pressure probe. EGTA causes the destruction of the cell membrane and leads to a complete loss of turgor pressure after several hours.

There was no change in the turgor in the adjacent compartment measured by the aid of a second pressure probe positioned in the adjacent compartment when the turgor pressure was changed in the other ligatured compartment under either of the above experimental conditions.

### RESULTS

#### A. Experimental Part

Figure 1A represents typical measurements of the volumetric elastic modulus of the cell wall, \( \epsilon \), of ligatured and intact internodal compartments of *C. corallina* cells. This shows the dependence of \( \epsilon \) on increasing turgor pressure. In the experiment the ratio, \( \beta \), of the cell surface area, \( A_c \), to the area of puncture (injury), \( A_n \), was 95 (\( \times \)), 200 (\( \bullet \), intact cell), and 10,000 (\( \circ \)). The area was calculated by measuring the radius, \( r \), and the length, \( l \), of the internode, and the radius, \( r_t \), of the microcapillary sticking through the cell wall and using the equations: \( A = \pi l (r + 2 t) \) and \( A_n = \pi r_t^2 \), for internode and puncture areas, respectively.

The typical increase of \( \epsilon \) with increasing pressure is independent of the ratio of the surface areas, \( \beta \) (Fig. 1A). The absolute values of \( \epsilon \) depend on the cell volume as has been demonstrated for intact cells of various species (17, 22). The absence of any influence of the size of the puncture on the \( \epsilon \) measurements is particularly evident when comparing the curves (denoted by \( \times \) and \( \circ \), respectively) (Fig. 1B). These measurements were performed on a given cell in which the two ligatured compartments were nearly equal in length and diameter, i.e., nearly equal in volume and surface area. The radius of the capillary tip was varied between 5 and 200 \( \mu m \). Both curves for \( \epsilon = f(\Pi) \) coincide as is expected when the volume dependence of \( \epsilon \) is experimentally eliminated in this way.

It can also be shown that the half-time of water flow, which is induced by changing the cell turgor by means of the pressure probe, is independent of the magnitude of the pressure change. In none of the pressure ranges (0–6 bar) in which it was determined, does the half-time show any dependence on magnitude and direction of the turgor changes (± 0.1 to ± 1 bar increase or decrease of cell turgor).

In Figure 2 the volume dependence of \( \epsilon \) is plotted from the data given partly in Figure 1 and from data not presented here. The values were taken at pressures between about 3.5 and 4 bar. The curve of \( \epsilon = f(\Pi) \) resembles curves obtained recently on intact cells of *Nitella flexilis*, *N. obtusa*, *M. crystallinum*, and *Capitatum annuum* (6, 12, 13). With higher plant cells the ratio, \( \beta \), between intact cell surface and area of puncture will be much greater (about 50–200) than that used in the experiments reported here.

The agreement between the values of \( \epsilon \) for ligatured cells of various sizes and intact cells of corresponding volume provides unequivocal evidence that the volume dependence does not arise from a change in the thickness of the cell wall with cell volume (or wall area in intact cells, since it can be assumed that the thickness of the cell wall does not vary within a given cell).

Figure 3A shows the dependence of the hydraulic conductivity on pressure in different ligatured cell compartments where the ratio, \( \beta \), was of the order of 50 and 10,000 and the corresponding volumes were 6.4 and 33.2 \( \mu l \), respectively. The values of \( L_p \) increase with decreasing pressure below 2 bar independently of the value of \( \beta \). In Figure 3B measurements on \( L_p \) were performed on the cell used in Figure 1B which was ligatured in two nearly identical compartments. There is no significant difference between the graphs of \( L_p \) as a function of pressure measured in the two compartments (Fig. 3B). This is expected since the geometric and

\(^3\) Abbreviation: EGTA: ethylene glycol bis(\( \beta \)-aminoethyl ether)-\( N,N' \)-tetraacetic acid.
FIG. 1. Dependence of volumetric elastic modulus, \( \varepsilon \), on cell turgor pressure, \( P \), in ligatured and intact cells of \( C. \) corallina. \( \text{Chara} \) cells were ligatured with a silk thread and divided into pressure-tight compartments. A: \( \varepsilon \) measurements on three different intact or ligatured cells with different volumes, \( V_c \), and different ratios, \( \beta \), of intact cell surface to the areas injured by the microcapillary of the pressure probe. (■): Intact cell, \( V_c = 18.9 \, \mu l, \beta = 200 \); (▲): ligatured cell compartment, \( V_c = 11.3 \, \mu l, \beta = 95 \); (○): ligatured cell compartment, \( V_c = 8.2 \, \mu l, \beta = 10,000 \). B: \( \varepsilon \) measurements in two pressure-tight compartments on the same cell with similar volumes, \( V_c \). In both compartments the values measured with different tip diameters of the pressure probe show the same dependence on cell turgor pressure. (▲): \( V_c = 17.5 \, \mu l, \beta = 10,000 \); (○): \( V_c = 18.9 \, \mu l, \beta = 200 \). Vertical bars represent the standard deviation of the \( \varepsilon \) measurement, horizontal bars are the pressure intervals in which \( \varepsilon \) was determined.

