Plant Physiol. (1988) 88, 131-138
0032-0889/88/88/0131/08/$01.00/0

Regulation of Electrogenic Proton Pumping by Auxin and Fusicoccin as Related to the Growth of *Avena* Coleoptiles[1]

Received for publication September 21, 1987 and in revised form April 1, 1988

ALFRED P. SENN[2] AND MARY HELEN M. GOLDSMITH*
Department of Biology, Kline Biology Tower, Yale University, P.O. Box 6666, New Haven, Connecticut 06511

ABSTRACT

The temporal relations between early responses to indoleacetic acid (IAA), proton secretion, hyperpolarization of the membrane potential, and growth change during the incubation of segments of oat (*Avena sativa* L.) coleoptiles in a low salt medium. When IAA is added after pretreatment of several hours, proton secretion increases after a latency of 7 minutes and reaches its maximum 10 to 15 minutes later. This timing coincides with both the increase in growth of the segments and the hyperpolarization of the membrane potential of parenchyma cells, consistent with the hypothesis that the change in membrane voltage reflects the activity of an electrogenic proton pump. The extent of IAA-induced hyperpolarization is substantially reduced by elevating $[KCl]_o$, most likely because this increases the passive conductance of the membrane. Neither growth nor proton secretion is affected by high $[KCl]_o$ (30 millimolar), indicating that neither process is limited by the magnitude of the membrane potential. These results are consistent with the acid growth hypothesis. Following short incubation times, however, IAA-induced hyperpolarization and growth are detected within 10 minutes, while acidification of the medium is delayed for more than 40 minutes. This result is seemingly in conflict with the acid growth hypothesis, but in freshly cut tissue, the pH of the external medium may not reflect the pH of the epidermal cell walls. The temporal coincidence of auxin-induced growth and hyperpolarization suggests that in freshly isolated segments the hyperpolarization is a more sensitive indication of proton secretion than is acidification of the external aqueous environment.

According to the acid-growth hypothesis (12, 22), cell elongation is caused by auxin inducing secretion of protons from the cell into the apoplast. The ensuing acidification causes loosening of the cell wall matrix and turgor-driven cell expansion. The observation that a drop in the pH of the apoplast precedes the change in growth rate in the case of both auxin (6, 13) and fusicoccin (5, 17) provided important support for the acid-growth hypothesis; however, recent more quantitative attempts to attribute auxin-induced growth to acidification of cell walls have indicated that the pH of the walls may not be the only factor involved (15, 27, 29). Brummer and coworkers (3, 4) have argued that auxin first causes cytoplasmic acidification, which in turn stimulates the electrogenic proton pump. They further suggest that the ensuing hyperpolarization of the plasma membrane, through secondary effects on essential transport processes and metabolism, may be more important in the stimulation of growth by auxin than is wall acidification.

Since protons are secreted from the cell against both a considerable concentration and electrical gradient, this process requires energy presumably furnished by H^+-translocating ATPases in the plasma membrane. Electrophysiological measurements have shown that both IAA and FC[3] cause the plasma membrane to hyperpolarize, indicating that the increased proton secretion is electrogenic (1, 9). Although a careful comparison has been lacking, the hyperpolarization should be coincident or even prior to measurable acidification of the external medium. The time required to acidify the inner portion of the wall where loosening occurs (26) is not known; however, the onset of hyperpolarization should be a sensitive indication of the time at which proton secretion and wall acidification actually begins.

The purpose of these experiments was to determine the temporal relations between the induction of membrane hyperpolarization, proton secretion, and growth by IAA. Furthermore, by adjusting the membrane potential and the auxin-induced hyperpolarization by varying $[KCl]_o$, we examined the suggestion that a highly negative membrane potential *per se* is important for the stimulation of growth by auxin.

MATERIALS AND METHODS

Plant Material. Seeds of *Avena sativa* L. cv Victory (Svalöf AB, Svalöv, Sweden) were rinsed under tap water, imbibed for 10 to 60 min, and grown in vermiculite moistened with distilled water. Seedlings were grown at 25°C in the dark and exposed to weak green light during watering or collecting the plant material. Experiments were performed under room illumination (daylight fluorescent lighting) unless otherwise stated. After 5 d, the seedlings had coleoptiles 4 to 5 cm long. These seedlings were more uniform in size than younger ones, and segments cut from them responded to IAA as did segments from shorter coleoptiles. Segments 12 mm long were cut 3 mm below the tip of the 5-d-old coleoptile, and the primary leaves were removed. For electrophysiological experiments and measurements of proton secretion, the segments were split longitudinally between the vascular bundles, but they were left intact for measurements of growth. The coleoptile sections were then floated on BSM (0.1 mM KCl, 0.1 mM $CaCl_2$, 1 mM Mes, or 1 mM Hepes adjusted to pH 6.0 with BTP). Unless otherwise indicated, the segments were pretreated for 3 h in this medium before adding IAA. In the case of experiments involving elevated concentrations of KCl, the BSM was replaced by test solutions containing different concentrations

[1] Supported by National Science Foundation grant DMB-8419759. The senior author was supported by a John Simon Guggenheim Fellowship while writing this paper.

[2] Present address: Biologiste, Maternité du Chuv, CH-1011 Lausanne, Switzerland.

[3] Abbreviations: FC, fusicoccin; BSM, basal salt medium; BTP, 1,3-bis[tris(hydroxymethyl)-methylamino]propane; V_m, membrane potential; $[KCl]_o$, external concentration of KCl.

of KCl (0.1–30 mM) after 2 h and then incubated for another hour prior to adding IAA.

Membrane Potential. After pretreatment, the split sections were mounted cut-side up in a shallow lucite chamber (0.5 ml) and bathed by a continuous flow (2 ml/min) of buffered BSM solution. The membrane potential of intact parenchymal cells 3 to 4 cells inside of the epidermis and in either the first or second layer below the cut surface was measured with an intracellular borosilicate glass (Frederick Haer & Co., Brunswick, ME or WPI, New Haven, CT) microelectrode (Ag/AgCl), backfilled by capillarity with 3 M KCl. The reference electrode (Ag/AgCl), containing 2% agar + 3 M KCl, was attached near the outlet of the superfusion chamber. Resistances of the microelectrodes varied between 6 and 15 MΩ. Their tip-junction potentials were less than −5 mV, as determined by breaking the electrode tip and did not vary more than 2 to 3 mV over the whole range of the $[KCl]_o$ studied. The voltage and resistance measurements were made as previously described (2). Although the microelectrode tip was likely to be in the vacuole (2, 11), selection of impalements with highly negative membrane potential and low resistance permitted us to monitor the plasmalemma primarily (2).

Solutions were changed in less than a minute using a manifold, while recording the membrane potential. The regime was to determine the membrane potential without IAA until it stabilized and then switch to a bath of the same salt composition containing IAA (10 μM).

