

Letters to the Editors

Comments on:

Effect of Turgor Pressure on Water Permeability
of *Allium cepa* Epidermis Cell Membranes

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We should like to comment on the paper by Palta and Stadelmann [3] concerning the measurement of the hydraulic conductivity, L_p , in onion epidermal cells. The authors determined L_p by eluting monocellular epidermal strips soaked with tritiated water and did not find an effect of the osmotic pressure of the medium on L_p . On the one hand, Palta and Stadelmann feel that this result contradicts the findings of Zimmermann and Steudle [5] who reported a remarkable increase of L_p for different *Characean* species and also for the marine alga *Valonia utricularis* in the range of low cell turgor pressures, P , on approaching the plasmolytic point. On the other hand, they feel their work is confirmed by the results of the same authors, who found no pressure dependence of the hydraulic conductivity in the bladder cells of *Mesembryanthemum crystallinum* over the whole range of P (0 to 5 bar) [4]. Possibly Palta and Stadelmann are not fully aware of the interpretation of these different effects of turgor pressure [6], which are by no means contradictory. For the discussion of the reliability of L_p values of higher plant cells obtained by different methods which may vary by 4 orders of magnitude, it is extremely important to know whether L_p is pressure-dependent.

Palta and Stadelmann stress the argument that in the experiments on *Characean* internodes and *V. utricularis* the cell membrane was damaged at low P (0 to 2 bar) due to the experimental procedure and that the increase of L_p is an artefact. This is certainly not true. Zimmermann and Steudle used their pressure probe technique [4, 5] to determine P and L_p directly from pressure relaxation experiments which could be induced either by changing the osmotic or hydrostatic pressure gradient. In these measurements (1) the cells behaved like ideal osmometers, (2) *Characean* cells showed cytoplasmic streaming over the entire pressure range, and (3) the membrane potential which was measured simultaneously in *V. utricularis* cells was not affected by the experimental procedure. This experimental evidence demonstrates that the algal cells were intact at low P and did not become leaky. Furthermore, it was shown [5] that both the concentration of the medium and the turgor pressure affect L_p of *Characean* cells in a different way. In osmotic experiments the increase in the external concentration caused a reduction of L_p which had also been found to be the case in transcellular osmosis measurements [1]. However, at low P the concentration effect could be compensated or even overcompensated by the inverse effect of turgor. This is shown in Fig. 1, where the pressure dependence of L_p of a *Nitella flexilis* internode is shown both in the presence and absence of an osmoticum (0.1 M sucrose) in the external solution. Due to the concentration dependence of L_p the graph of $L_p = f(P)$ obtained in artificial pond water (APW) is shifted to lower turgor pressures, but does not change its hyperbola-like shape. This result strongly supports pressure-depen-

