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Growth-induced Water Potentials in Plant Cells and Tissues[1, 2]

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ABSTRACT

A physical analysis of water movement through elongating soybean (*Glycine max* L. Merr.) hypocotyls was made to determine why significant water potentials persist in growing tissues even though the external water potentials were zero and transpiration is virtually zero. The analysis was based on a water transport theory modified for growth and assumed that water for growing cells would move through and along the cells in proportion to the conductivity of the various pathways.

Water potentials calculated for individual cells were nearly in local equilibrium with the water potentials of the immediate cell surroundings during growth. However, water potentials calculated for growing tissue were 1.2 to 3.3 bars below the water potential of the vascular supply in those cells farthest from the xylem. Only cells closest to the xylem had water potentials close to that of the vascular supply. Gradients in water potential were steepest close to the xylem because all of the growth-sustaining water had to move through this part of the tissue. Average water potentials calculated for the entire growing region were −0.9 to −2.2 bars depending on the tissue diffusivity.

For comparison with the calculations, average water potentials were measured in elongating soybean hypocotyls using isopiestic thermocouple psychrometers for intact and excised tissue. In plants having virtually no transpiration and growing in Vermiculite with a water potential of −0.1 bar, rapidly growing hypocotyl tissue had water potentials of −1.7 to −2.1 bars when intact and −2.5 bars when excised. In mature, nongrowing hypocotyl tissue, average water potentials were −0.4 bar regardless of whether the tissue was intact or excised.

The close correspondence between predicted and measured water potentials in growing tissue indicates that significant gradients in water potential are required to move growth-associated water through and around cells over macroscopic distances. The presence of such gradients during growth indicates that cells must have different cell wall and/or osmotic properties at different positions in the tissue in order for organized growth to occur. The mathematical development used in this study represents the philosophy that would have to be followed for the application of contemporary growth theory when significant tissue water potential gradients are present.

The growth of plant cells results from extension of the constricting cell wall (9). In order for growth to be maintained, water must move from some source of supply into the protoplast. As a result, the water potential of the protoplast must be below the water potential of the source. The protoplast water potential required to move this growth-sustaining water is what we call a growth-induced water potential (7, 27). Such water potentials must accomplish two things. First, they must be sufficiently low to move water from its source through various tissues to the growing cells; and second, they must cause the water to enter the cells. It can be calculated that only small water potential differences are required to cause a growth-sustaining water flux to enter a cell. For example, a cell having a membrane permeability representative of higher plant cells (*i.e.* 10^{-6} cm sec^{-1} bar^{-1}; 11, 31) and a very high growth-sustaining water flux across that membrane (*e.g.* 10^{-7} cm sec^{-1}) would require a water potential drop of $10^{-7}/10^{-6} = 0.1$ bar. Thus, for cells located within a tissue, there would almost be local water potential equilibrium between each protoplast and its immediate surroundings. Nevertheless, since most of these cells would be separated from the ultimate source of water by intervening cells, growth-sustaining water fluxes might involve significant water potential gradients through the tissue.

Most studies of tissue growth have assumed osmotic equilibrium between the entire tissue and the environment (9, 27). However, measurements show a persistent growth-induced disequilibrium in water potential between tissue and its aqueous environment (3, 4, 16). Initial efforts have been made to predict the magnitude of this disequilibrium in oat coleoptiles (29) and in leaf discs (24), but there are no studies that combine predictions and water potential measurements in the same system. The objective of the following work is to present a physical basis for growth-induced water potentials in a hypocotyl tissue system and to test the resulting predictions with direct measurements.

THEORY

The theoretical portion of this study will be based upon the steady-state form of the water transport equation derived by Molz and Ikenberry (23), with a term added to represent growth-induced water uptake (24; see also Appendix). Since the tissue involved in the experiments was in the form of a solid cylinder, application will be made in the geometry shown in Figure 1 (growth occurred while the tissue was intact and water moved radially inward and outward from the xylem in the tissue cylinder). In this case, the equation takes the form:

$$0 = D\frac{d^2\psi}{dr^2} + \frac{D}{r}\frac{d\psi}{dr} - (\epsilon + \bar{\pi})G \qquad (1)$$

where ψ = tissue water potential (bar), r = radial distance from stem center (cm), D = tissue free energy diffusivity (cm^2 sec^{-1}), ϵ = average elastic modulus of the cell wall (bar), $\bar{\pi}$ = average osmotic pressure equivalent (hereafter called average osmotic pressure) of cell contents (bar), and G = volumetric water uptake rate due to growth/unit volume of tissue (cm^3 cm^{-3} sec^{-1}). It should be noted that equation 1 considers the diffusivity of the tissue as a whole and therefore involves both the cell wall pathways and the vacuolar pathways for water transport (12).

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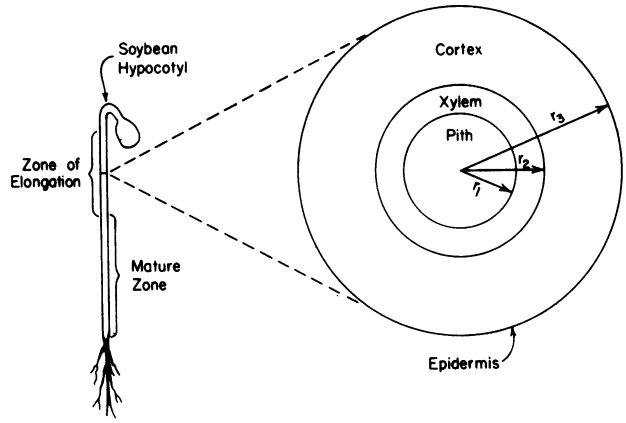

FIG. 1. Diagrammatic representation of cross-sectional anatomy of tissues from mid-region of zone of elongation of soybean hypocotyl. Radii r_1, r_2, and r_3 were 0.050, 0.075, 0.150 cm on average, respectively. During growth, water moved inward to the pith and outward to the cortex from the xylem tissue.

