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Water Relations of Growing Maize Coleoptiles[1]

Comparison between Mannitol and Polyethylene Glycol 6000 as External Osmotica for Adjusting Turgor Pressure

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ABSTRACT

Water relations of growing segments of maize (*Zea mays* L.) coleoptiles were investigated with osmotic methods using either mannitol (MAN) or polyethylene glycol 6000 (PEG) as external osmotica. Segments were incubated in MAN or PEG solutions at 0 to −15 bar water potential (ψ_0) and the effects were compared on elongation growth, osmotic shrinkage, cell sap osmolality (OC), and osmotic pressure (π_i). The nonpenetrating osmoticum PEG affects π_i in agreement with Boyle-Mariotte's law, *i.e.* the segments behave in principle as ideal osmometers. There is no osmotic adjustment in the ψ_0 range permitting growth (0 to −5 bar) nor in the ψ_0 range inducing osmotic shrinkage (−5 to −10 bar). Promoting growth by auxin (IAA) has no effect on the osmotic behavior of the tissue toward PEG. In contrast to PEG, MAN produces an apparent increase in π_i accompanied by anomalous effects on segment elongation and shrinkage leading to a lower value for ψ_0 which establishes a growth rate of zero and to an apparent recovery from osmotic shrinkage after 2 hours of incubation. These effects can be quantitatively attributed to uptake of MAN into the tissue. MAN is taken up into the apoplastic space and the symplast as revealed by a large temperature-dependent component of MAN uptake. It is concluded that MAN, in contrast to PEG, is unsuitable as an external osmoticum for the quantitative determination of water relations of growing maize coleoptiles.

Incubations in osmotic solutions are often used for experimentally changing the water relations of growing plant tissues. Ideally, osmotic solutes used for this purpose should produce quantitatively defined changes in the turgor pressure of the incubated tissues without interfering with other physiological properties, particularly with the osmotic pressure of the cells. Traditionally, defined turgor changes are induced by incubating the tissue in osmotic solutions of defined π_0[2] prepared with MAN or other solutes. This approach has certain technical advantages (*e.g.* rapid adjustment of water potential equilibrium between tissue and medium, maintenance of steady-state conditions with respect to the growth process) and does not necessitate sophisticated equipment. Moreover, since the tissue is incubated in a solution, a rapid and homogeneous administration of hormones, inhibitors, etc., is easy to accomplish. A disadvantage of this traditional method, which has repeatedly been emphasized in the literature (4, 6), is that turgor pressure is not directly determined but obtained by calculation and that one must therefore rely on the assumption that an experimental shift in the medium water potential ($\psi_0 = -\pi_0$) results in an equivalent shift of turgor pressure in the growing cells (as long as cell-volume changes are negligible). This assumption may only be valid if the osmotic pressure of the tissue (π_i) remains practically constant during the experimental period. Up-regulation of turgor by osmotic adjustment (as observed, *e.g.* in the growing zone of maize or barley leaves; see refs. 16, 29) or significant uptake of the external osmoticum into the cells would obscure the simple relationship between ψ_0 and turgor and would therefore lead to erroneous results.

In this paper we present a systematic investigation of the effects of osmotic stress on π_i of maize coleoptile segments with particular regard to auxin-induced growth. We compared two commonly used osmotica, MAN as a low mol wt solute penetrating into the apoplastic space of the tissue, and PEG, a high mol wt solute which cannot enter the apoplastic space (2, 11, 19). To characterize the physiological influences of these osmotica on the water relations of coleoptile segments, we investigated their effects on π_i not only in the ψ_0 range required to inhibit growth but also at lower ψ_0 inducing osmoelastic tissue shrinkage. This range offers the possibility of testing whether the tissue behaves as an ideal osmometer, *i.e.* whether the changes of π_i induced by external osmotica are quantitatively produced by changes of cellular water content at constant solute content as predicted by Boyle-Mariotte's law.

MATERIAL AND METHODS

Caryopses of *Zea mays* L., cv Brio (from Asgrow, Buxtehude, FRG) were sown on moist vermiculite in covered plastic boxes and the seedlings grown for 4 d in darkness at 25.0 ± 0.3°C. A 10-min pulse of red light (0.7 W m^{-2}) was applied 24 h before harvesting to obtain straight coleoptiles of 25 to 30 mm in length (12). Ten 10-mm segments dissected 3 mm below the coleoptile tip were, after removing the primary leaf, lined up on a 0.8-mm stainless steel tube and, after a 1-h preincubation in water, incubated in 20 mL of osmotic solutions (with or without 10 μmol L^{-1} IAA) under vigorous