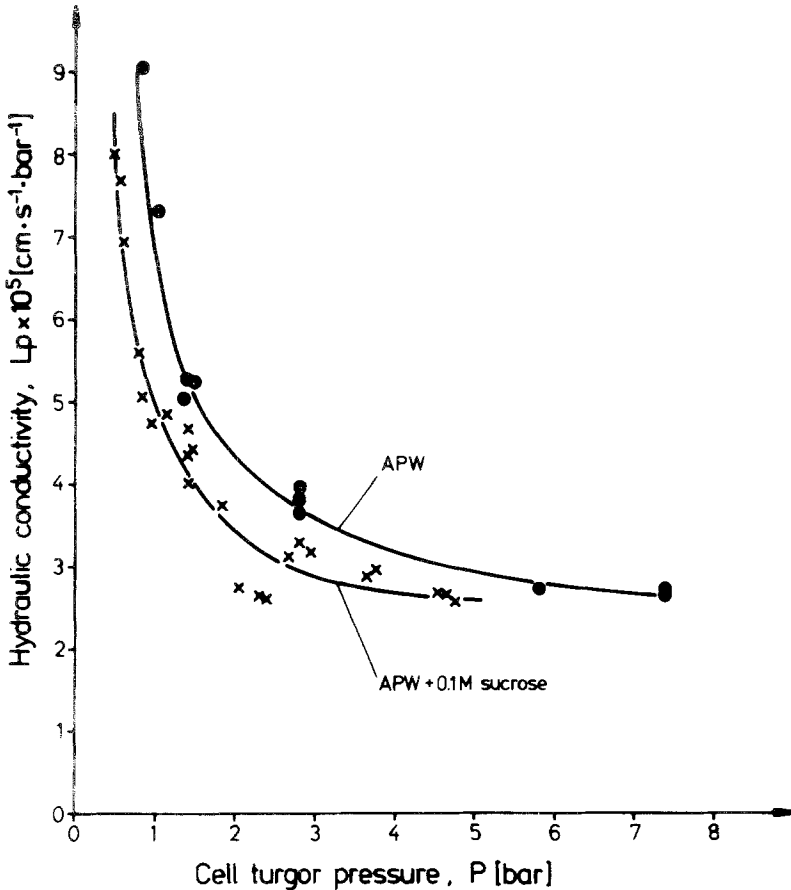


Fig. 1. Effect of cell turgor pressure, P , on the hydraulic conductivity, L_p , of a single internodal cell of *Nitella flexilis* in artificial pond water (APW) (composition: 1 mM NaCl; 0.1 mM KCl, CaCl₂, and MgCl₂, respectively) and in APW+0.1 M sucrose. The hydraulic conductivity was calculated from exosmotic water flows induced by hydrostatic pressure gradients with the aid of the pressure probe technique [4, 5]

dent changes of the hydraulic conductivity, rather than a mechanical rupture of the cell membrane, which would occur at the same P , independently of the nature and concentration of the solute in the medium.

The effect of turgor pressure on L_p has been discussed in terms of structural changes of the cell membranes [5] and in terms of an interaction between ion fluxes and water flow [6]. In *V. utricularis* the potassium uptake increases sharply in the low pressure range [cf. 6] and thus exhibits a pressure dependence very similar to that of L_p in this range. Theoretically, it can be shown for the steady state that in the presence of a net ion (solute) flux, J_s , an additional water (volume) flow, J_w , will be generated which may be substantial, if J_s is high and the volumetric elastic modulus, ϵ , of the cell is low. If the rate of exchange of the solutes across the cell membrane is much slower than

that of water, the relation between J_s and J_w will be given by [6]:

$$J_s = \frac{\varepsilon + \Pi^i}{RT} J_w \quad (1)$$

where Π^i is the osmotic pressure of the cell sap.

Eq. (1) was derived for an impermeable solute in the presence of an active stationary solute flow and a stationary water flow. Using Eq. (1) it is possible to evaluate the apparent resistance of the cell membrane to water, dP/dJ_w , under these conditions ([6], see also [2]):

$$\frac{dP}{dJ_w} = \frac{1}{L_p} - \frac{J_s \cdot RT}{J_w^2} \quad (2)$$

Eq. (2) states that dP/dJ_w depends on both L_p and on the direction and magnitude of J_s . At least qualitatively, Eq. (2) can explain the increase of the water conductivity in the low pressure range observed in *Valonia* under nonstationary conditions. J_s is inwardly directed (i.e., it is negative) and strongly pressure-dependent. Thus, the hydraulic conductivity calculated from dP/dJ_w will be higher, if L_p is calculated assuming that $J_s=0$. For *Characean spp.* a similar consideration may be valid. It should be pointed out that the apparent L_p value calculated for $J_s=0$ is the physiologically relevant parameter for the water relations of plant cells instead of the thermodynamic value of L_p .

If the hydraulic conductivity is independent of turgor pressure, this only indicates that there is either no coupling between water flow and active solute flow in these cells or that the coupling coefficient and the magnitude of solute flow are unaffected by P over the entire pressure range. In general, interactions between active solute (ion) transport and water transport have to be taken into account when the L_p of plant cells is determined in the presence of active solute flows. Some of the discrepancies as regards the absolute value of L_p in the literature may be due to the neglect of this important point. Furthermore, turgor-dependent structural changes of the plasmamembrane such as folding or compression may also result in changes of L_p . Therefore, if the effect of turgor pressure on the water permeability is to be studied, it is undoubtedly necessary to determine P directly and to separate the pressure effect from concentration effects (see Fig. 1).

