Solutes in the Free Space of Growing Stem Tissues

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ABSTRACT

The concentration of osmotically active solutes in the cell wall free space of young stem tissues was studied using a variety of extraction methods. When the intercellular air spaces of etiolated pea (Pisum sativum L.) internodes were perfused with distilled H2O, the resulting solution contained a solute concentration of about 70 milliosmoles per kilogram. A second procedure involving vacuum infiltration of segments followed by centrifugation to collect the free space solution gave similar results. Apical stem segments yielded free space extracts about twice as concentrated as those from basal portions of the stem. After correcting for dilution of the free space solution by the infused water, the osmotic pressure of the undiluted free space in pea stem tissue was estimated to be 2.9 bars for apical segments, 1.8 bars for basal regions. These values may be somewhat overestimated due to solute efflux from intracellular pools during the extraction procedure. Similar results were obtained for stem regions of etiolated soybean ( Glycine max [L.] Merr.) and cucumber ( Cucumis sativus L.) seedlings.

From measurements of the electrical conductivity and refractive index of free space extracts before and after ashing, it appears that 25% of the solutes are inorganic electrolytes and 75% are organic nonelectrolytes with an average size similar to that of glucose.

A significant osmotic pressure in the wall space offers an explanation for the frequent observation that nontranspiring plants have negative water potentials. Calculations of hydraulic resistance from water potential data must take into account solutes in the free space, else 'apparent,' but unreal, changes in resistance may be calculated.

In plant tissues at osmotic equilibrium, the \( \Psi^3 \) of water in the FS of the tissue will be the same as that of water inside the cells. Within cells, \( \Psi \) may be dissected into two components: the hydrostatic pressure (turgor pressure) and \( \pi' \). In the extracellular space, \( \Psi \) may similarly be divided into \( \pi'' \) and a hydrostatic pressure term. In relatively dry plant tissues, water in the cell wall may be under a significant tension (sometimes termed negative hydrostatic pressure) due to surface tension effects at air-water interfaces within the wall space. Under such conditions, \( \pi'' \) will be a relatively insignificant component of \( \Psi \). However, under wetter conditions, \( \pi'' \) may become the dominant term for \( \Psi \) of the FS. A tacit assumption in many studies of plant water relations is that \( \pi'' \) is negligible (3, 23). However, a few quantitative investigations indicate that \( \pi'' \) in leaf tissues may be in the range of 1 to 2 bars (1, 10). Knowledge of the quantity and identity of FS solutes is important not only for an understanding of plant water relations, but is equally important for studies of nutrient transport (8, 12), host-pathogen relations (9), the electrophysiology of cell membranes (10), and the physical chemistry of cell walls (21).

In this investigation, we have extracted solutes from the cell wall FS of etiolated stems of pea, soybean, and cucumber seedlings by three different techniques (forced perfusion, infiltration followed by centrifugation, and expression of fluid by the pressure bomb technique). We find significant \( \pi'' \) values, which are higher in apical than in basal regions of the stem. These findings have important ramifications for studies of the hydraulic resistances of plant tissues.

MATERIALS AND METHODS

Plant Material. Pea ( Pisum sativum L. cv Alderman), cucumber ( Cucumis sativus L. cv Burpee's Pickler), and soybean ( Glycine max [L.] Merr. cv Wayne) seedlings were grown in wet vermiculite in complete darkness at 27 ± 1°C and low RH (20–50%, not controlled). Plants were handled under dim green light obtained from a 40-w cool-white fluorescent lamp filtered through one amber and two green acetate filters (Roscolene No. 813 and No. 874; Roscoe, Port Chester, NY). Seedlings 6 to 8 cm tall were selected for experimentation; all subsequent operations were performed in the light. In some cases, pea seedlings were carefully removed from the vermiculite, the cotyledons excised with a new razor blade (under dim green light), and the seedlings replanted in vermiculite to grow for 2 d more in complete darkness.

