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Enlargement in *Chara* Studied with a Turgor Clamp[1]

Growth Rate Is Not Determined by Turgor

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ABSTRACT

A new method, the turgor clamp, was developed to test the effects of turgor on cell enlargement. The method used a pressure probe to remove or inject cell solution and change the turgor without altering the external environment of the cell walls. After the injections, the cells were permanently at the new turgor and required no further manipulation. Internode cells of *Chara corallina* grew rapidly with the pressure probe in place when growth was monitored with a position transducer. Growth-induced water potentials were negligible and turgor effects could be studied simply. As turgor was decreased, there was a threshold below which no growth occurred, and only reversible elastic/viscoelastic changes could be seen. Above the threshold, growth was superimposed on the elastic/viscoelastic effects. The rate of growth did not depend on turgor. Instead, the rate was highly dependent on energy metabolism as shown by inhibitors that rapidly abolished growth without changing the turgor. However, turgors could be driven above the maximum normally attainable by the cell, and these caused growth to respond as though plastic deformation of the walls was beginning, but the deformation caused wounding. Growth was inhibited when turgor was changed with osmotica but not inhibited when similar changes were made with the turgor clamp. It was concluded that osmotica caused side effects that could be mistaken for turgor effects. The presence of a turgor threshold indicates that turgor was required for growth. However, because turgor did not control the rate, it appears incorrect to consider the rate to be determined by a turgor-dependent plastic deformation of wall polymers. Instead, above the turgor threshold, the rapid response to energy inhibitors suggests a control by metabolic reactions causing synthesis and/or extension of wall polymers.

It is well accepted that ψ_p[2] is involved in cell enlargement. The evidence is based mostly on varying the ψ_p with osmotica or soil water deficits and observing changes in growth rates. These treatments also alter the solute and/or pressure environment of the cell walls (2, 11, 40), which raises the possibility that side effects on wall metabolism could complicate the conclusions. Some studies avoided the complications by applying unidirectional tensions to cells or isolated cell walls (9, 10, 20, 25, 31, 35, 42), but the tensions may not have completely simulated ψ_p, which is multidimensional (31, 40).

After the elegant investigations of isolated cell walls by Preston and coworkers (33, 35), it was proposed that ψ_p causes an irreversible deformation of the wall to a larger size by a time-dependent plastic yielding resembling a steady creep (27, 28, 33). In some studies, creep appeared proportional to the ψ_p above a minimum termed the yield threshold (8, 35). Lockhart (27, 28) formalized these concepts in an equation of the form:

$$G = M(\psi_p - Y) \quad (1)$$

where G is the relative growth rate ($m^3 \cdot m^{-3} \cdot s^{-1}$ or s^{-1}), ψ_p is the turgor (MPa), Y is the yield threshold turgor (MPa), and M is the wall extensibility ($m^3 \cdot m^{-3} \cdot s^{-1} \cdot MPa^{-1}$ or $s^{-1} \cdot MPa^{-1}$).

Equation 1 has been extensively used (2, 40) but often without distinguishing growth from elastic changes. In a central study in *Nitella* (18), the ψ_p was changed with osmotica, but growth often did not change and elastic changes were not reported. In *Phycomyces*, increasing the ψ_p often caused only transitory changes in enlargement (32). Shackel et al. (37) found little relationship between ψ_p and growth in grape leaves. However, elastic responses to ψ_p were observed in mature *Nitella* cells (23). These differences between theory and observation indicate the importance of distinguishing between elastic and growth responses and call into question the exact role of ψ_p.

In the present work, we reexamined the role of ψ_p in cell enlargement by developing a method to change and maintain the ψ_p at any level without altering the external environment. With the method, ψ_p in large cells was changed by injecting solution previously collected from other cells growing in the same medium. Also, by monitoring the length of the cells, we could distinguish between elastic effects and growth.

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[2] Abbreviations: ψ_p, turgor (MPa); Y, yield threshold turgor (MPa); M, extensibility ($m^3 \cdot m^{-3} \cdot s^{-1} \cdot MPa^{-1}$ or $s^{-1} \cdot MPa^{-1}$); ψ_w, water potential of a cell (MPa); ψ_s, osmotic potential of a cell (MPa); ψ_o, water potential of nutrient solution (MPa); ψ_{pf}, final turgor; ψ_{pi}, initial turgor; ϵ, volumetric elastic modulus of a cell (MPa); V, volume of a cell before injection (m^3); V_A, volume of cell solution injected into a cell to change turgor; n_{sA}, mol of solute in volume V_A; V_B, volume of cell solution injected into a cell to maintain turgor; n_{sB}, mol of solute in volume V_B; V_T, total volume of injected solution (= $V_A + V_B$); L_P, hydraulic conductivity of cell membranes ($m \cdot s^{-1} \cdot MPa^{-1}$), $t_{1/2}$, half-time for turgor relaxation(s); A, surface area of cell (m^2); R, gas constant ($m^3 \cdot MPa \cdot mol^{-1} \cdot K^{-1}$); T, absolute temperature (K); FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

THEORY

The technique is termed a turgor clamp because it (a) changes and (b) maintains (clamps) the ψ_p of cells by adding or removing cell solution from the vacuole using a pressure probe (Fig. 1). Assume that the cell is essentially in equilibrium with the water potential of the nutrient solution (ψ_o):

$$\psi_s + \psi_p = \psi_w = \psi_o \quad (2)$$

where ψ_s is the osmotic potential and ψ_w is the water potential of the cell. The following theory is based on concepts given by Steudle (38).

Changing the ψ_p

Cell solution is injected into the vacuole to increase ψ_p by an amount $\Delta\psi_p = (\psi_{pf} - \psi_{pi})$, where the subscripts f and i are the final and initial ψ_p, respectively (A in Fig. 1). The $\Delta\psi_p$ adds a small volume V_A of water to the cell. In general, $\Delta\psi_p = \epsilon(V_A/V)$, where ϵ is the bulk (volumetric) elastic modulus and V is the initial volume of the cell. Accordingly:

$$V_A = \frac{\Delta\psi_p V}{\epsilon} \quad (3)$$

Because the injected solution V_A has the same ψ_s as the cell, ψ_s is unchanged. However, the injection adds n_{sA} mol of solute to the cell. In general, $V\psi_s = -RTn_s$ for dilute solutions, where R is the gas constant, T is the temperature in Kelvin, and n_s is the number of mol of solute. Accordingly, the amount of solute added is:

