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Analysis of the Dynamic and Steady-State Responses of Growth Rate and Turgor Pressure to Changes in Cell Parameters[1]

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ABSTRACT

The physical analysis of plant cell enlargment is extended to show the dependence of turgor pressure and growth rate under steady-state conditions on the parameters which govern cell wall extension and water transport in growing cells and tissues, and to show the dynamic responses of turgor and growth rate to instantaneous changes in one of these parameters. The analysis is based on the fact that growth requires simultaneous water uptake and irreversible wall expansion. It shows that when a growing cell is perturbed from its steady-state growth rate, it will approach the steady-state rate with exponential kinetics. The half-time of the transient adjustment depends on the biophysical parameters governing both water transport and irreversible wall expansion. When wall extensibility is small compared to hydraulic conductance, the growth rate is controlled by the yielding properties of the cell wall, while the half-time for changes in growth rate is controlled by the water transport parameters. The reverse situation occurs when hydraulic conductance is lower than wall extensibility. The analysis also shows explicitly that turgor pressure is tightly coupled with growth rate when growth is controlled by both water transport and wall yielding parameters.

In growing tissue where the resistance to water flow is distributed throughout the tissue, the physical analysis is more complicated because gradients in water potential (and hence turgor pressure) are required to sustain high growth rates. However, the analysis of growth in such tissues shows that the turgor and time-course relations are similar to that in single cells. These turgor and time-course relations provide experimentally useful ways for determining (a) whether growth is limited by water uptake, and (b) whether an agent which alters the growth rate does so by affecting the water transport or wall yielding properties or both.

The enlargement of plant cells is considered to be due to the irreversible yielding of the cell wall to the stress produced by cell turgor (17, 26). In its simplest form, the theory states that two simultaneous processes are required for cell enlargement: (a) water uptake and (b) irreversible expansion of the cell wall. In principle, either one of these processes may limit growth. The theory was first put into explicit analytical form by Lockhart (20) who derived an expression relating the steady-state growth rate to the physical parameters which control water transport and wall expansion. This concept of turgor-driven wall extension has been supported by numerous experimental results (3, 7, 14, 15, 27).

The physical theory of cell enlargement is particularly relevant for studies on the mode of action of hormones and for studies on factors which limit growth under laboratory and field conditions. That the water status of plants under field conditions can affect growth has been shown in a number of studies (1, 3, 10, 18). While auxin has generally been assumed to affect growth by altering the yielding properties of the cell wall (8, 26), the possibility that it also increases water permeability is more controversial (4, 12). In order for a change in water permeability to influence growth, water uptake must at least in part be limiting for cell expansion. Several experimental studies have concluded or assumed that growing cells are essentially in osmotic equilibrium with the water source (8, 14, 15), while the others have concluded that growing tissues may be significantly away from osmotic equilibrium (3, 22, 27).

In this report, I present a further development of the biophysical theory of cell enlargement which overcomes three important limitations of the earlier analysis (20, 26). First, the previous derivation applied only for steady-state growth; it did not describe the time-dependent behavior of growing cells, *e.g.* the time course for (transient) changes from one steady-state rate to another. Second, the Lockhart equation for steady-state growth rate was derived by eliminating turgor pressure from the two equations which describe the rates of water influx and wall extension during growth. Thus, the coupling of turgor to growth rate, while implicit in the Lockhart equation, is obscured. Third, the Lockhart equation strictly applies only to single cells or to analogs of the single cell, *i.e.* where there is only one major barrier to water flow. It does not apply to tissues where the resistance to water flow is distributed throughout the tissue (22).

The analysis presented here describes the time-dependent behavior of growing cells when the growth rate is altered or perturbed, and explicitly shows the coupling of turgor to growth rate which is implicit in the Lockhart equation. The analysis is further extended to include the more complicated case of growing tissue where the resistance to water flow is distributed throughout the tissue. Finally, I point out ways in which the model's predictions may be experimentally useful for estimating the degree to which water transport limits growth and whether an agent such as auxin affects growth by increasing water permeability.

THEORY

Single Cell Case. The analysis of Lockhart (20) is based on the fact that during steady-state growth, the rate of water influx into a cell must equal the rate of irreversible volumetric expansion of the cell wall chamber. These two processes are described by the equations[3]:

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[3] Previous formulations of the growth equations have not included the solute reflection coefficient, σ, although it has been discussed by Penny and Penny (23). Omitting σ is equivalent to setting σ equal to one. It is included here in part to account for interactions between solute and solvent flows in the membrane (11).

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$$\text{relative rate of water influx} = \frac{1}{V}\frac{dV}{dt} = L(\sigma\cdot\Delta\pi - P) \quad (1)$$

$$\text{relative rate of irreversible wall expansion} = \frac{1}{V_0}\frac{dV_0}{dt} = \phi(P - Y) \quad (2)$$

(symbols used in this analysis are given in Table I). By setting equations (1) and (2) equal to each other, substituting for pressure, and solving for growth rate, the general equation for growth rate in the steady state, $\dot{v}_s$, is obtained (26):

$$\dot{v}_s = \frac{L\cdot\phi}{L+\phi}(\sigma\cdot\Delta\pi - Y). \quad (3)$$

Equation (3) is valid only during steady-state growth. During the transient adjustment from one steady-state to another, equation (1) does not equal equation (2), and so we must distinguish two (nonequal) rates: one for water influx, another for irreversible wall expansion. For example, an instantaneous doubling of wall extensibility (ϕ) will cause the rate of wall expansion to double instantaneously, but will have no immediate effect on the rate of water influx. The higher rate of wall expansion will necessarily cause the cell turgor to decrease, thereby increasing the driving force for water influx (and hence the rate of water influx). Turgor will continue to decrease until the rates of water influx and wall expansion again equal each other. At this point, a new steady-state growth rate is restored and equation (3) is again valid. The difference between the volume generated by water influx and the volume generated by irreversible wall expansion is accounted for by elastic (reversible) changes in the cell walls during the adjustment in turgor pressure.

