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PLANT CELL EXPANSION: Regulation of Cell Wall Mechanical Properties

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CONTENTS

"To make sure that all written records agree with the orthodoxy of the moment is merely a mechanical act."

George Orwell *1984*

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INTRODUCTION

To resist the tendency of plant protoplasts to take up water in response to large osmotic gradients, primary walls must be extremely strong. What is remarkable about primary walls, however, is not their rigidity, but their ability to undergo metabolically controlled yielding in response to turgor pressure, allowing plant cells to enlarge by an order of magnitude or more. Without this ability, growth and morphogenesis would be impossible. It is not surprising, therefore, that the mechanism of wall extension has been a primary concern of physiologists interested in development for over 50 years.

The last review in this series specifically on the topic of wall extension was by Cleland in 1971 (33). Since then, the field has advanced considerably and a voluminous literature has accumulated. Recent general coverage of cell expansion is available in articles by Cleland (47), Penny & Penny (274), Zeroni & Hall (440), and in books by Frey-Wyssling (95) and Preston (291). More specialized reviews have appeared on various aspects of cell expansion, including mathematical models (81, 356), biophysics (126, 130), the role of ion currents (422), turgor pressure and cell water relations (68, 441), cell wall architecture (335, 409), mechanical properties (354), chemical structure (70, 93, 94, 167, 186, 262, 263, 326), biosynthesis (263, 409), and turnover (93, 186), as well as the effects of auxin (39, 42, 50, 74, 212, 301, 311), gibberellic acid (164, 171, 174), ethylene (78), fusicoccin (206), and blue light (60).

This review will attempt to integrate some of the major biophysical and biochemical components of wall extension, with an emphasis on the events occurring within the wall itself. Because recent studies on the directionality of cell expansion in *Nitella* have shed light on the stress-bearing region of the wall, with important implications for the control of the rate as well, both the directionality and the rate of cell expansion will be considered. However, no attempt has been made at a comprehensive literature review of these two topics! The use of auxin and other growth regulators as probes for altering growth rates and wall properties will be discussed in detail, particularly the short-term effects which may be temporally correlated with events in the wall. However, metabolic processes involved in growth, including the primary sites of hormone action, will not be treated, nor will the recently revived controversy over the role of hormones in the regulation of growth in the intact plant [(136); see TIBS (1983) 8: 354–57) for an entertaining debate]. The discussion will be restricted to the type of extension typical of plant parenchyma and epidermal cells (26) which occurs uniformly over the cell surface, termed "surface growth" or "diffuse growth," as opposed to "tip growth" of pollen tubes, root hairs, and fungal hyphae (125).

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DIRECTIONALITY OF CELL EXPANSION

Dynamics of Wall Architecture

NITELLA The directionality of cell expansion is governed by the wall's structural and mechanical anisotropy and the prevailing pattern of wall stresses. Although turgor pressure is nondirectional, the stress pattern will vary with cell shape. Since filamentous plant cells such as *Nitella* approximate thin-walled cylinders, there is a 2:1 transverse to longitudinal stress ratio on the side walls (25, 195). The orthogonal stress on the end walls is nullified by the opposing force of the adjacent cells. *Nitella* internodes typically enlarge from shortened discs 20 μm in length to highly elongated cylinders 6 cm long and 0.5 mm wide. The length to diameter relative growth rate (RGR) ratio is about 5:1 throughout development (125). To overcome the unfavorable stress ratio, which would tend to promote growth in girth, the wall must be circumferentially ("hoop") reinforced. While some degree of order is evident in the matrix (229, 291), it is generally accepted that the overriding influence on the directionality of the wall's mechanical properties is the orientation of the cellulose microfibrils (130, 144), and more specifically, the microfibrils of the inner wall layer (322, 323). The crystalline microfibrils serve as a discontinuous filler, augmenting the strength of the matrix by introducing shear interactions. These shear forces are maximal when the wall stress is parallel to the orientation of the microfibrils (415), as has been found in advanced composites (59). The importance of microfibril angle in determining cell shape in *Nitella* has been demonstrated by Green and co-workers (125) using microtubule toxins such as colchicine or isopropyl-phenylcarbamate (IPC) to bring about the deposition of random microfibrils. Such cells tend to grow as spheres rather than as cylinders (125).

The three-dimensional organization of the microfibrils conforms to the classical "multinet" model of wall growth (121, 123, 295, 327, 328). New microfibrils are deposited by apposition in a scattered but predominantly transverse orientation (122, 291, 294, 322). As a consequence of cell expansion, an individual wall layer is thinned and the wall polymers, including the microfibrils, are passively realigned in the direction of maximum strain (123, 240, 241, 291). The degree of angular displacement will depend on the amount of strain (82, 291). At the same time, the extending layer is displaced toward the outer surface by the continued apposition of new wall material.

The evidence for multinet growth in *Nitella* walls is very strong. First, the microfibrils exhibit the predicted gradient in orientation (transverse at the inner surface, longitudinal at the outer surface). Apposition has been demonstrated both by autoradiography (122) and, more recently, by use of the inhibitor of cellulose synthesis, 2,6-dichlorobenzonitrile (DCB) (147, 322a). Cellulose-

poor wall layers deposited in the presence of DCB have a characteristic texture in the electron microscope. By pulsing *Nitella* cells with DCB, Richmond (322a) has been able to mark the newly deposited wall layer and to visually monitor it as it is displaced from the inner to the outer wall surface. As expected, the layer undergoes thinning because of wall extension. There is also strong support for the principle of passive realignment. Gertel & Green (108) showed by physically clamping living cells that reorientation of the microfibrils in the outer wall layers (as measured by the birefringence patterns of torn edges) did not occur in the absence of a longitudinal strain. Physically stretching living cells enhanced the realignment of the outer microfibrils. In contrast, neither clamping nor stretching altered the deployment of the inner microfibrils, consistent with the generally accepted view that the deposition angle is controlled by microtubules in the cytoplasm (134).

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HIGHER PLANTS At present there is no theoretical basis for estimating the stress patterns on the walls of cells within tissues. Until such an analysis is available, we can only speculate about the mechanical significance of primary wall architecture. One approach is to regard the epidermis as an outer limiting boundary (126). In stems, the transverse stress on the walls of cells in the central region is largely nullified by the opposing force of neighboring cells (195). Thus, in principle, the walls of cells in the central region should need little or no transverse reinforcement to expand predominantly in length. In reality, the walls of these cells do bear some of the transverse stress, as evidenced by the presence of intercellular air spaces where the transverse stress is not cancelled (D. Q. Fletcher, personal communication). However, the transverse to longitudinal stress ratio will still be less than 2:1. If the transverse stress of the stem were borne entirely by the epidermis, the stem would expand preferentially in girth because the orientation of microfibrils in the epidermis of many stems is longitudinal (28, 193, 379, 380, 406, 407). In pea stems it has been observed that excised epidermal layers (407) or bored out cylinders consisting of epidermal and outer cortical cells (391) expand preferentially in diameter. In Azuki bean hypocotyls there is a progressive shift from transverse to longitudinal microfibrils on the inner surface of the tangential wall of the epidermis as the cells elongate (379).

The classical observation that the two halves of longitudinally split stems and coleoptiles bow outward when floated in water (391) suggests that the central tissues are normally under compression while the epidermis is under longitudinal tension. The thickened outer epidermal wall and longitudinal orientation of the microfibrils are consistent with the longitudinal stress of the stem being borne primarily by the epidermis. At least some of the outward curvature, which develops slowly, must be the result of a water potential gradient introduced by exposing the central cells to the external solution. However, the fact

that auxin causes the split halves to bow inward is strong circumstantial evidence that the hormone alters the extensibility of the epidermal wall. In roots the situation is reversed, i.e. the two halves bow inward (21, 391). From this we might infer that the longitudinal stress of roots is borne by inner rather than by outer tissues. Further studies with split roots might help to delineate the tissue specificity of hormones and other factors.

In higher plants, the walls of the subepidermal parenchyma of *Avena* coleoptiles have been shown, by a variety of microscopic techniques, to be of the multinet type (289, 417–419). The "tubular" texture is also found in meristematic cells and the parenchyma of many elongating stems and hypocotyls (95). However, in some algae [e.g. *Chaetomorphia* and *Cladophora* (291)] as well as higher plant epidermis (28, 379), collenchyma (418, 419), and parenchyma (327) cells, alternating layers of transverse and longitudinal microfibrils have been observed. This pattern has been referred to as a "polylamellate," "multiply," or "cross-fibrillar" (323). Preston (293) has stated that authentic parenchyma have only multinet type walls, and that polylamellate walls are diagnostic for collenchyma or their precursors. The ultimate identity of such cells is not important for the present discussion. The extensive investigations of Roland, Vian, and co-workers (329, 331–336) documenting the presence of the cross-fibrillar wall architecture in elongating parenchyma have led to the "ordered fibril" model of wall architecture, in which the angle of microfibril deployment changes with time, creating the well-known herringbone pattern seen in electron micrographs. According to the new hypothesis, the directionality of expansion is controlled by the specific loosening of either the longitudinal layers (causing elongation) or the transverse layers (causing lateral expansion) throughout the thickness of the wall. The presence of the multi-ply organization through the wall thickness suggested that no passive realignment occurs during elongation, in contradistinction to the multinet model (333). However, it has now been mathematically demonstrated that the angular displacement of microfibrils during the elongation of higher plant cells may be too small to detect (293). More recently, the principle of passive realignment has been incorporated into the ordered fibril model, thus bringing it closer to multinet growth (331, 351). Sargent (351) has suggested that the crossed-fibrillar outer wall of barley epidermal cells results from extension strain acting on an initially helicoidal inner wall layer. In contrast, Roland et al (331) have proposed that the initial order of the wall is a prerequisite for cell wall extension, i.e. the microfibril organization is in some way involved in regulating wall extensibility. This order is lost ("dissipated") during the course of elongation, and the loss of order is correlated with the decline in the potential for growth. The wall is thus described as a "dissipative structure," which uses up its growth potential when the highly ordered wall structure is lost (331). However, these correlations do not establish cause and effect. There is no evidence that microfibril

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angle influences wall extensibility [with the possible exception of the so-called "neutral angle" at 54.7° (144)], although it is clearly the main determinant of wall anisotropy (144, 291). Probine & Barber (294) reported that only mature *Nitella* walls show the herringbone pattern, yet they have lost the ability to grow. Finally, the organization of the middle and outer wall layers may not be important for extension if the inner wall layer bears the stress.

The Stress-Bearing Region of the Wall

Roelofsen (327) originally proposed that the transverse stress is borne primarily by the inner wall layers containing transverse microfibrils, while the longitudinal stress is borne by the outer layers where longitudinal microfibrils are located. It has long been appreciated that the identification of the stress-bearing region of the wall is critical to a basic understanding of wall mechanics, but the problem went unsolved for lack of an experimental approach. The breakthrough came in the form of a careful study of the time-course of events during the shift from elongation to lateral expansion which occurs in *Nitella* cells treated with colchicine, IPC, or trifluralin. Richmond (322) monitored both elongation and lateral expansion by time-lapse cinemicrography; polarized light was used to monitor the distance between the two dark isotropic bands which are visible against the bright background of the birefringent wall. When expressed as a "birefringence index" (ratio of the distance between the bands to the cell diameter) a value proportional to the net microfibrillar orientation is obtained (322). Treatment of elongating cells with antimicrotubule drugs caused a rapid decrease in the index, indicating that deposition of a more isotropic wall layer begins soon after treatment. After about 15 h there is a complete reversal in the polarity of expansion, i.e. the RGR in diameter is 5 times the RGR in length. This is approximately what one would predict for a mechanically isotropic wall responding to a 2:1 transverse to longitudinal stress ratio, if Poisson ratio effects are also taken into account (323). Interestingly, the birefringence index increases slightly at this time and the overall transverse alignment persists. This is interpreted in terms of the multinet growth model as passive realignment of the outer microfibrils in the direction of maximum strain, i.e. their transverse alignment becomes slightly improved (322, 323). During the 15 h lag time the cells, which are growing at RGRs of about 1.5–2.0%, increase their surface area by roughly 25% without any apparent change in wall thickness. This implies that the inner 25% of the wall has been replaced while the outer 25% has been thinned to zero thickness. It follows that the directionality of expansion is determined by the properties of the inner 25% of the wall, which must therefore be the stress-bearing region (both transverse and longitudinal). The outer wall layers are too weakened by thinning to contribute significantly to wall rigidity. This can be demonstrated dramatically by treating young *Nitella* cells with DCB. After replacement of the inner portion of the wall with a cellulose-poor wall layer the cells burst (322).

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Recent studies in which ethylene and colchicine were used to perturb microfibril orientation (79, 316, 365, 410) support the importance of the inner wall layer in higher plants as well. The presence of polylamellate wall architecture in some cells raises questions about the basis of directional growth in stems. If the inner wall layer of a young cortical cell consists of longitudinally oriented microfibrils during part of the growth period, how is preferential growth in length maintained? It seems likely that tissue interactions, as described previously, may compensate for the lack of structural anisotropy in such cases.

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RATE OF CELL EXPANSION

Growth Equations

As first formulated by Lockhart (195, 196) and recently discussed by Cosgrove (61), steady state cell expansion is achieved when the rate of water uptake equals the rate of wall yielding. In terms of water potential (15), the equation for the rate of water uptake is:

$$dV/dt = -L(\Delta \psi_w) \qquad 1.$$

where V is cell volume, L is hydraulic conductance ($bar^{-1}\ s^{-1}$) and $\Delta\psi$ is the difference in water potential between the cell and the external environment. Molz & Boyer (234) have noted that a water potential difference of only 0.1 bar is sufficient to drive a growth-sustaining water flux across a typical plant plasma membrane.

Equation 1 can be made more detailed by replacing $\Delta\psi$ with the parameters which determine it, yielding the equation:

$$dV/dt = L(\sigma \cdot \Delta\pi - P) \qquad 2.$$

where V is cell volume at pressure P (cm^3), σ is the solute reflection coefficient (usually defined as 1.0), $\Delta\pi$ is the osmotic potential difference between the cell and the external medium (bars), and P is the turgor pressure (bars). Assuming that cell expansion occurs primarily by elongation, the equation for the rate of wall yielding is:

$$dV_0/dt = m(P - P_c) \qquad 3.$$

where V_0 is the cell volume at zero turgor (cm^3), m is the longitudinal wall extensibility (the reciprocal of viscosity) ($bar^{-1}s^{-1}$), and P_c is the critical turgor pressure, i.e. the minimum turgor for cell expansion (bars). This equation has been particularly useful in studies with *Nitella,* in which turgor pressure has been measured directly during growth (124). At steady state,

Equations 2 and 3 can be combined, giving the general expression for steady state growth rate (305):

$$v_s = \frac{L \cdot m}{L + m} (\sigma \Delta \pi - P_c) \qquad 4.$$

Note that P cancels out when Equations 2 and 3 are combined, which tends to obscure the role of turgor pressure in growth (61). As first pointed out by Lockhart (196), Equation 4 predicts that when hydraulic conductivity L is large (relative to wall extensibility m) Equation 3 reduces to:

$$v_s = m(\sigma \cdot \Delta \pi - P_c), \qquad 5.$$

which applies only to the case of a cell in osmotic equilibrium, where the difference in water potential between the cell and the medium ($\Delta \pi_w$) is negligible. Elongating *Nitella* internodes approximate such a situation since m has been estimated to be 2.8×10^{-5} s^{-1} bar^{-1} and L has been calculated from the hydraulic conductivity (68) to be 2×10^{-3} s^{-1} bar^{-1} (61). In higher plant tissues, the surrounding symplast and apoplast offer additional resistances to water movement not encountered by isolated cells. Thus, elongating cells which are some distance removed from the vascular supply may experience a marked decrease in water potential relative to the cells adjacent to the vascular supply (osmotic disequilibrium) (307). Under these conditions the growth rate may, in principle, be regulated by the tissue hydraulic conductance.

Measurements of tissue ψ_w in growing versus nongrowing tissues have led to the conclusion that rapid rates of growth are associated with lowered ψ_w's, for example, -0.8 to -2.5 bars in oat coleoptiles (307) and -1.7 to -2.1 bars in soybean hypocotyls (15). Boyer & Wu (15) further showed that the increase in the growth rate of decapitated soybean hypocotyls by auxin treatment was not associated with a change in the $\Delta \psi_w$ of the tissue. From the relationship in Equation 1, the authors concluded that auxin must have altered tissue hydraulic conductivity.

However, more direct evidence suggests that water transport does not normally limit growth. Abrasion of both the inner and outer cuticles of rye coleoptiles and perfusion of both the inner and outer surfaces greatly reduced the halftime of the osmoelastic transient in response to a turgor shift, yet the growth rate of such sections was not appreciably enhanced over nonabraded sections (128). Recent direct pressure probe measurements of hydraulic conductivity in the epidermal and cortical cells of intact pea stems indicate that auxin enhances neither the cellular nor tissue hydraulic conductivity when it promotes growth (63). The authors confirmed the low tissue ψ_w of the growing region (-3 bars) as well as its insensitivity to auxin, as noted by Boyer & Wu (15). However, the effect was apparently an artifact resulting from the high

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solute concentration in the free space of the growing region (62). Cosgrove and Cleland concluded that the transverse water potential gradient in growing pea stems was too small (no more than 0.5 bars) to be a rate-limiting factor in growth, given the high hydraulic conductance of the tissue. While the elegantly designed and executed experiments of Cosgrove and Cleland represent strong evidence against a role for hydraulic conductivity in controlling the growth rate, their pressure probe was inserted at a position 1 cm below the hook, and, given the presence of growth gradients (356), it is not clear that the impaled cell was growing at the same rate as the tissue as a whole, even if it was located in the middle of the growing region. No effect of auxin was detected on the volumetric elastic modulus of the cortical parenchyma, while others have reported rapid auxin-induced decreases in the Young's modulus of peas by the resonance frequency method (22, 23, 397). This discrepancy may indicate that the two moduli, obtained by different methods, are not measuring the same wall property.

