Cyanide Inhibition of Acid-induced Growth in *Avena* Coleoptile Segments

**ABSTRACT**

The comparative effects of metabolic inhibitors on acid- and auxin-induced growth in oat (*Avena sativa* L. var. Victory) coleoptile segments have been examined. Acid (pH 4)-induced growth in both peeled and unpeeled segments is inhibited by 1 millimolar KCN when added at the time of acidification. KCN inhibits total acid-induced growth by 59 and 76%, respectively, in peeled and nonpeeled segments during the first 60 minutes. The growth rate of cyanide-treated tissue drops to zero or near zero in both peeled and nonpeeled segments during this period. Cyanide inhibition of total acid-induced growth in peeled segments at pH 5 is even more severe, amounting to about 90% during the first 60 minutes. The possibility that inhibition by cyanide may be caused by some nonspecific effect of the inhibitor on a process other than respiration, e.g., turgor reduction due to membrane damage, has not been ruled out. Acid-induced growth is also inhibited by 3 millimolar sodium fluoride and by anoxia. In unpeeled segments total pH 4-induced growth is inhibited 73% by sodium fluoride and 38% by anoxia during the 1st hour. Possible corrections to the above inhibition percentages which may be necessary due to the sensitivity of basal growth to inhibitors are discussed. Cyanide was found to inhibit auxin-induced growth much more rapidly than acid-induced growth. These data suggest that acid growth may be dependent on respiratory metabolism but to a lesser degree than is auxin-induced growth. If the acid growth theory of auxin action is correct, it appears that there may be two steps in the growth process which are dependent on respiratory metabolism: (a) auxin-induced proton pumping which is highly sensitive to respiratory inhibitors; and (b) acid-mediated wall loosening which is moderately and perhaps indirectly sensitive to respiratory inhibitors.

The rapid elongation of stem and coleoptile segments in response to auxin has been described as an active process which is very sensitive to metabolic inhibitors (14, 15). According to the acid growth hypothesis, auxin is thought to activate a metabolically driven proton pump which excretes H⁺ into the cell wall (5, 18). The result is a decrease in wall pH which is thought to cause wall loosening which is followed by turgor-driven growth. The rapid suppressive effect (e.g., 2–10 min, see refs. 13 and 15) of respiratory inhibitors on auxin-induced growth may be caused by their ability to interfere with oxidative phosphorylation, thus deactivating the proton pump. The resulting increase in cell wall pH may be responsible for the observed cessation of wall loosening and growth.

In contrast, the rapid elongation of stem and coleoptile segments in response to external application of acid has been described as a passive process which is dependent upon turgor but insensitive to metabolic inhibitors and anaerobic conditions (14). More recently, it has been described as an enzymic wall-loosening process which is insensitive to metabolic inhibitors (18). The enhancement of growth by acid has been considered similar to that which occurs in the last steps of auxin-induced growth except that the source of protons is from the external solution instead of an internal pump. When acid is supplied externally, the deactivation of the putative proton pump by respiratory poisons or anoxia should have no significant effect on the concentration of H⁺ at the cell wall, i.e., acid-induced growth should be unaffected by metabolic inhibitors.

The characterization of acid growth as a phenomenon independent of respiratory metabolism is largely based on inhibitor studies with *Avena* coleoptile segments in which acid or CO₂-stimulated growth appears to be unaffected by respiratory inhibitors (7, 8) and upon studies of frozen-thawed coleoptile segments which exhibit acid-induced extension under applied stress (17). In this paper, we demonstrate that acid growth in *Avena* coleoptile segments is sensitive to the respiratory inhibitors, KCN and NaF, and to anoxia. These data are not consistent with the proposal that acid-induced growth is purely passive or independent of metabolism, and they justify further consideration of the possibility that acid growth may depend directly or indirectly on respiratory energy.