FIG. 2. Dependence of volumetric elastic modulus, \( \varepsilon \), on the cell volume, \( V_c \), both in intact and in ligatured cell compartments of \( C. \) corallina at a cell turgor pressure of \( P = 3.5 \) to 4 bar. Data are taken partly from Figure 1, A and B, and from experiments not presented here.

elastic parameters of the two cell compartments (ratio of the volume to surface area, cell wall thickness and elastic modulus, respectively) are completely identical.

A large number of analogous measurements with intact and ligatured cells has confirmed the finding (Fig. 3) that there is no observable dependence of the measured \( L_p \) values on the size of the puncture \( A_i \) caused by the microcapillary of the pressure probe. This is clear-cut evidence that the true half-time for water exchange between the cell and its environment is measured, and it follows that the pressure and volume dependence of \( \varepsilon \) is not
artificially induced by the pressure probe technique and further that the increase in the hydraulic conductivity at low pressures is not caused by leakage round the capillary.

The absolute value of \( L_p \) at higher pressure (1.2–1.6 \( \times 10^{-5} \) cm s\(^{-1}\) bar\(^{-1}\)) is in good agreement with the values determined by Dainty and Hope (3), and Dainty and Ginburg (2), but about 30\% lower than the values reported by Dainty et al. (4), although the same method, i.e. transectular osmosis, was used.

**B. Theoretical Considerations.** In this section the possible error in the determination of the function \( \epsilon = f(P) \), which might be caused by a change in the elastic properties of the membrane and wall around the size of puncture of the microcapillary, is estimated. If we make the plausible assumption that the elastic properties around the site of puncture of the microcapillary and those of the entire intact cell wall do not interfere, we can use the following equation for the measured volume change neglecting the correction term introduced by equation 4:

\[
\Delta V = \Delta V_c + \Delta V_i
\]

Dividing this equation by \( V_c \) and \( \Delta P \), and using the Philips equation (equation 2) yields:

\[
\frac{1}{\epsilon_M} = \frac{1}{\epsilon} \frac{V_c - V_i}{V_c} + \frac{1}{\epsilon_i} \frac{V_i}{V_c}
\]

\( \epsilon_i \) is the elastic modulus of the site of puncture around the microcapillary and \( V_i \) the corresponding operational volume of the site of injury. Equation 7 is based on the assumption that the disturbance caused by mechanical injury of the cell wall or the cell membrane could be described mathematically by a volume contribution, \( V_c \), and an elasticity, \( \epsilon_i \). It is also assumed that the ratio of the intact cell part to the injured part remains constant over the whole pressure range and that the dependence of the variables of the system on cell turgor pressure can be described by the variation of the elasticity. The mathematical description in terms of a pressure-dependent \( \epsilon_i \), i.e. a dependence of the elasticity of the injured cell part on turgor pressure, is analogous in its conclusions and equations to the description in terms of a pressure dependent value of \( \epsilon \), i.e. a dependence of the size of injury with turgor pressure.

In order to arrive at a first estimation of the magnitude of \( \epsilon_i \), we make the further assumption that:

\[
\frac{A_e}{A_i} \approx \frac{V_c}{V_i} = \beta
\]

from which it follows that:

\[
\epsilon_M = \frac{\beta \cdot \epsilon_i \cdot \epsilon}{\epsilon_i (\beta - 1) + \epsilon}
\]

If we further consider the worst case, that \( \epsilon \) is independent of cell turgor pressure whereas experimentally the \( \epsilon_M \) values are a function of pressure (see Fig. 1), it follows for the \( \epsilon_M \) value at \( P = 0.5 \) bar that:

\[
\epsilon_M = \alpha \cdot \epsilon
\]

The proportionality factor, \( \alpha \), is defined by the ratio of the extreme values of \( \epsilon_M \) at \( P = 0.5 \) bar and \( P = 6 \) bar measured experimentally.