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[2] Abbreviations: π_0, π_i, osmotic pressure of incubation medium and tissue, respectively; MAN, mannitol; OC, molal osmotic concentration; ψ_0, water potential of incubation medium.

aeration at 25.0°C in normal laboratory light. Osmotic test solutions were prepared by dissolving MAN or PEG (mol wt range 5600–7000; both from Roth, Karlsruhe, FRG) in distilled water using the formulas of Michel *et al.* (20, 21). The osmotic pressure of these solutions was checked by vapor pressure osmometry (model 5100 C, Wescor, Logan, UT). Elongation kinetics were determined by measuring the length changes of 10 segments with a ruler. For determination of osmotic pressure, segments were blotted, weighed, and frozen at −20°C. After thawing, samples of 10 segments were centrifuged at 15,000 min^{-1} for 15 min at 5°C and the osmolality of the supernatant determined in triplicate by vapor pressure osmometry. Osmolalities were converted into osmotic pressure (bar) by multiplying with a factor of 24.8 (according to van't Hoff's equation). These values were considered to represent the average osmotic pressure of the cell sap (π_i) without correction for dilution by apoplast solution. The MAN content of coleoptiles was determined by the method of Lewis and Smith (15). Samples of 10 frozen-thawed segments were homogenized with 5 mg powdered charcoal and the slurry centrifuged at 15,000 min^{-1} for 15 min at 5°C. Ten microliters of diluted (1:5) supernatant were added to 990 μL distilled water (to give 1 mL test solution), 1 mL of Na-acetate-buffer (1 mol L^{-1} [pH 4.5]) and 1 mL $NaIO_4$ solution (750 mg L^{-1}) in a 1-cm quartz cuvette. The blank contained 1 mL distilled water instead of $NaIO_4$ solution. The absorbance decrease at 260 nm caused by mannitol-dependent periodate reduction was recorded with a spectrophotometer. The corrected values were converted into molal concentrations using a standard curve (linear between 0 and 100 μg MAN per mL test solution). Sucrose, fructose, or glucose did not significantly interfere with this assay.

All experiments were repeated at least four times at different occasions. The figures show means (± estimates of standard error where appropriate). Standard errors of growth experiments were in the range of 1 to 2%.

RESULTS

The following experiments were performed with solutions of MAN and PEG in the absence of any other solutes in order to minimize the interference of osmoregulatory solute uptake (23, 26). The maize coleoptile segments were preincubated in distilled water for 1 h. This was sufficient for the run-off of residual growth and for establishing a water potential equilibrium between water and tissue ($\psi_0 = \psi_i = 0$ bar). Figure 1 shows the elongation kinetics of such segments after transfer to solutions of MAN and PEG adjusted to ψ_0 in the range of 0 to −10 bar. After about 2 h, the segments demonstrate a growth response which is assumed to be due to a derepression of endogenous auxin formation, although an increase of auxin-sensitivity cannot be excluded (12). Segment length and fresh-mass changes after 8 h of incubation are plotted as a function of ψ_0 in Figure 2. These data indicate that MAN and PEG affect segment elongation similarly in the ψ_0 range of 0 to −6 bar. After a small initial osmoelastic shrinkage (which is clearly detectable in Fig. 1, *e.g.* at $\psi_0 = -4$ bar), both osmotica produce similar decreases in the steady-state rates of elongation. As judged from the end-point determinations in Figure 2a, there is a nearly linear relationship between ψ_0 and elongation down to the point of zero growth (final length = initial length). However, the ψ_0 establishing zero elongation is significantly higher in PEG (−4.3 bar) than in