**MATERIALS AND METHODS**

**Plant Material.** One-cm segments were cut from dark-grown 4- to 6-day-old *Avena sativa* L. var. Victory coleoptiles which had been given a 0.5 to 1.5 h red light treatment on the 3rd day to suppress mesocotyl growth. The primary leaves were removed, and 10 of these segments were strung vertically on a wire and immersed in an aerated 5 mM succinate buffer (pH 4) in a glass chamber. In a few experiments corn (*Zea mays*, L. hybrid WF 9 × 38 from Bear Hybrid Corn Co., Decatur, Ill.) coleoptiles were used. The corn seedlings were obtained and segments prepared as described in (21).

**Growth Measurement.** An angular position-sensing transducer was used to produce continuous elongation versus time curves. The curves were analyzed by computer and replotted by an IBM 1130 line plotter as previously described (20). Solutions containing inhibitors were added as described under “Results.” The 5 mM succinate buffer (pH 4) was prepared by titrating 5 mM succinic acid to pH 4 with concentrated NaOH. In preparing these acid solutions which were to contain KCN, addition of alkaline KCN to the pH 4 buffer caused an increase in pH to 4.25 to 4.35. This slight increase in pH was ignored in all experiments involving the use of KCN at pH 4. Several experiments were done to determine whether there were any differences between KCN effects on acid growth at pH 4.35 versus pH 4. In all cases KCN inhibition was still noted at pH 4 although in two out of four cases the inhibition was somewhat less. However, because of variability in results it is difficult to say whether there was appreciably less inhibition at pH 4 than at pH 4.3. When KCN was added to pH 5 or buffers, the pH increased to about 5.4 or 7.2, respectively. These solutions were titrated back to pH 5 or 6 with dilute HCl. Occasional checks made at the end of experiments indicated no appreciable change in pH during the course of the experiments. All experiments were
carried out under low intensity incandescent light and were repeated three or more times. For experiments with peeled coleoptiles, the removal of the epidermis was done under cool-white fluorescent lighting. Illustrations show results from representative experiments.

Measurement of Extension in Vitro. In order to measure extension of frozen-thawed coleoptile segments a device similar to that described by Rayle and Cleland (17) was used. Coleoptile segments (1 cm) were frozen in dry ice and thawed twice and then mounted in a device designed to measure extension under a constant tension. One of the flaccid coleoptile segments was attached to a fixed platform in the bottom of a Plexiglas chamber using a small drop of Eastman 910 adhesive. The other end was attached to the arm of a position sensor transducer at a point 11 cm from the axis of rotation (modification of the apparatus described in ref. 6). A 10- to 20-g weight was hung from the opposite end (also 11 cm from the axis of rotation) of the transducer arm. The chamber was then filled with the appropriate test solution and segment extension was determined by recording the output of the transducer on a Sargent Welch model SRLG recorder.

Determination of Endogenous Solute Content. In order to test for inhibitor-induced solute leakage, short term changes in solute content were measured in the presence and absence of inhibitor. Coleoptile segments were prepared and mounted in the growth chamber and incubated at pH 4 with or without KCN just as in growth experiments. After 90 min the segments were withdrawn from the chamber and adhering liquid was removed by directing a stream of compressed air through the hollow interior of the coleoptile cylinder for 1 s and around the outer surface of the coleoptile for 2 s. The segments were then placed in a short length of Tygon tubing stoppered at each end and the tubing was submerged in liquid N2. The tubes were subsequently stored on dry ice until measurements of solute content could be made. Solute content was determined by expressing sap from thawed segments and measuring its osmolality using a Wescor model 1500 B vapor pressure osmometer.

Basil Growth Correction. Since part of the growth inhibition by KCN at pH 4 may represent inhibition of basal or endogenous growth rather than acid growth per se, one might contend that growth at pH 6 should be subtracted from the acid growth control before calculating per cent inhibition. The values obtained when per cent inhibition is calculated in this manner are shown in parentheses following the values obtained without this correction both in the text and in Table I.