Osmotic Pressure of FS (\( \pi'' \)). The solution which makes up the cell wall FS was collected by three different methods, and 8- to 10-ml samples were measured in a vapor pressure osmometer (Wescor model 5100; Wescor, Logan, UT). The osmometer was calibrated in the 0 to 100 mOs-kg\(^{-1}\) range before and after all measurements. Osmolality was converted to osmotic pressure (at 20°C) by multiplying the value in Os-kg\(^{-1}\) by 24.37 bar-Os\(^{-1}\) kg\(^{-1}\).

The FS solution was collected in the first method by forced perfusion of the intercellular air channels with water. In this method, the basal ends of five excised segments were sealed with quick-setting epoxy (Qwik-Stik; GC Electronics, Rockford, IL) into a pressure vessel completely filled with water (Fig. 1). The vessel was pressurized using a gas cylinder, and the fluid which exuded from the ends of the segments was collected with a capillary tube. Excision was made under water (so the contents of the cut cells would be immediately released to the water), and the segments were incubated on water for 30 to 40 min on a rotary shaker before being placed in the apparatus. In a variation of this method, intact pea seedlings were sealed with epoxy into the lid of the pressure vessel, such that 4 to 5 cm of the shoot extended outside of the vessel (Fig. 1). The seedlings were vacuum infiltrated...
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with water and blotted dry, the lid was mounted onto the vessel, and the pressure inside the root chamber was raised to 1 bar above ambient pressure with a pump. The droplets which exuded from the stomatal pores on the stem surface were collected with a capillary tube and measured with the osmometer.

In the second method, 2-cm-long segments were excised and washed as above, then vacuum infiltrated with water for 60 s. The infiltrated segments were quickly blotted dry, packed into the body of a 10-ml polyethylene syringe, and centrifuged for 3 min at 150g to 450g. The solution removed from the segments in this way was collected in a tube at the bottom of the syringe and measured with the osmometer.

In the third method, pea stems were cut under water either 1.5 cm below the apical hook or just above the cotyledons and washed with water for 30 min. Groups of three seedlings were sealed with epoxy into a pressure chamber (PMS Instrument Co., Corvallis, OR) with the cut ends extending about 3 to 5 mm outside of the chamber. The portions of the seedlings inside the chamber were covered with plastic wrap to reduce evaporative water loss. The pressure in the chamber was raised using a gas cylinder, and the fluid expressed from the tissue was collected directly onto 6.5-mm paper discs and measured in the osmometer.

Volume of Air Spaces. To determine the volume occupied by intercellular air space, stem segments 1.5 cm long were preincubated on water for 30 min to ensure good hydration, weighed, infiltrated with silicone oil (Fluid 200, 2 centistokes viscosity; Dow Corning) under vacuum, and re-weighed. The % air volume in the tissue was calculated from the increase in weight and the density of the oil. A similar procedure was followed using water as the infiltrating fluid. From the difference between the oil and water values, one could calculate the absorption of water by the cells during the infiltration with water.

Volume of FS Water. Two separate techniques were used to estimate the volume of water in the apoplasm of stem tissue. The first technique was the conventional measurement based upon efflux of $[^{14}C]$mannitol out of tissue preequilibrated in labeled mannitol (6). Ten segments (2 cm long) were excised from the apical or basal stem regions, and their cuticles were abraded with a slurry of carborundum powder and water. The segments were washed twice to remove the abrasive, blotted dry, incubated in 10 mM unlabeled mannitol for 30 min, then transferred to a 3.5-cm plastic Petri dish containing 5 ml of 10 mM mannitol with 5 μCi·ml$^{-1}$ of $[^{14}C]$mannitol (New England Nuclear). The segments were incubated on the radioactive mannitol solution for 120 min with constant agitation on a rotary shaker. After this period, the segments were blotted dry and impaled onto a pair of 30-gauge hypodermic needles. The mounted segments were dipped into water for about 3 s to remove the surface mannitol solution, blotted dry, and submerged into 200 ml of water which was stirred continuously. Periodically, 150-μl aliquots were removed from the wash water, placed into 3.0 ml of Aquasol scintillation counting cocktail, and subsequently counted with a liquid scintillation counter (model IS7800; Beckman). When efflux was complete, the segments were blotted dry and weighed. Mannitol diffused out of the segments over a period of about 45 min. The half-time for efflux was 5 to 10 min for the various stem tissues. Less than 1% of the total radioactivity remained in the tissues after washout. The appearance (translucence) of the tissue did not change noticeably during the course of the procedure, indicating that the tissue air spaces did not fill up with water, although partial infiltration cannot be excluded.

