Plant Physiol. (1966) 41, 1725-1735

Potassium Loss and Changes in the Fine Structure of Corn Root Tips Induced by H-ion[1]

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Received September 27, 1966.

Summary. The effects of H, Ca, and anaerobiosis upon loss of K to ambient solutions, upon changes in the fine structure, and upon the respiration of corn root tissue were studied. In the pH range 5.5 to 8.0 losses of K decreased with decreasing H concentration. Ca reduced K loss greatly in the lower part of the pH range but with increasing pH the effect of Ca declined. Losses of K under N_2 were much greater than those measured under air but the same effects of H and Ca were found. The effect of phosphate upon K loss was found to depend upon pH, temperature and the state of development of the tissue.

In pure H_2O or dilute HCl no obvious derangement of the fine structure of meristematic cells was found to occur in 3 hours above pH 4.4 except attenuation of the groundplasm. At pH 4.4 and below, serious injury was found. The presence of $CaCl_2$ or NaCl in the treatment solution greatly ameliorated the effect of H, $CaCl_2$ being effective at minute concentration (0.01 meq per liter). NaH_2PO_4 was without any great effect. Anaerobiosis at neutral pH produced severe tissue damage.

In contrast to anaerobic treatments, aerobic treatments (pH 5.8) resulting in large losses of K were not accompanied by any diminution of the respiratory rate.

Earlier studies concerning the loss of K from plant tissue to ambient solutions have shown a marked dependence of K loss upon the pH of the medium (4, 11, 13). While at a pH of 5.0 or higher, the K loss by 6 day old barley roots was found to be very small, at pH 4.0 a sharp increase in K loss was found to occur, some 40 % of the initial K content being lost in 3 hours to dilute HBr at this pH. In contrast to this, K is lost in large amounts by corn root tips placed in distilled water with a pH of 5.8 (8) and by 2 to 3 day old barley roots to NaCl solutions at a similar pH (16). The apparent discrepancy is most probably related to the age of the tissue used. Six day old barley roots are composed almost entirely of mature, fully vacuolated cells of relatively low cytoplasmic content. Corn root segments cut within a few millimeters of the tip and 2 day old barley roots have a much larger proportion of immature cells with a relatively high protoplasmic content. A high proportion of the endogenous K of these cells is located in the cytoplasm whereas K of mature tissue may be largely sequestered in vacuoles and thus less readily exchanged for H and other ions in the medium. Experiments measuring the K lost by barley roots as a function of age (16) have shown 2 day old barley roots to lose 40 % of their initial K content in 2 hours to dilute NaCl while the loss for 7 day old roots amounted to about 17 %. The initial K content of young tissue is generally higher than that of mature tissue. The corn root tissue used here and in earlier work (6, 7, 8) contains about 80 meq. K per kilo on a fresh weight basis. Two day old barley roots have about 50 meq. per kilo. Seven day old barley roots have initially only about 15 meq. per kilo. Since this latter tissue is almost fully vacuolated, it is clear that only a very small amount of K would be readily available for exchange. Reabsorption of eluted or displaced K would further limit K loss in the more mature tissue.

The effects of Ca upon ion uptake as well as upon loss of ions from plant tissue have been the subject of many investigations (2, 3, 4, 8, 10, 11, 13–27). Ca has been shown to reduce greatly the loss of K from 6 day old barley roots at pH 4.0 (13). The same effect has been noted with 2 day old barley roots (16) and with corn root tips (8) but in these cases the pH was about 5.8. This suggests that even above pH 5.0 the depletion of K from plant tissue is accomplished principally by the

[1] This report is based on work performed under Contract No. AT-(11-1) with the United States Atomic Energy Commission and partially supported by Deutche Forschungsgemeinschaft.

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agency of H. Besides replacing adsorbed K, H may further enhance loss of K by removing Ca from cell membranes, thus increasing their permeability.

To obtain further information about the role of H, K loss from corn root segments was measured under a variety of environmental conditions. Experiments were done in a pH range of 5.5 to 8.0, at 26° and 1° and under aerobic and anaerobic conditions. The effects of some treatments upon the subsequent respiratory rates were investigated in order to determine whether K losses are accompanied by noticeable changes in the overall metabolic rate.

In an electron microscopic study, the fine structure of tissue exposed to various environmental conditions was examined in an effort to relate K loss to changes in cell structure. It has been observed in Ca deficient barley roots that alterations in K loss and Na uptake induced by witholding Ca during development were well correlated with observable changes in the fine structure (17). In this case, supplying Ca to the deficient tissue restored both the normal fine structure and the normal pattern of Na uptake and K loss. Changes in the fine structure of shoot meristematic cells associated with Ca deficiency have been described by Marinos (15).