Comparing this equation with equation 9 yields the following equation:
\[ \alpha = \frac{\beta \cdot \varepsilon_i}{\varepsilon_i (\beta - 1) + \varepsilon} \] (11)

and, respectively, if the equation is solved for \( \varepsilon_i \):

\[ \varepsilon_i = \frac{\alpha \cdot \varepsilon}{\beta - \alpha \cdot \beta + \alpha} \] (12)

at \( P = 0.5 \) bar.

With Characean cells, the tip diameter of the microcapillary of the pressure probe is usually 20 to 50 \( \mu \)m, thus \( \beta \) is of the order of 10\(^4\), \( \alpha \) is calculated to be about 0.2 using the corresponding values of \( \varepsilon_M \) at \( P = 0.5 \) and \( P = 6 \) bar (100 and 500 bar, respectively) (Fig., A and B).

Introducing these values into equation 12 yields:

\[ \varepsilon_i = 25 \cdot 10^{-6} \cdot \varepsilon \] (13)

at \( P = 0.5 \) bar. The average value for \( \varepsilon \) is of the order of 100 bar at \( P = 0.5 \) bar so that we can estimate the value of the elastic modulus around the area of puncture of the microcapillary to be of the order of 2.5 \( \times \) 10\(^{-3}\) bar.

This low value indicates that small pressure changes should lead to a pronounced change in the area of injury. Taking equation 2 into account the relative change in this area can be readily calculated to be:

\[ \frac{dA_i}{A_i} \approx \left( \frac{dV_i}{V_i} \right)^{2/3} = \left( \frac{dP}{\varepsilon_i} \right)^{2/3} \] (14)

and therefore \( \frac{dA_i}{A_i} \sim 54 \) when \( dP = 1 \) bar, and \( \varepsilon_i = 2.5 \times 10^{-3} \) bar.

Equation 14 states that a pressure change of 1 bar should increase the area of injury by a factor of 54 which would certainly cause the wall and the coupled membrane to tear. The resulting decrease in pressure would be easily detected by the pressure probe (20).

In order to consider the objection that the measured dependence of the volumetric elastic modulus on pressure is attributable to a pressure dependence of the elasticity of the punctured cell wall area, the assumption is made that the elastic modulus, \( \varepsilon_i \), of the injury site decreases linearly with pressure:

\[ \varepsilon_i = \gamma \cdot P \] (15)

where \( \gamma \) is a constant factor.

If we further assume that in the high pressure range the influence of the elasticity changes in the cell wall close to the site of puncture is negligibly small we obtain the following equation for defining \( \gamma \):

\[ \gamma = \frac{\varepsilon_i}{P_N} \] (16)

where \( P_N \) is roughly the normal turgor pressure in the internodes (4.5–6 bar).

A low \( \varepsilon \) value implies by definition (equation 2) a high elasticity of the cell wall, thus, the change in cell volume in response to a given pressure pulse is larger at low \( \varepsilon \) values compared with that on observed at high \( \varepsilon \) values. Equations 15 and 16 state that the contribution of the injured cell wall part, defined by \( \varepsilon_i \), to the over-all wall elasticity of the intact cell increases with decreasing turgor pressure. Equations 15 and 16 therefore represent the best case that we can propose that the decrease of \( \varepsilon \) with increasing turgor pressure can be traced back completely to an artifact. Substitution of the value of \( \varepsilon_i \) from equation 15 into equation 9 yields:

\[ \varepsilon_M = \frac{\beta \cdot \gamma \cdot P \cdot \varepsilon}{\gamma \cdot P \cdot (\beta - 1) + \varepsilon} \] (17)

Figure 4 illustrates the function \( \varepsilon_M = f(P) \) calculated from equation 17 for different absolute values of \( \beta \).

It is evident that even for very unfavorable ratios of the punctured area to the total surface area, i.e. \( \beta = 50 \), a decline in the measured value of the elastic modulus would only be detectable below 1 bar. A value lower than 50 to 100 for \( \beta \) is not measurable because the tip diameter of the pressure probe capillary would have to be so large that the cell wall would be torn during its introduction into the vacuole resulting in cell destruction. Under the above mentioned assumptions \( \varepsilon_M \) would be constant over the entire pressure range although experimentally a quite different change of \( \varepsilon \) with turgor pressure is determined. Because of this discrepancy between the theoretical curve expected in the presence of mechanical lesions and the experimental findings, we are forced to conclude that the increase in the absolute value of \( \varepsilon \) with increasing turgor pressure is definitely an intrinsic property of the cell wall and not an artifact introduced by cell wall injury caused by the pressure probe.

