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Effect of Salt on Auxin-Induced Acidification and Growth by Pea Internode Sections[1]

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ABSTRACT

The capacity of excised internode sections of pea to grow and secrete protons in response to indoleacetic acid (IAA) and Ca^{2+} and K^+ treatments was examined. By incubating unpeeled and unabraded sections in rapidly flowing solutions, it was shown that acidification of the external medium in the presence or absence of IAA is dependent on the presence of Ca^{2+} and K^+. Similar results were obtained when unpeeled and unabraded sections were incubated in dishes with shaking. When peeled or abraded sections were incubated with shaking in IAA, H^+ release was also dependent on the presence of Ca^{2+} and K^+. The release of H^+ from sections incubated in Ca^{2+} and K^+ is not caused by displacement of H^+ from binding sites in the cell wall. Rather, the release of protons from sections is temperature dependent, and it is concluded that this is a metabolically linked process. Although Ca^{2+} and K^+ are essential for the release of H^+ from isolated stem sections of peas, these cations do not influence elongation. Despite the large increase in proton release induced by Ca^{2+} and K^+ either in the presence or absence of auxin, growth in the presence of these ions was never greater than it was in their absence. Furthermore, cations do not affect the neutral sugar or uronic acid composition of the solution which can be centrifuged from isolated sections. As is the case for growth, an increase in the neutral sugar and uronide composition of the cell wall solution is dependent only on IAA. It is concluded that IAA-induced growth of pea stem sections is independent of the secretion of protons.

The acid growth hypothesis first proposed by Hager *et al.* (10) states that auxin-induced growth is mediated *via* proton secretion from hormone-treated cells. The evidence cited in support of this hypothesis includes the observations that low pH stimulates extension growth in isolated plant parts (2, 21, 22) and that, under the appropriate conditions, auxin-treated tissues release H^+ (3, 10, 20, 27). Further credence has been attached to the acid growth hypothesis by the demonstration that H^+, like auxin (16), promotes the release of xyloglucan from pea stem sections (14).

The acid growth hypothesis has not received universal acceptance as a description of the mechanism underlying auxin-induced growth. By contrast with the sustained response of coleoptile and stem segments to auxin, the response of similar tissue segments to low pH is transient (29, 30). Proponents have argued that such criticisms do not invalidate the acid growth hypothesis (6). Rather, they argue that indirect evidence also implicates a protein, whose turnover is rapid, in the maintenance of auxin-induced growth (4, 20). In the presence of H^+ alone, this protein becomes limiting, while, in the presence of auxin, its level is maintained.

A more forceful argument against the acid growth hypothesis comes from investigators who have been unable to correlate H^+ secretion with auxin-induced growth (13, 15, 28). Several of these reports show that extension growth is auxin-dependent while proton release requires the addition of both auxin and cations (5, 13). Kholdebarin and Oertli (15) showed that there was no correlation between auxin-induced elongation of barley coleoptiles and pH; rather, auxin-enhanced elongation was correlated with an increase in medium pH. Cohen and Nadler (5) also found that the presence of salts of Ca^{2+} was necessary for H^+ release from auxin-treated *Avena* coleoptile segments. Even supporters of the acid growth hypothesis point out that, for acidification to occur in response to IAA treatment, sections must be either preincubated or incubated in the appropriate salt solution (4, 17). From what is known about H^+ extrusion from plant tissues, it is axiomatic that, if proton extrusion is not coupled with the release of an anion, there must be counter transport of a cation (11, 12).

In this paper, we report experiments designed to resolve the role of salts in the processes of auxin-induced elongation and medium acidification by internode sections of peas.

MATERIALS AND METHODS

Seeds of *Pisum sativum* L., c.v. Alaska, were grown in vermiculite for 7 days in the dark at 24 to 26 C. All further manipulations were carried out under fluorescent room light. Except for peeled sections, all sections were excised 1 cm below the apical hook of the third internode and incubated either in a rapid flow device or on a shaker. For the rapid flow method, 1.2-cm sections were cut using a harvesting rack designed for rapid cutting. Approximately 100 sections were packed into each of eight syringe barrels (26). It took 1 h to cut and pack 800 sections. The basal 1 to 3 mm of the sections were placed in distilled H_2O for an additional 30 min. A pump system (26) was used to circulate 175 ml of glass-distilled H_2O over four barrels of sections for 30 min at 180 ml/min·barrel. The water was then replaced with an equal volume of the appropriate incubation medium, which was circulated at the same rate. All solutions were vigorously bubbled with filtered air during the washing and incubation periods.

