Plant Physiol. (1989) 89, 184–191
0032-0889/89/89/0184/08/$01.00/0

Received for publication February 21, 1988
and in revised form July 12, 1988

Mechanism of Gibberellin-Dependent Stem Elongation in Peas[1]

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ABSTRACT

Stem elongation in peas (*Pisum sativum* L.) is under partial control by gibberellins, yet the mechanism of such control is uncertain. In this study, we examined the cellular and physical properties that govern stem elongation, to determine how gibberellins influence pea stem growth. Stem elongation of etiolated seedlings was retarded with uniconozol, a gibberellin synthesis inhibitor, and the growth retardation was reversed by exogenous gibberellin. Using the pressure probe and vapor pressure osmometry, we found little effect of uniconozol and gibberellin on cell turgor pressure or osmotic pressure. In contrast, these treatments had major effects on *in vivo* stress relaxation, measured by turgor relaxation and pressure-block techniques. Uniconozol-treated plants exhibited reduced wall relaxation (both initial rate and total amount). The results show that growth retardation is effected via a reduction in the wall yield coefficient and an increase in the yield threshold. These effects were largely reversed by exogenous gibberellin. When we measured the mechanical characteristics of the wall by stress/strain (Instron) analysis, we found only minor effects of uniconozol and gibberellin on the plastic compliance. This observation indicates that these agents did not alter wall expansion through effects on the mechanical (viscoelastic) properties of the wall. Our results suggest that wall expansion in peas is better viewed as a chemorheological, rather than a viscoelastic, process.

Gibberellins were discovered because of their marked stimulation of shoot elongation. In recent years notable progress has been made in the genetics of gibberellin synthesis and its control of shoot elongation, particularly in pea and maize plants (see Ref. 20 for review). However, the mechanism of action of GA on shoot growth in these species is not fully understood. Two general, and complementary, approaches have been taken to examine GA action. The first views the stem as composed of cell files, and asks how GA affects the number and final size of the cells making up the stem. Various studies (*e.g.* 24, 26) have shown that GA may affect both cell division and final cell size. However, final cell size is a complex function of the patterns of cell expansion and cell division, which are themselves likely to be interdependent, so the causal mechanism of GA action is not clear from these studies. The second approach considers that stem elongation arises primarily from wall expansion and water absorption, and asks how these underlying processes are controlled. In this approach, cell division is immaterial because the formation of new crosswalls in a cell file does not add to the length of the cell file. In this physical view of growth, wall yielding properties and cell hydraulic properties govern tissue expansion (8). This approach is the one taken in the current study.

Two physical mechanisms have been proposed to account for enhanced shoot elongation by GA. From experiments in which pea stem segments were placed under a bending load, Lockhart (19) concluded that GA increased wall 'plasticity.' Conflicting evidence came from Yoda and Ashida (28) who used a similar method, but found that GA stiffened the wall in pea stems. This bending technique is subject to various criticisms; nevertheless, other mechanical techniques have provided additional evidence that GA makes the wall more extensible in oat internodes (1), lettuce hypocotyls (16, 18, 27), and pea apical hooks (22). However, the biochemical basis for the altered wall remains uncertain; it might result from enhanced wall loosening, reduced wall cross-linking, or altered wall composition (14, 15).

In other plant tissues, notably the cucumber hypocotyl, GA is thought to increase cell osmotic pressure, thereby increasing cell turgor pressure, raising wall stress, and accelerating wall extension (17). The evidence for this solute-mobilization hypothesis remains mostly indirect. Cleland *et al.* (2) found that GA did not alter the mechanical characteristics of the cucumber wall as measured by the Instron technique, and therefore suggested that turgor might be altered instead. Likewise, Katsumi and Kazama (17) failed to detect changes in the viscoelastic properties of cucumber hypocotyl walls after short-term GA treatment. They reported that osmotic pressure increased after GA treatment of cucumber hypocotyls, but they did not measure turgor. The solute-mobilization hypothesis might explain why GA stimulates elongation in many intact plants but is less effective in excised sections. Without normal translocation of solutes via the phloem, GA might be less capable of stimulating growth.

Despite the availability of pea mutants with lesions in GA synthesis, little work has been carried out on the mode of action of GA in peas. Evidence that GA acts on wall properties exists for peas, but it is not strong (19, 22, 28). Apparently, no one has examined whether solutes and turgor pressure in pea stems are affected by GA. Because these two mechanisms might simultaneously contribute to the GA response in peas, we have carried out a series of experiments to test for both mechanisms. One experimental problem with the use of GA-deficient mutants is that the dwarfism is expressed only in the light; multiple light-growth reactions complicate the analysis

[1] Supported by U.S. Department of Energy grant DE-FG02-84ER13179 and in part by United States National Science Foundation grant DMB-8351030.

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of such material, and make pressure-probe measurements more difficult because of the smaller cell size. To circumvent these problems, we retarded elongation of etiolated pea seedlings with a GA synthesis inhibitor and reversed its effects by applying exogenous GA. This allowed us to examine the action of both the retardant and GA. Turgor pressure was measured directly with the pressure probe, and cell wall properties were evaluated by *in vivo* stress relaxation techniques and stress/strain (Instron) analysis.