In order to apply equation 1, it is necessary to obtain a reasonable estimate for D. This can be done in two ways, either by evaluating the theoretical expression for D in terms of protoplast and cell wall hydraulic and elastic properties (component analysis), or by determining the kinetics of water sorption of the intact tissue (kinetic analysis). In the former case, one utilizes the expression given in references 17–19, and 23:

$$D = \frac{\Delta x\,(Pa + K\Delta x A/2)}{W_v S + V_0/(\epsilon + \pi_0)} \qquad (2)$$

where A = cross-sectional area of vacuolar pathway (cm^2), a = cross-sectional area of cell wall pathway (cm^2), π_0 = osmotic pressure at zero turgor (bar), Δx = diameter of cell (cm), K = permeability of cytoplasmic complex separating cell wall from vacuole ($cm\ sec^{-1}\ bar^{-1}$), V_0 = cell volume at zero turgor pressure (cm^3), W_v = volume of cell wall/cell (cm^3), P = hydraulic conductivity of the cell wall ($cm^2\ sec^{-1}\ bar^{-1}$), and S = specific water capacity of the cell wall material ($cm^3\ cm^{-3}\ bar^{-1}$). Equation 2 does not explicitly consider the effects of plasmodesmata, if any. However, a very similar expression would result if they were considered (19).

To estimate D from sorption kinetics, one makes use of standard diffusion theory as applied by Molz *et al.* (22). The equation used is D = slope/C_2 where "slope" is obtained from a semilog plot of 1 minus the fractional water uptake of the rehydrating stem, and C_2 is a constant depending on the geometry of the stem tissue. The details of the calculation will be given later.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* L. Merr. var. Wayne) seedlings were grown from seed in Vermiculite in a dark, humid chamber (temperature = 29 ± 0.5 C), as previously described (6). Experiments were begun 70 hr after planting. All tissue manipulation was done under a green safelight.

Cell and Tissue Dimensions. Tissue samples were taken from several locations along the zone of elongation of a hypocotyl. Thin slices were mounted in water so that protoplast and cell wall dimensions could be observed under the light microscope.

Micrometer measurements of the radius and length of the tissue were used to determine tissue volume. The water content of the tissue was related to tissue volume by drying tissue of known dimensions in an oven at 100 C for 24 hr.

Growth Measurements. Experiments were conducted under growth conditions to assure that transpiration was negligible and water movement involved only that for cell growth. Hypocotyl elongation was measured inside a thermocouple psychrometer chamber in which entire soybean seedlings could be placed (6). A mark was made with India ink on each hypocotyl base before the seedlings were inserted in the chamber. Upon completion of the water potential measurement with the psychrometer, the seedlings were removed and the difference in length of the hypocotyls was determined.

For excised hypocotyls, growth was measured similarly just before excision of the tissue for water potential measurements.

Water Potential Measurements. In intact seedlings, the water potential ($\bar{\psi}$) of various regions of the hypocotyl was measured by placing four seedlings in a thermocouple psychrometer chamber (3) large enough to hold entire plants (6). Petrolatum was placed in a ring around a 2.5-cm region of the hypocotyl to be exposed to the thermocouple. The petrolatum screened the thermocouple sensor from other portions of the plant. Outside the petrolatum screen, 10^{-4} M $CaCl_2$ bathed the roots. $\bar{\psi}$ was measured by the isopiestic technique (5). The thermocouple chamber had been coated with melted and resolidified petrolatum (2) and measurements were corrected for the heat of respiration (1).

For detached hypocotyl segments, $\bar{\psi}$ was measured with a thermocouple psychrometer for excised tissue (5). Four hypocotyl segments were placed in the bottom of the thermocouple chamber, and $\bar{\psi}$ was measured as in the intact system.

The osmotic pressure ($\bar{\pi}$) was determined frequently in the hypocotyl tissue immediately after $\bar{\psi}$ had been measured. The tissue was frozen in the covered psychrometer chamber on dry ice. After thawing, the sap was expressed from the tip of each hypocotyl by squeezing between the fingers. The expressed sap was stored in a capped vial and placed on a thermocouple for measurement of $\bar{\pi}$. All manipulations for measurement of $\bar{\psi}$ and $\bar{\pi}$ were carried out in a humid chamber.

Sorption Measurements. Growing regions of 20 hypocotyls were excised and threaded cross-wise on a thin wire (0.076-mm diameter). After partially desiccating the segments in air, they were shortened to 1.5 cm by cutting rapidly with a razor blade and then permitted to rehydrate in 10^{-4} M $CaCl_2$ at pH 7. Water uptake during rehydration was measured by weighing the hypocotyl segments after quickly blotting excess solution with sponges.