RESULTS

Inhibition of Acid-induced Growth by Respiratory Inhibitors. KCN (1 mm) inhibits acid (pH 4, 5 mm succinate buffer)-induced growth in peeled or unpeeled Vena coleoptile segments when added simultaneously with the acid buffer (Table I and Fig. 1, top). In unpeeled segments inhibition of low pH-induced elongation usually is obvious within 10 min and is complete within a little less than an h. On the average total acid-induced elongation is inhibited by 76% (64%) within 1 h (Table I) and to a greater extent thereafter (Fig. 1, top left).

In peeled segments inhibition by KCN becomes apparent within 15 min and is complete in a little more than an h. On the average, total acid-induced elongation is inhibited 59% (49%) within 1 h (Table I) and to a greater extent thereafter (Fig. 1, top right). KCN also inhibits acid-induced growth at pH 3 in unpeeled segments (data not shown). In peeled segments, KCN inhibition of acid-induced growth at pH 5 is substantially stronger (80% versus 59%) than at pH 4 (Table I).

When KCN is added after acidification, inhibition of acid growth in unpeeled segments is usually complete within 1 h whereas in control segments acid growth usually continues for at least 2 h and, in many cases, much longer (Fig. 1 top left). In peeled segments inhibition is complete in less than 2 h whereas in control segments acid growth continues for more than 3 or 4 h (Fig. 1, top right).

It is evident from the above experiments that cyanide inhibition of acid-induced growth in peeled segments is not as rapid as that in unpeeled segments. However, the acid-induced growth rate of peeled segments is greater than that of unpeeled segments and it appears that the removal of the cuticle has a general promotive effect on the acid growth rates, perhaps, by facilitating the entry of H+ as suggested by Cleland and Rayle (3).

Following the withdrawal of KCN, resumption of acid growth at a somewhat reduced rate is often observed in unpeeled segments, provided the KCN treatment has not exceeded 30 min (Fig. 1, top left). No emergent (10) or stored growth (13) is observed upon withdrawal of KCN. In peeled segments we were not able to determine whether there was a resumption of growth following KCN withdrawal because of the variability of results and because of the relatively high rate of acid growth in the presence of KCN (even though it was significantly lower than that of the acid controls).

Another respiratory inhibitor, NaF, also suppresses low pH-induced growth (Table I). When NaF (3 mm) is added to unpeeled segments at the time of acidification, acid growth is almost completely inhibited within 1 h. Total growth is inhibited by 73% (65%) in 1 h (Table I).

Effect of Cyanide on Auxin-induced Elongation. When 1 mm cyanide is added to unpeeled or peeled segments growing in the presence of auxin, inhibition begins within 3 min and is nearly complete within 30 min (Fig. 1, bottom). This inhibition of auxin-induced growth by cyanide is clearly more rapid than cyanide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Elongation after 60 Min</th>
<th>% Inhibition</th>
</tr>
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<tbody>
<tr>
<td>pH 4</td>
<td>0.63 ± 0.19 (N = 24)</td>
<td>76 (64)</td>
</tr>
<tr>
<td>pH 4 + 1 mm KCN</td>
<td>0.15 ± 0.11 (N = 14)</td>
<td>76 (64)</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.21 ± 0.05 (N = 28)</td>
<td>76 (64)</td>
</tr>
<tr>
<td>pH 6 + 1 mm KCN</td>
<td>0 (N = 3)</td>
<td>100</td>
</tr>
<tr>
<td>pH 4 + N2</td>
<td>0.39 ± 0.14 (N = 9)</td>
<td>38 (9)</td>
</tr>
<tr>
<td>pH 6 + N2</td>
<td>0.01 ± 0.01 (N = 4)</td>
<td>95</td>
</tr>
<tr>
<td>pH 4 + 3 mm NaF</td>
<td>0.17 ± 0.07 (N = 4)</td>
<td>73 (65)</td>
</tr>
<tr>
<td>pH 6 + 3 mm NaF</td>
<td>0.07 ± 0.05 (N = 5)</td>
<td>100</td>
</tr>
<tr>
<td>pH 6 10 μM IAA</td>
<td>0.67 ± 0.07 (N = 4)</td>
<td>100</td>
</tr>
<tr>
<td>pH 6 10 μM IAA + 1 mm KCN</td>
<td>0 (N = 3)</td>
<td>100</td>
</tr>
</tbody>
</table>

