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Growth Physics in Nitella: a Method for Continuous in Vivo Analysis of Extensibility Based on a Micro-manometer Technique for Turgor Pressure[1]

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Abstract. **The view that the plant cell grows by the yielding of the cell wall to turgor pressure can be expressed in the equation: rate = cell extensibility × turgor. All growth rate responses can in principle be resolved into changes in the 2 latter variables. Extensibility will relate primarily to the yielding properties of the cell wall, turgor primarily to solute uptake or production. Use of this simple relationship *in vivo* requires that at least 2 of the 3 variables be measured in a growing cell. Extensibility is not amenable to direct measurement. Data on rate and turgor for single *Nitella* cells can, however, be continuously gathered to permit calculation of extensibility (rate/turgor). Rate is accurately obtained from measurements on time-lapse film. Turgor is estimated in the same cell, to within 0.1 atm or less, by measurement of the ability of the cell to compress gas trapped in the closed end of a capillary the open end of which is in the cell vacuole. The method is independent of osmotic equilibrium. It operates continuously for several days, over a several fold increase in cell length, and has response time of less than one minute. Rapid changes in turgor brought on by changes in tonicity of the medium, show that extensibility, as defined above, is not constant but has a value of zero unless the cell has about 80 % of normal turgor. Because elastic changes are small, extensibility relates to growth. Over long periods of treatment in a variety of osmotica the threshold value for extensibility and growth is seen to fall to lower values to permit resumption of growth at reduced turgor. A brief period of rapid growth (5× normal) follows the return to normal turgor. All variables then become normal and the cycle can be repeated. The cell remains essentially at osmotic equilibrium, even while growing at 5× the normal rate. The method has potential for detailed *in vivo* analyses of "wall softening."**

The growth of the plant cell may be regarded, instantaneously at least, as the yielding of the cell wall to the stresses present in it. In the *Nitella* internode and other cells not subject to tissue tensions, the stresses can be assumed to be proportional to turgor pressure. This allows the rate of growth (R) to be viewed simply as the product of the yielding tendency of the cell (Ex_v or gross extensibility) and turgor pressure (T).

$$R \text{ (fraction/hr)} = Ex_v \text{ [fraction/hr)/atm]} \cdot T \text{ (atm)}$$

All changes in growth rate then become resolvable into changes in one or both terms. Such changes in individual terms, once clearly identified, can then be studied with regard to their origin in structure or metabolism.

Equation (I), despite its brevity, is remarkably difficult to solve in growing cells since only the rate term is readily measured while enlargement is occurring (*21, 22*). Extensibility is not amenable to direct measurement because unilateral stretching of the growing cell would alter the usual stress pattern and disturb the growth process. Extensibility can be calculated using equation (I), provided information on turgor can be combined with data on rate in the same material.

Turgor estimates for the growing cell are often equivocal because equilibrium methods such as plasmolysis and plasmometry give only an upper limit for the value during growth. In some tissues the value during enlargement is estimated to be as much as 2 atmospheres below the equilibrium figure (*32*). Even if near-equilibrium conditions do obtain during growth, as might be expected in single cells growing under water, the growth response of interest may be rapid compared to the rate at which diffusion brings

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concentrations to the new equilibrium required for a measurement (8, 32). Use of equation (I) to analyze growth in tissues is made particularly difficult by the possibility that the various cell types in the tissue are not equivalent with regard to generating pressure and yielding to it. For these reasons a method for turgor in single cells which is rapid, continuous, independent of osmotic equilibrium, and compatible with growth is of some utility. This report describes such a method based on measurement of a cell's capacity to compress gas trapped in a capillary (16). Data on turgor, when combined with information on the growth rate of the same cell, permit the continuous solving of equation (I) and the calculation of gross extensibility (Ex_g).

Note that while turgor clearly can be affected by changes in extensibility (*e.g.* increased extensibility under conditions of low permeability to water can reduce turgor (21, 22), this does not mean that the 2 variables cannot be measured independently. The cell's ability to compress gas gives its internal pressure regardless of the many factors governing this pressure. Extensibility can thus be defined simply as the quotient of the existing rate and the existing turgor pressure.

Ex_g is given a subscript to distinguish it from related expressions. Generally, extensibility relates the rate of yielding of a material to the applied stress. For many materials the relationship can be written so that extensibility is a constant. This often involves incorporating the complexity of yielding behavior into the stress term. For example, in quasi-plastic flow the stress above a certain threshold is raised to a power and then multiplied by a constant extensibility term to give the rate of yield (28). Because there is no *a priori* reason to expect the extensibility of a growing cell to fall into one of the rheological relationships established for inert substances, it seems reasonable here to keep the stress term a simple pressure and to express complex yielding behavior, when it appears, in some elaboration of the extensibility term.

Plant cells typically show constant growth rate at constant turgor, so Ex_g will of necessity have the characteristics of a reciprocal viscosity. The similarity to viscous flow may well be only formal because, if metabolism is allowed to play a role, one can envision a variety of complex yielding systems which show this kind of behavior (29).

Ex_g, when measured over short periods of time and over small deformations, will reflect the instantaneous yielding properties of the cell wall, properties which may be maintained by metabolism occurring in, or at least acting on, the wall (30). The behavior of Ex_g *in vivo* therefore can be compared with the yielding properties of killed cells or isolated wall strips subjected to known artificial stresses. Provided the physical data on isolated material derive from small deformations at physiological rates, and provided correction can be made for the fact that a unilateral force instead of a multi-lateral pressure is applied in isolated material, differences in yielding properties from those seen *in vivo* must reflect the absence of chemical or metabolic factors normally present in the cell.

By the present method Ex_g can be evaluated after rapid shifts in turgor to give information on the stress:strain-rate relationship in the growing cell. Continuation of the analysis over longer periods reveals sizable shifts in these properties.

Materials and Methods

Principle. When a fine glass capillary is fused at one end and submerged, air is trapped inside it. If the open end is then inserted into a cell and the wound sealed, the trapped gas is compressed in accord with pressure inside the cell. Because of the constancy of pressure times volume, cell pressure is readily calculated. Turgor pressure is 1 atmosphere less than the pressure in the bubble. The small tendency of capillarity to compress the gas can be measured beforehand and subtracted from final turgor values. Adaptation of this principle for use on the growing cell, under conditions where the growth rate of the same cell could be simultaneously followed, involved many problems. The major physical one was that the trapped gas dissolved into the capillary fluid, at varying rates depending on turgor, so that volume changes over long periods of time were not a reliable basis for pressure calculation. A short-term volume change, brought on by a vacuum pump, was used for calibration.

Tazawa's cell compression method for turgor (34) and Virgin's resonance frequency method (38) also do not require osmotic equilibrium. Arens (1) has described 2 methods to estimate turgor in *Nitella*. In one, the original compressibility of a cell was restored, after loss of turgor, by inflating the cell through a canula. The gas pressure required was equal to the original pressure of the cell interior. In the other, the mid-portion of the cell was gripped and the position of one protruding cell-end (a) carefully noted. The cell was allowed to wilt and gas pressure was then applied to the space around the other cell-end (b) so as to restore the original configuration at (a). Values of 3 to 10 atmospheres were obtained. Recently Villegas (37) has used a capillary inserted in a *Valonia* cell to balance cell pressure with gas pressure. Pressure changes associated with small volume changes could be estimated. Gutknecht (17) has altered turgor in *Valonia* by connecting a source of hydrostatic pressure to the cell interior by a capillary. Apparently none of these methods has been adapted for continuous use on growing material.

Detailed Method. The following considerations governed the adaptation of the capillary method to the growing cell. All data necessary for determination of both growth rate and cell pressure would be photographed (time-lapse). This meant that the

cell and capillary had to be in the same plane, preferably side by side. Calibration was to be done periodically and solution changing, over a period of 48 hours, was to be automatic. Because calibration involved a large drop below atmospheric pressure, the system, including automated valves, had to be vacuum tight.

The major biological problem involved the cell's reaction to the presence of the capillary which, at first, either resulted in failure of the cell to grow or in failure of the capillary to function (due to plugging). Success was obtained only after extreme measures were taken to insure mechanical rigidity of the cell and capillary both during insertion and the protracted growth period.

a) *Calibration of Cell Pressure.* The pressure in a bubble can be determined by its volume change in response to transitory changes in its pressure. Normally 1 atm of the pressure on the bubble is from the atmosphere, the rest being due to turgor. The former component can be altered by known amounts by placing the entire system, plant and capillary, in a strong vessel and applying a pump. From the change in volume one obtains the pressure (P_B) in the bubble.

$$P_B = \frac{\triangle P\ V_2}{V_1 - V_2} \qquad \text{(II)}$$

We gave a decrement in pressure, $\triangle P$, of minus two-third atm for 3 minutes every half hour. V_1 was taken as the mean of 2 volumes: 5 minutes before and 5 minutes after the pulse. V_2 was the maximum volume during the pulse.

Turgor pressure, T, is:

$$T = [P_B - C] - 1 \qquad \text{(III)}$$

where C is capillarity, roughly 0.05 atm, measured by the extent of water entry (gas compression) when the capillary was submerged. Turgor was computed from measurements punched on computer cards. For pressure values between calibration pulses, the volume of the bubble could be used because the shrinkage was steady for a given turgor. No variation in streaming rate or growth rate was associated with pump action.

The response of bubble length to pump action and to the addition of osmoticum to the medium is seen in figure 3 where length is measured at 2 minute intervals. Upon a fall in turgor the bubble increases both its length and its response to the pump. The bubble can accurately respond to a change in pressure of two-third atm within 1 minute. Accuracy of pressure drop was maintained by a Cartesian diver type manostat (Greiner Science Company, New York).