The second technique to measure the volume of apoplastic water in stem segments involved measuring the dilution by FS water of a dye solution introduced into the tissue FS by vacuum infiltration. This method requires that the dye not be taken up by the cell or bound to the cell wall. Dyes were initially screened by incubating bisected pea internodes in the dye solution for 10 min, and then looking for complete wash out of the dye into water. Three dyes passed this initial test and, upon further experimentation, Indigo Carmine proved most suitable. To measure the FS water volume, 15 to 20 stem segments (1.5 cm long) were vacuum infiltrated with the dye solution, carefully blotted dry, and centrifuged as described above. Approximately 5 min elapsed between the time of infiltration and the start of centrifugation. This time was the same as that used in extracting FS solutes and was judged sufficient to permit dye equilibration with the surrounding FS solution. A 5-μl sample of the collected fluid was diluted with 0.5 ml pH 7 buffer. The absorbance of this solution was measured with a Gilford model 250 spectrophotometer (650 nm wavelength). By comparing this absorbance value with a calibrated dilution curve, the dilution of the introduced dye solution by FS water was calculated.

For reproducible results, the stem segments had to be well hydrated by preincubation on water for 20 to 30 min. Preliminary tests showed that, upon vacuum infiltration, water was taken up by the cells of the tissue, concentrating the dye solution which remained in the FS. The amount of this concentration could be estimated from the ratio of air volume measured by water infiltration to the air volume measured by oil infiltration. Applying this correction factor to the original dye solution, one can estimate the dilution of FS solutes from the concentration of dye in the extract.

Respiration Measurements. To determine whether infiltration of the tissue led to $O_2$ deficiency, $O_2$ uptake by a group of five pea stem segments (1 cm long) was measured, before and after infiltration with water, using a model 53 Biological Oxygen Monitor (Yellow Springs Instrument Co.).

Solute Characterization. The electrical conductivity of the FS solution collected from several groups of pea segments by the centrifugation method was measured with a conductivity meter (Radiometer Copenhagen type CDM 2d meter with type CDC 114 conductivity cell). The refractive index of the solution was measured with an Abbe Refractometer (Fisher Scientific). The solution was evaporated to dryness at 60°C, ashed in a muffle.
furnace at 500°C for 1.5 h, brought back into solution in distilled H₂O, and remeasured for electrical conductivity and refractive index.

RESULTS

Perfusion Method. When water was forced under pressure to flow through young pea internodes (Fig. 1), the solution which exuded from the free ends contained a solute concentration of about 70 mOsm·kg⁻¹ (Fig. 2A). As more volume was forced through the tissue, the osmolality of the exudate decreased to a plateau value of about 40 mOsm·kg⁻¹. When the perfusion was halted for 20 min and then restarted, the osmolality of the exudate increased to the initial high value. Continued perfusion reduced the osmolality of the exudate to the 40 mOsm·kg⁻¹ plateau. A similar pattern was obtained when 1 mm CaSO₄ was used for perfusion.

Most of the water forced through the pea tissue by this method appears to flow through the intercellular air spaces in the cortex. This fact was ascertained by microscopic observations under UV light of pea stems perfused with a 0.1% sodium fluorescein solution. During perfusion, the tissue air spaces fill up with fluid, resulting in a translucent appearance of the tissue.

The results from the perfusion procedure, however, were quite variable, apparently due in part to the variation in the size of the air channels. Therefore, the alternative method of infiltration of the air space, followed by removal of the infiltrated solution by centrifugation, was tried. This method offered the potential of calculating the dilution of the FS solution by the infiltrated water.