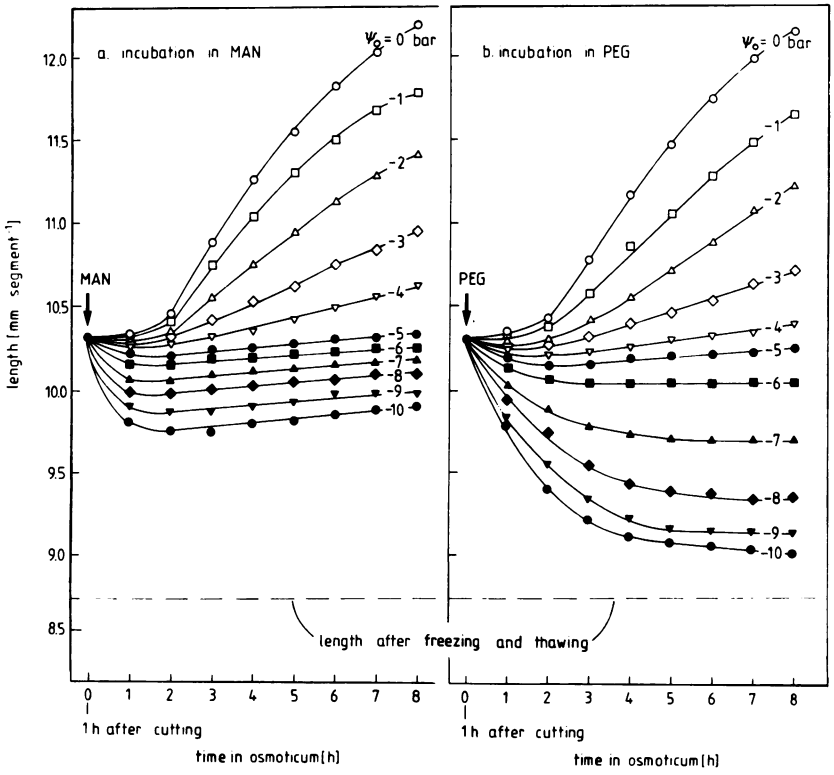

Figure 1. Effect of MAN (a) and PEG (b) on elongation and shrinkage kinetics of coleoptile segments. After cutting, the segments were preincubated in water for 1 h and transferred to osmotic solutions at time zero (ψ_0 = 0 to −10 bar). For comparison, segments were frozen in liquid nitrogen at time zero and measured after thawing and incubating in distilled water for 8 h.

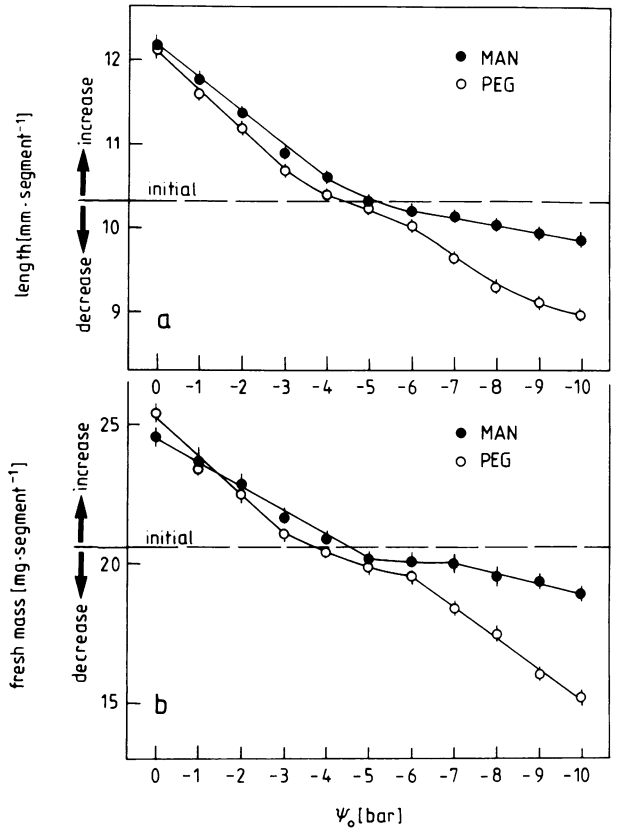

Figure 2. Effect of MAN (●) and PEG (○) on length (a) and fresh mass (b) of coleoptile segments after 8 h of incubation in osmoticum (ψ_o = 0 to −10 bar). The dashed lines indicate segment length and fresh mass before transfer to osmoticum. The data were obtained from the experiments shown in Figure 1.

MAN (−5.0 bar). The concomitant changes of segment fresh mass mirror the changes in segment length (Fig. 2b).

At lower values of ψ_o the effects of MAN and PEG are conspicuously different. Figure 2 shows that the decreases in length and fresh mass of segments shrinking at $\psi_o < -5$ bar are much more pronounced in PEG compared to MAN. The kinetics of segment shrinkage at $\psi_o \leqq -5$ bar MAN demonstrate a rapid reduction in length during the first hour of incubation followed by a slow increase indicative of osmotic recovery even at the lowest ψ_o used (Fig. 1a). Similar curves have been reported previously for *Avena* coleoptile segments incubated in MAN solutions (3, 23). In contrast, PEG produces a much stronger initial shrinkage without detectable recovery, ultimately leading to constant segment lengths (Fig. 1b).

