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# Water relations of growing pea epicotyl segments

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**Abstract.** The water relations of growing epicotyl segments of pea (*Pisum sativum* L.) were studied using the miniaturized pressure probe. For epidermal cells stationary turgor pressures of $P=5$ to 9 bar and half-times of water exchange of individual cells $T_{1/2}=1$ to 27 s were found. The volumetric elastic modulus ($\varepsilon$) of epidermal cells varied from 12 to 200 bar and the hydraulic conductivity, Lp=0.2 to $2\cdot10^{-6}$ cm $s^{-1}$ $bar^{-1}$. For cortical cells P=5 to 11 bar, $T_{1/2}=0.3$ to 1 s, Lp=0.4 to $9\cdot10^{-5}$ cm $s^{-1}$ $bar^{-1}$ and $\varepsilon=6$ to 215 bar. The $T_{1/2}$ of cortical cells was extremely low and the Lp rather high as compared to other higher plant cells. The $T_{1/2}$-values of cortical cells were sometimes observed to change from short to substantially longer values ($T_{1/2}=3$ to 20 s). Both short and long pressure relaxations showed all the characteristics of non-artifactual curves. The change is apparently due to an increase in Lp and not $\varepsilon$, but the reason for the change in cell permeability to water is not known.

In osmotic exchange experiments on peeled segments using solutions of different solutes, the half-time of osmotic water exchange for the whole segment was approximately 60 s. Water exchange occurred too quickly to be rate controlled by solute diffusion in the wall space. The data suggest that the short $T_{1/2}$-values in the cortical cells are the physiologically relevant ones for the intact tissue and that a considerable component of water transport occurs in the cell-to-cell pathway, although unstirred layer effects at the boundary between the segment and solution may influence the measured half-time. Using the theory of Molz and Boyer (1978, Plant Physiol. **62**, 423–429), the gradient in water potential necessary to maintain the uptake of water for cell enlargement can be calculated from the measured diffusivities to be approximately 0.2 and 1 bar for growth rates of 1% $h^{-1}$ and 5% $h^{-1}$, respectively. Thus, although the $T_{1/2}$-values are short and Lp rather high, there may be a significant osmotic disequilibrium in the most rapidly growing tissue and as a consequence the influence of water transport on the growth rate cannot be excluded.

**Key words:** Cell wall (elasticity) – Growth (elongation) – Epicotyl – Hydraulic conductivity – *Pisum* – Water transport.

## Introduction

Plant cell enlargement may in principle be limited by the uptake of water into the growing cells or by the yielding properties of the cell walls (Ray et al. 1972; Cleland 1977). It is often tacitly assumed in the literature that growth in higher plant tissues is entirely limited by the yielding of the cell wall. However, experimental estimates of the water potential necessary to sustain cell enlargement in higher plant tissues suggest that the assumption of osmotic equilibrium in growing tissues may not be correct (e.g., Ray and Ruesink 1963; Boyer 1968; Molz and Boyer 1978). To evaluate the possible limitation of growth by the water transport and the wall yielding properties in higher plant tissues, these properties for individual cells must be measured. The pressure probe technique (Hüsken et al. 1978; Zimmermann and Steudle 1978) allows quantitative measurements of the hydraulic conductivity of the cell membranes (Lp), the cell wall elasticity, and the half-time ($T_{1/2}$) of water exchange between an individual cell and its surroundings at a given change in water potential. Combining the pressure probe data with the theories available for

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*Abbreviations:* P=turgor pressure; $T_{1/2}$=half-time of water exchange of individual cell; $Lp$=hydraulic conductivity; $\varepsilon$=volumetric elastic modulus; $\bar{T}_{1/2}$=average half-time of water exchange of tissue

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water transport in tissues (Philip 1958; Molz and Ikenberry 1974), it should be possible to answer the question of the rate-limitation of growth by water transport and also, in principle, the question of the pathway of water flow (apoplasmic or cell-to-cell) in growing tissue. Both questions are involved with the controversial question of an effect of auxin on water permeability (cf. Boyer and Wu 1978; Dowler et al. 1974).

The objective of this paper was to work out the physical parameters of cellular water transport in growing epicotyl tissue. The data have been related to the overall osmotic properties of the tissue (i.e., to is shrinking and swelling characteristics), which have been determined from dimensional (length) changes of epicotyl segments using a sensitive extensiometer.

## Materials and methods

*Plant material.* Pea (*Pisum sativum* L. cv. Alderman and cv. Kelvedon) plants were grown from seeds in potting soil for 6 to 7 d at 28° C in a completely dark humid chamber. Except as noted, 1.5-cm long segments were excised from the epicotyl region (second internode) directly below the hook of seedlings 8–12 cm tall. Stem diameters varied from 1.3 to 2.0 mm, with an average of 1.6 mm. In some cases the epidermis of a segment was stripped from both ends using fine forceps, while in other cases the cuticle was abraded by gently rubbing the segment with a slurry made up of # 220 carborundum powder and water. Freehand cross and longitudinal sections were made of the region where pressure probe measurements were performed in order to estimate the dimensions of cortical cells.