RESULTS

Growth-induced Water Uptake. The radius R of the hypocotyls was found to vary between 0.143 and 0.158 cm, and the fraction of tissue volume occupied by water was 0.46. The remaining tissue volume was occupied by dry material and a large amount of intercellular space. If t is time, v is the volume of the elongation zone, L its length, and R its radius, then to a good approximation the following relationship holds, since the radius of the hypocotyl is virtually constant during growth:

$$\frac{dv}{dt} = \pi R^2 \frac{dL}{dt} \qquad (3)$$

Using an average R of 0.15 cm and a measured extension rate of 0.2 cm hr^{-1} yields 0.014 $cm^3\ hr^{-1}$ for volumetric growth rate. Since the tissue volume is 46% water, one obtains a total growth-induced water uptake of (0.46) (0.014) = 0.0065 $cm^3\ hr^{-1}$. At any one time, the length of the growing zone was approximately 3.2 cm (16). Hence, the growth-induced water uptake rate/unit volume of tissue is $0.0065/(\pi)(.15)^2(3.2) = 0.03\ cm^3\ cm^{-3}\ hr^{-1} = 8 \times 10^{-6}\ cm^3\ cm^{-3}\ sec^{-1}$. It is recognized that growth probably does not occur uniformly through the length of the zone of elongation of a hypocotyl. Therefore, the above value of G should be viewed as an average for rapidly growing cells of soybean hypocotyls.

Diffusivity Estimates. Table I shows average values measured for the various parameters needed to evaluate the diffusivity according to the component analysis (equation 2). The values that

were not measured directly in the experiments were obtained from the literature referenced in the table. The average hypocotyl cell was a cylinder with a radius of 2×10^{-3} cm and a length of 12.5×10^{-3} cm parallel to the stem (Fig. 2). Calculation of D from the data listed in Table I results in a tissue diffusivity of 8×10^{-7} cm^2 sec^{-1}.

From the kinetic analysis, an estimate for D was obtained from the sorption kinetics of intact hypocotyl stems. The effect of the pith was ignored since this tissue will rehydrate in about one-fifth of the time required for the cortex. Figure 3 shows 1 minus the fractional water uptake, Fu, of swelling hypocotyl stems as a function of time. (Fu is defined as the mass of water absorbed at any time divided by the total mass absorbed.) Ten min after rehydration began, sorption could be viewed predominantly as a radial flow of water from the xylem into the cylindrical cortex with an inner radius of 0.075 cm. Because water flux and free energy flux are both assumed proportional to water potential gradient in the derivation of equation 2, sorption of water should be proportional to sorption of free energy to a first approximation. This allows diffusivity to be calculated as described by Molz *et al.* (22) with an average $r_3 - r_2$ of 0.067 cm, which takes account of the diameters of the segments and the shrinkage of the cortical tissue due to desiccation. The relevant equation is D = slope/C_2, where "slope" = d(ln [1 − Fu])/dt is obtained from the linear portion of the curve in Figure 3. The constant $c_2 = \alpha_1^2 (\rho - 1)^2/(r_3 - r_2)^2$, with a value for α being obtained from tables (22). For our case, with $\rho = r_3/r_2$ equal to 2, α_1 is 1.361. The resulting value for D is calculated to be roughly 2.8×10^{-6} cm^2 sec^{-1}.

Table I. *Values for the Various Parameters Contained in Equation 2. The Cell was Viewed as Shown in Figure 2.*

Δx (cell diameter)	4×10^{-3} cm
V_0 (cell volume)	1.6×10^{-7} cm^3
A (protoplast area)	4.8×10^{-5} cm^2
a (wall area)	1.5×10^{-6} cm^2
W_v (wall volume)	6.3×10^{-9} cm^3
π_0 (osmotic pressure at zero turgor)	7 bar (Meyer and Boyer, 1972)
ε (cell wall elastic modulus)	78 bar (Steudle, Zimmermann, and Lüttge, 1977)
S (wall specific water capacity)	0.01 bar^{-1} (Molz, 1975)
P (wall conductivity)	2×10^{-7} cm^2 sec^{-1} bar^{-1} (Newman, 1974)
K (membrane permeability)	10^{-6} cm sec^{-1} bar^{-1} (Steudle, Lüttge, and Zimmermann, 1975)

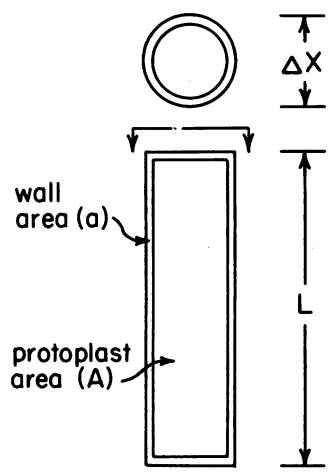

FIG. 2. Diagrammatic representation of a cortical cell from mid-region of zone of elongation of soybean hypocotyl. For a typical cell, Δx was 4×10^{-3} cm. Cell length, L, varied from 2×10^{-3} to about 24×10^{-3} cm depending on position of cell within zone of elongation. Average L was taken to be 12.5×10^{-3} cm. During growth, water transport is perpendicular to long axis of cell.

