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Growth Rate and Turgor Pressure

AUXIN EFFECT STUDIED WITH AN AUTOMATED APPARATUS FOR SINGLE COLEOPTILES[1]

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ABSTRACT

Because turgor pressure is regarded as the driving force for cell extension, any general theory of plant growth requires quantitative information on the relationship between steady irreversible growth rate and turgor pressure. To investigate contrasting views of this relation an automated apparatus was constructed which perfused both the outer and inner epidermis of a single coleoptile while its growth rate was continuously recorded. Turgor was altered abruptly by perfusing with solutions of varying tonicity. With specially grown rye coleoptiles the half-time of the osmo-elastic response was reduced to 2 minutes or less. After decay of this response, however, rate continued to change (so as to partially compensate the effects of the turgor shift in question) for 30 to 60 minutes. Only then could a steady rate be taken. A characterization of steady rate *versus* turgor covering five turgor values for a single coleoptile thus required many hours. The conclusions are as follows. (*a*) The change in steady rate, per unit change in turgor, was much greater +IAA than −IAA. (*b*) Both auxin and turgor act to reset an apparent stabilizing system whose presence is shown in the partial compensation of the initial response to turgor shifts. The above "extensibility" changes are operational only. They need not reflect changes in the immediate physical extensibility of the wall; they could reflect changes in a process acting on the wall. (*c*) The growth rate *versus* turgor relation shows some hysteresis.

In excised coleoptiles, it has been repeatedly shown that growth rate is promoted by the presence of auxin and by increase in turgor (achieved by reducing the amount of osmoticum in the medium). Any formal general statement about the mechanism of growth has to account for both kinds of promotion of rate.

Several possibilities for the joint action of pressure and auxin on growth can be seen in Figure 1 and the simple formula

$$r_s = (P - Y_s)\, m_s \cdot [r_s = 0 \text{ if } (P - Y_s) \leqslant 0] \qquad (1)$$

Here r is rate, P is turgor pressure (bar), Y is a yield threshold, a pressure (bar) below which no extension occurs and m is an "extensibility," the slope relating rate to $(P - Y_s)$. The subscripts indicate that the value is characteristic of steady rate, as distinguished from values accompanying transients between steady rates.

In equation 1 increasing pressure inevitably raises rate. Since auxin has not been found to raise the osmotic concentration of responding excised tissue (21), it appears not to act via changes in the P term. It could raise steady rate by either raising m_s (Fig. 1a) or lowering Y_s (Fig. 1b), or both could change. If r_s is a linear function of $(P - Y)$ as in equation 1 and Figure 1, the mode of action should be easily ascertained. If the true relation were concave upward, the mode of action of auxin action (curve shifting *versus* curve steepening) would be less obvious (12). Difficulty in answering this question centers on the determination of the rate to be considered typical for a given turgor value.

Treatments in Parallel. If sections are cut and floated for several hours on a concentration series of solutions, rate can be determined as the length increase observed, divided by time. Unfortunately the quotient is a function of (*a*) the course of osmo-elastic equilibration of the tissue's turgor pressure to the new value, (*b*) any transient adjustment of the irreversible growth rate to the turgor shift (as found in the present study), (*c*) time course of loss of endogenous IAA in the −IAA experiment, (*d*) effects of aging, and (*e*) possible slow osmotic adjustment of the tissue cells. Ordin *et al* (21) give the time course of the approach to a steady elongation rate, seen in a range of mannitol concentrations. Rate at a given apparent turgor pressure changes with time, and this drift is not parallel for the various solutions. The same is seen in the data of Bennett-Clark (1). When rate is taken as mean rate over the entire treatment in such studies the results indicate that steady rate is increased by auxin, in terms of Figure 1, through an increase in m_s (5, 17). That is, IAA increases the relative ability of turgor pressure to increase rate.

Treatments in Series. With continuous measurement of coleoptile length (singly or in the aggregate) one can attempt to measure the rate *versus* turgor relation by imposing a sequence of turgor shifts on the same tissue. This method could reveal details lost by averaging in the above method. A problem lies in choosing the time, after turgor shift, for taking steady rate. There is the possibility that an observed steady rate is really a composite phenomenon where, *e.g.*, a decaying elastic shrinkage is balanced by a decaying acceleration, this latter being part of the irreversible growth response to the shift (6). Data taken in this way have suggested, in contrast to measurements in parallel, that auxin increases steady rate primarily by the lowering of Y_s in equation 1 (8), discussed in Ray [25]; see Fig. 1b).