Centrifugation Method. This second technique gave results similar to that obtained by the perfusion method. The first extraction produced a relatively concentrated solution; subsequent extractions yielded progressively more dilute solutions until a plateau was reached (Fig. 2B). Similar values were obtained whether the stem segments were centrifuged at 150g, 240g, or 470g. Centrifugation at 240g was used routinely in the following experiments, since the volume of liquid obtained was sufficient for one to several osmometer measurements.

The osmolality of the solution extracted by infiltration and centrifugation depended on which region of the stem was used (Table I). Apical regions yielded solutions about twice as concentrated as did basal regions. This pattern was observed not only in pea internodes, but also in soybean and cucumber hypocotyls (Table I). These values, it should be noted, are minimum values. If solute efflux from cells is slow, then the extracted solution will have an osmolality which underestimates that of the original FS solution by the proportion of water introduced to the FS by infiltration. Furthermore, if the amount of dilution differed between apical and basal segments, this might explain the observed difference in osmolality of the extracted solutions. Therefore, to estimate the amount of dilution, the volume of air space and of water in the FS of the tissue were measured.

Air and Water FS Volumes. Air space volumes were measured by infiltrating tissues with silicone oil. From the increase in weight, the per cent air space in the various tissues was found to be 1 to 5% of the volume of the stem tissues (Table II). These values are in agreement with visual estimations made from cross-sections of the stems. Values obtained by infiltration of the tissues with water were always higher than those obtained with oil. Stems which were cut directly from seedlings without allowing the segments a hydration period had very high apparent air space volumes (up to 10%). Inasmuch as water infiltration will raise the water potential of the FS and, consequently, water will be absorbed by the cells, air space volumes will be overestimated in tissues infiltrated with water.

The volume of FS water was estimated by efflux of labeled mannitol from tissues preequilibrated with [¹⁴C]mannitol. FS water measured by this method occupied about 4% of the tissue volume of pea stems (Table II). Hence, we can estimate that infiltration of the air spaces with water reduced the original FS concentration about 50%.

An alternative method, based upon dilution of an infiltrated dye solution, was also used to estimate the FS water volume. This method gave values similar to the radioactive mannitol method (Table II), except in the case of cucumber stems. This discrepancy may indicate that the dye is absorbed or bound in some way by the cucumber tissue. Absorption or binding of the dye results in an overestimation of the FS water volume and, hence, an underestimation of the extrapolated original FS concentration. A reversible binding of [¹⁴C]mannitol by stem tissue, such that some of the bound label is released during efflux, will result in a similar error.

We used the average of the two dilution estimates to extrapolate back to the FS solute concentration before infiltration with water. Such an extrapolation (Table I, last column) shows that apical and basal tissues still differ in the osmolality of their FS solutions. This extrapolation assumes that there is no solute efflux during the extraction procedure to compensate for the dilution of the FS solution. The data in Figure 2A, however, indicate that there is at least some solute efflux during the procedure. On the other hand, some FS solutes would likely diffuse out of the tissue during the preliminary washing period after excision. Thus, the original concentration of FS solutes probably lies somewhere between the extrapolated value and the extracted value.

It might be argued that the fluid extracted from segments was contaminated with solutes released from damaged cells at the cut
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Table I. Osmotic Pressure of FS Solutions Extracted from Various Stem Tissues Infiltrated with Water
Except in experiment C, FS solutions were collected by centrifugation of excised segments. Reported values are means ± se of first extract; (n) is the number of measurements.

