Proc. Natl. Acad. Sci. USA
Vol. 74, No. 4, pp. 1565–1569, April 1977
Botany

Cell wall extension in *Nitella* as influenced by acids and ions

(cell elongation/acid growth/extensibility)

JEAN-PIERRE MÉTRAUX AND LINCOLN TAIZ

Division of Natural Sciences, Thimann Laboratories, University of California, Santa Cruz, California 95064

Communicated by Kenneth V. Thimann, January 19, 1977

ABSTRACT **The giant internode cells of *Nitella axillaris* exhibit acid-induced growth similar to that found in higher plants. The threshold pH is 4.5, with a maximum at 3.5. The acid growth effect is transient, lasting no more than 32 min. Extensibility measurements of isolated cell walls showed a similar pattern of acid enhancement. Prolonged boiling in water (12 hr) only partially inhibited the acid-induced wall extensibility and actually increased the extensibility at pH 6. It was concluded that physical, rather than enzymatic, processes were responsible for acid-enhanced continuous extension ("creep") in *Nitella* walls. A complex cation-sensitive mechanism that affects extensibility was also characterized. Among the stimulatory (wall-softening) cations, divalents were generally more effective than monovalents, with magnesium being the most stimulatory. The inhibitory (wall-hardening) cations included divalents and trivalents, aluminum being the most inhibitory. Ionic effects on extensibility were even less sensitive to prolonged boiling in water than acid effects.**

According to the acid growth hypothesis, cell wall loosening is at least partially regulated by the pH of the cell wall solution (1–3). In higher plants, cell elongation induced by auxin or fusicoccin is preceded by a rapid acidification of the cell wall free space (4, 5). The amount of acidification is sufficient to induce cell wall loosening and can account for the observed growth, by analogy to the growth promotion obtained with acidic buffers alone (6).

The mechanism of acid-induced wall loosening has been extensively studied, but is still unresolved (7, 8). One explanation that has been proposed involves cell wall hydrolases or transglycosylases which become active when the wall is acidified (1, 9–11). Another explanation is based on acid-labile bonds in the wall, either covalent or noncovalent, that are nonenzymatically cleaved in an acid environment (3, 7, 8). Since the acid effect can be observed in frozen-thawed coleoptile sections or tissue strips subjected to an externally applied tension, one method that has been used to distinguish between the above two alternatives has been to compare the mechanical extensibility of the tissue before and after treatment with Pronase, detergent, or boiling methanol (3, 11). These experiments have yielded conflicting results (3, 11). However, because of the multicellular nature of the material being extended, it is not possible to distinguish between the mechanical properties of the primary wall and those of the middle lamella, which normally is also under compression.

The giant internodal cells of the Characean alga, *Nitella*, have been shown by Green (12–17) and Preston and coworkers (18–21) to be useful objects for studies of the growth and mechanical properties of cell walls. Their filamentous structure eliminates complications caused by tissue interactions, their lack of a cuticle insures uniform penetration of test solutions, and their size (up to 1 mm in diameter and 7 cm in length) makes it possible to measure the extension of cell walls uninterrupted by a middle lamella. We have therefore investigated the acid growth response in living cells and isolated cell walls of *N. axillaris*. The extensibility of walls boiled in methanol or in water was compared with that of freshly isolated walls. In addition, the ability of various cations to enhance or inhibit wall extensibility was determined.

Abbreviations: LOLA, laser optical lever auxanometer; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

MATERIALS AND METHODS

Cultures of *N. axillaris* Braun were grown under fluorescent lights (Norelco cool white) with an incident light intensity of 35 microeinsteins/m^2·sec at the culture surface in a medium of 2% soil mixture in glass-distilled water. The soil mixture consisted of one-third commercial potting soil and two-thirds of a blend containing loam, leaf mold, sand, steer manure, and bone meal in the ratios: 16:5:6:4:0.05 (vol/vol) (B. Palevitz, personal communication). The soil suspension was autoclaved for 1 hr, and the medium, which had a pH of 7.0, was changed monthly. Internode cells about 2 cm in length were selected from cultures that were visually free of epiphytes. All cells were rinsed thoroughly in distilled water prior to use.

