Plant Physiol. (1974) 54, 835-839

Effect of pH and Auxin on Chloride Uptake into *Avena* Coleoptile Cells[1]

Received for publication April 29, 1974 and in revised form July 12, 1974

BERNARD RUBINSTEIN
Department of Botany, University of Massachusetts, Amherst, Massachusetts 01002

ABSTRACT

The effect of pH on $^{36}Cl^-$ movement into coleoptile cells (*Avena sativa* L. cv. Garry) was investigated and compared with effects of indoleacetic acid. $^{36}Cl^-$ uptake, but not efflux, is stimulated when coleoptile sections are placed in media adjusted to pH levels from 5 to 3 after a preincubation period at pH 6.5. The enhancement is seen within 2 minutes, is not correlated with growth, and is completely erased by respiratory inhibitors. In comparison to the acid-induced stimulation, the stimulatory effect of indoleacetic acid on $^{36}Cl^-$ uptake is also not accompanied by accelerated efflux, and indoleacetic acid does not further stimulate $^{36}Cl^-$ uptake into 1-millimeter sections beyond that seen at pH 3.5 without auxin.

One of the experimental findings which suggests an interaction between a hormone and the plasma membrane is the auxin-stimulated uptake of $^{36}Cl^-$ into *Avena* coleoptile cells (22). This effect occurs 15 to 30 min after IAA addition and seems to be energy-dependent. In order to investigate this interaction further, the effects of pH must be examined.

H^+ concentration has long been recognized as an important factor during anion uptake by higher plants (7, 8, 15, 25). Uptake of Cl^- was shown to be inhibited by pH levels below 5 in carrot (15) and rye roots (14), and Br^- uptake decreased as pH was decreased in barley roots (7) and potato discs (25). These studies are difficult to interpret, however, because of the long uptake periods (hours to days) during which injury might have occurred. A 10- to 60-min uptake period using maize roots resulted in a steady increase in NO_3^- uptake as the pH was changed from 8 to 5 (26). An optimum of pH 5 has been found for Br^- and an optimum of about pH 4 was found for Cl^- movement into barley roots (4, 9).

Another link between H^+ concentration and anion uptake is the pH decrease occurring in the bathing medium when barley roots are actively taking up salts (8, 18). Related to this phenomenon, perhaps, is the interesting finding in *Nitella* that Cl^- uptake occurs only at nonencrusted regions of the cell; such smooth areas are sites of acid extrusion (24).

Since H^+ levels may be an important regulator of ion fluxes, it is of interest to compare the effect of auxin on Cl^- uptake with that of low pH. This is because of recent evidence suggesting that auxin stimulates H^+ secretion (1, 6, 20) and that the pH decrease outside the cell may be as much as 1.3 units after 150 min (1).

I will attempt to show that Cl^- uptake into coleoptile sections is increased as external pH is lowered to 5 and below, and that an energy source is required for the stimulation. The relationship between this finding and an auxin effect on H^+ secretion is also considered.

MATERIALS AND METHODS

Oat seedlings (*Avena sativa* L. cv. Garry) were grown on vermiculite in covered plastic boxes in complete darkness at 23 C. The seeds were watered with tap water at time of planting. After 84 to 90 hr, plants with coleoptiles between 1 and 2 cm were selected, the coleoptiles were isolated, and 1- to 5-mm sections were cut 3 mm from the tip of each coleoptile. The sections were floated on 10 mM Na-K phosphate buffer (pH 6.5) on a reciprocal shaker for 2 to 4 hr before use. Additions of substances before the ion uptake period were made by aspiration and readdition of the new solution.

For measurement of Cl^- uptake, the coleoptile sections (usually 15 per treatment in 25-ml flasks) were floated on 2.5 ml of a desired solution containing $^{36}Cl^-$ (ICN Corp., 7 mCi/g Cl; final concentration approximately 0.5 mM). The incubation period was usually 15 min on the shaker, after which the radioactive solution was aspirated and replaced with ice cold 0.2 M KCl for 10 min. The sections were then placed directly into scintillation vials (1 dram vials, Opticlear glass, Rochester Scientific Co.) containing 4 ml of Multisol (Interex Corp.) for extraction and counting. Replicates rarely varied by more than 10% from their mean. All manipulations were performed under a dim green safelight except for the $^{36}Cl^-$ uptake period which was carried out under incandescent lights in the laboratory.