Materials and Methods

Five day old corn seedlings (*Zea mays* L. var. Peoria) were grown in 0.00025 N $CaCl_2$ and sections cut from the primary roots as previously described (6). Root segments cut 0 to 1.8 mm and 1.8 to 3.8 mm from the tip are designated sections 1 and 2 respectively. For investigation of K loss, 30 root segments (ca. 30 mg fr wt) were used with a solution volume of 300 ml. Before dry ashing (550° for 1 hr) the roots were sulfated with a few drops of 5 % H_2SO_4 in methanol. The ashed samples were taken up in 10.00 ml 50 % (v/v) methanol, 0.01 N in HCl. K was determined by flame photometry. Respiration was measured by conventional Warburg technique using 30 root segments and a fluid volume of 4.00 ml. Recrystallized NaCl, glass redistilled water and polyethylene ware were used. Numerical values represent averages of at least 3 determinations.

Considerable variability in loss of K under any particular experimental conditions was found when results obtained with different batches of roots were compared. In the pH range studied minor variations in pH are associated with relatively large changes in K loss (fig 1). The variability found between different experiments is probably due mainly to our failure to reproduce pH values with sufficient accuracy. Other factors such as variable Ca status of the tissue may have contributed. Variation between replicate values obtained using a single batch of root material was, however, very small. The graphs (for K loss) each represent experiments done with a single batch of roots.

For electron microscopy, the root segments were placed in the Palade fixative (veronal buffered 1 % OsO_4) at 1° for 90 minutes, dehydrated with an acetone series, stained with 1 % uranyl nitrate in acetone, and embedded in Epon. After sectioning, the tissue was post stained with lead citrate. Photographs were taken with an RCA EMU-3 electron microscope.

Results and Discussion

In the pH range 5.5 to 7.0, decreasing H ion concentration is associated with a marked reduction in the amount of K lost to the external medium (fig 1). This is true for both sections. Less K is lost from the second section (1.8–3.8 mm from the tip) than from the first (0–1.8 mm) probably reflecting sequestration of K in vacuoles. Raising the pH from 5.5 to 7.0 resulted in an approximately 90 % reduction in the 3 hour K loss. In this experiment the solutions were buffered with 0.50 mM Na phosphate. The influence of phosphate upon K loss is shown in figure 2. It is apparent that at 26° (and at a pH near neutrality) phosphate reduces K loss significantly, the loss at pH 6.9 in the presence of phosphate being about two thirds that found when the pH was raised simply by removal of CO_2 from the aeration stream. This is probably the result of a beneficial effect of phosphate upon metabolism. At 1.0°, i.e. with a low metabolic rate, the phosphate effect was not observed. At pH 8.0

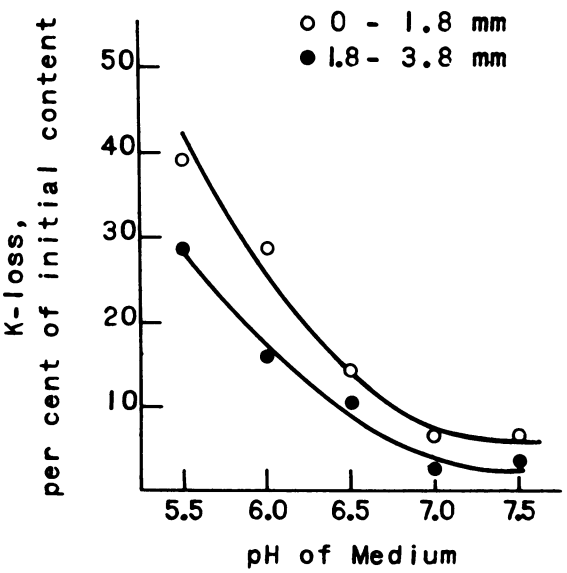


FIG. 1. Effect of H upon K loss in 1.0 m M NaCl. 26°; 3 hours; aeration with CO_2-free air; pH adjusted with Na phosphate (0.05 m M).

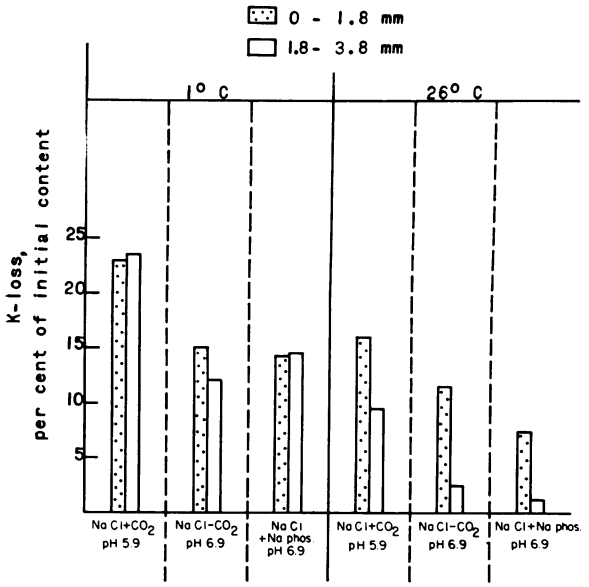


FIG. 2. Effect of temperature upon K loss to 0.001 N NaCl. 3 hours; Na phosphate 0.0005 M.

and at 1.0° (data not shown) phosphate had an opposite effect, increasing the loss of K by both sections well above that observed in its absence. Perhaps in this case formation of insoluble $Ca_3(PO_4)_2$ resulted in removal of Ca from cell membranes and therefore in increased leakage of K.