Recent methods for amplifying the elongation of plant materials so as to make short-term measurements possible have utilized long columns of stem or coleoptile segments, or displacement transducers whose outputs are amplified and plotted on chart recorders (22). We have found an optical lever, somewhat similar to that described by Bose (23), both simpler and potentially more sensitive when used in conjunction with a laser. Growth and extensibility of *Nitella* walls were therefore monitored with a laser optical lever auxanometer (LOLA), which was capable of resolving increments as small as 0.1 μm. The optical lever arm consists of a small glass tube attached perpendicularly to a razor blade in such a way that the lever balances on the razor's edge (Fig. 1*A*). A front surface mirror is cemented to one side of the razor blade, and the edge of the blade is positioned in a groove on an horizontal axis beam. This arrangement allows friction-free rotation in the vertical plane, while restricting lateral shifting. Internodal cells from intact filaments were anchored to the base of a perfusion chamber and to the optical lever arm with two Teflon-coated silver wires [0.005 inch (125 μm) diameter]. The wire itself had no deleterious effects on the growth of the alga. To secure the cell without causing damage the wires were looped and knotted on the proximal sides of the upper and lower nodal branch cells, which prevented the loops from slipping. A counterweight of 50 mg introduced a slight tension in the system without stretching the cell. During measurements the cells were illuminated by dim light (6–9 microeinsteins/m^2·sec) from an incandescent lamp.

Isolated cell walls, obtained by delicately scraping out the cytoplasm of an excised internode with a hair loop, were clamped onto two hooked glass rods by means of polyethylene rings (Fig. 1*B*). This method of attachment is similar to the method of Kamiya *et al.* (24) and avoids the use of glue, which requires that the material be air-dried and possibly altered. The apparatus for applying a constant load to isolated cell walls is

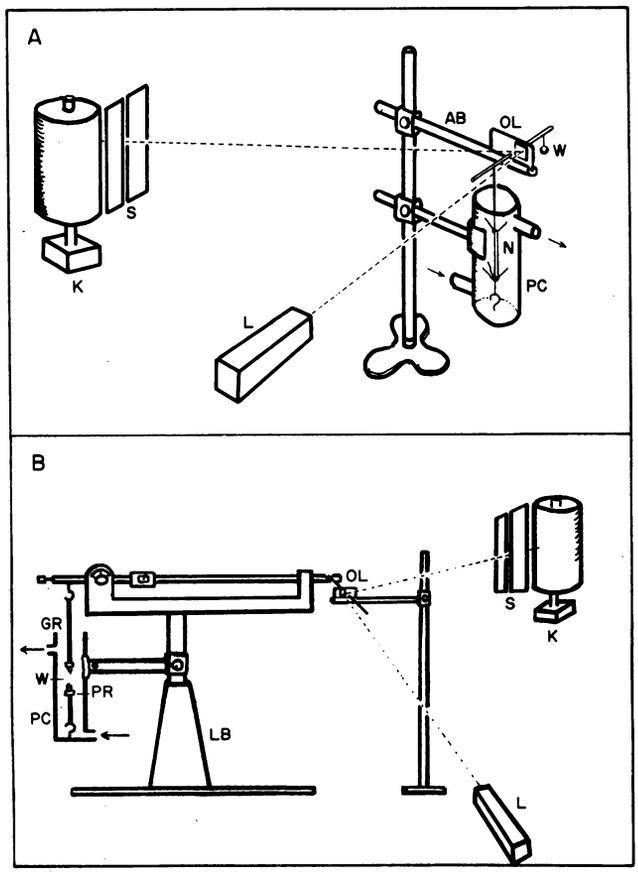

FIG. 1. (*A*) LOLA system for measuring cell growth. L, laser; K, kymograph; S, slit; PC, perfusion chamber; N, *Nitella* cell; OL, optical lever; AB, horizontal axis beam; W, counterweight. (*B*) LOLA system for measuring cell wall extensibility. LB, laboratory balance; PC, perfusion chamber; W, cell wall cylinder; PR, polyethylene ring; GR, glass rod; OL, miniature optical lever; L, laser; K, kymograph; S, slit.