The time-dependent turgor response of a growing cell following a perturbation or change in any of the parameters which govern growth rate is given by the differential equation (see Appendix A for full derivation):

$$\frac{dP}{dt} = P^2(-L-\phi) + P(L\cdot\sigma\cdot\Delta\pi + \phi\cdot Y - L\cdot\epsilon - \phi\cdot\epsilon) + (L\cdot\epsilon\cdot\sigma\cdot\Delta\pi + \phi\cdot\epsilon\cdot Y). \quad (4)$$

Integration of equation (4) gives the solution (see Appendix A):

$$P(t) = P_1 - (P_2 - P_1)(1 - e^{t/t_c}), \quad (5)$$

Table I. *List of Symbols and Units*

P—cell turgor pressure (bar).
ϵ—volumetric elastic modulus of the cell (bar).
V_0—cell volume (cm^3) at zero turgor. This quantity is not constant but may increase in time (due to growth).
V—cell volume (cm^3) at turgor P and at a particular moment of growth. It too is a function of time.
t—time (s).
L—hydraulic conductance of the cell ($bar^{-1}\ s^{-1}$). This parameter differs from the usually defined conductivity Lp in that it incorporates the volume and area geometries of the cell. That is, it is equal to the membrane hydraulic conductivity times the membrane surface area (A) and divided by the volume (V) of the cell ($Lp\cdot A/V$).
σ—solute reflection coefficient (dimensionless).
$\Delta\pi$—difference in osmotic potentials between the inside of the cell and the external medium (bar).
ϕ—wall extensibility ($bar^{-1}\ s^{-1}$).
Y—yield threshold (bar). The minimum turgor required for wall expansion.
ψ—water potential (bar).
r—radial distance from the center of the cylinder (cm).
$\bar{\pi}$—average osmotic potential of the tissue (bar).
D—tissue free energy diffusivity of water ($cm^2\ s^{-1}$).

where $P(t)$ is the turgor pressure at time t, P_1 is the negative root of the quadratic on the right half of equation (4), P_2 is the positive root, and t_c is the time constant, which is a function of all the parameters in equation (4). One can see that the pressure, and thus the growth rate, will approach its steady-state value with an exponential time course having a half-time ($t_{1/2} = \ln(2)\cdot t_c$) dependent on the parameters for both water influx and wall extension.

The significance of these equations can perhaps be best understood graphically. The time course for the change in turgor following an (instantaneous) decrease in wall extensibility, as predicted by this analysis, is shown in Figure 1A. Substitution of these pressure values into the rate equations for water influx and irreversible wall expansion demonstrates that initially the two rates are unequal, but approach equality as the pressure reaches its steady-state value (Fig. 1B).

This analysis of growth (represented in equations [3] and [5]) allows us to define three quantities as a function of the biophysical parameters governing water transport and irreversible wall expansion: steady-state growth rate ($\dot{v}_s$), steady-state turgor pressure (P_s) and the half-time ($t_{1/2}$) for changes from one steady-state rate to another. The influence of wall extensibility, ϕ, on these three quantities is graphed in Figure 2A. The growth rate curve shows the results of the Lockhart equation (*i.e.* equation [3] here). When wall extensibility, ϕ, is small (relative to hydraulic conductance, L), a change in ϕ has a great effect on the steady-state growth rate, $\dot{v}_s$, but when ϕ is larger than L, its influence diminishes. At the extreme case where ϕ is much larger than L, growth is completely limited by the ability of the cell to take up water and changes in the yielding properties of the cell wall are insignificant for growth.

Intuitively, one can see that at the left extreme of Figure 2A, where growth is limited by the ability of the cell wall to expand, the steady-state turgor pressure, P_s, rests very close to the full turgor value (*i.e.* $P_s = \sigma\cdot\Delta\pi$, which is the nongrowing equilibrium

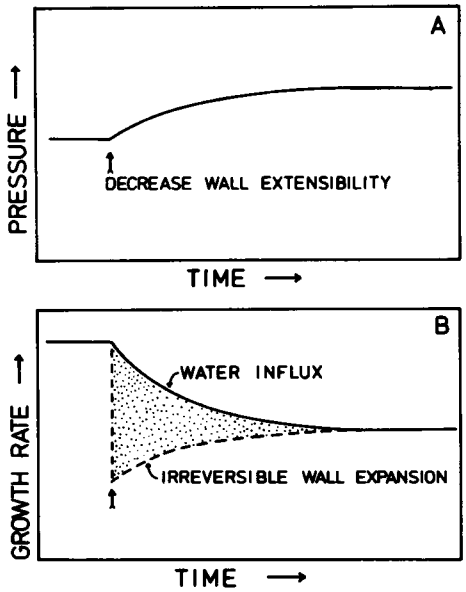

FIG. 1. Time course for changes in turgor pressure and growth rate in single cells. A, change in cell turgor following an instantaneous decrease in wall extensibility. Calculated from equation (A11) in Appendix A, this curve shows that the pressure readjustment to the new steady-state value follows an exponential time course. B, change in the rates of water uptake and wall expansion following an instantaneous decrease in wall extensibility. The pressure from (A) was substituted into equations (1) and (2) to obtain the rates of water influx and wall expansion. Stippled area shows the change in volume due to elastic changes in the cell wall.

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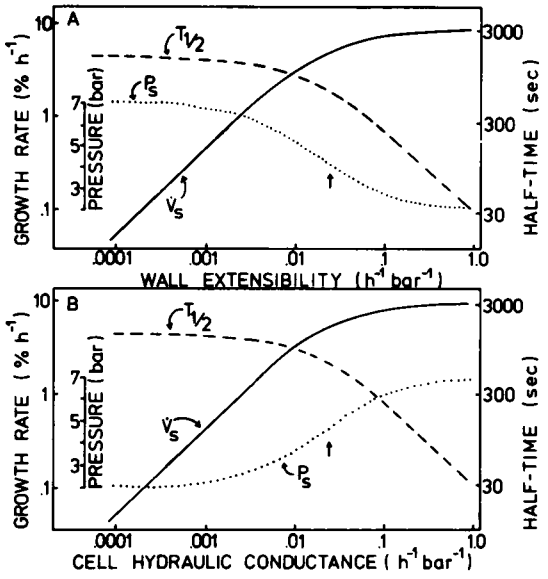

FIG. 2. Growth rate ($\dot{v}_s$), pressure (P_s) and half-time ($t_{1/2}$) for growth transients as a function of (A) wall extensibility and (B) hydraulic conductance. Note that the axes have logarithmic scales. The parameters were initially assigned the following values: $\phi = 0.02$ bar^{-1} h^{-1}, $L = 0.02$ bar^{-1} h^{-1}, $\sigma \cdot \Delta\pi = 7$ bar, $Y = 2$ bar, and $\epsilon = 80$ bar. One parameter was varied, with the others held constant, and the steady-state growth rate, pressure and half-time were calculated. The arrow in the graph indicates the point where $L = \phi$.