FC was either added directly to the solution in the recording chamber while the flow was stopped or to the preincubation during the 15 min immediately prior to making the electrical recordings. In the first case, the flow of bathing medium was restored after the membrane potential reached a steady value (5–10 min). Although FC was not present continuously, the hyperpolarization was not reversed and persisted unchanged during a further 2-h incubation in flowing, FC-free bathing medium.

Proton Secretion. All pH measurements were performed with a Zeromatic pH meter (Beckman Instruments, Fullerton, CA), equipped with a flat-surface combination pH electrode (Ingold, No. 6020, One Burtt Road, Andover, MA). Solutions were either stirred or shaken vigorously or bubbled with a continuous stream of O_2 to ensure adequate oxygenation.

The cuticle is a significant barrier to the diffusion of protons into the medium (22); however, IAA-induced acidification from longitudinally split segments proved to be equivalent to that from peeled ones (data not shown), and thus the split sections were used for both proton secretion and electrophysiology. In order to increase the sensitivity of detecting protons, we lowered the buffer capacity at pH 6 by using a buffer with a higher pK_a (Hepes: $pK_a = 7.5$) and also reduced the volume of solution to 1 ml per 20 split half segments.

The methods used to follow the proton secretion process depended on whether short- or long-term responses were investigated. For short-term experiments, the pH of the medium was monitored continuously with the pH electrode connected to a recorder. In some experiments, the actual amount of protons secreted into the medium was determined by back titrating each minute with 0.01 N NaOH from a calibrated microburette (S-1100 A, Gilmont Instruments, 401 Great Neck Road, Great Neck, NY). For longer term studies, the incubating solutions were titrated back to pH 6.0 every 45 to 60 min. In this way, proton secretion could be followed while maintaining the external pH within 0.5 unit of the initial pH 6.

Elongation Assays. Following the preincubation period, short-term growth was monitored with an angular position transducer (Metripak, Gould Inc., Model 33-06, Instruments Systems Division, Cleveland, OH) positioned on the top of three 12 mm long coleoptile segments. The segments were mounted on a string in a small chamber (1 ml) and continuously bathed with fresh medium (2 ml/min). For longer term measurements, eight unsplit 12 mm long sections were threaded along a nylon string. Following the usual preincubation period, the groups of segments were transferred to media with and without IAA and the total length of the segments was measured at 45-min intervals to the nearest 0.5 mm by aligning the segments on the string end to end in a grooved piece of Lucite against a mm scale. Elongation of the segments (in %) was expressed relative to their initial length at the time of transfer to the test solutions.

RESULTS

Effect of Length of Preincubation on Responses to IAA. The membrane potential of parenchymal cells as well as the proton secretion and elongation of coleoptile segments, were monitored for 7 h in BSM following excision. In the absence of added IAA (*open circles*), there was little proton secretion (Fig. 1B) or growth (Fig. 1C). During the initial 90 min, however, the membrane

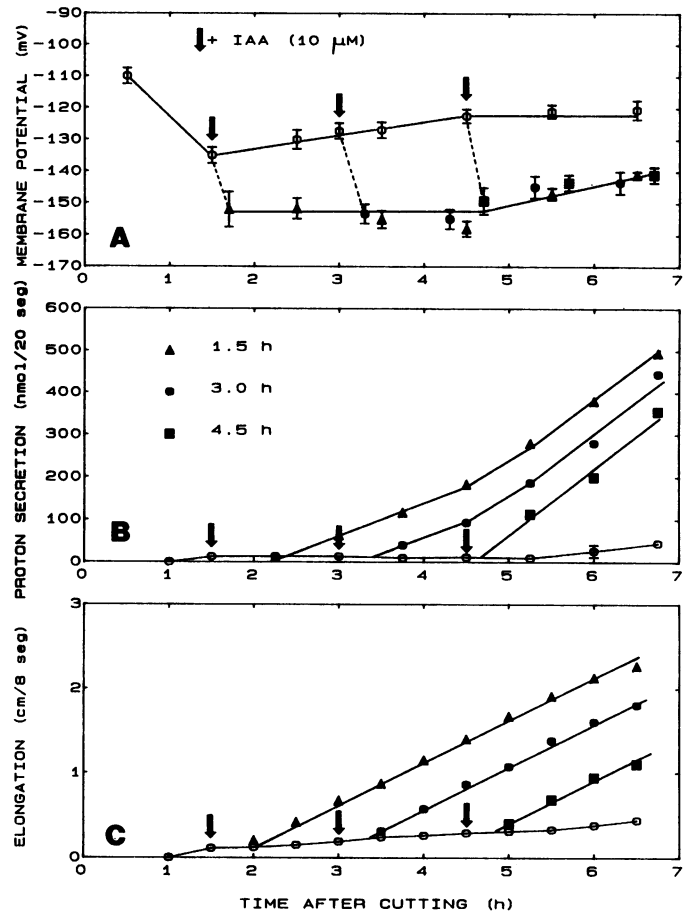

FIG. 1. Long-term effects of IAA (10 μM) on membrane potential (A), proton secretion (B), and growth (C) of oat coleoptiles. At zero time, coleoptile sections were excised and incubated in BSM (pH 6.0) (○). IAA was added at the arrows after 1.5 (▲), 3.0 (●), or 4.5 h (■); the recorded responses are given by filled symbols. A, Membrane voltages are means ± SE of 10 to 40 measurements performed on 20 (control: *open symbols*) and 10 (IAA-treated: *filled symbols*) different segments. Only the stable negative membrane potentials achieved after 20 min in IAA are plotted here. *Dashed lines* connect the mean values of V_m at the time of and 20 min after IAA addition. B, Net proton secretion by split segments determined by back-titration. C, Elongation is expressed as increase in length (cm) of eight coleoptile segments. Results in B and C are means of three separate experiments, and the standard errors are equal or less than symbol size.