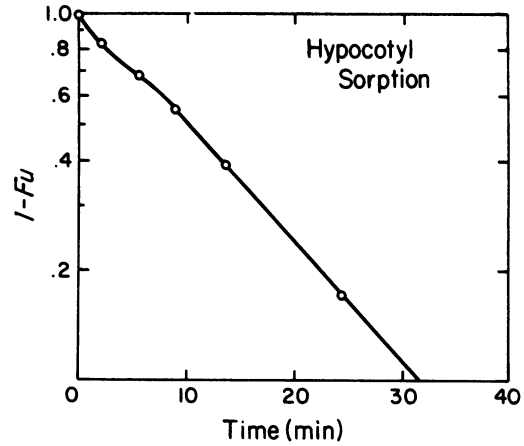

FIG. 3. Sorption kinetics of 1.5-cm lengths of soybean hypocotyl tissue from zone of elongation. Sorption occurred in water. Dimensionless parameter 1 − Fu represents 1 minus the fraction, at time t, of total water absorbed at end of sorption period.

The kinetic analysis is based on the assumption that local equilibrium existed between the cell walls and vacuoles of the soybean cortex cells during the rehydration process. The sensitivity analysis performed by Molz and Ikenberry (23) suggests that this assumption is valid. In terms of their notation for dimensionless quantities, we have $C_2/C_1 = 29.8$, $D_1/D_2 = 97.7$, $L^2/D_2RC_2 = 2{,}720$, and $L^2/D_2RC_1 = 83{,}812$. These parameter values are well within the range which resulted in a prediction of local equilibrium.

The D values obtained from the component and kinetic analyses differed by a factor of 3.5. There are at least two reasons why the kinetic analysis would be expected to result in a slightly high estimate for D. First, it is possible for water to leak through the cut edges and epidermis. Second, the xylem, which we assumed to be a cylinder of thickness $r_2 - r_1$, was actually distorted slightly into a cube shape with rounded corners. This would shift the sorption kinetics toward the plane sheet case and away from that of a hollow cylinder. Both effects would tend to increase the sorption rate above that predicted by the theory (10). Therefore, the D value obtained from the kinetic analysis probably can be viewed as an upper bound for the actual tissue diffusivity.

Measured and Predicted Water Potentials. Water forced through a hypocotyl with pressures similar to the measured water potential of growing tissue gives exudation rates about 10 times the rate of water uptake for cell enlargement (6). Therefore, the roots and xylem of the hypocotyl do not constitute significant barriers to water movement of the growing cells. The barrier is the growing tissue and is encountered when water moves outward from the xylem.

The water potential difference between the solution bathing the roots (10^{-4} M $CaCl_2$) and various portions of the stem was measured. (The water potential of the bathing solution was essentially zero.) In the elongating zone (average elongation rate = 0.17 cm hr^{-1}), tissue $\bar{\psi}$ was −1.7 to −2.1 bar when measured with the thermocouple psychrometer for intact tissue. In the mature zone (average elongation rate = 0.0004 cm hr^{-1}) tissue $\bar{\psi}$ was −0.3 to −0.5 bar. Because the measurements were made in the thermocouple psychrometer for intact seedlings, transpiration was zero, and there were no excision effects. $\bar{\psi}$ was correlated with the rate of elongation, lower $\bar{\psi}$ being associated with faster rates. A similar observation was made when the measurements of $\bar{\psi}$ were repeated with tissue excised from hypocotyls. The elongation zone had average $\bar{\psi}$ of −2.5 bar while the mature zone had average $\bar{\psi}$ of −0.4 bar.

In order to calculate the radial water potential distribution at a

given position in the zone of elongation, equation 1 was solved in the domains between 0 and r_1, and r_2 and r_3 shown in Figure 1. If one defines the constant $\alpha = (\epsilon + \bar{\pi})G/D$, then equation 1 becomes:

$$\frac{d^2\psi}{dr^2} + \frac{1}{r}\frac{d\psi}{dr} = \alpha \qquad (4)$$

We will first solve equation 4 in the cortex. Substituting $U = d\psi/dr$ reduces equation 4 to a linear first order equation in U, and a general solution is given by:

$$\psi = \frac{\alpha r^2}{4} - \frac{r_3^2}{2}\ln(r) + B \qquad (5)$$

wherein it is assumed that $d\psi/dr$ at $r = r_3$ is zero (no water crossing the epidermis). Because the xylem resistance of hypocotyls is quite low (6), the water potential of the xylem fluid in our experiments must have been between the water potential of the soil surrounding the roots (approximately −0.1 bar) and the measured water potential of the mature stem (approximately −0.4 bar). If we estimate the xylem water potential to be −0.3 bar, the constant B in equation 5 can be evaluated to give the particular solution:

$$\psi = \frac{\alpha}{4}(r^2 - r_2^2) - \frac{\alpha r_3^2}{2}\ln\left(\frac{r}{r_2}\right) - 0.3 \qquad (6)$$

A similar solution procedure can be followed for the pith. Using boundary conditions $d\psi/dr = 0$ at $r = 0$ and $\psi = -0.3$ bar at $r = r_1$ results in the solution:

$$\psi = \frac{\alpha}{4}(r^2 - r_1^2) - 0.3 \qquad (7)$$

For the diffusivities and growth estimate discussed previously, α was calculated to be approximately 250 based on the kinetic analysis and 850 based on the component analysis. Figure 4 shows plots of ψ as a function of r for $\alpha = 250$, 550, and 850, respectively. The curves show that cells closest to the vascular system have water potentials similar to that of the vascular tissue, but water potential gradients between cells are relatively large. Cells farthest from the vascular system have water potentials considerably lower than the vascular water, but relatively small water potential differences exist between these cells. The potential gradients (slope of the ψ *versus* r curves) are steepest next to the xylem because all of the growth-sustaining water must cross this tissue. Near the epidermis or pith center, most of the water has already been absorbed, and the water potential gradient required to move the remaining water approaches zero.

