Reexamination of the Acid Growth Theory of Auxin Action

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ABSTRACT

Some crucial arguments against the acid growth theory of auxin action (U Kutschera, P Schopfer [1985] Planta 163: 483–493) have been re-investigated by simultaneous measurements of proton fluxes and growth of maize (Zea mays L.) coleoptiles. Special care was taken to obtain a mild, effective, and reproducible abration of the cuticle. Proton secretion rates were determined in a computer-controlled pH-stat. In some experiments, equilibrium pH was measured. Growth rates were determined simultaneously in the same vessel using a transducer-type auxanometer. It was found that (a) the timing of auxin and fusicoccin-induced (FC) proton secretion and growth matches well, (b) the equilibrium external pHs in the presence of IAA and FC are lower than previously recorded and below the so-called ‘threshold-pH,’ (c) neutral or alkaline unbuffered solutions partially inhibit FC and IAA-induced growth in a similar manner, (d) the action of pH, FC, and IAA on growth are not additive. It is concluded that the acid-growth-theory correctly describes incidents taking place in the early phases of auxin-induced growth.

The acid growth theory of auxin action (2, 9) states IAA-induced apoplastic acidification (3, 4, 19) causes an increase in extensibility of the cell wall (6). Extensibility limits hydraulic extension driven by turgor pressure. Four predictions for the validity of this theory and the function of an acidic pH in growth control have been critically investigated by Kutschera and Schopfer (11, 12) as follows:

1. Auxin must cause the cells to excrete protons quantitatively related to growth. The cell wall pH must decrease prior to the change in growth rate. Kutschera and Schopfer (11, 12), however, found no correlation in the kinetics between the elongation of segments and medium acidification.

2. Exogenous protons must be able to substitute for auxin causing cell wall loosening and growth, but Kutschera and Schopfer found the elongation elicited by external buffers pH 4.5 to 5.0 was significantly less than by IAA.

3. Neutral or alkaline solutions infiltrated into the cell wall must inhibit auxin-induced growth, but no influence of alkaline buffers on the auxin-induced component of growth was detected (11, 12).

4. The fungal toxin FC, known to be a potent stimulator of proton secretion, promotes growth similar to auxin (16, 17). Kutschera and Schopfer tried to quantify these two effects. Compared to FC, IAA was less effective in stimulating proton secretion, but this small increase of acidification by IAA was accompanied by a strong promotion of growth rate (14).

The data presented by Kutschera and Schopfer (11–13) seem to indicate that the auxin-, but not fusicoccin-induced, promotion of growth fails to meet all above criteria. In the present paper, we inspected and re-investigated the pros and cons. Many arguments of Kutschera et al. (11–13) require that the pH in the important layers of the cell wall does not differ significantly from the pH of the external bathing medium. If this assumption is not true, the absence of a correlation between pH in the bathing medium and growth response does not necessarily contradict the acid-growth theory. Furthermore, the following basic problems influence any experimental strategy for testing the theory: (a) The cuticle constitutes a barrier for protons (7). Mild and effective abraison is therefore one of the most central requirements for any investigation of this kind (4, 7, 8, 11, 12, 19). Even with optimal abraison, however, there remains some apoplastic diffusion resistance (22). (b) Particular attention has to be paid to the buffer, its pK, and uptake mechanism. Metabolic effects of the buffer cannot be excluded (29). A careful quantitative comparison of various buffers has been performed by Kutschera and Schopfer (12) in order to evaluate this problem. Nevertheless any buffer will be effective only near its pK. Exhaustion of the buffer in the cell wall may occur because its diffusion rate into the wall is significantly lower than the mobility of the protons. Pretreatment in buffers was recently suspected to cause artifacts when the acid-induced growth is going to be investigated (21). (c) It has to be taken into account that the sensitivity to auxin is different in epidermis and inner tissue (14, 15, 24, 26). This complicates the interpretation of pH drops and their correlation with growth response. The fact that IAA seems to act exclusively on the outer epidermis complicates comparisons between the effects of auxins and other agents affecting proton secretion like FC or sugars (12). The lower apparent auxin-induced proton secretion in media containing Na+ and no K+ may also be due to tissue sensitivity, but our knowledge on differential effects of Na+ and K+ is still limited.