Excised sections (1 cm) were also incubated in 5-cm plastic Petri dishes in 6 ml of solution on a rotary shaker at 120 rpm. These sections were rinsed for 30 min in rapidly flowing H_2O in the apparatus described above prior to incubation in Petri dishes.

Peeled sections were prepared from 2-cm-long sections excised immediately below the apical hook by removing the epidermis with fine forceps. The 2-cm, peeled sections were incubated in H_2O for 45 to 75 min before excising a basal 1-cm portion. These peeled, 1-cm sections were rinsed for 30 min in rapidly flowing H_2O prior to incubation in Petri dishes on the shaker.

Abraded sections (1 cm) were excised after intact stems were

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abraded by gently pulling the subapical region of the stem between thumb and forefinger previously wetted with silicon powder grit (Buehler Ltd., Evanston, IL). Abraded sections were rinsed for 30 min in rapidly flowing water and incubated on the shaker.

Unless otherwise stated, all sections were incubated in H_2O with the addition, where appropriate, of 0.5 mM $CaCl_2$, 2.0 mM KCl, and 17 μM IAA.

Growth of sections was measured by monitoring fresh weight or length changes. For sections incubated in rapidly flowing medium, fresh weight changes were determined by weighing separately each barrel of 100 sections after centrifugation at 1,000*g* for 5 min and comparing this weight to an initial fresh weight of 3 g per 100 sections (26). Changes in section length were monitored by measuring the shadow images of sections projected by a 14× lens system (24).

Medium pH was measured using a glass combination electrode (No. 39507; Beckman Instruments, Irvine, CA) and a Beckman digital pH meter on a sample of the medium which had been vigorously bubbled with N_2 for 3 min. Except in those experiments where the medium was buffered, the pH of the initial media after bubbling with N_2 was between 6.8 and 7.3.

Cell wall solution was removed from excised internode sections using the procedures described in detail by Terry and Bonner (26). Sections were incubated as described for the rapid flow method, infiltrated with H_2O under vacuum, and centrifuged for 5 min at 1,000*g* (26). The neutral sugar composition of the water-soluble, alcohol-insoluble components of the cell wall solution was determined by GC using procedures described previously (26). Uronic acids were determined colorimetrically using the method of Blumenkrantz and Asboe-Hansen (1).

The experiments reported in this paper were repeated at least three times, and the data reported are the average of three replicate treatments.

RESULTS

Using the technique for rapidly flowing incubation medium past excised sections, acidification of the medium by unpeeled, unabraded sections can be detected only in the presence of 0.5 mM Ca^{2+} and 2.0 mM K^+ (Fig. 1). When Ca^{2+} and K^+ are present, proton release from sections can be detected in both the presence and the absence of 17 μM IAA. In the absence of these cations, no protons are released without IAA, and only small amounts are released in the presence of auxin (Fig. 1). When pH data are expressed as proton concentration, it can be seen that Ca^{2+} and K^+ stimulate a 30-fold increase in H^+ release after 3 h of incubation in IAA and a 10-fold increase in medium acidification in the absence of IAA (Fig. 1B). Similar results are obtained when sections are incubated in 6 ml medium in 5-cm Petri dishes on a rotary shaker (Fig. 2). The release of protons into the external medium is also dependent on the presence of Ca^{2+} and K^+, and, by comparison with the rapid flow method, more protons are released from sections which are not IAA-treated.