Cosgrove (61) has developed the growth equation further to describe the nonequilibrium situation which obtains when the growth rate is perturbed by suddenly altering one of the parameters in Equation 4. It is shown that an increase in the growth rate caused by an increase in wall extensibility will be accompanied by a decrease in turgor pressure, while an increase in the growth rate caused by an increase in hydraulic conductance will be associated with an increase in turgor pressure. In principle, such predictions can then be used to determine the mode of action of growth regulators. Experimentally, growth rate-coupled changes in turgor pressure may be difficult to detect over short time periods (63). However, the fact that turgor pressure declines during long-term auxin-enhanced elongation of coleoptile sections (15) is consistent with the well-established enhancement of wall extensibility by auxin. In the intact plant, turgor pressure may play an important role in the decline of the growth rate along the developmental gradient (290).

The In Vivo Cell Wall Parameters

The walls of mature, nongrowing cells are, by definition, fully elastic. Direct pressure-probe measurements of the volumetric elastic modulus (basically, the ratio of the change in pressure to the increment of volume) of mature algal and higher plant cells suggest that wall elasticity varies with cell volume and turgor pressure (376).

Additional complexities arise during irreversible cell expansion. The wall parameters m and P_c are difficult to define. P_c is functionally equivalent to a physical wall yield point, but, as will become apparent, it is profoundly influenced by metabolism. Extensibility m has the units of fluidity, while the isolated cell wall behaves as a strain-hardening viscoelastic-plastic material (143, 197). One manifestation of this discrepancy is that growth is linear with

time, while the extension of isolated walls is linear with log time (42). The missing element in the in vitro extension is, of course, metabolism. Blocking metabolism of growing sections with inhibitors or low temperatures causes a rapid decline in the growth rate, even when turgor pressure is not significantly affected (306). This simple experiment indicates two things: first, wall-loosening processes require constant metabolic input; second, wall stiffening seems to be well accounted for by physical strain-hardening properties, although this, too, may be be regulated by metabolism. During steady growth, metabolic wall-loosening events promoting acceleration are balanced by metabolic as well as physical wall-stiffening processes promoting deceleration, as illustrated in Figure 1 (127, 129).

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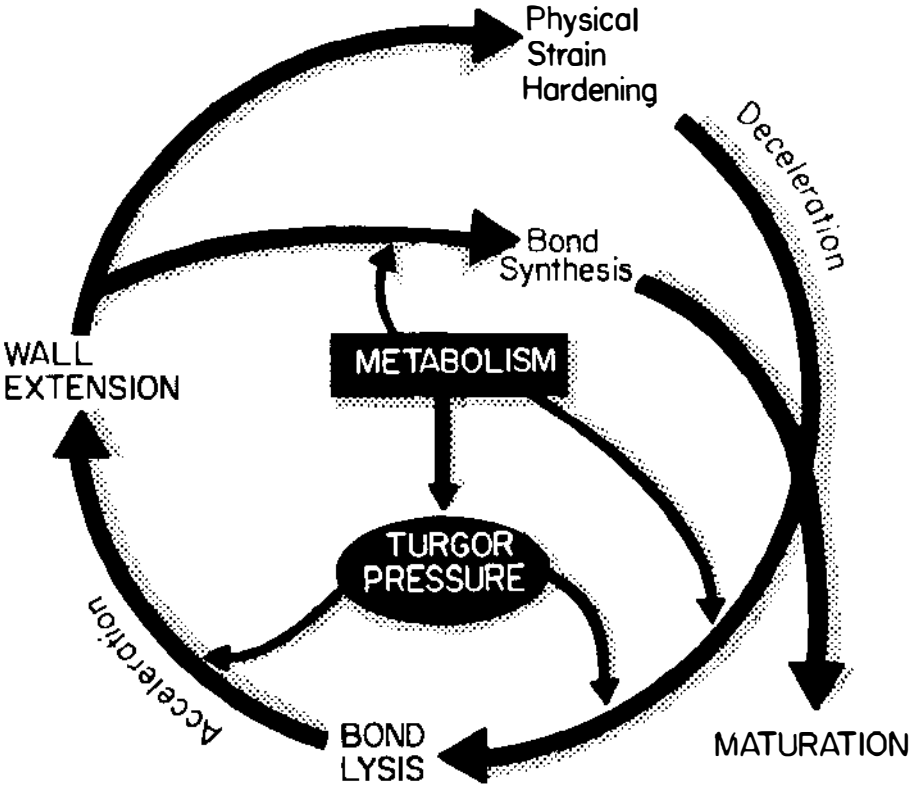

Figure 1 Diagram of a model for wall extension. Each turn of the cycle represents a microscopic, independent viscoelastic-plastic extension step. Note the central role for metabolism in maintaining turgor pressure, mediating wall-loosening as well as in causing wall-stiffening through bond synthesis, ultimately leading to cell maturation. Turgor pressure is required for both bond lysis and for driving extension. Each cycle includes a deceleration factor of physical and/or metabolic origin.

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The elegant and detailed studies of Green and his associates (124, 129) have provided strong evidence that the growth rate of *Nitella* internode cells is regulated primarily by changes in the critical turgor pressure (reviewed in 377). Addition of external osmotica to the culture medium of young, rapidly growing cells caused an immediate cessation of growth, even when the reduction in turgor was only 0.2 atmospheres. This suggests that P_c must be around 4.8 atm. Cleland (33) has questioned this interpretation and has suggested that the apparent cessation of growth may actually represent a retarded elastic contraction (creep recovery) (De_2 in Figure 3) superimposed on a slightly reduced extension rate. However, in multiaxial extensibility studies, using mercury-inflated *Nitella* walls, Kamiya et al (166) and Richmond et al (323) have shown that the creep recovery phase is essentially complete by 5 min. This contrasts with the growth cessation which occurs in response to a turgor step-down, which may persist for an hour or more (124). Using multiaxial stress, Metraux et al (228) have detected a P_c at about 4.8 atm for the plastic deformation of isolated walls. Thus, P_c as measured in *Nitella* may well represent a physical property of the wall.

Provided that P does not fall below 2.0 atm, growth resumes after a lag and gradually accelerates to nearly the initial rate. The resumption in growth proceeds without a concomitant increase in turgor pressure, indicating that one of the cell wall parameters, m or P_c, has been changed. Reasoning that m could have no effect on the growth rate when $P-P_c <= 0$, Green at al concluded that P_c must have been lowered. The P_c-lowering process was shown to be sensitive to azide and therefore metabolic. The limit of P_c-lowering appears to occur at 2.0 atm, based on the observation that no growth resumption occurs below this value. It has been speculated that this value represents the true physical yield stress Y detected in uniaxial stress experiments with *Nitella* wall strips (291). However, it is also possible that metabolic factors are involved. For the remainder of the review, the adjustable P_c at 4.8 atm will be called P_{ca}, while the nonadjustable P_c at 2.0 atm will be called P_{cx}.

When the turgor is shifted upward there is an immediate growth burst followed by a logarithmic decay back to a steady rate. The growth burst is far in excess of a simple elastic recovery both in magnitude and duration. Frequently it is observed that the growth burst returns the cell to the length it would have achieved had the turgor step-down not been administered, and in some cases overcompensation occurs (124). This phenomenon has been termed "stored growth" (297). The deceleration following the growth burst is considered to be a physical strain-hardening process, since it is not inhibited by azide. Green et al (127) give reason to believe that strain-hardening is equivalent to an increase in P_{ca} rather than a decrease in m.

It has long been recognized that a critical turgor pressure is required for

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growth in higher plant tissues (29, 33). This P_c appears to be of the nonadjustable type (P_{cx}) since no growth resumption occurs and auxin does not lower it (33, 128). Recently, a novel method for measuring the P_{cx} of stem tissues has been developed in which turgor pressure is monitored directly by means of a pressure probe in excised stem sections isolated from a water source. The turgor decreases rapidly due to cell expansion until equilibrium is reached at a value equivalent to P_{cx} (D. J. Cosgrove, E. Van Volkenburgh & R. E. Cleland, unpublished results). Analysis of the P_c term in higher plant tissues by means of osmotically induced turgor shifts is experimentally difficult because the slower penetration of water extends the period of osmoelastic adjustment. If the new growth rate of the tissue is measured prior to reaching equilibrium, it may be artificially low or high, depending on the direction of the turgor shift. During the period of osmoelastic adjustment, the wall parameter being measured may be changing (274). Green & Cummins (128) were able to shorten the osmoelastic response of rye coleoptiles to 10 min by carefully abrading the inner and outer cuticles and continuously perfusing both surfaces during the experiments. When such coleoptiles were challenged with various concentrations of pentaerythritol, an initial large osmoelastic contraction was immediately followed by a slow readjustment to a new steady rate, the entire response taking about 40 min. Unlike the response of *Nitella* internodes to small turgor step-downs, there was no phase of growth cessation preceding the acceleration to a new steady rate. However, the new steady growth rate approached zero as the turgor was lowered by 4–5 bars. Assuming a turgor pressure of about 10 bars, the authors concluded that the steady state P_{cx} value was 4–5 bars (128). Auxin had no effect on P_{cx}, but did increase the slope of the growth rate vs turgor pressure curve, interpreted as an increase in the extensibility term m. Hysteresis was observed in this curve when the turgor was shifted either upward or downward and the "sign" of the relationship was such as to indicate that adjustment to lower turgor somehow promotes extra extension during a subsequent step-up. This phenomenon may be related to stored growth (127). In auxin-treated sections, stored growth has been observed by some workers (119, 128, 198, 300) but not by others (48, 297), although stored growth is consistently observed in control sections. Two factors which may be critical for detecting stored growth in higher plants are: (*a*) speeding osmoelastic equilibration by removing cuticular barriers to water uptake (128); (*b*) selecting material with slow endogenous growth rates (119). In wheat coleoptiles, Göring et al (118) have reported that turgor reduction leads to stored growth in control and slow-growing auxin-treated sections, and the stored growth has been correlated with enhanced rates of proton extrusion. Similar effects of water stress have been obtained by Cleland (38) and Marrè et al (207). Further studies on stored growth under conditions similar to those of Green & Cummins (128) are needed.

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The results of Green and Cummins suggest that coleoptiles have only a P_{cx} and no P_{ca} and that auxin alters the steady state extensibility term, m. However, another explanation is possible. If, as suggested by Hettiaratchi & O'Callaghan (143), P_c is a complex function of three factors—the initial cell dimensions, wall thickness, and a wall modulus—it is doubtful that all the cells of the coleoptile would have the same P_{ca}. Thus, for any given turgor step-down only a portion of the cells might be below their P_{ca} value. P_{ca} might only be detectable in single cells. Furthermore, in their turgor shift studies Green et al (127) showed that the curve of the initial, instantaneous growth rate vs turgor pressure was concave upward. The effect of auxin was to cause the curve to shift to the left, toward lower turgor pressures. Thus, the effect of auxin on the "instantaneous" wall properties is somewhat different from the hormone's effect on the steady state properties, and a shift in the rate vs turgor curve to the left could be interpreted as a lowering of P_c.

To emphasize the self-stabilizing nature of the growth rate, Green et al (127) have proposed the following equation:

$$dr/dt = A - D \cdot r \qquad 6.$$

where A is a growth-accelerating tendency and $D \cdot r$ is a decelerating tendency coupled to the growth rate r. During steady state growth, $A = D \cdot r$. When $r = 0$, an increase in the rate will be entirely due to changes in A, whereas when r is infinite, the action of D predominates. Since A, D, and r can all be measured directly in response to turgor shifts, this equation is particularly useful in studies of the action of hormones in higher plants. Analysis of the responses of rye coleoptiles to turgor shifts led Green et al (127) to conclude that auxin causes a 4.5-fold increase in a metabolism-dependent acceleration process *(A)*, and only a 50% reduction in a putative rate-dependent deceleration factor *(D)*. It was suggested that the deceleration factor might represent physical strain hardening. Self-stabilization is a property of both m and P_c.

Physiological Control

HIGHER PLANT HORMONES AS PROBES Excision of sections from the elongating regions of coleoptiles, stems, or hypocotyls results in a drastic decline in the growth rate. The response of such sections to auxin and other hormones provides a convenient model system for studying the mechanism of cell expansion. A variety of sensitive auxanometers have been employed to monitor the short-term kinetics of the growth response (83, 274). Typically, enhancement of growth rates are detectable after a 10–20 min lag period, reaching a maximum (about fivefold relative to the control rate) by 30 min (274). The first maximum is frequently transient, and growth soon decelerates to a somewhat reduced steady rate which declines gradually over a period of 5–24 h (depend-

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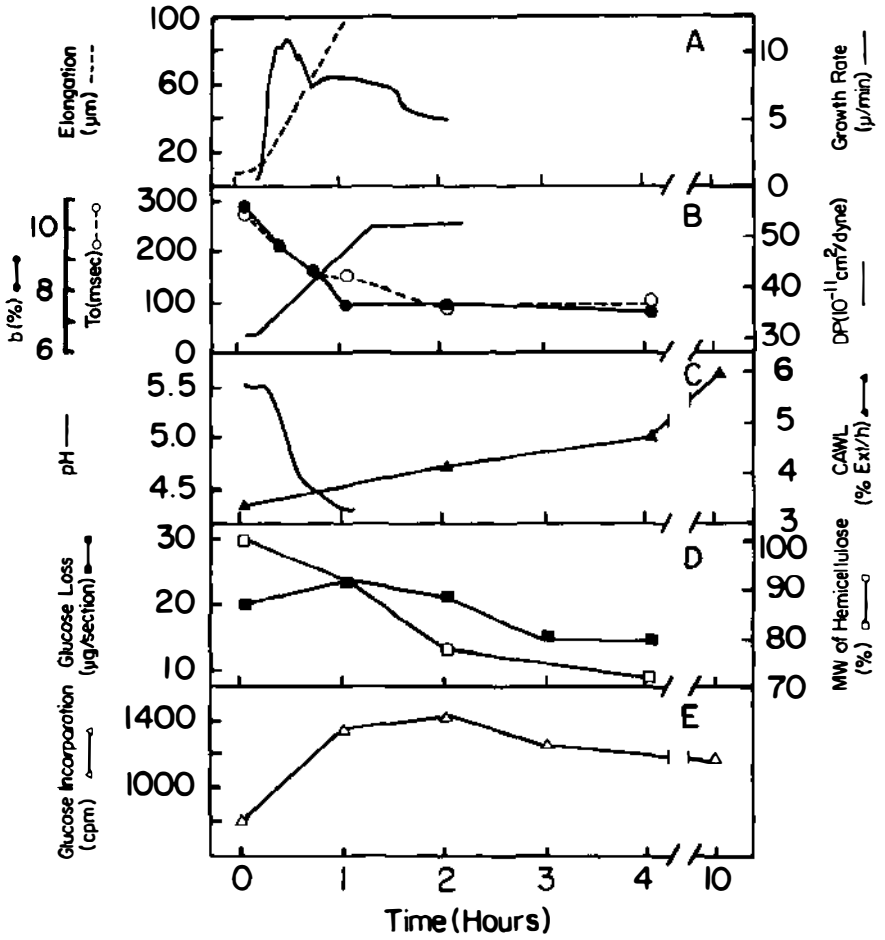

Figure 2 Summary time-course of the biomechanical and biochemical events during auxin-induced elongation of *Avena* coleoptiles. The data was compiled from the following sources: A (395), B (45, 343), C (37, 43), D (211, 349), E (300).

ing on the presence or absence of an energy source). Further oscillations leading to a second maximum have also been observed (see 274 for detailed description). An example of the short-term growth kinetics of *Avena* coleoptile sections is shown in Figure 2A. In the absence of sucrose the auxin-induced rate declines to that of the controls after 6 h (274).

Another example of a rapid growth response to a hormone is the GA-induced elongation of lettuce hypocotyl sections. Moll & Jones (232) have shown that the response to GA begins within 10 min and reaches a new steady rate by 40–50 min (232). The effect of GA is primarily to antagonize the inhibitory

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effect of blue light (262). Blue light inhibits the elongation of intact sunflower and cucumber hypocotyls very rapidly [within 1 min; (64)], although the effect is much diminished in sections.

The "biphasic" shape of the auxin growth rate curve has been interpreted in various ways, as follows: 1. the deceleration after the first maximum might reflect a negative feedback mechanism on the protein synthesis required for growth (270, 274); 2. the two maxima could represent two separate overlapping responses to auxin (274, 403); 3. the deceleration phase could indicate a decrease in turgor which would occur if osmoregulation lags behind cell expansion (50). However, it should be emphasized that section growth represents a summation of the expansion of hundreds of cells. Auxin growth kinetics have never been determined for single cells, and it is not known whether such tissue growth oscillations are characteristic of individual cells or whether they represent sequential responses of cells along developmental gradients (356–358). It is therefore premature to interpret the growth kinetics of sections at the cellular level. Computerized video-microscope systems are now available for monitoring the growth of single cells. There are further reasons for caution regarding growth rate curves. The precise shape of the growth curve varies with the species, but it may also vary from segment to segment of the same species (274). Spontaneous growth bursts have been reported for corn coleoptiles (89) and mung bean (288). The kinetics of auxin-induced growth depends on the timing of hormone addition relative to the growth burst (288). Developmental stage may also affect the growth rate curve. In mung beans, only the most rapidly growing regions of the hypocotyl exhibit biphasic kinetics (13, 112–115). Clearly, numerous factors other than those directly involved in auxin action may influence the growth rate curves of sections.