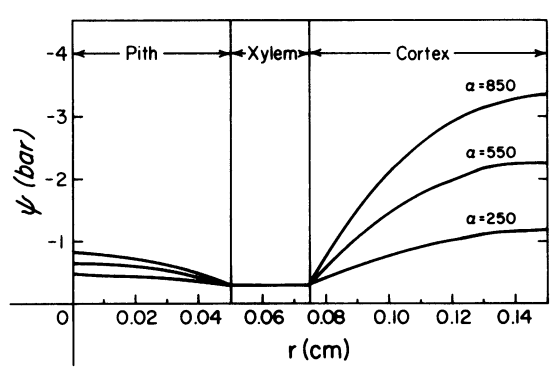

FIG. 4. Water potential profiles in elongating soybean hypocotyl tissue supplied with water from vascular system. Each profile is drawn along a radius of tissue starting from center of pith. The three curves for differing $\alpha = (\epsilon + \bar{\pi})G/D$ represent values for D determined from kinetic analysis ($\alpha = 250$), component analysis ($\alpha = 850$), and an average D ($\alpha = 550$). Growth was considered to be 0.2 cm hr^{-1} in each profile. To account for osmotic content and frictional resistance xylem, water potential of xylem sap was assumed to be −0.3 bar in enlarging region.

Relatively small potential gradients are required to supply water to the pith as compared to the cortex (Fig. 4). This is because the pith is of less volume and surrounded on the outside by the xylem. Thus, the relatively large area of the water supply coupled with the smaller water requirements of the pith result in lower water potential gradients.

It is clear from equation 7 that the magnitude of ψ at any position in the tissue depends primarily on α. The parameter α is directly proportional to G and inversely proportional to D. Increasing G will lead to lower tissue water potentials and steeper gradients. Increasing the diffusivity does the inverse. Increases in $\epsilon + \bar{\pi}$, which would ordinarily be expected to increase α, would increase D by almost the same amount. Thus, ψ is relatively insensitive to $\epsilon + \bar{\pi}$ (see Appendix). If in the analysis $d\psi/dr$ were not zero at the epidermis (such as would be expected if transpiration occurred across the epidermis), the cortex water potential distributions of Figure 4 would be steeper everywhere by an amount equal to 1/r times $d\psi/dr$ at $r = r_3$.

DISCUSSION

The theoretical analysis of water movement through hypocotyl tissue began with the assumption that water would flow along any path through the tissue at a rate proportional to the conductivity of the path. Therefore, no specifications of the particular type of flow between cells was required (Appendix). From this assumption and the anatomical properties of the tissue, it was possible to calculate water potentials at various locations within the tissue as water moved radially outward from the xylem into the tissues of the growth region.

The results suggest strongly that significant growth-induced water potentials arise in plant tissue even though the individual cells of the tissue are almost in equilibrium with their local osmotic environment. These water potentials are required despite the slow rates of water movement because the flow pathway through the tissue has a relatively low hydraulic conductivity. For individual cells, the differences in water potential are too small to measure but, over macroscopic distances, they become detectable. In the present work, the thermocouple psychrometer indicated water potentials ranging from −1.7 to −2.5 bars in rapidly growing hypocotyl tissue.

Since the tissue diffusivity, D, calculated from the component analysis had a major effect on the ψ gradients predicted for soybean hypocotyls, it is important to consider the factors contributing most to the magnitude of D. Anatomical features of the tissue (ΔX, a, A, W_v, and V_0) contributed to D, although the water capacity of the cell walls (W_wS) was small when compared to that of the vacuoles. This, in turn, caused D to be a strong function of $\epsilon + \pi_0$, but the effect of $\epsilon + \pi_0$ on ψ was virtually canceled by $\epsilon + \bar{\pi}$ elsewhere in equation 7. Except for the anatomical features of the tissue, the effect of D on ψ was dependent primarily on P and K. There are only a few values for P in the literature (25, p. 375) and these center on 2×10^{-7} cm^2 sec^{-1} bar^{-1}, which was used here. A somewhat larger number of estimates of K are available, and for higher plants, these are on the order of 10^{-6} cm sec^{-1} bar^{-1} (11, 31). For a number of reasons, both P and K are probably only reliable within a factor of 2- to-3 fold. Consequently, D and, in turn, α are likely to vary by about this amount. The values of ψ for soybean were calculated for a range of α (Fig. 4) to account for this variability in the components making up the estimate. The kinetic analysis of diffusivity, which should have integrated the entire complex of factors influencing D (including plasmodesmata, if any), supported the calculations based on P, K, and the anatomical features of the tissue. It is important to note that the kinetic analysis involved the rehydration of tissue freshly cut under degassed water, so that a large amount of water should have entered the tissue via the xylem, as assumed in the calculations and as surely occurred during the growth of the intact seedling.

It is also significant that our estimates of D in soybean hypocotyls ($0.8–2.8 \times 10^{-6}$ cm^2 sec^{-1}) are similar to those measured previously (22) for stem tissues external to the xylem in cotton ($1.3–1.8 \times 10^{-6}$ cm^2 sec^{-1}). Apparent diffusivity estimates for disks of leaf tissue made by Molz *et al.* (24) are not comparable to the values mentioned above because the rather large leaf disks contained xylem strands which greatly increased the apparent diffusivity of the bulk tissue.