To avoid many of these problems, we used a computer-assisted pH-stat for the measurement of proton secretion rates (1). This technique eliminates any problems with buffer strength and uptake mechanism (21, 29), because the measurements can be performed in an unbuffered solution. Another difficulty of pH-drop measurements, the dependence of
proton secretion rates on external pH, is avoided. We furthermore developed a new method for the removal of the cuticle that does not damage the cells and yields quantitative reproducible results. Growth rates could be simultaneously measured in this apparatus with a transducer auxanometer. Additionally, this technique allows the simultaneous measurement of proton secretion and growth.

In the present paper, we will demonstrate striking correlations between the rates of proton secretion and growth.

MATERIALS AND METHODS

Plant Material

Seeds of *Zea mays* L., cv Goldprinz, obtained from C. Sperling, Lüneburg, FRG, were soaked in running tap water for 2 h and then sterilized by treating with 10% *H₂O₂* for 10 min. The seedlings were grown in moist vermiculite No. 4 in a climate-controlled room in the dark at 27°C (94% RH). They were not subjected to red light. Coleoptiles were harvested after 4 d. They had a length of about 3 cm. The apical 3 mm and the primary leaf were removed. The apical 10 mm of the remaining coleoptiles were cut and used in the experiments. The harvested coleoptiles were put into distilled water prior to abrasion.

Abrasion of the Cuticle

Two g of tissue were placed in a 25 mL flask with 10 mL distilled water containing 2 to 4 g of SiC powder (1200 mesh, obtained from K. Schriever, Hamburg, FRG). The flask containing coleoptiles and abrasive suspension was vortexed at a rate of 400 impulses min⁻¹ in order to achieve a more reproducible abrasion of the cuticle than is obtained by conventional rubbing between SiC-coated fingers. Scrubbing time was 10 min if not otherwise indicated. After abrasion, the coleoptiles were washed and transferred into distilled water until insertion into the pH-stat and the auxanometer.

For comparison, abrasion with emery cloth (180 mesh) was performed according to a procedure described elsewhere (11, 12, 13).

SEM

SiC-scrubbed and untreated coleoptiles were fixed for 2 h in a mixture of 30% ethanol and 96% acetic acid (3:1 v/v), dehydrated in a graded acetone series, mixtures of 100% acetone and amylacetate (3:1, 1:1, and 1:3 v/v), and finally in pure amylacetate. The coleoptiles were subjected to critical point drying using liquid CO₂. Dried segments were coated with gold and kept in a desiccator. A Cambridge stereoscan S4 (Cambridge Instruments, Cambridge, UK) was used for examination of surface structures.

Staining Procedures

The effect of abrasion on the percentage of cells made accessible to the outer medium was tested by incubating coleoptiles in a solution of neutral red for 2 min. The percentage of accessible cells was estimated by visual inspection in a binocular microscope. To check for cells killed by the abrasion, coleoptiles were stained in Evans' blue as described elsewhere (11, 25). Photographs were taken on Kodak 2415 technical pan film.

Measurement of H⁺-Efflux

Proton fluxes were measured by means of a computer-assisted pH-stat, consisting of dilutors (Microlab, M. Hamilton, Bonaduz, Switzerland), AD Converters (type pH 530 D WTW, Weilheim, FRG), and a processor (Apple Europlus, 48K). Two g of coleoptile segments were incubated in a medium containing 10 mM KCl and 1 mM CaCl₂ that was vigorously bubbled with air. The pH was held constant by the pH-stat to ±0.01 pH units by continuous titration with KOH solutions. Since large amounts of K⁺ were present in the incubation medium, the K⁺ concentration was not significantly changed by the titration. Experiments were carried out at pH 6 if not otherwise indicated. From the amount of KOH consumed, the net proton efflux rate could be automatically determined. The experiments were performed in a climate controlled room at 26°C and 75 μM photons ⋅ m⁻² ⋅ s⁻¹. The ultrahigh resolution determination of proton secretion rate shown in Figure 7 was performed with another pH-stat, in which 1 g of plant material was incubated in 10 mL of solution.