<table>
<thead>
<tr>
<th>Exp. Series</th>
<th>Plant Material (n)</th>
<th>Region of Stem</th>
<th>Extracted Solutiona</th>
<th>Corrected for Dilutionb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mOs·kg⁻¹</td>
<td>bar</td>
</tr>
<tr>
<td>A</td>
<td>Pea (8)</td>
<td>Apical</td>
<td>73 ± 7.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>44 ± 5.0</td>
<td>1.1</td>
</tr>
<tr>
<td>B</td>
<td>Pea, cotyledon excised (4)</td>
<td>Apical</td>
<td>25</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>16</td>
<td>0.4</td>
</tr>
<tr>
<td>C</td>
<td>Pea, intact† (5)</td>
<td>Upper half</td>
<td>70 ± 7.3</td>
<td>1.7</td>
</tr>
<tr>
<td>D</td>
<td>Cucumber (3)</td>
<td>Apical</td>
<td>47</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>20</td>
<td>0.5</td>
</tr>
<tr>
<td>E</td>
<td>Soybean (10)</td>
<td>Apical</td>
<td>64 ± 5.2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>16 ± 1.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

a Osmolality of first extract.
b Using average dilution factors from Table II.
c FS solution was forced out of infiltrated air spaces in stem by pressurizing the root chamber.

Table II. Volume of Air and Water in the FS of Various Plant Tissues, as Per Cent of Tissue Volume

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Region</th>
<th>Air Space</th>
<th>FS Water¹</th>
<th>FS Conc. after Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mannitol method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Pea</td>
<td>Apical</td>
<td>2.8</td>
<td>4.1</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>3.2</td>
<td>4.0</td>
<td>56</td>
</tr>
<tr>
<td>Soybean</td>
<td>Apical</td>
<td>3.6</td>
<td>3.6</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>5.2</td>
<td>4.6</td>
<td>47</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Apical</td>
<td>1.2</td>
<td>4.1</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>1.1</td>
<td>4.6</td>
<td>81</td>
</tr>
</tbody>
</table>

¹ Mean of eight to ten values, measured by oil infiltration.
² Mean of two values, measured by radioactive mannitol efflux.
³ Using 0.2% Indigo Carmine solution.

surface of the segments, despite the precautions taken to wash out these solutes. This possibility is unlikely to give a higher osmolality in apical segments than in basal segments; i.e. the basal cells are several-fold larger than the apical cells and, thus, would release a greater amount of solutes. However, we tested for the influence of cut surfaces in the following way. Intact pea seedlings were sealed into a pressure vessel (Fig. 1) and vacuum infiltrated with water. The fluid in the air channels was then driven out of the stem through the stomatal pores by raising the pressure in the root chamber by 1 bar. The exuded solution had an osmolality of 70 mOs·kg⁻¹ (Table I, exp. C). Since the tissue from which this solution was obtained was uncut and undamaged, the solutes could not have come from cells damaged by cutting, nor are they likely due to an artifactual efflux in response to wounding. This value is the same as that found in excised, washed segments. Note that dilution of the FS solution would still be expected to occur because of vacuum infiltration of the air spaces. If the procedure is attempted without infiltrating the air spaces, no fluid is expressed from the seedling using low root pressures.

We were also concerned that infiltration of the air spaces might cause anaerobiosis, and consequently solute leakage from O₂-starved cells. The following observations argue against this possibility. When pea stems were perfused with water which was previously bubbled with 100% O₂, the solute concentration of the exudate remained as high as when distilled H₂O was used. In the centrifugation technique, the time between infiltration and centrifugation was about 5 min. It is unlikely that the tissue would become anaerobic in such a short period. When the rate of O₂ uptake by pea segments was measured with a Warburg type apparatus, the respiration rate remained essentially the same before and after infiltration with water. Furthermore, when the centrifugation method was applied to pea seedlings which 2 d previously had their cotyledons excised, the extracted solution had a solute concentration only one-third that found in normal seedlings (Table I). The osmolality of expressed cell sap from such seedlings, however, was reduced only about 20% from normal seedlings. From these results, it seems unlikely that infiltration and centrifugation caused gross leakage of cell contents. Finally, when fluid was expressed from pea stems, which were not infiltrated with water, by a Scholander-type pressure bomb, the solute concentration was higher when the fluid was exuded from the apical end of the seedling than from the basal end (Table III). These data are in agreement with those obtained by the centrifugation procedure, and argue against an artifact produced by O₂ deficiency. From these considerations, it appears that young stem