The Growth Vessel and Automated Solution Changing. The plant grew in a continuously illuminated pressure vessel and was photographed through a flat window. The vessel was made from a large "0-ring" joint by glass-blowing. It was clamped to a heavy lathe tool-holding device to permit precise and stable adjustment of the vessel's position. An outlet above the fluid level in the vessel went to a vacuum pump, an inlet was provided below the 0-ring, and a drain was at the base (fig 1). Solutions were changed by draining and refilling, all movements by gravity. Valves were operated by air-driven linear actuators controlled by solenoid valves. Automation of both calibration and solution change involved combined use of a 1 rev/hr multiple cam ("gang") timer and a stepping switch. One cam activated a vacuum pump for 3 minutes every half-hour for calibration (see above). During every hour a second cam closed 1 of 2 gaps in a circuit which could open the drain valve, and shortly thereafter a third cam closed a similar gap in an inlet valve circuit for a few minutes. The valves would actually open only during those hours when the stepper closed the second gap. The stepper was advanced 1 step per hour by a fourth cam on the gang timer. Thus the major functions, being all timed by the same rotating shaft, could not get out of phase. Typically, normal growth medium was alternated with growth media of increased tonicity, using various osmotica such as sugar or polyethylene glycols (Carbowax). The Carbowax (Union Carbide Company) had been passed through an ion-exchange column. Concentration of growth medium was kept constant, independent of osmoticium concentration. A large flask of normal medium was repeatedly used to fill the growth vessel during 1 run. Smaller flasks of altered medium (used once each) were drained completely to fill the growth vessel. This arrangement permitted 7 or 8 solution changes with only 4 flasks and valves. Timing had to be adjusted in advance to allow for variation in viscosity of the various solutions, particularly those containing Carbowax.

Toxicity was avoided by using polyethylene for tubing, valves, and fittings and by making all glue junctions with DeKhotinsky cement (Central Scientific Company, St. Louis). The 0-ring itself was toxic so the solutions were kept from it.

The Capillary. A fish-hook shaped capillary was used in order to bring the compressed bubble alongside the growing cell surface for photography. At full turgor only about one-sixth of the capillary was filled with gas. To have the length of the bubble roughly correspond to the length of the young cell in order to maximize accuracy, the capillary was made much longer than the cell, then bent. An internal diameter of about 40 μ was found to make the bubble readily visible on the film and to transfer only a modest volume of cell sap into the capillary. Cell diameter was about 200 μ so the capillary cross-section was about 4 % that of the young cell. Initial sap dilution ran about 3 to 6 %. One mm diameter thin-wall glass capillary (Corning Glass Works, Corning, New York) was hand-drawn over a broad flame to give several cm of thin capillary of essentially constant bore. The glass was then treated with Siliclad (Clay, Adams, Inc., New York, New York) to prevent later segmentation of the gas

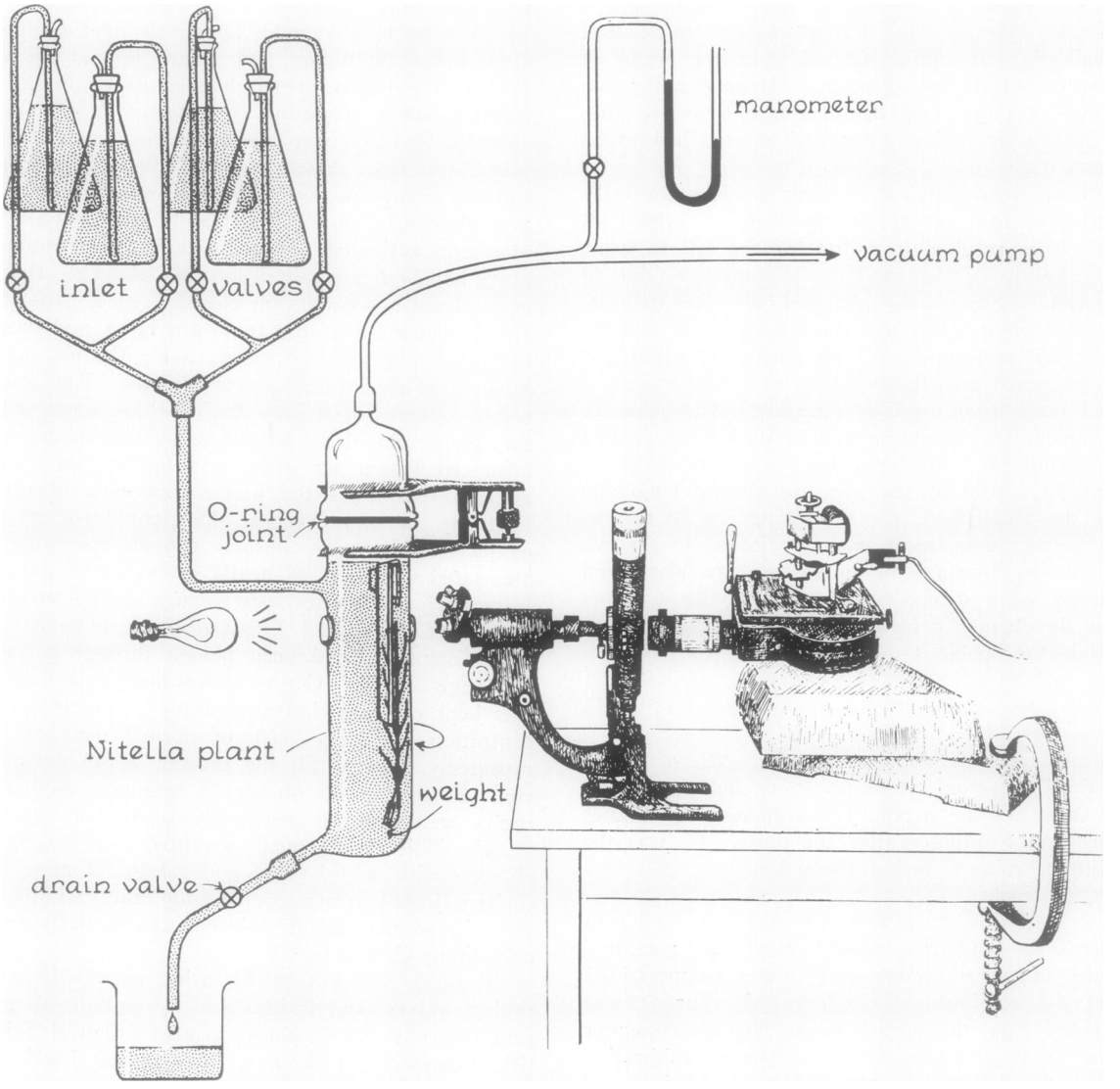

FIG. 1. General experimental set-up to permit continuous time-lapse photography (right) of the plant while it is growing in the pressure vessel. The vessel is held by a clamp (not shown) fixed to a machinist's tool holder with screw adjustments in 3 dimensions. The vacuum pump acts at one-half hour intervals for calibration of cell pressure (see text). Solution changing was on a drain-and-refill basis, automated by circulating electric valves through a gang timer and stepping switch. The mercury manometer was occasionally used to check the level of vacuum delivered by the pump.

bubble. The 40 μ bore was ideal for the reading part, but was too large for insertion. A special tip was fashioned with a DeFonbrune microforge (Aloe Science Company, St. Louis). The platinum filament was coated with glass to prevent toxic vapors from being deposited on the capillary. The capillary was fused at 2 points 12 mm apart and a region just above the lower fusion was heated to form an extremely thin-walled (3 μ) swelling. A weight was added below and the swelling stretched, by heating, into a delicate, and thin-walled extension. After bending into a hook shape, the capillary was glued to a cover-glass chip. The tip was broken with tweezers to give a roughly oblique cut tip of diameter 20 μ. The mounted capillary was then returned to the forge and the broken tip touched to the filament and stretched and smoothed to a form like that of a syringe needle. Just before insertion it was dipped in water, then 20 % "Bio-nox" detergent (Triton X-100, Rohm and Haas Chemical Corporation, Philadelphia, Pa.) to minimize the cell's reaction to insertion.

The Holder. A complex holder was used for both the insertion, carried out in a large Petri dish under a dissection microscope, and for prolonged observation in the pressure vessel. See figure 2.