Peeld segments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Elongation after 60 Min</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td>0.98 ± 0.34 (N = 26)</td>
<td>59 (49)</td>
</tr>
<tr>
<td>pH 4 + 1 mm KCN</td>
<td>0.40 ± 0.18 (N = 16)</td>
<td>59 (49)</td>
</tr>
<tr>
<td>pH 5</td>
<td>0.55 ± 0.15 (N = 7)</td>
<td>59 (49)</td>
</tr>
<tr>
<td>pH 5 + 1 mm KCN</td>
<td>0.11 ± 0.09 (N = 7)</td>
<td>80 (69)</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.19 ± 0.07 (N = 5)</td>
<td>80 (69)</td>
</tr>
<tr>
<td>pH 6 + 1 mm KCN</td>
<td>0 (N = 3)</td>
<td>100</td>
</tr>
<tr>
<td>pH 6 10 μM IAA</td>
<td>0.34 ± 0.10 (N = 7)</td>
<td>100</td>
</tr>
<tr>
<td>pH 6 10 μM IAA + 1 mm KCN</td>
<td>0 (N = 3)</td>
<td>100</td>
</tr>
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inhibition of acid-induced growth. In contrast to the results with acid-treated tissue, however, the growth rate of auxin-treated tissue is smaller in peeled segments than in unpeeled segments. When KCN is added simultaneously with auxin, subsequent auxin-enhanced growth is eliminated.

Inhibition of Acid Growth by Anaerobic Conditions. When N₂ instead of air is bubbled through the chamber containing unpeeled coleoptiles, acid growth is inhibited by 38% (9%) within 1 h (Table I) and inhibition is complete within 1.5 to 2 h (Fig. 2). When air is replaced by N₂ during an acid response, inhibition of acid growth usually occurs within 10 min. Inhibition by N₂ would probably be more rapid if it were not for the fact that residual air bubbles attach to the sides of the coleoptile segments and are eliminated only slowly after N₂ gassing begins. When air is added to coleoptile segments immersed in N₂-saturated pH 4 buffer, the control rate of acid growth resumes almost immediately; sometimes stored growth appears to occur. In some cases it is possible that emergent growth (10) also occurs but due to the quantitative variability in results this is difficult to confirm.

**DISCUSSION**

The question of metabolic dependence is fundamental in discussions of the mode of action of acid in the enhancement of wall loosening and growth. Efforts toward elucidation of the nature of acid effects should be founded on a firm initial determination of whether the mechanism is active or passive. However, there appears to be conflicting evidence on this important issue (11).

In a previous study (19) we showed that 0.1 mM KCN inhibited acid (pH 4.5) growth of *Avena* coleoptile segments. Abscisic acid partially inhibited acid growth at the same pH. The present paper represents an extension of this work. A pH of 4 was employed here in order to duplicate more closely the conditions used by others in reports showing no sensitivity of acid-induced growth to respiratory inhibitors.

The data presented here indicate that the metabolic poisons KCN and NaF suppress acid-induced growth in *Avena*. Anaerobic conditions also appear to inhibit acid growth. The fact that the effect of these respiratory inhibitors on acid-induced growth is relatively rapid, though less rapid than with auxin-induced growth, indicates that there may be at least an indirect dependence of acid growth on respiratory energy. If so, why has the notion developed that acid growth is insensitive to metabolic inhibitors?