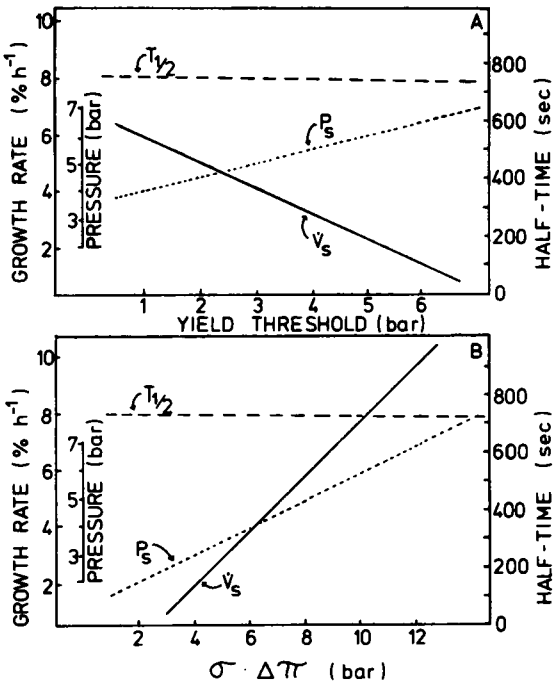

FIG. 3. Growth rate ($\dot{v}_s$), pressure (P_s) and half-time ($t_{1/2}$) for growth transients as a function of wall yield threshold (A) and the product of reflection coefficient and osmotic potential difference, $\sigma \cdot \Delta\pi$ (B). These curves were calculated as in Figure 2.

value of turgor). At the right extreme, where growth is limited by water uptake, the cell expands whenever turgor exceeds the yield threshold, so that turgor rests very near the yield threshold for wall expansion (*i.e.* $P_s = Y$). This relation is explicitly shown in Figure 2A, where steady-state turgor, P_s, is plotted as a function of wall extensibility, ϕ. When $\phi = L$, the cell turgor balances exactly at the midpoint between full equilibrium turgor ($\sigma \cdot \Delta\pi$) and the yield threshold (Y). Note that in the range where the ratio L/ϕ is between 0.1 and 10, the steady-state pressure is coupled with the steady-state growth rate, $\dot{v}_s$. Thus, a change in growth rate is necessarily accompanied by a change in turgor.

The half-time for changes in pressure and thus growth rate is also a function of wall extensibility. Figure 2A shows that the relation of ϕ to $t_{1/2}$ is exactly complementary to that for steady-state growth, $\dot{v}_s$. That is, $t_{1/2}$ is most sensitive to the larger of the two parameters (L and ϕ), contrary to the case for $\dot{v}_s$ where the smaller of the two values has the greater influence.

The plot of $\dot{v}_s$ and $t_{1/2}$ as a function of hydraulic conductance (L) shows similar results (Fig. 2B). However, note the important difference in the turgor pressure curve. Whereas in Figure 2B the turgor rises as the growth rate rises, in Figure 2A the turgor falls. Thus, the turgor response to a decrease in growth rate is completely different for the two cases where a change in ϕ or L is responsible for the lower growth rate.

Figure 3, A and B, shows the influences of the yield threshold (Y), solute reflection coefficient (σ) and osmotic potential difference ($\Delta\pi$) on $\dot{v}_s$, $t_{1/2}$ and P_s of the growing cell. Changes in any of these three parameters significantly affect the growth rate, $\dot{v}_s$, and pressure, P_s, but hardly alter the half-time (since $\epsilon \gg \Delta\pi$, in this analysis). On the contrary, a change in the volumetric elastic modulus (ϵ) has virtually no effect on $\dot{v}_s$ or P_s, but does alter the $t_{1/2}$ of growth responses (data not shown).

Whole Tissue Case. The analysis presented above can be applied to single cells and to tissues where there is only one barrier to water flow. Such tissues include, for example, cases where water transport through the cell wall pathway is very rapid compared with the cell-to-cell pathway (*i.e.* across membranes and through plasmodesmata) or in tissues where the cuticle forms the major resistance to water flow, *e.g.* in excised *Avena* coleptiles (27). For tissues where the resistance to water flow is distributed throughout the tissue, the transport of water will follow diffusion kinetics (25) and a different analysis must be used. As shown below, however, the major conclusions from the single cell analysis are also valid for the case with distributed resistance.

As Molz and Boyer (22) have shown, the equation describing radial water transport (*i.e.* from a central vascular bundle to the epidermis) during growth in a cylindrical stem is given by the partial differential equation:

$$\frac{\partial \psi}{\partial t} = D\frac{\partial^2 \psi}{\partial r^2} + \frac{D\partial \psi}{r\partial r} - (\epsilon + \bar{\pi})\left(\frac{1}{V_0}\frac{\partial V_0}{\partial t}\right). \quad (6)$$

This equation simply states that the rate of change of ψ in each cell is a function of the rate of wall expansion and the rate of water influx. Assuming all cells (in a radial direction) have the same osmotic potential, then equation (6) is equivalent to:

$$\frac{\partial P}{\partial t} = D\frac{\partial^2 P}{\partial r^2} + \frac{D\partial P}{r\partial r} - (\epsilon + \bar{\pi})\left(\frac{1}{V_0}\frac{\partial V_0}{\partial t}\right). \quad (7)$$