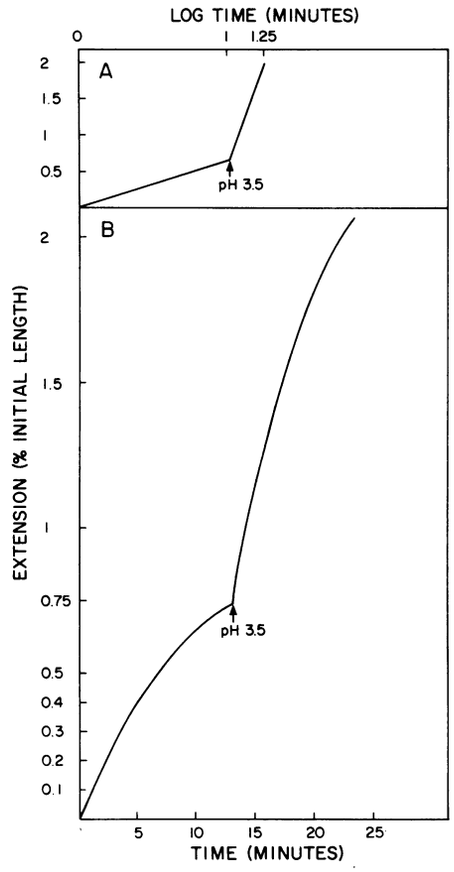

FIG. 2. (*A*) Replot of LOLA trace versus logarithm of time to obtain a linear curve. Creep rates were calculated from the slopes. (*B*) LOLA trace of the creep of an isolated *Nitella* wall. Arrow indicates time of pH transition from pH 6 to pH 3.5.

shown in Fig. 1*B*. The perfusion chamber is clamped in place at the pan end of a single pan balance and the optical lever arm rests on the beam at the other end. The cell wall preparation is hooked to the bottom of the perfusion chamber and to the balance. By adjusting the weights a specific load can be applied to each wall. The applied load was calculated from the turgor pressure and the cell size. Since the internode is cylindrical, the longitudinal force on the wall *in vivo* is approximated from the equation $F = P\pi r^2$, where P is the turgor pressure and r is the cell radius. Turgor pressure was taken as 5 bars (50 MPa) (15) and the radius was measured by means of a microscope equipped with an ocular micrometer.

To record growth a beam of light was directed at the mirror from a laser (0.5 mW, Metrologic) and the reflected beam was passed through a 2-mm slit onto a kymograph drum approximately 10 meters away. The use of a coherent light source rather than ordinary light made it possible to project the beam over distances without any appreciable spreading of the spot. The position of the light spot was recorded on Kodak polycontrast photographic paper wrapped around the drum. The system was calibrated by moving the lever arm through a known distance and measuring the deflection of the spot.

Since the growth of *Nitella* cells is linear with time, the growth rate was calculated from the slope of the LOLA trace. During the experiment the cell was perfused with growth medium containing 1 mM citrate/phosphate or sodium succinate buffer. After a 30-min equilibrium period in the perfusion chamber, cell elongation was recorded at pH 6 for 15 min. The solution was then changed to a lower pH, and the growth rate in the new medium was recorded. Each cell was subjected to only one pH transition. Control experiments demonstrated that changing the solution, which required 15 sec, did not affect growth rate. The response to pH was expressed as the percent increase over the rate at pH 6. The rapidity of the response (within 20 sec) ruled out any indirect effect of acid on surface bacteria.