This second method, with its potential for use with single coleoptiles, needs only to have the above ambiguity with regard to osmo-elastic transients removed to be fully workable. Thus

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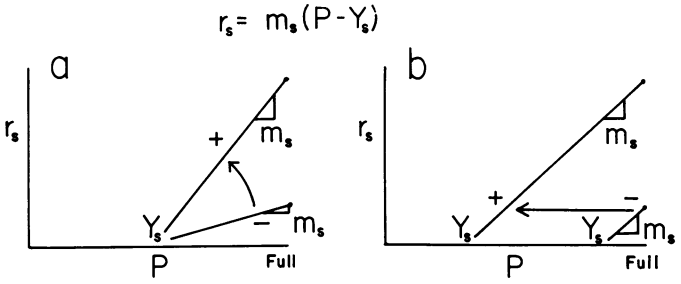

FIG. 1. Generalized formula for relating steady growth rate, r_s, to both turgor pressure, P, and to the action of auxin. a: Growth occurs only above a threshold turgor, Y_s, and increases above that threshold linearly with a slope of m_s. The action of auxin, to give high rate at full turgor, is to increase m_s while Y_s remains constant. b: The same relative increase in rate at full turgor is attained by lowering Y_s and leaving m_s constant. This diagram shows only extreme possibilities. Naturally auxin could affect both m_s and Y_s and it is possible that rate is not linear with $(P - Y_s)$. To a rough approximation, the present experiments favor the model in a.

to determine a steady rate of irreversible growth after a turgor shift one need only monitor growth rate continuously, sampling the value until it is demonstrably stable and show that osmoelastic changes can be effectively excluded from affecting this rate. The apparatus and procedures to be described were designed to meet these ends, and resolve the question of auxin action via m_s *versus* Y_s in equation 1.

Coleoptiles are the material of choice for such a study because they offer both an inner and outer epidermis for water exchange. Studying single coleoptiles, rather than aggregates, is advantageous for distinguishing elastic and irreversible extension on kinetic grounds. Direct continuous read-out of rate allows easy judgment on the attainment of steady rate. Because of the need for repeated shifts in turgor, and the long time required for attainment of a new steady rate after turgor shift (despite demonstrable rapid water movement and elastic change), experiments ran for 14 or more hours. For convenience and to ensure reproducible protocol, an automated apparatus was developed over a period of 2 years. In brief, the cavity perfusion offers quick response to turgor shift, while the apparatus gives high resolution of rate over indefinite periods.

MATERIALS AND METHODS

The major technical problem was attainment of effective perfusion without generation of excessive vibrational noise impairing accurate rate measurement. The apparatus is diagrammed in Figure 2.

Perfusion Chamber. The coleoptile was held vertically in a small flow chamber, the continuous flow refilling the chamber several times per minute. We have recently found a metering pump (Flow Metering, Inc., Oyster Bay, N.Y.) to be more reliable than any peristaltic pump arrangement. Fluid left by two routes is shown in Figure 2c. Most overflowed into a drain; the remainder was drawn by suction down the coleoptile cavity to perfuse the inner epidermis. To achieve the latter, the base of the coleoptile cavity was penetrated by a small glass tube, this junction being made tight by dropping a small elastic band (small disk of rubber with a central hole) over the coleoptile causing it to grip the tube (Fig. 2d). This lower tube was connected to a syringe needle which passed through a gasket in the bottom of the chamber. Suction was applied to the needle, the fluid going to a drain. For purposes of perfusion, the top of the coleoptile could be free. This end, however, had to be the site of physical connection to the transducer and hence a fitting was required. Again an elastic band caused the coleoptile to grip a section of glass tubing partly penetrating its cavity. This glass tube was glued (epoxy cement) to a short section of syringe needle which had had a small hole filed in one side. This hole became the entry port for fluid perfusing the cavity. The other end of the needle was the attachment site for a rod connected to the transducer core. The coleoptile and the two syringe needles were assembled into a single "tube" under a dissecting microscope. Curved forceps were used to push the lower syringe needle through the gasket at the chamber base to position the coleoptile. Perfusion over all surfaces could then proceed.

Transduction-Rate Measurement (Fig. 2a). The transducer used was a Linearsyn Sanborn 595 DT-100, amplifier-indicator 311A (Hewlett Packard, Palo Alto, Calif.) with a maximum range of 2.5 mm. The soft iron core of the transducer was glued to the top of a glass rod, and the lower end of the rod was glued to the upper (short) syringe needle affixed to the coleoptile. This was done after the coleoptile was in the chamber. A thermal cement (De Khotinsky cement, Fisher Scientific Co., Pittsburgh, Pa.) was used for this junction. A major problem in long term growth studies was the gradual bending of the coleoptile. This leads to rubbing on the side of the chamber generating great mechanical noise. Bending was prevented by exerting a small positive tension on the transducer core and