Since the cells at the center of the stem grow at the same rate as the cells at the outer part of the stem (or else there would be telescoping of the stem during growth), then $(1/V_0)(dV_0/dt)$ may be replaced using the equation for wall growth by $\phi(\bar{P} - Y)$, where $\bar{P}$ is the average turgor pressure for the whole tissue, obtaining:

$$\frac{\partial P}{\partial t} = D\frac{\partial P^2}{\partial r^2} + \frac{D\partial P}{r\partial r} - (\epsilon + \bar{\pi})(\bar{P} - Y)\phi. \quad (8)$$

Equation (8) was solved by numerical analysis on a PDP-11 computer (Digital Computer Corp.) using standard finite difference methods (9, see also Appendix B). The growing stem was modeled as a cylinder having ten "cells" along the radius (Fig. 4). For initial conditions, the tissue was assumed to be nongrowing and in osmotic equilibrium with a medium of $\psi = 0$ bar. Growth

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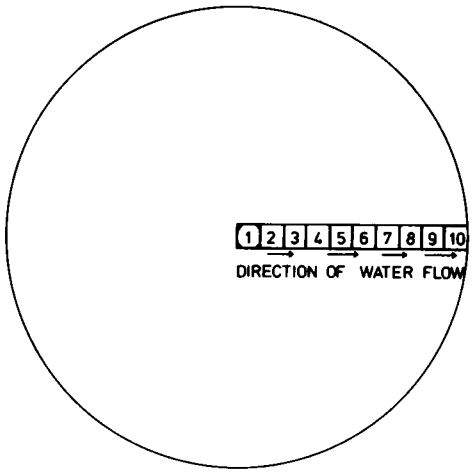

FIG. 4. To calculate the pressure relations of growing tissues, a stem was modeled as a cylinder of constant width in which growth was purely longitudinal. The central 'cell' was assumed to be the source of water for growth (*i.e.* xylem). See Appendix B for mathematical details.

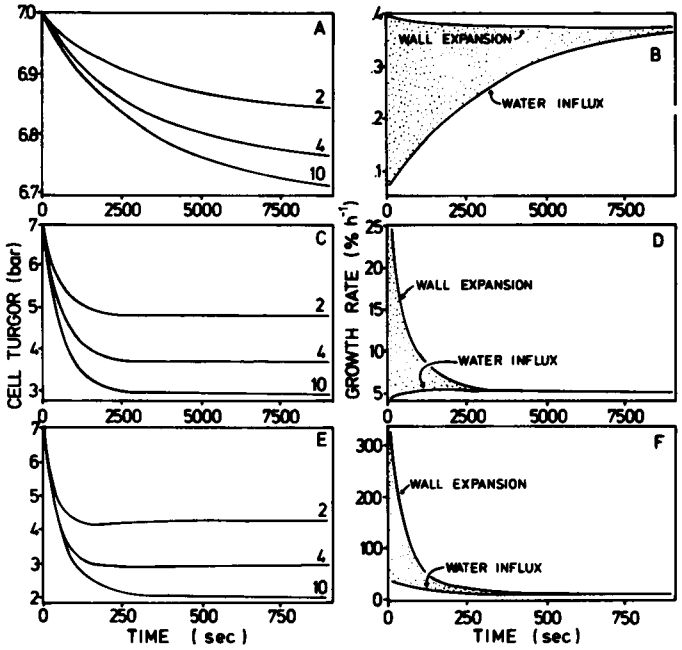

FIG. 5. Time course for changes in cell turgor and growth rate in tissues with distributed resistance to water flow. For these calculations, $D = 2.8$ 10^{-6} cm^2 s^{-1}, $\sigma \cdot \Delta\pi$ 7 bar, $Y = 3$ bar, and $\epsilon = 78$ bar. At zero time, ϕ was increased from zero to 0.001 bar^{-1} h^{-1} (A, B), 0.07 bar^{-1} h^{-1} (C, D), and 1.0 bar^{-1} h^{-1} (E, F). A, C, and E, changes in turgor in cells 2, 4, and 10 (see Fig. 4); B, D, and F, changes in rates of wall expansion and water influx. Stippled areas indicate elastic changes in volume.

was initiated by setting ϕ to some positive value and the consequent changes in pressure in each of the ten cells were calculated.

Before steady-state growth rate is attained, the cell turgor is a function of both position and time (Fig. 5A). At steady state, turgor is a function of position only. The rates of water uptake and wall expansion for the whole tissue are equal only at steady state (Fig. 5B), as in the analysis of growth in the single cell. When growth of the tissue is limited by the ability of the cell wall to expand, *i.e.* ϕ is small, then the pressure curves for all cells along the radius behave like single component exponentials with the same half-time (Fig. 5A). Likewise, the curves for the rates of water uptake and wall expansion (Fig. 5B) are exponential with the same $t_{1/2}$ as the cell turgor pressure curves. When water uptake and wall expansion are about equally limiting for growth, this simple one-exponential characteristic is still maintained (Fig. 5, C and D). Only when wall extensibility becomes so large that it no longer can influence growth rate (*i.e.* water uptake is completely limiting) do the curves deviate from this simple one-exponent case (Fig. 5, E and F). In this case, the pressure curves are not exponential, and the inner cells attain steady state faster than do the cells further from the water source. Similarly, the curves for water uptake and wall expansion are not exactly exponential. In this case, it may still be useful to speak of a half-time, but it does not carry with it the implication of an exponential curve.