The functioning of the vascular system in the growing region of the epicotyl was ascertained by placing the cut end of a young shoot in a vial containing a methylene blue solution (0.5 mg $ml^{-1}$). The solution was allowed to ascend for 2 to 4 h at room temperature in the light. The epicotyl was then sliced by hand with a razor blade and the cut surface examined under a stereomicroscope for the presence of the dye. With a second method, the cut end of a young shoot was fitted snugly into a piece of water-filled rubber tubing which could be pressurized with a syringe. The top of the epicotyl was cut off, the stump was dried with a paper towel, and the cut surface was examined under a stereomicroscope while the syringe was pressurized.

The osmotic pressure ($\pi^i$) of expressed sap from frozen/thawed segments excised from the growing region of the pea epicotyl was determined cryoscopically with a Knauer osmometer. In three determinations the sap had an osmolarity of 450 to 470 mOsmol, which is equivalent to an osmotic pressure of 11.0 to 11.5 bar at 20° C.

*Elongation and pressure probe measurements.* An apparatus was built to measure continuously the change in length of a single segment, while simultaneously making pressure probe measurements (Fig. 1). The basal part of an epicotyl segment was mounted in a vertical Plexiglas chamber by two nylon screws, while the other end of the segment, located 1 cm above the mounting screws, was connected to a position transducer. Changes in the segment length or its derivative (growth rate) were recorded with a strip chart recorder (see Cosgrove 1981). The segment was bathed in a continuously flowing solution with a usual flow rate of ca. 0.5 ml

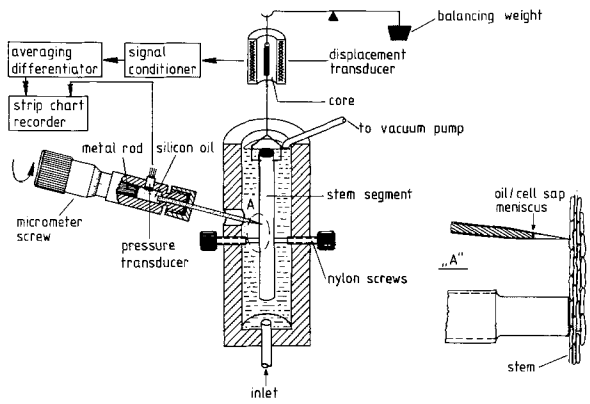


**Fig. 1.** Experimental set-up for measuring simultaneously water relation parameters of individual cells and the elongation (growth) rate in segments of pea epicotyl. For further explanation see text

$s^{-1}$. The chamber volume was 1.2 ml (0.4 ml in special cases). Solutions could be rapidly changed by switching a valve connected to the inlet port. The segments were bathed in 1 mM KCl solution which was exchanged alternatingly with 50 mOsmol solutions of different osmotica (KCl, urea, sucrose) to determine the average half-time for the length changes of the tissue which should be the same as the half-time of swelling and shrinking of the tissue ($\bar{t}_{1/2}$). The diffusivity of the tissue, $D_t$, is related to $\bar{t}_{1/2}$ and the radius ($R$) of the cylindrical segment (cf. Philip 1958) by:

$$\bar{t}_{1/2}=\frac{0.062\cdot R^2}{D_t}. \quad (1)$$

The absolute changes in length for 1-cm segments were 10 to 30 µm (=0.1 to 0.3% of the segment length) for 50 mOsmol solutions (≙1.22 bar). All experiments were performed under normal room lighting.

For pressure probe measurements, the capillary of the pressure probe was inserted into the chamber through a hole on one side of the chamber (Fig. 1). The hole was sealed with vaseline which stopped leakage of the circulating solution but permitted movement of the capillary. A glass plate covered one side of the chamber and allowed for observation of the capillary with a horizontal microscope (160×). The pressure probe technique has already been extensively discussed (Hüsken et al. 1978; Zimmermann and Steudle 1978). In our experiments the position of the boundary between the oil and the cell sap was directly observed through the microscope. The boundary was controlled manually by turning the micrometer screw (Fig. 1) and not by an electronic feedback, as described presiously (Hüsken et al. 1978). This manual technique was used because water exchange in pea cortical cells was so rapid and because it permitted a more precise regulation of the oil/cell sap boundary.

Cell half-times of water exchange ($T_{1/2}$) were obtained from pressure relaxation experiments. The hydraulic conductivity of the cell membrane (Lp) can be calculated from $T_{1/2}$, if the cell surface area ($A$), the cell volume ($V$), and the volumetric elastic modulus ($\varepsilon$) are known $\left(\mathrm{Lp}=\frac{V}{A}\frac{\ln 2}{T_{1/2}(\varepsilon+\pi^i)}\right.$; cf. Zimmermann and Steudle, 1978$\left.\right)$. $\varepsilon$ relates instantaneous changes in cell volume ($\Delta V$) to the corresponding changes in turgor ($\Delta P$) $\left(\varepsilon\equiv V\frac{\Delta P}{\Delta V}\right)$. $\varepsilon=V\frac{\Delta P}{\Delta V}$ is only valid if the meniscus is moved very rapidly so that there is no substantial water flow out of the cell during the movement. If $T_{1/2}$ is short $\varepsilon$ may be underestimated (Steudle et al. 1980). Since the movement of the meniscus could be made very rapidly, this

underestimation could have been 5–10 % at most for the rapidly exchanging cortical cells.