In an isopiestic thermocouple psychrometer, the thermocouple determines the vapor pressure of water in equilibrium with the liquid water in the plant tissue. For excised tissue, gradients in water potential gradually disappear in the psychrometer chamber, and the water potential is one that existed at an intermediate point in the water potential distribution before excision. In intact tissue, water flow continues and the water potential recorded at vapor equilibrium is one present at some intermediate point in the distribution that persists in the cortex inside the thermocouple chamber. Since most of the cells of the cortex are in contact with air in the intercellular spaces, air within this tissue has a vapor pressure representing some average for all of the cells, and the thermocouple sensor indicates that value. From the theoretical standpoint, these values should not differ greatly. The data showed this to be true, since measured water potentials averaged −2.5 bars for excised tissue and −1.9 bars for intact tissue that had been growing rapidly. In both cases, water potentials approached zero if growth approached zero, as would be expected if growth-induced gradients in water potential had approached zero.

It is important to note that the growth-induced water potentials observed in excised and intact hypocotyl tissue are similar to those predicted by the analysis presented here when an average water potential is calculated for the tissue as a whole. For the intact tissue, it would seem that the correct average may be $\bar{\psi}_c$, the average for the cortex alone. For excised tissue, the relevant average would include both the pith and cortex weighted according to their relative volumes, *i.e.* $\bar{\psi}_{pc} = (V_p\bar{\psi}_p + V_c\bar{\psi}_c)/(V_p + V_c)$. The xylem probably would have little effect on $\bar{\psi}_{pc}$ because it is quite rigid, has a relatively low water capacity, and would contribute little water to the equilibration process (21). For the three α values shown in Figure 4, the corresponding average water potentials are −0.95, −1.7, and −2.4 bars if the cortex alone is used, and −0.88, −1.6, and −2.2 bars if the pith and cortex averages are combined according to their relative volumes. The averages based on the component estimate for the diffusivity (−2.2 and −2.4 bars) are in good agreement with thermocouple measurements. Those based on the kinetic analysis (−0.88 and −0.95 bars) tend to be low by a factor of 2 to 3 but still well above the water potential differences expected between an individual cell vacuole and the cell exterior.

In view of the foregoing, it is likely that water potential gradients exist in rapidly growing plant tissues. Several consequences arise from this situation. First, water movement through the tissue could be enhanced if some agent increased the hydraulic conductivity of cell membranes or cell walls. Auxin has been shown to have this effect (6). Second, the growth-controlling properties of cells must differ according to their position within the tissue. If such properties could not adapt to local conditions, disorganized growth patterns would result in the face of water potential and turgor pressure gradients.

A simple example will illustrate the second situation. There is evidence that turgor must be above a threshold (Y) in order for growth to occur (9). Although the relationship between turgor and cell enlargement appears somewhat variable (14), we will adopt for convenience the simple expression relating turgor and growth:

$$\frac{1}{V_0}\frac{dV_0}{dt} = \phi(T-Y) \qquad (8)$$

where V_0 is cell volume (cm^3), t is time (sec), ϕ is cell wall extensibility (bar^{-1} sec^{-1}), T is turgor pressure (bar), and $T - Y \geq 0$.

The steady growth process in a cylinder of hypocotyl tissue external to the xylem would then be given in the Appendix:

$$D\frac{d^2\psi}{dr^2} + \frac{D}{r}\frac{d\psi}{dr} - (\epsilon + \bar{\pi})\phi(T-Y) = 0 \qquad (9)$$

which is simply equation 1 with G replaced by $\phi(T - Y)$.

If ϕ and Y in equation 9 are approximated as constant average values ($\bar{\phi}$ and $\bar{Y}$) for the tissue as a whole, and growth is assumed to occur at constant osmotic pressure $\bar{\pi}$ (13), then equation 9 becomes:

$$D\frac{d^2\psi}{dr^2} + \frac{D}{r}\frac{d\psi}{dr} - (\epsilon + \bar{\pi})\bar{\phi}(\psi + \bar{\pi} - \bar{Y}) \qquad (10)$$

In equation 10, the growth-induced water uptake function is given by $G = \bar{\phi}(\psi + \bar{\pi} - \bar{Y})$. Since everything in this function is assumed constant except ψ (*i.e.* growth is a linear function of tissue water potential), equation 10 could be solved. However, growth rate would be a maximum at small values of ψ near the xylem and a minimum or zero at the epidermis. This clearly does not occur. If it did, a stem would grow in a "telescoping manner" with cylinders of tissue near the xylem slipping ahead of tissue cylinders located closer to the epidermis. Since the stems of hypocotyls are not observed to extend in such a manner, it is likely that whatever parameters control growth, shift in such a way that uniform growth occurs throughout the tissue. The attainment of an orderly growth pattern in the face of water potential and turgor pressure gradients may be one of the major functions of the growth-adjusting mechanism (28).