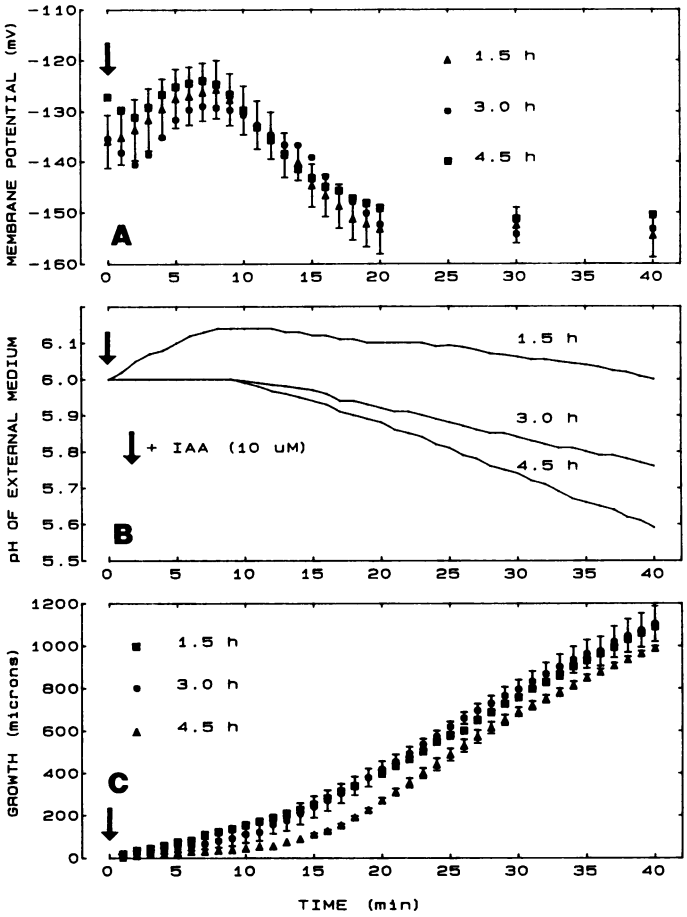

FIG. 2. Short-term effects of IAA (10 μM) on membrane potential (A), net proton secretion (B), and growth (C) of oat coleoptiles. Tissue sections were preincubated in BSM (pH 6.0) for different times (1.5, 3.0, or 4.5 h) before IAA was applied at zero time (*arrows*). A, Each value was derived from continuous recordings of individual cells and represents the mean response ± SE of 5 (1.5 h), 10 (3.0 h), and 3 (4.5 h) cells. In the absence of IAA, V_m remained at the time zero value over the 40-min recording period. B, Kinetics of change in the external pH induced by adding IAA to 10 longitudinally split coleoptiles in 1 ml of BSM. For segments incubated without IAA, the external pH rose to 6.15, remained close to 6.0, and declined to 5.9 for 1.5, 3.0, and 4.5 h pretreatments, respectively. Short-term recording method was used. C, Elongation (μm) of 3 (1.2 cm) coleoptile segments monitored with a position transducer. Growth rates of segments without IAA were comparable to those between 0 and 5 min in the presence of IAA. Data shown are means ± SE of four experiments.

potential (Fig. 1A) became 25 mV more negative. Over the next several hours, V_m underwent a gradual depolarization, after which it remained stable at a mean value of −120.0 ± 3.0 mV for 24 h (data not shown).

The long-term changes in membrane potential, proton secretion, and growth in response to addition of IAA after different periods of preincubation are also shown in Figures 1, A to C (*filled symbols*), and the initial 40 min of the responses to IAA are shown in Figures 2, A to C. Twenty minutes after IAA addition, the membrane potential had stabilized at a more negative value that then persisted for many hours (Fig. 1A). As observed previously, the electrical response is actually biphasic (1, 9, 20); during the first 8 min, IAA induces a transient depolarization of 7.5 to 10 mV. This is succeeded by a repolarizing phase during which the membrane becomes from 14 to 22 mV more negative than its initial value (Fig. 2A).

Table I. *Mean Latent Periods for IAA-Induced Responses as Function of Presoaking Time of Tissues*

Latency for the specific electrical response is the time after adding IAA when the membrane starts to repolarize as in Figure 2A. Latencies for the proton secretion and growth determined from the intersection of linear regressions on data from incubation of segments with and without IAA. Values are means ± SE. Number of experiments in parentheses.

Presoaking Time	Lag Times before Start of		
	Hyperpolarization	Proton secretion	Elongation
h		*min*	
1.5	9.8 ± 0.6 (6)[a]	38.8 ± 0.8 (4)[a]	9.2 ± 0.6 (2)
3.0	8.3 ± 1.0 (6)	10.2 ± 0.6 (9)	10.4 ± 0.6 (5)[a]
4.5	7.0 ± 0.6 (6)[a]	9.2 ± 0.6 (4)[a]	13.0 ± 0.9 (4)[a]

[a] Values significantly different at 95% level.

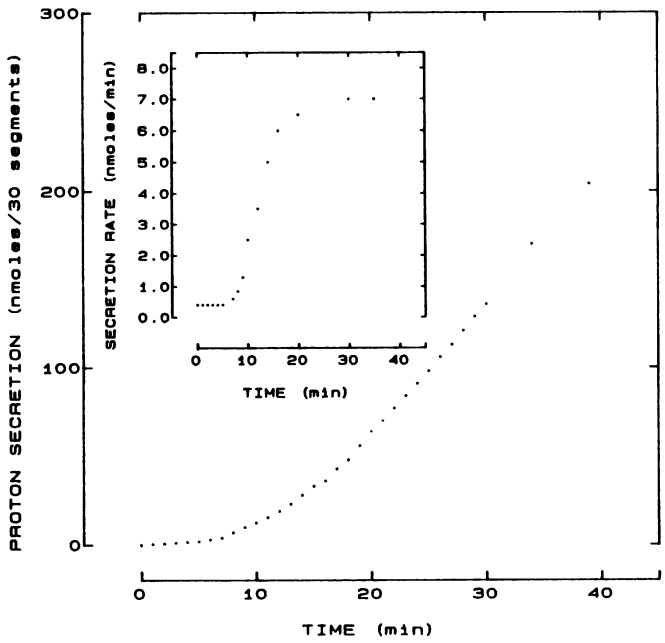

FIG. 3. Short-term effects of IAA (10 μM) on net proton secretion of oat coleoptiles. Tissue sections were preincubated in BSM (pH 6.0) for 3.0 h before adding IAA (0 time). Net proton secretion by 10 split segments in 1 ml of medium was estimated by backtitration to pH 6.0 every minute using the short-term recording method. *Inset* shows the first derivative of the proton secretion, the rate of secretion *versus* time.

Although the timing of the electrical response did not vary with different pretreatments, this was not true for the acidification (Fig. 2B). Both the latency between IAA addition and the start of acidification (Table I) and the time for secretion to achieve a constant rate (Fig. 1B) decreased with longer preincubations. For example, when IAA was applied at 1.5 h, acidification was not apparent for more than 40 min (Fig. 2B), and net proton secretion did not reach a final steady rate for several hours (Fib. 1B). After a 4.5 h preincubation, however, acidification was detectable in 10 min (Fig. 2B) and proton secretion reached a final steady rate in 30 min (Fig. 1B). Segments incubated 1.5 h in the absence of IAA consistently increased the external pH to between 6.10 and 6.3. (This uptake of protons is not apparent in Fig. 1B because in this experiment such small increases in the pH were not readjusted to 6.)