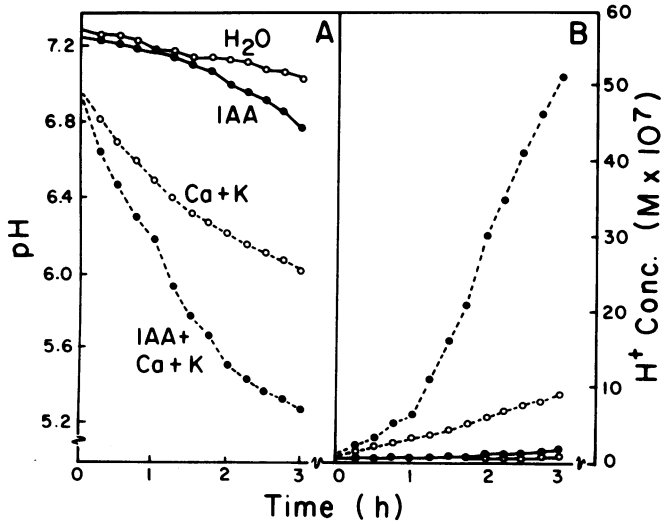

FIG. 1. Effect of 0.5 mM Ca^{2+} and 2 mM K^+ in the presence and absence of 17 μM IAA on H^+ release, expressed as pH (A) and H^+ concentration (B) of the medium from 400 pea internode sections incubated in 175 ml rapidly flowing medium at 25 C.

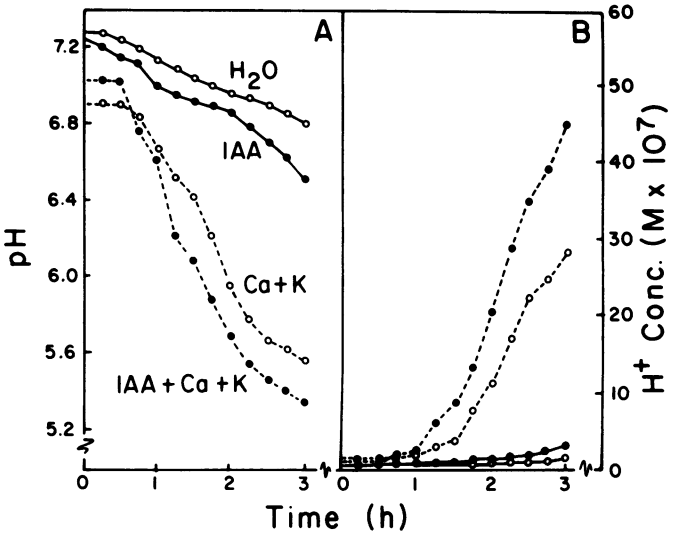

FIG. 2. Effect of 0.5 mM Ca^{2+} and 2 mM K^+ in the presence and absence of 17 μM IAA on H^+ release, expressed as pH (A) and H^+ concentration (B) of the medium, from 14 pea internode sections incubated in 6 ml medium with shaking at 25 C.

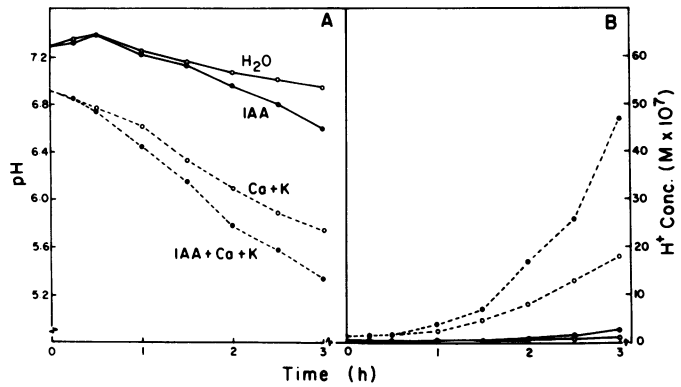

FIG. 3. Effect of 0.5 mM Ca^{2+} and 2 mM K^+ in the presence and absence of 17 μM IAA on H^+ release, expressed as pH (A) and H^+ concentration (B) of the medium, from 14 abraded pea internode sections incubated in 6 ml medium with shaking at 25 C.

Medium acidification by peeled and abraded sections is also dependent on the presence of Ca^{2+} and K^+. When abraded sections are incubated with shaking, proton release is observed only in the presence of Ca^{2+} and K^+ (Fig. 3). Proton release from abraded sections incubated in Ca^{2+} and K^+ without IAA is 7-fold greater than that from sections incubated with IAA alone and only 40% of that from sections treated with salts and IAA (Fig. 3). Similarly, when peeled sections are incubated with shaking, significant medium acidification occurs only in the presence of Ca^{2+} and K^+ (Table I). As is the case for abraded sections, peeled tissue incubated in Ca^{2+} and K^+ without IAA releases considerably more H^+ (almost 40-fold) than do sections incubated in IAA without Ca^{2+} and K^+ (Table I).