To compare the analysis for single cells with the analysis for multicellular tissues, the influence of D, ϕ, Y, and $\sigma \cdot \Delta\pi$ on $\dot{v}_s$, $\bar{P}_s$ and $t_{1/2}$ were calculated. Figure 6 shows that changes in D and ϕ in the whole tissues have the same effect on $\dot{v}_s$, $\bar{P}_s$ and $t_{1/2}$ as changes in L and ϕ in the single cell case. That is, when ϕ is small, it controls growth, but has no influence on $t_{1/2}$ while the reverse is true when ϕ is very large. In between these extremes, $\bar{P}_s$ is tightly coupled with the growth rate, $\dot{v}_s$. In this middle range, a decrease in tissue diffusivity causes a decrease in $\bar{P}_s$, while a decrease in wall extensibility causes an increase in $\bar{P}_s$. Changes in Y and $\sigma \cdot \Delta\pi$ have large effects on the steady-state growth rate, $\dot{v}_s$, and on the steady-state pressure, $\bar{P}_s$, but only minor influence on $t_{1/2}$ (Fig. 7). As in the single cell case, a decrease in the growth rate by changes in Y or $\sigma \cdot \Delta\pi$ will be accompanied by an increase or decrease in $\bar{P}_s$, respectively. Changes in the volumetric elastic modulus ϵ do not significantly affect $\dot{v}_s$ or $\bar{P}_s$, but do affect $t_{1/2}$ (data not shown).

This analysis does not take into consideration that, in a tissue like a stem or root, the rate of cell enlargement varies with distance along the axis. Silk and Wagner (29) have recently published a steady-state model of the growing corn root which includes such spacial heterogeneity in growth rate. Refinement of the dynamic analysis presented above to include such growth distributions

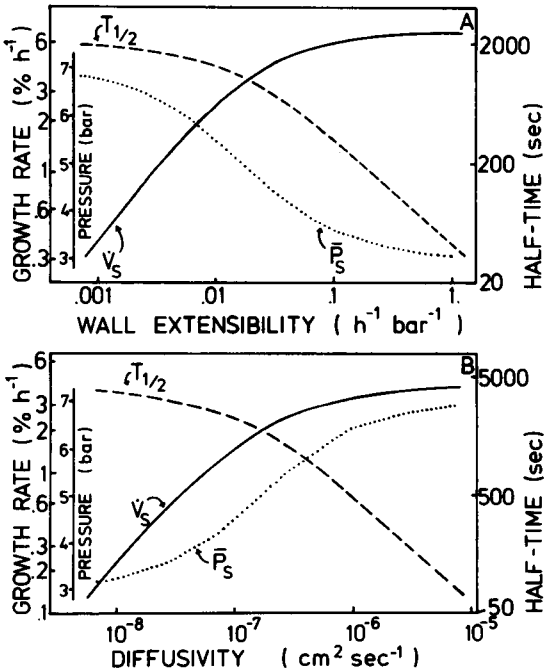

FIG. 6. Growth rate ($\dot{v}_s$), average pressure ($\bar{P}_s$) and half-time ($t_{1/2}$) for growth transients as a function of wall extensibility (A) and tissue diffusivity (B). Note the logarithmic axes. Initially $D = 2.8 \times 10^{-6}$ cm^2 s^{-1}, $\phi = 0.01$ bar^{-1} h^{-1}, $\sigma \cdot \Delta\pi = 7$ bar, $Y = 3$ bar and $\epsilon = 78$ bar. Either D or ϕ was varied, all others kept constant.

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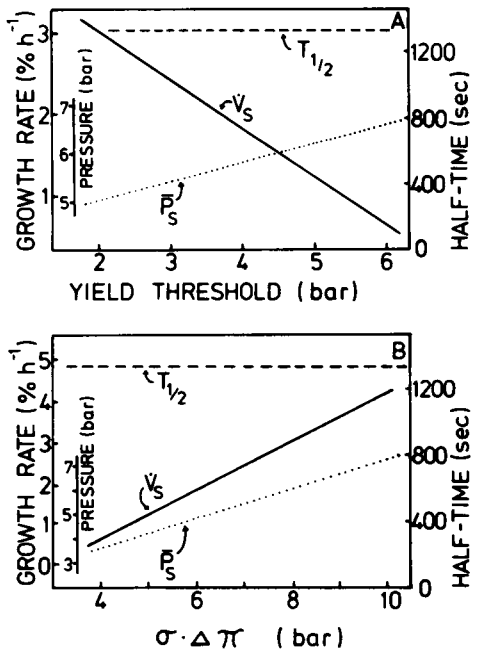

FIG. 7. Growth rate ($\dot{v}_s$), average pressure ($\bar{P}_s$), and half-time ($t_{1/2}$) for growth transients in tissues as a function of (A) yield threshold (Y) and (B) the product of reflection coefficient and osmotic potential difference ($\sigma \cdot \Delta\pi$). The same procedure as in Figure 6 was used to calculate these curves.

would be expected to quantitatively alter some of the relationships presented here, but qualitatively the results would be the same.

DISCUSSION

The analysis of cell enlargement presented here shows for the first time that both the water transport and wall yielding parameters of a cell regulate the time course for the attainment of steady-state growth rate following perturbation or alteration of the growth rate. Furthermore, the coupling between growth rate and turgor is explicitly shown for the first time.

In principle, it is easy to determine whether growth in the single cell is limited by the rate of water uptake or the rate of wall expansion, since L and ϕ have the same units and may be directly compared. The values for these two parameters, however, are available for only one species: the giant-celled alga *Nitella*. Green *et al.* (15) found that ϕ of growing *Nitella* internode cells is approximately 2.8×10^{-5} s^{-1} bar^{-1}. The hydraulic conductance of a *Nitella* cell may be calculated by multiplying the membrane hydraulic conductivity ($Lp = 2 \times 10^{-5}$ cm s^{-1} bar^{-1}, see ref. 11) by the volume to area ratio (10^{-2} cm), giving a value of 2×10^{-3} s^{-1} bar^{-1}. Since ϕ is two orders of magnitude smaller than L, the growing *Nitella* cell will be essentially in osmotic equilibrium, the growth rate will be controlled completely by the wall yielding process, and the time course for adjustment to a new steady-state rate will be controlled completely by the cell's water transport parameters. This conclusion probably holds for growth in other single cells, although data are not available to make this evaluation.

For tissues where the resistance to water flow is distributed throughout the tissue, a similar evaluation is not as straight forward. Water transport in such tissues is a function of both D and tissue geometry (shape and thickness) and the hydraulic resistance varies with distance of the cell from the water source. Boyer and Wu (4) have calculated an effective hydraulic conductance for growing soybean hypocotyls by taking the ratio of the growth rate over the difference in ψ of the tissue and the water source. However, an analytical expression for such an average L based on tissue D and geometry has not been derived up to now.