Measurement of Equilibrium pH

One g of well-abraded coleoptile segments was incubated in a cuvette containing 10 mL well aerated incubation medium as described above. pH was traced with a chart recorder connected to a WTW pH meter. FC or IAA were added after a stable equilibrium pH had been achieved.

Measurement of Growth

Five coleoptile segments 5 mm in length were strung on a glass rod and inserted in the incubation medium of a pH-stat (10 mM KCl, 1 mM CaCl₂). The rate of elongation was measured with an angular position transducer (TWK Electronic, Düsseldorf, FRG) and monitored by the same processor as for determination of the net H⁺ efflux rate. This method allowed simultaneous measurements of growth and H⁺ efflux.

Growth Regulator Solutions

IAA was used as potassium salt, since it dissolves rapidly. FC was stocked as methanolic solution. Aliquots were taken, the methanol was evaporated, and the residue solved in a small volume of water and applied.

Incubation Medium

In all experiments, the incubation medium was 10 mM KCl and 1 mM CaCl₂. The pH of this unbuffered solution could be held constant to ±0.01 pH by means of the pH-stat.
RESULTS AND DISCUSSION

Effect of Abrasion on Integrity of Epidermis and Cuticles

The epidermal cuticle is a strong barrier for protons (7). Before measurement of proton secretion it must be removed without destroying the cells. We therefore compared various scrubbing techniques by inspecting the effect on the cells using SEM and by staining in neutral red for permeability and Evans' blue for dead cells.

On SiC-abraded coleoptiles, there was practically no staining by Evans' blue except at the cut surfaces (Fig. 1f). This shows that the cells survived the treatment. Neutral red stained about 90% of the cells (Fig. 1h), indicating free access between the cells and the external medium. However, scrubbing with wet emery cloth made 30 to 50% of the cells accessible to the external medium and killed 20 to 35%, depending on the force used to press the cloth onto the coleoptile (Fig. 1, e and g). Many cells abraded with emery cloth are actually damaged and killed. Moreover, it is known that SEM pictures of emery-cloth treated coleoptiles show massive destructions quite evidently (Fig. 10b in ref. 11). Furthermore, this method is difficult to standardize. On the other hand, SEM does not reveal any damage in cells of SiC coleoptiles (Fig. 1, a, b, c, and d).

These findings clearly show that the method of scrubbing is of great importance for the result that can be expected from ion flux measurements. We conclude that our technique abrades more cells and kills fewer cells than other procedures.

Effect of Scrubbing on Proton Efflux

The aim of scrubbing is to lower the diffusion barrier for protons by removing the cuticle. Figure 2 shows some important characteristics of SiC-abraded coleoptiles: (a) The SiC method clearly enhanced the rates of proton secretion. (b) A maximal effect is obtained after 10 min of scrubbing, prolongation up to 20 min did not have any effect on proton secretion rate. (c) At pH 5 there is a significant rate of proton efflux in the absence of FC or IAA. Kutschera and Schopfer, however, could achieve pH 5 only after treatment with 10^{-5} M IAA.

Well abraded coleoptiles acidified the incubation medium to a pH of about 4.5. They respond to hormonal treatment with an increased proton efflux after short lags and their growth strongly depends on external pH (Fig. 3b).

Effect of pH on Growth Rate

As expected, there was a clear dependence of growth rate on external pH (Fig. 3a). The lower the pH of the bathing medium, the higher was the growth rate. Scrubbing considerably increased the influence of external pH on elongation rate (Fig. 3b). The maximal action on the pH-elongation curve could be detected after a scrubbing time of 5 to 10 min. In contrast to other reports, lowering pH from 6.0 to 5.0 caused a small but significant promotion of growth. After an acidification from pH 6.0 to 4.5, the elongation rate was stimulated by a factor of 2.5 in scrubbed coleotepiles. Very high growth rates could be achieved at pH values lower than pH 4.5. A pH of about 3.5 induces a growth burst comparable to that caused by IAA and FC in unscrubbed coleoptiles. If a 10 mM citrate incubation buffer was used and adjusted to pH 4, the acid-induced growth is quite comparable to the action of an unbuffered solution in a pH-stat at the same pH. Summing up these results we can conclude that any treatment affecting the apoplastic pH also changes growth rate.