Table I. *Effect of IAA and Cations on Medium Acidification by Peeled Pea Internode Sections Incubated with Shaking*

Peeled sections (20) were incubated 3 h in 6 ml medium ± 17 μM IAA and ± 0.5 mM $CaCl_2$ ± 2 mM KCl.

Treatment	pH	$[H^+]$
		μM
H_2O	7.11	0.078
IAA	7.04	0.091
$Ca^{2+} + K^+$	5.44	3.6
IAA + Ca^{2+} + K^+	5.30	5.0

Table II. *Effect of IAA and Cations on the Fresh Weight of Pea Internode Sections Incubated in Rapidly Flowing Medium*

Acidification data are presented in Fig. 1.

Treatment[a]	ΔWt	ΔWt[b]
	g	%
H_2O	1.339	
IAA	2.548	90
$Ca^{2+} + K^+$	1.243	
IAA + Ca^{2+} + K^+	2.167	74

[a] Sections (400) incubated 3 h ± 17 μM IAA and ± 0.5 mM $CaCl_2$ and 2 mM KCl.

[b] %ΔWt = ([ΔWt IAA] − [ΔWt − IAA])/(ΔWt − IAA).

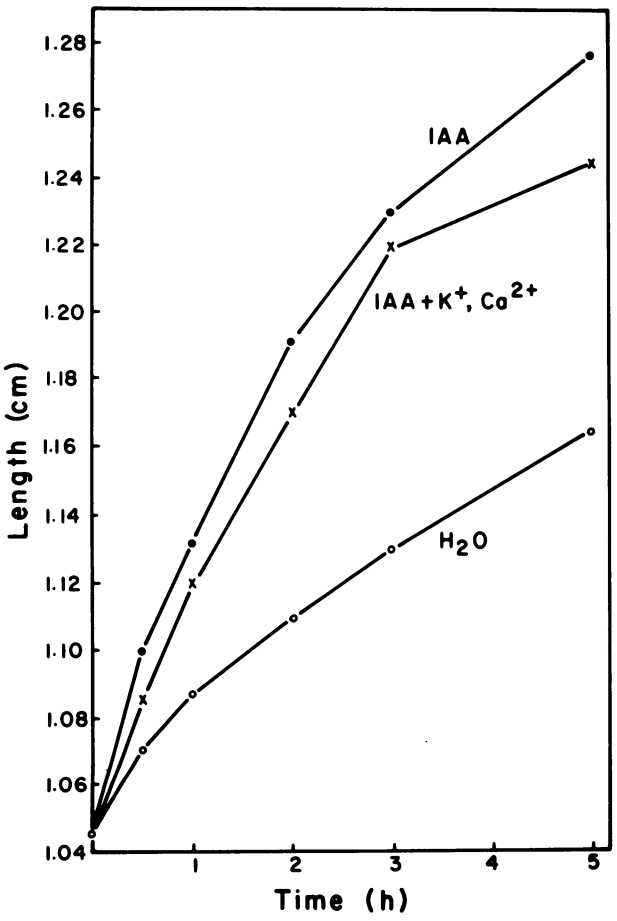

FIG. 4. Effect of IAA in the presence and absence of 0.5 mM Ca^{2+} and 2 mM K^+ on elongation of pea internode sections incubated by shaking in 6 ml medium at 25 C.

Table III. *Effect of an Acidic Medium on the Elongation of Pea Internode Sections Incubated by the Rapid-Flow and Shaker Methods*

Incubation[a]		ΔL	ΔL[b]
Method	Medium		
		mm	%
Rapid flow	H_2O (pH 6.2)	0.74	
	H_2O (pH 4.5)	1.42	92
Shaker[c]	H_2O (pH 6.2)	0.74	
	H_2O (pH 4.5)	1.11	50

[a] Sections cut at same time from same tray of seedlings and incubated for 1 h in a buffer consisting of 5 mM KH_2PO_4 and 5 mM CH_3COONa with 10 mM NaCl adjusted to either pH 4.5 or 6.2.

[b] %ΔL = ([ΔL pH 4.5] − [ΔL pH 6.2])/(ΔL pH 6.2).

[c] 14 sections per 6 ml medium.