The length of preincubation had relatively little effect on the growth in response to auxin (Fig. 1C). In each case, a lag of about 10 min occurred between addition of IAA and an increased rate of elongation (Table I and Fig. 2C). The sensitivity to auxin of oat coleoptile segments does not undergo as dramatic a change

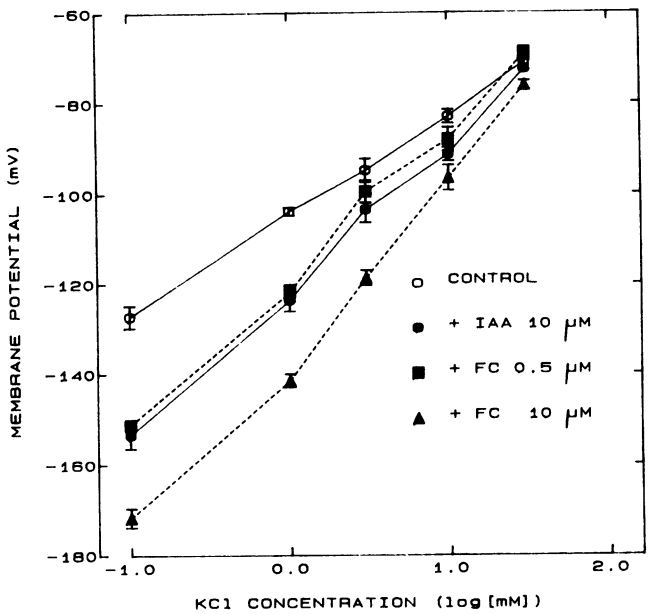

FIG. 4. Dependence of the steady-state membrane potential (mV) on the extracellular KCl concentration (mM) for control segments or for segments treated with IAA (10 μM) or FC (0.5 and 10 μM). $[KCl]_o$ was varied stepwise from lower to higher concentrations. The regime was to determine the membrane potential without IAA or FC and then either add FC directly to the bath with the flow stopped or switch to a bath of the same composition but containing IAA. Measurements were done 20 to 30 min after adding FC or IAA. Results are means ± SE of 13 to 19 impalements for control cells, 17 to 29 for IAA-treated, and 4 to 12 for FC-treated cells.

during incubation in salt solutions as has been reported for maize (30). The results can be summarized by observing that the latency prior to a steady rate of net proton secretion depends more strongly on the time between cutting the tissue and adding IAA than does the lag prior to either hyperpolarization or enhanced elongation (Table I).

To ensure that unrelated ionic adjustments of the tissue to the medium did not confound the results, we selected a standard 3 h equilibration period in BSM for all subsequent experiments. Under these conditions, the various responses to auxin—membrane hyperpolarization, medium acidification, and increased growth rate—were chronologically related. Presumably, this is because after longer preincubations processes that slow the appearance of secreted protons in the medium, such as their reentry into cells through cotransport processes or their interaction with anionic groups in the walls, were at a minimum.

Relationship between Proton Secretion and Membrane Hyperpolarization. In an attempt to determine the relation between the electrical hyperpolarization of the plasma membrane and proton secretion, the protons secreted into the medium every minute were titrated with NaOH (Fig. 3). The net rate of proton secretion was enhanced within 7 min after IAA addition (*inset*, Fig. 3) and gradually increased to a maximum 20 to 30 min after IAA addition. The membrane repolarized during this same period (Fig. 2A). The coincidence between the kinetics of proton secretion and membrane hyperpolarization after equilibration in low external salts supports the hypothesis that the hyperpolarization reflects an increased activity of an electrogenic proton pump.

Alteration of the IAA and FC Responses by $[KCl]_o$. The steady-state membrane potential of coleoptile cells depolarized about 25 to 30 mV per decade between 0.1 and 30 mM $[KCl]_o$ (Fig. 4). The cells depolarized monotonically, reaching the new resting potential within a minute. Both IAA and FC caused cells in low (0.1 to 1 mM) $[KCl]_o$ to hyperpolarize but were less effective with cells in 10 to 30 mM $[KCl]_o$. These results indicate that K^+ plays an important role in establishing the membrane potential of both control and treated tissues and that the proton pump is more able to hyperpolarize the membrane when $[KCl]_o$ is low.

The FC-induced hyperpolarization and proton secretion depend on the concentration of FC (24). At all $[KCl]_o$, 10 μM FC caused significantly greater hyperpolarization than 10 μM IAA. When the concentration was reduced to 0.5 μM FC, however, the response to FC was no longer significantly different from that to 10 μM IAA (Fig. 4). Presumably, this is because on a molar basis, FC is about twenty times more effective than IAA in stimulating the proton pump.

Short-term kinetics of the responses to IAA are presented in Figure 5, A and B, at several $[KCl]_o$. After control cells had achieved a stable mean potential, IAA was added to the tissues ($t = 0$), and the changes in membrane potential were monitored until a new stable value was reached, usually 20 to 30 min later. Then several more cells were impaled in the same $[KCl]_o$ in order to obtain the mean potential of treated cells. In 0.1 mM KCl, the IAA-response had the usual biphasic shape (Fig. 2A); but with increasing $[KCl]_o$ in the bathing medium, both phases of the response to IAA decreased, becoming insignificant at 30 mM KCl (Fig. 5A). A similar effect of increasing $[K^+]$ on the hyperpolarization induced by 1 mM butyric acid has been observed by Marrè *et al.* (19).

Having discovered that increasing $[KCl]_o$ decreased the electrical response to IAA, we investigated whether the IAA-induced proton secretion and growth were also affected. Although in the absence of IAA the basal level of proton secretion increased as $[KCl]_o$ was raised (Fig. 6, A to D), the latency and amount of IAA-induced acidification, determined from the difference between control and IAA-treated tissues (Fig. 6, A to D), was similar for all $[KCl]_o$ (Table II, Fig. 7A). Furthermore, the proton secretion was independent of the accompanying anion, either Cl^- or SO_4^{2-}. The latency for IAA-induced acidification coincided with the time that the segment started to elongate at a faster rate (Table II, Fig. 7, A and B).

IAA-induced growth was also independent of $[KCl]_o$. The reduced growth in 30 mM $[KCl]_o$ (Fig. 7B) can be attributed to the increased osmolarity of the medium. When a constant osmolarity was maintained at all salt concentrations by adding an impermeant solute such as mannitol, IAA-induced growth was similar at all $[KCl]_o$.

These results lead to the conclusion that both IAA-induced proton secretion and growth are independent of the $[KCl]_o$ between 0.1 and 30 mM (Fig. 7, A and B). Given the level of variation in the data, proton secretion is relatively insensitive to the membrane potential between −70 and about −160 mV, and the nearly twofold increment in proton secretion due to IAA is virtually independent of the membrane potential (Fig. 8).