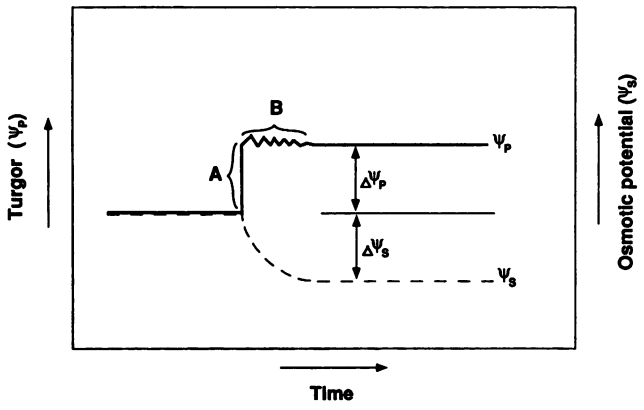

Figure 1. Schematic diagram of the turgor clamp. A, Changing the ψ_p. After the ψ_{pi} is obtained in a cell, an injection of cell solution of volume V_A will induce a sudden increase in ψ_p by $\Delta\psi_p$ but no change in ψ_s. Without any further manipulation, the ψ_p would relax toward the original level. B, Maintaining the ψ_p. To prevent the ψ_p from relaxing, repeated small injections are given. Because the cell is not in equilibrium with the surrounding water, water flows out of the cell. Eventually, the water loss concentrates the injected solution so that the ψ_s of the cell changes by an amount $\Delta\psi_s = -\Delta\psi_p$. This brings the cell back into equilibrium with the surrounding water. No further injection is required. The cell is at a permanent new ψ_p. A similar approach can be used to decrease the ψ_p except that cell solution is removed from the cell.

$$n_{sA} = \frac{-\psi_s V_A}{RT} \quad (4)$$

and the new cell volume is $(V + V_A)$.

Maintaining the ψ_p

Increasing the ψ_p in A in Figure 1 causes ψ_w to increase. Water flows out of the cell and causes ψ_p to decrease. More cell solution is injected whenever ψ_p decreases, which maintains the ψ_p (B in Fig. 1). Each small injection adds solute, and because all the injected water is lost (volume V_B), the cell solution becomes more concentrated. Eventually:

$$\Delta\psi_s = -\Delta\psi_p \quad (5)$$

and the cell returns to equilibrium with the external medium:

$$\psi_p + \Delta\psi_p + \psi_s + \Delta\psi_s = \psi_w = \psi_o \quad (6)$$

The volume of the cell has not changed in B and remains $(V + V_A)$. The amount of solute necessary to generate the $\Delta\psi_s$ of Equation 5 is thus:

$$n_{sB} = \frac{-\Delta\psi_s(V + V_A)}{RT} \quad (7)$$

Total Effect of Injections

From Equations 4 and 7, the total injected cell solute is $n_{sA} + n_{sB}$ and:

$$\psi_s V_A + \Delta\psi_s(V + V_A) = \psi_s V_T \quad (8)$$

where V_T is the total volume $(V_A + V_B)$ of cell solution injected into the cell. Substituting into Equation 8 the $-\Delta\psi_p$ from Equation 5 and the V_A from Equation 3, then rearranging, gives:

$$\frac{V\Delta\psi_p}{\psi_s\epsilon}(\psi_s - \Delta\psi_p - \epsilon) = V_T \quad (9)$$

This equation indicates that, for ϵ much larger than ψ_s or $\Delta\psi_p$, the total injected solution is determined mostly by $-V\Delta\psi_p/\psi_s$.

The volume of small injections to maintain ψ_p is $V_B = \Delta\psi_s(V + V_A)/\psi_s$ and is related to the volume V_A initially injected to change ψ_p (Eq. 3) by:

$$\frac{V_B}{V_A} = \frac{-(\epsilon + \Delta\psi_p)}{\psi_s} \quad (10)$$

Thus, for ϵ much larger than $\Delta\psi_p$, V_B/V_A is approximated by $-\epsilon/\psi_s$.

By this method, all the new solute remains in the cell, and only water flows out, thus making the concentration higher than before the injections. The cell returns to equilibrium with the external solution, and the ψ_p is permanently changed. To reduce ψ_p, a similar procedure is used, but solution is removed rather than injected, and water enters the cell rather than flowing out.

MATERIALS AND METHODS

Plant Materials

Chara corallina Klein ex Willd., em. R.D.W. (= *Chara australis* R. Br.) was cultivated in plastic containers (60 L) with 50 mm of soil at the bottom. The nutrient solution, changed monthly during the experiments, had a pH of 8.0 to 8.5 and was comprised of 1 mM NaCl, 0.1 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, and the micronutrients: 1.5 μM FeNa EDTA, 0.7 μM $ZnCl_2$, 0.4 μM $NaMoO_4$, 0.35 μM H_3BO_3, 10 nM $MnCl_2$, 8.4 nM $CoCl_2$, 2.3 nM $CuCl_2$. The temperature was 22 to 24°C, and continuous fluorescent light (PAR = 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was given at the top of the containers. The first or second internode cell from the apex of a branch of the thallus was used for determinations. The cells were 10 to 45 mm in length and 0.6 to 0.8 mm in diameter. The cells were taken from branches whose apical cells were no longer than 3 mm to ensure that the first and second internode were growing rapidly. For in situ determinations, the cells remained attached to whole plants. For single-cell determinations, the internode cells were removed by cutting on the other side of the node at each end. All measurements were conducted in the growth medium at 23°C and 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PAR.

In Situ Determinations

To relate the results to intact plants, elongation first was monitored in intact plants in the culture medium. Using an instrument similar to that used for soybean seedlings (31), we held the lower end of the internode rigidly with a fork made of Plexiglas and the upper end was hooked with a stainless steel wire (0.16 mm diameter) that was led to one end of an arm of a radial displacement transducer (Schaevitz, Pennsauken, NJ) situated above the culture medium. On the other end of the arm, a weight was hung from a thin plastic line that applied a force of 0.05 g to the cell. The length change of the cell was recorded by a strip chart recorder.

Single-Cell Determinations

In most of the experiments, isolated internode cells were used. The experimental cell was excised from the plant and mounted in a horizontal Plexiglas chamber after the branches were trimmed away at each node (Fig. 2). The basal node was held in a wedge-shaped slit by an adjustable wedge plug (Fig. 2). The apical node was attached by a hook to a stainless steel wire (0.16 mm diameter) that went out of the other end of the chamber through a thin slit and connected to the arm of a horizontally arranged growth transducer (the same type as above). The chamber was covered by a piece of Plexiglas, and the interface between the chamber and the cover was sealed with petrolatum (Vaseline). The culture solution at the growth temperature flowed constantly through the chamber. The solution could be changed rapidly (3 s) by switching from one reservoir to another. The transducer was attached to a micromanipulator that does not appear in Figure 2. The system was calibrated by attaching the transducer directly to the wedge plug and varying the position of the transducer with the micromanipulator. The calibration was linear when the arm of the transducer was within ±7.5° of the central position for the growth transducer.