Two procedures, were used to estimate cell volume and surface area which must be known for the evaluation of $\varepsilon$ and Lp. Epidermal cells were marked after pressure probe measurements with a small amount of waterproof ink applied to the outside of the glass capillary. The cell could then be identified and measured, in about 75 % of the cases, by examining an epidermal peel of the segment under a microscope. For cortical cell dimensions, we measured 100 cortical cells at random from freehand longitudinal sections. The size distribution of these cells was not normal but righthand-skewed. Therefore, we used a modal cell volume ($V$ = 250 pl; range: 40 to 2,700 pl) and cell surface area ($A$ = 27,000 μm$^2$; range: 9,000 to 120,000 μm$^2$) rather than mean values to calculate Lp and $\varepsilon$.

## Results

*Pathway for water flow during growth.* The growing region of the pea epicotyl contains one main vascular bundle and four smaller peripheral ones (Fig. 2) which are embedded in large parenchymatous cortical cells composing 90% of the cross sectional area of the epicotyl. During normal growth of the seedling, water must travel from the active xylem elements to the cells furthest away. Since this distance (and consequently the hydraulic resistance to water flow) would depend strongly on which of the vascular bundles transport water, we investigated this question first. When the basal end of an excised seedling was placed in a methylene blue solution for 2 to 4 h, the dye was found only in the central bundle of the growing region, i.e., in the most apical 2 cm. Cross sections made at more basal parts of the epicotyl showed that the dye appeared in the peripheral bundles ca. 4–5 cm below the hook. This result indicates that only the central vascular bundle provides water for the most actively growing region. This conclusion is further supported by experiments in which the basal end of an epicotyl was pressurized using a water-filled syringe. When the apical cut surface was made 1 cm below the hook, water welled up at several points in the central bundle, but not in the peripheral bundles. When the cut surface was made at more basal parts of the epicotyl, the water welled up more abundantly. At about 5 cm below the hook, water also began to appear at the surface of the peripheral bundles. Thus, in the growing region of the epicotyl, the central vascular bundle is the source of water, which must then flow radially across a layer of 15 to 20 large cortical cells. These cells vary considerably in their diameter ($2r$ = 15 to 100 μm; mean ± SD: 47 ± 16 μm; $n$ = 100 cells).

*Pressure probe measurements.* When pea segments were first mounted in the apparatus and bathed in aerated distilled water or 1 mM KCl, they elongated rapidly (ca. 5% h$^{-1}$) for the first 45 min, then settled down to a stable slow growth rate varying from 0.5 to 2.5% h$^{-1}$. Pressure probe measurements were started after this slower growth rate was attained (60 to 90 min after mounting). The introduction of the probe into the cells did not alter the growth rate.

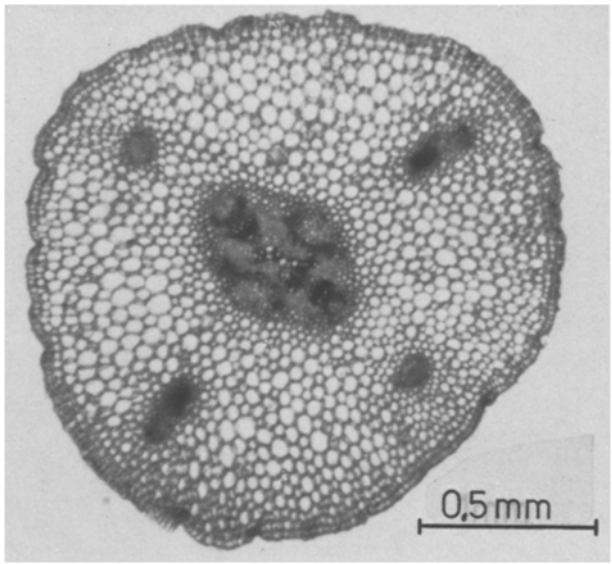


**Fig. 2.** Freehand cross-section of the growing region of pea epicotyl (about 1 cm below hook)

In about 20 epidermal cells successfully measured (each cell in a different segment), the stationary turgor varied from 5 to 9 bar and the $T_{1/2}$ from 1 to 27 s. The values of $\varepsilon$ calculated for the individual cells varied from 12 to 200 bar, while Lp was found to range from between 0.2 to $2 \cdot 10^{-6}$ cm s$^{-1}$ bar$^{-1}$. Figure 3 A shows a typical pressure relaxation experiment and $\Delta P/\Delta V$ determination for an epidermal cell.

When cortical cells were measured, the cell half-times were found to be much faster than those for the epidermal cells, ranging from 0.3 s to 1 s with a mean at 0.6 s (see Fig. 4 A). Turgor pressures were found to range from 5 to 11 bar. These values are smaller than the osmotic pressure of expressed sap ($\pi^i \simeq 11$ bar), which might indicate that the osmotic pressure of the free-space solution in the cell wall is not negligible. Using the modal cell volume and surface area, Lp was estimated to be 0.4 to $9 \cdot 10^{-5}$ cm s$^{-1}$ bar$^{-1}$ (Fig. 4 B).