MATERIALS AND METHODS

Plant Material

Seedlings of *Pisum sativum* L. cv Alaska were grown in moist vermiculite in darkness at 26 to 28°C. To retard growth, seedlings were treated with uniconozol from the start of seed imbibition. Uniconozol was obtained as a commercial preparation (Chevron XE-1019; synonym: Sumitomo Chemical Co. S-3307) containing 10% of the retardant (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, which interferes with gibberellin synthesis by blocking oxidation of kaurene to kaurenoic acid (13). Forty-five mg of the 10% powder was dissolved in 100 mL of water and sprayed evenly over the seeds in a flat 27 cm long, 19 cm wide, and 6 cm deep, filled with wet vermiculite. In a few cases, the seeds were soaked for 30 min in the retardant solution before sowing. Inhibited seedlings typically were used on d 8 or 9 after sowing and were termed I[3] plants. GA was applied as a 5 μL drop to the plumule of the I plants on d 7. The solution contained 2 mM gibberellic acid (Calbiochem) and 0.1% Tween 20 as a surfactant. These plants were termed I+GA plants, and were used 1 d after GA application (2 d in some indicated experiments). Control plants were either untreated pea seedlings, 4 or 5 d old, or I plants treated with 0.1% Tween. In the evening prior to most experiments, plants were gently transplanted into polyethylene vials (5 cm tall, 2.8 cm diameter) filled with vermiculite. Plants were handled under dim green light obtained from a 40-W cool-white fluorescent lamp filtered through one amber and two green acetate filters (Roscolene No. 813 and 874, Roscoe, Port Chester, NY).

Growth

For some experiments, elongation rates of intact and excised epicotyls were measured continuously with position tranducers and recorded with a microcomputer-based data acquisition system (6).

Elongation rate was also measured as a function of position along the stem. Epicotyls were marked at fixed intervals with black oil-base ink (Speedball, Hunt Manufacturing Co., Statesville, NC) using a fine eyebrow hair and a micromanipulator. Plants were photographed after 6 h (I+GA and untreated control plants) or 23.5 h (I plants). The displacement of the marks was analyzed with a digitizing tablet, microcomputer, and custom software.

To measure the effect of GA on cell dimensions, thick epidermal strips were peeled by forceps from the mature regions of the epicotyl and lengths of 100 cells from the outer cortical layer were measured under a microscope. Cell diameters were measured from free-hand cross-sections of different seedlings. Cell dimensions are likely to be slight underestimates because of release of turgor pressure and tissue tensions during preparation.

Turgor Pressure and Osmotic Pressure

Thirty to 60 min before turgor measurement, the apical 3 cm of the epicotyl of an intact plant was mounted and sealed in a humid plastic chamber (7) to reduce evaporation. Turgor pressure was measured in cortical cells 5 to 6 mm below the hook (the region of maximum growth rate), using the pressure probe technique described previously (7, 10). Typically, 10 to 15 cells were measured in each plant; standard errors were usually 0.1 bar or less for each plant.

To determine osmotic pressure, cell sap was expressed from the apical 6 to 8 mm of the epicotyl below the hook and measured with a vapor pressure osmometer (model 5500, Wescor, Logan, UT). Osmolality was converted to osmotic pressure at 25°C by dividing by 41 mOsmol kg^{-1} bar^{-1}.

In Vivo Wall Relaxation

Two relaxation methods were used. Turgor relaxation was measured with the pressure probe by excising the growing portion of the epicotyl, isolating the tissue from water, and measuring the time-dependent reduction in turgor pressure, caused by continued wall loosening and relaxation. This method has been described in detail previously (7). A second method, termed the pressure-block technique (9), measured wall relaxation without excision. In brief, the apical 1-cm of the stem was sealed into a custom-made pressure chamber using 5-min epoxy (Devcon Corp., Danvers, MA). Stem elongation was monitored with a position transducer mounted within the pressure chamber (for details, see Ref. 9). Wall relaxation was induced by applying just sufficient chamber pressure to prevent stem elongation. As the wall is loosened but is prevented from expanding, wall stress is reduced, turgor pressure falls, and additional pressure must be applied to the chamber to prevent the tissue from absorbing water and elongating. Thus, the chamber pressure is both the means to induce wall relaxation and the measure of the rate and magnitude of relaxation.

Volumetric Elastic Modulus

To avoid wall relaxation, which will confound this measurement, segments approximately 1 cm in length were excised from the region immediately below the growing portion of the stem. After incubation on water for 10 to 15 min, they were blotted dry and mounted in the plastic chamber for pressure probe measurements. After equilibration for 15 min, turgor pressure was measured in 8 to 12 cortical cells with the pressure probe (7). The tissue was removed from the chamber and immediately weighed to the nearest 10 μg. The tissue was allowed to lose 0.5 to 1.0% of its weight by evaporation, then was sealed back in the chamber. After 10 min equilibration, turgor pressures were again measured and the plant reweighed. This step-wise evaporation and turgor measurement was re-

[3] Abbreviations: I, inhibitor-treated; I+GA, inhibitor plus GA treated; P, turgor pressure; ϕ, wall yield coefficient; ϵ, cell volumetric elastic modulus; Y, yield threshold; π, osmotic pressure.

peated until turgor dropped below 1 bar. When the relative weight is plotted against turgor pressure, the local slope of the curve is a measure of the tissue-averaged volumetric elastic modulus.

Stress/Strain (Instron) Analysis

Apical 1.2-cm epicotyl regions were excised and frozen at −20°C. The tissue was thawed, pressed slowly between two glass slides to remove excess water, and mounted between two clamps of a custom-made stress/strain analyzer (3). Water extrusion was found necessary to eliminate artifacts arising from water extrusion during the measurement. The instrument was interfaced with a microcomputer for control and data analysis. The 5-mm portion between the clamps was extended at 3 mm min^{-1} until a 30-*g* force was attained. The clamps were rapidly returned to their original positions and the tissue was reextended until 30-*g* force was again attained. Slopes of the endpoints of the two force/extension curves were calculated by a least-squares fit, and are expressed as % extension per 100 *g* force. The second stress/strain curve was taken as a measure of the elastic characteristics of the tissue, whereas the plastic characteristics were taken as the difference between the first and second extensions (3). After stress/strain measurement, the 5-mm section between the clamps was excised, dried at 70°C, and weighed to the nearest 10 μg. Elastic and plastic compliances were calculated by multiplying the dry weight per unit length times the density (assumed 1 g cm^{-3}) times the strain/stress slopes. This corrects for differences in wall cross-sectional area (3).