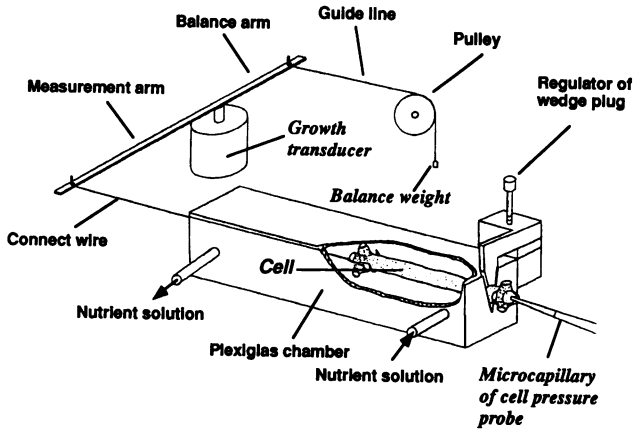

Figure 2. Apparatus for simultaneously determining elongation and ψ_p in a single internode cell of *C. corallina*. Elongation is detected with the growth transducer, and ψ_p is detected with the cell pressure probe. The ψ_p can be permanently changed (clamped) with the pressure probe, as described for Figure 1. For details, see "Theory" and "Materials and Methods."

The tip of the microcapillary of the cell pressure probe (39) was inserted into the cell at the stationary node by using a micromanipulator. The orifice of the tip of the microcapillary was opened and sharpened with a grinder (Narashige, Optical Apparatus, Ardmore, PA) to give a short, sharp tip with a diameter of 30 to 50 μm. The large tip prevented plugging and caused little wounding. During grinding, the microcapillary was held by a micromanipulator and kept at an angle that made a sharpened face at about 45° to the long axis. The sharpened microcapillary was dipped in silicone oil and exposed to hot air from a hair dryer to make a dry but hydrophobic outer surface. The shape and hydrophobic treatment reduced leakage when the tip was inserted into a cell (leakage detected by a decrease in the ψ_p).

A large cell pressure probe was used for the turgor clamp. We used 3.5 mm^3 of the inner regulatable reservoir for cell solution. The remaining internal volume was occupied by silicone oil. For measurements involving increased ψ_p, cell solution was first obtained from another similar cell. The tip was inserted in the test cell, and the ψ_p was measured by returning the oil/solution boundary to the position before insertion. The ψ_p was then raised as described before (see "Theory" and Fig. 1). For measurements involving decreased ψ_p, the ψ_p was measured and the tip was withdrawn slightly from the cell to allow a leak. It was resealed into the cell when the ψ_p decreased to the desired level. The ψ_p was kept at this level by removing small quantities of cell solution until there was no further change (see "Theory" and Fig. 1).

The inner diameter of the microcapillary was 0.56 mm, and the position of the meniscus could be observed to at least 0.1 mm at ×50 magnification with the stereomicroscope. This allowed a volume to be detected of less than ×0.0003 to ×0.001 of cell volume. Both the elongation and the ψ_p were continuously recorded with a two-pen chart recorder.

ϵ, L_p, and ψ_s

The ϵ and L_p of growing and mature internode cells were measured by the methods of Zimmermann and Steudle (43).

The cell volume and surface area were determined from the lengths and widths of the cells determined microscopically. After determining these parameters, we cut off one end of the cell, a drop of cell solution was quickly collected, and ψ_s was measured using a microliter isopiestic psychrometer (3). The osmotic potential of the culture medium also was measured with the same psychrometer.

RESULTS

Growth-Induced Water Potential, ϵ, and L_p

Table I shows that the ψ_p essentially equalled $-\psi_s$ in growing internode cells of *Chara*. Also, the ψ_w of the cells was almost the same as the ψ_o of the culture medium. This indicates that the growth-induced water potential ($\psi_o - \psi_w$) was negligible (2), the cells were in near-equilibrium with ψ_o, and the conditions of Equation 2 were satisfied. The growth-induced water potential was small because the L_p of the cells was large, 0.87 to 1.4 $\times 10^{-6}$ $m \cdot s^{-1} \cdot MPa^{-1}$, and the rate of water influx for growth was slow (about 10^{-10} $m \cdot s^{-1}$), which gives a calculated ($\psi_o - \psi_w$) on the order of only 0.0001 MPa. The ϵ was large and ranged between 21 and 83 MPa whether or not the cells were growing. The ψ_s was −0.60 to −0.62 MPa, the cell volume was 6 to 18 $\times 10^{-9}$ m^3, and the surface area was 3 to 12 $\times 10^{-5}$ m^2.

Excision Effects

Before excision, growth rates in the intact plants were measured in the culture medium and were steady for 20 h (Fig. 3A). Excising, transporting, and puncturing the internodes with the pressure probe shown in Figure 2 had little effect on their growth rate. Growth ordinarily was slightly faster for about 20 min and then recovered to a rate similar to that before excision (Fig. 3, B and C). Growth remained constant for at least 8 to 10 h (Fig. 3, B–D). The rate varied widely from 30 to about 200 μm/h, depending on the cell, but was not related to cell length. Puncturing the cell with the pressure probe sometimes reduced the growth rate (Fig. 3C) and sometimes did not (Fig. 3D), but the rate was always stable afterward. Because growth was similar to that of the intact plant and remained stable for a long time in the single-cell apparatus, all the following experiments were conducted in the single-cell apparatus (Fig. 2).

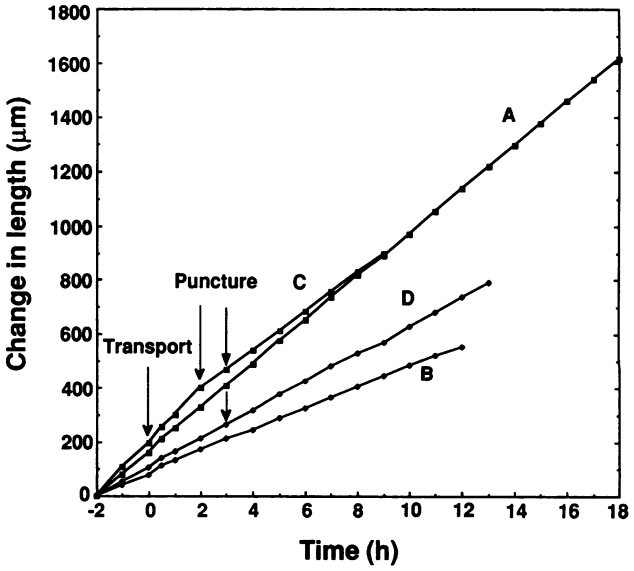

Figure 3. Growth of *Chara* internode cells in situ (A), after excision and transport to the single-cell apparatus described in Figure 2 (B–D), and after puncturing with the microcapillary of the pressure probe in the single-cell apparatus (C and D). The transport of the cells from in situ to the apparatus took 20 to 30 s. The original cell lengths were 32, 40, 28, and 21 mm, respectively, for cells A, B, C, and D. Transport and puncture took place at the arrows.