ACID GROWTH HYPOTHESIS: PRO AND CON In 1971 it was proposed by two laboratories independently that auxin stimulates the activity of a plasma membrane proton pump, probably an H^+-ATPase, which acidifies the cell wall and causes wall loosening (33, 135). The major evidence in support of the model has been reviewed previously (39, 50, 206, 311) and can be summarized as follows: 1. The response to auxin occurs within 15 min, suggesting a membrane-mediated event (87). 2. Acid buffers in the physiological pH range ($<$pH 5.8) promote elongation of living sections and cell wall loosening of frozen-thawed sections under constant load with almost no lag period (provided the cuticle, which is impermeable to protons, has been abraded or removed) (50, 308). 3. Neutral buffers inhibit auxin-induced growth as well as the extension of frozen-thawed sections (76, 310, 314). 4. Auxin enhances the rate of proton extrusion, and the kinetics of proton extrusion parallel the time course of auxin-stimulated growth (37, 159; Figure 2). 5. Other agents which stimulate proton extrusion, such as the fungal phytotoxin, fusicoccin (40, 206), as well as

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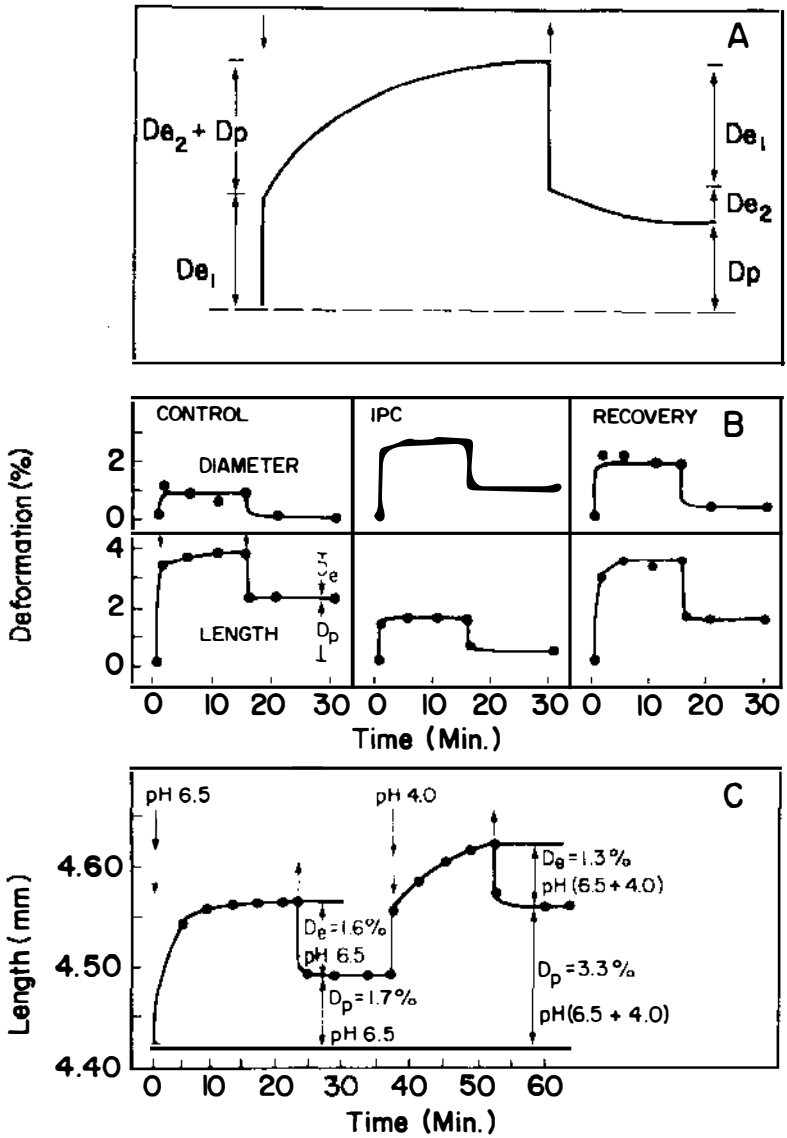

Figure 3 A. Schematic of typical in vitro wall extension under constant load. De_1, instantaneous elastic deformation; De_2, retarded elastic deformation; Dp, plastic deformation; De_2 + Dp, creep. After Ward (416). B. Deformation responses of mercury-filled *Nitella* wall tubes to multiaxial stress (5 atmospheres applied at 0 min). The diameter expansion is shown on the upper pannels and the length on the lower panels. The first pair shows the control cells. Note the marked anisotropy of extension. The second pair illustrates the response of a wall treated with IPC long enough to replace the inner 20–50% of the wall with random microfibrils. The wall has become more isotropic. The third pair is a recovery cell, showing partial restoration of mechanical anisotropy (323). C. The effect of acid buffer (1 mM Mes-Tris, pH 4.0) on the multiaxial extension of a young *Nitella* cell wall.

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the nonhormonal compound, α-naphthyl acetate (408), also stimulate elongation. 6. The amount of auxin- and fusicoccin-stimulated growth is directly proportional to the amount of protons released (38, 207). 7. Auxin and FC induce membrane hyperpolarization with similar kinetics to their effects on proton extrusion (7a, 47, 206, 209). 8. Metabolic inhibitors (50) and ATPase inhibitors (160) which block proton extrusion also block growth. Wall acidification has also been implicated in the growth and gravitropic curvatures of hypocotyls and roots (77, 85, 86, 245, 246), the light- and NaCl-induced expansion of leaves (262a, 405), and the rapid closure movements of the leaves of the Venus flytrap, *Dionea muscipula* (423). Barley roots may be an exception, however (265).

The acid growth hypothesis of auxin action has been investigated intensively over the past decade and, not surprisingly, a number of discrepancies which appear to contradict the model have been reported. Many of the early criticisms concerned the inability to detect rapid auxin-induced proton extrusion (50). The responses of dicot stems and hypocotyls have been the most controversial, perhaps because of the critical importance of the outer cell layers (16, 17, 76, 218, 225, 273, 306, 385). Thus, peeling the epidermis from pea stems strongly inhibits auxin-induced acidification and growth, while coleoptiles are less sensitive to peeling (50, but see 285). The consensus of opinion now seems to be that when carried out under the optimal conditions, which include permeabilizing the cuticle (312), proper aeration (160), and inclusion of potassium ions (386), rapid auxin-induced acid secretion can be readily detected in those tissues that show an auxin growth response. Vesper & Evans (408) made the serendipitous discovery that α-naphthyl acetate (NA), a nonhormonal agent, promoted both growth and proton release in peeled coleoptile sections of *Avena* and *Zea mays,* as well as in lentil roots. It was proposed that hydrolysis of NA in the cytoplasm released protons which were then extruded from the cell. The elongation so induced was longer lived than acid-induced growth, presumably because the release of protons from the cell more closely mimicked auxin-stimulated proton extrusion. A threshold rate of proton efflux necessary to induce growth was determined by means of a dose-response curve to NA. Auxin treatment resulted in a disproportionately large amount of growth relative to the rate of proton efflux. The authors suggested that the discrepancy between the growth rate and proton efflux in auxin-treated coleoptiles could be caused by an increased sensitivity of auxin-treated coleoptiles to protons (408). It should be noted that the rate of auxin-induced proton extrusion reported by Vesper and Evans was extremely low (from pH 6.0 to 5.8 in 90 min) compared to other published rates (37, 159). However, their explanation has received indirect support from Cleland, who showed that auxin does in fact enhance the capacity of coleoptiles for acid-induced wall loosening ("CAWL") (43; Figure 2C).

A persistent criticism of the acid growth model has been that acid-induced

growth is transient, usually lasting no more than 1–2 h, while auxin-induced growth can continue for 6 to 24 h, depending on the presence of an energy supply (274, 401). However, it is unlikely that exogenously applied acid adequately mimics proton extrusion, since proton extrusion, unlike exogenous acid, will generate a membrane potential (47, 212). The membrane potential is essential for a variety of metabolic processes (18), particularly solute transport involved in turgor maintenance (282, 364, 370). Cleland & Rayle (50) have shown that application of an external tension to acid-treated, living coleoptile sections can sustain an elevated extension rate for at least 6 h, suggesting that the growth rate of the sections becomes turgor-limited after the initial growth burst. This interpretation is supported by the observation of Göring & Bleiss (117) that the rate of decrease of cell sap osmotic concentration in excised, unabraded wheat coleoptiles treated with acid buffers is about 2 times greater than the rate of decrease in auxin-treated sections, even though auxin-induced growth is 25% greater than acid-stimulated expansion over the 3 h incubation period. Considerable cell damage was apparent near the cut surfaces, which increased with the duration of the incubation in acid (117). On the other hand, if permeant solutes are included in the the medium surrounding peeled *Avena* coleoptiles, the cell sap osmotic concentration remains nearly constant for 4–7 h despite a large increase in volume (368). Auxin enhances total solute accumulation, but this is thought to be a consequence of auxin-induced cell expansion rather than a direct effect of auxin (368). The effect of acid buffers on cell sap osmolality in the presence of permeant solutes has apparently not been examined. However, it seems safe to assume that osmoregulation during cell expansion would be inhibited by exogenously applied acid but not by auxin (50).

A related criticism of the acid growth model is the observation by Perley et al (277) that in lupin hypocotyls acid-induced elongation is accompanied by shrinkage in diameter, whereas auxin stimulates lateral expansion. Taiz & Metraux (376) confirmed the occurrence of acid-induced lateral contraction in etiolated pea stem sections and provided evidence that it was caused by a reduction in turgor pressure since it could be prevented by preincubation in permeant solutes or by a turgor step-up. Consistent with the acid growth mechanism, the early acceleration phase of auxin-induced elongation is also accompanied by a turgor-related lateral contraction which can be prevented by preincubation in permeant solutes (376). This suggests that osmoregulation lags slightly behind the auxin stimulation of cell expansion, as proposed by Cleland & Rayle (50). Interestingly, FC-induced growth in pea stems was associated with a dramatic lateral expansion (376).

In unabraded sections it has been observed that upon completion of the acid growth effect, when the tissue has become refractory to further acid treatment, the section will still grow in response to auxin (283, 284, 309, 402). One

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interpretation of this finding is that auxin and acid act via different mechanisms (284). However, it is not clear that the same cells are responding to acid and to auxin. Since unabraded sections were used, protons would enter the tissue mainly through the cut ends (162). Göring (personal communication) has shown that unabraded sections can respond repeatedly to acid if the damaged cells near the cut surfaces are excised. He suggests that auxin readily penetrates the cuticle to promote the growth of the interior cells which have not yet been exposed to acid. Even assuming that the same cells are responding sequentially to acid and to auxin, it can be argued that wall acidification is only one of many essential events and that other auxin-mediated processes are required for steady state growth (50). As discussed above, turgor pressure may become limiting during growth induced by exogenous acid. Auxin may help to protect the osmoregulatory and pH-stat mechanisms against the toxic effects of external acid. And, as previously noted, the capacity of the wall to be loosened by acid is itself promoted by auxin (43). The acid growth hypothesis must include the qualifier that growth can only be regulated by acid *when acid is the limiting factor* (42).

Other workers have been able to "uncouple" proton secretion from growth by use of inhibitors (109, 287, 342), anoxia ("emergent growth") (269), or by omitting potassium from the medium (373, 390). Auxin-induced growth of unpeeled *Avena* coleoptile sections is inhibited within 10 min by CH (cycloheximide) (32, 34), and proton extrusion by peeled sections is inhibited even sooner by a variety of protein synthesis inhibitors (7). The growth, proton extrusion, and potassium uptake of maize coleoptiles which were preincubated in CH, puromycin, or canavanine for 1 h and then treated with IAA plus inhibitor for a further 3 h were strongly inhibited (54). Canavanine did not block incorporation of labeled leucine in protein (54), suggesting that specific proteins are required rather than the utilization of a cofactor involved in protein synthesis. However, other studies with unabraded *Avena* coleoptile sections indicated that some IAA-induced growth persisted for at least 2 h after CH treatment (287, 420). It is likely that in these studies the delayed inhibition may reflect the time required for the inhibitor to diffuse through the cut ends (7).

Recently, Pope found that abraded *Avena* coleoptile sections also exhibited, for about 60 min, a residual response to auxin, even when detectable proton extrusion and about 60% of the total growth was inhibited by CH (286). CH inhibited the percent IAA stimulation of growth by 30–100%, depending on the CH concentration and the seed lot. These results suggest either that the residual IAA-induced growth is being mediated by undetected cell wall acidification (e.g. by inner parenchyma cells) or that auxin can promote some growth by an alternate CH-insensitive mechanism. An example of a CH-insensitive mechanism in coleoptiles is "O_2-sensitive growth" (104–106, 168). At low auxin concentrations, 8% oxygen promotes the growth of coleoptile

sections, even though ATP synthesis and proton extrusion are inhibited relative to the 20% O_2 control (104). The growth stimulation by 8% O_2 is insensitive to CH (104). Thus, under these conditions, growth and proton extrusion have also been "uncoupled" by a CH-insensitive mechanism. The low O_2-induced increase in the growth rate has been correlated with a decrease in wall hydroxyproline-rich protein (104), consistent with the hypothesis of Cleland & Karlsnes (46) that extensin synthesis contributes to the cessation of growth. Since Pope's experiments were carried out at 20% O_2, the relationship to O_2-sensitive growth is unclear. The auxin-regulated decrease in wall-bound extensin in air is inhibited by CH (104), but low concentrations of CH may selectively inhibit proton secretion.

Potassium salts are generally included in the incubation media for auxin-induced acidification, although they are not required for short-term growth. Terry & Jones (388) measured growth and acidification in unpeeled, unabraded pea stem sections submerged in aerated water in a flow-through system. Significant auxin-induced proton extrusion was only obtained in the presence of calcium and potassium ions, although inclusion of ions did not enhance the growth rate over a 5 h period (388). In fact, their data show a consistent, slight inhibition. Similar results were obtained by Parrish & Davies (268), except that peeled sections were used. They suggested that potassium may exchange protons from the wall, causing a discrepancy between wall pH and medium pH. Terry and Jones argued against a physical exchange mechanism on the basis of the temperature dependence of ion-induced acidification. They suggested that ions stimulated an electrogenic H^+-pump. Stuart & Jones (373) showed that in lettuce hypocotyls fusicoccin stimulated the release of protons only in the presence of external potassium. Since the potassium effect was inhibited by the uncoupler CCCP, they also proposed an active mechanism involving membrane transport. However, inhibition by low temperature or an uncoupler does not rule out a physical exchange mechanism, since medium acidification might require a continuous exchange of newly transported protons from the wall by ions. Blocking transport would limit the efflux of exchangeable protons. In the absence of ions the protons may accumulate in the free space, feedback inhibiting further proton efflux (50, 364). The addition of ions may have two effects, stimulating the plasma membrane proton pump and facilitating the release of protons into the medium.

The relationship between exogenous cations and auxin-induced growth and acidification clearly needs to be resolved. In *Avena* coleoptiles it has long been known that omission of potassium ions from the medium reduces both the growth rate and the duration of enhanced growth in response to auxin (391), and there is evidence that auxin promotes H^+/K^+ exchange at the plasma membrane (41). In pea stems (209) and corn coleoptiles (27, 189a), both FC-induced acidification and growth appear to be proportional to the concen-

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tration of external potassium. One variable may be the ionic composition of the free space solution. Cosgrove & Cleland (62) have shown that the free space solution of pea stem sections contains a solute concentration of 70 milliosmoles per kg, 25% of which is unidentified inorganic electrolytes. If adequate salts are present in the free space, why are exogenous salts required to detect medium acidification? Are the free space salts rapidly depleted during auxin treatment or lost by diffusion into the incubation medium? The fact that abraded sections can come to equilibrium with a large volume of medium (relative to the free space) suggests that proton efflux in vitro may greatly exceed proton efflux in the intact plant.

The rapid inhibition of elongation by neutral buffers has been a mainstay of the acid growth hypothesis. Neutral buffers inhibit growth whether administered early or during steady state elongation. No deleterious effects of neutral buffers on the metabolism of growing sections have been reported. The only other possibility is that growth declines because of an inhibition of auxin uptake (116). B. Bonner and co-workers (personal communication) have recently shown that neutral buffers inhibit the growth of pea stems treated with IAA-methyl ester whose uptake is insensitive to pH. Thus, in peas at least, auxin uptake is not limiting growth during treatment with neutral buffers. It is still possible that neutral pH inhibits some other process. At this point, however, we can only conclude that in most cases wall extension requires an acid pH.

Auxin is the only hormone for which there is abundant evidence that proton extrusion is one of the rate-limiting factors involved in the promotion of cell expansion. GA-stimulated elongation of lettuce hypocotyls (371, 373), zeatin-induced expansion of cucumber cotyledons (337), and ethylene-enhanced elongation in an aquatic *Ranunculus* (66) have all been correlated with increases in wall extensibility in the absence of enhanced rates of proton extrusion. However, in the case of cucumber cotyledons, short-term measurements indicated that neutral buffers inhibited cotyledon expansion in response to zeatin (337). *Ranunculus* is an interesting case because it also seems to respond to auxin via an acid-secretion mechanism (66). Ethylene actually inhibits medium acidification by *Ranunculus* while promoting elongation (66). In contrast, Malone & Ridge (203a) report that ethylene and auxin both promote proton excretion and elongation in petiole segments of *Nymphoides*, consistent with the acid growth hypothesis. In lettuce hypocotyls, calcium uptake rather than proton extrusion has been implicated in the rapid growth response (233). Calcium transport has recently been reviewed in relation to phytochrome action and calmodulin activation (205, 337a). It has been proposed that calcium influx may regulate metabolic processes, including the release of secretory vesicles essential to tip growth (318) and to auxin-induced growth (18, 142a). The recent discovery of calmodulin in the osmotic shock fluid of maize roots (185a) raises the possibility that this protein may be associated with the outer surface

of the plasma membrane, in close proximity to the wall. These examples illustrate the need to understand more about factors other than protons which regulate extension.