RESULTS

Morphological Effects of Uniconizol

Figure 1 illustrates that uniconozol greatly stunts elongation and causes stem thickening. Uniconozol also modified other aspects of growth: the plumule was partially expanded, had a deep yellow color, and did not form an apical hook. Seedlings treated with uniconozol had a greater tendency to wilt after transplanting as compared with untreated controls, which suggests that the retardant may also affect transpiration.

Growth

Uniconozol greatly retarded the rate and extent of elongation along the stem, and this growth inhibition was largely reversed by GA application to the plumule (Fig. 2). The region of elongation was shortened by half in the inhibited (I) plants, and the maximum relative elongation rate was reduced to 20% of untreated controls. GA application increased the maximum extension rate to 75% of untreated controls and extended the region of elongation, although this latter effect was reduced somewhat by the second day after GA treatment (Fig. 2). Stem thickness was greater in the I plants, but even factoring in this difference, volumetric growth was still greatly reduced in I plants (Table I).

Table II shows that cell length was reduced almost by half by uniconozol treatment. By dividing the elongation rate by the cell length, we may calculate that uniconozol reduced the production of cells (in the subepidermal layers) to one-fourth

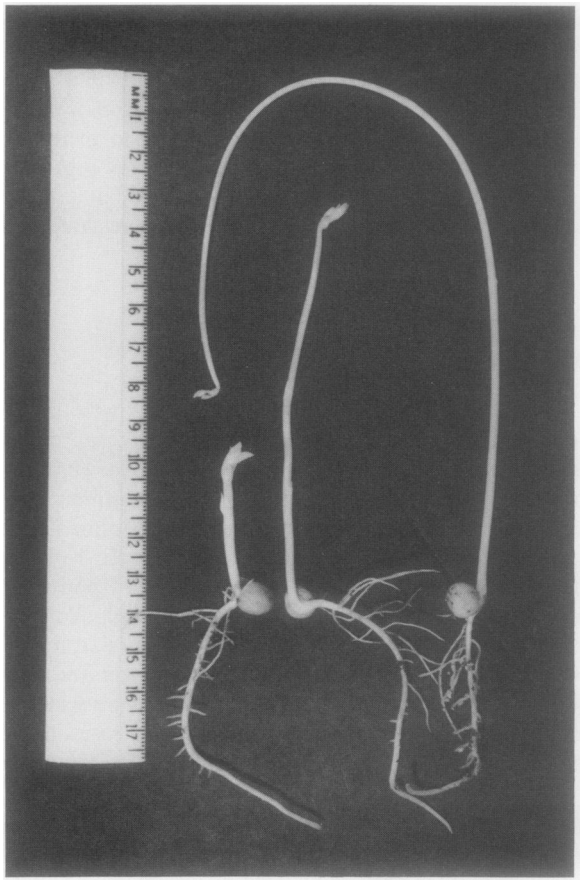

Figure 1. Photograph of I, I+GA, and untreated control pea seedlings (from left to right). All seedlings were 8 d old; GA was applied 2 d prior to the photograph.

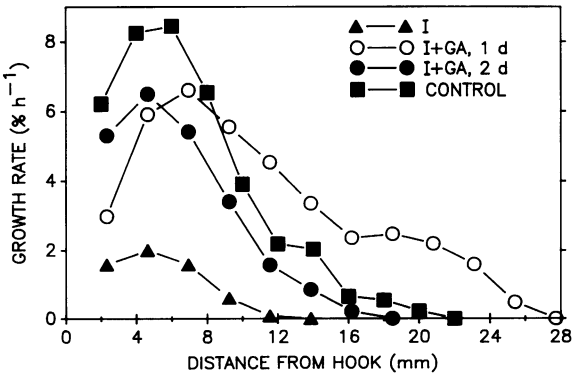

Figure 2. Growth distribution along the stem axis for I, I+GA (1 and 2 d plants) and untreated controls.

that of untreated controls. These effects on cell size and division rate were largely or entirely reversed by GA application.

To determine the response kinetics, stem elongation was measured continuously before and after application of GA to the plumule in inhibited plants (Fig. 3). Stem elongation slowly began to increase within an hour, noticeably accelerated at 8 h, and reached a maximum rate 15 to 17 h after GA treatment.

Osmotic Pressure and Turgor Pressure

To test whether these growth alterations were caused by altered solute uptake and turgor pressure, bulk cell sap was

Table I. *Characteristics of the Growing Regions of I, I+GA and Control Plants*

SD and sample number are in parentheses.