Efflux was determined by the method of Pitman (17). Experiments were conducted in the dark or green safelight and on the shaker. Forty-five sections were floated on 2 mM $^{36}Cl^-$ in 10 mM phosphate buffer (pH 6.5) for 3 hr. The sections were then washed for 1 min with the phosphate buffer at pH 6.5 and bathed in 3 ml of buffer containing 2 mM unlabeled KCl. At various time intervals, the buffer was removed and fresh buffer was added. The $^{36}Cl^-$ released to the medium was determined on 2-ml aliquots by first acidifying with HNO_3 (final concentration 0.4 N) and then precipitating with $AgNO_3$ (final concentration 12.5 mM). After centrifugation at 1700*g*, for 15 min, the supernatant was aspirated, and the precipitate was suspended in scintillation fluid. At the end of the experiment, the sections were placed in scintillation fluid and counted.

Growth of the sections was measured with a Metripak position transducer (Clevite Corp.) as described by dela Fuente and

[1] This work was supported in part by Grant GB 37663 from the National Science Foundation and by a Faculty Research Grant from the University of Massachusetts.

Leopold (3), except that recordings were made on a Beckman 10-inch recorder. The chamber surrounding the sections was constructed so that aerated test solution flowed in from the bottom and out through a side arm to waste. A continuous flow system was thus effected.

RESULTS

The effect of pH on $^{36}Cl^-$ uptake into 5-mm coleoptile sections is shown in Figure 1. After preincubation for 2 to 4 hr at pH 6.5, the tissue was floated for 15 min on buffers of various pH containing $^{36}Cl^-$. Solutions at pH 7 or 6 produced no differences, but further reductions in pH led to an almost linear increase of $^{36}Cl^-$ in the tissue. No attempts were made to examine pH levels below 3.

To determine the kinetics of the low pH stimulation, sections were removed from either pH 6.5 or 3.5 buffer at time periods up to 15 min, washed, and counted (Fig. 2). A difference between tissues at the two pH levels was apparent by 6 min, and

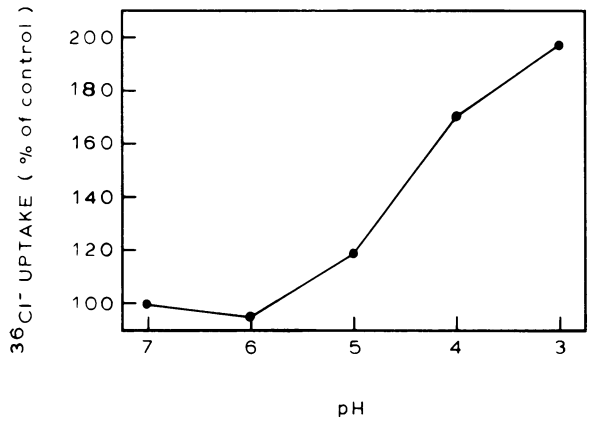

FIG. 1. Effect of pH on $^{36}Cl^-$ uptake into 5-mm coleoptile sections. Na-K phosphate buffer was used at pH 7 to 5 and citrate-phosphate buffer at pH 4 and 3. The sections were at the indicated pH during the $^{36}Cl^-$ uptake period (15 min). Controls at pH 7 took up 65 cpm·section^{-1} or 24 nmoles $^{36}Cl^-$·g fresh weight^{-1}.

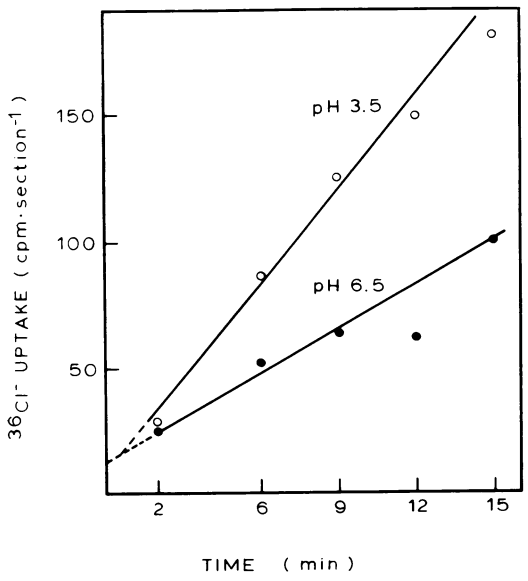

FIG. 2. Time course of $^{36}Cl^-$ uptake into coleoptile sections. The sections were in 10 mM phosphate buffer (pH 6.5) or 10 mM citrate-phosphate buffer (pH 3.5) for the times indicated before being removed and washed in cold 0.2 M KCl for 10 min.