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Reply to *Comments on*:
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The comments on our paper (Palta and Stadelmann, 1977) by Steudle and Zimmermann do not provide any convincing arguments to support their earlier conclusions (Steudle and Zimmermann, 1974). Following are the reasons for this assertion:

I. The variability of L_p values with cell species and/or physiological conditions should not be confused with the variability of L_p caused by errors in the various experimental methods used on the same cell material under the same physiological conditions. The claimed variability of L_p values for higher plant cells by 4 orders of magnitudes cannot be corroborated in the literature, where reliable values indicate a 15- to 20-fold maximum variation of L_p between different types of cells (Stadelmann, 1963, p. 698*f*). Variations in L_p values for different cell material must be expected because of the differences in the composition and structure of the membranes of different cell species.

II. Cytoplasmic streaming, an established criteria for cell viability, is frequently observed in injured cells, where it may be even more intense. Likewise perfect semipermeability of the protoplasm layer for the vacuolar solutes does not exclude changes in water permeability and other transport parameters. Steudle and Zimmermann are probably not aware that membrane alterations can occur without affecting cytoplasmic streaming and semipermeability. It was recently shown by Palta *et al.* (1977*a*, 1977*b*) that freeze injured cells behave like ideal osmometers and exhibit cytoplasmic streaming just like uninjured cells, whereas the active transport systems were damaged.

The factors causing the membrane damage in the punctured *Nitella* cell cannot yet be assessed, partly because of lack of details about the puncturing procedure. The sharp increase in L_p measured at zero turgor in these cells could be caused by membrane rupture produced by the sudden pressure increase (1-2 atm pressure applied by a pressure probe used by Steudle and Zimmermann) required for the L_p measurement. Such rupture may occur instantaneously and thereby result in a very short relaxation time (very high value of L_p). A temporary membrane rupture may repair very quickly and will not affect semipermeability and protoplasmic streaming. Osmotically induced breaking of red blood cells for preparing ghosts is a very good example of membrane rupture under pressure followed by a quick repair. Another possible reason for the increase in L_p would be a lower structural stability of the cell membrane and/or the vacuolar membrane because of the drastic change

of the ionic content of the cell sap vacuole by the lowering of the cell sap concentration using cell puncture procedure.

III. It will be quite a coincidence that in the experiments conducted by Dainty and Ginzburg (1964), Tazawa and Kamiya (1966), and Palta and Stadelmann (1977), the concentration of the external solution always accurately compensated the corresponding increase in L_p . The L_p is expected to increase sharply at the lower turgor pressure according to Steudle and Zimmermann which was not found by any of these authors. Different methods and different plant materials were used by these authors to measure L_p . Furthermore, water permeability constant values for *Allium cepa* inner epidermis cells found at plasmolyzing concentrations (Url, 1971) are very close to the diffusional water permeability values found by Palta and Stadelmann (1977).

IV. There is no evidence for membrane folds at low turgor pressure. Electron microscopic pictures of plasmolyzed cells showed absence of any fold (Sitte, 1963). Furthermore, if folding is the explanation, it should have the same effect in bladder cells of *Mesembryanthemum crystallinum*. No effect on L_p was found at low turgor in these cells (Steudle *et al.*, 1975). Active transport has been found to be sensitive to turgor pressure changes, and this experimental evidence can be used to explain the well-known phenomenon of osmoregulation in algal cells (Bisson & Gutknecht, 1975). This however, does not necessarily mean an effect of turgor on L_p . Turgor changes induce active transport of ions which can generate some water flux in the direction of the ion flux. This water flux coupled to the ion flux does not indicate that the hydraulic conductivity of the membrane (L_p) has changed also.

V. The graph in the letter of Steudle and Zimmermann is no unequivocal evidence for a pressure dependence of L_p . The lower L_p values for the same turgor pressure in 0.1 M sucrose + APW supports rather the suggestion of Palta and Stadelmann (1977) that the increase in L_p with lowering P is caused by membrane alteration. Sucrose is known for its protective action on cell membranes; thus, L_p will be lower at the same pressure P in presence of sucrose in the medium. Tests with solutions of substances which do not have such membrane effect, or more generally, experiments using a different method for measuring L_p would be required to demonstrate unequivocally any pressure dependence of L_p in *Nitella* cells.

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For all other references, see:

- Palta, J.P., Stadelmann, E.J. 1977. Effect of turgor pressure on water permeability of *Allium cepa* epidermis cell membranes. *J. Membrane Biol.* **33**:231