The extension or "creep" of isolated *Nitella* walls is viscoelastic and exhibits linearity with logarithm of time (10). This means that when extension is plotted against time the slope of the curve decreases continuously. But when extension is plotted against the logarithm of time the curve is linear, and this is the case for the first two decades of logarithm of time (7). The response to acidification was therefore expressed as the percent change in the slope, compared with the slope at pH 6, of a replot of extension against logarithm of time of the LOLA trace. An example of the procedure is shown in Fig. 2. The extension (ΔL) was arbitrarily adjusted to zero at 1 min. The perfusion medium was either the same as that used in the growth experiments or 1 mM Tris/2-(*N*-morpholino)ethanesulfonic acid (Mes)/succinic acid buffer prepared in glass-distilled water. Each wall was subjected to only one pH transition. When the effect of boiling on the absolute strain rate was measured, the following procedure was used. A single internode cell was isolated and cut in half transversely with a razor blade. One of the halves was placed in the constant load system, and the strain rate (d/dt) ($\Delta L/L_0$), was determined. The strain rate of the second half was

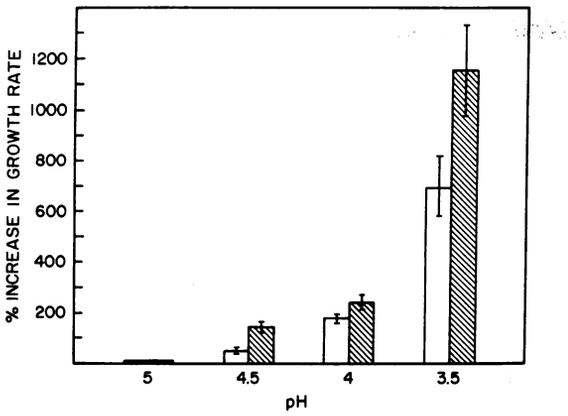

FIG. 3. Effect of pH on the elongation rate of *Nitella* internode cells. Open bars represent succinate buffer, shaded bars represent phosphate/citrate buffer.

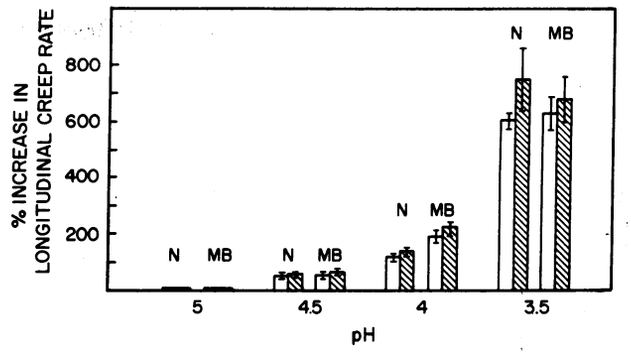

FIG. 4. Effect of pH on the longitudinal creep rates of *Nitella* cell walls boiled in methanol. N, not boiled; MB, boiled in methanol for 15 min. Open bars represent succinate buffer, shaded bars represent phosphate/citrate buffer.

measured after boiling (see *text* for conditions), and the effect of boiling was expressed as the percent increase over the unboiled half.

All experiments were repeated at least five times. The vertical bars on all histograms are the standard errors of the mean.

RESULTS AND DISCUSSION

Acid Growth Response of Living Cells. Preliminary experiments established that the growth rate of single *Nitella* internode cells was constant over the range of pH 8 to 5. Significant stimulation was observed, however, as the pH was lowered below 5. Fig. 3 shows the percent increase in growth rate relative to the rate at pH 6. Acid-induced growth begins near pH 4.5 and peaks at pH 3.5. The duration of the response was 32 ± 2 min at pH 4.5 and 4.0, and 6 ± 2 min at pH 3.5. Below pH 3.5 growth could not be measured due to rapid losses in turgor pressure. It can be seen from Fig. 3 that citrate/phosphate buffer was more stimulatory than succinate buffer at the same pH. Citrate has also been shown to promote growth in corn root segments (25). At the end of the growth response at pH 4.5 or 4.0, the growth rate usually returned to the pH 6 rate. However, the response at pH 3.5 was always followed by a loss of turgor and cell shrinkage.

As in higher plants, the acid growth response in single *Nitella* internode cells is transient. The shorter response time for *Nitella*, compared to 1–2 hr for coleoptiles (3), may reflect a more efficient penetration of the buffer rather than a qualitative difference in the growth mechanism. The peak of the acid growth response in *Nitella* is also substantially lower than the pH 5.5 peak reported for peeled coleoptile sections (6). However, it is identical to the peak found in corn root sections by Edwards and Scott (26).