DISCUSSION

Significance of IAA-Induced Hyperpolarization. The correlation between the kinetics of the hyperpolarization of the membrane potential of the parenchyma cells and the increased acidification of the medium by the coleoptiles following several hours pretreatment in BSM is consistent with an electrogenic transport of protons. The electrical response indicates that parenchyma cells of oat coleoptiles do respond to auxin; this conclusion differs from that of Kutschera *et al.* (14), who reported that when the epidermis was removed maize coleoptiles neither secreted protons nor elongated when IAA was added.

In principle, the hyperpolarization caused by IAA could reflect either an increase in the current of protons through the pump or a decrease elsewhere in the membrane of the passive back flux

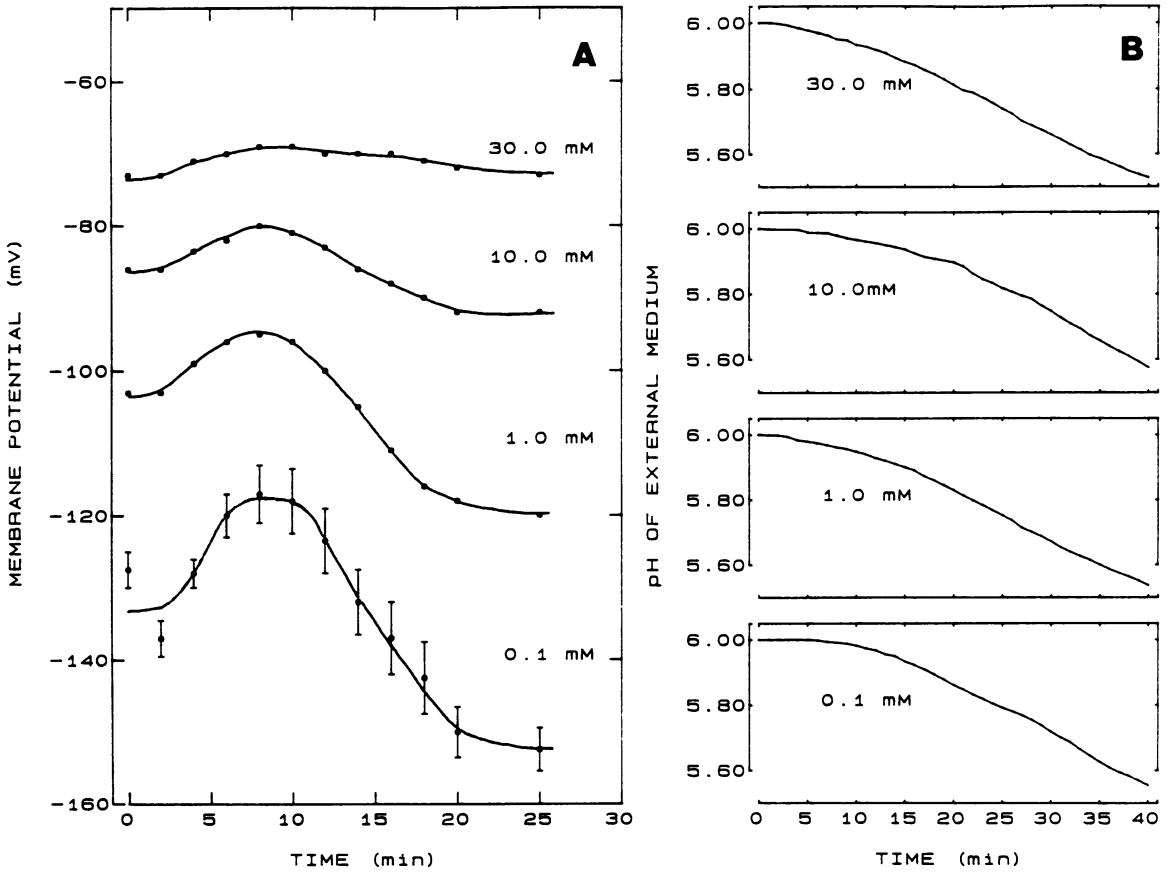

FIG. 5. Short-term effects induced by IAA (10 μM) on the membrane potential (A) and on the pH of the medium (B), when coleoptiles are incubated in different $[KCl]_o$. IAA was added at time 0, 3 h after cutting. Experimental conditions as in Figure 2, A and B. A, Each value is the mean of 3 to 10 individual recordings. The SE for all curves are similar to those indicated for 0.1 mM KCl. After impalements in the absence of IAA stabilized, V_m did not vary significantly. B, Curves shown are representative of four comparable experiments. An apparent shortening of the latency with increasing $[KCl]_o$ resulted mainly from an increase in the basal level of proton secretion. This is indicated by the decrease in the pH of the medium after 40 min in the absence of IAA to 5.95 ± 0.05 in 0.1 mM KCl; 5.91 ± 0.04 in 1.0 mM; 5.84 ± 0.03 in 10 mM; and 5.8 ± 0.03 in 30 mM KCl.

of cations. Only a decreased permeability to protons (as opposed to any other cation), however, would also account for the increased net efflux of protons which was observed. The occurrence of greater acidification together with hyperpolarization is therefore an indication of more effective pumping.

Effect of Presoaking. The observation that segments presoaked for only 1.5 h begin to elongate in response to IAA after the usual 10 min latent period but without any apparent acidification for more than 40 min (Fig. 2, B and C) is difficult to reconcile with the acid growth hypothesis. Although acidification of the medium is not apparent when tissues are pretreated for only 1.5 h, these cells hyperpolarize with the same time course as tissue soaked for longer times. Furthermore, the onset of auxin-induced growth in tissues pretreated for different periods correlated more closely with the timing of this hyperpolarization than with observed acidification (Table I). These results suggest that the cells are secreting protons after a pretreatment of only 1.5 h, even though the pH of the medium does not change. Elongation of the epidermal cells is thought to be limiting the extension of coleoptile segments. Since we are using longitudinally split segments with an intact cuticle, the diffusion of protons from the apoplast into the medium is limited to the cut surface of the section. It is quite possible that the pH of the epidermal cell walls of the intact segments used for growth may be lower than the medium because diffusional exchange is hindered by the cuticle. Acidification of the medium depends on the gradient of protons at the cut surface of the segment which in turn depends not only on the amount of protons secreted by the epidermal cells, but also on the amounts reentering other cells of the tissue in cotransport processes and associated with anionic groups within the apoplast. Longer presoak periods, perhaps by providing sufficient time for cations to diffuse in from the medium, might reduce either the retention of protons by the cell walls or their uptake by other cells and thereby increase the acidification of the medium. To summarize, we infer that for segments presoaked for only 1.5 h, the onset of hyperpolarization may be a better indication of when acidification of epidermal cell walls begins than is a drop in the pH of the bathing medium. In the case of segments presoaked for 1.5 h, confirmation of this inference by measurements of the apoplastic pH with a pH microelectrode would rescue the acid growth hypothesis.