There are several possible explanations which may help to resolve the discrepancies in results reported by various investigators. Some of the earliest data cited as evidence for an apparent lack of dependence of acid-induced growth on metabolism are
those of Hagar et al. (8) which indicate that anaerobic conditions have little or no effect on acid growth. However, in a more recent paper (9) from that laboratory, Krauss and Hager have presented data showing that anoxia does appear to inhibit acid (pH 4)-induced growth in *Avena* coleoptile segments by about 50%.

Other papers cited as evidence that acid-induced growth is insensitive to metabolic inhibitors are papers by Evans et al. (7) and by Rayle and Cleland (16). In the paper by Evans et al. (7) it was shown that various metabolic inhibitors had little or no effect on the growth response to CO₂-saturated solutions. However, it is not certain that the metabolic sensitivities of acid- and CO₂-induced growth are comparable or that CO₂ and acid have strictly comparable modes of action (16). In the paper by Rayle and Cleland (16) it is stated that auxin-induced growth is sensitive to a “number of inhibitors” which have no effect on the response initiated by hydrogen ions. However, only cycloheximide is specified and no data are given. In a subsequent review (10) by the same authors, it is stated that acid-induced growth is not inhibited by cyanide or dinitrophenol but no data are given.

In a study of the acid sensitivity of the leaf sheath base in *Triticum*, Bridges and Wilkins (1) showed data indicating that growth induced by citrate-phosphate buffer at pH 3 is unaffected by anaerobic conditions. Whether this is also true for wheat coleoptile tissue has apparently not been tested. Phillipson et al. (12) reported that cyanide had little inhibitory effect on acid-induced growth in *Avena*. However, we have used a 10-fold lower concentration of cyanide than that employed here and exposed the tissue to more acid (pH 3.5) conditions. Even under these circumstances they noted that cyanide caused a slight but significant inhibition of acid-induced growth.

Thus, there is little direct evidence in the literature in support of the notion that acid-induced growth in *Avena* is independent of metabolism, although the data of Rehm and Cline (19) and the data presented in this paper provide evidence to the contrary. Vesper (21) has recently reported that acid-induced growth in corn coleoptile segments is largely insensitive to KCN. She found that a 40-min pretreatment with 1 mM KCN inhibited the acid (pH 4 citrate-phosphate buffer)-induced growth rate by not more than 24%. Since it seemed unlikely that *Avena* and Zea should vary greatly in sensitivity to KCN, we tested the cyanide sensitivity of the acid response in corn coleoptile segments using the same growth-recording apparatus and conditions which we used for *Avena*. The results (data not shown) confirmed Vesper’s findings, i.e. that the acid response is much less sensitive to cyanide in corn coleoptile segments than in oat coleoptile segments.

We considered the possibility that the reduced cyanide sensitivity of acid growth in corn coleoptile tissue might be due to lesser cyanide sensitivity of that tissue. This was tested by measuring the cyanide sensitivity of respiration in corn versus oat coleoptile tissue at pH 4 using a YSI model 53 O₂ monitor and polarographic O₂ electrode. Surprisingly, the cyanide sensitivity of respiration in corn coleoptile tissue at pH 4 appears to be considerably less than that of oat coleoptile tissue. Inhibition by 1 mM KCN was greater than 90% in oat tissue and only about 50% in corn tissue (data not shown). The difference does not appear to be due to a difference in cyanide penetration since the inhibitory effect in corn, though only partial, was detected as rapidly as that in oats. Preliminary experiments also indicate that auxin-induced growth in corn is not as sensitive to KCN as is auxin-induced growth in oats (data not shown). The difference in cyanide sensitivity of acid-induced growth in corn and oats may be due to an intrinsic difference in respiratory sensitivity to KCN rather than to differences in the degree of dependence of acid growth on respiratory metabolism in the two tissues.