Acid Enhancement of Longitudinal Creep. When isolated plant cell walls are placed under a constant tensile stress, the wall undergoes a rapid stretching followed by a prolonged extension. The continuing extension under constant load is termed "creep," and the longitudinal creep rate of *Nitella* cell walls has been shown to be proportional to the growth rate of the living cell (19). Thus, creep is considered to reflect processes occurring during growth.

Isolated *Nitella* cell wall cylinders exhibited typical viscoelastic extension when placed under a constant load (Fig. 2*B*). By replotting the LOLA trace as a function of logarithm of time, a linear relationship was obtained from which the creep rate could be determined (Fig. 2*A*). The effect of pH on the creep of freshly isolated walls and walls boiled 15 min in methanol is shown in Fig. 4. The values were calculated from the logarithmic time plot of the creep rates as shown by Fig. 2*A*. The effect of pH on the creep rate is similar to its effect on the growth rate. Boiling in methanol did not inhibit the response. This result is inconsistent with an enzyme-mediated mechanism for creep, and suggests a direct effect of acid on wall structure. Citrate/phosphate buffer did not significantly promote creep to a greater extent than succinate buffer at the same pH, except possibly at pH 3.5. This suggests that there is a metabolic component in the enhancement of growth by citrate/phosphate.

Since boiling in methanol may not be sufficiently drastic to rule out the involvement of cell wall enzymes, isolated walls were boiled in water for 15 min and for 12 hr, and their subsequent response to pH was determined. The results are presented in Fig. 5. The absolute values for acid stimulation in Fig. 5 are not directly comparable to those in Fig. 4 because a nonionic buffer was used in Fig. 5. The acid enhancement of creep was slightly greater in the nonionic medium than in the medium containing soil extract. Boiling in water for 15 min had no effect on the response at pH 4.5 and 4.0, and reduced the response by 50% at pH 3.5. If the inhibition at pH 3.5 were due to the inactivation of an enzyme, it would be necessary to postulate separate mechanisms for acid-induced creep at pH 4.0 and 3.5. Boiling in water for 12 hr significantly reduced the acid effect (50–75%), but failed to eliminate it. Although it is highly

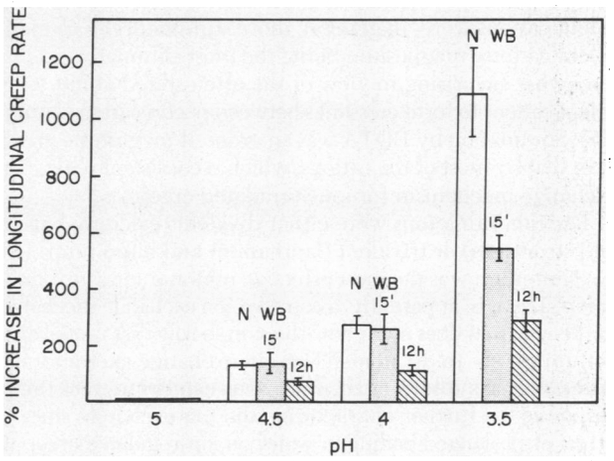

FIG. 5. Effect of pH on the longitudinal creep rate of *Nitella* cell walls boiled in water. N, not boiled; WB 15′, boiled in water for 15 min; WB 12h, boiled in water for 12 hr. The perfusion buffer was 1 mM Tris/Mes/succinic acid.

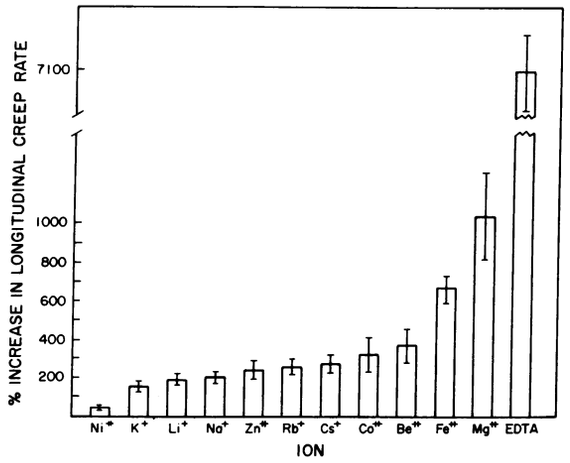

FIG. 6. Enhancement of *Nitella* wall extensibility by cations. The perfusion buffer was 1 mM Tris/Mes/succinic acid, pH 6. The ion concentration was 10 mM.