With 1.5 h pretreatment, the latencies for both hyperpolarization and elongation are about 9 min. After 4.5 h, however, the hyperpolarization begins after 7 min, significantly before IAA-induced elongation, which is delayed to 13 min (Table I). This increase in latency for IAA-induced growth during long pretreatments is consistent with a decreased capacity for cell wall loosening (7).

IAA-Induced Proton Secretion Develops Gradually. Following long presoaks, the timing of the auxin-induced proton secretion (Fig. 3, *insert*) coincided with the hyperpolarization (Fig. 2A) providing further evidence of the electrogenic nature of the auxin-induced secretion of protons. The sigmoidal increase in proton secretion in response to auxin occurring over 15 to 20

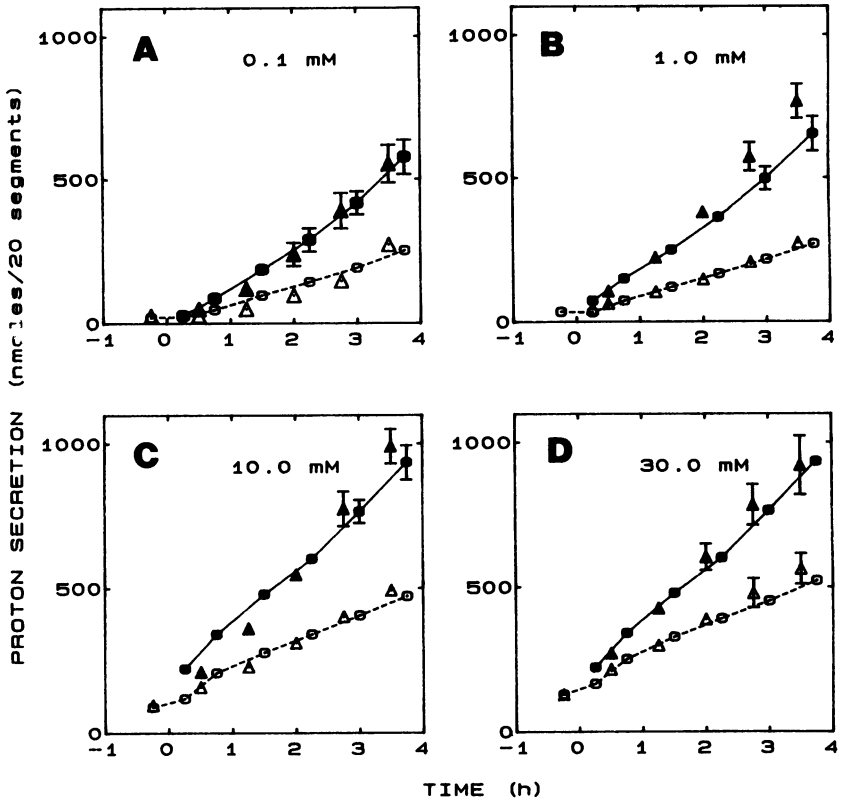

FIG. 6. Long-term kinetics of proton secretion, titrated with NaOH, by oat coleoptiles incubated in different concentrations of KCl (○, ●) or K_2SO_4 (△, ▲). Experimental conditions as in Figure 1B. IAA (10 μM) was added (time 0) 3 h after cutting. *Dashed lines* show the basal level of proton secretion in the absence of IAA (*open symbols*), continuous lines after addition of 10 μM IAA (*closed symbols*).

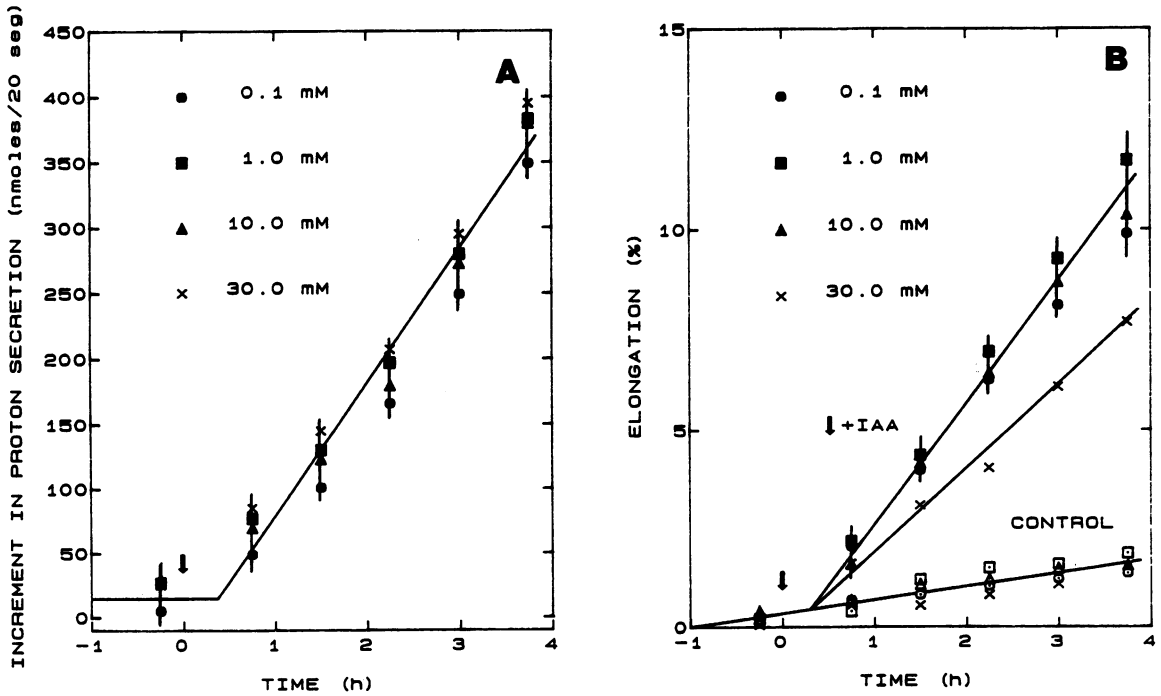

FIG. 7. A, The auxin-induced proton secretion in different $[KCl]_o$. The proton secretion of control tissues was subtracted from that of the auxin-treated segments in Figure 6, A to D to obtain the auxin-induced proton secretion. *Vertical bars* indicate the magnitude of the SE. The slopes ± SE are 95.0 ± 13.2, 88.6 ± 8.2, 87.0 ± 10.1, 91.8 ± 7.1 nmol h^{-1} 20 $segments^{-1}$, respectively, for 0.1, 1.0, 10.0, and 30 mM KCl. B, The growth of control (*open symbols*) and IAA-treated segments (*closed symbols*) in different $[KCl]_o$. Elongation expressed as a percentage of the length at time −1 h (2 h after cutting), when segments were transferred to the various $[KCl]_o$. SE are given by the *vertical bars*.