Timing of Auxin- and FC-Induced Proton Extrusion and Growth Matches Well

Even individuals who do not accept the acid-growth theory do not question that the promotion of growth by FC is mediated by protons (13). This fungal toxin is therefore an ideal reference substance permitting us the study of more or less pure proton-induced growth (17).

Fusicoccin strongly stimulated net proton secretion (Fig. 4, dots). There was a simultaneous promotion of growth rate (Fig. 4, asterisks). The lags were very short. These observations are well in line with experiments published elsewhere (13).

For IAA, Kutschera and Schopfer (12) reported long lag phases (40–60 min) for proton secretion but short ones for growth (15 min). They concluded that the acid growth theory does apply for FC, but not for IAA. These findings are not consistent with our data. Our experiments with SiC-abraded coleoptiles show a fine correlation of the kinetics of proton secretion and growth (Fig. 5). Both have a lag phase around 10 min. Growth rate and proton secretion peak synchronously 20 min after application. After this peak, growth rate drops, but 40 to 60 min after treatment a second maximum occurs. The dual-peak response has also been described in earlier papers (18, 27, 28). The discrepancy with the data reported by Kutschera and Schopfer may be due to some limitations of their pH measurement technique. It may be that long lag phases (and high equilibrium pH) are caused by an insufficient abrasion technique. It is interesting to note that in Kutschera and Schopfer (13) the lag phases of IAA and FC are even longer.

Recently, Ikoma and Okamato (10) found a quantitative and chronological relationship between the peaks of membrane potential and growth rate after an IAA treatment. They explain delays observed in conventional pH-drop measurements with the high buffer capacity of the wall. Measurements in a pH-stat are independent of buffer capacity and thus our results are well in line with their data. The recent paper of Senn and Goldsmith (23) also indicates a close correlation of both processes.

Drops in Equilibrium External pHs in Presence of IAA and FC Are Sufficient to Cause Significant Extension

A controversial matter in the literature is the question whether IAA is able to induce a drop of pH in the cell wall to values that can explain the bursts of growth observed. Unfortunately, there is no appropriate method to measure pH directly in the cell wall. Being limited to indirect evidence, several investigators determined the equilibrium pH after IAA treatment and implied that this value is more or less equal to the actual pH in the important layers of the wall. In Kutschera

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and Schopfer's measurements (11, 12), the pH equilibrated at pH 6 before and at pH 5 after IAA treatment. This pH would not be low enough to explain the effects of IAA on growth (11, 12). We decided to measure equilibrium pH with SiC-abraded coleoptiles. As shown in Figure 6, coleoptiles lowered the pH of the incubation medium to pH 4.4 to 4.7 within 40 to 60 min in the absence of IAA. This value is 1.5 pH units lower than the one observed by Kutschera and Schopfer (11, 12), who also used maize coleoptiles. According to their data, pH 4.5 represents the 'threshold pH,' the maximum pH able to induce a significant increase of growth rate and of cell wall extensibility.

In our system, any treatment with FC or IAA induced a quick pH drop much below the equilibrium pH of 4.5 to 4.7. With 10^{-5} M FC, a pH of 3.7 could be achieved. Coleoptiles treated with 10^{-3} M IAA acidified the solution to pH 4.0 to 4.1. In comparison, Kutschera and Schopfer report a pH of 5 with 10^{-3} M IAA and pH 3.8 with 10^{-5} M FC (12, 13).