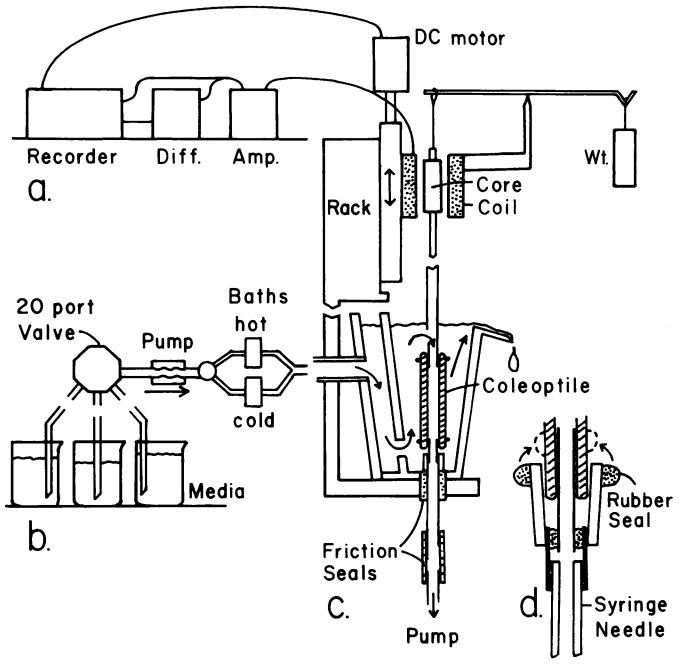

FIG. 2. Schematic of the apparatus. Part c is the small flow chamber (enlarged) showing the flow of solution (arrows) from the pump to the left. Part of the flow is pulled through the coleoptile cavity by a second pump (below). The coleoptile is affixed to tubes, at both its ends, by the arrangement whose section is enlarged in d. A hole in the upper tube allows both cavity perfusion and direct physical connection to the transducer core in a. Growth of the coleoptile raises the core, generating voltage in the coil which is both recorded directly and differentiated, over 6 sec, to give direct recording of rate. A circuit based on limit switches of the recorder resets the pen whenever it reaches the edge of the chart. This circuit uses a D.C. motor to raise the coil to compensate for growth. A small counterweight enhances straightness of growth. Patterns of solutions b are set up to feed a 20 port valve which delivers them to the pump at intervals fixed by a timer and stepper-switch arrangement (not shown).

hence the coleoptile. A thread or rod connected to the core went up to the end of a horizontal lever, a counterweight (net pull 0.9*g*) was applied to the other end. The fulcrum for the lever was affixed to the transducer coil.

To produce the desired rate resolution, it was necessary to amplify the transducer output 10 times. At this amplification, a transducer core displacement of only 0.25 mm produced full scale deflection of the pen monitoring the coleoptile length (on a Hewlett Packard Model 7100 BM, 25-cm strip chart recorder). Thus, for protracted studies, the transducer coil had to be repositioned upward (downward) after each 0.25 mm of growth (shrinkage) to prevent the recorder pen from going off scale. Limit switches on the chart recorder, which signal off-scale pen movement, were used to trigger a circuit which drove a reversible D.C. motor connected to the displacement screw of a "positioner" (Prior & Co., Bishop's Stortford, England) to raise or lower the transducer coil until the pen reached a standard position (a third switch breaking the D.C. circuit at that position. The range of the screw, and hence the ultimate range of the apparatus, was 10 mm. This circuit is shown diagrammatically in Figure 2a.

Voltage from the transducer gave length *versus* time directly on the strip chart. A custom-made box (by Mr. Payne Freret, Department of Electrical Engineering, Stanford University) took the derivative of voltage (averaged over 6 sec), which is a measure of rate of growth. Continuous read-out of rate allows clear assessment of the approach to equilibrium rate during an experiment.

Solutions and Automation. A 20-port valve (Chromatronix, Inc., Berkeley, Calif.) whose collecting tube advanced one port per pulse, was used to determine the sequence in which various solutions perfused the coleoptile. Potentially a pulse could be delivered every 10 min (or any other prescribed interval). By the use of a stepping switch and patch board the valve-advance circuit was completed only on selected cycles of the 10-min motor (Industrial Timer, Parsippany, N.J.). Thus the duration of perfusion by a given solution could be any multiple of 10 min, as desired. Valve advance interrupted flow for only 5 sec. A solenoid valve switched the moving solution through one of two constant temperature baths—one above ambient, the other below. Control of temperature pattern for anticipated studies can be governed by a second channel on the stepping switch, and a second patch board. Within the constraint of the fixed repeating timing unit (set for 10 min as described), one could program any pattern (up to 20) of solution or temperature changes at selected 10-min intervals (up to 51). A final position on the stepper was used to shut off the apparatus.

Safety Devices. The apparatus was designed for all night operation and a variety of potentially dangerous or expensive failures had to be anticipated (or designed for in retrospect). Various relays interlocked the system. One shut off all pumps while the valve advanced, to prevent surging. The travel of the coil was electrically limited by a microswitch kept in the conducting state only over a fixed range. Unlimited travel of the coil downward broke the chamber. As the unattended apparatus moved solutions in close proximity to 115-v lines, a flood detection-prevention system was constructed. Strategically placed sensors, microswitches held in the nonconducting state by a sugar cube, would close when wetted to activate a battery powered circuit which opened a relay supplying power to the entire unit. All current was taken from Ground Fault Interrupters (Rucker, Inc., Concord, Calif.) which shut off all power if any current leaks to ground.

Plant Material and Pretreatments. Initially, *Avena* coleoptiles were used, water exchange being speeded by stroking the inner epidermis with a rod coated with wet abrasive. Comparably rapid exchange was obtained, without physical treatment, with Balbo and Merced rye. For the present experiments, seeds were soaked in aerated water for 1 hr, then set on filter paper wicks which were saturated with a 1.5 bar solution of penta-erythritol. Seeds imbibed overnight at 4 C, were then given 3 hr dim red light and 2 days for growth in darkness, at 24 C. Plants were held at 9 C overnight for use the next day. Growth in solute appeared to suppress cuticle development since the rate of attainment of osmo-elastic equilibrium was enhanced. Growth in the apparatus was comparable for oat and rye but more consistent in the latter.