Table II. *Mean Latent Periods for IAA-Induced Responses as Function of [KCl]$_o$*

Latencies for the specific electrical response are the times when the membrane begins to repolarize in IAA. See Figure 5A. Latencies for proton secretion and growth were determined from data such as in Figures 6 and 7 from the intersection of linear regressions on data from control and IAA-treated tissues. Segments were presoaked 3 h in BSM. Values are means ± SE. Number of experiments in parentheses.

[KCl]$_o$	Lag Times before Start of		
	Hyperpolarization	Proton secretion	Elongation
mM		*min*	
0.1	8.3 ± 1.0 (6)	10.2 ± 0.6 (9)	10.4 ± 0.6 (5)
1.0	8.0 ± 1.2 (3)	10.0 ± 0.4 (5)	10.0 ± 0.6 (3)
10.0	9.0 ± 1.5 (3)	11.4 ± 0.9 (5)	10.6 ± 1.2 (3)
30.0	Not detectable	10.4 ± 1.1 (4)	13.6 ± 1.5 (3)

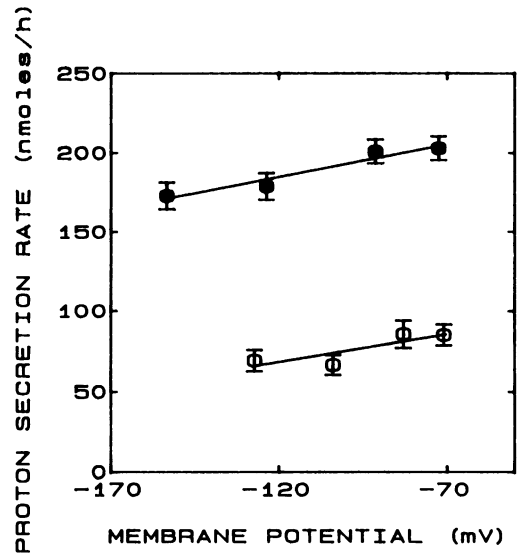

FIG. 8. The rate of proton secretion is nearly independent of the membrane potential. The net rate of proton secretion by control and IAA-treated segments was estimated by the slopes of the curves of Figure 6, A to D. The membrane potentials for the corresponding [KCl]$_o$ are taken from Figure 4. The dependence of the H^+-secretion rate on membrane voltage, estimated from the slopes, is 0.35 ± 0.15 (nmol h^{-1} mV^{-1}) for the controls and 0.41 ± 0.24 for the IAA-treated cells.

min could result from (a) increase in the supply of metabolically derived protons, (b) activation of preexisting ATPases, or (c) increase in the synthesis and incorporation of new transport sites into the membrane.

Initial Transient Depolarization Is Not an Auxin Response. The first phase of the electrical response to IAA, during which the cells depolarize by +8.5 ± 1.6 mV, lasts about 7 min (Table II, Fig. 2A). As has been reported (1), this depolarization is not specific to IAA and can be attributed to an increase in the passive permeability of the membrane to Cl^- relative to K^+. FC may be more effective than IAA in hyperpolarizing the membrane because it stimulates the pump without affecting the passive permeability of the membrane.

Effects of Elevated [K^+]$_o$. Since, at the steady state, there is no net current through the plasma membrane, the outward current generated by the pump must be balanced by an equal inward current. As the pump is electrogenic, this return current must be carried by passive fluxes of ions. The contribution of protons to this return current is likely to be less than that of K^+ or Cl^-, because the proton concentration is usually two or more orders of magnitude lower than the concentration of these other ions. The reason the IAA-induced hyperpolarization is less in high [K^+]$_o$ is probably because increased passive shunting in higher [K^+]$_o$ decreases the effectiveness of the electrogenic pump in hyperpolarizing the membrane. The observation that neither IAA-induced proton secretion nor growth is affected by changes in [K^+]$_o$ is consistent with this interpretation.

In higher [KCl]$_o$ (10 or 30 mM), the rate of proton secretion in the absence of IAA was similar to that caused by IAA at lower salt concentration (Fig. 6); however, increased salt alone did not stimulate growth as much as IAA did (Fig. 7B). Similarly, in pea stem segments, Ca^{2+} and K^+ stimulated more proton secretion but less growth than did IAA (27). These results indicate that wall acidification, although necessary, may not always be a sufficient condition for growth.

Uptake of Cl^- is usually against its electrochemical gradient and is thought to involve a co-transport with protons (25). Since the proton secretion at different [K^+]$_o$ was independent of whether the accompanying anion was Cl^- or SO_4^{2-} (Fig. 6, A to D), reentry of protons with chloride does not appear to be a significant factor in these experiments. According to Coccuci *et al.* (10), the anion also does not influence the extent of the depolarization obtained with a 10-fold increase in [K^+]$_o$.

Absence of an Effect of Membrane Voltage on Proton Secretion and Growth. Both the basal and auxin-induced proton secretion appear relatively insensitive to change in the operating voltage (Fig. 8). Although in this experiment, the voltage span was limited to that obtained by varying [K^+]$_o$, this is the range of membrane potentials that a non-green plant cell, such as these oat parenchyma cells, would normally experience. The results suggest that proton ATPases in the plasma membrane are delivering a near saturating current that increases only 10 to 20% as the membrane depolarizes from −160 to −70 mV, but that auxin increases this maximum current by more than 200%.

Similarly, neither the timing nor the rate of growth are affected when the cells are depolarized by elevated external potassium. Contrary to the suggestion of Brummer and Parish (3), this indicates that a highly negative membrane potential is not necessary for auxin-induced growth.

Is K^+ Required by the Proton Pump? The basal rates of proton secretion in the absence of added auxin were increased by raising [K^+]$_o$ (*dashed lines*, Fig. 6, A to D). This has also been observed by others (18, 27) and could be attributed to a H^+-K^+ ATPase; however, the existence of such a chemical coupling between H^+-efflux and K^+-influx by an ATPase has never been convincingly demonstrated for plant cells (16, 23). A plausible alternative explanation would be that passive return of protons is decreased when cells are depolarized by increased [K^+]$_o$. Several authors have suggested that a H^+-K^+ ATPase might be responsible for the stimulation of K^+ uptake by FC (9, 18, 21). In the case of IAA, however, not only are the kinetics of H^+-secretion and K^+-uptake quite different (8), but also auxin-induced proton secretion is independent of [KCl]$_o$ (Figs. 6, 7A, and 8). In the present experiments, the independence of auxin-induced proton secretion of [K^+]$_o$ between 0.1 and 30 mM indicates that such a H^+-K^+ ATPase would have to be saturated at [K^+]$_o$ below 0.1 mM. This seems unlikely since auxin-induced proton secretion is most electrogenic at low [K^+]$_o$. The observation that the auxin-induced proton secretion does not increase when the cells are depolarized by increasing [K^+]$_o$ is consistent with shunting of an electrogenic H^+-ATPase through passive ion channels rather than a H^+-K^+ATPase.