Table I. *Uptake of $^{36}Cl^-$ by Coleoptile Sections Bathed in Various Solutions of Low pH*

Measurements were made following a 2 to 4 hr preincubation in pH 6.5 potassium phosphate buffer; the sections were transferred to the appropriate solutions containing $^{36}Cl^-$ for 15 min; they were then washed and counted.

Solution (10 mM)	pH	Promotion over Control[1]
		%
Citrate buffer	3.7	+36
Succinate buffer	3.7	+42
Glycine buffer	3.4	+42
H_2SO_4-NaOH	3.4	+49
Acetate buffer	3.7	−21

[1] Control tissue in pH 6.5 potassium phosphate buffer took up 150 cpm/section during the 15 min.

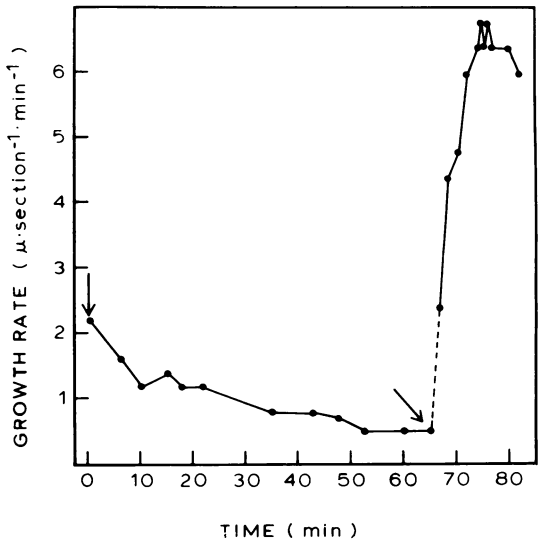

FIG. 3. Growth of 5-mm coleoptile sections as measured by a position transducer. The sections were bathed in 10 mM phosphate buffer (pH 6.5) at 0 time (first arrow). After 65 min, the buffer was changed to 10 mM acetate buffer pH 3.7 (second arrow).

the difference was maintained at a similar value (as per cent of pH 6.5 control) up to 15 min. Extrapolation of a line drawn through the points suggests a lag period between 0 and 2 min. Variation in the assay method prevents a better resolution of lag time.

The pH was regulated with a variety of substances to see if the type of buffer had an effect on $^{36}Cl^-$ uptake. As shown in Table I, any material which adjusted the pH from 3 to 4 resulted in an increased uptake of $^{36}Cl^-$. An exception was acetate buffer (10 mM, pH 3.7) which consistently reduced $^{36}Cl^-$ content 20% to 30% below tissue in phosphate buffer (pH 6.5) and 50% below tissue in the other acidic solutions.

If the stimulation of $^{36}Cl^-$ uptake were related primarily to growth, the possibility existed that acetate was not stimulating growth in this system as were the other acidic solutions. The acid pH regulated by acetate buffer, however, can still stimulate growth as shown in Figure 3. The kinetics show an almost immediate acceleration of growth thus confirming the stimulatory effect by this buffering system first reported by Rayle and Cleland (21).

A further attempt to separate the effects of growth (including the concomitant water influx) from effects on $^{36}Cl^-$ uptake is

Table II. *Effect of Mannitol on $^{36}Cl^-$ Uptake by Coleoptile Sections at Low pH*

Measurements were made following a 2 to 4 hr preincubation at pH 6.5; the sections were transferred to the above treatments containing $^{36}Cl^-$ for 15 min. They were then washed and counted. Solutions were maintained at pH 6.5 by 10 mM phosphate buffer and at pH 3.5 by 10 mM citrate buffer. Mannitol concentration was 0.3 M.