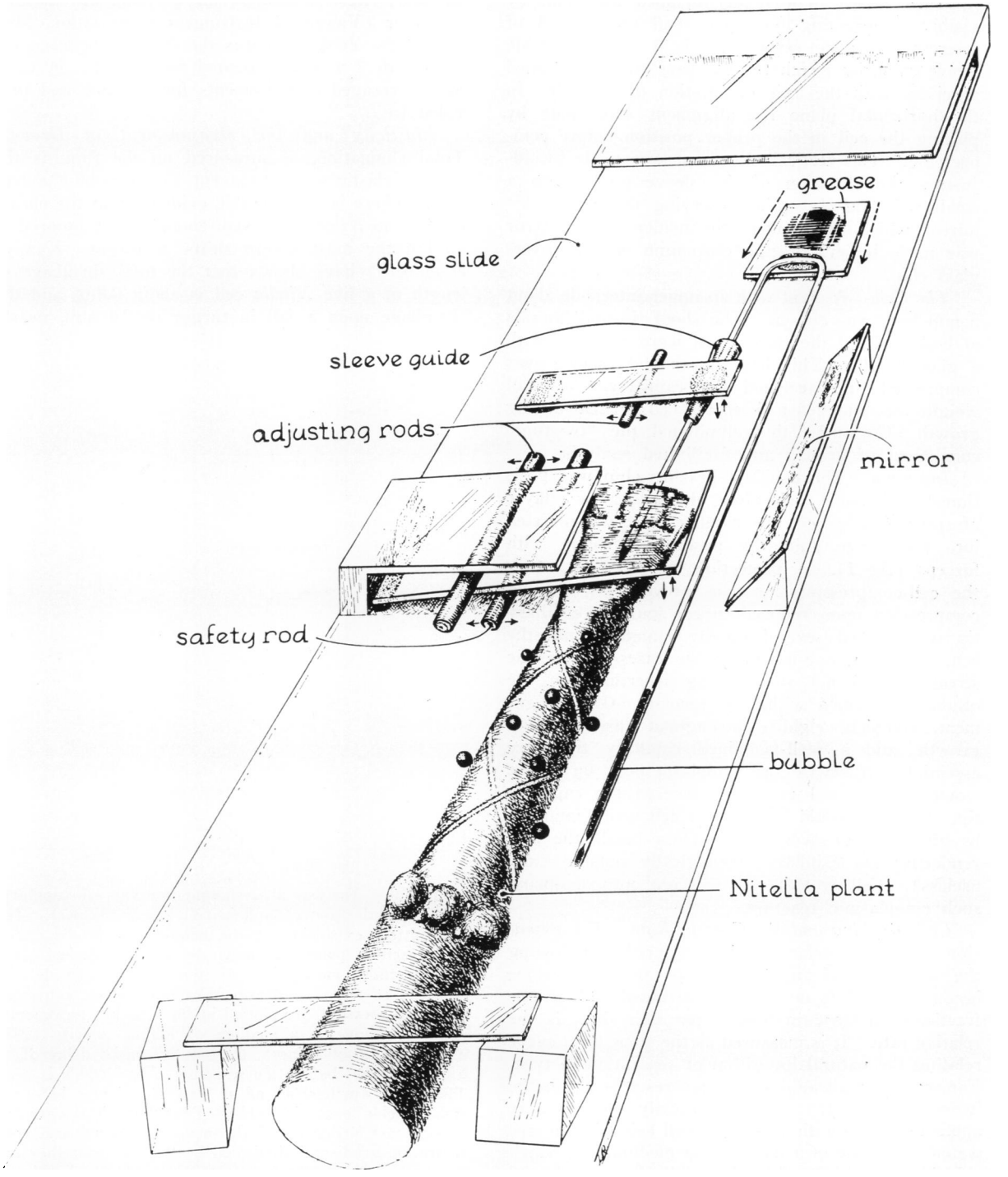

FIG. 2. A glass holder, assembled from a slide and cover glass pieces, used to hold the cell during insertion of the capillary and during prolonged growth in the pressure vessel. Adjustment of the position of the cell and the sleeve guide permitted advance alignment of the capillary tip with the central axis of the cell. The cell was firmly compressed at full turgor as shown; further compression upon reduction of turgor was prevented by the safety rod. When the holder and plant were in the vessel (fig 1), the field of the camera included the region of the cell showing the marking beads (on cell, left of bubble) and the adjacent part of the capillary containing the bubble. The structure at the base aids in the straightness of growth of the cell.

It hooked onto a horizontal bar glued inside the pressure vessel. The holder gripped the plant by visibly compressing the upper end of the cell of interest. It also served to hold an adjustable sleeve-guide by which the capillary tip was aligned precisely with the axis of rotation of the cell. In the horizontal plane the alignment was made by placing the cell in the proper position before compressing. The vertical alignment was made by adjusting the elevation of the sleeve guide with a small rod (fig 2) while observing through a 45° mirror which was part of the holder. The mirror was made by evaporating chromium onto a coverglass chip.

The Cell. A plant with an upper internode about 5 mm long was chosen. The shoot tip and laterals at both ends of the chosen cell were removed with a glass needle. This bare upper end (node) was compressed and accepted the capillary. A small weight was attached to keep the cell vertical during growth. Curiously, this eliminated the "twisting" component of growth and facilitated analysis.

Insertion. The capillary-bearing chip was positioned so it could slide along the holder on a layer of stopcock grease. The capillary tip was directed into the sleeve guide by pushing the chip with forceps (fig 2). A protection bar was put under the cell-compressing lever to prevent any further compression upon reduced turgor and the capillary tip was pushed several hundred microns into the cell. In about one-fourth of the cases cytoplasmic streaming did not stop during insertion. In the others it resumed within 30 seconds. Good alignment, complete rigidity throughout insertion and growth, and a well-fashioned capillary tip were essential. Otherwise the cytoplasm built up at the wound region and gradually covered the capillary tip. Often it would lay down a cell wall (found to be birefringent) over all the glass inside the cell, rendering the capillary topologically outside (and useless). Cell growth rate was not optimal during such cytoplasmic reaction.

Cell Marking and the Growth Rate. The extension of the cell surface can be measured by following the separation of any 2 points on the cell surface because growth is uniformly distributed (12). The fractional increase in length, per unit time, is the relative rate. It is measured as the slope on a curve relating the natural logarithm of separation to time. For marks, small anion-exchange resin beads in the hydroxyl form (15) are ideal optically. Marks were applied by hand with a hair, the cell being in ion-free water. The use of a defined salt medium (in which chloride competed with the wall for the beads' binding groups) along with periodic draining and filling of vessel, lead to frequent loss of marks. An excess had to be applied initially. Those beads that remained on throughout were seen in the movie to remain firmly fixed to the wall during growth. The camera took 1 frame per minute, recording behavior of the cell surface and the bubble. Key dimensions on the developed film (Kodachrome II, 16 mm) were measured on a Vanguard Motion Analyzer (Vanguard Instrument Corporation, Melville, New York). L was the distance between the centers of 2 widely separated resin beads. A computer averaged measurements for 1 frame and provided ln L.

Elasticity and the Measurement of Growth. Total elongation, as measured on the film, is the sum of elastic and permanent (irreversible) extension. There is considerable evidence that the elastic changes in *Nitella* are small enough to be ignored in all but the most severe shifts in turgor. Kamiya *et al.* (18) have shown that the total shrinkage in length of a live *Nitella* cell is about 0.8 % and the shrinkage upon a fall in turgor of 2.4 atm, nearly

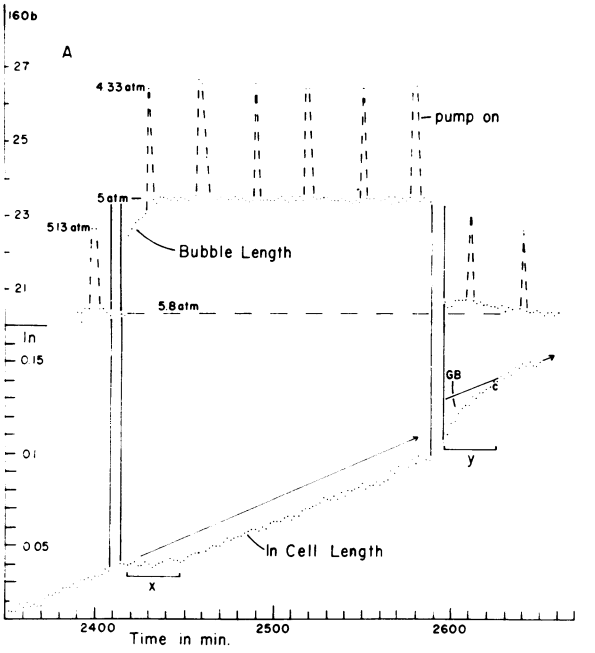

FIG. 3. Analysis at 2 minute intervals of capillary-bubble length (above) and in cell length (below). The intermittent expansion of the bubble, due to the action of a vacuum pump, is shown by vertical dashed lines. This permits calculation of the pressure inside the bubble (see text). (These calibration values, converted to turgor pressure, are plotted in fig 5, middle treatment). After a change to a medium of higher tonicity (2410 min, vertical solid lines), there is an initial delay of at least 2 minutes before the response of the bubble is seen. There is a further lag of at least 10 minutes before a stable bubble length is reached. That these delays are due to slow establishment of the new trans-membrane concentration gradient is indicated by the fact that they are long compared to the response time of the bubble to hydrostatic pressure change. A similar delay is seen upon return to higher turgor (2590 min). Growth halts for a brief period (x) at the start of inhibition, but then normal rate is rapidly restored. Upon return to essentially full turgor, a growth burst (GB) ensues, leading to a net increase in cell length over that anticipated in the absence of inhibition (long arrow). Duration of the burst (y) is about equal to the initial lag (x).

equal to the largest drop imposed by us, is only 0.1 %. Elastic changes took about 2 to 3 minutes to be three-fourths complete. In our material shrinkage could not be detected upon a fall in turgor of 0.8 atm (fig 3). A fall in turgor of about 3 atm did lead to about 0.4 % shrinkage over about 10 minutes on some trials, but to no shrinkage on others (fig 6). These shrinkages are small compared to length changes used to determine rates and are ignored unless otherwise specified.