If the cells of a tissue shrink in osmotic solutions by water extraction without change in intracellular solute content, their osmotic pressure changes in inverse proportion to the relative decrease in symplast volume ($\pi = \pi_{zero}$ (V_{zero}/V), Boyle-Mariotte's law). Since symplast volume is difficult to determine accurately, fresh mass is used as a substitute. This approximation leads to an underestimation of calculated OC values in the range of 0 to 5%, depending on ψ_o and the type of osmoticum used. Using this approximation, the validity of the above prediction is tested for MAN and PEG as external osmotica in Figure 3. These data indicate that the OC—and therewith π—of the cell sap expressed from coleoptile segments incubated in PEG increases in close agreement with the theoretical values calculated according to Boyle-Mariotte's law (Fig. 3b). The deviations between measured and calculated OC values exceed the 5% level only at the highest PEG concentrations used. In contrast, coleoptile segments incubated in MAN solutions increase in OC much more strongly than expected from their decrease in fresh mass caused by osmotic shrinkage (Fig. 3a). This deviation increases from 12% at −2 bar MAN to 60% at −10 bar MAN. The striking difference between the effects of the two osmotica is also revealed by comparing the OC of the cell sap with the OC of the incubation medium. The segments incubated in PEG approach an osmotic equilibrium at $\psi_o \leqq -7$ bar, whereas segments treated with osmotically equivalent concentrations of MAN demonstrate a higher measured OC and a lower calculated OC than the medium in which they were incubated for 8 h (Fig. 3). In control experiments, segments were subjected to dry air instead of subjecting them to PEG solutions and the resulting fresh mass and OC changes were recorded. Figure 4 shows that the OC increase measured closely agrees with the OC calculated from the water loss produced by

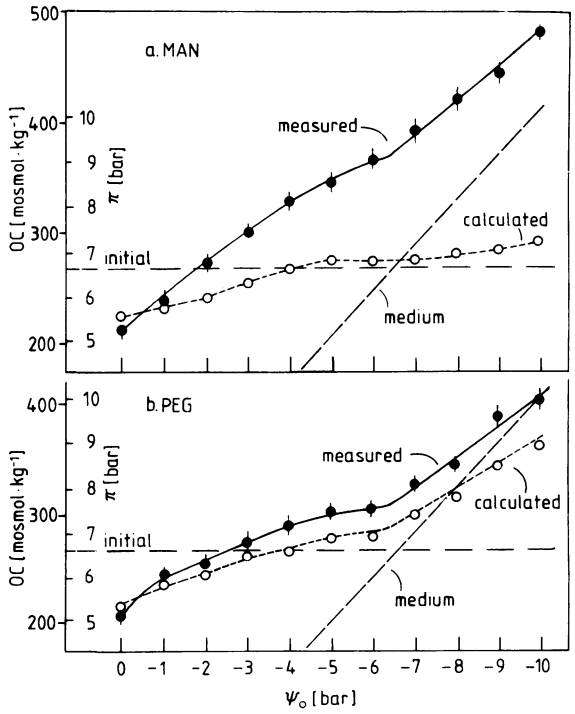

Figure 3. Effect of MAN (a) and PEG (b) on OC (π) of cell sap of coleoptile segments after 8 h of incubation in osmoticum (ψ_o = 0 to −10 bar). The osmolality was either determined in expressed cell sap by vapour pressure osmometry (measured) or derived from the initial osmolality (before transfer to osmoticum, 265 mosmol kg^{-1}, corresponding to 6.6 bar) and the fresh mass change according to OC (8 h) = OC (0 h)·fresh mass (0 h)/fresh mass (8 h) (calculated). OC of incubation media are included for comparison. OC of PEG solutions is plotted as 'effective osmolality' including the matric component.

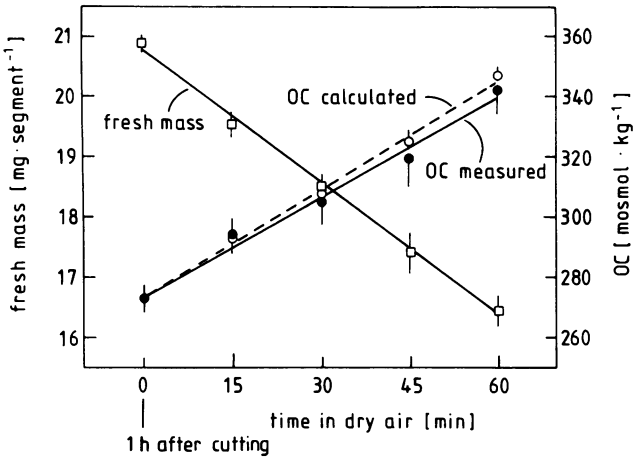

Figure 4. Effect of desiccation in dry air on water content (loss of fresh mass) and OC of coleoptile segments. After cutting, segments (cuticle abraded with a polishing cloth to facilitate water vapour loss) were preincubated in water for 1 h, blotted, and placed over activated silica beads in a closed box. OC was either measured (●) or calculated (○) as in Figure 3.

desiccation under these conditions. Thus, extracting water from the tissue by PEG solutions leads to similar results as extracting water by dry air. In summary, PEG produces fresh mass (volume) and OC changes in agreement with the assumption that osmotically expanding and shrinking maize coleoptile segments can be regarded with good approximation as ideal osmometers. On the other hand, if MAN is used instead of PEG for adjusting the water potential of the incubation media, the results indicate an apparent increase in the amount of osmotic solutes in the tissue such that the OC of the expressed sap is always higher than the OC of the medium.