Table IV. *Effect of IAA and Cations on Elongation and Medium Acidification by Pea Internode Sections Incubated with Shaking*

Treatment[a]	Elongation		Acidification	
	ΔL	ΔL[b]	pH	$[H^+]$
	mm	%		μM
H_2O	0.92		7.20	0.06
IAA	1.99	81	6.95	0.11
$Ca^{2+} + K^+$	1.08		6.40	0.40
IAA + Ca^2 + K^+	2.01	63	5.84	1.40

[a] Sections (14) incubated for 3 h in 6 ml medium ± 17 μM IAA and ± 0.1 mM $CaCl_2$ and 2 mM KCl.

[b] %ΔL = ([ΔL IAA] − [ΔL − IAA])/(ΔL − IAA).

Table V. *Effect of IAA and Cations on Elongation of Abraded Pea Internode Sections Incubated with Shaking*

pH data are presented in Fig. 3.

Treatment[a]	ΔL	ΔL[b]
	mm	%
H_2O	0.74	
IAA	1.65	124
$Ca^{2+} + K^+$	0.70	
IAA + Ca^{2+} + K^+	1.45	107

[a] Sections (14) treated 3 h in 6 ml medium ± 17 μM IAA and ± 0.50 mM $CaCl_2$ and 2 mM KCl.

[b] %ΔL = ([ΔL IAA] − [ΔL − IAA])/(ΔL − IAA).

Table VI. *Effect of IAA on Elongation of Peeled Pea Internode Sections Incubated with Shaking*

Treatment[a]	ΔL	ΔL[b]
	mm	%
H_2O	0.35	
IAA	0.85	143

[a] Peeled sections (20/6 ml) incubated for 3 h ± 17 μM IAA.

[b] %ΔL = ([ΔL IAA] − [ΔL H_2O])/(ΔL H_2O).

Although Ca^{2+} and K^+ cause a marked enhancement of proton release when included in the incubation medium of unpeeled, peeled, or abraded sections, the presence of H^+ secreted by this tissue into the incubation medium has no significant effect on the growth of these sections. When growth of unpeeled sections incubated in rapidly flowing solution is measured by determining fresh weight change, it is found that both the total amount of

Table VII. *Effect of IAA and Cations on Fresh Weight, Medium Acidification, and Composition of the Cell Wall Solution of Pea Internode Sections Incubated in Rapidly Flowing Medium*

Treatment[a]		Section		Medium pH	Sugars of Cell Wall Solutions[c]				
Cations	IAA	ΔWt	ΔWt[b]		ARA	XYL	GAL	GLC	UA
		g	%		*μg/100 sections*				
None	−IAA	1.04		7.20	24	40	56	91	14
	+IAA	2.32	123	6.90	28	58	65	115	51
	+IAA/−IAA				1.17	1.45	1.16	1.26	3.6
Ca^{2+} + K^+	−IAA	1.04		5.90	23	37	57	75	14
	+IAA	1.82	76	5.30	31	51	70	101	48
	−IAA/−IAA				1.34	1.38	1.23	1.35	3.4

[a] Sections (400) incubated for 3 h ± 17 μM IAA and ± 0.5 mM $CaCl_2$ and 2 mM KCl.
[b] % ΔWt = ([ΔWt IAA] − [ΔWt − IAA])/(ΔWt − IAA).
[c] ARA, Arabinose; GAL, galactose; GLC, glucose; UA, uronic acids; XYL, xylose.

growth and the auxin-induced increase in growth are greater in the absence of Ca^{2+} and K^+ (Table II). For unpeeled sections incubated in Petri dishes on the shaker, growth determined by monitoring changes in section length in the presence of IAA is not promoted by Ca^{2+} and K^+ (Fig. 4). It should be emphasized that the two techniques of section incubation (namely, shaking sections in a small volume of solution and rapidly flowing medium past stationary sections) result in comparable levels of medium acidification (Figs. 1–3) as well as in similar growth rates in response to IAA treatment (data not shown). Elongation is also elicited by incubation of sections at pH 4.5 in both rapid-flow and shaker procedures; however, the total amount of growth in response to acid is greater by the rapid-flow method (Table III).

Both acidification and section elongation proceed in the presence of lowered Ca^{2+} concentration (0.1 mM Ca^{2+}, 2 mM K^+; Table IV). Growth at 0.1 mM Ca^{2+} is not markedly different from growth at 0.5 mM Ca^{2+}, suggesting that Ca^{2+} is not inhibiting section elongation.