Perhaps the strongest indirect evidence that acid growth is independent of respiratory metabolism is the data of Rayle and Cleland (17) which indicate that frozen-thawed tissue under tensile stress exhibits acid-induced extension. It seems unlikely that such tissue would remain metabolically active. We have considered the possibility that cyanide may interfere directly with the activity of the putative acid-sensitive wall-loosening enzyme. If so, cyanide would be expected to inhibit both auxin- and acid-induced growth as observed in our laboratory. If cyanide interferes directly with acid-induced wall loosening, one would also expect cyanide to prevent acid-induced extension of frozen-thawed *Avena* coleoptile segments. This possibility was tested using the constant stress device described under “Materials and Methods.” KCN (1 mM) was found to have little or no inhibitory effect on acid-induced extension *in vitro* (data not shown). These results indicate that acid-induced wall loosening *in vitro* is largely insensitive to direct inhibition by cyanide. This conclusion leads to an apparent logical impasse. Our results with living material indicate that acid-induced growth is sensitive to inhibitors of respiration. Yet we know that frozen-thawed tissue (presumably inactive metabolically) retains its sensitivity to acid and that acid-induction of extension in frozen-thawed tissue is not inhibited by cyanide. Perhaps these data may be taken as an indication that acid induction of extension *in vitro* is a phenomenon different from acid-induced growth *in vivo*.

We have considered the possibility that inhibition of acid growth by some of the inhibitors employed here may result from a general toxic effect rather than from a specific inhibition of respiration. It may be, e.g. that exposure to anaerobic conditions leads to a nonspecific membrane damage. This may allow solute leakage resulting in reduced growth. Parrish and Davies (personal communication) have observed efflux of electrolytes from N₂-treated pea stem tissue. However, significant solute leakage was not observed until several hours after the beginning of N₂ treatment. In the present study (and also in that reported by Hager’s group [9]) inhibition of acid growth usually begins within 10 min after the start of nitrogenation. Hence, it seems unlikely that inhibition of acid growth by anoxia is a result of membrane damage.

The possibility that inhibition of acid growth by cyanide is caused by membrane damage leading to solute leakage has also been considered. KCN has been found to induce leakage in plant cell membranes in times as brief as 20 to 30 min (R. Spanswick, personal communication). In order to test this possibility we attempted to measure changes in endogenous solute content during the 30-min period following exposure of the coleoptile tissue to cyanide. Our data (not shown) indicated that cyanide treatment caused no detectable loss of solute from the tissue. Inasmuch as the method employed measures total tissue solute content, including solutes which may have leaked into the free space, we cannot rule out the possibility that solutes leaked from the cells into the free space without leaving the tissue.

In Table 1 the values in parentheses represent per cent inhibition as calculated by subtracting the inhibitor-sensitive component of basal (pH 6) growth from total growth at pH 4 or 5 before determining per cent inhibition in the usual manner. When KCN or NaF is added at the time of acidification, inhibition is large and the use of this correction does not decrease the values by more than 10 or 15% (Table I). The qualitative results remain the same. However, in the case of the N₂ experiments the application of the correction results in a significant decrease (from 38 to 9%) in inhibition. If this correction is assumed to be necessary, then the possibility exists that the apparent inhibition of acid growth by anaerobic conditions is actually the result of N₂ inhibition of basal (pH 6) growth which may accompany acid growth. This might also explain the N₂ inhibition of acid growth found by Krauss and Hager (9).

Whether basal growth accompanies acid-induced growth is not known nor is it known whether so-called basal or endogenous growth at pH 6 is dependent upon H⁺ secretion. Cleland (2) suggested that in *Avena* coleoptile segments there is basal proton pump which is inactivated when exposed to an external pH less...
than 5.7. Hence, for these pH 4 experiments, there would be no basal growth and the above correction would be unnecessary. If basal growth at pH 6 is due to H+ secretion, then at pH 4 with peeled segments the wall region would be swamped with externally supplied H+ and the fact that KCN might inactivate the H+ pump would be of no significance. Hence, there would be no need for making the basal growth correction. However, if basal growth is not due to H+ secretion then the need for applying the basal correction would be more likely. Regardless of whether or not H+ secretion is responsible for basal growth it would not appear that KCN inhibition of basal growth could account for KCN inhibition of acid growth. The fact remains that we do not understand the relationship between basal growth and acid- and auxin-induced growth.