Coleoptiles of small diameter showed faster equilibration and were therefore selected for use. To obtain the −IAA state, coleoptile sections 14 mm long were preincubated on distilled water or dilute medium for 90 min. Clamping in the apparatus reduced the effective length of the coleoptile to about 10 mm.

Turgor Shift. All shifts were "nominal" in that turgor was not measured during the experiment. Solutions of mannitol differing in osmotic value by 1 or 2 bars were used for perfusion, and it is assumed that cell turgor departed from its "full turgor" value by the corresponding amount. Because of the rapid osmo-elastic changes recorded, it is likely that the tissue was close to osmotic equilibrium throughout. Solutions typically contained 1% sucrose and 10 mM phosphate buffer, pH 6.3. In the majority of cases the +IAA rate seen after stimulation could be reattained after 7 to 9 hr at lower turgor (Fig. 5). This compares well with the stability seen in batches of floating sections (2).

RESULTS

Attainment of Steady Rate after Osmo-elastic Transient. Under conditions of minimal growth (low temperature, nearly mature tissue, or metabolic inhibition), the time-course of rate change following an imposed turgor shift reflects osmo-elastic adjustments of the tissue. These may accompany the pressure change (in concurrent elasticity) or, in principle, lag behind the shift (retarded elasticity). If both kinds are present, one would expect a biphasic character to the course of compensation of the turgor shift. Following the initial immediate deflection there would be an initial rapidly decaying phase for concurrent elasticity, then a slow phase for the retarded component.

In Figure 3, a to c, a pattern of four turgor shifts is applied to nearly mature tissue which has minimal growth rate. Curves for length change (Fig. 3b) and rate change (Fig. 3c) are given. The exponential character of the approach to steady rate (the last being essentially zero) is seen in a semilogarithmic plot of the time course of rate change for the turgor shifts (Fig. 3d). Use of the absolute value of the difference between present rate and ultimate rate, $|r - r_s|$, allows similar plots for the adjustment to turgor step-up as well as step-down.

The semilog plots are nearly linear, with half times slightly more than 1 min. Half-times of 5 to 15 min are reported for *Avena* coleoptiles without perfusion of the cavity (28). With a half-time of 1.2 min, osmo-elastic features of the response to turgor should be over in about 10 min (Fig. 3, b and c) and this should suffice for the attainment of steady rate after turgor shift in growing tissue. When comparable treatments are applied to a growing coleoptile, the expected rapid response and subsequent rapid exponential decay of rate are seen initially. Adjustment of rate is not completed, however, under the rapid regime. Rate continues to change, but does so with a half-time of 6 to 10 min. This is seen in Figure 3, f and g. The course of rate change in response to the turgor sequence in Figure 3e is given in Figure 3f. Note that steady rate, r_s, is not attained