Treatment	^{36}Cl Uptake	Promotion over Control
	cpm/section	%
pH 6.5	55	
pH 6.5 + Mannitol	73	+33
pH 3.5	108	+96
pH 3.5 + Mannitol	135	+146

Table III. *Influence of Respiratory Poisons on Acid-stimulated Uptake of $^{36}Cl^-$*

After 2 hr preincubation at pH 6.5, the inhibitors were added for 1 hr. The pH was then changed and $^{36}Cl^-$ added for 15 min. All sections were then washed in 0.2 M KCl and counted.

Treatment	pH[1]		Change from pH 6.5
	6.5	3.5	
	cpm/section		%
Control	105	144	+37
10 μM CCP[2]	69	53	−24
1 mM KCN	62	49	−21
1 mM NaN_3	52	43	−17

[1] pH was adjusted with 0.01 M potassium phosphate (pH 6.5) or 0.01 M citrate buffer (pH 3.5).

[2] CCP: carbonyl cyanide *m*-chlorophenylhydrazone.

shown in Table II where the sections were floated on a hypertonic solution of mannitol (0.3 M) during the uptake period. There was no alleviation of the low pH-induced stimulation of $^{36}Cl^-$ uptake. Movement into the tissue was, in fact, accelerated by the osmoticum at both pH levels.

The importance of metabolism and, in particular, the availability of ATP was examined using the inhibitors of oxidative phosphorylation, cyanide and azide, and the uncoupler, carbonyl cyanide *m*-chlorophenylhydrazone (Table III). The results were similar for all three inhibitors. At pH 6.5 the uptake of $^{36}Cl^-$ was reduced by 50% or more, and at pH 3.5 the amount of $^{36}Cl^-$ in the tissue was even less than that seen when the inhibitors were present at the higher pH. The counts in the tissue after inhibitor treatments may represent isotope trapped in central hollows, diffusion across the plasma membrane, or an incomplete inhibition due to slow uptake of the poisons.

Kinetics of $^{36}Cl^-$ efflux from coleoptile sections has been reported by Pierce and Higinbotham (16). The effect of reducing the pH on this efflux is shown in Figure 4. The pH change was made 60 min after the $^{36}Cl^-$ labeled sections were transferred to unlabeled buffer so that the efflux being measured would not include isotope efflux from free space (16). A 20% inhibition of efflux was seen 15 min after reducing the pH to 4.5.

Since auxin as well as low pH stimulates $^{36}Cl^-$ uptake, it is of interest to see if any interaction exists between IAA and increased H^+ concentration. The results in Table IV show that auxin concentrations at saturating levels (22) produced an added stimulation of $^{36}Cl^-$ uptake at pH 3.5, but only when 5-mm sections were used. In an effort to reduce delays due to diffusion and to alleviate some of the possible influence of unstirred layers, similar experiments were performed using 1-mm sections; in these cases, the addition of IAA to the citrate buffer (pH 3.5) no longer stimulated $^{36}Cl^-$ uptake rates above those seen with the acid pH alone. The effect of low pH on 1 *versus* 5-mm sections further shows that the acid-induced stimulation of $^{36}Cl^-$ uptake is not related to the number of cut surfaces since tissue mass was approximately the same in all treatments.

The effect of 30 μM IAA on efflux of $^{36}Cl^-$ from coleoptile sections is seen in Figure 5. The IAA was added 30 min after transfer to unlabeled buffer so as to eliminate efflux from the free space compartment (16). The data show that IAA produced an inhibition of efflux which was first apparent 15 min after auxin addition; the inhibition of efflux rate continued up to 150 min reaching about 70% of the control rate.