A further complication appears in trying to measure ϕ in whole tissues. Two approaches have been used in the past. One is to measure the growth rate of excised stem or coleoptile segments bathed in solutions of different osmotic potentials (8, 14). The slope of the line relating growth rate to osmotic potential of the solution is then taken as ϕ. This method, however, tacitly assumes that growth rate is completely limited by wall extensibility. In the more general case, the slope of this line is a function of both water transport and wall yielding parameters, exactly analogous to the single cell case where the slope is given by $(L \cdot \phi)/(L + \phi)$. Only when tissue diffusivity D is very large (not limiting growth) is the slope of this line equal to ϕ. A second approach used to estimate wall extensibility is to measure the plastic compliance, creep or stress relaxation of (usually) dead cell walls by various mechanical means (see ref. 23 for review). However, the parameters measured by such devices are clearly not equivalent to ϕ (8, 23). First, the changes observed in these parameters after growth stimulation by auxin seem to be a result of the higher growth rate, rather than a cause of it (23, 24). Second, their units are not the same as the units for ϕ, and cannot be converted into meaningful, equivalent units. For these theoretical and technical reasons, it is not easy to evaluate directly the limits placed on growth by the water uptake and wall expansion processes in growing tissues.

Fortunately, the analysis of growth in whole tissues shows that three quantities of growing systems ($\dot{v}_s$, $\bar{P}_s$, and $t_{1/2}$) may be used diagnostically (as in the single cell case) to determine (a) whether water uptake or wall expansion limits growth and (b) whether an agent which alters the growth rate does so by affecting D, ϕ, Y, or $\sigma \cdot \Delta\pi$.

When growth is limited by water uptake, $\bar{P}_s$ of the growing tissue will be lower than that in the nongrowing tissue. This condition has been experimentally demonstrated in rapidly growing oat coleoptiles (27), sunflower leaves (3), and soybean hypocotyls (21). Recently, Cutler *et al.* (10) found that they could increase the growth rate of rice leaves by applying a hydrostatic pressure to the roots of plants, from which they concluded that water uptake was limiting for growth. This conclusion, however, is based on erroneous reasoning. Application of hydrostatic pressure to the roots is equivalent (as they demonstrate) to an increase in $\Delta\pi$, and would increase the growth rate even when growth is completely limited by a low wall extensibility. This situation is

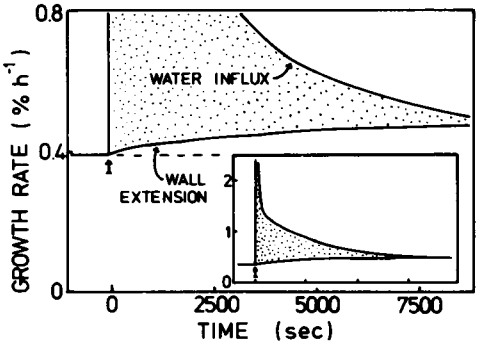

FIG. 8. Theoretical change in the growth rate of a stem induced by changing the water potential (ψ) of the water source. At the time indicated by the arrow, ψ of the xylem was increased from 0 bar to 1 bar by application of pressure, as in Cutler *et al.* (10). Following the elastic changes in stem length (stippled area), the growth rate is higher than before the change. Inset shows the full response: a very rapid change in rate is followed by a slower, exponential adjustment, from which $t_{1/2}$ of the stem may be calculated. Values of D (2.8×10^{-6} cm^2 s^{-1}) and ϕ (0.001 bar^{-1} h^{-1}) were chosen such that growth was completely controlled by wall yielding. ϵ was 78 bar, $\sigma \cdot \Delta\pi$ was 7 bar.

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Table II. *Effects of Changes in ϕ, D, Y and $\sigma \cdot \Delta\pi$ on Growth Rate ($\dot{v}_s$), Steady-State Pressure ($\bar{P}_s$) and Half-Time ($t_{1/2}$) in Growing Tissue*

Listed below are the predicted changes in four measurable quantities when the growth rate is *doubled* by altering the specified parameter(s). The first two rows refer to an experiment in which the growth rate is plotted against the water potential of the source (ψ^0).

Predicted Change In:	Altered Parameter(s)				
	D	ϕ	$\sigma \cdot \Delta\pi$	*Y*	Both *D* and ϕ
Slope of $\dot{v}_s$ *versus* ψ^0	Steeper	Steeper	No change	No change	Steeper
Intercept of $\dot{v}_s$ *versus* ψ^0	No change	No change	Higher	Higher	No change
$\bar{P}_s$	Higher	Lower	Higher	Lower	No change
$t_{1/2}$	Slight or no change	Slight or no change	No change	No change	Decrease by ½

demonstrated in Figure 8 where such an experiment has been modelled. It should be noted that measurements of length during such osmotic transients measure the rate of water influx, not the rate of irreversible wall expansion. Equivalent experiments have been performed by decreasing the growth rate with osmotic solutions (7, 10, 14) or with application of hydrostatic pressure to the shoot (3), but the results do not bear on the question of the limiting roles of water uptake and wall expansion during growth.

The physical mechanism by which any agent affects the growth rate may be determined by measurements of $\dot{v}_s$, $\bar{P}_s$, and $t_{1/2}$ (Table II). Turgor pressure measurements provide the key for determining whether an agent affects the water transport parameters (*D*, $\sigma \cdot \Delta\pi$) or the wall yielding parameters (ϕ, *Y*). If both processes are affected, the change in turgor will indicate which process is most significantly altered for the growth effect. For example, Boyer and Wu (4) present data that auxin alters both the hydraulic conductivity and wall extensibility of soybean hypocotyls, but their measurements show that turgor decreases in the auxin-stimulated tissue. This result indicates that auxin stimulation of growth is accomplished mainly by an increase in the yielding properties of the wall.