Auxin produces a 12-fold increase in the growth rate of maize coleoptile segments after a lag-phase of 15 min (12). The elongation and shrinkage kinetics of coleoptile segments incubated in PEG solutions in the ψ_0 range of 0 to −10 bar in the presence of IAA are shown in Figure 5. Similar experiments performed with MAN instead of PEG revealed the same typical differences between the two osmotica as those shown in Figures 1 and 2 (data not shown). In order to test whether IAA has any effect on OC besides cell sap dilution by osmotic water uptake, the effect of PEG on OC of auxin-treated coleoptile segments was determined at $\psi_0 = -5$, −10, and −15 bar. Figure 6 shows that the OC values calculated under the assumption of constant solute content (broken lines) closely agree with measured OC values (solid lines); only in strongly shrinking segments ($\psi_0 = -15$ bar) small deviations of 6 to 9% are apparent after ≧ 4 h of incubation. Thus, there is no indication of a physiologically significant change in the amount of osmotic solutes in the cell sap of IAA-treated coleoptiles incubated in PEG solutions for up to 6 h. In contrast, similar experiments with MAN again demonstrate a striking increase in OC which exceeds the calculated concentration increases through osmotic water loss manyfold (Fig. 7).

The strong increase of OC of expressed sap in MAN-incubated coleoptile segments could result either from the

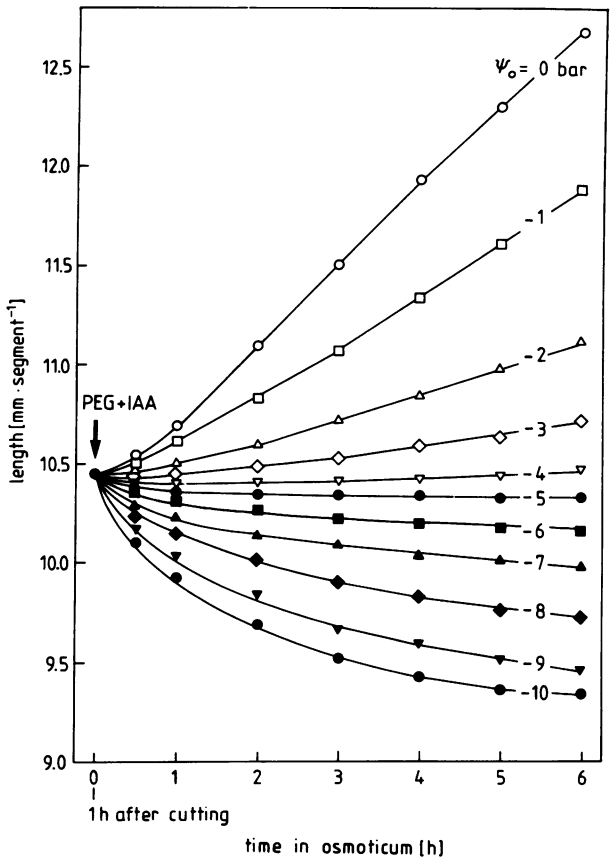

Figure 5. Effect of PEG on elongation kinetics of coleoptile segments in the presence of IAA. After cutting, the segments were preincubated in water for 1 h and transferred to PEG solutions at time zero (ψ_0 = 0 to −10 bar, containing 10 μmol L^{-1} IAA).