The $\varepsilon$ values were calculated from $\Delta P/\Delta V$ measurements to be 6 to 215 bar (Fig. 4 C). These values refer to high turgor pressures ($P$ = 3 to 13 bar) and represent average values over this pressure range. The pressure-dependence of $\varepsilon$ has not been thoroughly investigated up to now, although there are some indications that $\varepsilon$ decreases at lower $P$, e.g., $T_{1/2}$-values are frequently larger for endosmotic pressure relaxations than for exosmotic ones (see Fig. 3 B, C).

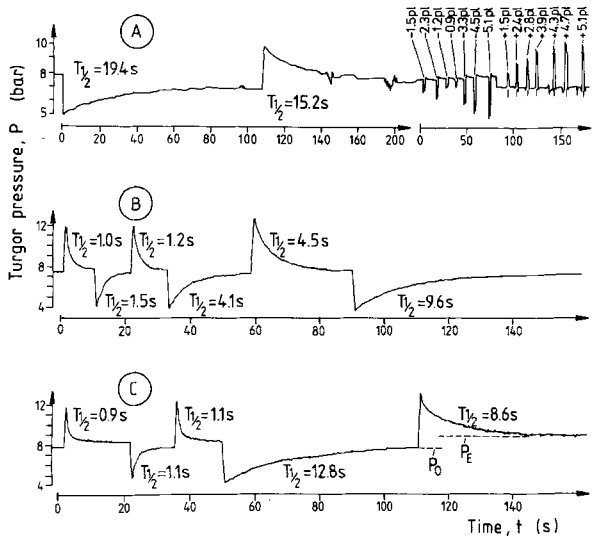


**Fig. 3. A** Pressure-relaxation curves for an epidermal cell of pea epicotyl. On the right side of the trace $\Delta P/\Delta V$ measurements for the estimation of $\varepsilon$ are shown. Values for $\Delta V$ are given in picoliters (*pl*). **B, C** Change from short to longer $T_{1/2}$ values in cortical cells of the pea epicotyl. In B the change occurs gradually over 2–3 pressure relaxations, while in C the change in $T_{1/2}$ is very abrupt. Note that the pressure after exosmotic pressure relaxation ($P_E$) is larger than the initial pressure ($P_0$), which is a criterion for membrance integrity. The difference in $T_{1/2}$ for endosmotic and exosmotic pressure relaxations is probably due to a dependence of $\varepsilon$ on pressure

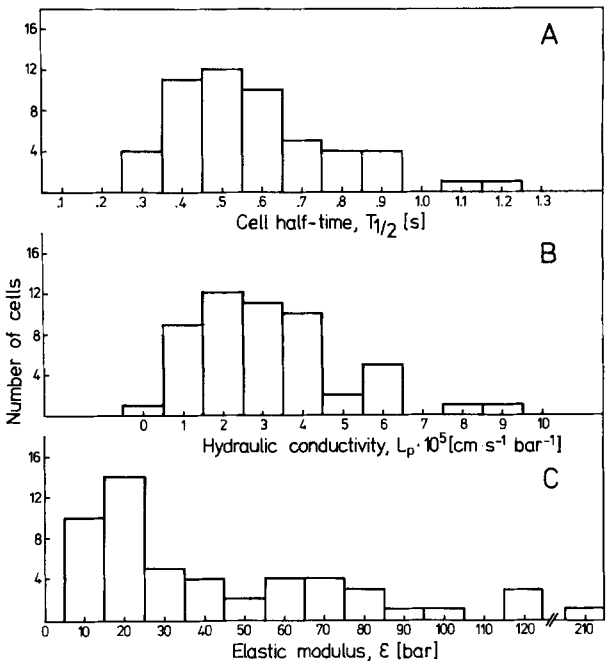


**Fig. 4.** Frequency distribution of 52 cortical cells of the pea epicotyl for cell half-time ($T_{1/2}$), hydraulic conductivity ($Lp$) and elastic modulus ($\varepsilon$)

The half-times of cortical cells are the fastest that have been observed in higher plant cells to date, and the Lp values are correspondingly larger than those previously reported for other higher plants (Zimmermann and Steudle 1978). $\varepsilon$ values are in the same order as found for other plant cells. It should be noted that the ranges for $\varepsilon$ and Lp of the epicotyl cells are rather large. This is due to a variation between cells and to the uncertainties in determining the volume and surface area of individual cells. The $T_{1/2}$ values for individual cells are accurately measured with the pressure probe (SD of 5–30%) and the reported range in $T_{1/2}$ reflects the natural cell variation in the tissue. In contrast, Lp and $\varepsilon$ are derived quantities whose errors are determined principally by the uncertainty in cell volume and surface area. Estimates of Lp and $\varepsilon$ for individual cells are thus only reliable within an order of magnitude, and the extreme values in the ranges probably are due to the use of the modal rather than real volume and area. It should be noted that the response time of the pressure probe ($\simeq$30 ms) was much faster than the cell $T_{1/2}$ and did not contribute a significant error to the measurements. For an extensive discussion of errors, the reader is referred to recent papers (Steudle et al. 1980; Tomos et al. 1981).