Culturing. Stock cultures were maintained by alternate growth (2 wk periods) in autoclaved soil extract (100 ml garden soil in 6 l distilled water autoclaved 20 min) and in a modified Forsberg (10) medium. It contained, per liter, $Ca(NO_3)_2$, 80 mg; $MgSO_4 \cdot 7\,H_2O$, 100 mg; $NaHCO_3$, 60 mg; KNO_3, 100 mg; NH_4Cl, 20 mg; Na_2SiO_3, 12.5 mg; KCl, 100 mg; K_2HPO_4, 1 mg. The tris buffer used by Forsberg was omitted because it supported bacterial growth. Because the plants grew in large volumes which were replaced every 6 hours or less, buffering was not important. pH was adjusted to 6.5 with HCl. Trace elements, individually chaelated with sodium nitrilotriacetate were added, at 1 ml/l from stock solutions: $FeSO_4$, 2.5 g/l; $ZnCl_2$, 526 mg/l; $MnCl_2$, 1.15 mg/l; $CuCl_2$, 21.3 mg/l; H_3BO_3, 295 mg/l; Na_2MoO_4, 60 mg/l; $Co(NO_3)_2$, 30 mg/l. The modified Forsberg medium supported growth for 2 weeks and was used for experiments. It did not sustain good growth indefinitely as did soil. The Forsberg solution did not support growth of any of the visible contaminants, including *Oedogonium*, so by innoculating soil medium with the terminal 10 cm tips of Forsberg medium plants, the cultures were kept free of visible contamination.

Osmotica. To alter turgor it is desired to add a solute whose sole action is to lower the water potential of the medium. There is always the possibility that any soluble organic molecule will enter the metabolism of the cell to either stimulate or inhibit growth. In non-sterile systems such as the present one, effects mediated by bacterial activity are hard to exclude. The traditional osmotica, mannitol and polyethylene glycols, were used in the present study and it was found that when incorporated into growth medium they could support bacterial growth after days of standing.

Certain inorganic molecules cannot serve as energy sources but may have other disadvantages. KCl would be expected to effect the resting potential of the membrane. Further, potassium and calcium ions are known to have direct "loosening" and "hardening" effects on *Nitella* cell walls (27). None-the-less, the cell response to KCl does not differ markedly from that to organic osmotica. (KCl does not enter unusually rapidly to alter turgor).

Limitations. The method is capable of resolving any growth response into turgor and extensibility components. The major limitation is that the growth response caused by the presence of an osmoticum may be due to changes other than the simple change in the colligative properties of the medium. The most striking example of this was found with Carbowax 400 (A low mol wt polyethylene glycol, Union Carbide Company, New York). Presence of this compound reduced turgor and initially stopped growth. The cell then not only recovered its original rate but exceeded it by 80 %, still at reduced turgor! The data to be presented will therefore primarily illustrate the method and only tentatively characterize the response of the cell to turgor change. Some confidence is justified in the immediate aspects of a response and in those slower aspects that are common to osmotica of diverse chemical nature.

Results

Possible Departure From Osmotic Equilibrium — Delays in Mixing. A rough test for whether the growing cell is in osmotic equilibrium is whether a decrease in tonicity of the medium surrounding a non-growing cell is fully matched by an increase in turgor inside the cell as it resumes growth. A discrepancy would show a departure from equilibrium. In figure 6, below, the length of the vertical arrows gives the change in tonicity of medium, the vertical shift in the large dots, the shift in cell turgor. The differences are small fractions of an atmosphere and, after considerable growth has occurred, could be due to rapid sap dilution. It was thought possible that detailed study of bubble length might detect finite departures from osmotic equilibrium during the bursts of rapid growth after the end of inhibition. The detailed record in figure 3 does show slightly reduced pressure (by 0.08 atm) during the growth burst compared to values for steady elongation. This discrepancy apparently has its origin however in the delay required for the establishment of a new concentration gradient across the cell membrane. This general problem is discussed by Dainty (8). There is a roughly 20 minute delay in fully establishing a new turgor pressure when osmoticum is added and growth stops (fig 3, 2412–2432 min). This delay exists despite the presence of considerable differences in density of the solutions being changed; these differences would promote mixing by convection. Here the pressure is "too high" to roughly the same extent for about the same amount of time as it was "too low" above. Part of the slightly reduced pressure during the growth burst could be the result of rapid sap dilution during the growth burst (see fig 6, third treatment). It is concluded that departure from osmotic equilibrium is certainly less than 0.08 atm and probably infinitesimal. This result is not unexpected in light of the high water permeability (7–12 μ/min/atm) of mature *Nitella* cells (35).

Growth Response. In all experiments a growing cell was alternately placed in normal medium and in normal medium supplemented with osmoticum. The periods in normal medium were 4 to 6 hours.

The data are presented with the 2 directly measured variables, ln L and turgor pressure, plotted against time. This gives an over-all view of the course of the experiment, the magnitude of the growth response, *etc.* The growth rate is the slope on the ln L curve and is given at representative points as r. Cell extensibility, Ex_9, is the quotient: rate/turgor and is given at the same points as e. In figure 6 the variable ln diameter is also given.

Initially a detailed study using one osmoticum was planned. Concern with the potential side effects of all osmotica has lead, however, to a broader and less intensive effort. The response to various osmotica will be briefly described and the common features summarized.

In figure 4 a cell is periodically subjected to increasing concentrations of Carbowax 4000 in Forsberg medium, reducing its turgor by 0.3, 1.0, and 1.8 atm respectively. In all cases the cell's initial response is to stop elongating. Then, after periods of time which increase with larger loss of tugor, growth resumes. This recovery is primarily due to

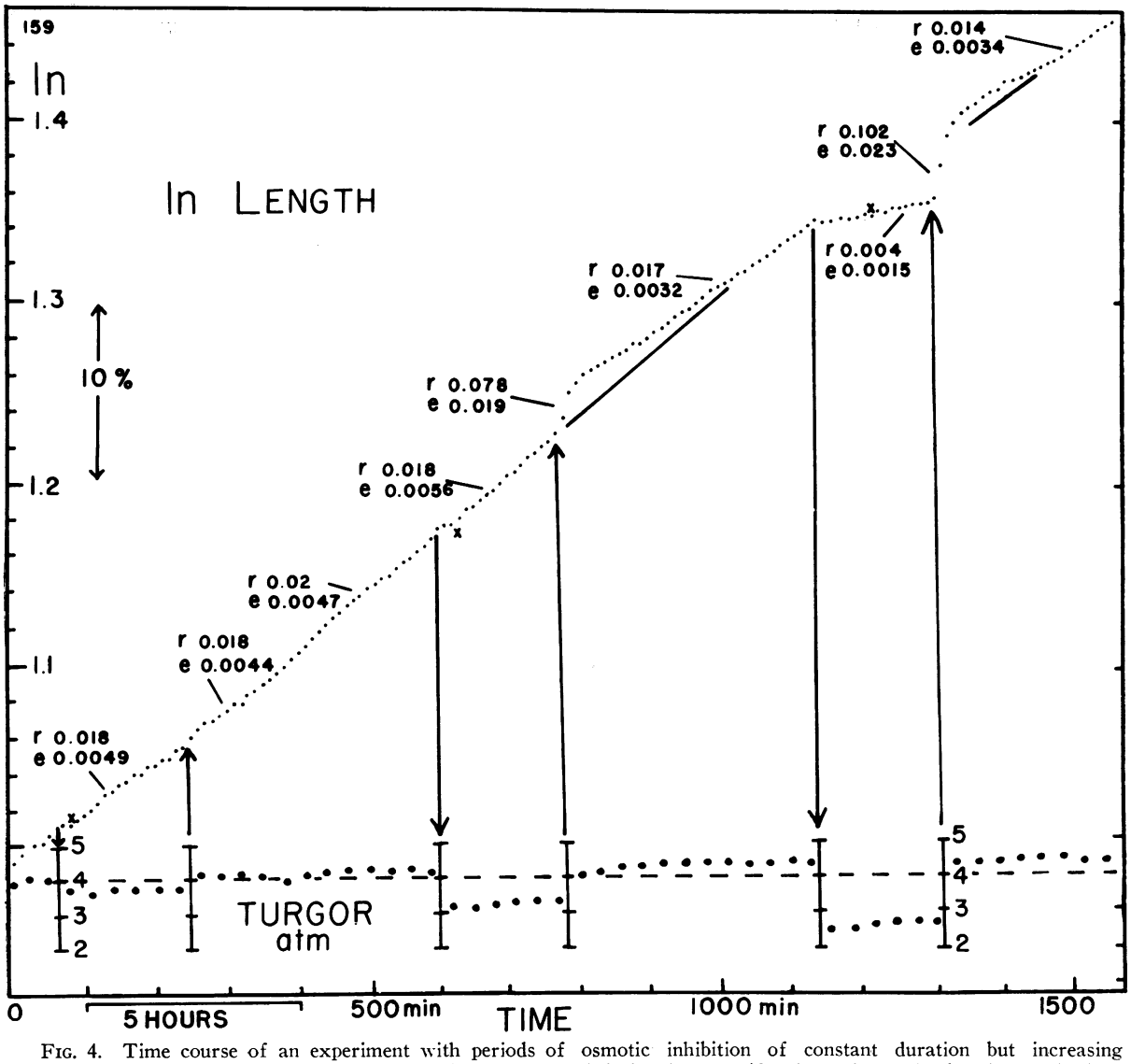

FIG. 4. Time course of an experiment with periods of osmotic inhibition of constant duration but increasing severity. The natural logarithm of length (between 2 marks) is given at 10 minute intervals, by the small dots. Use the upper vertical scale. The large dots and the small inserted scales give the turgor of the same cell at the same time. The relative rate of growth is the slope of the ln length curve and is given as r at various points. The gross extensibility (e) of the cell is also given. Descending arrows show start of osmotic inhibition, ascending arrows, its release. Note slight sensitivity to a small drop in turgor, resumption of normal growth rate upon moderate reduction of turgor (2nd inhibition) which is followed by a growth burst. A greater drop in turgor (3rd inhibition) almost totally inhibits growth, but is followed by a growth burst. Osmoticum: Carbowax 4000. Small x's show resumption of growth.

a change in Ex_v, not turgor. After the 2 smaller losses in turgor the normal rate is regained but after a loss of 1.8 atm only partial recovery is seen during the time available. In all cases a rapid elongation follows the return to osmoticum-free medium. The wall is seen to be highly extensible. The maximum rate attained and the amount of added length achieved during these "growth bursts" appear to increase as a function of the time the cell has spent at reduced (or zero) growth rate. A similar record is seen in figure 5 where the osmoticum was the same but the medium was soil extract. Here a pronounced net increase in length, due to inhibition plus recovery, is seen after the second inhibition. The over-shoot was very striking in runs with Carbowax 400, where a bacterial bloom eventually appeared, so its origin may be in extraneous metabolic factors combined with the change in turgor.