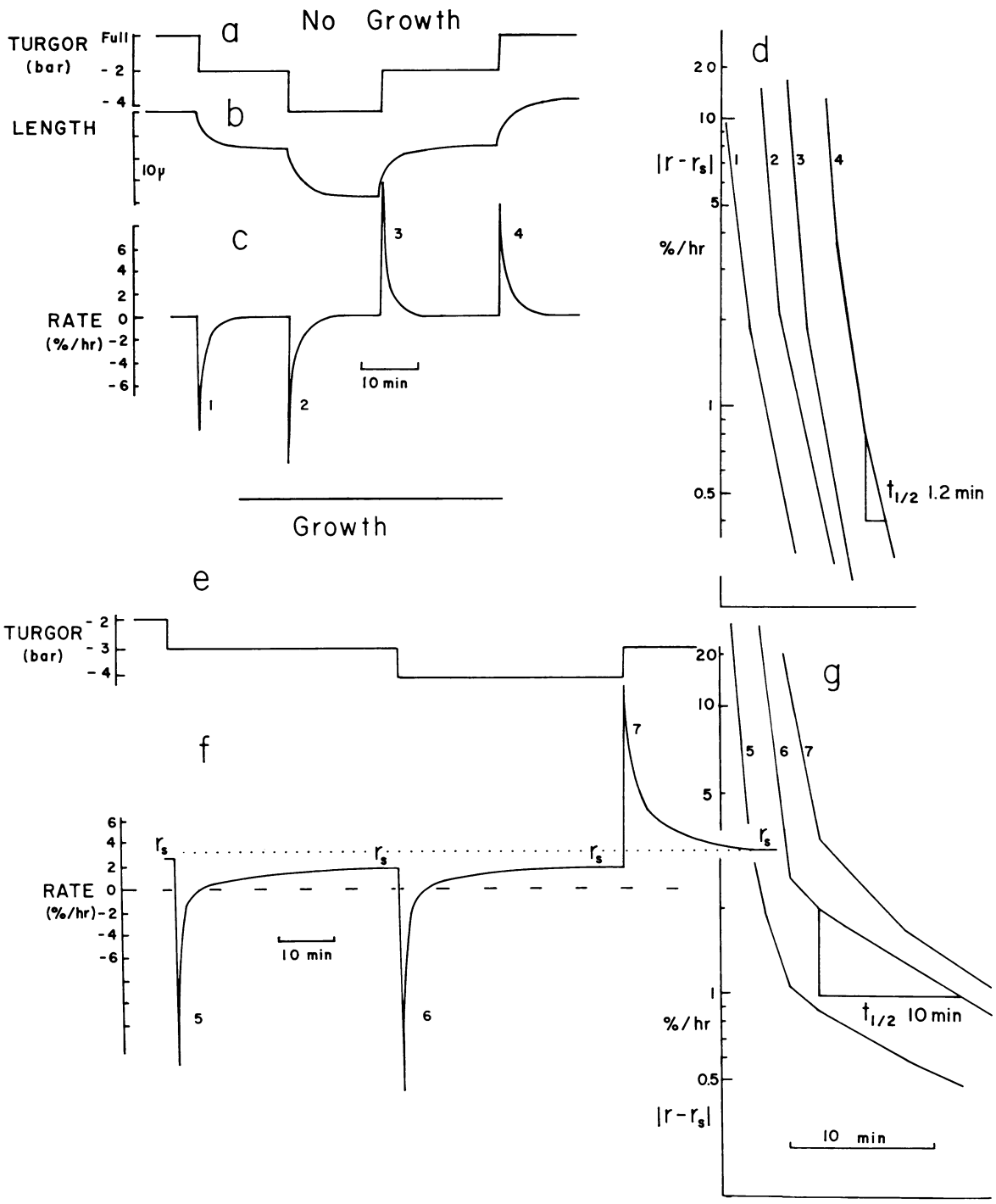

FIG. 3. a to d: Time course of the osmo-elastic response in nearly mature tissue. a: Sequence of nominal turgor changes, as brought on by changes in the osmotic value of the perfusion medium; b: corresponding length changes in a single coleoptile. These apparently reflect the course of turgor change, being complete in about 10 min. c: Parallel tracing of the rate pen for the above. Note that the tissue responds almost immediately to the new solution, almost all of the transient effect involves the decay of this initial response. The nearly linear decay characteristic of these numbered transients is seen in d where the absolute value of the difference between rate at any moment and final rate (here nearly zero) is plotted semilogarithmically. The simplest interpretation is that a water potential difference is rapidly set up across cell membranes and is then eliminated by the flux of water across the membrane. As this flux is driven by the water potential difference, an exponential decay is expected. e to g: Determination of r_s; nature of hysteresis. Format as in a, c, d (b eliminated). Note that the immediate rate shift and rapid subsequent compensation are similar to that seen in the nongrowing material. Rate does not come to a new steady value, however, for more than half an hour whereupon r_s is taken. The slow, second, compensatory response is particularly prominent after turgor step-up (transient 7). The two phase decay is evident in the semilog plots in g. Note similarity of slope of the initial phase of decay. This indicates that osmo-elastic behavior typical of the nearly mature state is still present. The initial magnitude of the rate shift is relatively greater in the growing material, but the decay characteristics are comparable for the first few minutes. If irreversible rate were an immediate function of turgor, one would expect the rate vs time curve to show osmo-elastic transients of the type in a to c superimposed on a simple stair-case pattern for rate. The protracted adjustment of rate to turgor shift, after osmo-elastic transients should be completed, is unexpected. The sequence in f, also shows strong hysteresis. Note that the r_s decreases less in the two turgor step-downs than it increases in the single step-up shown at right.