In a number of cortical cells we observed that after 10 to 30 large pressure relaxations, the $T_{1/2}$ changed from short (1 s or less) to much longer (3 to 20 s). Such a change is shown in Fig. 3B, C. In most cases the change in $T_{1/2}$ occurred within one or two seconds. Occasionally the change occurred gradually over a 5- to 30-s period. The change was also essentially irreversible: Only in one case (which was peculiar for other reasons) out of more than 40 such cases observed did the $T_{1/2}$ shorten again after becoming long. A similar switching from short to long $T_{1/2}$ was found in excised epicotyls of Kelvedon pea. The difference between the short and the long half-times could not have been due to a leaky membrane, since the cell turgor was exactly the same before and after the switching. Furthermore, the stationary pressure after an exosmotic pressure relaxation was slightly higher than the preceeding stationary turgor (Fig. 3), again indicating cell membrane integrity. In all respects which we could observe, both the short and the long half-times were valid, not artifactual, measurements.

In principle, the change from a short to a long $T_{1/2}$ could be due to a decrease in Lp or in $\varepsilon$. From $\Delta P/\Delta V$ measurements, $\varepsilon$ of a cell was found not to decrease after switching, but, on the contrary, sometimes increased by a small amount. Certain observations suggest that the short half-times are the physio-

logically relevant ones. First, it was observed several times that the $T_{1/2}$ of cells in a fresh segment (that is, one in the apparatus for 1 to 2 h) were at first all very short, but after the segment was in the apparatus for over 5 h, the half-times were all long, even when first penetrated. Second, the change from short to long half-times could usually (but not always) be rapidly induced by making large pressure relaxations. The change in $T_{1/2}$, however, occurred only in the single cell, for when the capillary was advanced into underlying cells, the half-times were short. Finally, when the epidermis was stripped off, the cortical cells on the segment surface usually had long half-times, even at first, while the deeper cells had short half-times. These observations suggest that the cell may have two different states with respect to its water permeability. Aging and rapid changes in turgor seem to facilitate the irreversible switching to longer half-times. In a few cases tissues were incubated in 1 mM $NaN_3$ or in 4° C water. The cells had short half-times and the change in $T_{1/2}$ was still occasionally observed. This shows that the switching does not require a high respiration rate (e.g., for healing processes or active transport).

*Osmotic exchange experiments.* To obtain direct evidence for the significance of the short or the long cell half-times, we measured the rate of swelling or shrinking of epicotyl segments when the osmotic pressure of the bathing solution was changed. Peeled or abraded segments quickly responded to changes in the osmotic pressure of the medium, with a half-time[1]), $\bar{t}_{1/2}$, of approximately 60 s (Fig. 5A), while very little change in length could be measured using segments with an intact cuticle. If the ends of the segments were covered with vaseline, the $\bar{t}_{1/2}$ remained unchanged, but if the cylinder surface was covered with vaseline, the rate of shrinkage or swelling was very low ($\bar{t}_{1/2} > 40$ min). These results show that water was exchanged primarily across the radial surface in peeled or abraded segments, and not across the ends.

In principle, the rate of swelling or shrinking could be limited by solute diffusion into the tissue (wall space) or by water transport through the cell-to-cell and apoplasmic pathways. In a series of experiments in which different solutes were used as osmotic agents, we found that the $\bar{t}_{1/2}$-values varied between 40 and 180 s for different solutes. At least for some segments (see segments 2, 3 and 4 of Table 1) there seemed

1 It should be noted that water exchange between the segment and the medium follows diffusion kinetics and is not exponential. The cells in different positions in the segments should have different time courses for swelling or shrinking. $\bar{t}_{1/2}$ represents the sum of the osmotic changes in all of the cells

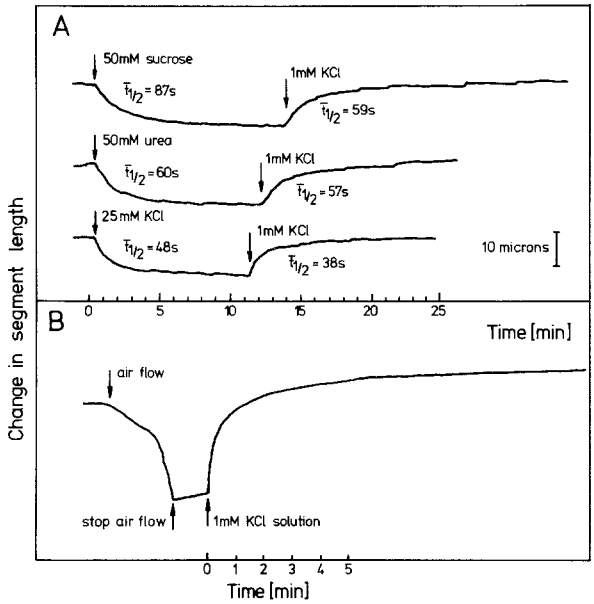


**Fig. 5. A** Osmotic shrinking and swelling of peeled segments of pea epicotyl (segment length: 1 cm) after treatment with 50 mOsmol solutions of sucrose (diffusion coefficient $D = 0.52 \cdot 10^{-5}$ cm$^2$ s$^{-1}$), urea ($D = 1.2 \cdot 10^{-5}$ cm$^2$ s$^{-1}$) and KCl ($D = 1.9 \cdot 10^{-5}$ cm$^2$ s$^{-1}$). Half-times of length changes ($\bar{t}_{1/2}$) decrease with increasing D of the solute. Length changes were reversible when the test solution was replaced by the original medium (1 mM KCl). **B** Rehydration of air-dried peeled segment in 1 mM KCl solution. The segment was partially dehydrated by passing an air stream through the chamber. When solution was again added, the segment swelled with a half-time of about 25 s. This experiment was repeated on four different segments with similar results