Table III. Osmolality of Exudates Expressed from Pea Internodes Using a Scholander-Type Pressure Chamber
Sequential samples (8 µl) were taken from either the apical or basal cut ends of a group of three seedlings sealed into the chamber. Data sets A, B, and C are apical exudates; D, E, and F are basal exudates.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>From apical ends</th>
<th>From basal ends</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>mOs·kg⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>67</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>75</td>
</tr>
</tbody>
</table>

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tissues have a significant $\pi^w$ (Table I).

**Nature of FS Solutes.** A quantity of FS solution was collected by the centrifugation technique and its electrical conductivity and refractive index was measured before and after ashing. These two physical characteristics, combined with the osmometer measurements, can help to characterize the type of molecules contributing to $\pi^w$. As shown in Table IV, about 75% of the osmolality and essentially all of the change in refractive index was lost by ashing. Most of the electrical conductivity, in contrast, survived ashing. The simplest, although not the only, interpretation of these data is that 75% of the solutes making up the FS solution are organic nonelectrolytes. The remaining 25% appear to be inorganic electrolytes. From the refractive index and osmolality data, we can say that, on average, the size of the solute contributing to $\pi^w$ is the same size as a sugar monomer such as glucose (Table IV).

**DISCUSSION**

The data presented in this paper point to two important conclusions; first, the apoplast of young stem tissues contains a significant concentration of osmotically active solutes; second, the concentration of these solutes is higher in the growing region of the stem than in the basal non-growing region. Because similar estimates of $\pi^w$ were obtained using three different methods, we deem it unlikely that the the solutes extracted from the stem tissues were artificially produced by the extraction procedures. Terry and Bonner (24) used an infiltration and centrifugation method, similar to that used here, to extract cell wall polysaccharides from pea stems, and showed that leakage of the cell contents only began at centrifugation forces greater than 3,000g. In our experiment, we routinely spun the segments at 240g. High solute concentrations were also found in our perfusion experiments (Fig. 2A). The observations that intact pea seedlings (i.e. no cut surfaces) contained 70 mOsm kg$^{-1}$ in their infiltrated wall space (Table I, exp. C), and that FS solutes (but not intracellular solutes) were greatly diminished by previous excision of the cotyledons, further supports the conclusion that young stem tissues normally have a significant $\pi^w$. Additional evidence to support this conclusion comes from direct turgor pressure measurements of pea cortical cells which implicate a $\pi^w$ of about 3 bars (4, 5).

Although it has long been suggested that the apoplasm of plant tissues contains substantial pools of both organic and inorganic solutes (15), there have been few quantitative studies. Using an equilibrium perfusion method, Bernstein (1) estimated the $\pi^w$ of cabbage leaves to be 1 to 2 bars. Jacobson (10) extracted extracellular fluid from the leaves of Venus'-flytrap by a centrifugation method, and measured an osmolality of 63 mOsm kg$^{-1}$ ($\pi^w = 1.5$ bar). Steudle et al. (22) have proposed that the low turgor pressure measured in *Kalanchoe* leaf cells might be due to a high malate concentration in the wall space. The presence of carriers for sucrose and other metabolites in the plasmalemma of various leaf, stem, and root tissues implies that the FS contains these solutes, and this is confirmed by washout studies (8, 9, 12). Furthermore, Satter and her colleagues have documented massive changes in the potassium concentration in the cell walls of the pulvini of nyctinastic leaves (see 18 for review). Unfortunately, no attempt has been made in these studies to relate the changes in absolute potassium concentrations to changes in free potassium levels. Only freely diffusible solutes will have an influence on the FS $\Psi$ (16, 17).