Auxin also induces elongation of both abraded and peeled sections incubated by shaking. For abraded sections, both net elongation and the stimulation of growth by IAA are greater in the absence of Ca^{2+} and K^+ than in their presence (Table V). Elongation of peeled sections is also stimulated by IAA in the absence of Ca^{2+} and K^+ (Table VI). Although IAA stimulates elongation of abraded and peeled sections, the net amount of elongation obtained from these sections is not as great as that obtained from sections which are neither peeled nor abraded (compare Table IV with Tables V and VI). Nonetheless, the percentage of increase in length with IAA over that of controls is greater in peeled and abraded sections (Tables V and VI) than in nonpeeled and unabraded sections (Table IV).

There is also no correlation between proton release from internode sections and the neutral sugar and uronide composition of the solution centrifuged from the cell wall free space (Table VII). Thus, in the absence of Ca^{2+} and K^+, IAA stimulates the release of alcohol-insoluble polymers rich in uronic acids as well as arabinose, xylose, galactose, and glucose. In particular, the presence of IAA causes a marked stimulation of uronic acid levels and, among the neutral sugars, a greater stimulation of xylose and glucose levels relative to arabinose and galactose (Table VII). In the presence of Ca^{2+} and K^+, a similar stimulation of uronic acid and neutral sugar levels in acid-insoluble components of the wall solution is observed. The level of arabinose and galactose is elevated in the presence of Ca^{2+} and K^+ relative to controls without salt. Growth, as measured by fresh weight of sections, correlates with IAA treatment of tissue, but proton secretion correlates with the presence of Ca^{2+} and K^+ (Table VII).

To test whether the role of Ca^{2+} and K^+ was to displace H^+ from proton binding sites in the cell wall, the following experiment was performed. Sections were incubated by the rapid-flow method for 3 h in IAA in the absence of Ca^{2+} and K^+ at 25 C. After a 3-h incubation, sections were transferred to medium containing IAA and 0.5 mM Ca^{2+} and 2 mM K^+ at either 26 or 4 C, and proton release was measured at 30-min intervals for a further 3.5 h (Fig. 5). Incubation in IAA alone does not cause significant proton release, confirming our previous observation; however, addition of Ca^{2+} and K^+ and incubation at 26 C promotes rapid acidification. Proton release has a lag time of less than 30 min and proceeds linearly with time for 3 h. Addition of Ca^{2+} and K^+ and incubation at 4 C does not enhance the low rate of H^+ efflux from sections (Fig. 5).

DISCUSSION

A review of the experimental conditions which investigators have used when studying proton release from excised tissue sections indicates that, in addition to auxin or FC[4] treatment, certain conditions must be satisfied in order for H^+ release to be detected. Among the conditions of incubation described are the inclusion of salts of Ca^{2+}, Mg^{2+}, K^+, Na^+ or mixtures of these ions in the incubation medium (3, 4, 8, 14, 18, 20, 23). Although the addition of salts of Ca^{2+}, Mg^{2+}, K^+, or Na^+ influences medium acidification by auxin-treated tissues, there are marked differences in the effectiveness of these ions. In suspension-cultured sycamore cells, medium acidification is enhanced by Mg^{2+} and Na^+ but not by Ca^{2+} or K^+ (8). In *Avena* coleoptile sections, on the other hand, Cohen and Nadler (5) found that auxin-enhanced medium acidification was dependent on the presence of Ca^{2+} alone. In contrast to most reports which emphasize increased medium acidification with auxin and salts, Kholdebarin and Oertli (15) have shown that, with coleoptiles of *Hordeum*, inclusion of K_2SO_4 or KCl at 10 mM reduces acidification relative to controls without salts. These differences between species may reflect their tolerance for an ion, *e.g.* Na^+, or could reflect competition between ions or the nutritional status of the tissue. It is clear that a generalization cannot be made concerning the relationship between medium acidification by auxin-treated tissue and the requirement for simple salts.

The degree of medium aeration or shaking also influences the extent of auxin-induced acidification. We have examined H^+ secretion from internode sections of dark-grown peas using different incubation and tissue preparation methods. When the incu-

[4] Abbreviation: FC, fusicoccin.