Turgor Clamp and Elongation

A stable ψ_p of 0.5 to 0.7 MPa was obtained when the tip of the microcapillary was inserted into an internodal cell. If cell solution was injected or removed, the ψ_p quickly returned to near its original level (half-time of 3–6 s, Table I, and Fig. 4, right). If the injection was followed with many small injections, ψ_p was maintained at the new level, and no further injections were required after about 20 min (Fig. 4, left) as predicted from "Theory." During the injections, ψ_p initially varied around the mean by ±0.005 MPa but varied less as injections proceeded.

After a ψ_p change, growth and the new ψ_p remained stable for hours. Following a ψ_p step up, there was an immediate increase in length followed by a steady elongation monitored for almost 5 h in the experiment of Figure 5. The rate of

Table I. *Potentials, Hydraulic Parameters, and Dimensions of Growing and Mature Internode Cells of Chara Typical of Those Used in the Experiments*

The $\psi_o - (\psi_p + \psi_s) = (\psi_o - \psi_w)$ = water potential difference across cell membranes that in growing cells is induced by growth, and n = number of cells. Growth was 35 to 180 μm/h in growing cells and 0 μm/h in mature cells. The ψ_p and $t_{1/2}$ were determined with the cell pressure probe. The ψ_s and ψ_o were measured with a microliter isopiestic psychrometer. Data for potentials are means ± 1 SD. Data for hydraulic parameters and dimensions are ranges.

	ψ_p	ψ_s	ψ_o	$\psi_o - (\psi_p + \psi_s)$	ϵ	L_p	$t_{1/2}$	V	A	n
	MPa	*MPa*	*MPa*	*MPa*	*MPa*	$m \cdot s^{-1} \cdot MPa^{-1}$	*s*	m^3	m^2	
Growing	0.58 ±0.06	−0.60 ±0.05	−0.01 ±0.00	0.01 ±0.01	21 to 80	0.87–1.4 × 10^{-6}	3.5–6.0	5.7–17.3 × 10^{-9}	2.8–11.3 × 10^{-5}	5
Mature	0.61 ±0.05	−0.62 ±0.05	−0.01 ±0.00	0.00 ±0.00	33 to 83	0.90–1.2 × 10^{-6}	3.0–6.0	14.3–17.6 × 10^{-9}	7.6–11.7 × 10^{-5}	4

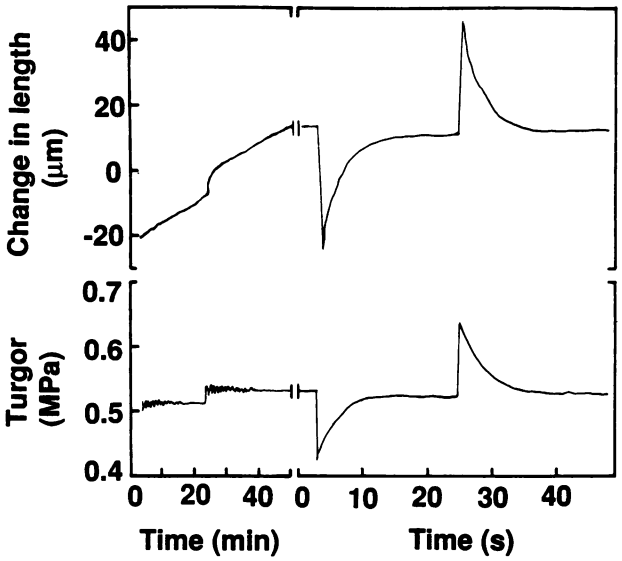

Figure 4. Turgor clamp and elongation (left) and ψ_p and length relaxation after single injection/uptake (right) in the same *Chara* internode cell in the apparatus in Figure 2. No turgor clamp was used in the experiment on the right. Note the change in the *x* axis at 50 min. Initially, cell length was 25 mm, growth rate was 35 μm/h, and ψ_p was 0.62 MPa.

elongation after the step up was the same as before the step up. After about 20 min of injections, the ψ_p continued at the new level without the necessity for further injections and thus had been "permanently" changed.

Because of the limited capacity of the pressure probe, the turgor clamp was operated in two ψ_p ranges: 0.2 to 0.5 and 0.4 to 0.8 MPa. In the lower range, there was a switch-like threshold ψ_p for growth, normally approximately 0.3 to 0.4 MPa (Fig. 6). Below this threshold, no growth was observed (Fig. 6, steps 2–4). ψ_p downshifts caused an initial elastic shrinkage, and equivalent upshifts caused an equivalent elastic stretching (Fig. 6, steps 2–4). Above the threshold, the same elastic behavior was observed, but it was combined with a steady elongation observable after 5 to 25 min (Fig. 6, steps 0, 1, 5–8). The elongation resumed at the same rate after the initial extension/shrinkage regardless of the ψ_p as long as ψ_p was above the threshold (Fig. 6, steps 0, 1, 5–8). Less time was required between the initial elastic response and the final growth response when ψ_p was slightly above the threshold than when it was far above. We were unable to detect any evidence of plastic deformation in this ψ_p range.

In the higher ψ_p range, growth also was unaltered by variations in ψ_p up to those slightly above the maximum obtainable by the cell (slightly above 0.56 MPa, Fig. 7, curve A, steps 1 and 3). The cells elongated rapidly when ψ_p was at its original level (Fig, 7, step 0) and resumed at the same rate following the elastic response to ψ_p steps up or down (Fig. 7, steps 0–2). When the ψ_p was stepped up to a level substantially higher than the maximum attainable by the cell (Fig. 7, step 4), the growth increased above the original rate as though plastic deformation was beginning. However, the growth often was not steady (data not shown), and growth did not occur when the ψ_p was returned to the prior level (Fig. 7, step 5, observed for 6 h). Despite repeated attempts, we never observed a growth recovery after exposure to ψ_p high enough to cause plastic deformation.