Parameter	I	I+GA	Control	Units
Elongation rate[a]	0.16	0.95	1.15	mm h^{-1}
Weight/length[b]	4.80 (0.14; 15)	2.46 (0.14; 12)	2.44 (0.08, 15)	mg mm^{-1}
Volume growth rate[c]	0.77	2.34	2.81	mm^{-3} h^{-1}
% Dry weight	7.1 (0.10; 15)	6.8 (0.30; 12)	9.0 (0.14, 15)	% fr wt[d]
Osmotic pressure	7.38 (0.14; 10)	7.33 (0.12; 10)	8.0 (0.1; 10)	bar
Turgor pressure	5.8 (0.13; 10)	5.1 (0.15; 10)	5.5 (0.14; 10)	bar
Yield threshold[e]	3.8 (0.08; 8)	3.1 (0.08; 8)	3.0[f]	bar
P-Y[e]	2.0	2.0	2.5	bar
ϵ[g]	45 (7.9, 6)	42 (6.3, 5)	45 (3.4, 3)	bar

[a] Calculated by integrating the growth distributions shown in Figure 2; the rate shown for I+GA is for 1 d after GA application. [b] Proportional to cross-sectional area; calculated as fresh weight divided by length of 8-mm section. I+GA measured 2 d after GA application. [c] Calculated by multiplying elongation rate and weight/length, and assumes density of 1 g cm^{-3}. [d] Percent of fresh weight. [e] Measured by the turgor relaxation technique, in which a 6- or 8-mm section was cut from the growing region (within humid glovebox) and allowed to undergo *in vivo* stress relaxation (7). [f] Data taken from (9). [g] Measured by the technique shown in Figure 5.

Table II. *Cell Dimensions of I, I+GA, and Control Plants, Measured 3 to 4 cm below the Apical Hook*

Listed are averages of 100 measurements taken from 10 plants (10 cells per plant); SE are shown in parentheses.

Parameter	I	I+GA	Control
Length, μm	134 (3.5)	277 (9.0)	232 (6.7)
% of control	58	119	100
Width, μm	32 (1.0)	29 (1.5)	28 (0.6)
% of control	114	104	100
Volume, 10^4 μm^3	11.1 (0.8)	18.7 (1.8)	14.2 (0.6)
% of control	78	132	100

expressed from the growing regions and measured by vapor pressure osmometry. There was no difference in π between I and I+GA plants (Table I). We used the pressure probe to measure directly the P of individual cells within the growing region of intact plants. Inhibitor-treated plants had somewhat higher turgor pressures than I+GA plants (Table I), while untreated pea seedlings had turgor pressures between the values for I and I+GA plants.

Yield Threshold and Effective Turgor for Growth

To measure the Y, we attempted to use *in vivo* stress relaxation techniques, in which wall loosening is allowed to reduce turgor pressure to the yield threshold (11). A 6- to 7-mm segment was excised from the stem region of most rapid growth, isolated from an external water supply, and cell turgor

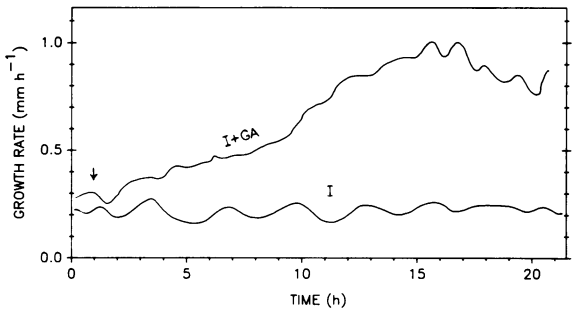

Figure 3. Time course for stimulation of stem elongation by GA. Each curve is the average of 8 or 9 individual plants, measured continuously with an LVDT-type position transducer and recorded by a microcomputer. At the point designated with the arrow, the GA solution was applied to the plumule.

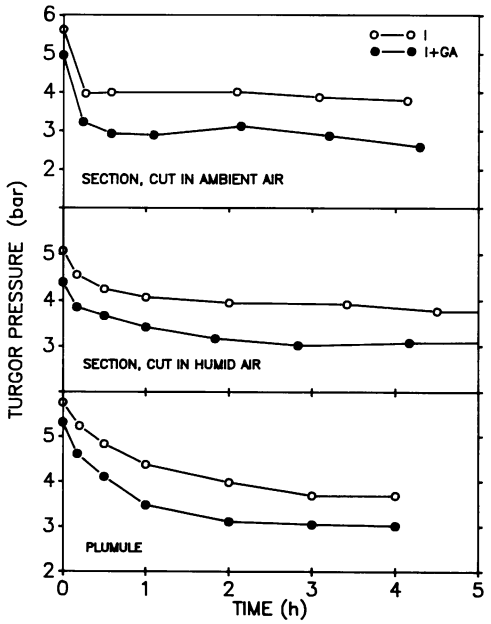

Figure 4. Turgor relaxation experiments for I and I+GA stems, under different conditions. In top panel, sections about 6 mm long were excised in ambient air, then resealed (each curve is mean of four experiments). In middle panel, 6-mm segments were excised in a humid glovebox (each curve is mean of eight experiments). Initial turgor pressures were atypically low in this set of experiments, for unknown reasons. In lower panel, the upper 1-cm of the epicotyl was excised, with plumule intact (mean of five experiments). A few segments, both I and I+GA, showed continued relaxation beyond 2 h, and were excluded from this summary. Standard errors were in the range of 0.1 to 0.3 bar.

pressure was measured as a function of time. In our first experiments, we found that turgor pressure decreased by 1.5 to 2 bars rapidly (within 15 min) after excision and remained steady thereafter (Fig. 4A). This immediate drop in turgor was mostly due to evaporative water loss during the short period in which the chamber was opened to excise the growing region. We found that uniconozol-treated plants had a much greater propensity to lose water than untreated plants; this may be related to their tendency to wilt during transplantation, as noted above.

To avoid water loss, we excised the stem segment inside a

humid glove box, applied a drop of water to the excised surfaces, and quickly blotted the surfaces dry. This procedure reduced evaporation and removed some of the solutes which were released from the cut cells. Figure 4B shows the relaxation time courses, in which I and I+GA plants relaxed to different asymptotic values. Inhibited plants relaxed to an average Y of 3.8 bars, whereas I+GA plants relaxed to 3.1 bars (Table I). Untreated controls were shown previously to relax to 3.0 bars (9).