What is the reason for these discrepancies? One possible difference between our data and theirs is that many of their pH drop experiments were performed in a solution containing Ca^{2+} but no K^{+}. However, some of their data show that they achieve similar equilibrium pH values in media containing 1 mM K^{+} (Fig. 72 in ref. 11). Moreover we found that there is no effect on the rate of proton pumping if the potassium concentration is increased from 1 mM to 10 mM as used in our experiments (data not shown), and the IAA-induced proton secretion is independent of potassium concentration in a range of 0.1 to 30 mM in Avena (23). The difference in abrasion techniques employed is probably the reason for the different results. Still, pH 4.0 to 4.1 is higher than the cell wall pH expected from the pH-growth curve (pH 3.5), but by using our scrubbing technique the discrepancy shrunk from 1.5 pH units to 0.5 pH units. The data obtained with FC, however, are well in line with Kutscher and Schopfer's results (12); FC-induced protons derive mainly from the inner cells (14). It seems that in contrast to the epidermal cells the inner cells are made more accessible by the emery cloth procedure. The rapid equilibration through the holes in the tissue (11) may explain the good match between our data and theirs in the case of FC.

Furthermore, the pH of the incubation medium is probably not identical to the pH at the surface of the plasma membrane. For maize roots, surface pH is reported to be up to 1.2 pH units below the medium pH (20). Other investigators predict the cell wall pH to be 0.3 pH units below equilibrium pH due to the Donnan potential (4, 5). These more conservative estimates would predict the pH in the wall of our coleoptiles to be at 3.7 to 3.8 after IAA-treatment and at 3.4 to 3.5 after FC treatment. Obviously, the differences between prediction and measurement shrunk.

Furthermore, it should be noted that this kind of estimation is very conservative. If we compare, for instance, the observed growth of abraded coleoptiles with the one predicted by the pH growth curve, the match is nearly perfect for both FC and IAA. This finding, however, is difficult to interpret because the auxin- and FC-induced growth in abraded coleoptiles is strongly depending on external pH.

Another factor that will cause a systematic overestimation of the apoplastic pH is tissue sensitivity. Only the epidermis responds to auxin with an increased proton efflux (14). After auxin treatment, the proton efflux from the outer cells will cause a pH drop also in the apoplast of the inner cells, which are not responding to auxin. The apoplastic pH of the inner cells thus will be permanently held below their equilibrium pH. A fraction of the protons excreted by the outer cells will end up in the proton influx of the inner cells. In other words, the equilibrium pH measured will be a mean equilibrium pH of all cells in the assay. Since only some cells are affected, and since only the extensibility in the outer epidermis is relevant for growth control (14, 15, 24), this will cause another systematic overestimation of the pH in the outer epidermal wall if it

Figure 1. Validation of the abrasion technique used in the experiments: SEMs of abraded and unabraded maize coleoptile surfaces, ×60 (a+b) and ×1000 (c+d). Note that on the scrubbed coleoptile there is no perforation or damage in the cell walls. e-h, Results of various staining experiments: e, coleoptile abraded with emery cloth and stained with Evans blue; f, same as e but coleoptile abraded with SiC powder; g, emery cloth-abraded coleoptile stained with neutral red; h, same as g but coleoptile abraded with SiC powder.

Figure 2. Effect of abrasion on proton secretion rate at fixed external pH. The maximal increase in proton secretion rate occurs after an abrasion time of 10 min. Further scrubbing does not influence epidermal permeability significantly, nor does it inhibit proton secretion due to wounding effects. Even at pH 5, there is a significant rate of net proton efflux. Bars indicate twice so of four experiments.
Three experiments. Coleoptiles (abscissa) and growth effectors. The measured (ordinate) of maize coleoptiles is shown in Figure 3. A, Effect of external pH (abscissa) on the maximal growth rate (ordinate) of maize coleoptiles. Bars indicate twice of at least three experiments. 'IAA' and 'FC' indicate the pH (abraded coleoptiles) and growth rates (unabraded coleoptiles) achieved with these effectors. The pH drop induced by IAA accounts for at least 50% of the measured growth rate. B, Comparison of the pH dependence of basal growth rate for scrubbed and unscrubbed coleoptiles: scrubbed coleoptiles are far more sensitive to external pH.

Is simply considered to equal the equilibrium pH of the bathing medium.