The duration of the initial extension/shrinkage could be determined by subtracting the steady growth rate (Fig. 7B, steps 0–3) from the observed changes in length (Fig. 7A). This subtraction indicates that the extension/shrinkage was complete in about 25 min at high ψ_p (Fig. 7C).

Figure 8A shows the relationship between growth and ψ_p in a single cell in which the entire range of ψ_p was possible to obtain because of an unusually small difference between the original and threshold turgors (0.11 MPa). Cell growth remained independent of ψ_p over the entire range. Figure 8B shows similar behavior in four different cells, one of which covered almost the whole ψ_p range. In every case, growth began abruptly at the ψ_p threshold but was unaffected over the range of ψ_p encountered by the cells. When ψ_p was substantially (0.08 MPa) above the original, however, a ψ_p dependency appeared (Fig. 8B), but growth was often transitory and did not resume when ψ_p was lowered (as in Fig. 7, steps 4 and 5).

We explored more fully the significance of the transition between the initial elastic stretching and the subsequent steady growth by investigating nongrowing cells. Nongrowing cells were obtained by exposing growing cells to various inhibitors. Figure 9 shows that inhibitors of photosynthesis (DCMU) and energy-dependent phosphorylation (FCCP), when added together (5 μM + 5 μM) in the light, inhibited growth immediately. After 20 min, growth became zero. ψ_p was not altered for about 2 h. After 2 h, ψ_p decreased, and the cells shrank presumably because of losses in membrane selectivity.

When growth was inhibited but the membrane had not yet lost selectivity, ψ_p jumps resulted in an initial rapid deformation followed by a slow approach to a stable length

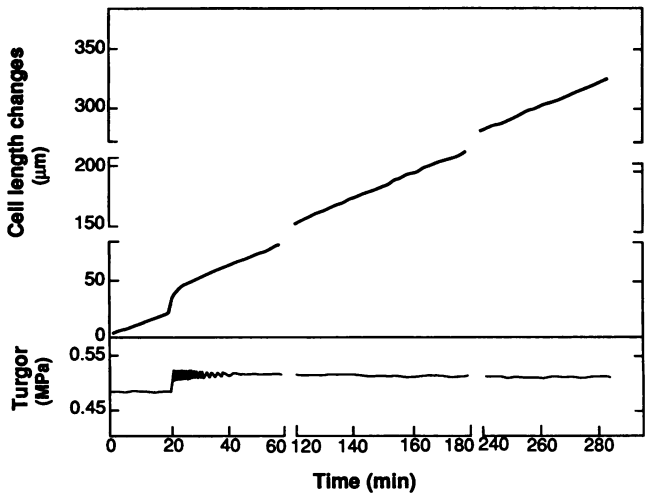

Figure 5. Elongation of *Chara* internode cells at various times after a step up in ψ_p with the turgor clamp in the apparatus shown in Figure 2. Note breaks in *x* and *y* axes. The cell initially was 34 mm long.

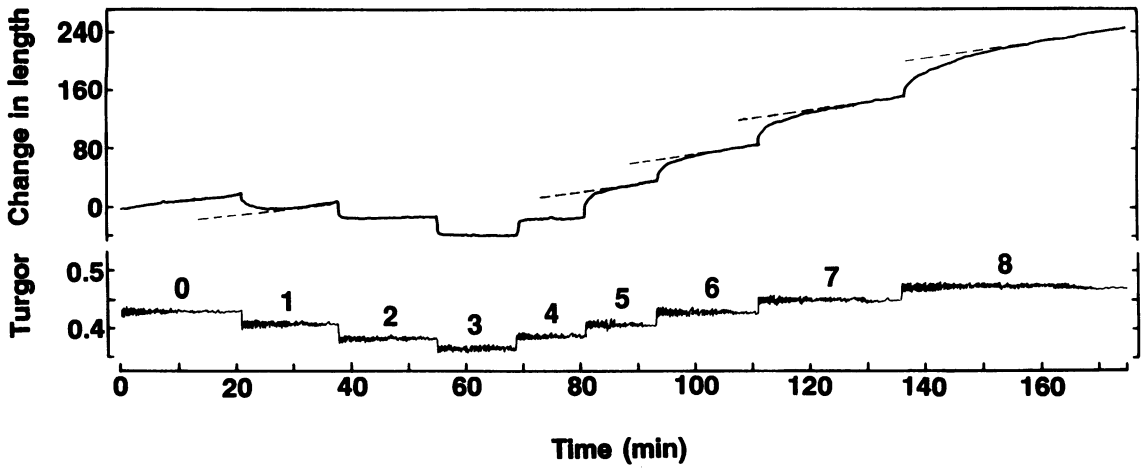

Figure 6. Elongation of *Chara* internode cells in response to various ψ_p step changes with the turgor clamp in the low ψ_p range. Before time zero, several steps down were used to decrease the ψ_p to about 0.43 MPa with the turgor clamp. Dashed lines show the resumption of steady growth after each subsequent ψ_p step. Initially, the cell length was 42 mm, growth rate was 64 μm/h, and ψ_p was 0.5 MPa.

resembling the kinetics seen in growing cells below the ψ_p threshold (Fig. 10A). Similar responses were seen in mature cells (Fig. 10B) and in growth-inhibited cells whose ψ_p was varied with an osmoticum (Fig. 10C).

The respiratory inhibitor dinitrophenol (5–10 μM) in the dark generally caused effects similar to DCMU and FCCP in the light. Other inhibitors such as monensin (5–10 μM) caused immediate irreversible ψ_p loss and were not used further.

ψ_p Changes Induced by Osmotica and Turgor Clamp

Because previous investigators often used osmotica to vary the ψ_p in growing cells (18), we compared the effects of

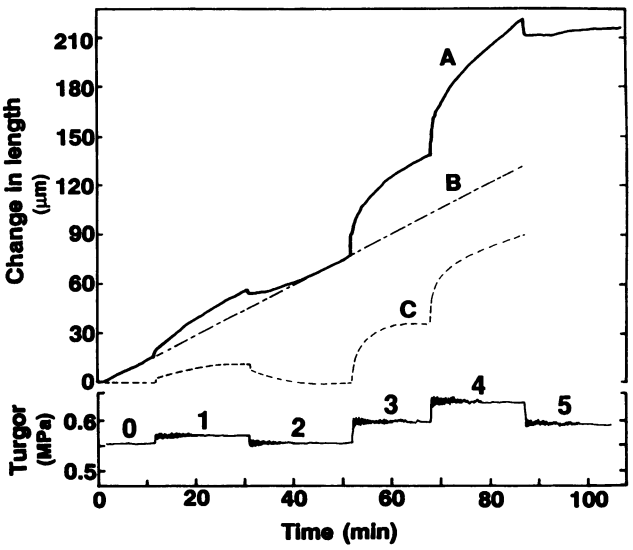

Figure 7. Elongation of *Chara* internode cells in response to ψ_p step changes with the turgor clamp in the high ψ_p range. Measurements were made around and above the normal ψ_p of the cell. A shows changes in cell length, B shows the slope of the length change in A (from ψ_p steps 0–3), and C is A − B. Initially, the cell length was 29 mm, growth was 92 μm/h, and ψ_p was 0.56 MPa.