These results indicate that uniconozol raises the yield threshold for growth, and that this effect is reversed by GA application. However, the driving force for wall expansion, defined as the quantity (P-Y), appeared to be the same in I and I+GA plants (Table I). To look for differences in the wall yield coefficient, we examined the kinetics of relaxation, in which P should decay to Y exponentially with a rate constant given by $\phi\epsilon$, where ϕ is the wall yield coefficient and ϵ is the volumetric elastic modulus (7).

Volumetric Elastic Modulus (ϵ)

Pea sections were allowed to lose water in small steps, and cortical cell turgor pressure was measured at each of these steps (Fig. 5). The slope of the P *versus* volume curve was linear until P fell below 2 bars. The local slope of this curve is ϵ, and the values for I and I+GA seedlings were nearly the same (Table I). This meant that the rate of relaxation in the two treatments should be directly proportional to the yield coefficients (ϕ).

Turgor Relaxation

A difficulty inherent in the turgor relaxation technique is that tissue excision, necessary to prevent water uptake, may inhibit stem growth processes, particularly wall relaxation (7, 9). When the top and bottom of the growing region was excised, I and I+GA tissues showed little difference in the initial rate of relaxation or in the halftime for relaxation (Fig. 4, middle panel). To minimize excision effects, we excised the apical 1-cm of the epicotyl by a single cut (the plumule was left intact) and allowed it to undergo relaxation (Fig. 4, bottom panel). Shoots from I and I+GA stems relaxed to different yield thresholds, yet the effective turgor (P-Y) was nearly the same (Table III). The I+GA stems relaxed at a faster rate, as indicated by the larger initial slope of the relaxation and by the larger rate constant. However, these differences in turgor relaxation were not sufficiently large to account for the differences in intact growth.

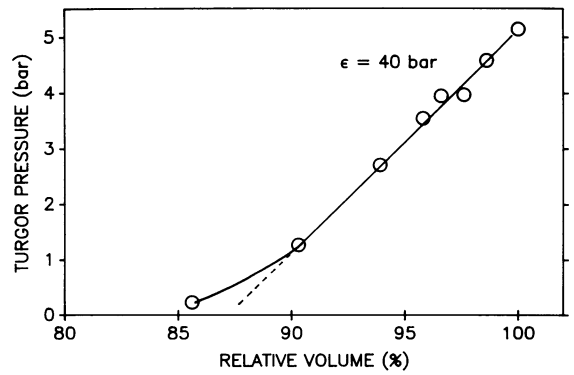

Figure 5. Measurement of the volumetric elastic modulus. Turgor pressure was measured after step-wise water loss by evaporation, measured by weighing. The slope of the curve provides a measure of the tissue-averaged volumetric elastic modulus.

This apparent discrepancy seems attributable to the wounding effect of excision. We found that after excision the large difference in growth rate between I and I+GA stems (Figs. 2 and 3) was greatly reduced. For these measurements, the top 2-cm of the epicotyl was excised (with plumule intact) and the cut base was placed in water. The upper 1-cm was marked with ink to monitor growth. During the first 3 h after excision, I+GA stems elongated at 3.7% h^{-1} whereas I stems elongated at 2.0% h^{-1} (means of 67 or 69 samples). These values represent a 27% decrease in the growth rate for I+GA plants, and a 50% increase for I plants (compare with the rates averaged over the apical 1-cm regions in Fig. 2). Thus, it seemed likely that excision diminished the differences in growth and wall relaxation between I and I+GA plants.

Pressure Block Measurements

To avoid excision entirely, we used the pressure-block technique (9), which allows stress relaxation of intact plant tissues. Figure 6 shows typical relaxations for I and I+GA stems. Untreated controls relaxed somewhat faster than I+GA plants (data not shown, but see Ref. 9). I+GA plants relaxed more quickly and to a larger extent than I plants (Table III).

We have not attempted to characterize these relaxations with a rate constant because they were nonideal in two respects. First, they did not always reach a plateau, but often continued to relax at a slow rate. This might be caused by a slow downward shift in the yield threshold or by solute accumulation in the relaxing tissue. Second, the relaxation was not a simple decay, in that its rate increased 4 to 8 min after the start of pressure-block, apparently in response to the forced stoppage of growth (9). Further work will be needed to understand these complex behaviors.

Table III. *Kinetics of in Vivo Stress Relaxation, as Measured by the Turgor Relaxation and Pressure-Block Techniques*

Shown are means, with standard errors in parentheses (n = 5 for the turgor relaxation data, n = 10 for the pressure-block data).

Parameter	I	I+GA	Units
Turgor relaxation[a]			
Initial slope[b]	2.6 (0.28)	3.5 (0.71)	bar h^{-1}
Initial P	5.75 (0.24)	5.31 (0.02)	bar
Yield threshold[c]	3.7 (0.2)	3.0 (0.1)	bar
P-Y	2.1 (0.2)	2.4 (0.1)	bar
Rate constant	0.94 (0.068)	1.30 (0.036)	h^{-1}
Pressure-block			
Slope at 5 min	2.1 (0.26)	9.85 (0.85)	bar h^{-1}
Slope at 60 min	0.6 (0.08)	1.2 (0.22)	bar
P at 5 min	0.45 (0.07)	1.3 (0.14)	bar
P at 60 min	1.1 (0.10)	3.0 (0.21)	bar

[a] *In vivo* stress relaxation of the apical 1-cm epicotyl (plumule intact). [b] Calculated from the difference between the intact turgor pressure and the turgor pressure at 10–15 min after start of relaxation. [c] Estimated as P when P stabilized or at 2 h, whichever came first.