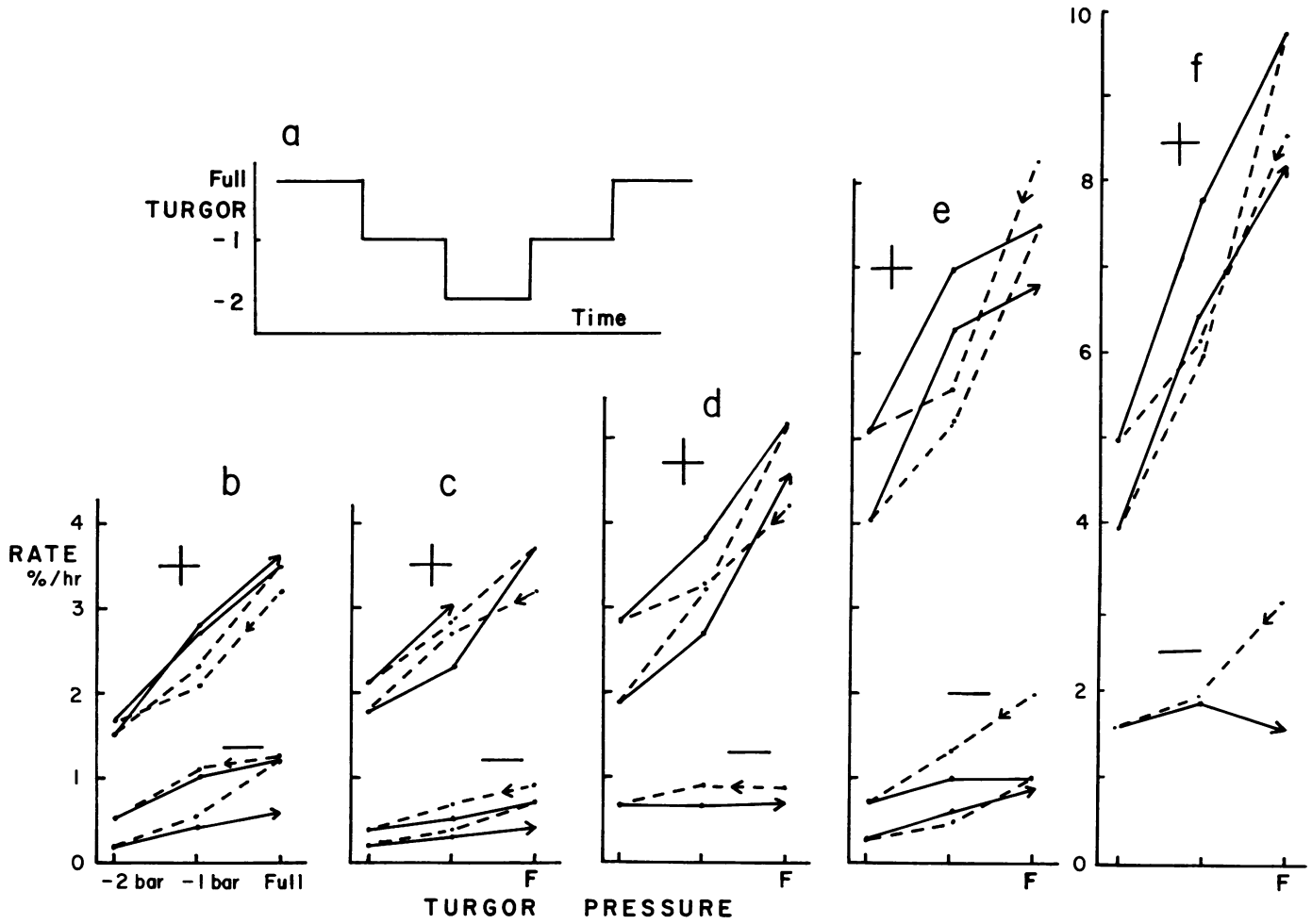

FIG. 4. a: Nominal turgor pattern imposed, in most cases, twice before application of auxin, then twice afterwards; b to f: patterns of steady rate, r_s, in coleoptiles of varying mean rate in auxin. In each case the −IAA pattern is low on the graph. The sequence of turgor shift is made evident by the arrows and by the showing of values taken after reduction in turgor in dashed lines, values for ascending turgor in solid lines. Note that (*a*) slope of the relation is greater +IAA than −IAA and (*b*) it is greater, for the same departure from full turgor, in the faster growing material. The variations came from differences in preincubation conditions but were not consistent enough to report on except that short preincubations gave faster, but more erratic behavior, especially −IAA, as in f.

until 40 min after the turgor shift. In Figure 3g, the shift from a rapid to a slow half-time for rate changes in growing material is evident. Because there is no indication of a comparable slow rate-decay component in nongrowing material at 20 C (nor in originally rapidly growing tissue chilled to 0 C, data not shown), we conclude that the slow adjustment pertains to the irreversible component of growth. It thus appears that while osmo-elastic changes are essentially over in 10 min, additional growth transients, always compensatory to the rate change brought on by the shift, persist much longer. The higher the growth rate the more prominent these transients, so duration of time in a given solution was adjusted to allow attainment of steady rate. This required that "steady rate" be evaluated only 30 to 60 min after a 1 bar shift in turgor.

Steady Rate *versus* Turgor Relation. Two general protocols were followed. In the first, the same small cycle (0, 1, 2, 1, 0 bar reduction in turgor as in Fig. 4a) was given twice prior to stimulation by auxin. After stimulation it was given twice again to the same coleoptile. In the −IAA condition, there was typically a gradual rate decline through the cycles, presumably due to the continued loss of IAA from the section. This was seen in runs not involving turgor shift. The relative sensitivity of r_s to turgor was low, changes in P of one bar leading to changes in r_s of roughly 0.3%/hr or less. The same cycles applied later to the same piece of tissue after auxin stimulation to higher rate typically revealed a much higher sensitivity of r_s to P. One bar shifts gave rise to changes in r_s of 1%/hr or more. It was found that the higher the mean rates in question, the higher the sensitivity to turgor shift. Compare the series of graphs in Figure 4, b to f. Graphs shown are selected from runs where the final +IAA rate was about equal to the initial steady +IAA rate.