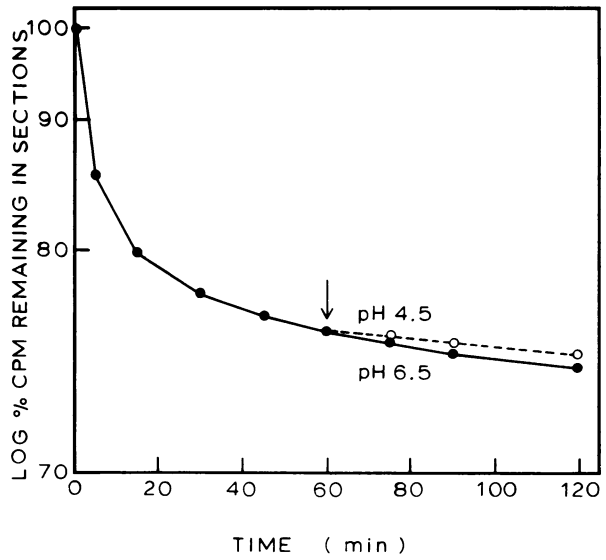

FIG. 4. Effect of pH on $^{36}Cl^-$ efflux from coleoptile sections. Following a loading period of 3 hr in $^{36}Cl^-$, the sections were transferred to 10 mM phosphate buffer containing 2 mM KCl. Sixty min later (arrow), half of the sections were bathed in 10 mM KH_2PO_4 (pH 4.5) with 2 mM KCl.

Table IV. *Effects of Section Size and pH on Auxin-stimulated $^{36}Cl^-$ Uptake*

After 2 hr preincubation at pH 6.5, sections were placed in buffer with or without IAA for 1 hr. The pH was then changed (containing IAA where appropriate) and $^{36}Cl^-$ added for 15 min. All sections were then washed and counted. IAA concentration was 30 μM; pH adjusted with 10 mM phosphate (pH 6.5) or citrate buffer (pH 3.5).

Treatment	Section Length[1]	
	5 mm	1 mm
	% of pH 6.5 control	
IAA + pH 6.5	+40	+58
pH 3.5	+47	+64
IAA + pH 3.5	+89	+52

[1] Each datum represents the mean of at least two experiments, each experimental treatment consisting of duplicate samples containing 15 sections. The 5-mm sections took up 10 nmoles/mg fresh wt for 15 min while 1-mm sections accumulated 18 nmoles/mg fresh wt.

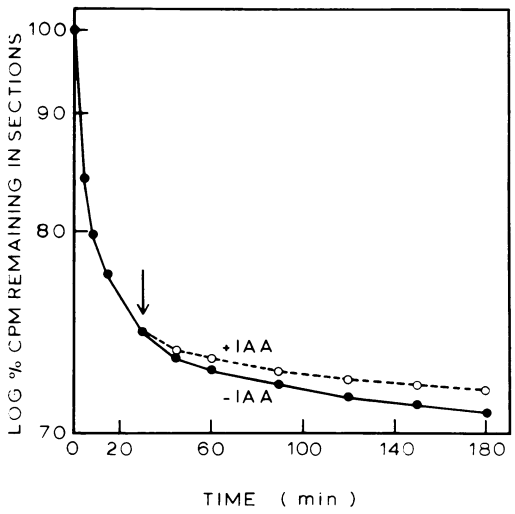

FIG. 5. Effect of auxin on $^{36}Cl^-$ efflux from coleoptile sections. Following a loading period of 3 hr in $^{36}Cl^-$, the sections were transferred to 10 mM phosphate buffer containing 2 mM KCl. Thirty min later (arrow), the sections were bathed in buffer containing 2 mM KCl either with or without 30 μM IAA.

DISCUSSION

Based on the time of incubation in $^{36}Cl^-$ and the time of the subsequent wash, the uptake being measured is probably occurring across the plasma membrane of the coleoptile cells (2), and from the data presented (Fig. 1), the stimulation of this uptake is linearly related to the external pH from 5 to 3. This effect is not accompanied by an accelerated exchange with Cl^- inside the cell since $^{36}Cl^-$ efflux is inhibited under acidic conditions (Fig. 3).

The mechanism of acid-stimulated $^{36}Cl^-$ uptake across the plasma membrane cannot yet be determined, but several possibilities do seem unlikely. Low pH is not destroying general permeability properties of the membrane. If destruction were occurring, acetate buffer would be as effective as citrate buffer (Table I), the effect would not be seen at high osmotic concentrations (Table II), and uptake would not be inhibited by respiratory poisons (Table III). It is also unlikely that acid-stimulated uptake is related to water influx accompanying growth since acetate buffer stimulates growth (Fig. 3) but eliminates the acid effect (Table I), and $^{36}Cl^-$ uptake is enhanced when sections are in hypertonic concentrations of mannitol (Table II).