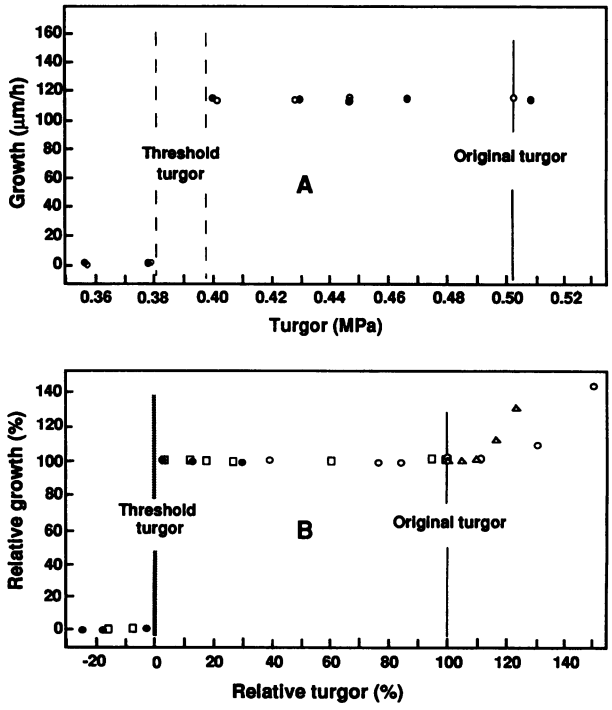

Figure 8. Growth at various ψ_p in internode cells of *Chara*. A, Data from a single cell, in which O was measured during ψ_p steps down and ● was measured during ψ_p steps up in the same cell. The x axis shows the actual ψ_p, and the y axis shows the absolute growth rate. B, Data from four different cells (O, □, ●, Δ). The x axis shows the relative ψ_p with the threshold as 0% and the original ψ_p (before turgor clamping) as 100% for each cell. The y axis is the relative growth rate (as percentage of the initial elongation before the turgor clamp was used).

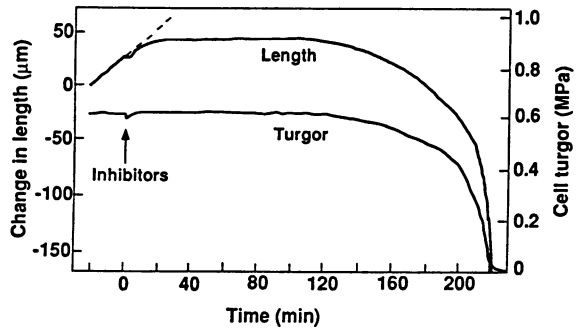

Figure 9. ψ_p and elongation in *Chara* internode cell at various times after inhibitors DCMU and FCCP (5 μM + 5 μM) were added to the external medium in the light. Dashed line shows elongation expected if inhibitors had no effect on rate.

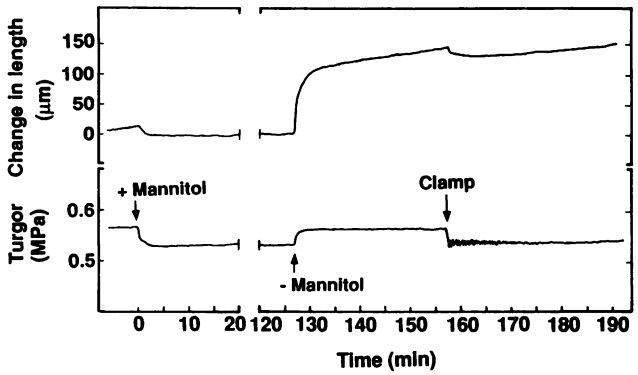

Figure 11. Comparison of turgor clamp and osmoticum (mannitol) with the same *Chara* internode cell. Cell was first exposed to −0.05 MPa mannitol for about 2 h (note break in x axis) and then returned to original culture medium after which the ψ_p was decreased 0.05 MPa with the turgor clamp (cell length was 44 mm).

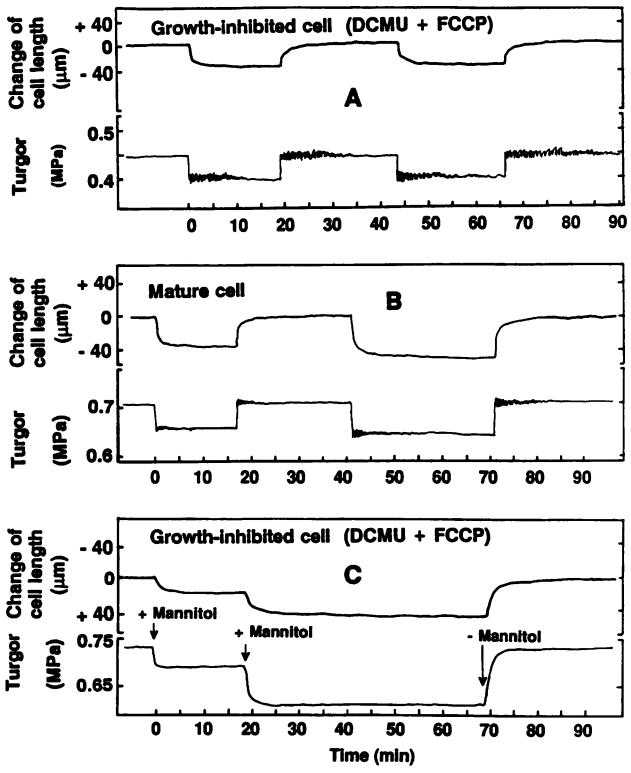

Figure 10. Length and ψ_p in nongrowing internode cells of *Chara*. A, Growing cell after inhibiting growth with 5 μM DCMU + 5 μM FCCP in the light and changing ψ_p 0.05 MPa with the turgor clamp (growth before inhibition was 92 μm/h, length was 28 mm). B, Mature cell having ψ_p changed 0.05 MPa with the turgor clamp (growth before clamping was 0 μm/h, length was 45 mm). C, Growing cell after inhibiting growth with 5 μM DCMU + 5 μM FCCP in the light and changing ψ_p with mannitol (growth before inhibition was 68 μm/h, length was 41 mm). This cell was first exposed to −0.03 MPa mannitol (+mannitol), then to −0.06 MPa mannitol (+mannitol), and finally was returned to the original medium (−mannitol).