Even for those systems that respond to auxin in vitro, it is not clear whether the growth rate is regulated by the free space pH in the intact plant. Lurrson (202) measured the surface pH of unabraded *Avena* coleoptiles and detected a pH gradient which became more acidic from base to tip. In old, nongrowing coleoptiles the apical regions were not acidic (202). A similar pH gradient along maize coleoptiles and *Helianthus* hypocotyls was detected by Mulkey & Evans (245) using an agar-indicator dye method. [In barley roots, unlike corn and mung bean, the elongation zone appears to be basic (264, 421).] In contrast, Goldberg (111, 112) has reported that unabraded sections taken from nongrowing regions of *Phaseolus* stems and mung bean hypocotyls acidified the medium at about the same rate or greater than sections taken from the growing region. More recently, Johnson & Jacobs (162) have obtained evidence using a microelectrode that the free space solution pH of intact, etiolated peas is about 5.9 in both growing and nongrowing regions of the stem. Previously, Jacobs & Ray (158) had detected cell wall pH values around 5.0 using the same technique in auxin-treated pea stem sections. Since the sections were incubated in a potassium-containing buffer, it is possible that the lower pH in sections was caused by potassium (388). The results with intact peas may indicate that the in vivo wall pH is: (*a*) only marginally low enough to induce acid growth; and (*b*) constant throughout the elongation and maturation region. Thus, although the evidence is still sketchy, the available data suggest that in the intact plant wall pH may be a limiting factor in some systems, e.g. coleoptiles, but perhaps not in others, e.g. barley roots and pea stems. When proton extrusion is not limiting, other factors must regulate the rate of cell expansion.

NITELLA: ACID GROWTH AT THE CELLULAR LEVEL The acid growth model of auxin action prompted an investigation by Metraux at al (228, 377) into the role of wall acidification in the growth of *Nitella* which would allow single cells to be studied. *Nitella* is a particularly convenient organism for studying acid growth because of the presence of acid and base "bands" along the length of each internode cell. Although proton extrusion probably occurs uniformly, localized hydroxyl transport raises the wall pH to ~9.0 in some regions compared to ~5.5 in others (see references in 377). The pH banding pattern is seen in cells grown in dilute salt solutions and is suppressed by autoclaved soil extract media. In marking studies using anion exchange resin beads it was determined that growth was primarily restricted to the acidic regions of the wall. The bands migrated over a period of hours, and growth was consistently correlated with wall acidity over time. Moreover, alkaline buffers prevented

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growth in the acid bands, while acidic buffers promoted growth in the base bands. When cells were grown in autoclaved soil extract, no regionalized growth occurred, consistent with the absence of a pH banding pattern and in agreement with the earlier marking studies of Green (125).

The above studies strongly implicated wall acidification in regulating growth in *Nitella*. What is the relationship between acid growth and the P_c-lowering process documented by Green? Recent unpublished experiments suggest that acid may be involved in P_c-lowering. As described in a previous section, when *Nitella* cells are subjected to a turgor step-down followed by a turgor step-up, a growth burst occurs ("stored growth"), the magnitude of which is a direct measure of wall loosening during the step-down period. It has been found that alkaline buffers administered during the step-down period strongly antagonize the subsequent growth burst during the turgor step-up, while acid buffers have no effect (P. S. Richmond and L. Taiz, unpublished data). This suggests that wall loosening during the turgor step-down requires an acidified wall.

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CELL WALL MECHANICAL PROPERTIES

Measurement and Interpretation

The mechanical behavior of plant cell walls has been described previously (33, 196, 274, 291, 354), and the reader is referred to these articles for background. Two useful volumes on biomechanics (412, 415) are now available in addition to standard texts on rheology (92, 416).

There is no agreed way to measure the elusive physiological cell wall parameters m and P_c in vitro. Mechanical tests on isolated walls have been performed using either uniaxial or multiaxial stress. Uniaxial mechanical testing has been of three types: Instron analysis (constant strain rate), stress relaxation (constant strain), and creep (constant load). Of these, creep most closely resembles in vivo extension. Usually, analyses of higher plant walls have been based on the Instron or stress relaxation methods because of the ease and rapidity of the measurements (45). To eliminate the undefined effects of enzymes, higher plant wall preparations traditionally have been boiled in methanol and rehydrated, although this treatment may profoundly alter the wall's native gel structure (31). Such preparations behave as purely physical systems, with a Q_{10} of ~ 1 for extension (33).

Extension of a viscoelastic solid is, by definition, fully reversible (92). In practice, this is not always the case for the first extension; such materials are also influenced by their previous strain history (416). The first extension of isolated cell walls contains a large irreversible component, i.e. there is only partial recovery of the original dimensions upon removal of the load, as illustrated in Figure 3A (p. 600). Loading causes an instantaneous elastic deformation De_1, followed by a slow, time-dependent deformation termed

creep, which consists of a retarded elastic component De_2, and a plastic component Dp (416). Unloading results in an instantaneous elastic contraction (De_1), succeeded by a retarded elastic contraction (De_2). Dp is the residual strain. Because of the large plastic component, the cell wall can be described as a viscoelastic-plastic material. Typical of viscoelastic materials, wall extension is linear with log time, and the same general pattern is observed both uniaxially and multiaxially (228, 323). This clearly differentiates mechanical extension from steady state growth. It is the irreversible component of the first extension (the "percent plasticity") which, since the work of Heyn (145), historically has been correlated with auxin-stimulated growth rates, although it is the least understood from a biomechanical viewpoint (33). For conventional rheological analysis of the viscoelastic properties, the irreversible component is eliminated by "mechanical conditioning" prior to testing, i.e. the walls are pre-extended by a small amount (1–5%). Mechanical conditioning effectively "erases" the long-term memory of the material, substituting a new short-term memory. (In Orwellian terms, the past record of the wall is made to conform to "the orthodoxy of the moment"!) Subsequent extensions are reproducible and 100% reversible, provided: (*a*) the pre-extension strain is not exceeded, and (*b*) the direction of the applied stress is the same (45, 354). The ratio of stress to strain of conditioned walls provides a measure of wall elasticity (Young's modulus). Exceeding the pre-extension strain or changing the direction of the force vectors will result in a further increment of irreversible extension, i.e. beyond the elastic limit, both an elastic and plastic component are present (45). The increase in stress with strain beyond the elastic limit is attributed to the elastic component and is referred to as "strain-hardening." A viscoelastic material is said to be linear if the ratio of stress to strain is a function of time only and is independent of the value of stress or strain (92). Plant cell walls have been shown to exhibit nonlinear viscoelastic behavior (35, 139), for which there is, unfortunately, no satisfactory theoretical treatment (416).

Uniaxial creep at neutral pH differs from growth in two important respects: it is linear with log time rather than time and the rates are about 10 times greater than growth rates (33, 291). The excessive extension is an artifact of uniaxial stress because it is not observed under multiaxial stress conditions. When *Nitella* wall cylinders are inflated with mercury at 5 atm (equivalent to the in vivo turgor pressure), the walls extend at an exponentially decaying rate but they do not stretch beyond the original length prior to excision, at least during the first 15 min (228). The multiaxial creep rate after 15 min is too low to be measured accurately, and clearly lower than the growth rate, even for young walls.

The Instron technique, in which wall stress is measured at a constant rate of strain, allows determination of the plastic compliance, DP, and the elastic compliance, DE, corresponding to the percent extension per unit stress. These

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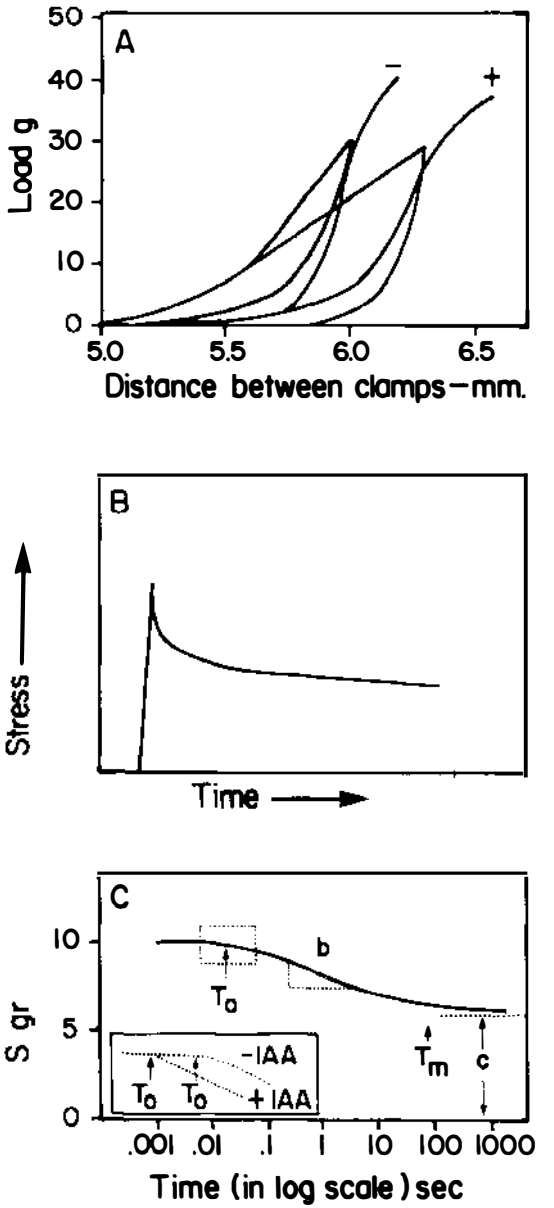

Figure 4 A. Instron extension experiment on *Avena* coleoptile walls obtained from control (−) or auxin-treated (+) coleoptiles (31a). B. Stress relaxation of *Avena* coleoptile walls (198). C. Stress relaxation vs log time, illustrating various stress relaxation parameters and the effect of auxin (insert) (198).

values can be calculated from the slopes of the stress/strain curves, examples of which are shown in Figure 4 (31). In practice, the percent extension per unit force (%PE) has been found to be equivalent to the plastic compliance (45).

In stress relaxation the wall is rapidly stretched to a predetermined strain and held constant while the decay in tension is measured (Figure 4). The advantage of stress relaxation is that information on the strengths (in energy units) of the bonds which limit extension can be obtained. Sellen (354) has recently reviewed the detailed stress relaxation studies with *Nitella* walls. Activation energies for stress-bearing bonds were estimated by fitting the curves for stress relaxation carried out at different temperatures into a single smooth function, or master curve (138–140). Based on this method, the resistance to stress of *Nitella* walls was suggested to be caused by the cooperative action of many weak interactions rather than a few strong covalent bonds (354).

The pattern of stress relaxation in higher plant walls appears to be similar to that of *Nitella*. Cleland & Haughton (44) used the "relaxation modulus" $E(t)$ (the ratio of strain to stress at time t), and the "relative relaxation" [the percent of decrease in $E(t)$ per decade of log time] to characterize the stress relaxation behavior of oat coleoptiles. Cell wall stress relaxation curves have also been analyzed using an empirically derived equation:

$$S = b \cdot \log \frac{t + T_m}{t + T_o} + c, \qquad 6.$$

where S is the stress, b is relaxation rate (slope of the curve $\Delta S / \Delta \log t$), T_o is the minimum relaxation time, T_m is the maximum relaxation time and c is the residual stress at the end of the relaxation period (212; see also Figure 4). In molecular terms, T_o is thought to represent the average molecular weight of the viscoelastic flow units, b is equated with the number of flow units per cross-sectional area of wall, and c is equivalent to the number of cross-links between adjacent polymers (100–103). While a theoretical basis for these assignments is still needed, this approach is potentially fruitful for wall extension analysis because of the detailed information obtainable. The ability to detect auxin effects on T_o is, however, influenced by the rate of the initial stretching (103). If the pre-extension rate is too slow, auxin effects are obscured because creep is occurring prior to the measurement. This has led to the development of a new equation which defines the parameter K as being equivalent to the pre-extension time in the stress-relaxation experiment (103). Using this equation, it has been possible to predict mathematically the effect of auxin pretreatment on a load extension curve at a constant strain rate for both oat coleoptiles and pea epidermis (103). This suggests that stress relaxation parameters, compliance values obtained with the Instron, and creep are directly comparable and reflect similar wall properties.

Viscoelastic-plastic materials may be characterized by a yield stress which

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must be exceeded before an irreversible extension can occur (92, 416). The existence of a minimum yield stress in isolated plant cell walls has not yet been definitively established. The data of Cleland (35) shows an apparent uniaxial yield stress for the creep of nonconditioned *Avena* walls at 10–12 g of load. A definite yield stress could be artificially induced by mechanical conditioning (35). The fact that the yield stress shifts to higher values with increasing stress, and that this appears to be a time-independent process, is further indication that cell walls are a strain-hardening material (44). Penny et al (271) observed a yield stress for the creep of lupin hypocotyls, which was not altered by pretreatment with auxin. Probine & Preston (291, 296) detected a possible uniaxial yield stress for creep in *Nitella* wall strips at 10^8 dynes m^{-2}, equivalent to about 1.7 atm pressure, although there was no distinct inflection point in the curve. This is close to the P_{cx} measured by Green et al in vivo (129). Multiaxially stretched *Nitella* walls, however, show a sharp increase in wall extension between 4.0 and 5.0 atm (228), which approximates the P_{ca} of the living cell. In sequential loading and unloading cycles, it was shown that the increase was entirely the result of the plastic component of extension (228). If Y, the uniaxial yield stress, is near 1.7 atm, what is the significance of the multiaxially measured P_c value? As previously mentioned, this pressure could represent the "point of dimensional instability" in the balloon model of wall extension proposed by Hettiaratchi & O'Callaghan (143). The expansion rate of a balloon increases suddenly when the balloon's radius and the thickness of the membrane reach critical values. If the much more complex cell wall exhibits similar mechanical properties, geometrical considerations may be as important as the mechanical properties in determining the P_{ca} of the wall.

Large uniaxial extensions ($>10\%$) may induce a type of plastic flow resembling "necking" and "cold drawing" phenomena, in which localized strain occurs and the polymers realign parallel to the direction of strain (412, 416). This is readily detected in cell walls viewed in polarized light. During uniaxial extensions of *Nitella* walls (323) or *Avena* coleoptile walls (11) the birefringence changes from negative to positive, indicating that the microfibril orientation has shifted from transverse to longitudinal. Auxin-treated coleoptiles exhibit a smaller shift in birefringence than controls for the same amount of strain (11, 12), demonstrating that auxin treatment reduces the cross-linking between the microfibrils and the matrix. However, the matrix polymers can also reorient in the direction of strain both in vitro and in vivo, as shown by Morikawa and Senda for the pectic fraction in *Nitella* (240, 242, 243), *Avena* coleoptile (241), and pea epidermal (141) walls. Such large-scale rearrangements can cause crystallization, an extreme form of strain hardening (416). The mechanism of strain hardening of a cylinder under multiaxial stress is less clear, inasmuch as few molecular rearrangements occur in the plane of the wall. Since, as recently pointed out by Sellen (355), radial compression of wall

thickness is the only negative strain in a multiaxially extending cylinder, strain hardening could result from such radially compressive forces.

The polarized light micrographs of Richmond et al (323) show clearly that the side walls of *Nitella* internode cells neck in irreversibly after uniaxial extension. It can be calculated that a 10% increase in length is accompanied by a 35% decrease in diameter, resulting in a net *decrease* in total surface area of the side wall. A decrease in surface area could only be accounted for by an increase in wall thickness or density. Lockhart et al (198) monitored the geometry of mung bean hypocotyl sections during uniaxial extension. When living tissue (in 0.25 M mannitol) was extended uniaxially, the increase in length was associated with only a small decrease in diameter, and the total surface area increased, as is normal growth. However, the surface area of killed tissue irreversibly decreased during uniaxial extension because of large lateral contractions, and the decrease was greater in frozen-thawed tissue than in methanol-boiled tissue (198). Thus, rather than thinning the wall, uniaxial extension leads to tangential contraction. In contrast, multiaxially extended *Nitella* walls do not contract laterally, and their surface areas increase during extension as in normal growth.

As noted above, there is no adequate theory to account for the wall's irreversible mechanical properties, yet it is this aspect of extension which is most analogous to growth. If the wall is being loosened in vivo by metabolic "wall-loosening events," and metabolism is required continuously for extension to occur, what part of this can be measured by in vitro mechanical tests of isolated walls? Cleland early proposed that the Instron method measures a strain-hardening function of the wall (31), which, according to the biophysical analyses of Green and co-workers (127), represents a minor component of in vivo extensibility. According to Cleland (33, 42), the initial in vitro plastic deformation represents "unused" wall-loosening events which are expressed when the force vectors are changed during the experiment (i.e. applying a uniaxial stress). During multiaxial extension of *Nitella* walls it is possible to simulate closely the in vivo turgor conditions. It was found that application of 5 atm pressure to *Nitella* walls, which was equivalent to the in vivo turgor, extended the wall to its initial length prior to excision (228). Even though the original length was not exceeded, a residual strain was noted when the pressure was released. The origin of the plastic component is obscure in this case, since the wall did not stretch beyond its original length prior to excision. One possibility is that during excision an "irreversible contraction" can occur which effectively erases the long-term memory of the wall (mechanical conditioning in reverse!). It may be that higher plant walls also "forget" their original extended lengths upon plasmolysis. This would account for the occurrence of plastic deformations even at low strains. Alternatively, partially incorporated wall polymers might contribute to plastic extension. At any point in time the

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stress-bearing region of the wall consists of a gradient ranging from newly deposited, partially incorporated polymers on the inner surface to fully extended material in the wall interior. The continuous flux of wall material during cell expansion implies that at a given instant the wall has properties both of a conditioned and a nonconditioned material.

Based on the mechanical properties of isolated walls, several models of in vivo wall extension have been suggested (33). The prevailing view is that linear extension with time during growth occurs by means of a series of independent viscoelastic extension steps (33). The P_c could represent a requirement for a critical amount of elastic strain before irreversible extension can take place (42). Opinions differ as to the rheological nature of the individual extension steps. According to one view, continuous extension of walls in vivo is by some type of "chemical creep" (33, 42) in which covalent bonds are being broken and reformed continuously under the influence of metabolic "wall-loosening factors" (50). Each viscoelastic extension event resembles the in vitro extension of a nonconditioned wall in that it contains both reversible and irreversible elements. In this view, each extension step is a viscoelastic-plastic extension. Masuda (212) and Burström (22, 23) have suggested that each extension step is strictly viscoelastic and reversible, like the in vitro extension of a conditioned wall. Each reversible extension must then be converted to a permanent strain by a metabolic fixation process, e.g. wall synthesis (212).