It will be noted (fig 2) that losses of K are greater for both sections at 1.0° than at 26°. This has been observed previously (8) and may be related to better maintenance of membrane stability in actively metabolizing tissue as well as to metabolic reabsorption of displaced K. The temperature effect was more pronounced in the second section in which metabolic absorption of ions is vigorous (8). At pH's low enough to induce injury an opposite temperature effect has been observed (11, 12, 18). This will be discussed later.

It is known that the presence of Ca in the medium reduces the loss of K by this tissue (8). Figure 3 depicts the influence of pH upon the efficacy of Ca in this regard. It is apparent that where as at pH 5.9 Ca greatly reduces the loss of K, at pH 8.1 its effect becomes small. In this experiment the pH was adjusted to 8.1 with NaOH and maintained by aerating with CO_2-free air. All solutions contained NaCl (1.0 meq per liter). These results indicate that K loss is regulated by the interaction of H and Ca. H increases cell permeability by removal of Ca from membrane sites rendering K bound in the cytoplasm more easily exchangeable. Removal of Ca may also result in loss of organic cytoplasmic constituents capable of binding K. As will be shown, the appearance of

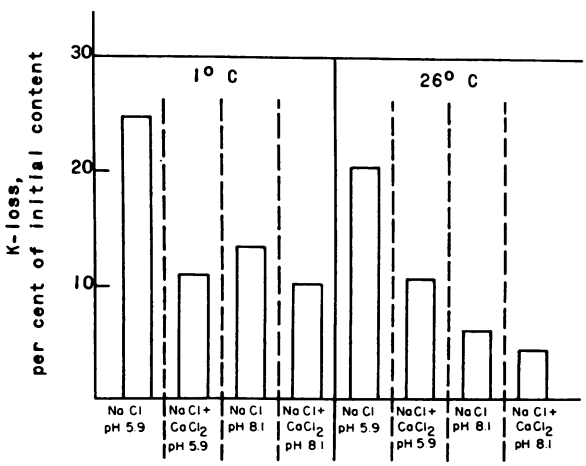


FIG. 3. Effect of H and addition of Ca on loss of K to 0.001 N NaCl at 1° and 26°. 3 hours; $CaCl_2$, 0 001 N; pH adjusted to 8.1 with NaOH and maintained with CO_2-free aeration; section 1 (0–1.8 mm).

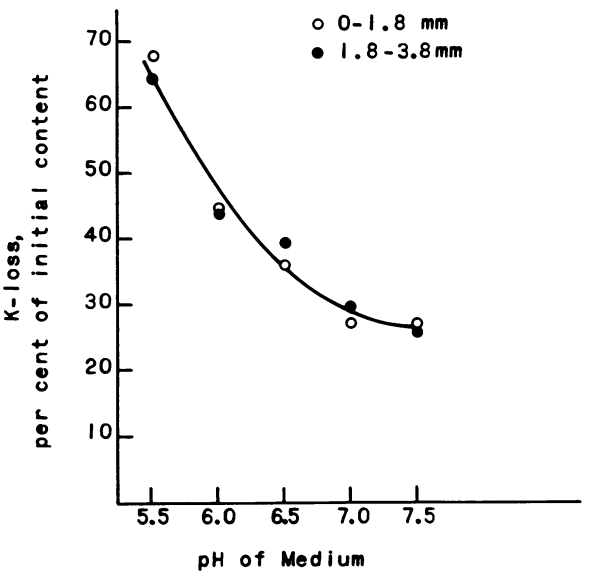


FIG. 4. Effect of H upon K loss under N_2 to 0.001 N NaCl. 3 hours; 26°; pH adjusted with Na phosphate (0.0005 M).

the groundplasm of meristematic cells after exposure to Ca-free solutions supports this possibility. Ca in the medium is antagonistic to both these effects.

Under anaerobic conditions losses of K are much greater than under normal aeration but the pattern of pH dependence remains the same (fig 4). Under anaerobiosis K loss from the second zone (1.8–3.8 mm) is about equal to that of the first zone suggesting that K normally sequestered in the vacuole is lost when aerobic metabolism cannot be maintained. As under aerobic conditions Ca reduced K loss considerably at pH 5.8 but was less effective at higher pH values (fig 5).