osmotica and turgor clamping. Figure 11 shows that the methods gave different results. Growth resumed partially or not at all when ψ_p was stepped down in a growing cell with mannitol (Fig. 11, note break in *x* axis). On the other hand, the same ψ_p step down with the turgor clamp in the same cell was not inhibitory, and growth resumed at a rate similar to that before clamping (Fig. 11). Sucrose showed comparable effects (data not shown). With KCl, the result was intermediate between mannitol and the turgor clamp. Growth was inhibited but eventually resumed after longer times than with the turgor clamp (Fig. 12). With both mannitol and KCl, removal of the osmoticum caused a large burst in enlargement not seen with the turgor clamp (Figs. 11 and 12, see also Figs. 6 and 7). Green et al. (18) reported the same kind of inhibition with exposure to osmoticum and a similar burst of elongation with removal of osmoticum.

DISCUSSION

The turgor clamp showed that small ψ_p changes could start or stop growth with almost a switch-like action. The start/stop signal could operate repeatedly. The ψ_p range for the signal was narrow, about 0.01 MPa, and invariant. Thus, ψ_p needed to be above a threshold that was essential for the growth process. We will call this ψ_p, the turgor threshold for growth.

In mature cells, this response was absent. There was only an initial immediate deformation (elastic) followed by a gradual deformation that required 5 to 25 min for completion (viscoelastic). When the ψ_p was returned to the previous level, the cell dimensions returned to their previous level. A similar response was observed if growing cells were exposed to inhibitors that prevented growth or if growing cells were subjected to ψ_p below the threshold. The elastic effects were in every way like those of the mature cells. Thus, regardless of whether or not the cells could grow, their walls exhibited reversible elastic behavior typical of many solid materials (12).

Elastic/viscoelastic behavior is generally attributable to a reversible straightening of individual polymer molecules un-

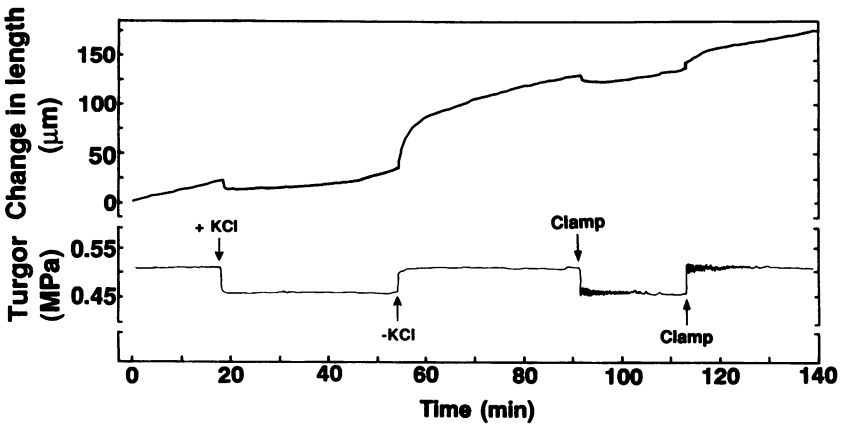

Figure 12. Comparison of turgor clamp and osmoticum (KCl) with the same *Chara* internode cell. Cell was first exposed to −0.05 MPa KCl and then returned to original culture medium after which the ψ_p was decreased 0.05 MPa with the turgor clamp (cell length was 23 mm).

der tension that is instantaneous in an ideal polymer but may require time in a polymer having complex coiling or intermolecular bonding. The longer times to complete the effects at high ψ_p than at low ψ_p is consistent with the straightening of more complex coiling as the tension increases. The reversibility of the effects is diagnostic for elastic/viscoelastic phenomena and was clearly evident here.

Above the threshold, elasticity was apparent, but a steady growth was superimposed on it and could be observed after the elastic responses were completed. Regardless of the direction of the ψ_p change, steady growth resumed at the rate before the pressure jump. Because the elastic/viscoelastic changes were rapidly reversible but growth was not, growth could be clearly distinguished from elastic behavior.

With osmotica, the two effects were more difficult to separate. Osmotica caused an elastic shrinkage followed by a large spurt in cell length when the solutes were removed. Previous workers also observed this effect (33), and some considered it to be caused in part by growth occurring but unexpressed when solute was present (33). On the other hand, Green et al. (18) and Ortega et al. (32) suggested that there was an immediate yield point just below the ψ_p of the cell (about 0.03 MPa) that changed in a way that could compensate for some of the ψ_p changes and in this way cause a growth spurt when the solutes were removed. They observed no elastic shrinkage when ψ_p decreased (18). Because the elastic and viscoelastic properties of cell walls are well known (6, 11, 23, 33, 41) and were confirmed by the turgor clamp, it seems impossible that there is no shrinking when a cell experiences a ψ_p step down unless growth occurs at the same time and cancels the shrinkage. The net effect would be a temporary apparent cessation of growth similar to that suggested by Cleland (11), but, because growth would be occurring but unseen, the cells would not be at an immediate yield point. The hidden growth would be apparent when ψ_p recovered, as we observed with the turgor clamp. Therefore, the early cessation of growth after a ψ_p step down was not consistent with a changing yield threshold. In addition, no growth burst was observed with the turgor clamp after a ψ_p step up. Therefore, the subsequent resumption of growth also was not consistent with a changing yield threshold. These results make it unlikely that the yield threshold varied.

Osmotica inhibited growth, which was unmistakable when close comparisons were made in the same cell with the same ψ_p change caused either by osmoticum or the turgor clamp. The only differences between the two experiments were the presence or absence of external solute and the direction of change in the internal osmotic potential. As ψ_p decreased with exposure to an osmoticum, the cell contents became more concentrated. As ψ_p decreased with the turgor clamp, the cell contents became more dilute. Because the ψ_p was identical in the two cases, the only possible effect of these changes was on metabolism. However, the turgor clamp gave the same growth rates during ψ_p steps down (dilution) and steps up (concentration), which indicates that any metabolic effects were too small to affect the measurements. Thus, external osmotica appeared to cause direct interactions with the wall or plasmalemma that were absent when ψ_p was changed with the turgor clamp. This concept is strengthened by the solute-specific nature of the inhibition. Sucrose and mannitol were more inhibitory than KCl. Sugars and their analogs can change wall properties and inhibit wall enzymes (22, 29). These possible side effects indicate that external osmotica should be avoided when studying ψ_p effects on cell growth.