Other indirect evidence for a significant $\pi^w$ in plant tissues comes from measurements of the fluid expressed by the pressure bomb technique. Although many studies have assumed that the exudate was very dilute, several (but not all) studies in which the measurements were made have found osmotic pressures in the range of 1 to 3 bars (7, 11, 19, 27). These values, however, are not necessarily the same as $\pi^w$. If the cells are very effective at removing solutes from the transpiration stream, the FS solution will be more dilute than the contents of the xylem. On the other hand, if water evaporates faster than solutes are taken up into the cell, then solutes will accumulate in the wall space. An additional complication in the pressure bomb technique is that pure water is expressed from the cells inside the chamber (if the cell membranes are ideally semipermeable), so the fluid expressed from the tissue should be more dilute than the undisturbed FS solution. Evidence that this is the case can be seen in Table IV, where the fluid expressed from pea stems gradually becomes more dilute as more volume is removed from the tissue.

The presence of FS solutes has important consequences for studies of plant water relations. First, pressure bomb measurements of plant water potentials will be in error by the amount $\pi^w$, since this method measures only the pressure component of $\Psi$ of the FS water (17). This problem has been recognized by some investigators (2, 27). The error usually seems to be small, especially in plants having low water potentials. However, the dilution of the FS solution which occurs during the pressure bomb technique has generally been ignored or unrecognized.

A more serious problem arises when one tries to measure hydraulic resistance at or near the point of zero transpiration. An equation commonly found in the water relations literature attempts to characterize a resistance (R) by relating the water flux (F) to the drop in water potential along the pathway, as follows:

$$F = \frac{\Delta \Psi}{R}$$

where $\Delta \Psi$ is the transpirational water flux (although the water flux for cell enlargement is also sometimes included), and $R$ is usually the difference between the $\Psi$ of the plant tissue (\(\Psi^p\)) and of the water supply (i.e. soil or xylem water). However, it should be clear that solutes in the apoplast will lower $\Psi^p$, and that $\Psi^f$ will stay depressed as long as the solutes remain in the cell wall space (assuming the wall space cannot support a positive hydrostatic pressure, which is reasonable since it is in open contact with the atmosphere through the intercellular air space network). By equation 1, under these circumstances one would then calculate an

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**Table IV. Physical Characteristics of FS Solutes Removed from Pea Internodes by Infiltration/Centrifugation**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Sample</th>
<th>EC$^a$</th>
<th>Osmolality</th>
<th>$\Delta R^b$</th>
<th>$\Delta R$/Osm.$^c$</th>
<th>EC/Osm.$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu$mho cm$^{-1}$</td>
<td>mOsm kg$^{-1}$</td>
<td>$\Delta \eta$ $10^4$</td>
<td>mOsm$^{-1}$ kg$^{-1}$ $10^4$</td>
<td>$\mu$mho cm$^{-1}$ mOsm$^{-1}$ kg$^{-1}$</td>
</tr>
<tr>
<td>1</td>
<td>Before ashing</td>
<td>1,310</td>
<td>60</td>
<td>16</td>
<td>0.27</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>After ashing</td>
<td>838</td>
<td>14</td>
<td>1.0</td>
<td>0.07</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Before ashing</td>
<td>996</td>
<td>51</td>
<td>12.5</td>
<td>0.25</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>After ashing</td>
<td>821</td>
<td>13</td>
<td>1.0</td>
<td>0.08</td>
<td>63</td>
</tr>
</tbody>
</table>

*a* Electrical conductivity.  
*b* Change in refractive index at 20°C, compared with pure water.  
*c* Compare with values of 0.48 for sucrose, 0.25 for glucose, 0.23 for citric acid, 0.15 for glycerol, 0.08 for urea, 0.055 for KCl. Data obtained from Ref. 26.  
*d* Compare with values of 65 for KCl, 51 for NaCl, 37.5 for citric acid, 34 for Na acetate. Data obtained from Ref. 26.
ever increasing hydraulic resistance as the transpirational flux went to zero; i.e. \( \Psi \) will not approach zero at zero flux. An example of such a case may be seen in Figure 2 of Reference 3. Such an 'apparent' change in resistance, however, has no physical reality, but is due to the lack in equation 1 of a \( \pi^m \) term to account for the non-zero intercept of the equation. A modified equation to include the effect of FS solutes would be:

\[
F = (\Delta \Psi + \pi^m)/R
\]