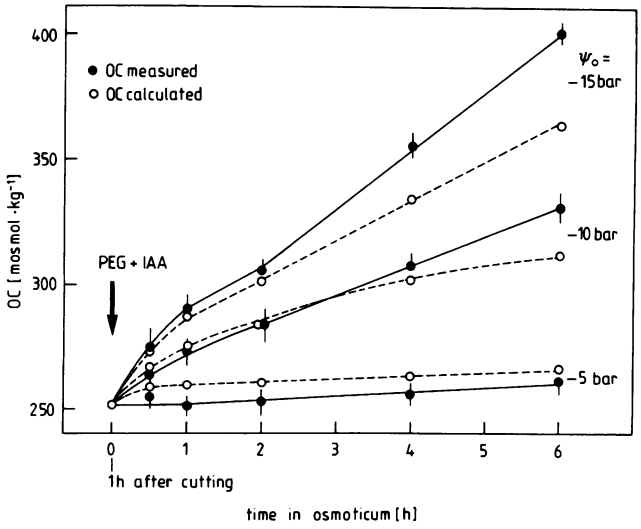

Figure 6. Effect of PEG on OC of cell sap of coleoptile segments in the presence of IAA. After cutting, the segments were preincubated in water for 1 h and transferred to PEG solutions at time zero (ψ_0 = −5, −10, −15 bar, containing 10 μmol L^{-1} IAA). OC was either measured (●) or calculated (○) as in Figure 3.

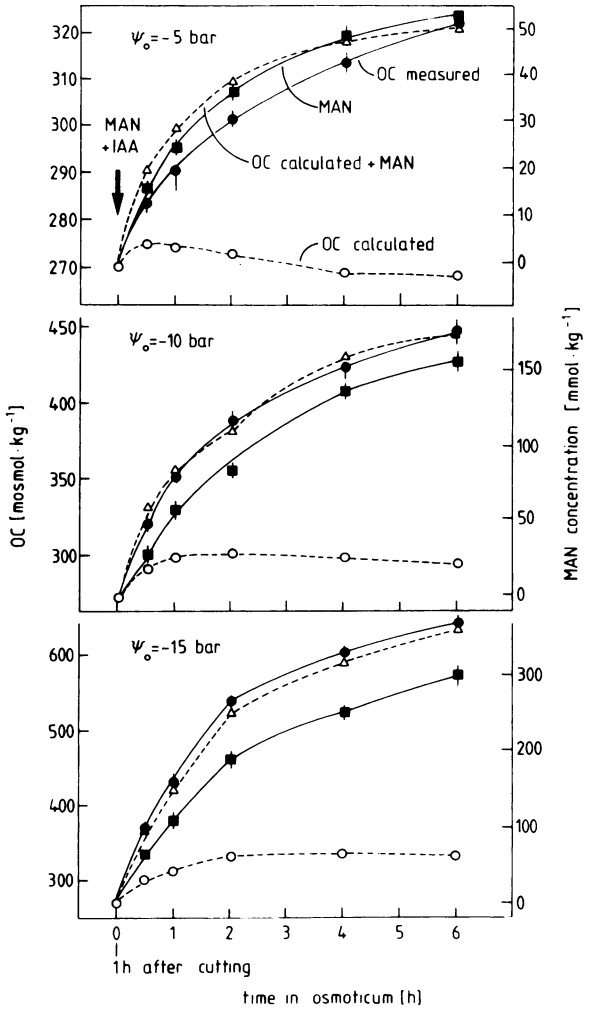

Figure 7. Effect of incubation in MAN on OC and MAN concentration of expressed sap of coleoptile segments. After cutting, the segments were preincubated in water for 1 h and transferred to MAN solutions at time zero (ψ_o = −5, −10, −15 bar, containing 10 μmol L^{-1} IAA). OC was either measured (●) or calculated (○) as in Figure 3. Molal MAN concentrations of expressed sap (■) were determined photometrically as described in "Materials and Methods." The sum of calculated OC and measured MAN concentration (△) is included for comparison. Note the different scales used for plotting OC and MAN concentrations in the three panels.

production of internal solutes (osmotic adjustment, *e.g.* through breakdown of starch) or from the uptake of MAN from the incubation medium. Figure 7 shows that the latter possibility is in fact borne out by direct determination of MAN in the sap. Under all conditions tested, the concentration of MAN in the sap quantitatively accounts for the discrepancies between measured and calculated OC. These results indicate that the apparent differences in osmotic behavior of maize coleoptile segments incubated in MAN or PEG solutions can be explained by an uptake of MAN into the tissue. This conclusion was further examined by measuring the temperature dependence of osmoelastic shrinkage in segments incubated either in MAN or PEG solutions. Figure 8 shows that at 5°C MAN affects shrinkage very similarly to PEG at 25°C except for a more rapid adjustment of the water flux equilibrium. This means that the reduction of membrane permeability by low temperature eliminates the anomalous effects of MAN on segment length changes. This is to be expected if at least a part of the MAN entering the tissue at 25°C is taken up into the symplast. Figure 9 confirms this supposition. In nonshrinking segments (ψ_o = −5 bar) about 50% of MAN uptake is symplastic as revealed by the difference in uptake at 25 *versus* 5°C. The contribution of apoplastic uptake (at 5°C) to total uptake (at 25°C) of MAN increases strongly if ψ_o is lowered to −10 or −15 bar, indicating that the apoplastic space available for MAN solutions is increased under these conditions.