Neutral or Alkaline Solutions Partially Inhibit IAA-Induced Growth

If acid growth theory is correct, auxin induced growth should be dependent on external pH. Conditions preventing a drop of pH in the apoplast of the outer epidermal walls should abolish auxin-induced growth. The use of buffers to stabilize pH is problematic because most buffers interact in metabolism and furthermore change the osmolarity and ion strength of the medium. Furthermore, pretreatment in buffers can cause artifacts suggesting acid-induced growth (21). In a pH-stat, it is possible to stabilize pH without any buffer. We investigated the effects of a neutral or alkaline unbuffered solution of constant pH.

Although the pH was held constant, the reference substance FC induced strong growth (Figs. 4 and 7) indicating that either the acid-growth theory does not apply to FC-induced growth or, much more likely, that the pH in the apoplast was not equal to the pH of the external medium. The identity of bathing medium pH and cell wall pH, however, is an assumption inherent in many arguments against the acid growth theory of auxin action. Nevertheless, external pH did affect the FC-induced growth component. Abrasion lowered FC-induced growth, since the exchange of protons to the external solution is facilitated (Fig. 7A). A part of this inhibition may be also due to physical action, although no damage can be seen. Figure 7B shows that the growth rate with and without FC is inhibited by a neutral or alkaline pH. The pH dependent difference of growth rates is always higher in presence of FC (a<<b in Fig. 7 A and B). This shows that the effects of pH and FC are not simply additive and is well in line with experiments by Kutschera and Schopfer (13) supporting the acid-growth theory of FC-induced growth.

In the same manner, IAA-induced growth can be partially prevented by abrasion (Fig. 7C) and by alkaline pH (Fig. 7D). Again, the pH-dependent difference increases after IAA treatment. This shows that alkaline conditions do inhibit auxin-induced growth. In contrast to Kutschera and Schopfer, we failed to demonstrate a total inhibition of FC-induced growth with alkaline solutions. This is probably due to our unbuffered solution. It has to be emphasized that the pH in the cell wall is not equal to the pH in the outer medium, especially if the pump rate is high. It is known that the surface pH shift is more pronounced in unbuffered solutions than in strong buffers. However, to prove that a significant component of IAA- and FC-induced growth is dependent on external pH, it is not required to inhibit growth quantitatively. Therefore, we did not use buffers and thus avoided the problems recently discussed by Schopfer (21), who found that without a change in pH, a transfer from buffer to distilled water induces an increased elongation, thus mimicking acid growth. This finding, however, may reflect true acid-induced growth induced by a change in surface pH by the removal of buffer. Whatever the reasons for this effect might be, it shows that experiments in strongly buffered solutions may further complicate the matter.

We tried to increase the inhibitory effect of neutral pH on IAA-induced elongation by employing a buffer containing 2 mM potassium phosphate and, to cover a broad range of pKa 2 mM potassium succinate and citrate. There was still some IAA-induced growth, the first peak was delayed, and there

![Figure 4](image-url) Action of FC on proton secretion (dots) and growth (asterisks). Typical result selected from 10 experiments, abraded segments (pH 5.5).
was no sustained IAA-induced growth. In our eyes, these data should be taken with caution, since metabolic effects probably play an important role in modifying the kinetics of growth.

**IAA Action on Growth Is Not Additive to FC- and Acid-Induced Growth**

The data mentioned above are in disagreement with those observed by Kutschera and Schopfer, who reported the effects of IAA and pH additive and therefore independent (11). They furthermore postulated that FC increases growth via the scheme of the acid-growth theory and IAA via some different unknown mechanism. This theory of additive and independent effects implies the following predictions:

1. If plants are treated with FC and subsequently with IAA and the pH in the medium is held constant, there should be a fully developed IAA-induced growth burst. The net increase in growth rate should be the same as without FC-treatment.

2. If plants are treated with IAA and subsequently with FC, and if FC would be able to increase proton efflux from the coleoptiles under these conditions, there should be a fully developed FC-induced growth burst additive to that induced by IAA.