Two physical states or conditions are recognized by this relation. In the first state, \( \Delta \Psi \) equals \( -\pi^m \). For \( \Delta \Psi \) to approach zero bar would require either the cells to take up solutes from the FS or the solutes to diffuse away. But diffusion over macroscopic distances is a very slow process and could easily be compensated by efflux of solutes from cells. As a consequence, there could be a standing gradient in \( \Psi \) between the water source and the plant tissue, without net flux of water.

In the second physical state, \( \Delta \Psi \) falls below \( -\pi^m \) (say, by transpirational water loss). In the wall space, this entails the development of a tension (negative hydrostatic pressure) in the FS water. Concomitantly, water will flow through the tissue and the rate of flow will be governed by the real hydraulic resistance of the pathway for water flow, which is determined by the combined cell-to-cell and cell wall pathways for water flow (14).

Standing gradients in water potential under conditions of zero transpiration, as well as apparent changes in hydraulic resistance, have been observed by various investigators (3, 23) who have offered other interpretations to explain the apparent anomaly. This question of real versus apparent changes in hydraulic resistances, as well as the nature of the low \( \Psi \) in growing tissues, is dealt with further in an accompanying paper (4). Up to now, theoretical analyses of water transport through plant tissues have not included the effects of solutes in the FS (14). Such an analysis is needed for a fuller understanding of the consequences of such FS solutes on water transport.

The nature and role of the FS solutes found in young stem tissues is not yet clear. Bernstein (1) found that only 22% of \( \pi^m \) in sunflower leaves was due to electrolytes (this is similar to the value of 25% found for peas in this work) and he suggested that FS solutes are in dynamic equilibrium with intracellular solutes. This appears also to be true at least for part of the FS solutes in peas (Fig. 2B). Hypocotyls were observed to be very 'leaky' to a variety of metabolites by Hancock (9), who suggested that the 'leakiness' commonly observed in tissue slices is not an abnormality, but rather a general characteristic of the original condition, reflecting the dynamic equilibria between solutes inside and outside of cells. A similar leakiness was recently reported in maize roots (20). Terry et al. (25) extracted water-soluble polysaccharides from the FS of pea segments using a centrifugation technique; the amount of carbohydrate (about 1.5 g l\(^{-1}\)), however, was insufficient to account for \( \pi^m \) found in this study. Furthermore, the refractive index data (Table IV) show that the solutes which make up \( \pi^m \) in pea are about the size of a monosaccharide, on average. Interestingly, McNeil (13) found that the principal osmotic substances in sunflower hypocotyls were hexoses and organic potassium salts.

The FS solutes in young stems might be related to the rapidly growing nature of these tissues. Rapidly expanding cells require a massive influx of solutes to maintain turgor in the face of dilution of the osmotic content of the cell by water uptake during growth. A high FS concentration of metabolites, in transit from the phloem to the expanding cells, might assist in solute accumulation and thus the maintenance of a high osmotic pressure within the growing cells. This hypothesis would be in accord with the observed higher FS solute concentration in apical segments compared with basal segments (Table I). A second possibility is that transpirational water loss is very rapid compared with solute uptake into the cells of the tissue. In such a case, solutes would build up in the wall space of the tissue. Transpirational flow would also work against the passive tendency of the solutes to diffuse towards the (less concentrated) water source. The fact that pea stems continue to efflux solutes in the perfusion experiments (Fig. 2A) argues against transpirational build up of solutes as being the sole process contributing to \( \pi^m \). Another possibility is that the FS solutes are part of a mechanism for regulation of turgor pressure by control of the gradient in solute concentration across the cell membranes. In this sense, control of \( \pi^m \) would act as a mechanism to buffer the cells from changes in the external water conditions, within certain limits. Further work is necessary to determine the identity of these FS solutes, and the role they play in plant tissues.

LITERATURE CITED