**DISCUSSION**

Theoretical and experimental evidence is presented which allows the conclusion that both the pressure dependence of \( L_p \) observed in some species in the low pressure range and the pressure and volume dependence of the elastic modulus of the cell wall are attributable to intrinsic cellular properties. The same conclusion can be drawn for the measurements of the water transport parameters in tissue cells of higher plant cells, using the new version of the pressure probe (6) for measuring water relations in higher plants. In such cells the ratio between intact cell surface to the area of injury, \( \beta \), is much worse, about 50 to 200 compared with the pressure probe used in this paper for giant algal cells. These results support the experimental findings that the \( L_p \) values increase at low pressures, as recently reported by Steudle and Zimmermann (14, 19). These authors found that the increase in the \( L_p \) values can be partially compensated for by raising the concentration of sucrose added to the artificial pond water. The graph \( L_p \) as a function of \( P \) in Characean cells is shifted to lower pressure values when sucrose is present. It is well established that increasing the sucrose concentration decreases the hydraulic conductance of cell membranes, probably due to a dehydration effect on the membrane (2, 15).

Errors in the calculation of the absolute values of the hydraulic conductance from turgor pressure relaxation processes might, however, arise from reasons other than those discussed above. The elastic modulus of the cell wall describes the elastic response of the cell to osmotic stress. In the pressure probe technique the pressure changes and the corresponding volume changes are measured instantaneously after disturbing the stationary state hydrostatically or osmotically; thus, the elastic properties of the cell wall are determined, whereas viscoelastic (plastic) properties of the cell wall play a minor role. When determining the parameter, \( \varepsilon \), from measurements of the pressure-volume curves of plant cells recorded under stationary conditions both elastic and viscoelastic properties of the cell wall influence the slope of the pressure-volume curve, and in turn, the absolute value of \( \varepsilon \) (see also Dainty et al. [4]). A comparison of \( \varepsilon \) values for cells of *Haliocystis parvula* obtained by the two different methods reveals that the absolute values can differ by an order of magnitude (unpublished results, see also Graves and Gutknecht [5]). \( \varepsilon \) values determined with the pressure probe technique in *H. parvula* are of the order of \( \varepsilon = 3 \) to 15 bar depending on turgor pressure, whereas \( \varepsilon \) values of about 0.5 to 2 bar are calculated from the slope of the stationary pressure-volume curve. The question is whether the half-time of water exchange of a cell with its environment is determined more by the elastic modulus measured instantaneously or by the modulus...
obtained under stationary conditions. The elastic properties should dominate as far as half-times of the order of seconds are concerned, such as in *Nitella* and *Chara* cells, so that it seems feasible to include the ε values measured with the pressure probe technique in the calculation of $L_p$. On the other hand, in *Valonia utricularis* cells, in the bladder cells of *M. crystallinum* (12, 20), and in the tissue cells of *C. annuum* (6) where the half-time is of the order of several minutes, the viscoelastic properties of the cell wall could exert an influence possibly leading to a slight overestimation of the ε values taken into account in the equation of the half-time of water exchange (equation 1) and in turn an underestimation of the value of $L_p$ when using the pressure probe technique. These errors, however, should be small since measurements of the hydraulic conductivity in *Valonia*, *Nitella*, and *Chara* cells, using both perfusion techniques and the method of transcellular osmosis have given the same values for $L_p$.

The increase in $L_p$ values on approaching the plasmolytic point can be explained in terms of a coupling of water flow with active ion transport. The derivation of equation 1 is based on the phenomenological equations of the thermodynamics of irreversible processes for water and passive solute transport (7). Active transport is not included in these equations. Steudle and Zimmermann (14) were able to show in a semiquantitative estimation for *Valonia* cells that the apparent resistance of the cell membrane to water flow in the presence of active transport processes is described by the following equation (14, 22):

$$\frac{dP}{dJ_v} = \frac{1}{L_p} - \frac{J_s \cdot RT}{J_v^2}$$  (18)

The resistance to water flow, $\frac{dP}{dJ_v}$, is the parameter determined in the measurement of water transport parameters, regardless of the method used. It is evident from equation 18 that $L_p$ is overestimated when the second term is neglected. The size of the error...