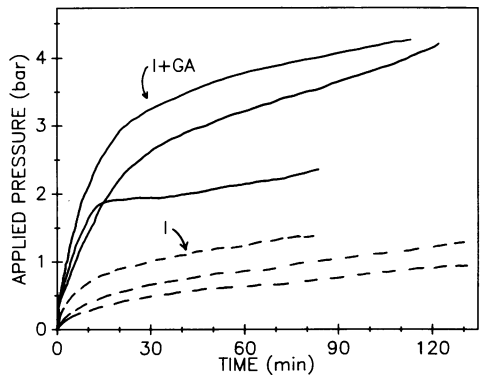

Figure 6. Stress relaxation of I and I+GA plants, as measured by the pressure-block technique. Three curves are shown for I+GA plants (solid lines), and three curves for I plants (broken lines). These curves represent the typical range of relaxation behavior for the two treatments. The peculiar I+GA curve, in which relaxation abruptly ceased at 10 to 15 min, was observed twice out of 10 trials. The other two curves show the more typical relaxation time courses for I+GA plants.

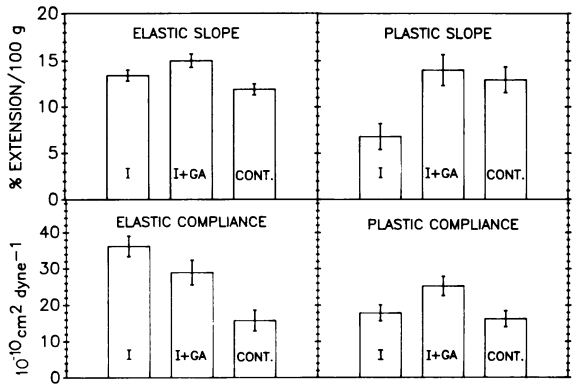

Figure 7. Mechanical properties of cell walls, as determined by stress/strain (Instron) analysis of frozen/thawed pea epicotyls. Top two panels show the raw slopes of the strain/stress curves; lower two panels show the elastic and plastic compliances, which are the strain/stress slopes corrected for the differences in cross-sectional wall area by multiplying the slopes by the dry weight per unit length (3). Mean dry weights per unit length, in mg per cm, were 2.72 for I stems, 1.91 for I+GA stems, and 1.35 for untreated controls. These measurements were carried out on three sets of plants, with similar results. Means and 95% confidence limits are shown for the combined data; n = 39 for I, 27 for I+GA, and 39 for controls (CONT.). Means and 95% confidence limits are plotted.

Nevertheless, the differences in relaxation of I and I+GA plants are large and may be summarized by the chamber pressures and slopes (dP/dt) at 5 min and at 60 min (Table III). I+GA plants exhibited nearly 5 times faster initial relaxation rate, and by 60 min had relaxed nearly 3 times more than I plants. As a first approximation, we have taken the chamber pressures at 60 min as a measure of (P-Y). Combining these pressure-block results with the turgor measurements, we estimate Y to be about 2 bars in I+GA plants and 4.5 bars in I plants. These values for Y differ from the turgor relaxation results, apparently because of the different relaxation behavior of intact *versus* excised tissue.

For the reasons explained above, the rate constant of the pressure-block relaxations was not calculated, and so it cannot be used to estimate the wall yield coefficient ϕ. However, ϕ may be estimated from the initial rate of relaxation, using the formula $dP/dt = \phi\epsilon(P-Y)$ (7). Using ϕ = 45 bar (Table I), (P-Y) = 1.1 bar for I plants and (P-Y) = 3.0 bar for I+GA plants (Table III), we estimate ϕ to be 0.042 bar^{-1} h^{-1} for I plants and 0.073 bar^{-1} h^{-1} for I+GA plants. Thus, the apparent yield coefficient ϕ is greater, by about 70%, in the I+GA stems. Note that this method of estimating ϕ includes the dynamic increases in relaxation, apparently brought on by growth blockage.

Instron Measurements

Figure 7 summarizes the stress/strain properties of pea stem segments. In the top panels, the mechanical extensibilities of the stem segments are shown without correction for the differences in wall cross-sectional area. Inhibited plants showed less plastic deformation per unit force, when compared with untreated controls and I+GA plants. However, because I stems were substantially thicker (see Table I and Fig. 7 legend), the force was distributed over a larger cross-sectional area. When differences in cross-section are taken into account, as shown in the lower panels, we found that the plastic compliance of the I plants was the same as that of untreated controls. I+GA plants exhibited a plastic compliance about 50% greater than I or control groups. Elastic properties showed even poorer correlation with growth properties than did the plastic properties (Fig. 7).

DISCUSSION

These experiments provide new data on the mechanism by which gibberellins enhance stem elongation. Our results indicate that the GA-synthesis inhibitor uniconozol causes dwarfing in pea plants by inhibiting wall yielding. This conclusion is supported most directly by *in vivo* stress relaxations, particularly with the pressure-block technique. Retarded plants exhibited greatly reduced wall relaxation; the yield threshold was raised and the wall yield coefficient (ϕ) was reduced after treatment with uniconozol. These effects were largely reversed by exogenous GA application. By direct measurements we found that these treatments had little or no effect on turgor pressure or osmotic pressure.

In previous studies it has sometimes been argued that GA increases shoot growth by stimulating solute transport into the expanding tissue. By raising π, so the argument goes, cells would draw in more water, increase their turgor pressure, and consequently enhance the rate of wall expansion and cell enlargement. Our results show that this mechanism does not operate when GA stimulates elongation of inhibited pea seedlings. By direct measurement, turgor pressures of the I+GA plants were found to be slightly less than that of I seedlings. Osmotic pressures were not affected. We may calculate net osmoticum import into the growing region by multiplying volume growth rate by osmotic pressure (Table I). Uniconozol reduced osmoticum import by 75%, and this effect was largely reversed by GA application. The same conclusion applies for dry mass import. However, these effects appear to be consequences of growth because π and % dry weight remained constant after GA-stimulation of growth. This constancy,

despite the accelerated growth, implies that solute uptake and maintenance of π is closely coordinated with cell expansion. However, the mechanism of such coordination is not known.