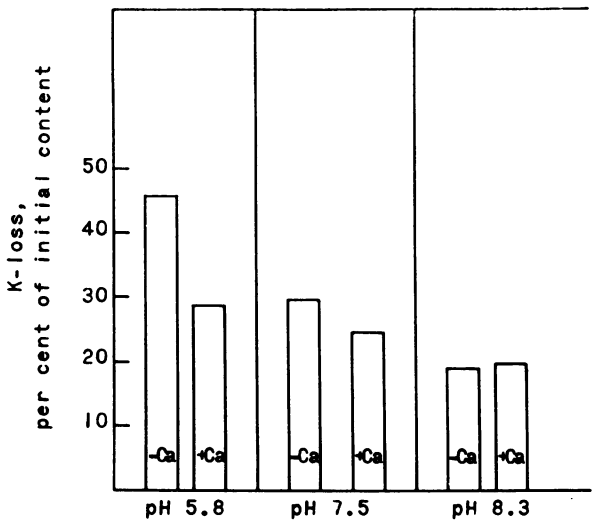


FIG. 5. Effect of H and Ca upon K loss under N_2 to 0.001 N NaCl. 3 hours; 26°; $CaCl_2$, 0.001 N; pH adjusted with HCl and NaOH; section 1.

The effect of various pretreatments upon respiration are shown in table I. Aerobic pretreatments which induce large losses of K have little effect upon the subsequent respiration rate. It would thus appear that loss of K per se in the pH range studied here has no serious effect upon the overall metabolic rate. When the pretreatment was anaerobic, however, the subsequent respiration rate was seriously affected in all cases. Under N_2, reducing the H concentration or adding Ca to the medium during pretreatment resulted in higher respiratory rates, the effects being similar to those exerted upon loss of K. Ca similarly reduces loss of dry matter by root tissue under anaerobiosis (18). To be effective Ca must be present during the anaerobic treatment. Addition of Ca to the Warburg vessel after anerobic pretreatment in a Ca-free medium does not increase the respiratory rate.

The effects of various treatments upon the fine structure of cells of the root tip are shown in figures 6 to 14. It is important in studies of this kind that only cells similar in age and morphology be compared. For this reason, the study was restricted to cells of the third to sixth cortical tier in the zone 0.4 to 0.6 mm from the tip.

Figure 6 illustrates a cell of an untreated root segment. The groundplasm of this tissue typically appears dense (in OsO_4 fixed preparations) with a large number of mitochondria and other organelles visible. No evidence of vacuolation was found in the region studied.

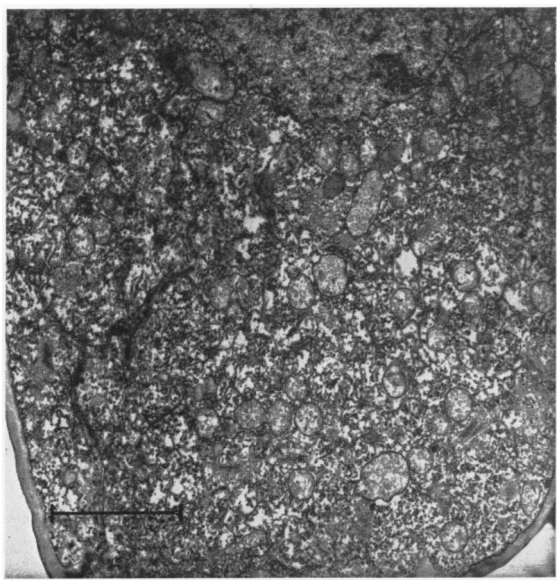
Figure 7 shows the effect of a 3 hour anaerobic treatment in distilled water (pH about 6.8). Here, the cytoplasm, as might be expected from the losses in dry weight found to occur under N_2 (18), appears much less dense than that of the control (fig 6). Moreover, the groundplasm in this case is not uniform. Areas of very low density occur. These are reminiscent of the structureless areas found by Marinos in Ca deficient cells of the shoot apex of barley (15). The most striking evidence of damage induced by anerobiosis is the presence of swollen bodies with little or no internal structure evident. These evidently arise from both mitochondria and proplastids. In some cases remnants of the cristae are visible. Mitochondria of normal appearance occur but rarely. In this region (0.4–0.6 mm) no obvious derangement of the endoplasmic reticulum as reported by Whaley et al. (28) in root cap cells of *Zea mays* was found. The effects of anaerobiosis upon the groundplasma and mitochondria shown in figure 7 are consistent with the effects produced by this treatment upon K loss (fig 4) and respiration (table I).