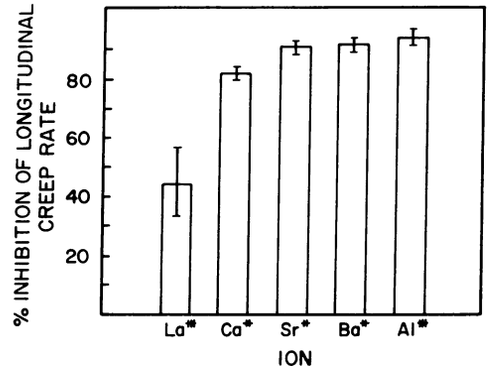

FIG. 7. Inhibition of *Nitella* wall extensibility by cations. The perfusion buffer was 1 mM Tris/Mes/succinic acid, pH 3.5. The ion concentration was 10 mM.

unlikely that the residual acid-induced creep is caused by an enzyme, it is necessary to account for the inhibition caused by prolonged boiling in water. An examination of the strain rate at pH 6 reveals that boiling in water for 15 min actually increased the strain rate by 24%, while boiling in water for 12 hr increased it by 31%. Thus, the inhibition in water for 12 hr increased it by 31%. Thus, the inhibition of acid-stimulated creep is accompanied by a general increase in the extensibility of the wall. Since prolonged boiling in water is known to extract pectins from plant cell walls (27), it seems reasonable to conclude that water-soluble compounds, probably pectins, participate in acid-stimulated creep in isolated *Nitella* walls. Morikawa and Senda (28, 29) have suggested that acid alters the orientation of the pectin carboxyl groups. However, the persistence of the pH effect, even after 12 hr of boiling in water, clearly indicates that water-insoluble compounds are also involved. It is perhaps significant that *Nitella* cell wall protein is known to be rich in carboxyl groups, and it is water-insoluble (30).

Effects of Ions on Creep. The ion exchange capacity of *Nitella* walls has been noted by others (19, 28). It has been observed that potassium, sodium, and magnesium ions increase *Nitella* wall extensibility, while calcium ions reduce it (19, 29). We tested a broad range of cations for their ability to stimulate creep at pH 6, or to inhibit it at pH 3.5. As shown in Fig. 6, divalent cations were, in general, more stimulatory than monovalent cations, magnesium being the most stimulatory. This is somewhat surprising in view of the often stated ability of divalent cations to form crosslinks between pectin carboxyl groups (27). Stimulation by EDTA was an order of magnitude greater than that by most of the cations, which is consistent with an ion exchange mechanism for ion-stimulated creep.

The inhibiting ions were either divalent (calcium, barium, and strontium) or trivalent (lanthanum and aluminum) (Fig. 7). Aluminum was the most effective inhibitor of acid-induced creep. There is, apparently, a complex ion exchange mechanism in the wall that does not follow the simple rule that monovalents act oppositely to divalents. The ion exchange sites and acid-labile sites are interrelated, since ions can counteract the pH response. To further characterize the ion exchange sites, the effect of prolonged boiling in water on ion-regulated creep was examined. After 12 hr of boiling in water, magnesium-stimulated creep was reduced by 17% and calcium inhibition of creep was reduced by 24%. Thus, at least some of the creep-limiting ion exchange sites appear to be water-soluble. There are, in fact, similarities between the relative effectiveness of ions to stimulate or inhibit creep and their ability to reduce or increase the viscosity of pectin solutions (31–33). Smidsrød and Haug (32) reported that the modulus of stiffness of calcium pectate gels was almost 30 times greater than that of magnesium pectate gels, while Kohn and Tibensky (33) reported that both the viscosity and the affinity of cations for the carboxyl groups of pectate increased in the order calcium < strontium < barium. However, prolonged boiling in water interferes less with ionic effects than with acid effects, suggesting a greater role for water-insoluble compounds in ion induced creep. The identity of the water-insoluble ion exchange sites remains to be determined. Morikawa and Senda (28), using different methods, also concluded that the ion exchange sites differ from the acid-labile sites in the wall.