An example of growth by an elastic extension was provided by Burström et al (23). The initial outward expansion of split pea stems placed in water is entirely reversible, but if left in water the extension becomes irreversible. Another interesting example is the expansion of guard cells, which is normally reversible. Meidner (223) has recently suggested that a metabolic reduction of the elastic modulus of guard cell walls during the *Spannungsphase* facilitates opening of the stomata of *Tradescantia* and *Commelina*. Removal of wall calcium by exchange with potassium was suggested as a wall-loosening mechanism. As opening proceeds the volumetric modulus apparently increases because of wall stiffening (223), but the process is reversible and no fixation of the strain occurs. However, E. Zeiger (unpublished preliminary results) has observed that *Commelina* guard cells treated with fusicoccin over long periods ultimately lose turgor and die, yet remain in the open position. Thus, some type of fixation of the elastic strain can occur.

To this reviewer, the elastic model is unlikely to explain wall extension during growth for the following reasons. If "fixation" means that the elastic strain is locked into place by covalent cross-links or held by an opposing force, such as a rigid new layer of wall, then the old wall layer is under tension and no stress-relaxation has occurred. Subsequent wall loosening, to allow for the next viscoelastic extension step, should then lead to a contraction rather than an expansion, since the wall is in a prestressed state. To prevent this, the turgor

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pressure would have to increase continuously during growth, which it does not do (291). If, on the other hand, "fixation" is envisaged as a cleavage of elastically stretched, stress-bearing bonds, this would seem to be equivalent to biochemical creep. In the examples cited above in which a reversible extension was converted directly into an irreversible one, the extension was terminal and was not associated with further growth. Thus, this type of extension may well be characteristic of the final stages of maturation.

A third view of wall growth currently championed by Burström (21) holds that the extension of living walls is independent of turgor pressure and is totally driven by the force of intussusception. The dependence of growth on turgor pressure is attributed to a nonspecific inhibition of wall secretion by external osmotica, as has been demonstrated in tip-growing cells (353). However, the responses to turgor shifts occur too rapidly (within seconds) to be mediated by wall synthesis (127–129), and there is, in general, a poor correlation between growth rates and wall synthesis rates.

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Mechanical Anisotropy

Two mathematical analyses of wall mechanical anisotropy have recently appeared (144, 355). The first direct experimental evidence of wall mechanical anisotropy was provided by Probine & Preston (296). In uniaxial stress studies carried out on rectangular *Nitella* wall strips cut parallel or perpendicular to the cell axis, they were able to demonstrate that the ratio of the transverse to longitudinal elastic moduli (E_T/E_L) of conditioned walls decreased from 5 to 2 during cell maturation. The ratio changes because of a decrease in longitudinal elasticity; E_T does not appear to vary with developmental stage. Anisotropy of creep has also been noted in uniaxially extended *Nitella* walls (236). Transverse creep was measured by hooking short wall sections ("wall loops") into the extensometer, which avoids the necessity of drying and gluing the wall specimen to clamps. When the applied stress was equivalent to the calculated in vivo stress, longitudinal creep during the first 20 min was about 4 times the transverse creep in young walls (236), while the creep rates were about equal in the two directions for old cell walls, in approximate agreement with the elastic properties measured by Probine & Preston (296). The compliances (strain per unit stress) were proportional to the creep rates (236). Although definite yield points could not be identified, the transverse strain vs stress curve was shifted to higher stresses by a factor of about two relative to the longitudinal extension curve, consistent with the 2:1 transverse to longitudinal stress ratio (236).

The question arises whether transverse extension is limited by cellulose-matrix interactions or by interconnections between cellulose microfibrils. For example, Boyd & Foster (14) have proposed a trellis configuration consisting of an interconnected network of microfibrils. Such a model is difficult to reconcile with the rheological behavior of the wall, since the trellis structure

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should approximate continuous cellulose in the transverse direction, yet transverse and longitudinal compliances and creep rates are of the same order of magnitude. In addition, transverse creep, like longitudinal creep, is enhanced by mild acid buffers (pH 3.5) as well as by magnesium and potassium ions, and is inhibited by calcium ions (237). Since it is unlikely that continuous microfibrils would be affected by such treatments, the control of transverse extension by shear interactions between the matrix and discontinuous cellulose microfibrils seems the most feasible model at present (415).

Kamiya et al (166) developed the technique of mercury inflation to mimic the multiaxial stress of turgor pressure in extensibility studies of mature *Nitella* cell walls. The same technique was recently applied to investigations of the mechanical properties of young, growing *Nitella* walls (228). Kamiya et al demonstrated that the elastic extension of multiaxially stressed mature walls is one-third to one-fourth the extension in response to an equivalent uniaxial stress. In young cell walls, Richmond et al (323) found that the plastic component of extension (D_p) multiaxially was only one-tenth the amount of plastic extension obtained uniaxially, while the elastic component was reduced by only one-half. Thus, the error introduced by uniaxial stress is much greater for the plastic component of extension than for the elastic component. In mature walls, Kamiya et al found that the multiaxial transverse extension exceeded extension in length by nearly 2 times in some cases. This is the pattern one would expect for a nearly isotropic wall and a 2:1 transverse to longitudinal stress ratio. In contrast, the D_p of multiaxially extended young walls was infinitely greater longitudinally than transversely, while D_e was only 2.5 times greater (323). Anisotropy of creep could also be detected uniaxially and was found to be equivalent to the anisotropy of uniaxial D_p. Multiaxial creep rates in either dimension were too low to measure accurately.

The fact that the anisotropic behavior of isolated normal walls agrees well with the anisotropy of cell expansion strengthens the argument that in vitro mechanical testing measures physiologically relevant wall properties. The validity of in vitro extension experiments can be tested further by examining the anisotropic properties of walls whose microfibril orientation and pattern of expansion have been perturbed by microtubule toxins such as IPC (323). Since the inner 25% of the wall determines the directionality of cell expansion, the importance of the inner wall for controlling wall extension in vitro can also be tested. Walls were isolated from cells treated with IPC long enough to completely alter the polarity of expansion from length to diameter, equivalent to a 20–50% replacement of the inner transverse wall layer with a layer of randomly oriented microfibrils. Although results of both uniaxial and multiaxial experiments were qualitatively similar, the multiaxial studies showed a better quantitative correlation with cell expansion (see Figure 3B). In vivo, the RGR in diameter was 5 times the RGR in length for an IPC-treated cell (377). The ratio

of transverse to longitudinal D_p increases from zero to about 1.3 after IPC treatment. If the extension is carried out at acid pH, this ratio approaches 3.8. Thus, the anisotropy of the wall's mechanical properties agrees fairly well with the expansion pattern of the living cell, and the correspondence is improved at acid pH. Wall acidity may contribute to the weakening of the middle portion of the wall, shifting the stress to the inner layers.

Correlations with the Growth Rate

NITELLA Probine & Preston (295) early showed that the longitudinal uniaxial creep rates (averaged between 1 and 100 min) of isolated *Nitella* cell walls were proportional to the growth rates over the previous 24 h, provided the test stress imposed on the specimen was normalized to the in vivo stress. These results were confirmed by Metraux & Taiz (230), who also reported an apparent lack of correspondence between transverse creep and the growth rate. The latter observation is consistent with the fact that the transverse Young's modulus is constant throughout development (295). Since there is a constant proportionality between growth in length and diameter, failure to detect variations in transverse creep may be indicative of a fundamental difference between in vitro and in vivo transverse extension. However, the RGR in diameter is only one-fifth the RGR in length, and the variations in transverse extensibility may have been below the limits of resolution of the techniques employed.

Although correlations between multiaxial creep and growth rates have not been possible because creep rates are too low, a comparison of D_p and the growth rate was carried out (288). It was found that D_p correlated with the growth rate only in the case of older cells growing at a RGR of 1% h^{-1} or less, while D_p was not proportional to the RGR of young cells growing at 1–3% h^{-1}. This again reinforces the view that growth is not equivalent to physical extension and must involve metabolic regulation. There does appear to be a strong physical component in the deceleration of growth associated with maturation, and on a time axis the deceleration phase predominates. Thus, over most of the period of development, wall mechanical properties parallel the growth rate and presumably play a regulatory role.

HIGHER PLANTS As in *Nitella,* there is generally good agreement between wall mechanical properties and growth rates in higher plant tissues (see 42 for a summary). Monro et al (235) compared the growth rates and wall mechanical properties of basal (nongrowing), middle (slowly growing), and apical (rapidly growing) regions of lupin hypocotyls. Relative to the growth of the intact hypocotyl these regions were growing at 0%, 24%, and 76% respectively. The DP ratios for the three regions were 1:1.5:1.8, and the creep ratios were 1:1.6:3.6. Thus, creep seemed to correlate better with growth than DP (235). The rheological behavior of young vs senescent *Apium* collenchyma bundles

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was studied by Pilet and co-workers (154–157, 281, 330). The plastic strain remaining after a brief period of creep was greatest in the young, growing tissue and gave the best correlation with tissue age (154). The T_o and "extensibility" (strain/gram) of basal vs apical regions of rice coleoptiles correlated well with growth at different developmental stages and under various environmental conditions (107, 216). Low T_o values have also been correlated with rapid growth rates in intact pea stems and *Avena* coleoptiles (430). Sakurai et al (343) marked young intact *Avena* coleoptiles into six regions and monitored the gradient in growth rate over a 4 day period. Each region was then excised and tested for both auxin responsiveness and mechanical properties. The stress relaxation parameter *b* was inversely correlated with both the growth rate and auxin responsiveness (343). While good correlations between the growth rate and wall extensibility have generally been observed, the growth rate measurements have normally been carried out over long periods of time in which the relative growth rate at the time of excision has not been known (356).

Auxin increases both DE and DP in *Avena* coleoptile sections measured with an Instron, but changes in DP are larger and more closely correlated with growth (31). When measured over 5–6 h, growth and compliances increase linearly with the logarithm of the auxin concentration in *Avena* (31), pea stems (52), and soybean hypocotyls (52). At supraoptimal auxin concentrations in the presence of sucrose, large discrepancies arise. Thus, in the presence of sucrose (36), supraoptimal auxin inhibits growth while DP slightly increases (31); in soybeans, inhibition of elongation is greater than the decrease in DT (total compliance) (52); and in peas, elongation is slightly inhibited while DT is greatly enhanced (52). Provided that such discrepancies were not caused by changes in cell wall anisotropy, they confirm the fact that in vitro wall extensibility cannot be equated with in vivo extensibility.

As shown in Figure 2, increases in the DP of *Avena* coleoptiles are detectable within 10–15 min of auxin treatment, coinciding with the onset of growth stimulation (45). However, the maximum auxin-induced growth rate is attained by about 30 min (274), while the maximum for DP does not occur until 60–90 min of auxin treatment (45). Addition of KCN or CH to the medium inhibits growth within minutes (7, 306), but the DP value declines over a 60–90 min period (45). Thus, whether growth is being promoted or inhibited, the new equilibrium DP lags 60–90 min behind the new steady growth rate. The amount of stimulation of DP (2–3 times) is also less than the auxin enhancement of the growth rate (5 times) (31). Somewhat different kinetics have been noted for dicot tissues. In soybean hypocotyls, the maximum auxin-induced growth rate occurred at 90 min, while the maximum DP was delayed until 3 h of auxin treatment (45). Coartney & Morré (52) reported that 2,4-D increased the DT of soybean hypocotyls over an 8 h period, while the growth rate reached a maximum much earlier. Penny et al (272, 275) found that the auxin-stimulation

of the DT of lupin hypocotyls was not detectable until 15 min after the onset of the enhanced growth rate, and continued to increase over a 90 min period, long after the growth rate had stabilized. In general, it appears that extensibility changes measured by the Instron method, at least for the DP parameter, occur at or near the initiation of growth, and thus are likely to contribute to the enhanced growth rate. The lack of a precise temporal correspondence between DP and the growth rate indicates that DP cannot be equated with *m*, but probably represents one component. Cleland (45) has recently suggested that DP represents the average *m* over the previous 60–90 min in the case of *Avena* coleoptiles and the previous 3 h in the case of soybean hypocotyls. It was proposed that the time required to attain the maximum DP value reflects the time needed to replace the inner, stress-bearing region of the wall with a more extensible layer, by analogy to the control of the directional mechanical properties in *Nitella* (323). This interpretation, while plausible, raises questions about the role of wall synthesis in regulating extensibility. Increasing the rate of wall synthesis by the inclusion of sucrose in the medium does not increase DP (45). The relationship between wall synthesis and extensibility will be discussed in a later section.

Another way of looking at DP is to view it as a component of *m* at any given time rather than the average *m* over a previous period. For example, as previously noted, Green et al (127) demonstrated that auxin caused a 50% reduction in a deceleration term *D*, which they speculated was physical strain hardening, in rye coleoptiles. The largest effect of auxin was on the *A* term. Since the Instron apparently measures strain hardening, DP and *D* could be related. There is no reason to assume that the regulation of *A* and *D* would follow the same time course. Inasmuch as the growth rate is primarily controlled by *A* (127), *D* could contribute to the growth rate but might vary independently of it. A rough idea of the kinetics of the *A* factor might be obtained by subtracting the relative stimulation of DP from the relative stimulation of the growth rate by auxin. However, this approach has limited value because the growth rate will be regulated by turgor pressure as well as by wall properties during steady state growth.

The technique of stress relaxation has been widely applied to the analysis of auxin-induced changes in extensibility (44, 219, 343–346, 348, 374). Cleland & Haughton (44) measured the "relaxation modulus" *E(t)*, defined as the ratio of stress to strain at time *t*, in conditioned and nonconditioned walls of *Avena* coleoptiles. Mechanical conditioning increased the relaxation modulus, indicating that the walls had become stiffer, and decreased the "relative relaxation rate," defined as the percent of decrease in *E(t)* per decade of log time. Pretreatment of sections with IAA for 4–8 h resulted in a 30–40% decrease in *E(t)*, but had no effect on the relative relaxation rate, although it did increase the absolute relaxation rate (decrease in stress per decade of log time) (44). The results were similar for both conditioned and nonconditioned walls. The stress

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relaxation behavior of *Regnellidium* petioles was affected similarly by auxin (57).

The most detailed stress relaxation studies have been carried out by Masuda and his colleagues using nonconditioned wall preparations of *Avena* coleoptiles, various dicot stems, and epidermal strips. Of the parameters in their semi-empirical equation (Equation 6), auxin specifically decreases T_o by about 65% and b by about 25% (218, 219, 343, 432, 437). Note that b, the slope of the stress relaxation vs log time curve, is equivalent to the absolute relaxation rate of Cleland & Haughton (44). As noted previously, T_o is also influenced by the pre-extension rate, and the auxin effect on lowering T_o is obscured if the pre-extension rate is too slow (100, 101). The b parameter also varies with the time interval chosen for determining the slope of the relaxation curve. Auxin's effect on b is not consistently observed when b is determined during the first 7 sec of relaxation, but becomes consistent over longer periods (60 sec) (343).

The auxin-induced decrease in T_o and b begins as early as 5 min after auxin treatment in *Avena* coleoptiles, preceding the stimulation of growth (343, 432). Changes in T_o and b also differ from the growth rate in that they do not reach their minimum values for 1–2 h after hormone treatment (343; see Figure 2). However, when coleoptiles are treated with auxin in the presence of 0.25 M mannitol, the decrease in T_o is complete by 10 min (429). Thus, the kinetics of the change in T_o appear to be influenced by the presence of an osmoticum. The fact that T_o and b decline prior to the onset of enhanced growth has suggested to Masuda (212) that they represent the potential for future extension rather than present or past extensibility. This is supported by turgor step-up experiments in which it was shown that the amount of stored growth obtained after treatment of oat coleoptile or pea stem sections with auxin in the presence of 0.3 M mannitol (or less) was proportional to the decrease in T_o values, whereas the compliance correlated better with the length of the sections prior to the turgor step-up (344), suggesting that compliance measures the previous wall history. However, the results of the turgor step-up experiments are not directly comparable to normal growth. In the former, the potential for future extension accumulates because growth is osmotically prevented by mannitol, i.e. the "wall-loosening events" are not used up by extension. Restoring the turgor pressure allows the expression of that potential. On the other hand, extension in response to auxin-induced wall loosening in the absence of mannitol is not being prevented by low turgor pressure, yet the growth rate does not increase until 15 min after the decrease in T_o. It then becomes necessary to postulate that this "potential extensibility" of the wall can be expressed during a stress-relaxation experiment, but not in vivo. The magnitude of the effect of auxin on the stress-relaxation parameters is also not proportional to the subsequent auxin-induced growth rate (343). An alternative interpretation of T_o and b is that they represent minor components of m, as previously discussed in relation to DP. The kinetics for DP, T_o, and b are, in

fact, very similar (Figure 2). Thus it would seem that comparable, although not necessarily identical, wall properties are being measured by the various in vitro techniques.

The effects of auxin on the creep behavior of *Avena* coleoptiles was examined by Cleland (35). Auxin had no effect on the shape of the extension vs time curve, indicating that it does not alter the basic viscoelastic behavior of the wall. The creep rate (%L/decade of log time) of 2 h auxin-treated sections was about twice that of the controls, similar to the ratio of their DP values. KCN administered simultaneously with auxin inhibited creep and DP in parallel (35).

Rapid effects of auxin on the elasticity of pea stems have been detected by the resonance frequency technique (22, 23, 397). A 10% decrease in the tissue Young's modulus occurred over a 30 min period, beginning 2–3 min after auxin treatment, about 5 min prior to the increase in the growth rate (22, 23). Although the resonance frequency method is also affected by turgor pressure (90), it seems unlikely that turgor would decrease prior to the onset of growth. However, it is widely recognized that the outer tissue layers limit elongation in pea stems and that auxin primarily modifies the epidermis and outer cortical layers when it promotes growth (16, 17, 76, 218, 225, 291, 312, 382, 427, 431). The resonance frequency method, in contrast, measures the total tissue elasticity without distinguishing among the tissue layers. The early decrease in the tissue Young's modulus detected by the resonance frequency method might thus represent an effect on the central tissues, which may be more sensitive to auxin than the outer tissues (391). In wheat coleoptiles the decrease in the Young's modulus induced by auxin exhibits a 7–15 min lag period similar to the lag for auxin-induced growth (120).