It should not be inferred from this discussion that non-negligible water potential gradients are predicted to occur in all types of rapidly growing tissue. If the tissues are only a few cells in thickness, and/or a major resistance to water movement occurs at the boundary of the tissue where water enters, then an analysis similar to that reported herein would predict a nearly uniform potential distribution throughout the growing tissue. Ray and Ruesink (29) suggested that such a situation may exist in oat coleoptiles. In their study, the kinetics of water uptake did not depend on coleoptile length. They predicted that growth-induced water potentials (−0.8 to −2.5 bars) were situated across the epidermis and caused water to enter the coleoptile cells primarily through the epidermis.

It seems clear that growth-induced water potentials may arise in some rapidly growing tissue systems because of the necessity for water to move macroscopic distances through cells unmodified for water transport. Many plant tissues have anatomical features resembling hypocotyls and require water to move similarly long distances from the vascular tissue; *e.g.* leaves exhibit growth-induced water potentials (3, 4). Therefore, growth-induced water potentials may be of wide occurrence. Since the equations in the Appendix could be derived for tissues having geometries different from those of hypocotyls, the analysis presented here could be applied to other systems, at least in principle. Because of the generality of the approach, the present development represents the philosophy that would have to be followed for the application of the growth theory described by Lockhart (15), Green *et al.* (14), and Ray *et al.* (27), when significant tissue water potential gradients are present.

APPENDIX: PHYSICAL AND MATHEMATICAL BASIS FOR EQUATION 1

The complete derivation of equation 1 for the cell wall and vacuolar pathways in the absence of growth was first presented by Molz and Ikenberry (23). Two separate transport equations were derived, one for water moving primarily along the cell walls, and the second for water moving mainly from vacuole to vacuole. Simultaneous solution of the two equations showed that for a broad range of tissue parameters (hydraulic conductivities, water

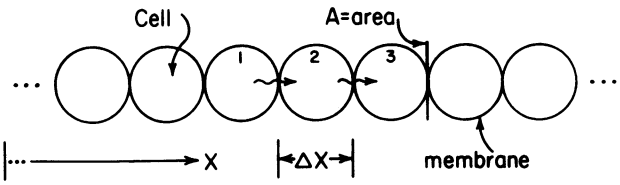

FIG. A1. Diagrammatic representation of cell geometry assumed in derivation of equation 1. Water moves from left to right across cells. Cells 1, 2, and 3 represent typical cells along pathway. Growth occurs perpendicular to plane of diagram in direction of long axis of cylindrical cells.

capacities, etc.), likely to include all physiologically reasonable values, the protoplasts of cells in a tissue containing more than a few cells in a series remained in water potential equilibrium with their walls even while water potential varied throughout the tissue. This condition was called local equilibrium and enabled one to combine the two separate transport equations into a single equation which applied to the tissue as a whole. The resulting expression for the diffusivity (equation 2) contained both cell wall and protoplast parameters.

Although the derivation process was somewhat elaborate, the final equation which resulted was similar to that derived earlier by Philip (26) except for the diffusivity. For simplicity, therefore, the outline of the derivation of equation 1 as it applies to this study will be made using the approach of Philip (26). The result will be conceptually valid, although the strictly correct expression for the diffusivity will not result. For a general review of the development of transport equations as they apply to plant tissue water relations, see reference 20.

The model selected for the derivation is shown in Fig. A1. It consists of a linear grouping of cylindrical idealized cells with water moving from left to right, and growth occurring along the long axis of the cylinders perpendicular to the direction of water flux. Otherwise, growth would have to be given more explicit consideration in the derivation from a coordinate viewpoint. If Q is the rate of water flow from vacuole to vacuole, then:

$$Q_{12} = -\frac{AK}{2}(\psi_2 - \psi_1) \text{ and } Q_{23} = -\frac{AK}{2}(\psi_3 - \psi_2) \qquad (A1)$$

where Q_{ij} = rate of flow from cell i to cell j ($cm^3\ sec^{-1}$), ψ_i = water potential of cell i (bar), K = permeability of the "effective membrane" consisting of the cytoplasm surrounding the vacuole (cm sec^{-1} bar^{-1}), and A = "effective cross-sectional area" of membrane perpendicular to flow (cm^2). (By "effective" we mean a somewhat ill defined quantity but still adequate for the purposes of the derivation.) Water volume will be conserved if the following relationship holds:

$$\frac{\partial V_2}{\partial t} = -\frac{AK}{2}(\psi_2 - \psi_1) + \frac{AK}{2}(\psi_3 - \psi_2) \qquad (A2)$$

where V_2 is the volume of cell 2 (cm^3), t is time (sec), and $\partial V_2/\partial t$ is the rate of change of cell volume ($cm^3\ sec^{-1}$). Equation A2 simply states that the rate of change of cell volume is equal to the difference between the rate that water flows into the cell and the rate that it flows out. Combining the first two terms on the right side of equation A2 and multiplying by $(\Delta x)^2/(\Delta x)^2$ yields:

$$\frac{\partial V_2}{\partial t} = \frac{(\Delta x)^2 AK}{2}\left(\frac{\psi_3 - 2\psi_2 + \psi_1}{(\Delta x)^2}\right) \qquad (A3)$$

where Δx is the diameter of the cells (cm).