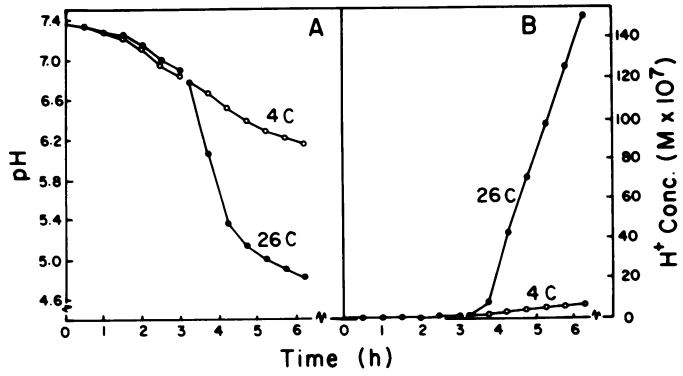

FIG. 5. Effect of Ca^{2+} and K^+ on IAA-induced H^+ secretion, expressed as pH (A) and H^+ concentration (B) of the medium, from pea internode sections at 4 and 26 C. Sections (400) incubated in 180 ml rapidly flowing 17 μM IAA for 3 h at 25 C then transferred to rapidly flowing 17 μM IAA plus 0.5 mM Ca^{2+} and 2 mM K^+ at 4 and 26 C.

bation medium is pumped rapidly past excised unpeeled and unabraded sections, IAA-induced H^+ secretion is optimized (Fig. 1). Under these conditions, IAA in the presence of Ca^{2+} and K^+ causes a 5-fold increase in medium acidification, whereas incubation in Petri dishes results in only a 64% increase in proton release (Fig. 2). When peeled or abraded sections are incubated in Petri dishes on a shaker, IAA enhances acidification by only 40% (Table I and Fig. 3). Acidification of the incubation medium is observed only in the presence of Ca^{2+} and K^+ (Fig. 1). When these ions are omitted from the incubation medium, there is negligible proton release from excised sections (Figs. 1, 2, and 3; Table I).

It could be argued that Ca^{2+} and K^+ facilitate the release of H^+ from the cell wall and that they do not play a direct role in H^+ secretion from the protoplast. It would follow from this argument that H^+ release does not occur in the presence of IAA alone because protons interact with the cell wall. The role of Ca^{2+} and K^+ in stimulating medium acidification would therefore be to displace protons from binding sites in the cell wall. For example, Rubinstein *et al.* (23) have shown that Ca^{2+} will stimulate proton release from frozen-thawed sections of oat coleoptiles, and they argued that Ca^{2+} caused acidification of the incubation medium by exchanging protons from sites in the cell wall.

Our experiments do not support the hypothesis that Ca^{2+} and K^+ serve to interact in an ion exchange capacity with the cell wall. Our evidence points to an interaction between salts and tissue which is temperature sensitive. Thus, medium acidification is induced by addition of Ca^{2+} and K^+ and incubation at 26 C but not by addition of salts and incubation at 4 C. If salts served merely to exchange protons from binding sites in the cell wall, then such exchange would be expected to occur equally at 26 or 4 C. Since this is not the case, it must be assumed that a physical interaction between salts and cell wall does not influence proton release. The dependence of H^+ release on temperature points to a metabolic basis for this phenomenon, and we propose that Ca^{2+} and K^+ play a role in a metabolically linked electrogenic proton pump.

There are numerous reports which implicate K^+ in an antiport model for H^+ transport from excised root (19) and coleoptile (11, 12) segments. Haschke and Lüttge (11) have shown that there is stoichiometry between malate synthesis, K^+ uptake, and proton release in auxin-treated oat coleoptile segments. These data clearly implicate K^+ in proton secretion, and, if the proposed antiport model is correct, K^+ or a similar cation would be essential for H^+ efflux from plant tissues.

Calcium has also been implicated in the process of proton release from auxin-treated coleoptile sections (5), and Epstein (7) believes that Ca^{2+} at 0.1 to 1.0 mM is essential for normal transport and retention of ions. It is significant that most investigators of the process of hormone-induced H^+ release have included salts of either Ca^{2+} or K^+ or both in their incubation media. In many cases, it has been reported that addition of these ions resulted in an enhancement of acidification. Cleland (4) has shown that, for optimal proton release from coleoptile segments in the presence of IAA, 1 mM $CaSO_4$ and 1 mM K_2SO_4 are required. Is H^+ release from auxin-treated tissue causally related to growth or can the processes of H^+ release and growth be separated? Our results clearly indicate that there is no causal relationship between medium acidification and auxin-induced growth.