DISCUSSION

Our data demonstrate that MAN is unsuitable as an external osmoticum for a quantitative determination of the water relations of growing maize coleoptiles by steady-state methods. MAN is rapidly taken up into the tissue in substantial

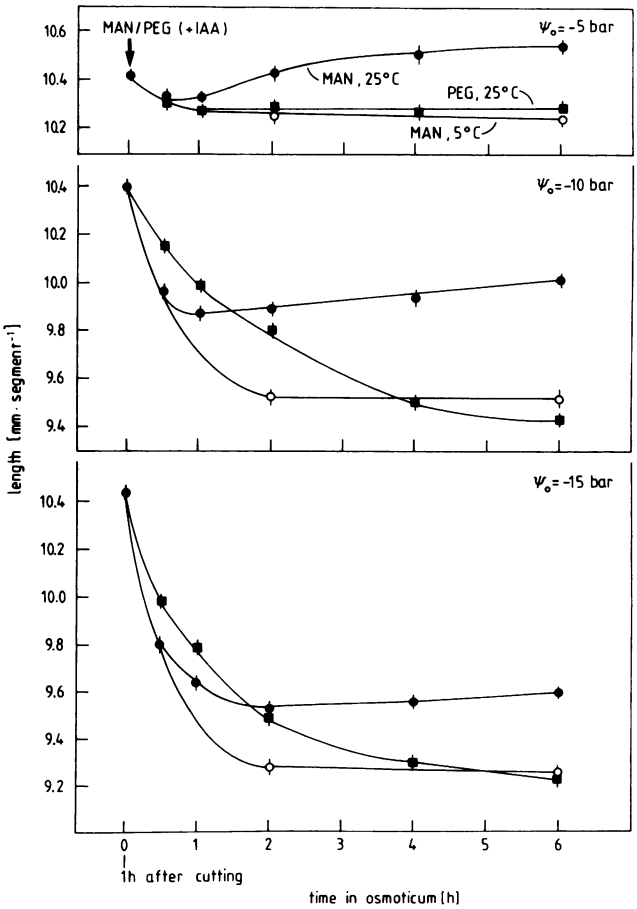

Figure 8. Kinetics of coleoptile shrinkage in MAN at 25°C (●), MAN at 5°C (○), and PEG at 25°C (■) in solutions of various water potentials. After cutting, the segments were preincubated in water (25°C) for 1 h and transferred to osmotic solutions at time zero (ψ_o = −5, −10, −15 bar, containing 10 μmol L^{-1} IAA) at 25 or 5°C.

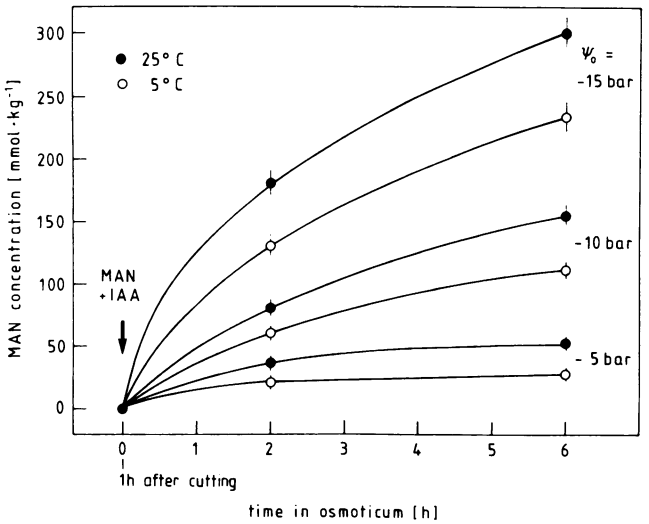

Figure 9. Kinetics of MAN uptake into coleoptile segments at 25°C (●) and 5°C (○). After cutting, the segments were preincubated in water (25°C) for 1 h and transferred to MAN solutions at time zero (ψ_0 = −5, −10, −15 bar, containing 10 μmol L^{-1} IAA) at 25 or 5°C. Molal MAN concentrations of expressed sap were determined as in Figure 7.