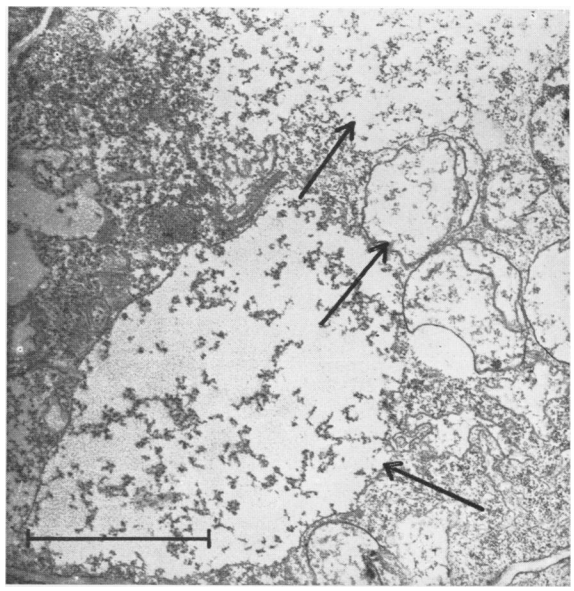
The effects of H at 26° under aerobic conditions upon the fine structure are shown in figures 8 to 14. At pH 4.75 (fig 8) although large amounts of K are undoubtedly lost (fig 1) no obvious damage results beyond a well-marked lessening in

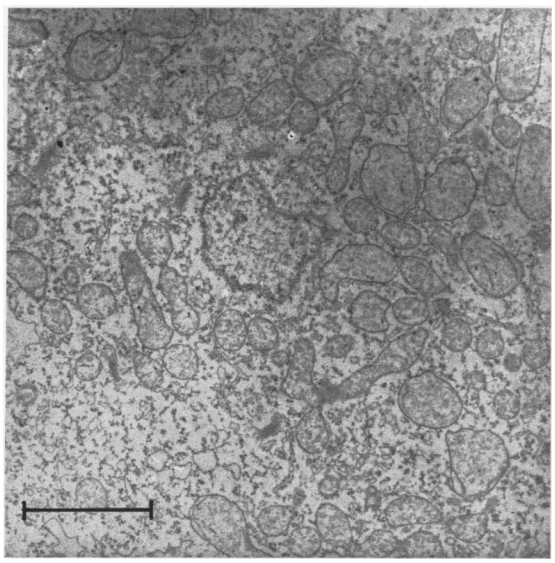
Table I. *Effect of Various Pretreatments upon Respiration of Corn Root Tips (0–1.8 mm)*

Pretreatment*			Respiration		
Solution	Gas phase	pH	Solution	Resp rate μl O_2/gm × hr	Resp. rate % control
Control**	...	...	NaCl + $CaCl_2$	989	100
NaCl***	Air	5.8	NaCl + $CaCl_2$	977	98
NaCl + $CaCl_2$	Air	5.8	NaCl + $CaCl_2$	911	92
NaCl + $CaCl_2$	Air	8.0	NaCl + $CaCl_2$	896	90
NaCl	N_2	5.8	NaCl + $CaCl_2$	497	50
NaCl + $CaCl_2$	N_2	5.8	NaCl + $CaCl_2$	704	71
NaCl	N_2	5.8	NaCl	503	50
NaCl	N_2	8.0	NaCl + $CaCl_2$	687	69
NaCl + $CaCl_2$	N_2	8.0	NaCl + $CaCl_2$	744	75

* Pretreatment, 3 hrs, 26°.
** No pretreatment other than brief washing with distilled H_2O.
*** Concentrations of NaCl and $CaCl_2$, 1.0 meq. per liter.







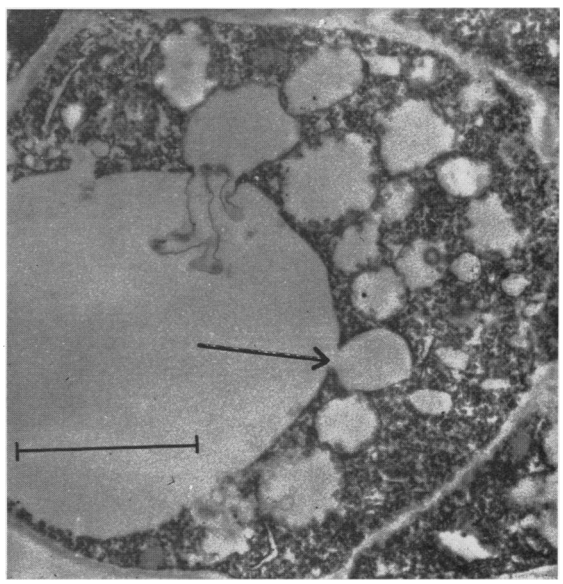


FIG. 6 (top, left). Detail of untreated cortical cell 0.4 to 0.6 mm from corn root tip. Dark lines on this and succeeding electronmicrographs indicate 1.0 μ. Arrows indicate areas of interest discussed in text.

FIG. 7 (top, right). Detail of cortical cell after 3 hour anaerobic (N_2) treatment in water at 26°.