Gibberellic acid increases the plasticity of *Avena* stem sections within 1 to 2 h, as measured by the Instron and constant load methods (2). This appears to precede GA-induced growth in this system, which has a lag time of about 3 h (174). GA also enhances growth and causes a decrease in the T_o of pea hook (249) and lettuce hypocotyl (178) walls, but does not enhance wall extensibility in cucumber hypocotyls (50a, 171). In cucumber hypocotyls, GA appears to promote cell expansion by lowering the tissue water potential, increasing the rate of water uptake (164, 171). Jones (164) has suggested that only in those tissues which show a dramatic response to GA does the hormone enhance wall extensibility.

Cytokinin-stimulated expansion of excised radish and cucumber cotyledons is associated with an increase in the Instron-measured DP of the cell walls (394). Ethylene-enhanced elongation of the aquatic plants *Ranunculus* and *Regnillidium* (56, 57, 66) is also accompanied by enhanced wall extensibility. Inhibition of pea stem elongation by ethylene is paralleled by a decrease in the longitudinal extensibility of the walls (78, 378). At the same time, a promotion of transverse extensibility is detectable, but only at acid pH (78, 378).

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Effects of pH and Ions

NITELLA Probine & Preston (296) first demonstrated that isolated *Nitella* walls were stiffened by calcium and loosened by potassium. These results were confirmed by Morikawa (240, 242) and by Metraux & Taiz (229), who also showed that magnesium and a number of other divalent and monovalent cations could cause wall loosening, while La^{3+}, Ca^{+2}, Sr^{2+}, Ba^{2+}, and Al^{3+} antagonized acid-induced wall loosening in ascending order of effectiveness. Consistent with a mechanism of wall stiffening based on physical cross-linking, EDTA causes massive wall loosening, suggesting that calcium is an important structural component of *Nitella* walls. Evidence has also been presented that the acid-labile sites in *Nitella* are distinct from the ion exchange sites (231, 242) and that the ion exchange sites have some specificity as far as the ability to regulate wall loosening is concerned (229). Transverse extension shows the same qualitative response to protons and ions as longitudinal extension, although higher concentrations are required, presumably because of the reinforcement by the transverse microfibrils (231). Since magnesium-stimulated *Nitella* wall loosening and calcium inhibition of acid-induced wall loosening were not significantly diminished by boiling in methanol or water, ion-mediated extensibility changes are most likely based on physical rather than enzymatic reactions. The effects of ions on *Valonia* cell walls parallel those of *Nitella* in many respects (384).

The uniaxial extensibility of *Nitella* walls is also strongly promoted by acid pH. Because of the abundance of nonesterified polygalacturonic acid (240), the wall has a considerable buffering capacity; hence the observed threshold pH for acid-stimulated creep depends on the concentration of the buffer. With 1 mM buffers acid-induced longitudinal creep begins at around 4.8 and transverse creep is first detectable at around 3.5 (229, 231). When 10 mM buffers are used, the pH threshold for acid-induced creep is 5.3 in the longitudinal direction and 4.0 in the transverse direction (226). The capacity for acid- or ion-induced creep is roughly proportional to the growth rate longitudinally, but does not seem to vary with developmental stage transversely (231).

Under multiaxial conditions, both D_t and D_p are stimulated by acid pH (see Figure 3), and the effect is greater in young walls than old walls (228). The P_c measured at 4.5 atm for walls extended at pH 6.5 is lowered to 2 atm (the lowest pressure measured) at pH 4.0 (377); the slope of the D_p vs pressure curve from 1–3 atm at pH 4.0 is about the same as the slope at pH 6.5 above the yield stress (>4.5 atm). This suggests that the primary effect of acid in *Nitella* is to lower the critical turgor, since "extensibility," defined as the slope of the strain/stress curve above the yield stress, is not increased by acid (377). These results can be compared with the observation, noted previously, that alkaline buffers block the metabolic P_{ca}-lowering process in vivo (P. A. Richmond and L. Taiz, unpublished data).

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Acid pH alters the shape of the creep curve as well as the magnitude. Our first short-term multiaxial creep studies with *Nitella* indicated that creep at both neutral and acid pH exhibited exponentially decaying kinetics. It was concluded that acid changed the magnitude of the creep, but did not fundamentally alter the shape of the curve (228). More recent studies carried out over longer periods of time have presented a radically different picture. Following the exponential phase there is a phase of rapid extension which is linear with time and continues until a strain of about 5% has occurred, at which point the wall bursts (H. Morrison and L. Taiz, unpublished data). This rapid linear extension is seen only at acid pH and is comparable in rate to the growth rate (about 2% h^{-1}). The extension is still viscoelastic, since removal of the pressure is accompanied by a retarded elastic contraction, although as much as 80% of the extension is irreversible. Thus, acid pH can induce a type of chemical creep in isolated walls, hypothesized to occur during in vivo wall extension. Similar results have been obtained in uniaxial experiments carried out on coleoptile walls (43a). The premise that isolated walls are incapable of linear extension with time must now be revised, although it is still not proved that the linear extension in vitro is by the same mechanism as occurs in vivo. The fact that the isolated wall weakens to the point of bursting suggests that continued wall repair and synthesis is required to counter the effects of protons.

HIGHER PLANTS The acid growth hypothesis first gained momentum when it was demonstrated that the extension of frozen-thawed coleoptiles (314) and plasmolyzed *Helianthus* hypocotyls (135) under constant load was greatly enhanced by acidic solutions. The unusual Q_{10} pattern for acid-induced extension is the same as for acid growth, i.e. 3–5 between 15° and 25°, 1 between 25° and 35° (212). This provided for the first time an in vitro system which simulated growth, or at least acid growth. To respond to acid, the wall must be close to its native state. Boiling in methanol (314), digestion with pronase (50), or treatment with 8 M urea (384) either eliminate or greatly reduce the sensitivity to acid. These results suggest that wall proteins are necessary for acid-induced extension. In contrast, pretreatment of *Helianthus* hypocotyl epidermal strips with 15% formaldehyde for 30 min had no effect on acid-induced extension (363). Since formaldehyde covalently cross-links proteins (although this has not actually been tested for cell wall proteins), protein mobility apparently is not required for acid-induced extension. However, during the extension, the pH of the medium was lowered continuously down to pH 2.0, with most of the extension occurring over the pH range 3.5–2.0. Acid-induced extension in this pH range may be by a different mechanism than the mechanism in the physiological pH range (428). Further studies with formaldehyde are warranted.

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The pH threshold for the acid-induced growth and extension of unpeeled, unabraded sections is around 4.0 (309). Removing the epidermis or abrading the cuticle raises the pH threshold to ~5.8 in *Avena* coleoptiles (76) and soybean hypocotyls (312). The cuticle has been demonstrated to be relatively impermeable to protons (312). The maximum extension rate occurs at around pH 3.0. In *Avena* coleoptiles the maximum was initially reported to be 4.8 (308), but under slightly different conditions the maximum occurs at pH 3 (381).

Acid effects on wall extensibility have been studied in parallel with auxin. Pretreatment of living, unpeeled *Avena* coleoptiles with pH 3 buffer causes an increase in the %PE as measured on methanol-boiled walls by the Instron method (310). The %PE increases within 15 min and nearly doubles by 150 min of acid treatment, comparable to the effects of auxin. It would be interesting to know whether the changes in %PE of acid-treated frozen-thawed sections under tension would show the same kinetics. Incubation of frozen-thawed coleoptiles for 2 h in the *absence* of tension led to only a 40% increase in the %PE (384). When acid-induced growth is inhibited by pH 7 buffer, the decline in %PE roughly parallels growth (310).

Yamagata et al (428) investigated the effects of acid on wall extensibility by stress relaxation. When bored out, hollow-cylinder pea stem sections were treated with acid for one hour and the mechanical properties of the methanol-boiled epidermis were tested, the T_o was lowered to the same extent as by auxin. Pretreatment with a combination of acid and auxin lowered the T_o still further, but the effect was less than additive. This suggests that at least part of the auxin effect was caused by wall acidification. In a second series of experiments, frozen-thawed epidermal strips were tested and acid was administered in vitro during the extension. However, since tension is required for acid to loosen the wall, there was a lag time of 1 sec after the wall was stretched before an acid-induced increase in the rate of stress relaxation was observed, which represents the lag time for the action of acid on the wall (428). This delayed action of acid introduced an inflection in the curve which made it impossible to calculate the effect of in vitro acid treatment on the T_o value. However, in an earlier study by the same group (220), epidermal walls were preconditioned in either neutral or acid buffer prior to the stress relaxation. The data show an acid-induced increase in the relaxation rate but little effect on T_o (220). Thus the effects of acid in vivo may differ from its effects in vitro.

The majority of studies on acid-induced wall extension have concerned acid-stimulated creep. It has been shown that the walls of higher plants must be under a minimum tension for acid to have an effect. In *Avena* coleoptiles the applied load must exceed 10 g (310), while for pea epidermis the minimum yield stress was about 0.7×10^7 dynes/cm^2 (4.8 g) (428). Since the creep rate of methanol-boiled *Avena* coleoptile walls also appears to show a yield stress at

10–12 g of applied load (35), acid does not seem to lower this yield stress of coleoptile walls. In contrast to coleoptiles *Valonia* walls do not require tension for acid-induced loosening (384).

Recently, Cleland et al (43a) have mathematically analyzed the acid-induced extension of *Avena* coleoptiles. At a 20 g load, an exponential phase predominates over the first hour, but results in only a 5% increase in length. Subsequently, extension becomes linear with time and can continue for 10 hours, causing a 50% increase in length. The ability of acid to cause linear wall extension with time in uniaxially stressed higher plant walls as well as in multiaxially stressed *Nitella* walls suggests that wall acidification may be the critical metabolic wall-loosening process responsible for linear wall extension during growth. This was seen, but not commented upon, by other workers (e.g. 155, 439). In the original extension curves of Hager et al (135) for *Helianthus* hypocotyls plasmolyzed in 50% glycerol, it can be seen that acid induces linear extension after the initial exponential phase.

The capacity for acid-induced wall loosening (CAWL) declines with age (113–115). In *Avena* coleoptiles, the CAWL of excised sections decreased in the presence or absence of sucrose over a 10 h period (43). Treatment with auxin causes an increase in the CAWL which reached a maximum at 10 h, and sucrose had no effect. FC did not enhance CAWL, and CH rapidly caused a decline (43–51). It is interesting that the kinetics of the CAWL property differ distinctly from those of any of the other wall parameters and of the growth rate (see Figure 1). CAWL thus represents another important component of auxin-enhanced wall extensibility. When evaluating a possible causal relationship between wall acidification rates and growth, the capacity of the tissue to respond to acid must be taken into account.

Wall extensibility in higher plants is also influenced by ions (375). Treatment of living stems or coleoptile sections with Ca^{2+} inhibits auxin-induced growth and causes a decrease in the compliances of isolated walls (49, 52), while K^+ induces wall loosening as measured by a bending technique (375). Stiffening by calcium was initially considered to be a direct cross-linking reaction with the pectin carboxyl groups of the wall, but this interpretation has recently been questioned. For example, it has been shown that treatment of isolated walls, either methanol-boiled or frozen-thawed, with calcium does not decrease the extensibility of the walls as measured by the Instron (49, 52). Therefore, it has been proposed that calcium does not stiffen the wall directly by forming cross-links as at first believed. Consistent with this observation, EDTA is less effective in promoting wall extension in *Avena* walls than in *Valonia* walls (384). In *Valonia,* 10 mM EDTA buffered at pH 7 induces greater extension than pH 4 buffer, while the opposite is true for *Avena* walls. It was further demonstrated that the major effect of calcium is to antagonize

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acid-induced extension, which is thought to be enzyme-mediated (45, 49, 384, 385). This can be interpreted in two ways: either calcium specifically blocks the action of a wall enzyme activated by acid pH (49) or calcium directly cross-links the wall, but the binding sites are accessible only at acid pH, a possibility suggested by the results of Bates & Ray (8; discussed later).

Other evidence supports a direct stiffening action for calcium. The creep studies of Nakajima et al (248) on frozen-thawed pea epidermal tissue indicated that 50 mM calcium stiffened the wall, while the same concentration of magnesium caused wall loosening. Whether or not such high concentrations have any physiological relevance is an open question. It is of interest, however, that growing regions of pea stems are characterized by higher Mg^{2+}/Ca^{2+} ratios in their walls than nongrowing regions (248). Soll & Böttger (363) found that EDTA strongly promoted the extensibility of frozen-thawed epidermal strips of *Helianthus,* while Ca-EDTA had no effect. EDTA-induced extensibility was shorter lived than acid-induced extension. Soll and Böttger argued that at pH 6.5 the concentraion of the active chelating species $EDTA^{4-}$ is lower than the $[H^+]$ of an equimolar HCl solution. When corrected for the active species, EDTA was as effective as protons in causing wall loosening (363). However, this does not account for the greater sensitivity of *Valonia* walls to EDTA than *Avena* walls. Further aspects of this question will be considered later. Chelators have also been implicated in wall loosening in suspension-cultured cells (80).

Proton Magnetic Resonance

An important new approach to the study of wall physical properties has been the use of broadline proton magnetic resonance to monitor the molecular motions of cellulose, pectin, and isolated *Phaseolus* hypocotyl walls as a function of pH (pD) (383). The material was first equilibrated with D_2O to eliminate the contribution of water to the measurements. Whether D_2O itself affects the wall's properties has not yet been determined. The analysis showed that about 60% of the wall was relatively rigid and 40% more mobile (203). Since cellulose comprised only 30% of the dry weight, much of the matrix, probably the hemicellulosic xyloglucan, is bound to the surface of the microfibrils, in agreement with earlier wall models (291). Lowering the pD from 6 to 2 had no significant effect on the ratio of rigid to mobile components in the wall, but it did decrease the motional frequency of the rigid components. The authors speculated that this might indicate a weakening of the more labile associations between the hemicellulosic matrix polymers, using the analogy of the harmonic motions of a stretched string (383). Clearly, proton magnetic resonance is a potentially powerful tool for monitoring the dynamic molecular events associated with wall extension.

THE BIOCHEMISTRY OF WALL EXTENSION

Recent Views on Wall Molecular Structure

Considerable progress has been made in the chemical analysis of cell walls over the past decade (3, 70, 167). Preliminary wall molecular models have been proposed for dicots (181) and monocots (19a, 71, 72), discussion of which is beyond the scope of this article. Two salient features of these early models are the hydrogen bonding of the major hemicellulosic consitutuents to the microfibril surface (xyloglucan in dicots, arabinoxylan in monocots) and the covalent attachment of the hemicellulose to the pectin and extensin fractions, forming a tightly cross-linked matrix network. It was originally proposed that wall extension involved the disruption of the hydrogen bonds at the xyloglucan-cellulose interface and that this was promoted by auxin-induced wall acidification (180). This idea was dropped when it was later shown (398) that the binding of xyloglucan to cellulose was not affected by weak acid treatments. However, xyloglucan turnover could perform the same function, as will be discussed below. The existence of a single covalent network interconnecting all the matrix components was challenged by Monro et al (237) on the basis of chemical extraction of lupine hypocotyl walls. At least two separate networks were proposed, and the pectic uronic acids were considered to be noncovalently bound to the wall (237). Jarvis (161) has presented evidence for two classes of uronic acids, covalently and noncovalently bound. More recent studies on sycamore suspension cell walls indicate that the neutral and acidic pectin polysaccharides are covalently interconnected, but the extent of covalent attachment to the hemicelluloses is undetermined (70). Nor does there appear to be any covalent attachment between the wall glycoprotein, extensin, and any of the other wall polymers. (70)

Recently there has been a tendency to avoid specific molecular models and to concentrate on detailed characterization of the subfractions obtained by enzymatic or chemical extraction. In dicots, five noncellulosic polysaccharides have been extensively purified and characterized: xyloglucan, glucuronoarabinoxylan, homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II (70). Monocot walls differ from dicot walls in several respects: the content of pectic polysaccharides is much lower, arabinoxylan is the dominant hemicellulosic component rather than xyloglucan, and the walls contains mixed β-1,3-β-1,4-linked glucans (70).

At present, the general wall model proposed by Preston (291), in which the cellulose microfibrils are coated by hemicellosic polymers and are separated by the pectic polysaccharides, is still considered valid (70). However, there are two schools of thought regarding the nature of the stress-bearing bonds within the matrix. One view is that wall extensibility is limited by covalent bonds which require enzymatic cleavage for loosening to occur. These include glyco-

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sidic linkages between sugar residues or between arabinose and hydroxyproline residues of the wall protein (191, 192), linkages formed by the oxidation of covalently bound ferulic acid residues to yield diferuloyl (biphenyl) bridges (98, 99, 361, 362), or interpolypeptide cross-links formed by isodityrosine (58, 98). An alternative view, recently discussed in detail by Preston (292) and favored by Sellen (354) for *Nitella* walls, is that the matrix owes its mechanical properties to the combined strength of many weak interactions among the various wall polymers and ions. In this case wall loosening is effected by enzymatic or physical means or a combination of the two.

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Cell Wall Enzymes and Polysaccharide Turnover

The possible role of wall enzymes in polysaccharide turnover and wall extension has been reviewed recently (42, 93, 186, 212, 213, 274, 440). As discussed earlier, acid-induced wall extension resembles an enzymatic process because of its sensitivity to denaturing conditions, its high Q_{10}, and its high pH threshold (~5.8). To account for the fact that neutral buffers can reverse auxin- and acid-induced wall loosening, and that growth and wall extensibility are rapidly blocked by a variety of inhibitors (50) it is necessary to postulate that the enzyme is both pH-regulated and rapidly reversible. Three other conditions which could be incorporated into the mechanism are the requirement for a minimum yield stress (309), the low Q_{10} between 25–35°C (310), and inhibition by calcium (49, 52). One type of enzyme which could, in principle, satisfy many of these criteria is a transglycosylase (3, 42). Such an enzyme would cleave stress-bearing glycosidic cross-links, allowing wall extension under turgor stress, and resynthesize the bond at a new location. This type of extension has been likened by Cleland to "chemical creep" (42).