to be a dependence of $\bar{t}_{1/2}$ on the diffusion coefficient of the solutes, although there was considerable variability in the data. $\bar{t}_{1/2}$ of sucrose, which has the lowest diffusion coefficient, was larger than that of urea and KCl (Table 1 and Fig. 5A). Furthermore, the $\bar{t}_{1/2}$ depended on the flow rate of the solution (Table 2). There was a noticable tendency in these experiments for the $\bar{t}_{1/2}$ to increase gradually during the course of the experiment although the dependence of $\bar{t}_{1/2}$ on the type of solute and flow rate occurred regardless of the order in which the treatments were given. These observations suggest that water flow was partly limited by the rate of diffusion of the solute molecules across a boundary layer of solution external to the segment, and perhaps also by solute diffusion into the cell wall space. Thus, a 60-s estimate for the $\bar{t}_{1/2}$ is an upper limit. In fact water flow may be faster. An indication of this is given by experiments in which the segment was allowed to shrink by air drying and then rehydrated with distilled water. The $\bar{t}_{1/2}$ of the rehydration was 25 to 35 s (Fig. 5B).

Half-times of 60 s or less for the osmotic exchange of segments are too short to be accounted for by solute diffusion in the wall space (see Discussion).

**Table 1.** Swelling and shrinking of peeled pea epicotyl segments in response to changes in the osmotic pressure of the medium. $\bar{t}_{1/2}$ is the half-time of the change in length of the segment which should be equal to the $\bar{t}_{1/2}$ for volumetric changes; $\frac{1}{\Delta\pi}\frac{\Delta L}{L}$ is the relative change in length of the segment per bar change in the osmotic pressure of the medium. The diffusion coefficients of the solutes are (in $10^{-5}$ cm$^2$ s$^{-1}$): sucrose: $D$=0.52, urea: 1.2, KCl: 1.9

| Segment No. | Starting solution | Transferred to | $\bar{t}_{1/2}$ (s) | $\frac{1}{\Delta\pi}\frac{\Delta L}{L}$ $\cdot 10^3$ bar$^{-1}$ |
|---|---|---|---|---|
| 1 | 1 mM KCl | 50 mM sucrose | 54 | 0.93 |
| | 1 mM KCl | 50 mM urea | 78 | 0.90 |
| | 1 mM KCl | 50 mM urea | 84 | 0.60 |
| | 1 mM KCl | 25 mM KCl | 123 | 0.84 |
| | 1 mM KCl | 25 mM KCl | 66 | 0.90 |
| | 50 mM urea | 1 mM KCl | 57 | 1.04 |
| | 50 mM urea | 1 mM KCl | 42 | 0.69 |
| | 50 mM urea | 1 mM KCl | 39 | 0.54 |
| | 25 mM KCl | 1 mM KCl | 45 | 0.76 |
| 2 | 1 mM KCl | 50 mM sucrose | 87 | 1.05 |
| | 1 mM KCl | 50 mM sucrose | 90 | 1.04 |
| | 1 mM KCl | 50 mM urea | 60 | 1.00 |
| | 1 mM KCl | 50 mM urea | 60 | 1.01 |
| | 1 mM KCl | 25 mM KCl | 69 | 0.80 |
| | 1 mM KCl | 25 mM KCl | 48 | 0.87 |
| | 50 mM sucrose | 1 mM KCl | 59 | 1.01 |
| | 50 mM sucrose | 1 mM KCl | 84 | 1.01 |
| | 50 mM urea | 1 mM KCl | 57 | 0.87 |
| | 50 mM urea | 1 mM KCl | 69 | 0.98 |
| | 50 mM KCl | 1 mM KCl | 38 | 0.94 |
| 3 | 1 mM KCl | 50 mM sucrose | 177 | 0.87 |
| | 1 mM KCl | 50 mM urea | 102 | 1.05 |
| | 1 mM KCl | 25 mM KCl | 93.6 | 0.72 |
| | 50 mM sucrose | 1 mM KCl | 144 | 0.82 |
| | 50 mM urea | 1 mM KCl | 93 | 0.87 |
| | 25 mM KCl | 1 mM KCl | 102 | 0.90 |
| 4 | 1 mM KCl | 50 mM sucrose | 60 | 0.81 |
| | 1 mM KCl | 50 mM sucrose | 90 | 1.02 |
| | 1 mM KCl | 25 mM KCl | 42 | 1.03 |
| | 50 mM sucrose | 1 mM KCl | 69 | 0.78 |
| | 50 mM sucrose | 1 mM KCl | 75 | 0.95 |
| | 25 mM KCl | 1 mM KCl | 48 | 1.10 |