The growth effects of gibberellin in pea stems appear to be mediated through increases in wall yielding, not via increases in P or π. In their studies of lettuce hypocotyls, Stuart and Jones (27) came to a similar conclusion, although with less direct evidence. However, their measurements were with excised segments, and it is possible that solute transport plays a greater role in the intact plant. Zack and Loy (29) suggested that GA increases wall extensibility because they found that osmotic pressure decreased after GA application; they did not, however, measure wall properties. Zack and Loy further reported that turgor pressure (and water potential) fell after GA application. However, their estimates of turgor pressure were probably confounded by stress relaxation during their measurement of water potential, so the lower turgor they observed after GA treatment might represent changes in the yield threshold, as reported here.

One study suggesting that GA might induce stem elongation via a solute effect is that of Cleland *et al.* (2), who found that GA enhanced the growth of cucumber hypocotyls but did not influence the plastic compliance, measured by the Instron technique. In contrast, auxin caused a marked increase both in growth rate and in the plastic compliance of the walls. Cleland *et al.* suggested, therefore, that GA acted not on the wall but on the osmotic pressure of the growing cells. Katsumi and Kazawa (17) have argued similarly.

Our measurements indicate that in pea stems the plastic compliance is not a reliable indicator of the wall properties that govern growth. For instance, I stems showed the same plastic compliance as the untreated controls, yet the pressure-block method showed that wall loosening and relaxation were greatly diminished in these plants. These results weaken the idea that the plastic compliance is correlated with a time-averaged value of ϕ (4). One might argue that the raw extensibilities might better serve as indicators of wall properties, since they correlated better with growth than did the compliances (Fig. 7). When wall cross-sectional areas differ, as in the current case, this argument is unacceptable because an imposed force results in less wall stress in the thicker specimens. Another caveat is that dry weight per length is assumed to be proportional to wall cross-section (3); if this proportionality does not hold, error may be introduced into the compliance calculation.

We conclude that GA, like auxin, affects the wall yielding characteristics of pea stems, but in different ways. Auxin enhances the rate of relaxation, does not affect the yield threshold, but does typically alter the mechanical characteristics of the wall, as measured by stress/strain analysis. GA and GA-synthesis inhibitors, on the other hand, affect both the rate of relaxation and the value of the yield threshold in peas, but do not always affect the mechanical characteristics of the wall.

Because of the difference between GA and auxin action on the plastic compliance of the wall, Cleland *et al.* (2) concluded that GA does not promote growth in cucumber hypocotyls through any effect on auxin metabolism. Other studies have supported this idea for oat internodes (1) and lettuce hypocotyls (25). Our results extend this conclusion to pea epicotyls, because GA alters the yield threshold, whereas auxin does not (7). However, the conclusion of earlier work (2, 17), that GA does not act on wall yielding in cucumber, is made questionable by our results and deserves reexamination.

Fry (12) has proposed that GA enhances growth in suspension-cultured spinach cells by preventing phenolic cross-linking of the wall, catalyzed by extracellular peroxidases. Jones (14) also supported this hypothesis, but the evidence is circumstantial. Our results bear indirectly on this hypothesis. If GA affected the degree of wall crosslinking, then GA and GA-synthesis inhibitors should have substantial effects on the viscoelastic properties of the wall and these effects should be discernible by stress/strain analysis. Specifically, I plants should have low elastic and plastic compliances, and I+GA plants should have high compliances. Lack of such effects in peas (this study) and in cucumber hypocotyls (2, 17) argue against this cross-linking hypothesis in these plants.

Wall expansion has been viewed as a viscoelastic extension arising from biochemical modification of the wall (for review, see Refs. 5, 23). The control of such wall expansion can be thought of as falling somewhere between two extremes, with pure physical extension (viscoelasticity) at one extreme and biochemical reaction-dependent extension (chemorheology) at the other extreme. When viscoelastic processes limit growth, the wall yield threshold (Y) and yield coefficient (ϕ) should correlate with mechanical measures of the wall, as provided by Instron analysis (3, 4), physical stress relaxation analysis (4, 18) and other stress/strain techniques. When growth is more tightly dependent on biochemical processes, it takes on the character of a chemorheological process (21) and mechanical properties of the wall may not correlate with growth behavior.

In this study, the poor correlation between wall growth and wall compliances suggests that cell expansion in pea stems is closer to a chemorheological process. Moreover, in other studies, hormones and other agents often affect growth rate to a much greater extent than they affect wall mechanical properties. In these cases too, it may be that the major effect of these agents is on the chemorheological, rather than viscoelastic, processes leading to wall expansion. In this respect, *in vivo* stress relaxation techniques are advantageous because they can measure metabolism-dependent biochemical processes that lead to wall loosening and relaxation. It should be noted these methods will measure relaxation whether it is induced by synthesis and intussusception of new polymers into the wall or by wall loosening without such incorporation. Thus, possible side effects of uniconozol on, for example, wall synthesis, do not confound these stress relaxation measurements.

Finally, in this study the pressure-block technique showed larger differences in relaxation behavior between I and I+GA plants than did the turgor relaxation technique. Apparently, excision at the start of turgor relaxation diminished the differences in wall relaxation. The pressure-block results provide the strongest evidence that GA acts on wall yielding characteristics; both Y and ϕ are affected. However, the complex dynamics of relaxation seen with the pressure-block method will require further study before they are understood.