FIG. 8 (bottom, left). Detail of cortical cell after 3 hour aerobic treatment in dilute HCl at 26°, pH 4.75. Large body near center of photograph is the nucleus.

FIG. 9 (bottom, right). Detail of cortical cell after 3 hour aerobic treatment in dilute HCl at 26°, pH 4.40.

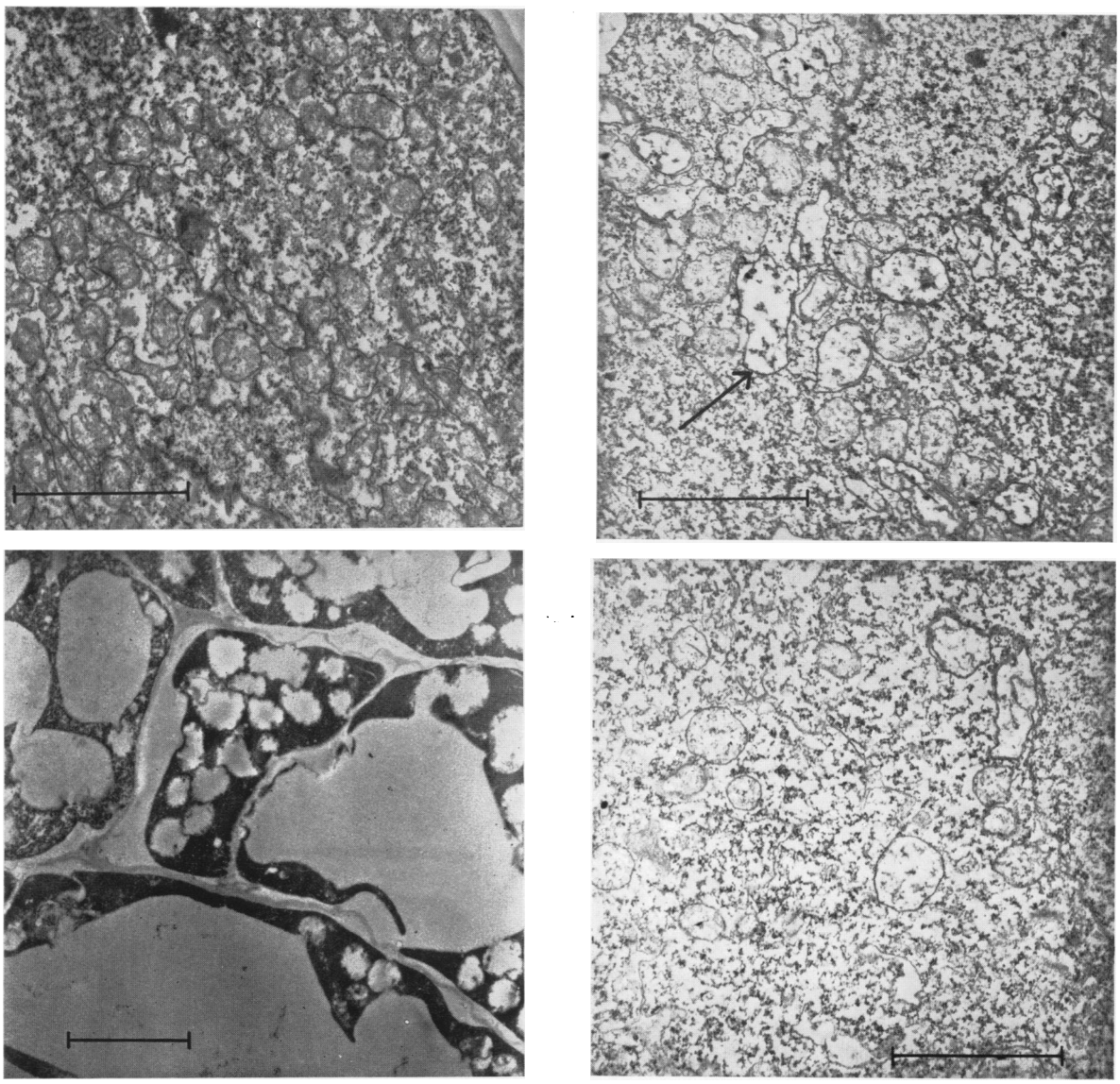


FIG. 10 (top, left). Detail of cortical cell after 3 hour aerobic treatment in dilute HCl plus 1.0 meq. $CaCl_2$ per liter, pH 4.40, 26°.

FIG. 11 (top, right). Detail of cortical cell after 3 hour aerobic treatment in dilute HCl plus 0.01 meq. $CaCl_2$ per liter, pH 4.0, 26°.

FIG. 12 (bottom, left). Detail of cortical cells after 3 hour aerobic treatment in dilute HCl, pH 4.0, 26°.