**Table 2.** Effect of solution flow rate on the half-time ($\bar{t}_{1/2}$) for shrinking and swelling of peeled pea epicotyl segments. The solution was changed from 1 mM KCl to 25 mM KCl to induce shrinking and back again to induce swelling. The chamber volumes were 1.4 ml for segments No. 5 and 6, 0.4 ml for segment No. 7

| Segment No. | Type of experiment | Flow rate ml s$^{-1}$ | $\bar{t}_{1/2}$ (s) |
|---|---|---|---|
| 5 | shrinking | 0.35 | 126 |
| | shrinking | 2.4 | 99 |
| | shrinking | 0.45 | 138 |
| | swelling | 2.4 | 72 |
| | swelling | 0.45 | 120 |
| 6 | shrinking | 1.0 | 69 |
| | shrinking | 0.17 | 210 |
| | swelling | 1.0 | 78 |
| 7 | shrinking | 0.5 | 174 |
| | shrinking | 2.5 | 54 |
| | shrinking | 0.6 | 234 |

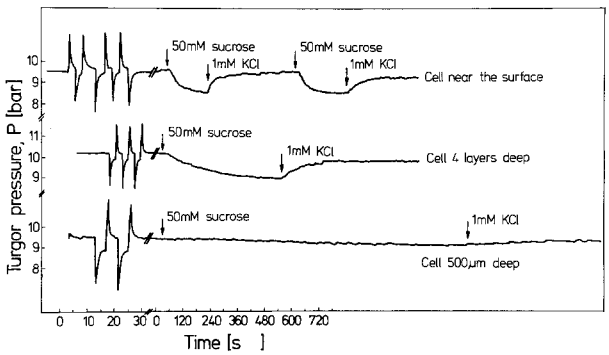


**Fig. 6.** Dependence of cell turgor response in cortical cells of peeled pea epicotyl segments on the position of the cell in the segment in osmotic experiments (right part of the traces). A cell close to the surface of the cylindrical segment reacts much faster to a change in the osmotic pressure of the medium (upper trace) than cells deeper in the tissue. In contrast, the half-times of pressure-relaxations after changing the cell turgor with the pressure probe (hydrostatic experiments; left part of the traces) are similar in all three cases

This result, however, still allows two interpretations for osmotic water flow in the segment: (a) cortical cell half-times could be very short (0.6 s) and water flows primarily cell-to-cell; or (b) cortical cell half-times could be long (10–20 s) and water flows readily through the cell wall pathway, but is mainly impeded by cell membranes. If alternative (a) were true, the response times for individual cells to a change in the osmotic pressure of the external medium would be strongly dependent on the position of the cells. To test these predictions, we measured the time course for pressure changes in individual cells at varying depths beneath the surface of the epicotyl during osmotically-induced shrinking and swelling. As shown in Fig. 6, the time course for the pressure change is strongly dependent on the position of the cell. At the surface, the pressure changes very quickly (faster than $\bar{t}_{1/2}$); three or four cells deeper, the pressure change is slower (about equal to $\bar{t}_{1/2}$); and deep inside the segment the pressure change is very slow (much slower than $\bar{t}_{1/2}$). Clearly the cells do not behave like isolated cells. This strong dependence of the osmotic response on the position of the cell supports the interpretation that cell half-times are very short and water flows through the cell-to-cell pathway to a large extent.

## Discussion

A striking observation in this work is that the half-time of water exchange of individual cortical cells is very short (0.3 to 1.0 s) when the measurements are started, and in some cases changes to substantially larger values (3 to 20 s). This could be caused (a) by an artifact due to the technique used, (b) a substantial decrease in Lp, or (c) by a decrease in $\varepsilon$. The latter possibility can be excluded, since $\varepsilon$ was found to remain constant or even increase after the switch to longer half-times occurred. We think that artifacts can also be excluded. Both the short and long relaxation curves show all the requirements of valid curves so that artifacts from the equipment as well as leakages in the cell membrane can be excluded. The effect could be due to a wound effect resulting from puncturing the cell which could lead to a transient increase in Lp in the beginning. However, this explanation is not likely because the area punctured by the tip of the microcapillary is only ca. $5 \cdot 10^{-2}$% of the cell surface area. This is unlikely to cause changes in $T_{1/2}$ (Lp) by more than one order of magnitude (see Zimmermann and Hüsken 1979).

It should be noted that switching occurs in a rather short time (often in 1–2 s) and in the presence of azide and at 4° C, which seems unlikely for a healing process. Furthermore, osmotic exchange experiments (in which no probe was introduced into the tissue) independently point to short half-times (see below). Therefore, we feel that the initially measured $T_{1/2}$-values are those which govern the water relations of the tissue under "normal" conditions.