In the case where turgor does not change significantly during a change in the growth rate, three possibilities must be considered: (a) growth is completely controlled by low *D*; (b) growth is completely controlled by low ϕ; and (c) both *D* and ϕ change equally. Case (c) may be distinguished from (a) and (b) by measuring $t_{1/2}$. For example, when the growth rate is doubled by doubling both *D* and ϕ, the $t_{1/2}$ is halved, but in tissue where growth is completely limited by low *D* or ϕ, doubling the growth rate will have no effect on $t_{1/2}$. Case (a) may be distinguished from case (b) by comparing the value of $\bar{P}_s$ with the value of $\sigma \cdot \Delta\pi$. When growth is limited by ϕ, $\bar{P}_s$ will almost equal $\sigma \cdot \Delta\pi$. When growth is limited by *D*, $\bar{P}_s$ will almost equal *Y*, which in higher plant tissues is significantly lower than $\sigma \cdot \Delta\pi$ (3, 7, 14, 21). It is important to note that the changes in $\dot{v}_s$, $\bar{P}_s$, and $t_{1/2}$ (Table II) would occur regardless of the absolute values chosen for the cell parameters (*D*, ϕ, *Y*, $\sigma \cdot \Delta\pi$); only the magnitude of $\dot{v}_s$, $\bar{P}_s$, and $t_{1/2}$ would be affected by selecting a different set of cell parameters.

A variety of possible methods have been developed which may be used to measure $\dot{v}_s$, $\bar{P}_s$, and $t_{1/2}$. Growth rates have been measured by a number of mechanical, optical, and electronic techniques (see ref. 23 for a review). Turgor may be measured (a) directly with the pressure probe (19), (b) by calculation from water potential and osmotic potential measurements (22), and (c) indirectly by measurement of tissue rigidity (13). Each method has its own advantages and disadvantages which must be carefully considered. It should be pointed out that estimates of turgor from water potential measurements of excised growing segments which are not in contact with liquid water may give misleading results. After a growing tissue is excised from the plant and placed in the psychrometer chamber, water is no longer available to enter the tissue and so expansion stops. However, if cell turgor is above the yield threshold for wall expansion, then stress relaxation of the cell wall will occur (26) and turgor will decrease until the pressure equals the yield threshold. Thus, it is questionable whether the water potentials measured by Boyer and Wu (4) in excised hypocotyl segments represent the water potential at the time of excision. Their calculations of hydraulic conductivity and auxin effects on hydraulic conductivity should therefore be regarded with caution. Furthermore, measurements of water potential gradients developed during growth (3, 4, 25) have tacitly assumed that the concentration of osmotically active solutes in the cell wall free space is negligible. If the osmotic potential of the free space solution is different for growing and nongrowing tissues, serious errors in the estimates of the water potential difference necessary to sustain growth may result. The magnitude of these potential errors from stress relaxation and solutes in the cell wall solution should be examined.

To measure $t_{1/2}$, the growth rate must be abruptly displaced from steady state and the time course for the return to steady state observed. Since $t_{1/2}$ of most growing tissues will be fast (a few minutes at most), very sensitive growth sensors must be used. The growth rate may be disturbed by application of tension (23), osmotic solutions (14, 27) or hydrostatic pressure (3, 10). The use of tension has the disadvantage that the uniaxial wall stress produced by a tension on the growing tissue is difficult to compare quantitatively with the multiaxial stress produced by cell turgor. The use of osmotic solutions may not be a valid way to measure $t_{1/2}$, since the time course measured with osmotic changes in the medium may reflect the time required for diffusion of the solutes into the cell wall space, rather than the water transport process itself (11, 27). The use of hydrostatic pressure (*i.e.* by applying pressure to the roots of a plant to increase shoot growth, or applying pressure to the shoot to decrease shoot growth) may be more useful than these other methods, providing the water flow induced by pressure follows the same pathway as that taken by growth-induced water influx.

Finally, it should be mentioned that Burström (5, 6) has criticized the concept that turgor is involved in growth. However, it has been repeatedly demonstrated that wall expansion does not occur without a minimum stress in the cell wall and that the growth rate is a function of the tension above this minimum value (3, 7, 10, 14, 27). This tension is normally provided by cell turgor, but may be replaced or augmented by application of an external tension to the tissue (16, 23, 28). Thus, although the exact relation between wall expansion and turgor is only empirically known, some equation similar to equation (2) must be employed to account for such experimental results.

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APPENDIX A

The derivation of the equations describing the pressure relations in a growing cell starts with the relation:

$$P = \epsilon \frac{V - V_0}{V_0}, \tag{A1}$$

which is a linear extension (11) of the equation defining the volumetric elastic modulus:

$$dP = \frac{dV}{V}\epsilon. \tag{A2}$$

Differentiating both sides of (A1) yields

$$\frac{dP}{dt} = \epsilon \frac{\frac{V_0 d(V - V_0)}{dt} - (V - V_0)\frac{dV_0}{dt}}{V_0^2}, \tag{A3}$$

which on rearrangement gives

$$\frac{dP}{dt} = \epsilon \left[\frac{1}{V_0}\frac{dV}{dt} - \frac{V}{V_0}\frac{1}{V_0}\frac{dV_0}{dt} \right]. \tag{A4}$$

As an aside, note that an elastic steady state, dP/dt is zero and assuming constant ϵ, equation (A4) can be rearranged to

$$\frac{V}{V_0}\frac{1}{V_0}\frac{dV_0}{dt} = \frac{1}{V_0}\frac{dV}{dt}.$$

Dividing both sides by V/V_0, we obtain

$$\frac{1}{V_0}\frac{dV_0}{dt} = \frac{1}{V}\frac{dV}{dt}, \tag{A5}$$

which is the expression derived by Lockhart (20) for steady-state growth. The expression on the left side of equation A5 is the relative rate of irreversible expansion of the cell walls, expressed in terms of cell chamber volume (unstretched), while the expression on the right side is the relative rate of water uptake. At steady state growth these two must be equal. Returning to equation (A4), we multiply the right side by V/V to obtain

$$\frac{dP}{dt} = \epsilon \left[\frac{V}{V_0}\frac{1}{V}\frac{dV}{dt} - \frac{V}{V_0}\frac{1}{V_0}\frac{dV_0}{dt} \right]. \tag{A6}$$