FIG. 13 (bottom, right). Detail of cortical cell after 3 hour aerobic treatment in dilute HCl plus 0.50 meq. NaCl per liter, pH 4.0, 26°.

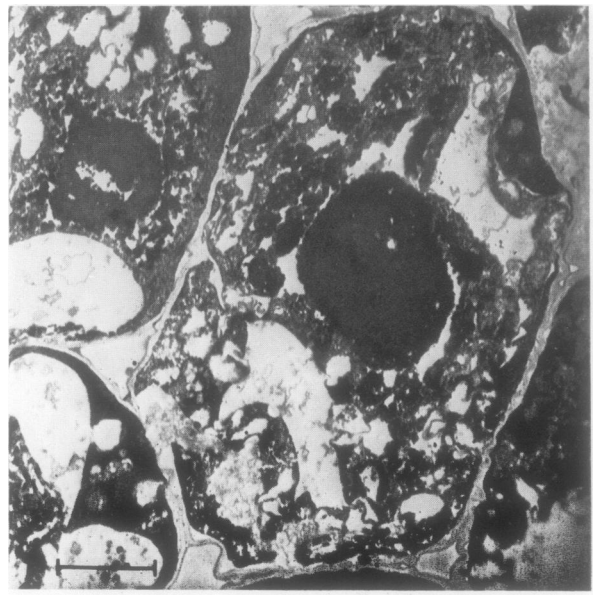


FIG. 14. Detail of cortical cells after 3 hour aerobic treatment in dilute HCl plus 0.50 mmole NaH_2PO_4 per liter, pH 4.0, 26°.

the density of the groundplasm. The appearance and numbers of mitochondria, proplastids and other organelles appear similar to those of the control. Treatment at pH 6.0 (distilled water) produced similar results. The failure of pretreatment at pH 5.8 to affect the subsequent respiration (table I) is confirmed by the lack of any discernable effect upon the appearance and numbers of mitochondria found in tissue treated in H_2O at pH 4.75 to 6.0. The attenuation of the groundplasm found may be related to the substantial loss of K occurring in this pH range. It would appear likely that the K lost from this tissue is associated with organic material derived from the groundplasm.

When the pH was lowered to 4.4 (HCl) very serious damage to the cells occurred (fig 9). In tissue treated at this pH for 3 hours, vacuole-like structures are prominent having well defined membranes. These are evidently derived from the swelling of mitochondria and proplastids. As in N_2 treated tissue, remnants of cristae are visible. These grossly swollen bodies appear to merge forming fewer and larger vesicles in a process superficially resembling normal vacuolation. The membranes of these bodies and the cell membrane appear to be surprisingly stable. Plasmolysis of many cells was observed. The swollen condition of the mitochondria of course implies a stable diffusion barrier. The rise in osmotic pressure within these bodies may occur as a result of protein hydrolysis. Formation of pseudo vacuoles results in concentration of the groundplasm. Also coagulation of groundplasm material is evident in its much coarser appearance. As a result of these factors, the groundplasm of acid injured material is typically very densely staining. In even more severe acid injury (fig 12) the groundplasm becomes entirely structureless and appears as extremely deeply stained homogeneous material.

The foregoing results were obtained using media (either H_2O or dilute HCl) to which no salts were added. It is apparent that in these cases the onset of acid injury occurs over a very narrow pH range. No obvious change in appearance (other than attenuation of groundplasm) is caused by a pH as low as 4.75 whereas catostrophic disturbance of cell structure results when the tissue is exposed to a pH of 4.4. Jacobson et al. (12) found losses of K, Ca and organic constituents of barley roots to increase sharply at about this pH when the tissue was placed in HCl solutions. The efficacy of Ca in overcoming deleterious effects of H upon ion uptake is well established (4, 10, 13, 20, 22). Losses of K and dry matter from barley roots occurring at pH 3.5 have been found to be sharply reduced by the addition of Ca to the medium (18). These results are consistent with the beneficial effect of Ca in preserving the fine structure shown in figure 10. The tissue shown was exposed to HCl at pH 4.4 to which was added 1.0 meq. $CaCl_2$ per liter. It is readily apparent that the addition of Ca completely overcame the damaging effect of H. The groundplasm appears as dense as that of the control and considerably denser than that shown in figure 8.

The reduction in groundplasm density occurring at pH 4.75 and above in the absence of Ca is not a dilution brought about by growth of the excised segments during treatment. Roots treated at pH 4.4 either in the presence or absence of Ca showed the same growth rate. Segments originally 4.0 mm long were in both cases 2.0 mm longer at the end of the 3 hour treatment. At higher pH (6.0) much less growth occurred, the final length being about 5.0 mm, but attenuation of the groundplasm was pronounced. Burstrom (1) has noted a similar effect of H upon root elongation. It appears therefore that even at pH 6.0 the tissue experiences losses of material derived from the groundplasm unless Ca is present. These losses are well correlated with losses of K, which are likewise reduced by addition of Ca (fig 3).