An increase in size of the growth burst with increasing time of growth inhibition (but no overshoot) is seen in figure 6 where the osmoticum was mannitol and where the duration, not the severity, of osmotic inhibition was varied. Recovery of rate appears only after about 4 hours into the longest inhibition. During this long inhibition an increase in turgor pressure is clearly seen. This increase is about that expected if solute increase had continued undiminished during the inhibition of volume increase. Extremely high rate and extensibility are noted upon return to normal medium. A fourth period of inhibition (at 2200 min) shows that the growth burst does not increase as a function of the number of inhibitory periods (a possibility not otherwise excluded) and that the rapid increase in length of the large growth burst is inelastic, at least after several hours.

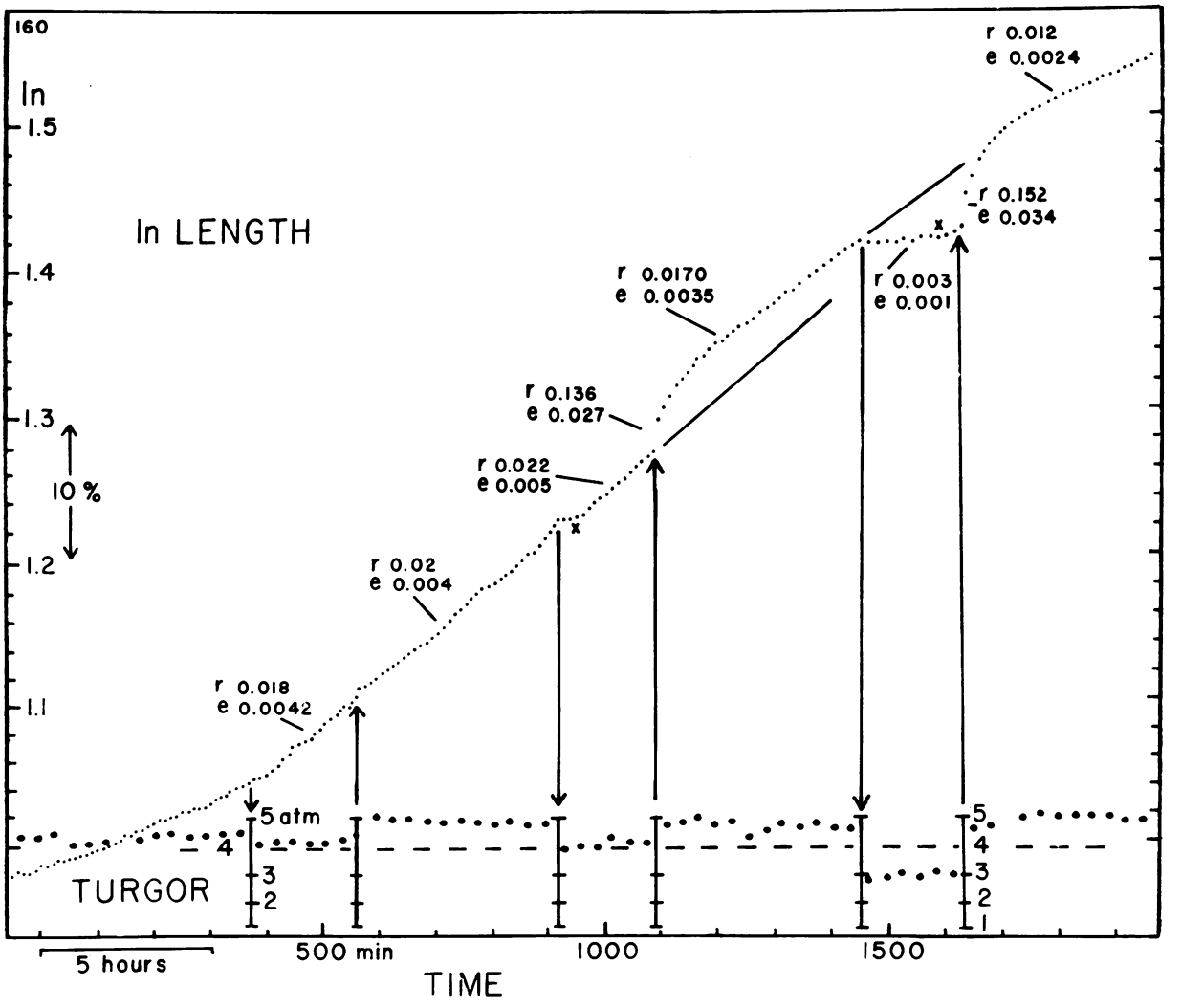

FIG. 5. Legend and experimental procedure the same as in figure 4. The second inhibition led to a pronounced net increase in cell length over that which would have been reached if the cell had been undisturbed. Note that the growth burst of the third inhibition gives a cell extensibility about 10 times that typical for the steady growth periods before and after the inhibition. Osmoticum: Carbowax 4000.

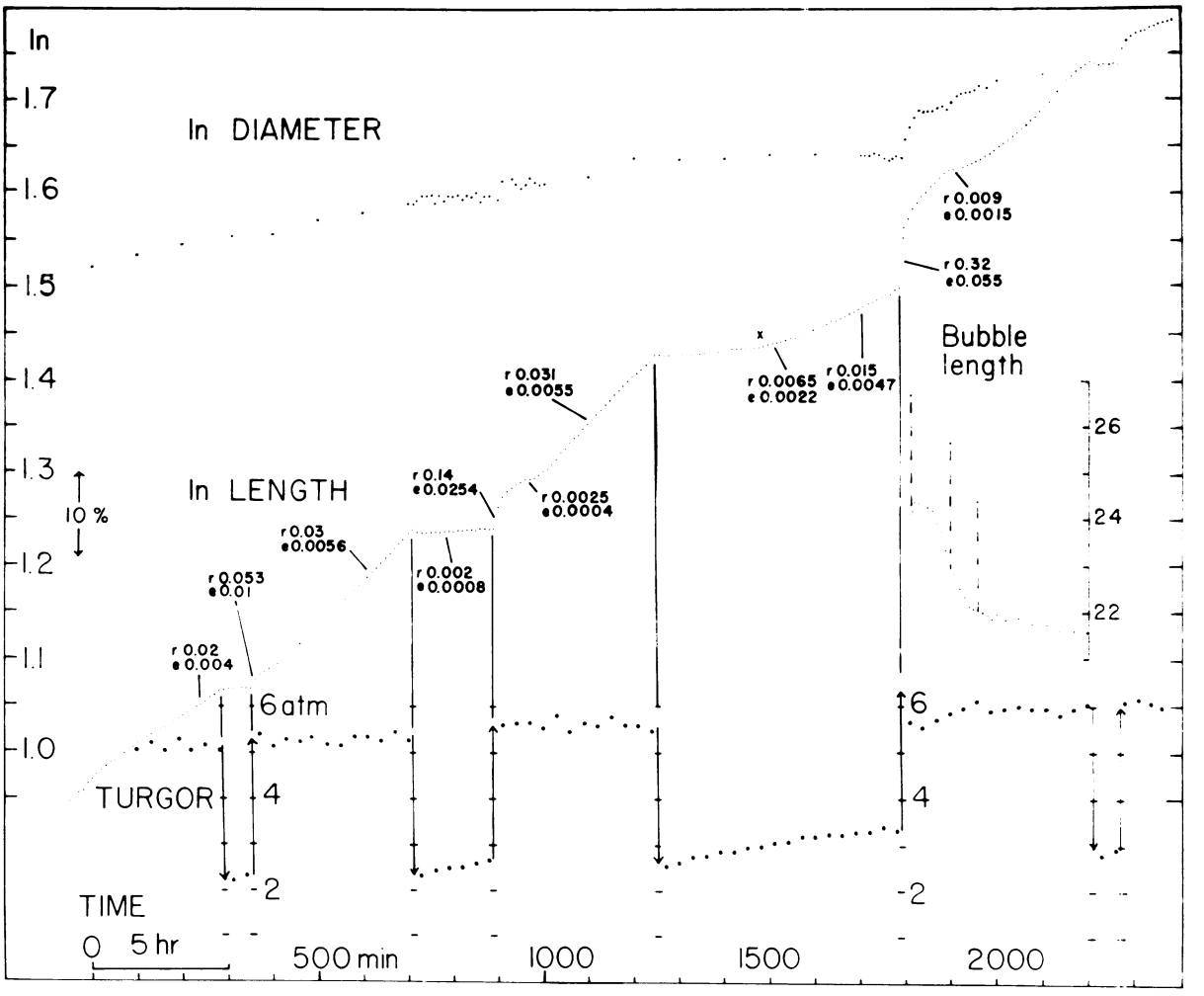

FIG. 6. Symbols and scales as in figure 4 and 5. An experiment where duration, not severity, of osmotic inhibition is varied. Here 1, 3, 9, and 1 hour of the same osmotic inhibition is given, with 6 hours of growth in normal medium between treatments. Note the progressive increase in size of the growth burst with increasing time of inhibition. The maximum slope at the start of each burst also increases. The turgor slowly increases throughout, but much more rapidly during inhibition. The turgor increases as if solute accumulation were continuing at the normal rate while volume increase was inhibited. Note partial recovery of growth rate after 4 hours of the third inhibition. ln Diameter is plotted above. Note the sharp increase during the largest growth burst (1800 min). Normally the increase in ln diameter is about 0.2 that of ln length. Over the first 40 minutes of the growth burst, it is about 0.5. The length of the vertical arrows corresponds to the change in turgor predicted by change in the tonicity of the medium (mannitol). That the change in cell turgor nearly corresponds to this, after a period at osmotic equilibrium during inhibition, indicates that a near-equilibrium condition persists even during the growth bursts. Bubble length measurements show lag in attainment of the steady trans-membrane concentration gradient after a change of solution.