An equation like A3 could be written for every cell of the series grouping. However, if there are several cells in the series, one can think of ψ as varying continuously along the x coordinate. Then all of the individual cell equations of the form:

$$\frac{\partial V_i}{\partial t} = \frac{(\Delta x)^2 AK}{2}\left(\frac{\psi_{i+1} - 2\psi_i + \psi_{i-1}}{(\Delta x)^2}\right) \qquad (A4)$$
$$i = 2, 3, \ldots, n-1$$

where n is the number of cells in series, can be viewed as the finite difference representation of the partial differential equation given in reference 30:

$$\frac{\partial V}{\partial t} = \frac{AK(\Delta x)^2}{2}\frac{\partial^2 \psi}{\partial x^2} \qquad (A5)$$

where V is cell volume as a function of position and time.

If growth is occurring in the grouping of cells, the change in cell volume with respect to time will be due to both growth and turgor (elastic) effects. To take both of these phenomena into account explicitly, we will begin with the identity $V = V_0 + (V - V_0)$ where V_0, the cell volume at zero turgor, is considered a function of time due to growth. At zero turgor, there will be no deformation of the cell wall and V will be equal to V_0. In general, however, equation A5 is written:

$$\frac{\partial V_0}{\partial t} + \frac{\partial (V - V_0)}{\partial t} = \frac{AK(\Delta x)^2}{2}\frac{\partial^2 \psi}{\partial x^2} \qquad (A6)$$

The quantity $\partial V_0/\partial t$ is what we consider to represent growth and will be treated as a known function of time. Therefore, in order to obtain an equation in the single dependent variable ψ, one must express $\partial(V - V_0)/\partial t$ in terms of water potential. To simplify the process, we will assume that growth occurs at a constant osmotic pressure $\bar{\pi}$. This seems reasonably true over short times for the hypocotyls used in the present work (6) as well as for many other tissues (13). Constant osmotic pressure would only need to be approximated for a few min since the analysis is not sensitive to variations of a few bars. Because cell water potential is the difference between turgor pressure and osmotic pressure, one can write:

$$\psi = T - \pi \approx \epsilon\left(\frac{V - V_0}{V_0}\right) - \bar{\pi} \qquad (A7)$$

In obtaining the first term on the far right side of A7, we followed Philip (26) by assuming that turgor pressure was proportional to cell volume deformation at a given time. Recent studies have indicated that the elastic modulus ϵ varies considerably with turgor pressure (8, 32). There are three reasons why this observation should not cause serious problems in the present study. First, in our experiments, the cells were at the highest turgidity obtainable during growth (average turgor pressures of 3.5–4.5 bars), and in the higher turgidity range, ϵ tends to be nearly constant (8). Second, the turgor pressure differences between cells probably were not large, and the variation in ϵ throughout the tissue would be correspondingly small. Third, the parameter α in equation 4 is only weakly dependent on $\epsilon + \bar{\pi}$. (This is because $\epsilon + \bar{\pi}$ in α nearly cancels with $\epsilon + \pi_0$ in D when $W_v S \ll V_0/(\epsilon + \pi_0)$ which is normally the case.) Therefore, equation A7 represents a reasonable approximation for our case.

Solving A7 for the quantity $(V - V_0)$ and differentiating with respect to time yields:

$$\frac{\partial (V - V_0)}{\partial t} = \frac{V_0}{\epsilon}\frac{\partial \psi}{\partial t} + \frac{\psi}{\epsilon}\frac{\partial V_0}{\partial t} + \frac{\bar{\pi}}{\epsilon}\frac{\partial V_0}{\partial t} \qquad (A8)$$

Substituting equation A8 into A6 and rearranging results in:

$$\frac{\partial \psi}{\partial t} = \frac{AK(\Delta x)^2 \epsilon}{2V_0}\frac{\partial^2 \psi}{\partial x^2} - \frac{1}{V_0}\frac{\partial V_0}{\partial t}(\epsilon + \psi + \bar{\pi}) \qquad (A9)$$

The elastic modulus for moderately turgid cells is on the order of at least 50 to 100 bars (8, 32) and ψ will average only 1 to 3 bars. Therefore, it is safe to neglect ψ in the last term of equation A9. Defining $D = AK(\Delta x)^2\epsilon/2V_0$ and $G = (1/V_0)\partial V_0/\partial t$ gives the one-dimensional cartesian form of equation A9 as:

$$\frac{\partial \psi}{\partial t} = D\frac{\partial^2 \psi}{\partial x^2} - (\epsilon + \bar{\pi})G \qquad (A10)$$

(Note that the ratio of $A\Delta x/V_0$ in the expression for the diffusivity removes the time dependence from the diffusivity to within the

approximation of the derivation.) Using standard techniques, equation A10 can be transformed to one-dimensional radial geometry to yield:

$$\frac{\partial \psi}{\partial t} = D \frac{\partial^2 \psi}{\partial r^2} + \frac{D}{r} \frac{\partial \psi}{\partial r} - (\epsilon + \bar{\pi}) G \qquad (A11)$$

For steady-state conditions with growth perpendicular to the radial coordinate r, $\partial\psi/\partial t$ will be zero and equation A11 reduces to the ordinary differential equation:

$$0 = D \frac{d^2 \psi}{dr^2} + \frac{D}{r} \frac{d\psi}{dr} - (\epsilon + \bar{\pi}) G \qquad (A12)$$

which is identical to equation 1 of this communication except for the detailed expression for the diffusivity. The diffusivity defined by equation 2 contains cell wall as well as protoplast parameters, with the cell wall parameters playing a major role in determining a numerical value.

A nonlinear form of equation A12 could be developed which would allow for variable diffusivity. However, the quality of existing data does not appear to justify such an extension at the present time, although this may change in the near future.

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