To date, the only reported case of transglycosylase activity in plant cell walls appears to be a reversible endo-dextranase (α-1,6-glucanase) associated with cell wall pellets from *Avena* coleoptiles (146). The activity exhibits a twofold increase in response to auxin and the effect is detectable within 1.25 h. These kinetics are too slow for the rapid growth response to auxin, but could be involved in long-term growth (see Figure 2). In the presence of low molecular weight dextran substrate, the wall pellets released glucose and isomaltose without increasing the total reducing sugars of the reaction medium. This was interpreted as evidence for transglycosylase activity (146). However, other explanations might account for the loss of reducing sugars in the medium. Nor is it clear that only one enzyme is involved. Regarding the native substrate for *Avena* dextranase, Heyn demonstrated that the wall pellets can degrade commercial preparations of arabinogalactan, but the significance of this finding for coleoptile walls is uncertain.

Darvill et al (71, 72) studied wall metabolism in corn coleoptile sections. Treatment with acid buffer or growth-promoting concentrations of auxin were

strongly correlated with the turnover of *t*-arabinose and 3,4-xylose residues of the wall. Glucuronic acid and galactose were also released from the walls of auxin and acid-treated tissues. These effects were not evident until 1–3 h of auxin treatment. No turnover of the wall β-glucan was observed (76, 183). The authors suggested that auxin-induced wall loosening is enzymatically mediated and is promoted by acid pH. The mechanism was postulated to involve: (*a*) a decrease in viscosity caused by the loss of arabinose side chains from the xylan backbone of the glucuronoarabinoxylan; (*b*) cleavage of the arabinogalactan side chain connecting the xylan to the extensin network; (*c*) decreased molecular interactions possibly of an ionic nature traceable to the loss of glucuronic acids.

Glycosidases have been identified in the cell walls of living tissues and in isolated wall preparations (91, 163, 247, 250, 270, 279, 381). Auxin stimulation of β-galactosidase activity in living *Avena* coleoptiles was detectable within one hour, and the enhancement was prevented by neutral buffers (162). The same enzyme was reported to be promoted by auxin in pea stems (381), but in frozen-thawed sections which probably included cytoplasmic contaminants. Auxin had no effect on either β-galactosidase or β-glucosidase activities in the cell walls of cucumber hypocotyls (172). Attempts to correlate glycosidase activity with growth rates in intact stems have generally been unsuccessful (73, 172, 247, 279), at least when expressed on a per cell basis rather than on a fresh weight basis (247). Specific aldonolactone inhibitors of various glycosidases failed to inhibit auxin-induced growth even though the activity of the wall enzymes was reduced (84, 111, 276). Taken together, the results do not support a role for glycosidases in wall extension.

The autolytic enzyme system of isolated coleoptile walls (149, 150, 173, 194) has been investigated extensively in relation to auxin-induced growth. The major constituent lost during autolysis of isolated walls is a mixed β-1,3-, β-1,4-linked glucan (149, 183, 436). Although auxin pretreatment does not enhance the autolytic capacity of isolated walls (149), treatment of *Avena* coleoptile sections with auxin in the absence of sucrose leads to an enhanced rate of glucan disappearance, and the effect is detectable after 2–4 h (199, 201, 211, 436; Figure 1). No decrease occurs in the presence of sucrose, possibly because of its being masked by new synthesis. Pulse-chase experiments (187, 188) would help to resolve this question. Net loss of glucan is not a prerequisite for cell expansion, since sucrose does not inhibit auxin-induced growth. However, only a change in the molecular weight may be required; degradation may perform a scavenger function for the metabolic retrieval of wall fragments (24). In accord with this concept, auxin causes a decrease in the molecular weight of hemicellulose B (mainly high molecular weight β-glucan) within 30 min (349; Figure 2).

There does not appear to be an consistent correlation between glucan loss and

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changes in wall mechanical properties. On the one hand, auxin-induced decreases in T_o and wall glucan are both observed in the presence of growth-inhibiting levels of mannitol and are prevented by mannitol concentrations in excess of 0.25M, indicating that both effects require a minimum turgor (200, 344). On the other hand, the data also indicates that T_o is not a reliable indicator of wall glucan levels. For example, the T_o value of walls from coleoptiles treated with auxin plus 0.15 M mannitol is the same as the T_o of the control in the absence of mannitol, yet the glucan content of the auxin-treated walls is much lower (Figures 2 and 3 of 344). Similarly, cycloheximide inhibits the auxin-induced decrease in T_o even when added 2 hours after auxin treatment, whereas CH does not inhibit glucan loss after the 2 h period (347). Thus, after 2 h glucan loss is insensitive to CH while T_o (and growth) can still be inhibited.

Nojirimycin, a potent inhibitor of β-glucosidases and exoglucanases (but not endoglucanases) has been shown to inhibit growth, glucan loss, and the decrease in T_o of auxin-treated coleoptiles (251–253, 344, 348) and pea epidermis (426). This has been cited as evidence for the involvement of an exoglucanase in coleoptile growth (254). However, it now appears that the inhibition may be a nonspecific one, since nojirimycin can inhibit tissue β-glucosidase within 10 min (75), while its inhibitory effect on growth requires a 60–90 min pretreatment (254).

Early interest in the degradation of β-glucan was stimulated by the discovery that a fungal exo-β-1,3-glucanase derived from *Sclerotinia libertiana* can induce growth in *Avena* coleoptiles [relative to a boiled enzyme control (210, 215, 217, 434)]. The response to the enzyme is transient, lasting about 60 min (434). Since unabraded sections were used, the enzyme may have been limited by its ability to penetrate through the cut ends. The growth-promoting properties of the enzyme were correlated with its ability to degrade the mixed linked wall β-glucan (434) and with its tranglycosylase activity (433). Treatment of corn coleoptile walls with 3 M LiCl solubilizes an exo-β-1,3-glucanase and an endo-β-glucanase, and simultaneously removes the walls' autolytic capacity (151, 153). These two enzymes appear to act in concert to degrade the mixed linked glucan during autolysis, but they were reported to be inactive in promoting the growth of corn coleoptiles even when the cuticle is abraded (152). Nor is the ability of living coleoptiles to hydrolyze external laminarin enhanced by auxin. These results do not support a role for these enzymes in cell expansion. On the other hand, antiserum raised against the LiCl-solubilized wall proteins inhibit auxin-induced growth and block wall autolysis (152). Control antiserum pretreated with isolated walls loses much of its inhibitory action. Since the antiserum did not inhibit the activity of the solubilized β-glucanases, Huber and Nevins proposed that they were immobilizing the enzymes, thus restricting their ability to degrade the wall. More recently, an exoglucanase isolated from

corn coleoptile walls, which decreases the viscosity of oat β-glucan, has been shown to be able to stimulate the growth of corn coleoptiles in the presence or absence of auxin (189). Whether or not this enzyme has the appropriate properties of a putative wall-loosening enzyme remains to be determined.

Although the autolysis of isolated coleoptile walls as well as the activities of the solubilized glucanases have acidic pH optima, acid-induced growth and T_o reduction are not accompanied by a decrease in wall glucan (348). Nor does nojirimycin, which blocks auxin-induced growth and glucan loss, block acid-induced wall extension (348). The effects of strong neutral buffers on glucan loss has apparently not been tested. Viscosity changes may be more significant than glucan loss. The periodate oxidation studies of Coartney & Morré (53) provided no evidence of large-scale increases in wall-reducing sugars indicative of massive depolymerization in auxin-treated corn or oat coleoptile walls. More subtle changes, such as selected attack by a glucanase which lowers the viscosity of the glucan without causing net loss, are indicated.

The pulse-chase homogenization experiments of Labavitch & Ray (187, 188) provided evidence that xyloglucan was specifically released from the walls of pea stems during auxin treatment. This has since been confirmed by Gilkes & Hall (110), Terry & Bonner (387), and Terry et al (389). The latter used a wall centrifugation method to spin out soluble products in the free space solution. Since xyloglucan release is detectable within 15 min of auxin treatment (188), it is a prime candidate for the early wall-loosening step in auxin-induced cell elongation. Jacobs & Ray (158) further showed that acid buffers also caused xyloglucan release. This is in contrast to the glucan loss of coleoptiles which is not promoted by acid. Acid-induced xyloglucan solubilization in pea stems was also confirmed by Terry et al (389), and the effect was inhibited by low temperatures, suggesting that enzymes are involved. Pea stem cellulase (133) is capable of degrading xyloglucan and could possibly be involved in xyloglucan turnover. Enzymes capable of degrading xyloglucan have also been detected in soybean cell walls (185). However, acid does not release xyloglucan from isolated pea stem walls in vitro, although it promotes the solubilization of pectin under these conditions (8). Bates and Ray speculated that the xyloglucan released by acid treatment in vivo may already have been soluble. They favor a physical mechanism of release rather than an enzymatic one (discussed in a later section). The in vitro acid-induced solubilization of pectin from isolated walls may be significant, since Terry et al (389) noted a 150–200% enhancement by auxin of soluble polyuronides in the free space solution after auxin treatment, compared to only a 50% increase for xyloglucan. As early as 30 min after auxin addition there was a 60% auxin-stimulation of pectin release, at which time xyloglucan release was only 10% greater than the controls. Thus pectin release seems to show a better quantitative correlation with growth than xyloglucan release. Inhibiting elongation and

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promoting lateral expansion with either kinetin (110) or ethylene (390) blocks xyloglucan turnover. Gilkes and Hall have proposed that xyloglucan turnover may be required for regulating wall mechanical anisotropy.

Goldberg & Prat (115) suggested that pectin metabolism may be important for the initial stage of auxin-induced growth, while glucan metabolism is needed for long-term growth in mung bean hypocotyls. They found a correlation between the sensitivity to auxin and acid in different zones along the hypocotyl and the pectin content of the walls. The pectin viscosity and wall calcium content was inversely correlated with the growth rate, although no direct effect of auxin was tested. The in vitro activity of wall-associated exo-β-1,3-glucanase was also correlated with the growth rate. Since the activity of the enzyme was the same in auxin-treated and control walls after solubilization, the authors suggested that the enzyme was regulated in situ by auxin-induced modifications of wall structure, possibly pectin metabolism. Mung bean hypocotyls are the only dicot tissue reported to contain mixed β-glucans in their cell walls (19).

In *Azuki* bean stems, auxin and acid cause about a 20% decrease in the average molecular weight of the hemicellulosic xyloglucans, although there is no net decrease in total xyloglucan from the wall (258, 259). The acid-induced MW decrease begins without a lag, is linear with time over a 3 h period, and is rapidly reversed by neutral buffers. Evidence was presented that a high MW xyloglucan was being degraded to a lower MW xyloglucan during auxin treatment (259) and that covalent bonds were being broken. Unlike the situation in pea stems and possibly mung beans, auxin causes an *increase* in the average molecular weight of the wall pectins within 30 min of treatment (257). Such an effect could be brought about either by transglycosylation reactions between the neutral sugar side chains attached to the rhamnogalacturonan backbone (338) or by the formation of phenolic cross-links between the two components (99). A concomitant auxin-induced decrease of hemicellulosic arabinose and galactose was also observed during this time (257), but only in the absence of sucrose (260).

In peas, lateral cell expansion induced by supraoptimal auxin concentrations is associated with a rise in cellulase activity, although the greatest increase occurs during the cell division stage (91). Ethylene-induced lateral expansion is not associated with an increase in cellulase activity in pea stems (324), although it has been implicated in the formation of aerenchyma tissue (179, 180). Cellulase does not induce elongation in *Avena* coleoptiles (339).

Establishing a causal relationship between the activity of wall enzymes and wall extension has proved to be extremely difficult. In yeast, a mutant deficient in β-1,3-glucanase has been isolated which shows normal budding behavior (350). Similar genetic approaches are needed in plant growth studies. A preliminary report by Catt (26a) on the isolation of cell wall mutants from carrot

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tissue cultures is a promising start in the search for mutants which are either deficient or constitutive for specific wall enzymes.

Noncovalent Interactions

HYDROGEN BONDS The various types of noncovalent interactions in the wall have been reviewed recently by Preston (292). Calculations of the activation energies during stress-relaxation experiments indicate that *Nitella* wall extension does not involve "heavily bonded units" and corresponds to "one or two covalent bonds or five to ten hydrogen bonds" (354). Because of the broad distribution of activation energies, Sellen considers it unlikely that covalent bonds are involved in wall extension, but rather the cooperative resistance caused by many weak interactions. Sellen speculates that the biochemical event which promotes linear extension may act by narrowing the activation energy spectrum (354). Although hydrogen bonds are believed to be abundant in cell walls, and may be important for xyloglucan binding to cellulose, wall loosening caused by acid is not mediated by the breaking of hydrogen bonds between xyloglucan and cellulose (398). Chaotropic agents, such as 8 M urea, which disrupt hydrogen bonds have surprisingly little effect on wall extensibility (384). These results do not support a wall-loosening mechanism based on the weakening of hydrogen bonds.

IONS It is well documented that the cell walls of algae (69, 240, 242, 399, 400) and higher plants (67, 182, 244, 321, 385) are high-capacity cation exchangers, primarily because of the presence of nonesterified pectin uronic acids. However, the contributions of other charged groups, e.g. proteins, plus the steric hindrance offered by the three-dimensional network, make the ion exchange properties of the wall matrix more complicated than those of soluble pectin (400). In both *Nitella* (400) and higher plant (321) walls there is evidence for at least two types of ion exchange sites with different affinities and specificities. Site specificity may influence the relative effectiveness of various ions to alter the wall's mechanical properties (229, 231).

In *Nitella,* Morikawa (240, 242) and Metraux & Taiz (229) suggested that ions may be acting on the gel structure of the pectins, which resemble higher plant pectins except that the uronic acid residues are almost entirely nonesterified (240). The order of wall-stiffening ability agrees with the effects of these ions on the stiffness and viscosity of pectate gels (see 229 for references). According to the "egg box" model of pectin gels (315), the cross-linking ions are bound in coordination spheres between adjacent polyuronide chains. However, the pectin gel model of ion action has been disputed by Tepfer & Taylor (385). They point out that the results of Metraux and Taiz are inconsistent with the relative ability of certain ions to form pectate gels. For example, they show that Co^{2+} and Zn^{2+} form weak pectate gels, while these ions are

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slightly more effective than K^+ (which does not form a pectate gel) in promoting wall loosening in *Nitella* (229).

An early model of auxin action postulated that auxin interfered with calcium cross-links in the wall (9). This model was abandoned when it was shown by Cleland (30) that $^{45}Ca^{2+}$ was not removed from coleoptile walls by auxin treatment. However, the homogenization technique used would only have measured a change in the wall's binding affinity for calcium (which was proposed in the models of that time) and would not have detected a change in wall calcium caused by displacement (for example, by protons). $^{45}Ca^{2+}$ in the tissue would not have escaped into the medium because the cuticle was left intact. Recent histohemical studies have indicated apoplastic redistribution of calcium during the gravitropic curvature of coleoptiles, with more calcium on the upper nongrowing side (360). Cohen & Nadler (55) presented evidence for an H^+/Ca^{2+} exchange during auxin-induced proton extrusion by *Avena* coleoptiles. These results suggest that the question of auxin's effects on cell wall calcium should be reexamined. Since protons release calcium from higher plant walls (363), it would be expected that auxin-induced acidification would cause a similar exchange to occur.

Tepfer & Taylor (385) found that the ability of divalent cations such as Ca^{2+}, Zn^{2+}, and Mn^{2+} to antagonize acid-induced growth and wall extensibility of bean hypocotyls was unrelated to their ability to bind pectin and to form pectate gels. However, there was an exact correspondence between the ability of ions to release protons from cell wall preparations and their ability to inhibit acid-induced wall extension (385). This suggests that calcium interacts with proton-binding sites in the wall, possibly structural glycoproteins. In contrast, Soll & Böttger (363) found a close correspondence between the effects of pH and ions on the extension of *Helianthus* epidermal strips and their effects on pectate gels. These apparently conflicting reports might be reconciled if there is a tripartite interaction between calcium, pectin, and glycoproteins, as described by Bates & Ray (8).

Yamaoka and co-workers (438–439a) have identified pectin methylesterase (PME) as the proteinacious factor required in addition to Ca^{2+} for pectin gel formation. Of particular relevance to the acid growth model, such gels are readily dissolved by weakly acidic solutions immediately after gelation (438). Analysis of the pectin fraction of soybean hypocotyls showed changes in the coagulating ability with cell age. The pectin of the growing region was rich in neutral side chains and tended to remain soluble in the presence of PME and Ca^{2+}, while the pectin of the mature zone was readily precipitated (439a).

Wall-bound calcium may be one of the factors which contribute to the parameter "CAWL" (43). From a regulation point of view, the H^+/Ca^{2+} ratio in the wall may be more important than the actual wall pH or calcium concentration, and tissues may differ according to their endogenous wall H^+/Ca^{2+}

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ratios. As previously noted, gravitropic curvature of coleoptiles is accompanied by asymmetric distributions of calcium (360) as well as by the generation of pH gradients across the tissue (246). Growth regulators might act by regulating the wall proton-calcium ratio rather than the wall pH. In principle, extensibility can be directly influenced by increasing the proton concentration when the wall calcium level is low, but may require calcium uptake if the level of wall calcium is high. In lettuce hypocotyls, calcium inhibition of GA-induced growth is reversed by EGTA, but not by protons (233). Ruthenium red, which blocks Ca^{2+} transport, inhibits GA-induced growth, although the specificity of the inhibition is not known (164, 233). It is tempting to speculate that blue light, which inhibits hypocotyl growth, may promote calcium efflux, while GA promotes calcium influx (60). Extension in lettuce may be limited by wall calcium rather than by wall pH, consistent with the observation that GA does not promote proton extrusion in lettuce (373).