Figure 7 shows the response to turgor changes where the osmoticum was KCl. The typical initial halt in growth, the eventual recovery of much of the previous growth rate, and the rapid elongation following an increase in turgor are evident.

The similarity of response among ionic and uncharged osmotica is somewhat unexpected, particularly if the resting potential of the cell membrane is significant in the growth process. This is not improbable since membrane fusions, particularly of Golgi vesicles, are implicated in the growth response. A fairly direct demonstration of this is found in geotropic response of the rhizoids of *Chara* (33). The concentration of KCl used would virtually abolish the resting potential if artificial pond water were the normal medium (19, 20). Because our normal medium contained KCl in sizeable amount, the resting potential would be reduced by roughly half, according to a simplified equation for resting potential given by Giese (11). Relatively non-polar

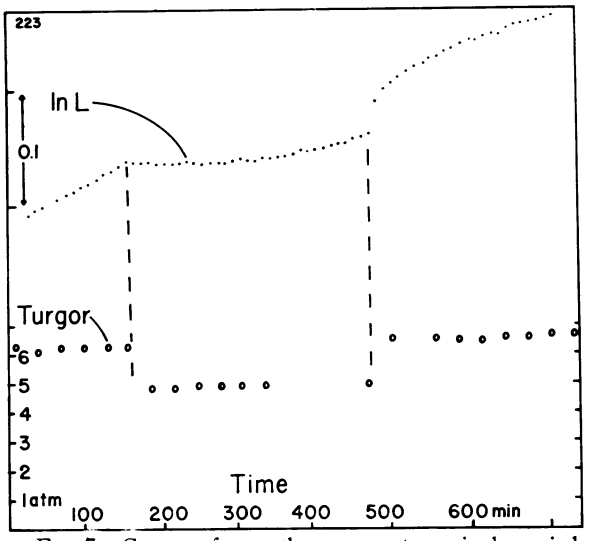

FIG. 7. Course of growth response to a single period of osmotic inhibition where the osmoticum is KCl. The time required for resumption of growth is somewhat longer than typical for other osmotica and the growth burst is somewhat reduced. Otherwise the response is similar to uncharged osmotica.

solutes, even ethanol, can also lower the resting potential. Because KCl and Polyethylene glycol, (a fat solvent) give a response similar to that elicited by mannitol, it appears that the value of the resting potential may not be a highly significant factor in the growth process. This could be confirmed by adapting the micro-manometer to serve as a micro-electrode to monitor resting potential concurrently with pressure, but this modification has not yet been tried.

The well-known "wall loosening" properties of potassium are not evident in the comparison of responses, perhaps because our normal medium contained sufficient KCl to saturate those wall components responsible for the effect.

In light of the similarity of response to various added solutes, there is some justification in concluding that the typical growth reactions reflect the cell's adjustment to a change in turgor *per se* rather than to various chemical and electrical properties of the osmotica. The adjustments, which tend to restore normal growth rate after shifts in turgor in either direction, are made primarily in extensibility, not turgor.

The Directed Character of Expansion. In *Nitella* the ratio of relative rate of increase in length to that in diameter is normally about 4.5 (13). During the largest growth burst observed (fig 6) changes in diameter were also measured. The ratio temporarily fell to about 2.0, so it appears that high Ex_θ correlates with reduced physical anisotropy of yield in the wall. That extension occurring far above the minimum yield stress should show reduced anisotropy was predicted in the model for *Nitella* wall growth developed by Probine and Barber (28).

Inhibition of Helical Growth. The extension of the *Nitella* cell is normally accompanied by a pronounced rotation of the top of the cell relative to its base (12). In the present experiments a small weight (0.07 g when submerged) was tied to the base of the plant to keep it vertical. Because the cell was gripped at its top, this tension spanned the observed cell. This vertical tension, small compared to the 3.5 g exerted by turgor on the end walls of the growing cell, virtually eliminated the rotational component. This facilitated the following of individual marks. That such a small weight is effective is remarkable because it acts at a small angle (about 15°) to the direction of helical growth. One of the osmotica tested, 1,2 di-methoxy ethane, when present at 0.1 M, virtually eliminated twisting in unclamped cells.

Discussion

The results permit preliminary examination of the working relationship: relative growth rate = extensibility × turgor, as set forth in equation (I). The equation implies that shifts in turgor should lead to proportional shifts in growth rate. This clearly does not happen because the cell responds to a small drop in turgor by cessation of growth then a gradual resumption of the normal rate. This and other behavior shows that the variables in equation (I) are interdependent. The observed behavior could be the result of complexity in either extensibility or turgor (or both). Fortunately, the variation in turgor, beyond that brought on by changes in tonicity of the external medium, is relatively small. A minor increase in turgor (due to continued solute uptake or production) can be seen during the resumption of growth after a turgor drop (fig 6). A small decrease in turgor due to rapid sap dilution during a growth burst probably contributes to the decline to normal growth rate. These changes, however, are small compared to the concurrent ones in extensibility. It will develop that the value of the extensibility term is a function of turgor, growth rate, and time.

Cell Extensibility and the Cell Wall. Extensibility of a cell has been shown by Lockhart (21, 22) to be a function of several variables. It is inversely proportional to wall thickness, and proportional to cell radius and cell wall extensibility. Wall thickness in *Nitella* is known to increase comparatively slowly (40 %/day) during mechanical inhibition of growth (14). Cell extensibility may change several fold in an hour. Since cell extensibility increases during inhibition in the present experiments, wall thickness change is actually in the wrong direction to explain the extensibility changes. It is therefore not considered a primary factor. Cell radius can be measured and its total variation over 2 days (increase of about 15 %) is very small compared to changes in cell extensibility. It is concluded that

cell extensibility is primarily a function of extensibility of the cell wall.

Two Stages of Growth Response to Turgor Shift. The first step in an effort to modify equation (I) to permit prediction of growth behavior is to look for broad characteristics common to all or almost all the responses observed. Study of the reaction of the growing *Nitella* cell to sudden shifts in turgor (fig 3, 4, 5, 6, 7) reveals that its growth rate can be characterized by 2 stages of response. The immediate change in rate following a shift in turgor in *either* direction appears as an exaggeration of the effect that would be predicted by equation (I). That is, a moderate (25 %) drop in turgor leads to much larger (100 %) drop in growth rate; a moderate increase in turgor (33 %) in a growing cell (fig 4, 5, middle) temporarily leads to a much greater (up to several hundred percent) increase in growth rate. This rapid response is followed by a slower compensatory adjustment which tends to restore the normal rate: normal growth may be resumed after being stopped by reduced turgor; the high rates of growth seen immediately after an upward shift in turgor are gradually restored to normal (fig 8A).

Somewhat similar rate changes, but without rate recovery at reduced turgor, are found in non auxin-treated *Avena* coleoptile sections temporarily exposed to osmoticum (29). A transient reduction in rate, a return to normal, and a brief recovery period at a rapid rate are associated with the temporary exposure of growing *Phycomyces* sporangiophores to longitudinal stretch (9). The same general kinetics are found upon exposure of a light-grown sporangiophore to a period of darkness (3). This sort of rate pattern, seen here in the response of *Nitella* to transitory osmotic inhibition, can be analyzed in terms of turgor and extensibility.

A Preliminary Model. The immediate rate response (either decrease or increase) seen upon change in turgor could be based on a single relationship between Ex_g (or rate) and turgor which has a threshold value and, above this, has a positive slope. See figure 8B. The slow response could involve a transposition of this relation. During the typical experimental cycle the rapid and slow responses alternate. A normally growing cell would be at position 1 in figure 8A, B, with a turgor of 5 atm. Upon a rapid loss of turgor to 3 atm (position 2) growth would stop because this turgor is below the threshold. One assumption sufficient to give restored growth at reduced turgor involves a lateral displacement of the original curve, to the left, until the normal rate is resumed (position 3). This is one aspect of the slow response. The sudden return to 5 atm of turgor would give, temporarily, a very high rate of growth (position 4) and then the slow response would shift the curve to the right until normal rate and extensibility were restored (position 5).