The stimulatory effect of reduced pH on $^{36}Cl^-$ uptake might be related to the increased amount of undissociated $H^{36}Cl$ which would then enter more rapidly in uncharged form. The pK of HCl is extremely low, however, and any change in undissociated $H^{36}Cl$ at pH 5 compared to pH 6, for example, would be negligible; the increase in $^{36}Cl^-$ uptake between these two pH levels is quite pronounced (Fig. 1). A consideration of charge may not be relevant at all since α-aminoisobutyric acid and 3-O-methyl glucose uptake are also stimulated by reduced pH (Bates and Rubinstein, unpublished data).

Due to the inhibitory effect of KCN, NaN_3, and carbonyl cyanide *m*-chlorophenylhydrazone, the possibility exists that at least one component of the acid-induced stimulation of $^{36}Cl^-$ uptake involves energy metabolism. The effect on metabolism could be triggered by the pH gradient. The importance of pH across a plant plasma membrane has been discussed by Lundegårdh (11), Robertson (19), and more recently by Smith (23) and in higher plants by Marré *et al.* (12). Parallels might also be drawn with the energy coupling which occurs during imposed pH gradients using chloroplasts (5, 10) or mitochondria (13).

The stimulation of $^{36}Cl^-$ uptake by auxin and acid have the following aspects in common: (*a*) both treatments stimulate uptake and inhibit efflux (Figs. 3 and 4); (*b*) increased uptake either after a pH drop or auxin addition is seen in media of high osmolarity (Table II; ref. 22); (*c*) increases in uptake caused by either treatment do not occur after coleoptile sections are preincubated in respiratory inhibitors or an uncoupler (Table II; ref. 22); (*d*) the effects of both auxin and acid are not restricted to cut surfaces (Table IV; ref. 22). This last can be seen by comparing uptake into 5- and 1-mm sections at pH 3.5. The tissue mass of both sized sections is approximately equal, but there are 5 times more cut surfaces in treatments with 1-mm sections. The acid-induced stimulation of $^{36}Cl^-$ uptake into 1-mm sections, however, is only 1.4 times greater than into 5-mm sections.

Rayle (20), Cleland (1), and Marré *et al.* (12) have presented evidence for an auxin-stimulated H^+ efflux. Their data indicate that the pH outside the plasma membrane could be lowered by auxin to within an order of magnitude necessary to stimulate Cl^- uptake as determined by externally applied H^+ (Fig. 1). Furthermore, the time required to record a significant decrease in pH after auxin addition to coleoptile sections, about 20 to 30 min (1), is close to the length of time which must elapse before an IAA-induced stimulation of $^{36}Cl^-$ uptake is seen (22).

Finally, floating 1-mm sections on IAA and citrate buffer at pH 3.5 does not stimulate uptake beyond that seen with the buffer alone. If auxin were acting by a mechanism unrelated to that of low pH, one would expect the stimulation by auxin to be apparent even at pH 3.5. The reason for additivity with 5-mm sections may be related to restricted diffusion. Due to the cuticle, H^+ cannot accumulate at the cell surface in sufficient quantities to maximize the response; thus, the simultaneous addition of auxin to the larger sections could account for even more H^+ ions by stimulating H^+ secretion. The level of H^+ ions at the cell surface of 1-mm sections in low pH would be higher due to the shorter diffusion path, and a further increase of H^+ by auxin-induced secretory processes would be less noticeable.

Acknowledgments—I am most grateful to Mr. James Bates for permission to include some data which were a part of his Senior Honors Thesis. Technical assistance has been provided by Ms. Susanne Müller-Eversbusch and Mr. Peter Mahar.