Summarizing, an acid-growth effect has been found in *Nitella axillaris* internode cells similar to that of higher plants. A corresponding acid stimulation of cell wall creep was also demonstrated which does not appear to be mediated by enzymes. A complex ion exchange mechanism in the wall is inferred from the extensibility response of isolated walls to a variety of cations. The acid-labile sites differ from the ion exchange sites in their susceptibility to prolonged boiling in water. Since living cells and isolated walls are affected by acid pH, normal growth in *Nitella* may be regulated by cell wall acidification as it appears to be in higher plants (4, 5). It has been reported that light-dependent, electrogenic hydrogen ion pumps are present in the plasma membrane of *Nitella* (34, 35), and alternating acid and alkaline regions have been detected along the lengths of mature internode cells (36). To our knowledge, the cell wall pH of actively growing *Nitella* cells has not been investigated.

We thank Prof. K. V. Thimann for his encouragement and many helpful suggestions during this work. We also thank Prof. P. Green for supplying us with a culture of *Nitella axillaris* Braun. This research was supported by the National Science Foundation (Grant BMS75-03391).

1. Hager, A., Menzel, H. & Krauss, A. (1971) "Versuche und Hypothese zur Primärwirkung des Auxins beim Streckungwachstum," *Planta* **100**, 47–75.
2. Rayle, D. L. & Cleland, R. (1970) "Enhancement of wall loosening and elongation by acid solutions," *Plant Physiol.* **46**, 250–253.
3. Rayle, D. L. & Cleland, R. (1972) "The *in vitro* acid-growth response: Relation to *in vivo* growth response and auxin action," *Planta* **104**, 282–296.
4. Jacobs, M. & Ray, P. M. (1976) "Rapid auxin-induced decrease