3. If plants are treated with acid pH 3.4, which induces a growth rate similar to that induced by IAA, there should be no further increase induced by FC but a fully developed IAA-growth response. We tested these predictions with the following experiments:

**First FC then IAA**

If plants were treated with an optimal concentration of FC and subsequently with 10 μM IAA, auxin did not at all affect growth. If 50 μM IAA were taken instead, it still did not induce an increase but, instead, a decline of proton secretion rate. Simultaneously there was a decline of growth rate (Fig. 8a). Obviously, IAA could not further stimulate proton secretion after an FC-treatment. At least in this case, there is no additive effect that could trigger IAA-induced growth. The absence of an IAA-induced response at optimal proton pumping rates is what is expected from the acid-growth theory; however, it may be due to toxicity of FC or due to a saturation of growth by FC regardless of the underlying mechanism.

**First IAA then FC**

If the plants were first treated with IAA and subsequently with FC, proton secretion was increased by FC, probably by activation of the IAA-insensitive inner tissue. However, there was no effect on growth (Fig. 8b), probably because the pH in the epidermal wall could not be further lowered by FC. Perhaps the proton pump in the epidermal cells was already maximal stimulated by IAA. Alternatively, the extension rate might have already been maximally stimulated by IAA, preventing further growth promotion by FC. In any case, the actions of IAA and FC are obviously not additive.

**Low pH and IAA/FC**

Low pH induced, as expected, a massive peak of growth rate (Fig. 9). After this peak a slow and steady decline of growth rate was observed. Two h later, the elongation rate was quite independent of pH. If in this phase plants were treated with auxin, there was only a very weak effect on growth rate. The initial reaction to IAA was a pronounced
inhibition of growth, followed by recovery. The maximal growth rate occurred 40 min after auxin treatment and generally exceeded that of control segments not treated with IAA. This is approximately coinciding with the second peak of auxin action occurring at normal pH. The first peak was prevented under these circumstances. The second peak was small and in some experiments even missing. In no case did a fully developed IAA response, predicted by the theory of additive and independent effects, occur. A differential pH-dependence of the two peaks of auxin-induced growth (18, 27) has been reported by Vanderhoef and Dute (28). It has to be taken into account, however, that acidic pH might have induced a drop in turgor preventing a more pronounced auxin action. This, however, can only explain the weak response but hardly the inhibition or the long lag phase.

In agreement with Kutschera and Schopfer (13), we found that coleoptiles did not respond to fusicoccin if they were previously treated with pH 3.5 (data not shown). As could have been expected, this supports the acid growth theory of fusicoccin action.

Summarizing these data we conclude that, in any case, the predictions made by the theory of additive and independent effects were not confirmed. The results of our experiments are more compatible with the acid-growth theory of auxin action. The slight increase of growth by IAA at acidic pH, however, indicates that there might be some additional mechanisms modifying the kinetics of IAA-induced growth.

CONCLUSIONS

The acid-growth theory has been criticized by Kutschera and Schopfer. The findings in the present paper are, however, in marked contrast to their data. Our data suggest that at least under certain experimental conditions the timings of growth and proton efflux match strikingly, that alkaline solutions affect FC- and IAA-induced growth in a similar manner, and that the pH drop caused by IAA is sufficient to explain a substantial fraction of IAA-induced growth. The differences between our data and that of Kutschera and Schopfer are probably due to different experimental conditions, e.g. abrasion techniques. To a lesser extent, a slightly different procedure for growing the coleoptiles (in the dark instead under red light) may play a role.

Our data, although being much more in line with the acid-growth theory than earlier reports, do not rule out the possibility that factors other than protons significantly participate in the regulation of auxin-induced growth. Most probably,
protein biosynthesis and cell wall formation are factors of similar importance, especially for the second peak (27), and they are probably required for sustained growth in response to IAA. In that respect, our data do not prove the acid-growth effect to be the only relevant mechanism, but they suggest a significant role of protons in growth control. Proton secretion is surely no simple independent side effect; especially, it seems to dominate the early phases of auxin action.

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LITERATURE CITED