The reason for the change in $T_{1/2}$ is still unknown. In plant tissues, where cells are connected to neighboring cells and to intercellular spaces, the conducting area for volume flow may not be identical with the cell surface area, which was used here for calculating Lp. Since the switching effect seems to occur when pressure changes are imposed on individual cells, it is possible that these manipulations change the immediate cell surroundings and alter the conducting area. Alternatively, plasmodesmata may contribute significantly to the membrane Lp (Robards and Clarkson 1976) and the switching effect may be due to an occlusion of these pores caused by the volume flow induced during the experiments. However, we do not know if the contribution of plasmodesmata to volume flow is large or why the pores should occlude simultaneously after a certain number of relaxations. It should be noted that such an irreversible change in $T_{1/2}$ has not been found up to now for other cells using the pressure probe or other techniques. It remains open if this effect is special for growing cells.

Osmotic exchange experiments on peeled segments support the finding of short cell $T_{1/2}$-values. The interpretation of such experiments is complicated, however, by solute diffusion in the free space of the tissue. Using a segment diameter of 1.6 mm and a $\bar{t}_{1/2}$ of 60 s, the diffusivity $D_t$ for osmotic exchange may be calculated (see Eq. 1) to be $6.6 \cdot 10^{-6}$ cm$^2$ s$^{-1}$. This value is similar to the diffusion coefficient in free solution of the solutes used (see legend of Table 1). However, one would expect that solute diffusion in the cell wall space would be severely restricted. If the path length of solute diffusion through the cylinder in the cell wall space is doubled, as assumed by Kohn and Dainty (1966), the apparent diffusion coefficient would be quadrupled. Interactions of the solute with the cell wall should further reduce the rate of solute penetration, perhaps by an order of magnitude (see discussion in Walker and Pitman 1976). Thus, the observed $D_t$ is too large to be accounted for by solute diffusion. The $D_t$-value is similar, however, to the diffusivity calculated for the radial water transport in the cell-to-cell pathway ($D_c = 3.2 \cdot 10^{-6}$ cm$^2$ s$^{-1}$, with $T_{1/2} = 0.6$ s, $2r = 47$ µm and a shape factor equal to unity; see Philip 1958). The $D_c$-value may be accurate only within a factor of 2 because of the inhomogeneities in cell dimensions. The similarity of the values for $D_t$ and $D_c$ suggests that water transport in pea epicotyls occurs to a considerable extent via the cell-to-cell pathway. Unfortunately, we have no corresponding data for water transport in the cell wall (apoplasmic) pathway which is necessary for a full description of water transport in higher plant tissues (Molz and Ikenberry 1974).

Our conclusion that cell-to-cell (membrane-controlled) water transport contributes substantially to the overall osmotic properties of the pea epicotyl is different from that of Kohn and Dainty (1966) who worked with thin discs of *Beta* and *Helianthus* storage tissue. From elution experiments using radioactively labeled sucrose, they concluded that solute diffusion, not hydraulic conductivity, was the principal factor controlling water exchange in their osmotic exchange experiments. Kohn and Dainty also found that the half-time for weight changes was dependent on the square of the thickness of the discs and used this result to support the idea of control of osmotic exchange by solute diffusion. However, cell-to-cell water transport should also show diffusion-type kinetics and thus a dependence of half-time on the square of the tissue dimensions (Philip 1958). It is remarkable that discs only 3–12 cells thick had half-times of up to 12 min, while in our experiments entire, peeled epicotyls had half-times of ca. 1 min.

Our results bear on the question of whether water transport limits cell enlargement in higher plant tissues. Ray and Ruesink (1963) tried to estimate the

water potential difference necessary to support the rate of water uptake involved in elongation of oat coleoptile segments by determining the concentration of mannitol in which the tissue neither gained nor lost water. After correcting for the influence of mannitol diffusion in the cell wall space, they estimated that rapidly elongating coleoptile segments have water potentials of 0.8 to 2.5 bar below the surrounding solution. With the isopiestic psychrometer, Molz and Boyer (1978) measured water potentials of $-1.7$ to $-2.1$ bar in intact, growing soybean hypocotyls. These values agreed well with estimated values obtained from their theoretical analysis, although this analysis depended in part on values for parameters obtained from the literature and from plants other than soybean. Using the theory of Molz and Boyer, we can calculate that the water potential gradient between the central vascular bundle and the epidermis in pea epicotyls would be ca. 0.2 bar for a 1% $h^{-1}$ growth rate and ca. 1 bar for a growth rate of 5% $h^{-1}$. These values are smaller than those estimated for other rapidly growing tissue, (Ray and Ruesink 1963; Molz and Boyer 1978; Boyer 1968) but still support the view that at the highest growth rates water uptake may partially limit cell expansion.

From our results we can conclude that during growth the individual cells in the pea epicotyl are in water flux equilibrium with their close surroundings (up to a few cells), but water potential gradients may exist over longer distances. Our results suggest that radial water transport in the pea epicotyl could occur substantially through the cell-to-cell pathway. If this is true, the effects of auxin on water permeability reported in the literature (Boyer and Wu 1978) could be understandable as an effect of auxin on membrane-controlled water transport. Pressure probe measurements can show whether there is an effect on the Lp of cell membranes by auxin. In combination with measurements of the irreversible extension of the cells, they should also show how mechanical (elastic and plastic) properties of individual cells change during the induction of growth by plant hormones.

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