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**Fig. 4.** Theoretical calculation of the possible errors in ε measurements. Calculation of the function $ε_M = f(P)$ is based on equation 17:

$$ε_M = \frac{β \cdot γ \cdot P \cdot ε}{γ \cdot P \cdot (β - 1) + ε}$$

The model calculation is made with data ($ε = 400$ bar at $P = 5$ bar and $γ = 80$) taken from the experimentally determined function of $ε$ on pressure (O) in a *C. corallina* cell with $V_e = 8.2$ μl. The ratio, β, of the intact cell surface area to the injured area is varied from 50 (●) to 1,000 (△). A comparison of the theoretical calculation with the experimental results shows that the measured dependence of $ε$ on turgor pressure cannot be explained with an elasticity change of the cell wall close to the inserted microcapillary, because the discrepancy between the theoretical curve expected in the presence of mechanical injury introduced by the pressure probe and the experimental finding is quite obvious.
made in the calculation of \( L_p \) depends on the values of \( J_1 \) and \( J_2 \). In \( V. \ utricularis \) it is relatively easy to show that the contribution of these two parameters to the over-all membrane resistance to water flow is negligible above 1 bar. In pressure ranges below 1 bar where the active K influx is strongly dependent on pressure and increases markedly with declining pressure, the second term of equation 18 may become of the same order of magnitude as the first term \( \frac{1}{L_p} \). If the second term is taken into account in the calculation of the hydraulic conductivity of the cell membranes of \( V. \ utricularis \), it is possible to show that the thermodynamic hydraulic conductivity, \( L_p \), is constant over the entire range (22). It is the effective value of the hydraulic conductivity composed of both terms of equation 18 which is of crucial importance in the osmoregulatory response of the cell to osmotic stress, and not the thermodynamic value, \( L_p \). Any arguments about the accuracy of \( L_p \) values and the pressure dependence of the hydraulic conductivity in some species in certain pressure ranges could well be terminated if a sharp distinction were made between the effective membrane resistance to water flow determined experimentally and the thermodynamic resistance. These two parameters are identical only in the absence of active ion transport processes, which may be pressure-dependent as in cells of \( V. \ utricularis \). In order to explain the increase in the \( L_p \) values in Characean cells we have to assume that the magnitude of either the solute fluxes or of the coupling coefficient (17, 22) between water and solute flux changes by some unknown processes in the low pressure range. The pressure dependence of ion fluxes found in \( V. \ utricularis \) is not known to exist in Characean cells, but in these cells leakage of ions through the nodes is observed (12), which is evident, e.g. as a constant decline in cell turgor pressure (over about 10–20 h). In cells in which both nodes have been tied off, the cell turgor gives a constant reading with the pressure probe. Therefore, we cannot rule out the possibility that this natural process of intracellular ion loss is pressure-dependent.

The pressure dependence of the \( L_p \) values in the bladder cells of \( M. \ crystallinum \) can be explained on a similar basis to the increase of \( L_p \) in \( Valonia \) cells. The membrane of the bladder cells is very permeable to KCl and NaCl and apparently lacks an active transport mechanism (12). Palta and Stadelmann’s finding (8) that the value of \( L_p \) in onion epidermal cells is constant under various conditions of osmotic stress indicates that either there is no coupling between water and active solute flow in these cells or neither the coupling coefficient nor the magnitude of the flows is influenced by pressure over the entire pressure range.

Structural changes which are pressure-dependent might offer a possible explanation for the pressure dependence of the \( L_p \) values in some species. Dielectric breakdown measurements on cell membranes of plant cells, animal cells, and bacteria have shown that certain parts of the membrane can be compressed by mechanical and electrical forces (16, 17, 22). The hydraulic conductivity of a membrane or the membrane resistance to water flow should be crucially influenced by the compression state of the membrane.

Folding of the membrane at a molecular level could be a further pressure-dependent process leading to an apparent increase in the \( L_p \) values, since the phenomenological equation for water transport does not provide a correct description of the over-all force-flow characteristic of the membrane in a folded state. In a mathematical analysis of a simple membrane model Richardson et al. (10) showed that in a folded membrane the increase in water flow is larger (by a factor of 3) than the proportional increase in surface area. Simultaneously the solute flow increases only fractionally in relation to the increase in surface area. Folding the membrane creates an additional passive water flow. Palta and Stadelmann (8) excluded the formation of folds in membranes on the basis of electron micrographs of plasmolysed cells. However, one can argue first that folds occur relatively easily in the tonoplast membrane and have to be taken into account because the \( L_p \) value of the total barrier is measured, and second that the process of fixation used for the preparation of thin sections for the electron microscope does not easily allow any definite conclusions as to the natural occurrence of folds in the plasmalemma membrane.

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