To see if the above slopes could be legitimately extrapolated to lower turgor values, a second protocol involving four consecutive downward steps of one bar, then reversal, was applied. The sections had been preincubated −IAA and then responded to IAA while in the chamber. This was to assure that rapid growth was the result of auxin action. In such cases, the steady rate fell more gradually as the turgor was progressively reduced (see Fig. 5). This alone suggests a paraboloid relation between r_s and P. Retracing the turgor sequence, however, showed a strong rise in steady rate to the first ascending step. That a steady rate was achieved is seen in the rate pattern in Figure 3f. Such data reveal that r_s values are on a hysteresis loop, usually roughly symmetrical about a straight line. On a long enough time scale, it is possible that the hysteresis would diminish, as it

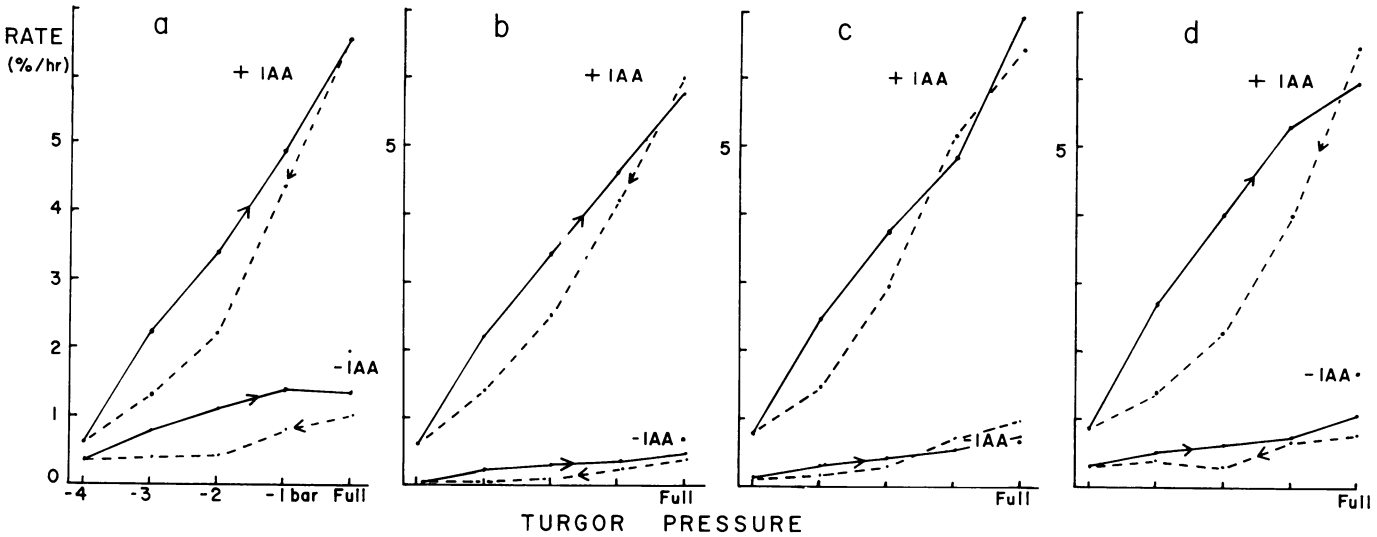

FIG. 5. Similar to Fig. 4 except that only one cycle of four steps down, four up, is given. Upper curves are after stimulation by IAA (from the rate shown as a dot on each graph). The cycle took 7 to 9 hr and runs were selected where the +IAA rate was about the same before an I after the cycle. Note that a threshold turgor is approached at 4 to 5 bars below full turgor and that the relation shows hysteresis. At a given turgor, the rate is generally higher when approached from below (——) than from above (- - -). Further reduction in P may well move the apparent Y_s further to left. This would lower the mean slope of the +IAA but not to the degree where it would be equal to the −IAA slope (Fig. 4). Lower curves are from comparable experiments with other coleoptiles where IAA was not added.

does in most systems showing hysteresis, but the time required to test this appears long compared with the growth period of the tissue.

Note that the hysteresis is not a result of aging. Rate is too high as turgor is increased in the latter half of the turgor cycle. The mean slope of the loops in IAA-treated material was always greater than slopes seen in comparable −IAA material (lower graphs from other coleoptiles). It is possible that extending the turgor range further might lower the mean slope of the +IAA loop. Hysteresis was occasionally seen in protocol No. 1, Figure 3, e and f. That rate approaches zero when P is reduced 4 to 5 bars below full turgor, as shown in the data of Cleland (5). With full turgor in rye being about 10 bar (by plasmometry), this gives Y_s a value of 4 to 5 bars. The hysteresis suggests, however, that more severe reduction in P might indicate a smaller Y_s.

A correlation of greater turgor sensitivity with the +IAA state (about proportional to the IAA effect on steady rate) is seen. This is tempered with the realization that the system shows hysteresis. Within hysteresis loops, the mean slope of small loops (small range of turgor treatment) differs from the mean slope of the full loop. The data clearly favor auxin action on steady rate via a change in m_s in Figure 1 (as against change in Y_s), but they also indicate that no simple algebraic statement will suffice to predict rate precisely, even steady rate. The characteristics of the time course of response to the various turgor shifts are under study in search of an analytical statement that will embody the main features of the response to turgor pressure, and auxin's effect on this response.

DISCUSSION

Technology. The value of precise continuous recording of growth rate of single coleoptiles was first evident in the publications of Ray (24) and Ray and Ruesink (27) where the time course of the IAA response and growth responses to metabolic inhibitors, turgor shift, *etc.*, were studied. Amplification was optical, and recording manual. Since that time many laboratories have used similar methods (20, 22). Some have added further amplification by lining up segments of the tissue of interest in a column and measuring aggregate length either optically (10) or with a transducer (7, 29). This, of course, averages the kinetic responses, a disadvantage when the objective is to distinguish rapid osmo-elastic responses from longer term growth adjustments. The major new features of the present apparatus are the continuous perfusion of both the inner and outer surfaces of the hollow tissue during rate measurement, and the differentiator which determines rate over a period as short as 6 sec. The recent history of growth measurement is covered in a review by Evans (9).