Since the model of ψ_p-driven growth was set up by Lockhart (27, 28), it has been thought that the growth of plant cells is mainly a plastic or viscoplastic deformation of walls caused by ψ_p. It is well known for polymers that plastic effects require time and are irreversible, often exhibiting a rapid phase followed by a slow steady "creep" or viscoplastic phase. Finally, strain hardening occurs that decreases creep.

Viscoplastic behavior is thought to result from a sliding of polymers past each other so that an overall lengthening of the material occurs. Because of the sliding, release of the external force does not reverse the lengthening. This nonreversibility can be diagnostic for plastic effects in a nonliving system. The rapid phase is probably caused by sliding of weak bonds and the creep phase by stronger bonds. Strain hardening indicates that slide-prone bonds have been exhausted.

There are two consequences of this mechanism. First, the rate of deformation must change as the ψ_p changes. Thus, it is noteworthy that growth did not change between the ψ_p threshold and the maximum ψ_p attainable at the ψ_s. Second, in the absence of metabolism, steady plastic deformation

(creep) should continue for a time but should eventually decrease as the polymer undergoes strain hardening. However, we were unable to detect unaltered growth rates when metabolism was inhibited. The immediate decrease in growth despite a constant ψ_p indicates that growth was more closely determined by energy metabolism than by detectable effects of creep. It follows that between the ψ_p threshold and the maximum cell ψ_p, plastic deformation played little if any role in growth.

Others also observed growth responses that are consistent with this conclusion. Shackel et al. (37) reported that leaves grew at a constant rate under environmental conditions that caused ψ_p to change in the epidermal cells. Green et al. (18) noted a considerable ψ_p range over which growth occurred at a constant rate. In both studies ψ_p was measured directly and thus especially compelling measurements were provided, although the osmotica used in one of them (18) may have caused side effects.

The constancy of growth at variable ψ_p and the dependence on metabolism imply that there could be a metabolically controlled differential sliding of wall microfibrils that only occurred under tension and required metabolism. There is evidence that wall enzymes are involved in growth (16, 17, 21, 24, 29). These activities probably create a demand for metabolic energy to supply new wall materials (polysaccharides and proteins) and also for the assembly of the materials into walls (4, 5, 7, 11, 14, 15, 24, 33, 36, 40). This energy could be essential for the metabolic reactions actually causing growth (17, 19, 34).

It is interesting that, when ψ_p was forced above the maximum attainable by the cell, there was an increase in elongation rate with increased ψ_p that resembled the behavior of a polymer undergoing plastic deformation. A similar response was noted by Ortega et al. (32). Thus, it was possible to observe wall behavior that conformed to the concept of ψ_p-driven plastic elongation with the turgor clamp. This gives confidence that we would have detected it if it had been present at the lower ψ_p in the normal range. Its absence except at abnormally high ψ_p indicates that experiments involving deformation of nonliving tissue (9, 10, 25, 42) or isolated cell walls (19, 35) may need to be reinterpreted because the observed plastic behavior may have been outside the possible ψ_p range for the cells. This suggests that experiments with one-dimensional force applied to ψ_p-containing cells and tissues (13, 31) also may display plastic behavior because they apply forces above the normal cell ψ_p.

At these abnormally high turgors, the plastic deformation inhibited growth after the initial deformation. This suggests that there could have been damage to a structural feature necessary for growth. Exceeding the normal stretch of cells may damage the fine structure of the membrane and/or microtubules likely to be involved in the biosynthesis of the walls (26). This wound effect did not disturb the ψ_p and thus was not a simple loss of membrane selectivity.

With the turgor clamp, we were able to explore the entire range of turgors from those too low to support growth to those so high that wounding occurred. There was some disturbance of growth when the cells were excised, transported, and punctured, but recovery was rapid, and growth resumed at rates similar to those of intact cells for long times (as long as 25 h). A similar response was observed in another laboratory (32). The possibility that the probe directly altered the metabolic activity of the cell seems remote in view of this growth response, but changing the ψ_p could have caused metabolic effects that would normally take place.

C. corallina was an ideal species for this work because it shows no activity for ψ_p regulation, and osmoregulation is slow (1). The turgor clamp permanently changed the ψ_p of the cells, which remained stable for many hours after injections ceased. A stable ψ_p was not achievable with only one injection and normally required several hundred repeated but diminishing injections to bring the ψ_s into balance with the new ψ_p (see "Theory"). Assuming the cells had a Ψ_s of -0.6 MPa and ϵ of 50 MPa (Table I), increasing the ψ_p by 0.1 MPa required the injection of a cell solution having a volume 0.2% of the cell volume (Eq. 3), which is negligibly small. To maintain the new ψ_p, an additional cell solution having a volume of 16.7% of cell volume needed to be injected (Eq. 10). Water moved out of the cell during these maintenance injections, but thereafter, no further water movement was caused by the turgor clamp, and the cell returned to its usual equilibrium state. In this equilibrium state with the external medium, a concentration difference remained between the cell solution in the probe and in the clamped cell, but no effects on cell behavior were detected. The injections served to indicate that the pressure probe was in hydraulic contact with the cell interior, and indeed, a stable pressure not requiring injections indicated that the microcapillary tip was blocked. This problem plagued the efforts of Ortega et al. (32) but was avoided by using a large orifice tip in the present work.

A central question concerning cell enlargement has been the role of water uptake, ψ_p, and metabolism. In higher plants, the hydraulic conductance of the path for water movement is complex and requires a significant water potential difference between the water in the xylem and the enlarging cells to supply water to the cells (2, 30, 31). In *Chara*, the water supply was not a limiting factor for the internodal cells, which were surrounded directly by the water source. The hydraulic conductivity of the cells was so high that water potential differences were negligible. ψ_p, although necessary for growth, did not drive it according to the rules of physical deformation. The role of ψ_p was restricted to placing the wall under a strain large enough to allow growth to occur, but the rate appeared to be controlled by factors other than ψ_p. The present results suggest that wall metabolism may supply these factors and, in the absence of a significant frictional resistance to water movement, may be the major determinant of the rate of growth.

NOTE ADDED IN PROOF

During a recent visit to our laboratory, Professor Mary A. Bisson observed protoplasmic streaming in *Chara corallina* internodes before, during, and after inserting the microcapillary. She also found that streaming continued during pressure steps with the turgor clamp. These observations further indicate that the manipulations with the turgor clamp caused minimal wounding.

ACKNOWLEDGMENTS

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