The major aspects of this scheme that seem established are the disproportionate shift in rate upon change in turgor (in both directions) and the resumption of growth at low turgor. Data are insufficient to show that relationship between Ex_g (or rate) and turgor is of any specific form (such as the linear one in the diagram) and that this form is unchanged as the threshold value is displaced

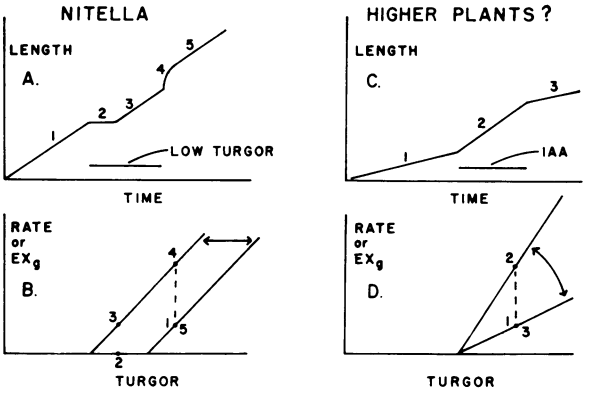

FIG. 8. A diagram to illustrate 2 types of growth response and their possible origin in different kinds of "wall softening." As a simplification, rate is considered roughly proportional to extensibility, Ex_g. *A* shows the typical sequence of length change in *Nitella* through a temporary reduction in turgor. This is accounted for in the model *B* where ordinary growth finds Ex_g near the base of an arbitrarily linear curve relating Ex_g to turgor (postion 1). A drop in turgor, position 2, reduces Ex_g and rate to zero. A shifting of the original curve to the left supports normal growth at position 3. Restoration of the original turgor causes very high extensibility and rate (position 4). A shift of the curve to its original position ultimately restores the normal growth rate (position 5). In C a transient stimulation of growth in higher plants by auxin is diagrammed. Its possible explanation, in terms of Ex_g and turgor, as inferred from the literature, is shown in *D*. The mechanism here is a change in slope of the curve without shifting the threshold. Note that the variation of rate and Ex_g is identical along the dashed lines in *B* and *D* but the underlying mechanism of rate change would be different The present method has the potential of describing the relation between Ex_g and turgor pressure through various growth responses.

Note added in proof: The work of Evans (Ph.D. Thesis, University of California, Santa Cruz, 1967) shows that auxin-grown *Avena* sections have a solute content of 8.2 atm compared to 10.1 atm for non-treated material. The threshold turgor for growth, however, was 3.4 atm and 7.8 atm respectively. Because the treatments were prolonged it is not known whether this reduction in minimum yield stress (and an apparent lateral shifting of the extensibility-*vs*-turgor relation) is the immediate action of IAA on wall properties or is the result of considerable elongation in auxin. Only in the latter case could diagrams C and D apply to *Avena* coleoptiles. The 2 model growth responses contrasted here never-the-less illustrate the potential utility of *in vivo* analysis of the extensibility-turgor relation in the study of wall softening.

along the turgor axis. This can be tested by breaking up the large turgor shifts into small steps. The various components of this tentative model can be examined for their generality and, where possible, for their physical or metabolic basis.

a) *A Threshold Value of Turgor for Growth and Extensibility.* All osmotica tested show that in *Nitella* a small drop in turgor leads to a cessation of growth and thus a reduction of Ex_g to zero. As seen at the left in figure 4 a drop in turgor of about 0.4 atm is sufficient. Since turgor was 4 atm the threshold value is roughly 90 % of full turgor. A threshold concentration (above the plasmolytic value) of osmoticum sufficient to stop growth, has often been reported for other material. Cleland (4) found that 0.25 M mannitol inhibited almost all growth in *Avena* coleoptile sections while 0.12 M inhibited almost all of the auxin-stimulated component of growth. Ray and Ruesink (32) showed that mannitol of 0.2 to 0.3 M stopped growth in tissues whose cell contents were about 0.4 osmolal. Bennet-Clark (2) found that KCl at a concentration of about 5.4 atm was sufficient to halt growth in material with an internal solute concentration of about 13 atm. While more dilute solutions may momentarily halt growth, this is not pertinent because the effect is probably a rapid elastic response which briefly compensates the extension accomplished by growth.

In a study of the gradual "creep" of excised *Nitella* cell wall strips Probine and Preston (27) found a threshold for extension at about 35 % of the stress normally experienced by the wall *in vivo*. Our data indicate a considerably higher threshold. It would be interesting to know if the *in vivo* shift in minimum yield stress would be reflected in the properties of wall strips from cells killed before and after adjustment to osmotic inhibition.

b) *Extensibility Above the Threshold.* The *in vivo* quantitative relation between increase in turgor and increase in Ex_g above the threshold, cannot be described by the data presently available. Upward shifts in turgor of varying amount, all starting from a given low turgor at which growth was occurring, could be used to discover the relation between increased turgor and increased extensibility, from that given turgor. Perhaps the curves would be similar for various turgors. At present no *in vivo* data is available. Probine and Preston, however, (27) have studied the mechanical properties of excised longitudinal strips of *Nitella* cell wall and found that, after a variable amount of "set" following application of a weight, the strips slowly yielded to the weight. Their data correlated well qualitatively with the normal growth process in that strips from rapidly growing cells crept relatively rapidly. In absolute terms the strips yielded more rapidly to the applied stress than they had been yielding to the equivalent longitudinal component of turgor stress. [*E.g.*, a 9 % yield at normal stress over 99 min was observed in a strip from cell that had grown 9.5 % over 1 day (1,440 min)]. The difference may reflect the fact that the strip could decrease its lateral dimension while the intact growing wall continuously increases its lateral dimension. A role for creep in the normal growth process was also supported by their finding that transverse strips showed negligible creep, correlating with the small increase in this direction *in vivo*.

Their data were scattered above the threshold but a plot of percent extension (ordinate) *vs.* load gave curves which rose roughly linearly. As already noted the threshold value was considerably lower than that seen *in vivo*. Such a relation, if reversibly shifted as in figure 8B, could give the typical response (fig 8A). Their data were later used in a theoretical model for *Nitella* wall growth that gave a linear increase in rate of movement of individual microfibrils, with stress, above a threshold stress (28).

The rapid "exaggerating" growth response to a shift in turgor in *Nitella* appears to have a counterpart in *Avena* coleoptile tissue. In auxin grown material, above a certain value for turgor, a shift in turgor will yield a relatively larger shift in growth rate. *E.g.*, a 14 % increase in apparent turgor (8.4–9.6 atm) gives a 39 % increase in rate over 4 hours as calculated from data of Cleland (4). Ex_g would also increase over this range. Unlike the data from *Nitella* there is growth below the break in the curve. This may reflect osmoregulation during the 4 hours. In osmotic inhibition of coleoptile section growth there appears to be little growth recovery by lowering of the yield threshold of the wall. In auxin-free medium, however, some action on the wall during inhibition is indicated by the rapid elongation that occurs upon restoration of full turgor (29). Because the yield point does not seem to be lowered, this softening may take the form seen in figure 8D. Here the increased extensibility would be due to a change in slope of the Ex_g-turgor relation rather than to a shift in the curve with constant slope as in figure 8B. In *Avena* the elastic component of elongation is greater than in *Nitella*; it would be included in Ex_g

c) *Movement of the Yield Threshold.* Movement of minimum yield stress to lower values of turgor is a widespread process. It undoubtedly occurs in many cases of initiation of expansion such as the branching of hyphae, rhizoid formation in the *Fucus* zygote, *etc.* Presumably the lowering involves a chemical breaking of cross-links in the wall so as to concentrate stress on the remaining links, causing them to give. If this process is reversible, a reduction in the concentration of the lytic agent would raise the yield value. An alternate mechanism is available, however, for the raising of the yield value. Higher plant tissues (and many inert materials) show "strain hardening", an increase in yield value coupled to the amount of stretch the material has undergone (5, 24). The metabolic nature of the lowering and the physical (or metabolic) nature of

the raising of the yield point is not known for *Nitella*. A rough similarity in the duration of the reduction and raising of the yield point in figure 3 suggests both might be metabolic.

Few higher plant tissue display yield point lowering, as evidenced in resumed growth at reduced turgor. In *Avena*, full restoration of growth rate at reduced turgor appears not to occur. After complete cessation, the amount of subsequent elongation over several hours is not much more than a recovery of elastic shrinkage (2, 26). The small amount of rate recovery after growth cessation, weakly enhanced by auxin at 5 ppm but not at 1 ppm (2), may be due to osmoregulation rather than to a change in wall properties because deplasmolysis is observed in strongly inhibited sections (26). Sucrose promotes increase in internal solute content and recovery.

Recovery of the full normal growth rate in a solution of osmoticum (0.19 M) strong enough to initially halt growth has been reported for potato by Thimann *et al.* (their table I) (36). This recovery only took place in the presence of auxin and appeared to require 2 days. Data on internal solute concentration were not given so it is not known if recovery was due to recovery of turgor and IAA-increased extensibility or a lowering of a threshold for extensibility (or a combination).

Two Modes of Wall Softening. One can recognize, even at the present abstract level of analysis, 2 extreme kinds of "softening." In terms of growth rate and turgor alone a shift from position 1 to position 4 in figure 8B would be indistinguishable from the shift from position 1 to 2 in figure 8D. The basis of the rate change in terms of the whole Ex_0-turgor relation could be quite different. Combinations of these 2 extremes can readily be imagined.