amounts even at moderate reduction of ψ_0 in the absence of plasmolysis. It can, therefore, be concluded that MAN not only penetrates into the apoplast but is also taken up into the symplast and thereby leads to a significant increase of π_i. This conclusion is supported by direct measurements of the MAN content of cell sap (Figs. 7, 9) and by the demonstration of a large temperature-dependent component of MAN uptake (Fig. 9) and its effects on segment elongation (Fig. 8). The 'safe' period for rigorous osmotic experiments with MAN not complicated by symplastic MAN uptake may be restricted to less than 1 h. The osmotic recovery due to symplastic MAN uptake is quite obvious in earlier work with *Avena* coleoptiles (3, 23) or cucumber hypocotyls (19). Rapid symplastic uptake of MAN and related physiological effects have been reported for numerous other plants (8, 9, 25, 27, 28). Preliminary experiments in our laboratory with other sugar alcohols such as sorbitol and pentaerythritol have led to similar results as those reported here for MAN. Our results demonstrate that caution is warranted in drawing quantitative conclusions from experiments with these osmotica.

In contrast to MAN, high-mol wt PEG represents a non-absorbable osmoticum which is excluded from both the apoplast and the symplast (2, 9, 11, 19) and, therefore, simulates the effect of water stress imposed in air more closely than MAN. Although deleterious effects of PEG in long-term experiments with transpiring plants cannot be excluded (14, 30) and toxic effects have been reported for impure batches of PEG (24), this compound appears to provide a useful alternative to MAN, at least for short-term incubation experiments in well-aerated solutions (17). Our experiments provide no hint for any nonosmotic effects of PEG on maize coleoptile segments during an experimental period of 8 h. Therefore, PEG appears to be much more suitable as an external osmoticum for quantitative work on water relations in this plant material.

PEG and MAN produce tissue shrinkage in very different manners. PEG cannot enter the free space of the cell walls and therefore leads to *cytorrhysis*, *i.e.* upon withdrawal of water protoplasts and cell walls contract without separating and the fresh mass decreases in proportion to symplast volume. In contrast, MAN penetrates the free space of the wall and therefore induces *plasmolysis*, *i.e.* the shrinking protoplasts partly separate from the cell walls whenever $\pi_i < \pi_{wall\ solution}$ (2, 22). Consequently, it has to be expected that MAN causes a greatly increased influx of external solution into the apoplastic space as soon as ψ_0 drops below the point of incipient plasmolysis (turgor = 0, $\pi_i = \pi_{wall\ solution}$). The sap expressed from the tissue under these conditions will be heavily contaminated with the external solution and the fresh mass will no longer decrease in proportion to symplast volume. This phenomenon, which is clearly detectable in Figure 2, may be responsible for the major part of the anomalous effects of MAN on the measured values of cell sap OC at ψ_0 below incipient plasmolysis (Fig. 3a). Figure 2 suggests that ψ_0 surpasses the point of incipient plasmolysis when the curves for MAN and PEG separate, *i.e.* between −6 and −7 bar. This agrees with the value of π_i = 6.6 bar (OC = 265 mosmol kg^{-1}) measured in the cell sap of coleoptile segments before incubation in osmotic solution (Fig. 3), and with the fact that distinct plasmolysis of mesophyll cells can be observed in tissue slices under the microscope after 10 min of incubation in MAN solutions of $\psi_0 \leqq -8$ bar. It should, however, be pointed out that the elimination of turgor pressure in the mesophyll cells at $\psi_0 \leqq -6$ to −7 bar does not eliminate turgor pressure of the segments completely. Figure 1b clearly indicates that the point of maximal cell-wall contraction obtained after freezing and thawing the segments is not reached even at $\psi_0 = -10$ bar PEG. This indicates that a tissue other than the mesophyll maintains a positive turgor pressure even at this low ψ_0 and must therefore possess a π_i higher than the average π_i as determined from the cell sap from the whole segments. Consequently, either the vascular tissues and/or the epidermis could be responsible for the turgor pressure observed at $\psi_0 \leqq -7$ bar. The phenomenon of residual turgor pressure in seemingly plasmolyzed coleoptile segments is included in several previous studies (3, 23) but has apparently never been explicitly noticed.

Using PEG solutions for modifying the turgor pressure of growing maize coleoptile segments, it can be shown that this material exhibits no rapid adaptive changes of water relation parameters by water stress as, for instance, reported for growing leaves (1, 16, 29), hypocotyls (18), and roots (10, 13). Thus, in this material the procedure of calculating the induced changes of turgor pressure from the water potential of osmotic solutions to which the tissue is exposed may be valid. The data obtained with this method are in agreement with the observation made in previous related studies with *Avena* coleoptiles (5, 23, 26) and *Pisum* internodes (7) that auxin does not cause growth by regulatory turgor or osmotic potential changes. Moreover, there are no indications of water-stress-induced changes in the cell-wall yielding parameters as inferred, *e.g.* in growing roots (10, 13).

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