The high effectiveness of Ca in reducing H injury is shown in figure 11. For this experiment, the pH was lowered to 4.0 (HCl) and only 0.010 meq. $CaCl_2$ per liter was added. Incipient H injury is apparent in the distension of some of the organelles but overall damage was slight compared to that shown by tissue exposed to pH 4.0 in the absence of Ca (fig 12).

While, as noted above, the effectiveness of Ca and other polyvalent cations upon the effects of relatively high H concentrations upon various aspects of plant metabolism has been extensively investigated, the role of monovalent ions is not as well recognized. Fawzy et al. (4) have shown that barley roots pretreated in HCl at pH 4.0 subsequently absorbed significantly more K when 1.0 meq. LiCl or NaCl per liter was added to the pretreatment solution. We have found a similar protective effect of NaCl upon the fine structure of corn root tips. Figure 13 shows the effect of addition of 0.50 meq. NaCl per liter to a treatment solution of HCl at pH 4.0. It is readily apparent that NaCl at this rather small concentration greatly reduces the destructive effect of H although the groundplasm is coarser and less dense than that of the control (fig 6). When the NaCl concentration was increased to 10.0 meq. per liter, no further effect of Na was found. We did not attempt further to define the lowest effective NaCl concentration.

Salt effects upon acid injury appear to involve not only the cation but also the accompanying anion. When, instead of NaCl, 0.50 mmole NaH_2PO_4 per liter was added to the HCl treatment at pH 4.0 (fig 14) the subsequent appearance of the fine structure resembled that of tissue exposed to pH 4.0 in the absence of salt (fig 12). An explanation of the failure of NaH_2PO_4 to protect the tissue against acid injury must await further study.

The foregoing results make apparent the difficulty of defining the physiological pH range. It

is clear that the pH range over which the possibility of gross injury may be discounted depends on several factors. Presence or absence of Ca is of course a major determinant but other cations and even anions are capable of modification of the H effect. In ion uptake experiments done at low pH and with variable substrate ion concentrations (5, 27) interpretation should take account of the protective role of the salts used. Whereas the higher concentrations may be effective in overcoming gross H ion injury, tissue exposed to very low substrate concentrations at the same pH may be seriously affected. Under these circumstances, interpretations made solely in terms of H competition at carrier sites or adsorption sites may not be satisfactory. For example, the results of Shone and Barber (23) indicate high elution of absorbed iodide when barley roots were placed in H_2O at pH 4.0. This was interpreted in terms of H effects upon positively charged adsorption sites. It seems at least possible, in view of the results reported here, that the elution may have been accompanied by serious derangement of cell structure. In our work, we have found very serious visible damage to fine structure to occur within one half hour at pH 3.5.

The influence of temperature upon H injury has been investigated by Jacobson et al. (11, 12) and by Marschner and Mengel (18). Injury, measured by loss of K and other constituents was shown to decrease sharply with decreasing temperature. This indicates that metabolic activity may play a role in the production of H injury. It may be suggested that H ions produced endogenously are important. When their production is increased by anaerobiosis or their diffusion out of the tissue is hindered by lowering the ambient pH, a tolerable level within the tissue may be exceeded. The data of Shone and Barber (23) showing a reduction of iodide elution to H_2O at pH 4.0 when the temperature was lowered to 0.2° may be interpreted as an illustration of this effect but further work is needed to substantiate this hypothesis. With this tissue and at higher pH levels (fig 2, 3) the temperature effect appears to be quite different, more K being lost at 1° than at 26°. Evidently metabolic activity, depending upon external pH and probably other environmental conditions, may either depress or enhance loss of K and other constituents.

In view of the large effect produced by minute amounts of Ca found in this work and the effects of endogenous Ca upon K uptake shown by Jacobson et al. (14) it is clear that, in addition to the factors discussed above, the ratio, weight of tissue: volume of solution must be taken into account when assessing the effect of H upon ion uptake or other activities of plant tissues used in experimental work.

These experiments were performed using very young corn root tissue and a very low tissue: solution ratio (0.15 gm per liter). The results can not be applied uncritically to other plant materials or experimental conditions. The results, however, generally parallel those obtained by Jacobson et al. working with barley roots at low pH and perhaps may be of fairly general application. Failure to find changes in fine structure of fixed material is of course no guarantee that a treatment had no serious effects upon cell structure. Damage may have occurred on the molecular level. Also, preparation of tissue for electron-micrography involves harsh treatment. Subtle but important changes in structure are apt not to be apparent after this treatment.

Acknowledgment

We are greatly indebted to Dr. James H. McAlear and the staff of the Electron Microscope Laboratory, University of California, Berkeley, and to Dr. Karl A. Grossenbacher of this department, for their advice and assistance in the preparation of the electronmicrographs.

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