Growth Physics in Higher Plant Tissues. In comparison to the situation in *Nitella* the physical properties of higher plant tissues, manifested in their resistance to imposed stretch, appear less adequate to explain, in themselves, the *in vivo* responses to shifts in turgor. There are major mechanical differences between growth and imposed stretch, especially in killed tissue. A *decrease* in volume and surface area may accompany stretch (24). At any event, the high threshold of turgor needed for rapid growth *in vivo* is not matched by a high minimum yield stress in the artificially stretched tissue (25). The minimum yield force for increase in area in nearly plasmolysed mung bean is only about 5 grams (with IAA) to about 30 grams (without previous incubation in IAA). The hypocotyl's longitudinal component of normal turgor was calculated to be 235 g (24). Further, the *in vivo* relation between rate and turgor, which might be expected to correspond to a strain-rate *vs.* stress relation found in imposed stretch, does not obtain. Strain-rate and stress are almost independent. Starting at a rate 15 × the normal one for *Avena*, an increase in rate of imposed extension of 100-fold is accompanied by roughly a 15 % decrease in the yielding tendency of coleoptile tissue not previously grown in auxin (6, fig 8). With previous growth in auxin the decrease is roughly 25 %. Lockhart (23) found only small viscous properties in mung bean. A 5× increase in rate of stretch gave only a 50 % increase in the work of deformation. Temperature effects were minimal. The failure to find prominent viscous properties in stretch tissue has lead Cleland (personal commun.) to the view that growth occurs in a series of pulses statistically spread throughout the wall.

A pulse is started by the metabolic lowering of the yield threshold of the wall. When this falls below the existing stresses generated by turgor, the wall yields. The process of yielding however is coupled to an increase in the yield threshold, the phenomenon of strain-hardening. This ultimately raises the threshold above existing stresses to end the pulse. This process is called strain-hardening plastic deformation (SHPD) (5, 6). The action of auxin, which could potentially increase rate in many ways (*e.g.* increasing the frequency of pulses, rate of lowering of yield threshold, *etc.*), may be to change the coupling between extension (not rate of extension) and increase in the yield threshold of the wall. In stretched tissues it is found that this coupling is changed by auxin treatment so as to give more extension per unit increase in the yield threshold. Even below its threshold value for growth, increased turgor promotes the increase in compliance brought on by incubation in auxin (7). The present method cannot distinguish between viscous flow and either SHPD or a chemorheological mode of wall yielding (29, 31). The major component of the pulse growth mechanism that is obviously present in *Nitella* is a capacity to lower the yield threshold. This is seen in the resumption of growth at reduced turgor, a phenomenon not prominent in *Avena*. In the coleoptile it is therefore assumed that yield point lowering can occur only above a very high threshold value of turgor (Cleland, personal commun.).

In summary, there appears to be a high turgor threshold for growth and Ex_0 in many plant systems, including *Nitella*. The physical nature of yielding above this threshold is not clear but stretching experiments on *Nitella* cell walls indicate it could be a viscous process while stretch studies on higher plant tissues find little viscosity and inappropriately small auxin effects on it. The prominent auxin effect in stretch experiments is a reduction in strain-hardening, a process essentially independent of rate. To incorporate strain-hardening as a rate limiting step *in vivo* a pulsed metabolic lowering of the wall's yield threshold during pulses of growth must be assumed. This lowering is, curiously, a striking feature of *Nitella's* slow *in vivo* response to reduced turgor but it is not noteworthy in the response of growing higher plant tissues to reduced turgor. It

is suggested that further *in vivo* characterization of the extensibility-turgor relationship, in the manner diagrammed in figure 8, can resolve "wall softening" into sub-processes and thereby promote understanding of the growth process in terms of structure and metabolism.

Acknowledgment

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Literature Cited

1. ARENS, K. 1939. Bestimmung des Turgordruckes an einer Einzelzelle mit dem Manometer. Planta 30: 113–17.
2. BENNET-CLARK, T. A. 1956. Salt accumulation and mode of action of auxin. A preliminary hypothesis. In: Chemistry and Mode of Action of Plant Growth Substances. R. L. Wain and F. Wightman, eds. Butterworth and Company, London.
3. CASTLE, E. S. 1966. Light responses of *Phycomyces*. Science 154: 1416–20.
4. CLELAND, R. 1959. Effect of osmotic concentration on auxin-action and on irreversible and reversible expansion of the *Avena* coleoptile. Physiol. Plantarum 12: 809–25.
5. CLELAND, R. 1967. Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta 74: 197–209.
6. CLELAND, R. 1967. Auxin and the mechanical properties of the cell wall. Ann. N. Y. Acad. Sci. 144: 3–18.
7. CLELAND, R. 1967. A dual role of turgor pressure in auxin-induced cell elongation in *Avena* coleoptiles. Planta 77: 182–91.
8. DAINTY, J. 1963. The polar permeability of plant cell membranes to water. Protoplasma 57: 220–28.
9. DENNISON, D. S. AND C. ROTH. 1967. *Phycomyces* sporangiophores: fungal stretch receptors. Science 156: 1386–88.
10. FORSBERG, C. 1965. Axenic culture of *Chara globularis* Thuill. and *Chara zeylanica* Wild. Life Sciences 4: 225–26.
11. GIESE, A. C. 1962. Cell Physiology. W. B. Saunders Company, Philadelphia, Pennsylvania.
12. GREEN, P. B. 1954. The spiral growth pattern of the cell wall in *Nitella axillaris*. Am. J. Botany 41: 403–09.
13. GREEN, P. B. 1963. Cell walls and the geometry of plant growth. In: Meristems and Differentiation. Brookhaven Symp. Biol. 16: 203–17.
14. GREEN, P. B. 1963. On mechanisms of elongation. In: Cytodifferentiation and Macromolecular Synthesis. M. Locke, ed. Academic Press, New York.
15. GREEN, P. B. 1965. Anion-exchange resin spheres as marking material for wet cell surfaces. Exptl. Cell Res. 40: 195–96.
16. GREEN, P. B. AND F. W. STANTON. 1967. Turgor pressure: direct manometric measurement in single cells of *Nitella*. Science 155: 1675–76.
17. GUTKNECHT, J. 1968. Salt transport in *Valonia*: inhibition of potassium uptake by small hydrostatic pressure. Science 160: 68–70.
18. KAMIYA, N., M. TAZAWA, AND T. TAKATA. 1963. The relation of turgor pressure to cell volume in *Nitella* with special reference to mechanical properties of the cell wall. Protoplasma 57: 501–21.
19. KISHIMOTO, U. 1959. Electrical characteristics of *Chara corallina*. Ann. Rep. Sci. Works Fac. Sci. Osaka Univ. 7: 115–46.
20. KISHIMOTO, U., R. NAGAI, AND M. TAZAWA. 1965. Plasmalemma potential in *Nitella*. Plant Cell Physiol. 6: 519–28.
21. LOCKHART, J. A. 1965. An analysis of irreversible plant cell elongation. J. Theoret. Biol. 8: 264–75.
22. LOCKHART, J. A. 1965. Cell extension. In: Plant Biochemistry. J. Bonner and J. Varner, eds. Academic Press, New York.
23. LOCKHART, J. A. 1967. Physical nature of irreversible deformation of plant cells. Plant Physiol. 42: 1545–52.
24. LOCKHART, J. A., C. BRETZ, AND R. KENNER. 1967. An analysis of cell-wall extension. Ann. N. Y. Acad. Sci. 144: 19–33.
25. OLSON, A., J. BONNER, AND D. J. MORRE. 1965. Force extension analysis of *Avena* coleoptile cell walls. Planta 66: 126–34.
26. ORDIN, L., T. H. APPLEWHITE, AND J. BONNER. 1956. Auxin induced water uptake by *Avena* coleoptile sections. Plant Physiol. 31: 44–53.
27. PROBINE, M. C. AND R. D. PRESTON. 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. Exptl. Botany 13: 111–27.
28. PROBINE, M. C. AND N. F. BARBER. 1966. The structure and plastic properties of the cell wall of *Nitella* in relation to extension growth. Australian J. Biol. Sci. 19: 439–57.
29. RAY, P. M. 1961. Hormonal regulation of plant cell growth. In: Control Mechanisms in Cellular Processes. D. M. Bonner, ed. Ronald Press, New York.
30. RAY, P. M. 1967. Radioautographic study of cell wall deposition in growing plant cells. J. Cell Biol. 35: 659–74.
31. RAY, P. M. AND A. W. RUESINK. 1962. Kinetic experiments on the nature of the growth mechanism in oat coleoptile cells. Devel. Biol. 4: 377–79.
32. RAY, P. M. AND A. W. RUESINK. 1963. Osmotic behavior of oat coleoptile tissue in relation to growth. J. Gen. Physiol. 47: 83–101.
33. SIEVERS, A. 1967. Elektronenmikroskopische Untersuchungen zur geotropischen Reaction. III. Die transversale Polarisierung der Rhizoidspitze von *Chara foetida* nach 5 bis 10 Minuten Horizontallage. Z. Pflanzenphysiol. 57: 462–73.
34. TAZAWA, M. 1957. Neue Methode zur Messung des Osmotischen Wertes einer Zelle. Protoplasma 48: 342–59.

35. TAZAWA, M. AND N. KAMIYA. 1965. Water relations of Characean internodal cell. Ann. Rep. Biol. Works Fac. Sci. Osaka Univ. 13: 123–57.
36. THIMANN, K. V., G. M. LOOS, AND E. SAMUEL. 1960. Penetration of mannitol into potato disks. Plant Physiol. 35: 848–53.
37. VILLEGAS, L. 1967. Changes in volume and turgor pressure in *Valonia* cells. Biochim. Biophys. Acta 136: 590–93.
38. VIRGIN, H. I. 1955. A new method for the determination of the turgor of plant tissues. Physiol. Plantarum 8: 954–62.