in the free-space pH and its relationship to auxin-induced growth in maize and pea," *Plant Physiol.* **58,** 203–209.
5. Cleland, R. (1973) "Kinetics of hormone-induced H^+ extrusion," *Plant Physiol.* **58,** 210–213.
6. Rayle, D. L. (1973) "Auxin-induced hydrogen ion secretion in *Avena* coleoptiles and its implications," *Planta* **114,** 68–73.
7. Cleland, R. (1971) "Cell wall extension," *Annu. Rev. Plant. Physiol.* **22,** 197–222.
8. Ray, P. M. (1973) on *Recent Advances in Phytochemistry,* eds. Runeckles, V. C., Sondheimer, E. N. & Walton, D. C. (Academic Press, New York), pp. 93–124.
9. Heyn, A. N. J. (1970) "Dextranase activity and auxin-induced cell elongation in coleoptiles of *Avena*," *Biochem. Biophys. Res. Commun.* **38,** 831–837.
10. Tanimoto, E. & Masuda, Y. (1968) "Effect of auxin on cell wall degrading enzymes," *Physiol. Plant.* **21,** 820–826.
11. Yamagata, Y., Yamamoto, R. & Masuda, Y. (1974) "Auxin and hydrogen ion actions on light-grown pea epicotyl segments II. Effect of hydrogen ions on extension of the isolated epidermis," *Plant Cell Physiol.* **15,** 833–841.
12. Green, P. B. (1954) "The spiral growth pattern of the cell wall in *Nitella axillaris*," *Am. J. Bot.* **41,** 403–409.
13. Green, P. B. (1958) "Structural characteristics of developing *Nitella* internodal cell walls," *J. Biophys. Biochem. Cytol.* **4,** 505–515.
14. Green, P. B. (1960) "Multinet growth in the cell wall of *Nitella*," *J. Biophys. Biochem. Cytol.* **7,** 289–295.
15. Green, P. B. (1968) "Growth physics in *Nitella*: A method for continuous *in vivo* analysis of extensibility based on a micromanometer technique for turgor pressure," *Plant Physiol.* **43,** 1169–1184.
16. Green, P. B. & Chapman, G. B. (1955) "On the development and structure of the cell wall in *Nitella*," *Am. J. Bot.* **42,** 685–692.
17. Green, P. B., Erickson, R. O. & Buggy, J. (1971) "Metabolic and physical control of cell elongation rate. *In vivo* studies in *Nitella*," *Plant Physiol.* **47,** 423–430.
18. Probine, M. C. & Preston, R. D. (1961) "Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. I. Wall structure and growth," *J. Exp. Bot.* **12,** 261–282.
19. Probine, M. C. & Preston, R. D. (1962) "Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. II. Mechanical properties of the walls," *J. Exp. Bot.* **13,** 111–127.
20. Probine, M. C. & Barber, N. F. (1966) "The structure and plastic properties of the cell wall of *Nitella* in relation to extension and growth," *Aust. J. Biol. Sci.* **19,** 439–457.
21. Haughton, P. M., Sellen, D. B. & Preston, R. D. (1968) "Dynamic mechanical properties of the cell wall of *Nitella opaca*," *J. Exp. Bot.* **19,** 1–12.
22. Evans, M. L. (1974) "Rapid responses to plant hormones," *Annu. Rev. Plant. Physiol.* **25,** 195–223.
23. Bose, J. C. (1906) *Plant Response as a Means of Physiological Investigation* (Longmans, Green, and Co., New York), pp. 5–7.
24. Kamiya, N., Tazawa, M. & Takata, T. (1963) "The relation of turgor pressure to cell volume in *Nitella* with special reference to mechanical properties of the cell wall," *Protoplasma* **57,** 501–521.
25. Edwards, K. L. & Scott, T. K. (1976) "Rapid-growth responses of corn root segments: Effect of citrate-phosphate buffer on elongation," *Planta* **129,** 229–233.
26. Edwards, K. L. & Scott, T. K. (1974) "Rapid growth responses of corn root segments: Effect of pH on elongation," *Planta* **119,** 27–37.
27. Northcote, D. H. (1972) "Chemistry of the plant cell wall," *Annu. Rev. Plant Physiol.* **23,** 113–132.
28. Morikawa, H. & Senda, M. (1974) "Nature of the bonds holding pectic substances in *Nitella* walls," *Agr. Biol. Cem.* **38,** 1955–1960.
29. Morikawa, H. & Senda, M. (1974) "Effects of acids and ions on mechanical properties of *Nitella* cell walls," *The 15th Annual Meeting of Japanese Society of Plant Physiologists,* 6–8 Apr., abstr. p. 107.
30. Thompson, E. W. & Preston, R. D. (1967) "Proteins in the cell walls of some green algae," *Nature* **213,** 684–685.
31. Smidsrød, O., Haug, A. & Whittington, S. G. (1972) "The molecular basis for some physical properties of polyuronides," *Acta Chem. Scand.* **26,** 2563–2566.
32. Smidsrød, O. & Haug, A. (1972) "Properties of poly(1,4-hexuronates) in the gel state II. Comparison of gels of different chemical composition," *Acta Chem. Scand.* **26,** 79–88.
33. Kohn, R. & Tibensky, V. (1971) "Exchange of calcium, strontium and barium ions on pectin," *Coll. Czech. Chem. Commun.* **36,** 92–100.
34. Kitasato, H. (1968) "The influence of H^+ on the membrane potential and ion fluxes of *Nitella*," *J. Gen. Physiol.* **52,** 60–87.
35. Spanswick, R. M. (1972) "Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance," *Biochim. Biophys. Acta* **288,** 73–89.
36. Spear, D. G., Barr, J. K. & Barr, C. E. (1969) "Localization of hydrogen ion and chloride ion fluxes in *Nitella*," *J. Gen. Physiol.* **54,** 397–414.