ACKNOWLEDGMENTS

We thank Melva Perich, Renee Flemish, and Daniel Durachko for their excellent technical assistance, and David Law for preliminary experiments on uniconozol and GA doses. Uniconozol was kindly provided by Loren D. Tukey, Prof. of Pomology, Penn State.

LITERATURE CITED

1. **Adams PA, Montague MJ, Tepfer M, Rayle DL, Ikuma H, Kaufman PB** (1975) Effect of gibberellic acid on the plasticity and elasticity of Avena stem segments. Plant Physiol **56:** 757–760
2. **Cleland R, Thompson ML, Rayle DL, Purves WK** (1968) Differences in effects of gibberellins and auxins on wall extensibility of cucumber hypocotyls. Nature **219:** 510–511
3. **Cleland RE** (1967) Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta **74:** 197–209
4. **Cleland RE** (1984) The Instron technique as a measure of immediate-past wall extensibility. Planta **160:** 514–520
5. **Cleland RE** (1987) The mechanism of wall loosening and wall extension. *In* DJ Cosgrove, DP Knievel, eds, Physiology of Cell Exansion during Plant Growth, Symposium in Plant Physiology, Penn State Univ, American Society of Plant Physiologist, Rockville, MD, pp 18–27
6. **Cosgrove D** (1982) Rapid inhibition of hypocotyl growth by blue light in *Sinapis alba* L. Plant Sci Lett **25:** 305–312
7. **Cosgrove DJ** (1985) Cell wall yield properties of growing tissues. Evaluation by in vivo stress relaxation. Plant Physiol **78:** 347–356
8. **Cosgrove DJ** (1986) Biophysical control of plant cell growth. Annu Rev Plant Physiol **37:** 377–405
9. **Cosgrove DJ** (1987) Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques. Planta **171:** 266–278
10. **Cosgrove DJ, Cleland RE** (1983) Osmotic properties of pea stem internodes in relation to growth and auxin action. Plant Physiol **72:** 332–338
11. **Cosgrove DJ, Van Volkenburgh E, Cleland RE** (1984) Stress relaxation of cell walls and the yield threshold for growth: demonstration and measurement by micro-pressure probe and psychrometer techniques. Planta **162:** 46–52
12. **Fry SC** (1979) Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth. Planta **146:** 343–351
13. **Izuma K, Kamiya Y, Sakura A, Oshio H, Takahashi N** (1985) Studies of sites of action of a new plant growth retardant (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3307) and comparative effects of its stereoisomers in a cell-free system from *Cucurbita* maxima. Plant Cell Physiol **26:** 821–827
14. **Jones RL** (1983) The role of gibberellins in plant cell elongation. CRC Crit Rev Plant Sci **1:** 23–47
15. **Jones RL** (1986) The control of plant cell elongation by auxin and gibberellin. *In* M Bopp, ed, Plant Growth Substances 1985, Springer-Verlag, Berlin, pp 274–283
16. **Kamisaka S, Sano H, Katsumi M, Masuda Y** (1972) Effects of cyclic AMP and gibberellic acid on lettuce hypocotyl elongation and mechanical properties of its cell wall. Plant Cell Physiol **13:** 167–173
17. **Katsumi M, Kazama H** (1978) Gibberellin control of cell elongation in cucumber hypocotyl sections. Bot Mag Tokyo **1:** 141–158
18. **Kawamura H, Kamisaka S, Masuda Y** (1976) Regulation of lettuce hypocotyl elongation by gibberellic acid. Correlation between cell elongation, stress-relaxation properties of the cell wall and wall polysaccharide content. Plant Cell Physiol **17:** 23–34
19. **Lockhart JA** (1960) Intracellular mechanism of growth inhibition by radiant energy. Plant Physiol **35:** 129–135
20. **MacMillan J, Phinney BO** (1987) Biochemical genetics and the regulation of stem elongation by gibberellins. *In* DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion during Plant Growth, Symposium in Plant Physiology, Penn State Univ, American Society of Plant Physiologists, Rockville, MD, pp 156–171
21. **Murakami K, Ono K** (1979) Chemorheology of Polymers. Elsevier, Amsterdam
22. **Nakamura T, Sekine S, Arai K, Takahashi N** (1975) Effects of gibberellic acid and indole-3-acetic acid on stress-relaxation properties of pea hook cell wall. Plant Cell Physiol **16:** 127–138
23. **Ray PM** (1987) Principles of plant cell growth. *In* DJ Cosgrove, DJ Knievel, eds, Physiology of Cell Expansion during Plant Growth, Symposium in Plant Physiology, Penn State Univ, American Society of Plant Physiologists, Rockville, MD, pp 1–17
24. **Reid JB** (1983) Internode length in Pisum. Do the internode length genes affect growth in dark-grown plants? Plant Physiol **72:** 759–763
25. **Silk WK, Jones RL** (1975) Gibberellin response in lettuce hypocotyl sections. Plant Physiol **56:** 267–272
26. **Stuart DA, Durnam DJ, Jones RL** (1977) Cell elongation and cell division in elongating lettuce hypocotyl sections. Planta **135:** 249–255
27. **Stuart DA, Jones RL** (1977) Roles of extensibility and turgor in gibberellin- and dark-stimulated growth. Plant Physiol **59:** 61–68
28. **Yoda S, Ashida J** (1960) Effects of gibberellin and auxin on the extensibility of the pea stem. Plant Cell Physiol **1:** 99–105
29. **Zack CD, Loy JB** (1984) Comparative effects of gibberellic acid and N6-benzyladenine on dry matter partitioning and osmotic and water potentials in seedling organs of dwarf watermelon. J Plant Growth Regul **3:** 65–73