A possible explanation of the inhibitory effect of KCN on acid-induced growth in peeled segments is that KCN may cause a rapid alkalination of the cell wall space either by inactivation of the postulated proton pump or by the alkalizing effect of CN- ions per se. The consequent pH increase in the wall region would be expected to decrease wall loosening and growth. This assumes that the penetration of the cuticle of an unpeeled coleoptile by external H+ is insufficient to cause wall alkalification in the presence of hydroxyl ions which may accumulate when the pump is inactivated. The results of our studies and the earlier work of Cleland and Rayle (3) with peeled segments indicate that the cuticle does constitute a significant barrier to H+ movement in and out of the tissue. We consistently obtained a stronger acid response in peeled tissue. We noted that removal of the cuticle led to some reduction in the inhibitory effect of KCN, apparently by allowing a more rapid entry of H+ ions from the external solution. Nevertheless, we feel that it is unlikely that cell wall alkalination due to inactivation of the H+ pump by cyanide inhibition can explain cyanide inhibition of the acid response. Evidence in support of this conclusion includes: (a) the observation that KCN treatment led to a 59% inhibition of acid-induced growth even in peeled segments; (b) our finding published elsewhere (ref. 4) that cycloheximide, which is known to inhibit auxin-induced proton pump activity within a few minutes, does not inhibit acid-induced extension for at least 3 h; (c) our observation that lowering the pH from 4 to 3 in unpeeled segments does not relieve inhibition by cyanide (data not shown); and (d) our unpublished study showing that cyanide strongly inhibits the acid growth response in intact corn roots, an organ in which there is no cuticle to restrict H+ penetration.

Although the mode of inhibition of acid growth by cyanide is of importance, the major issue being addressed here is whether or not acid growth is sensitive to cyanide. Our data indicate that it is.

After the bulk of the experiments for this study had been completed, analyses of the effects of KCN on peeled Avena coleoptile segments at pH 5 were carried out. Although acid-induced growth was significantly less at pH 5 than at pH 4 the per cent inhibition was greater (80 versus 59%). Since auxin-induced wall acidification presumably leads to a wall pH nearer to 5 than 4 (3), it may be concluded that the proposed acid-mediated component of auxin-induced growth is, if anything, more sensitive to KCN than growth induced at pH 4 as measured here.

A comparison of the effects of KCN on auxin- and acid-induced growth shows that the inhibitory effect is distinctly more rapid in the former case. In a recent review, Penny and Penny (11) concluded that acid growth is less sensitive than auxin growth to metabolic inhibitors and that there appear to be fewer metabolic events associated with the former than with the latter. Our results are consistent with that conclusion and suggest that if auxin does cause cell elongation via H+ secretion, then there appear to be two steps in the process which are sensitive to metabolic inhibitors. The first and most sensitive to KCN may be H+ secretion, whereas the second, and apparently less sensitive, step may be the wall-loosening action of H+. Our results indicate that the acid response is sensitive to metabolic inhibitors and the implication is that respiratory metabolism may be involved, at least indirectly, in maintaining the cell walls in a condition such that their extensibility can be enhanced by acid. Thus, the apparent requirement for ATP production in order to maintain sensitivity to acid may not be due to its direct involvement in acid-induced wall loosening. Most hydrolyase reactions are exergonic and do not require ATP. The role of ATP production in maintaining acid sensitivity may be in the support of wall biosynthesis leading to the incorporation or maintenance of stress-supporting polysaccharide-bonding patterns which are susceptible to acid or to an enzyme enhanced by acid.

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