Growth Rate. The dependence of steady rate upon turgor pressure complies with the general view that the cell is extended by the yielding of the cell wall to the pressure of the cell interior (26). Evidence for a finite minimum turgor (Y_s) for growth has been found in soybean by studies using osmotic inhibition ($Y_s = 4$ bars) and an elegant direct pressure inhibition technique ($Y_s = 2$ bars) by Meyer and Boyer (18). A Y_s has also been found in roots (11). That geotropism initiates growth in wheat leaf sheaths clearly shows that a yield threshold has been exceeded (3). In the present work, we find no absolute Y_s but, rather, an approach to zero rate when turgor is reduced by 5 bars.

Because the yielding cell wall must sustain a gradient of some 10 bars across a few μm of wall thickness while neither bursting nor becoming inextensible, it is reasonable to expect more than a simple yielding. An intimate role for metabolism is evident in the biochemical role for auxin and the very close temporal dependence of rate upon metabolism (25). Our understanding of how the gross physical yielding is linked to the various components of metabolism is relatively limited. A first step, however, is the realization that the yielding rate is not constant after the osmo-elastic transients have decayed (Fig. 3f). This is also seen in *Nitella* (14) where the slow compensation could be shown not to be due to turgor adjustment because internal cell pressure was monitored throughout.

Compensatory adjustment presumably reflects a self-stabilizing scheme for wall yielding. Auxin must reset the balance point of the stabilization; otherwise, its action would be can-

celed. Thus the extensibility/turgor values are operational only and need not directly reflect changes in the immediate physical properties of the wall. It can be concluded only that some component of the stabilization scheme has been altered. This component could be a process acting on the wall rather than a property of the wall itself.

Hysteresis. Normally the relation between a treatment and response can be represented by some kind of a linear plot. When the magnitude of the response is a function of directionality of the treatment (increasing *versus* decreasing its magnitude), the relationship can be portrayed only as a loop. Most systems showing such hysteresis are "reluctant to change."

The electromagnetization of iron is a well known example. Reversing the sign of the current has relatively little effect until a large change has been made because the iron crystallites are reluctant to change their state of magnetization. Hence response (degree of magnetization) lags behind variations in treatment. A similar lag is seen in the cyclic removal/re-entry of water into soils where the passage of menisci through small cavities is a barrier to change and leads to hysteresis (19). In the context of cell walls, hysteresis of this sort is found in the relation between cell volume and turgor pressure in *Nitella* where volume falls, initially "too slowly" as pressure drops, rises "too slowly" (initially) when pressure is increased (30). Asymmetry of water movement is found in *Nitella* where water enters the cells more easily than it leaves (15). Since water is entering the rye cells at all values of r_s, this asymmetry would not apply. Further, the osmo-elastic studies in nongrowing rye tissue, where shrinkage and expansion are compared (Fig. 3, a to c), give no indication of nonreciprocal patterns of water movement.

In cyclic stretching of mung bean tissue, Lockhart (16) found hysteresis in elastic behavior, again of the sort that the system was reluctant to change. Since he was measuring reversible length change and we are measuring rate of irreversible length change, no parallel need be expected. It is, nonetheless, worth noting that the *in vivo* rate of yielding properties seen in rye show hysteresis of the *opposite* sign. That is, the system is "anxious to change." This implies that adjustment to lower turgor somehow "primes" the cell (not necessarily the wall) for unusually rapid extension upon return to higher turgor. By the same token, increases in turgor appear to severely impair extension upon any drop in turgor.

The complex (biphasic) adjustment to turgor shift *in vivo*, not detected in mechanically stretched plasmolyzed tissue by Lockhart, appears to involve a stabilization process (13). Hence the observed hysteresis may be that of a regulating system rather than of any simple physical phenomenon. Understanding must await further *in vivo* characterization of the yielding process.

Nature of the Pressure Effect. The cell wall is strong and it is reasonable to expect that one role of turgor is to physically distend it (26). The complex response of growth rate to a single shift in turgor probably involves additional effects on metabolism. It is unlikely that these involve a thermodynamic action via volume changes in reactions because, in a variety of biological processes, increase in pressure inhibits (rather than promotes) and 50% changes in rate in even the sensitive systems involve 200 bars of pressure (23). Other modes of action on metabolism are indicated by Chrispeels' (4) report on inhibition of protein synthesis by reduction of turgor (only glycols were tested) in barley aleurone. In rye growth, the immediate response to turgor drop is an abrupt fall in rate which presumably reflects the consequence of a drop in stress on the wall. The subsequent readjustment of rate, potentially related to a change in protein synthesis, is upward and thus of the wrong sign to be explained in any simple way by the effects Chrispeels has noted. For the moment the rye turgor shift response can be viewed as the reaction of a stabilizing system to perturbation, one component of the system being reset by pressure. This component, or another, is reset by IAA so that rises in turgor are much more effective in raising rate.

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