LITERATURE CITED

1. CLELAND, R. 1973. Auxin-induced hydrogen ion excretion from *Avena* coleoptiles. Proc. Nat. Acad. Sci. U.S.A. 70: 3092-3093.
2. CRAM, W. J. AND G. G. LATIES. 1971. The use of short term and quasi-steady influx in estimating plasmalemma and tonoplast influx in barley root cells at various external and internal chloride concentrations. Aust. J. Biol. Sci. 24: 633-646.
3. DELA FUENTE, R. K. AND A. C. LEOPOLD. 1970. Time course of auxin stimulations of growth. Plant Physiol. 46: 186-189.
4. ELZAM, O. E. AND E. EPSTEIN. 1965. Absorption of chloride by barley roots: kinetics and selectivity. Plant Physiol. 40: 620-624.
5. FORGER, J. M. III. AND L. BOGORAD. 1971. Acid to base phosphorylation and membrane integrity in plastids in greening maize. Biochim. Biophys. Acta 226: 383-392.
6. HAGAR, A., H. MENZEL, AND A. KRAUSS. 1971. Versuche und Hypothese zur Primärwirkung des Auxins beim Strechungswachstum. Planta 100: 47-75.
7. HOAGLAND, D. R. AND T. C. BROYER. 1940. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. J. Bot. 27: 173-185.
8. HOAGLAND, D. R. AND A. R. DAVIS. 1923. The composition of the cell sap of the plant in relation to the absorption of ions. J. Gen. Physiol. 5: 629-646.
9. JACOBSON, L., R. OVERSTREET, AND J. A. CHASTAIN. 1957. The effect of pH and temperature on the absorption of potassium and bromide by barley roots. Plant Physiol. 32: 658-662.
10. JAGENDORF, A. T. AND E. URIBE. 1966. ATP formation caused by acid-base transition of spinach chloroplasts. Proc. Nat. Acad. Sci. U.S.A. 55: 170-177.
11. LUNDEGÅRDH, H. 1939. An electro-chemical theory of salt absorption and respiration. Nature 143: 203-204.

12. MARRÉ, E., R. COLOMBO, P. LADO, AND F. RASI-CALDOGNO. 1974. Correlation between proton extrusion and stimulation of cell enlargement. Effects of fusicoccin and of cytokinins on leaf fragments and isolated cotyledons. Plant Sci. Lett. 2: 139–150.
13. MITCHELL, P. 1966. Chemi-osmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. 41: 445–602.
14. OLSEN, C. 1953. The significance of concentration for the rate of ion absorption by higher plants in water culture. IV. The influence of hydrogen ion concentration. Physiol. Plant. 6: 848–858.
15. PETRIE, A. H. K. 1938. The intake of ions by carrot tissue at different hydrogen-ion concentrations. New Phytol. 37: 211–231.
16. PIERCE, W. AND N. HIGINBOTHAM. 1970. Compartments and fluxes of K^+, Na^+, and Cl^- in *Avena* coleoptile cells. Plant Physiol. 46: 666–673.
17. PITMAN, M. G. 1963. The determination of the salt relations of the cytoplasmic phase in cells of beet root tissue. Aust. J. Biol. Sci. 16: 647–668.
18. PITMAN, M. G. 1970. H^+ efflux from cells of low-salt barley roots during salt accumulation. Plant Physiol. 45: 787–790.
19. ROBERTSON, R. N. 1968. Protons, Electrons, Phosphorylation and Active Transport. Cambridge University Press, London.
20. RAYLE, D. L. 1973. Auxin-induced hydrogen ion secretion in *Avena* coleoptiles and its implications. Planta 114: 63–73.
21. RAYLE, D. L. AND R. CLELAND. 1970. Enhancement of wall loosening and elongation by acid solutions. Plant Physiol. 46: 250–253.
22. RUBINSTEIN, B. AND E. N. LIGHT. 1973. Indoleacetic acid-enhanced chloride uptake into coleoptile cells. Planta 110: 43–56.
23. SMITH, F. A. 1970. The mechanism of chloride transport in Characean cells. New Phytol. 69: 903–917.
24. SPEAR, D. G., J. K. BARR, AND C. E. BARR. 1969. Localization of hydrogen ion and chloride ion fluxes in *Nitella*. J. Gen. Physiol. 54: 397–414.
25. STEWARD, F. C. AND C. PRESTON. 1941. Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiol. 19: 481–519.
26. VAN DEN HOVERT, T. H. AND J. J. M. HOOGMANS. 1955. On the absorption of nitrate by maize in water culture. Acta Bot. Neérl. 4: 376–384.