Factoring out V/V_0 and making the substitutions:

$$\frac{1}{V}\frac{dV}{dt} = L(\sigma \cdot \Delta\pi - P) = \text{relative rate of water influx} \tag{A7}$$

and

$$\frac{1}{V_0}\frac{dV_0}{dt} = \phi(P - Y) = \text{relative rate of irrev. wall expansion}, \tag{A8}$$

we obtain

$$\frac{dP}{dt} = \epsilon \frac{V}{V_0}[L(\sigma \cdot \Delta\pi - P) - \phi(P - Y)]. \tag{A9}$$

Making the further substitution

$$V = \frac{V_0 P}{\epsilon} + V_0,$$

which is a rearrangement of equation (A1), we obtain

$$\frac{dP}{dt} = \epsilon \frac{\frac{V_0 P}{\epsilon} + V_0}{V_0}[L(\sigma \cdot \Delta\pi - P) - \phi(P - Y)].$$

This equation reduces to

$$\frac{dP}{dt} = (P + \epsilon)[L(\sigma \cdot \Delta\pi - P) - \phi(P - Y)].$$

Multiplying out the above equation and collecting terms, we finally obtain

$$\frac{dP}{dt} = P^2(-L - \phi) + P(L \cdot \sigma \cdot \Delta\pi + \phi \cdot Y - L \cdot \epsilon - \phi \cdot \epsilon) + (L \cdot \epsilon \cdot \sigma \cdot \Delta\pi + \phi \cdot \epsilon \cdot Y). \tag{A10}$$

Equation (A10) is of the form

$$\frac{dP}{dt} = \alpha P^2 + \beta P + \rho,$$

where: $\alpha = (-L - \phi)$, $\beta = (L \cdot \sigma \cdot \Delta\pi + \phi \cdot Y - L \cdot \epsilon - \phi \cdot \epsilon)$, and $\rho = (L \cdot \epsilon \cdot \sigma \cdot \Delta\pi + \phi \cdot \epsilon \cdot Y)$, and has the explicit solution (2)

$$P_{(t)} = P_1 + \frac{P_2 - P_1}{1 + ke^{\alpha(P_2 - P_1)t}} \tag{A11}$$

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where P_1 and P_2 are roots of the quadratic equation (A10) and k is a constant of integration.

Equation (A11) is transformed into the form of equation (5) by the following procedure. If P_1 is assigned as the negative root, then $ke^{\alpha(P_2-P_1)\cdot t}$ evaluates to a value much smaller than 1 for $t > 0$. Using the approximation that $1/(1 + x) = 1 - x$ for $x \ll 1$, equation (A11) becomes:

$$P(t) = P_1 + (P_2 - P_1)(1 - e^{\alpha(P_2-P_1)\cdot t})$$

which is the same as equation (5). If on the other hand we assign P_2 as the negative root, $ke^{\alpha(P_2-P_1)\cdot t}$ evaluates to much larger than 1. Using the approximation that $1/(1 + x) = x^{-1}$ for $x \gg 1$, an expression equivalent to equation (5) is obtained.

Equation (A11) shows that the time course for changes in pressure, and consequently changes in growth rate, is approximately exponential in form, with a rate constant determined by $\alpha(P_2 - P_1)$. Extraction of the roots of equation (A10) and substitution of the relevant biophysical parameters into the rate constant shows that

$$\alpha(P_2 - P_1) = \pm[L^2(\sigma\cdot\Delta\pi + \epsilon)^2 + \phi^2(Y + \epsilon)^2 + 4\cdot L\cdot\phi(\sigma\cdot\Delta\pi - \epsilon)(Y - \epsilon)]^{1/2}. \quad \text{(A12)}$$

Thus, the time course for growth rate changes is a function of both wall extensibility and water conductivity, as well as the other parameters. One can see that in a nongrowing cell, where wall extensibility is zero, the rate constant simplifies to

$$t_c = L(\pi + \epsilon) = \frac{A\cdot Lp}{V}(\pi + \epsilon)$$

assuming the cell is in distilled water and $\sigma = 1$. This is the same rate constant derived previously for water exchange in a single non-growing plant cell (11, eq. 2.28). Thus, equations (A10) and (A11) are more general expressions for the dynamic water relations of plant cells, including both growing and nongrowing cells.

APPENDIX B

The equation for the pressure relations of a cylindrically-shaped tissue growing in length (but constant radius) was solved by transforming it into an explicit finite difference formula and then iteratively calculating the pressure in each cell at each point in time. Equation (8) is equivalent to:

$$P_{i,j} = P_{i,j-1} + \Delta P_{\text{water influx}} + \Delta P_{\text{wall expansion}} \quad \text{(B1)}$$

where $P_{i,j}$ is the pressure in the i^{th} cell from the center at the j^{th} time interval. The water influx term in equation (B1) is given by:

$$\Delta P^{w_{i,j+1}} = \frac{tDb^2}{2ia^2}[(2i + 1)P_{i+1,j} - (4i)P_{i,j} + (2i - 1)P_{i-1,j}] \quad \text{(B2)}$$

where t is the time step (s) for each interaction, D is the tissue diffusivity ($cm^2\ s^{-1}$), a is the radius of the growing cylinder (cm) and b is the number of equally-spaced intervals ('cells') into which the radius is divided. The value of t was always chosen such that the quantity $t\cdot D\cdot b^2/a^2$ was less than the critical value of 0.5. See Crank (9) for details of the finite difference method.

The wall expansion term in equation (B1) is the same for all cells along the radius and is given by:

$$\Delta P^{\epsilon}_{i,j+1} = (\epsilon + \bar{\pi})(\bar{P}_j - Y)\phi \quad \text{(B3)}$$

where ϕ is given in ($bar^{-1}\ s^{-1}$) and $\bar{P}_j$ is the average turgor of all cells along the radius at the previous time point.

For all calculations presented in this report, $a = 0.075$ cm and $b = 10$. The first cell in the water pathway was assumed to be the source of the water for growth (*i.e.* the xylem) with a constant $\psi = 0$ bar.

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