It could also be argued that our inability to measure H^+ accumulation in the incubation medium of sections incubated in IAA alone is a result of proton uptake. Thus, in the absence of cations, H^+ produced by protoplasts would be reabsorbed, and a recycling of H^+ would prevent medium acidification. In the presence of cations, a H^+/cation antiport would be established, resulting in the accumulation of H^+ in the incubation medium. It is clear that, if this mechanism explains why H^+ release does not occur with auxin alone, it still holds that proton release cannot be causally related to auxin-induced growth.

Although the ion content of the incubation medium influences acid release from excised unpeeled and unabraded, peeled, and abraded sections, the concentration of H^+ in the incubation medium has little effect on growth. Thus, in the presence of IAA and Ca^{2+} and K^+, the pH of the incubation medium falls to pH 5.2 by 3 h of incubation, but the growth of sections is somewhat less than those incubated in IAA alone, whose medium pH is about 7.0. We observed similar correlations between medium composition and pH in all experiments; growth occurs only when IAA is present, and the amount of growth is not influenced by either low pH or the presence of Ca^{2+} and K^+. Furthermore, we have demonstrated that removal of the cuticle or epidermis of pea stem sections is not essential for H^+ release. Under all conditions of tissue preparation, H^+ release from auxin-treated sections was influenced only by the presence of Ca^{2+} and K^+. In peeled and abraded sections, Ca^{2+} and K^+ stimulated significant proton release in the absence of auxin (Table I and Fig. 3); however, the basis for this response is not known.

Other workers have also been unable to demonstrate a correlation between auxin treatment and acidification. Cohen and Nadler (5) have shown that, in excised coleoptile sections, Ca^{2+} is essential for auxin-induced acidification. A similar lack of correlation between medium acidification, elongation, and FC treatment has been demonstrated in excised hypocotyls of lettuce (25) and in coleoptiles of corn (9). In the case of lettuce hypocotyl elongation, KCl was found to be required for H^+ release but not for elongation of FC-treated sections. Although these data appear at variance with those experiments demonstrating a correlation between acidification of the incubation medium or tissue free space and auxin-induced growth, the differences may be explained by arguing that auxin or FC can stimulate both growth and acidification. When isolated segments of tissue are incubated with an auxin or FC alone, growth occurs; however, if cations are added to the incubation medium, both growth and medium acidification occur.

We have also been unable to demonstrate a correlation between proton release from stem segments and auxin-induced changes in the neutral sugar and uronic acid composition of the cell wall solution. Terry and Bonner (26) have shown that there is a correlation between auxin-induced growth and the neutral sugars which are found in the cell wall solution by centrifugation. Our data supports their findings and also demonstrates that changes in xylose, glucose and uronic acid correlate only with IAA treatment and are not influenced by the presence or absence of salts.

Although auxin alone does not allow for appreciable H^+ release from pea internode sections, it is clear that auxin facilitates H^+

secretion. Auxin-treated sections secrete protons rapidly when provided with salts, whereas sections not treated with salts secrete less H^+. It is possible, therefore, that one of the effects of auxin is to stimulate the synthesis of organic acids which exchange their H^+ for cations in an antiport fashion. The data of Haschke and Lüttge (11) with oat coleoptile sections support this suggestion, since IAA alone causes a 2-fold increase in malic acid synthesis over sections incubated in 1 mM KCl. Sections incubated in IAA and KCl produce twice as much malic acid as those sections incubated in IAA alone (11).

We conclude that, in pea stem sections, auxin-induced growth can occur in the absence of measurable acidification of the incubation medium. Our conclusion is based on experiments which demonstrate that H^+ release and growth can be experimentally separated. Thus, it is clear that, for H^+ release to occur, cations must be present in the external medium, and we propose that these cations serve to exchange for H^+ from the cytoplasm by a mechanism analogous to the metabolically linked proton pumps previously described for other H^+ transporting systems. Growth, on the other hand, is dependent on the presence of auxin.

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