Relations to Auxin-Induced mRNAs. Another early response to auxin is the appearance of new mRNAs within about 10 min (28). In the present experiments, the increase in proton secretion and the accompanying hyperpolarization of the membrane have a latency as short as 7 min. Thus, this is one of the earliest known responses to auxin, and its S-shaped kinetics differ from those of

transcription of mRNAs in other tissues (28). The timing of auxin-induced mRNA synthesis in oat coleoptiles is yet unknown, as is whether any of the early messages are involved in the activation of proton secretion by auxin.

LITERATURE CITED

1. BATES GW, MHM GOLDSMITH 1983 Rapid response of the plasma-membrane potential in oat coleoptiles to auxin and other weak acids. Planta 159: 231–237
2. BATES GW, MHM GOLDSMITH, TH GOLDSMITH 1982 Separation of tonoplast and plasma membrane potential and resistance in cells of oat coleoptiles. J Membr Biol 66: 15–23
3. BRUMMER B, RW PARISH 1983 Mechanisms of auxin-induced plant cell elongation. FEBS Lett 161: 9–13
4. BRUMMER B, A BERTL, I POTRYKUS, H FELLE, RW PARISH 1985 Evidence that fusicoccin and indole-3-acetic acid induce cytosolic acidification of *Zea mays* cells. FEBS Lett 189: 109–114
5. CLELAND RE 1976 Fusicoccin-induced growth and hydrogen ion excretion of *Avena* coleoptiles: relation to auxin responses. Planta 128: 201–296
6. CLELAND RE 1976 Kinetics of hormone-induced H^+ excretion. Plant Physiol 58: 210–213
7. CLELAND RE 1983 The capacity for auxin-induced wall loosening as a factor in the control of *Avena* coleoptile cell elongation. J Exp Bot 34: 676–680
8. CLELAND RE, T LOMAX 1977 Hormonal control of H^+-excretion from oat cells. *In* E Marrè, O Ciferri, eds, Regulation of Cell Membrane Activities in Plants. Elsevier, Amsterdam, pp 161–171
9. CLELAND RE, HBA PRINS, JR HARPER, N HIGINBOTHAM 1977 Rapid hormone-induced hyperpolarization of the oat coleoptile transmembrane potential. Plant Physiol 59: 395–397
10. COCCUCI M, E MARRÈ, A BALLARIN-DENTI, A SCACCHI 1976 Characteristics of fusicoccin-induced changes of transmembrane potential and ion uptake in maize root segments. Plant Sci Lett 6: 143–156
11. GOLDSMITH MHM, HR FERNANDEZ, TH GOLDSMITH 1972 Electrical properties of parenchymal cell membranes in the oat coleoptile. Planta 102: 302–323
12. HAGER A, H MENZEL, A KRAUSS 1971 Versuche und Hypothese zur Primarwirkung des Auxins beim Streckungswachstum. Planta 100: 47–75
13. JACOBS M, PM RAY 1976 Rapid auxin-induced decrease in free space pH and its relationship to auxin-induced growth in maize and pea. Plant Physiol 58: 203–209
14. KUTSCHERA H, R BERGFELD, P SCHOPFER 1987 Cooperation of epidermis and inner tissues in auxin-mediated growth of maize coleoptiles. Planta 170: 168–180
15. KUTSCHERA U, P SCHOPFER 1985 Evidence against the acid-growth theory of auxin action. Planta 163: 483–493
16. LEONARD R 1982 The plasma membrane ATPase of plant cells: cation or proton pump? *In* A Martonosi, ed, Membranes and Transport, Vol 2. Plenum Press, New York, pp 633–637
17. MARRÈ E, P LADO, F RASI-CALDOGNO, R COLOMBO 1973 Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation. I. Effects of fusicoccin, natural and synthetic auxins and mannitol. Plant Sci Lett 1: 179–184
18. MARRÈ E, P LADO, F RASI-CALDOGNO, R COLOMBO, MI DE MICHELIS 1974 Evidence for the coupling of proton extrusion to K^+ uptake in pea internode segments treated with fusicoccin or auxin. Plant Sci Lett 3: 365–379
19. MARRÈ MT, G ROMANI, E MARRÈ 1986 Transmembrane hyperpolarization and increase of K^+ uptake in maize roots treated with permeant weak acids. Plant Cell Environ 6: 617–623
20. NELLES A 1977 Short-term effects of plant hormones on membrane potential and membrane permeability of dwarf maize coleoptile cells (*Zea mays* L. d1) in comparison with growth responses. Planta 137: 293–298
21. NELLES A 1978 Evidence for a fusicoccin-stimulated potassium ion pump in cell membranes of dwarf maize coleoptiles. Plant Sci Lett 12: 349–354
22. RAYLE DL, RE CLELAND 1977 Control of plant cell enlargement by hydrogen ions. Curr Top Dev Biol 11: 187–214
23. RODRIGUEZ-NAVARRO A, MR BLATT, CL SLAYMAN 1986 A potassium-proton symport in *Neurospora crassa*. J Gen Physiol 87: 649–674
24. RUBINSTEIN B, RE CLELAND 1981 Responses of *Avena* coleoptiles to suboptimal fusicoccin: kinetics and comparisons with indoleacetic acid. Plant Physiol 68: 543–547
25. SANDERS D 1984 Gradient-coupled chloride transport in plant cells. *In* GA Gerencser, ed, Chloride Transport Coupling in Cells and Epithelia. Elsevier/North-Holland, Amsterdam, pp 63–120
26. TAIZ L 1984 Plant cell expansion: regulation of cell wall mechanical properties. Annu Rev Plant Physiol 35: 585–657
27. TERRY M, RL JONES 1981 Effect of salt on auxin-induced acidification and growth by pea internode sections. Plant Physiol 68: 59–64
28. THEOLOGIS A 1986 Rapid gene regulation by auxin. Annu Rev Plant Physiol 37: 407–438
29. VESPER MJ 1985 Use of a pH-response curve for growth to predict apparent wall pH in elongating segments of maize coleoptiles and sunflower hypocotyls. Planta 166: 96–104
30. VESPER MJ, ML EVANS 1978 Time-dependent changes in the auxin sensitivity of coleoptile segments. Plant Physiol 61: 204–208