LECTINS AND LECTIN-LIKE INTERACTIONS Extracted wall polymers can self-assemble into configurations which, at the ultrastructural level, resemble patterns seen in native walls (317). Several types of wall polymer interactions have been described in recent years which could be related to wall extension. Of particular interest is the extraction of lectin-like proteins from the cell walls of mung bean seedlings (175–177). On the basis of their high specific activity, these appear to be authentic wall proteins rather than cytoplasmic contaminants, and their levels are higher in nongrowing walls than in growing walls (161). The activity is specific for galactosyl residues and is strongly antagonized by D-galactose, pNP-α-D-galactoside and γ-D-galactolactone. It may be significant that the latter compound, a specific inhibitor of β-galactosidase, promotes growth in lupin hypocotyls (276), but wall lectins have not been characterized in lupin. There are no reports of growth stimulation in mung bean hypocotyls by galactose derivatives, and galactose actually inhibits growth in coleoptiles (435).

The lectin activity of mung bean walls is inhibited by acid pH and is inactivated by prolonged washing with EDTA. Mn^{2+} is partially effective in preserving activity (176). The mung bean lectin denatures easily, as evidenced by its tendency to form insoluble precipitates after ultrafiltration or lyophilization. Kauss and co-workers (175) have recently shown that mung bean lectin does not contain hydroxyproline and that it copurifies with β-galactosidase activity. The lectin and enzyme may be the same protein.

Bates & Ray (8) studied the release of polymers from radioactively labeled pea stem walls as a funciton of pH. At zero time the release of TCA- and ethanol-insoluble radioactivity was the same at pH 4.5 and 7.0. However, if the walls were preincubated in the two buffers prior to extraction, the amount of ethanol-insoluble material decreased and the amount of TCA-insoluble ma-

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terial increased at neutral pH. The material lost from the ethanol-insoluble fraction was identified as pectin. This was interpreted as a binding reaction at neutral pH between the pectin and a TCA-insoluble fraction, probably a protein. This binding, which could also be observed in aqueous extracts, had a Q_{10} of 1.6, could be prevented by boiling the wall, and was inhibited by acid, all of which suggest a lectin-like interaction. However, the binding was not prevented by galactose or galacturonic acid. Calcium had two effects: at acid pH it promoted binding of pectin to the insoluble wall residue; at neutral pH it prevented the binding between pectin and protein (8). The acid-induced release of pectin from the isolated walls is consistent with the observations of Terry et al (389) that auxin and acid cause the release of polyuronides into the free space solution. However, the failure to detect a similar acid-induced release of xyloglucan remains unexplained. One possibility is that the xyloglucan fraction being "turned over" is already soluble and is lost from the wall during the isolation procedure (8). If so, the release of xyloglucan may be a manifestation of changes in wall mechanical properties rather than a cause.

Wada & Ray (414) described another type of physical interaction between the hemicellulosic β-glucan and glucurono-arabinoxylan of *Avena* coleoptile walls. These two polymers are soluble as a mixture, but when separated the β-glucan has a tendency to form precipitates or gels due to intermolecular binding. If the β-glucan exhibits such a tendency in the wall, this might contribute to the wall's mechanical strength. The enzymatic cleavage of glucan chains (349) might help to reduce such intermolecular binding reactions. Secretion of glucurono-arabinoxylan into the wall might also interfere with this reaction, increasing extensibility. This model contrasts with the idea that arabinoxylan rigidifies the walls of monocots by hydrogen bonding to cellulose (222). The ability to form hydrogen bonds with cellulose is inversely related to the abundance of arabinosyl residues (69).

Extensin and Polymer-Bound Phenolics

The chemistry and possible functions of covalently bound wall glycoproteins and phenolics have been reviewed recently by Preston (292), Lamport (191), and Lamport & Catt (192), but significant progress has been made in the last few years. Extensin is a wall glycoprotein rich in the unusual amino acid hydroxyproline, which is also abundant in collagen, the extracellular matrix protein of animal cells (192). The levels of tightly bound wall hydroxyproline have been inversely correlated with growth rates in a number of cases (148, 165, 267, 280, 341, 424, 425). Inhibition of proline hydroxylation by low O_2 (104–106) or the iron chelator α-α-dipyridyl (6) can stimulate growth, presumably because it results in the production of a defective wall protein. However, the α-α-dipyridyl results should be interpreted with caution. Lang (193a) has reported that the chelator reverses light-induced inhibition of radish hypocotyl

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elongation, even though light does not increase cell wall hydroxyproline. Although the complete amino acid sequence is not yet known, physical analyses indicate that extensin is a rod-like molecule consisting of a protein core wrapped in a sheath of oligosaccharides composed of β-linked arabinofuranoside residues attached to hydroxyproline, as well as galactosyl serine linkages (191). It is speculated that the oligopolysaccharide coating may rigidify the protein, analogous to the collagen triple helix (191). The "sugar coating" may also make extensin more hydrophilic and also provide sites for glycosidic bonding to the matrix polysaccharides (191).

Exciting progress has recently been made on the mode of extensin's attachment to the wall, with implications for wall assembly as well as extension. It has long been known that tightly bound extensin is very insoluble and cannot be extracted by digestion with wall enzymes or even by anhydrous HF, which cleaves glycosidic linkages (192). The fact that the wall retains its shape even after HF digestion suggests that extensin may form it own independent network (192). However, extensin is readily solubilized by acidified chlorite, which causes delignification by cleaving phenolic polymers (264). This fact, and the report of an unidentified tyrosine derivative in extensin by Lamport (190), led to the discovery by Fry (98) of a new phenolic amino acid, isodityrosine, with cross-linking ability in potato cell walls. The dimer is connected by a diphenyl ether bridge in a reaction catalyzed by peroxidase (98). Cooper & Varner (58) have now demonstrated the presence of isodityrosine in carrot cell walls, and have shown that a variety of reagents which block the formation of isodityrosine by inhibiting the peroxidase reaction also prevent the incorporation of a salt-soluble hydroxyproline-rich protein into the tightly bound fraction of the wall. This suggests that peroxidase plays a critical role in the formation of the covalent extensin network and, by implication, in wall extensibility as well. Lamport & Epstein (192a) recently have proposed a new wall model based on two interdigitating networks of extensin and cellulose microfibrils.

The cinnamic acid derivatives, ferulic acid and *p*-coumaric acid, which are normally associated with lignin formation in secondary walls, have recently been found in the primary walls of monocots (362) and at least one dicot (96). Such phenols can also undergo oxidative coupling reactions, catalyzed by peroxidase, to produce dimers, and diferulic acid has been identified in cell walls (137, 204). Smith & O'Brien (361) found high levels of peroxidase, esterase, and wall-bound phenolics specifically associated with the outer wall of epidermal cells undergoing elongation in wheat roots. The authors speculated that peroxidase might be involved in cross-linking reactions between esterified ferulate moieties, while esterase might reverse such cross-links by cleaving the ester linkages to the polysaccharides (361). If, as the authors suggest, the epidermis regulates extension of the root as a whole, these enzymes might play an important role in regulating root growth. However, as

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previously noted, root growth appears to be limited by internal tissues rather than by the epidermis (21, 391).

In suspension-cultured spinach cells, Fry (99) has shown that feruloylation occurs specifically on the nonreducing termini of the neutral arabinose and/or galactose residues of the pectin fraction. The rate of expansion of spinach cells is promoted by gibberellic acid (about 2 times) after a lag of 48 h (96, 97). A GA-stimulated release of soluble pectin into the medium (but not an increase in total polysaccharides) precedes the enhancement of expansion by 24 h. The release of soluble pectins is accompanied by a GA-induced decrease in peroxidase secretion into the wall. Fry has suggested the attractive model that GA might promote cell expansion by inhibiting the secretion of peroxidase into the wall, thus inhibiting the formation of diferulate cross-linkages in the pectic fraction. This would explain the shedding of soluble pectins into the medium. However, it is difficult to establish a causal relationship between pectin release and cell expansion because of the long time periods involved, and such a mechanism would seem to be incompatible with the rapid growth response of lettuce hypocotyl sections to GA, which has a lag time of about 10 min (232). There is also no information on the actual number of diferulate cross-links in the wall, making it difficult to ascertain whether they are structurally important in vivo. Kinetic studies are needed on the changes in the number of diferulate cross-links after GA treatment. Thus far, diferulate has apparently not been detected in spinach cell walls (99). Fry has ruled out the possibility that GA acts via an increase in turgor, since GA did not alter the concentration of sorbitol needed to prevent growth (97). Nor did proton extrusion seem to be involved, inasmuch as the medium pH was not lowered by GA-treatment (96). However, since the culture medium was pH 4.4, and this is optimum for growth, it seems reasonable to assume that an acidified wall is a prerequisite for cell expansion, but that proton extrusion is not rate-limiting. The same conclusion seems to apply to the GA response of lettuce hypocotyls (373), and, perhaps, to *Avena* internodes (142, 174).

Another contributing factor to growth regulation in the spinach cell system might be a GA-induced decline in the accumulation of wall phenolic oxidation products, either polymers or quinoids, caused by the lowered peroxidase levels (96). Such compounds would increase wall hydrophobicity. Since water acts as a wetting agent and a plasticizer in cell walls (261), excluding water would encourage hydrogen bond formation and would decrease wall extensibility. An analogy can be made to the extensibility of insect cuticles, which consist of chitin microfibrils embedded in a protein matrix of varying hydrophobicity. Injection of 5-hydroxytryptamine into *Rhodinus* larvae resulted in an increase in the water content of the cuticle from 25.8% to 31.3%. This 6% increase in water content was accompanied by a tenfold increase in cuticle extensibility (319, 320). There is evidence that cuticle hydration is regulated by the degree

of phenolic cross-linking in the protein matrix (411, 413). Clearly, small changes in wall hydration could have disproportionately large effects on wall mechanical properties (65). The failure of chaotropic agents to cause wall loosening (381) argues against a role for hydrogen bonds in wall extension. The role of wall lipids in regulating hydration and mechanical properties does not seem to have been explored. Cutin in the epidermis and suberin in the endodermis could conceivably play a role in the regulation of the extensibility of these tissues.

Central to the isodityrosine and diferulate cross-link models of wall extensibility is the presence and activity of wall-bound peroxidase. There are many instances of a negative correlation between tissue peroxidase activity and growth rate (see references in 96, 97, 191, 192), but fewer studies on wall-bound peroxidase. Recent work suggests that the hydrogen peroxide required for the coupling reactions is generated in a reversal of the peroxidase reaction by extracellular NADH, which is produced in the wall during the conversion of malate to oxaloacetate by a wall-bound malic dehydrogenase (131, 132). Terry (390) has now found that GA causes a decrease in both wall-bound peroxidase and malic dehydrogenase when it stimulates growth in sugarcane leaves. Peroxidase secretion is promoted by Ca^{2+} in cultured sugarbeet cells (107a). Peroxidase secretion could conceivably play a role in Ca^{2+}-induced wall stiffening.

Wall Synthesis

Wall growth by intussusception of new wall material is a classic model for wall extension (291), most recently championed by Burström (20, 21). It has also been suggested that wall polymers can act as lubricants in the wall, promoting extensibility by reducing intermolecular shear stresses (127) [the "extensile synthesis" of Ray (300)]. Alternatively, wall polymers might act as friction elements, reducing extensibility ("intensile synthesis") (300). In growing corn root tips, the maximum rate of polyuronide production coincided with the peak of the relative growth rate (for a discussion see 356). Ray employed autoradiography to show that auxin promotes intussusception of wall material in the interior of the wall in *Avena* coleoptiles and pea stems, while wall deposition in the controls is mainly by apposition (299). This interesting observation has never been followed up, and the identity of the intussuscepted polymer is unknown. Although the inner layer, deposited by apposition, is believed to be the stress-bearing region of the wall (323), intussuscepted wall polymers may help in weakening the interior wall layers. A variety of polysaccharides, particularly arabinogalactan proteins (AGPs), are secreted through the cell wall into the media of suspension-cultured cells (192). Hence, AGPs are possible candidates for the intussuscepted polymer promoted by auxin.

Although intussusception has been correlated with auxin-induced growth,

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there is a poor correlation between rates of wall synthesis and rates of cell elongation, suggesting that while wall synthesis typically keeps up with wall extension so that wall thickness remains constant, the relationship is not always quantitative (see reviews in 33, 74, 274, 300, 440). When extension rates exceed rates of synthesis, marked wall thinning results (10, 278, 392, 393). In *Nitella* internode cells, incorporation of labeled glucose into the wall occurs uniformly over the cell surface, despite the fact that growth is occurring mainly in the acid band regions (226, 377). When the extensibility of the growing and nongrowing region of the wall was measured, no difference was detected, implying that similar types of wall polymers were deposited in the growing and nongrowing zones (228).

Enhancement of net wall synthesis by auxin has been observed in all cases where auxin increases the growth rate (42), and all polysaccharide fractions seem to be promoted (5, 24, 255, 256, 258, 298, 304). The stimulation by auxin is observed even when growth is inhibited by calcium (but not by mannitol), demonstrating that it is not an indirect result of growth (5, 255). Auxin also promotes the activity of glucan synthase I activity in pea stems within 15 min (302, 303). However, auxin-promoted wall deposition shows a 1 h lag period (Figure 2) and thus cannot contribute to the initial promotion of growth (1). Moreover, relatively high concentrations of exogenous sugars are needed for the promotion of wall synthesis by auxin (24, 300), while exogenous sugars have no effect on either growth or wall extensibility during the first 4–6 h (45). The rapid stimulation of the elongation of lettuce hypocotyl sections by GA is not associated with a GA-promotion of the incorporation of precursors into the wall (164, 366), although wall synthesis parallels growth in long-term experiments with lettuce and *Avena* stem sections (174, 238, 239).

Despite the poor correlation between bulk wall synthesis and growth, there is no case in which it has been demonstrated that growth can occur in the complete absence of wall synthesis, with the possible exception of Bonner's low-temperature studies (10). In vitro wall extension, of course, can occur without wall synthesis (314). At the opposite extreme, tip growth and wall deposition are practically synonomous, and it is not unreasonable to assume that wall deposition plays a role in diffuse growth as well. The fact that cotton fibers grow by both tip growth and diffuse growth (340) implies that the mechanisms of the two types of extension share features in common. Yamamoto (428a) has recently suggested that galactose inhibits the long-term growth of oat coleoptiles by interfering with cell wall synthesis. Galactose does not inhibit respiration or auxin-induced proton extrusion, and does not block short-term auxin-induced growth (428a). Wada & Ray (414) suggested that the secretion of glucurono-arabinoxylan into coleoptile walls could alter wall viscosity. Albersheim & Bonner (4) early reported that auxin enhanced the synthesis of polygalacturonic acid in *Avena* coleoptiles, and this has also been observed in

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Azuki bean hypocotyls (255, 258), even in the absence of exogenous sugars. The enhancement of pectin synthesis in coleoptiles could be observed within 15–30 min (12). It is possible that the auxin-induced release of soluble pectin from cell walls of pea stems represents newly secreted polymers rather than enzymatically degraded wall components (389). Taiz and Morrison (unpublished) have recently found that polyuronides can partially solubilize isolated *Nitella* walls, probably by acting as calcium chelators. The secretion of polyuronides into the wall might promote wall extension by competing with the stress-bearing inner wall layers for calcium.

The long-term effects of wall synthesis are apparent in the changing composition of primary walls during maturation. As previously discussed, the deposition and incorporation of extensin have been implicated in the modification of wall properties. Changes in wall polysaccharide composition with development have been investigated in Pinto bean hypocotyls (254, 404), *Azuki* bean hypocotyl (255), cotton fibers (224), and *Nitella* internode cells (227). The analysis of *Nitella* was carried out on single cells, and the composition was correlated with growth and surface area data. The neutral sugar content (TFA-hydrolyzable) gave the best correlation with the growth rate, and there was also a progressive decline in the percent dry weight of polyuronides and an increase in the percentage of cellulose during maturation (227). Similar patterns have been observed in maturing higher plant cells (224, 255, 325, 404). In Pinto bean hypocotyls, maturation was associated with an increase in noncellulosic glucan, probably xyloglucan (404). Such changes undoubtedly contribute to wall's mechanical properties and to CAWL (43).

BRAVE NEW WALL

Cell wall extension involves a multitude of physical and biochemical factors, the complexity of which we are only beginning to appreciate. The "acid growth hypothesis," which served as the dominant paradigm for wall extension studies of the 1970s, has been vigorously tested and (to this reviewer) it has survived, with certain qualifications. The recent demonstration that cell walls can extend linearly with time in vitro at acid pH strengthens this conviction. As indicated by the summary of the kinetics of auxin action shown in Figure 2, proton extrusion is still the best candidate for the "wall-loosening factor" involved in the early response to auxin, and other evidence suggests that wall acidification is required for long-term growth as well. Many problems remain concerning the mechanism of proton extrusion and, in particular, the role of exogenous ions. On the other hand, a number of cases have arisen (e. g. growth promotion by low O_2, GA, ethylene, and cytokinin) in which wall acidification is clearly not the limiting factor. Therefore, an important refinement of the acid growth model is that the capacity of the wall to be loosened at acid pH is itself under

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metabolic and hormonal control. This opens up a universe of biochemical events which potentially can regulate wall extensibility. These include the secretion of enzymes, wall polymers, lectins, phenolics, and lipids, as well as the transport of calcium and other ions into or out of the free space. The challenge of the future will be to sort out the possibilities. Genetic approaches may prove to be useful in this regard, especially if mutants deficient in wall enzymes or other wall components can be obtained. The spinach cell suspension system, which responds to GA, would seem to have potential for a genetic attack on the problem.

The spectacular advances in cell wall chemistry in the past decade raise our expectations that the partially abandoned wall models of the seventies will soon be replaced by more comprehensive ones. The hitherto underutilized technique of proton magnetic resonance is already providing insights into the molecular motions of wall polymers as a function of wall pH. Eventually, the biochemical and biomechanical approaches must merge for a full explanation of wall extension.

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