

Effects of Ca Upon Metabolic and Nonmetabolic Uptake of Na and Rb by Root Segments of *Zea mays*^{1, 2}

Raymond Handley, Abdel Metwally, and Roy Overstreet

Department of Soils and Plant Nutrition, University of California, Berkeley, California

Introduction

Ca exerts a strongly depressive effect upon the nonmetabolic diffusive entry of Na into the cells of the zone of cell division (0–1.8 mm from the root tip) of the primary root of *Zea mays* (9). Under the experimental conditions imposed in this previous work the maximum effect of Ca was achieved at a relatively very low concentration, about 0.10 meq per liter. It was postulated that Ca is active at the cell surface where it stabilizes and alters the permeability of a barrier to nonmetabolic ion entry, presumably the outer cell membrane. This supposition is based upon early work dealing with the penetration of dyes into animal cells (1) and recent work with plants (3, 4, 10, 12). The low amounts of Ca which are effective also suggest an involvement of Ca with the cell surface rather than competition of Ca with Na for adsorption sites deeper in the cell. Our previous paper (9) was concerned only with the effect of Ca (and Sr) upon nonmetabolic Na uptake since only the zone of cell division was investigated and this tissue displays no capacity for metabolic accumulation of Na, Ca, Sr or Cl ions (5, 6, 8). We are aware that none of the experimental criteria currently employed to distinguish between metabolic and nonmetabolic ion uptake is entirely free of objection. For instance, lowering the temperature drastically or administering metabolic poisons may well affect cell permeability as well as preventing operation of metabolic accumulation mechanisms. However, the simplest explanation of the following observations would seem to be that ion uptake by the tip section is entirely free of any direct connection with a metabolic ion pump: 1) Between 0° and 26° uptake is independent of temperature. 2) Labeled ions are eluted from the tissue into unlabeled solutions at about the same rate as that at which they were originally taken up. 3) Uptake of Cl is minute compared with uptake of cations. 4) Anerobiosis actually increases greatly the rate at which Sr (and probably other ions although we have not yet attempted to confirm this) in the medium comes to an apparent equilibrium with

that in the tissue. 5) Although Ca stimulates the respiration of this tissue (7) it depresses the uptake of Rb, an ion whose uptake into mature tissue is stimulated by Ca (12).

The fact that Ca decreases the permeability of cells to other solutes has been known for a long time. Equally well known is the seemingly incongruous fact first pointed out by Viets in 1944 (17) that Ca (and other polyvalent cations) often stimulate the uptake of K and some other alkali metal ions. It is clear that any reasonable hypothesis concerning the effect of Ca upon ion uptake must accommodate both of these facts. Our purpose here is to suggest or perhaps merely to reemphasize that Ca exerts 2 essentially distinct and antagonistic effects upon ion uptake. The first of these is a nonspecific depressant effect not directly linked with ion accumulation mechanisms and resulting from stabilization of the cell membrane with a consequent decline in permeability. The second effect we believe to be a specific one resulting from an involvement of Ca with the metabolic accumulation mechanisms of certain alkali cations and resulting in an increased rate of uptake of these ions. To this end we have investigated the effect of Ca upon Na and Rb uptake in both the zone of cell division investigated previously and the zone of cell elongation and vacuolation (1.8–3.8 mm from the root tip) where metabolic ion accumulation takes place vigorously (5, 6, 8). Rb and Na were chosen as ions which respectively do and do not display the “Viets effect” at physiological pH's (10). We have coupled this with a study of the loss of endogenous K during ion uptake.

Since the appearance of Viets' paper (17) the nature of the effect of Ca upon ion uptake has been the subject of many investigations (3, 4, 9, 10, 12, 14, 18). Like other aspects of ion uptake by plants it remains mysterious. Complete understanding must await identification of the carriers or other agencies responsible for metabolic ion uptake. A few papers which are especially pertinent should be mentioned. In 1957 Kahn and Hanson (12) reported that whereas Ca stimulated K uptake by excised maize roots it depressed K uptake by soybean roots. They analysed their data by the kinetic treatment of Epstein and Hagen (2). This analysis indicated that in both species Ca exerted a stimulatory and a depressant effect upon K uptake. The stimulatory effect predominated in maize. It was ascribed to an increased

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affinity between K and an hypothetical carrier substance because, in both maize and soybean roots, Ca lowered the calculated dissociation constant (K_s) of the carrier-K complex. The depressant effect predominated in soybean roots and was reflected (in both species) in lower calculated values of maximum uptake velocity. The analytical method employed did not permit a definite interpretation of the depressant effect. The authors proposed that it might arise in either of 2 ways: Ca might in some way lower the concentration of carrier or it might decrease the rate of K-carrier dissociation. They did not consider possible effects of Ca upon the rate of diffusion of K to sites of metabolic uptake.

Waisel (18) on the other hand has presented data in support of the idea that Ca affects uptake of monovalent ions mainly through its effects upon the permeability of the plasmalemma. He noted that at low concentrations of Na and Rb in the medium the Q_{10} of the uptake of these ions by barley roots was relatively low, about 1.4 for Na uptake and 1.8 for Rb uptake. Since Q_{10} 's of enzymatic reactions are commonly much higher he inferred that the velocity of the metabolic uptake reactions of both of these ions is normally limited by their rate of diffusion through the plasmalemma. Higher concentrations in the external solution yielded higher measured Q_{10} values, representing according to Waisel's interpretation, a shift away from a diffusion limited reaction towards one limited by the metabolic uptake process itself. The presence of Ca in the medium lowered the Q_{10} of Na uptake significantly at all concentrations of Na. The Q_{10} of Rb uptake was perhaps very slightly higher in the presence of Ca but a statistical treatment would be required to establish this. Waisel concluded from this and other evidence that Ca causes an increase in the non-metabolic diffusion rates of K and Rb while decreasing the diffusion rates of Na, Li, and probably H. It is difficult to imagine a barrier whose permeability to similar ions such as Na and Rb could be altered in opposite ways by the presence of Ca. Our data indicate that Ca reduces the permeability to both Na and Rb. However, Waisel's suggestion that the depressant effect of Ca upon Na uptake is due to decreased permeability of the cell membrane and his further suggestion that diffusion through the cell membrane is the rate limiting step in Na uptake are in agreement with our results.

In a recent paper by Foote and Hanson (4) it was demonstrated that uptake of K by soybean roots was increased by pretreatment with K-EDTA to remove part of the endogenous Ca. The increased uptake was largely due to stimulation of an initial phase of K uptake interpreted as representing a metabolically controlled diffusion of K through the outer cell membrane into the cytoplasm. A second phase of accumulation identified by the authors with deposition of K into vacuoles was little affected by short-term (30 min) pretreatment with K-EDTA. Longer exposure to K-EDTA caused depression of

this phase. Foote and Hanson believe that both phases are metabolically mediated. Our results favor the view that penetration of the cytoplasm may take place passively although the permeability of the membrane is undoubtedly influenced by metabolism as well as by the presence or absence of Ca. Except for this difference in interpretation, our results and conclusions are in accord with theirs.

Kinetic studies made by Tanada (16) indicate that the stimulatory effect of Ca upon Rb uptake is due to an increase in the affinity of Rb for an hypothetical carrier when Ca is present. He notes also that some time is needed for sufficient Ca to penetrate to Rb uptake sites to produce an observable stimulation. The implication is that the site of metabolic accumulation of Rb lies within the plasmalemma rather than at its outer surface. His data represent only short absorption periods (30 min) but appear to be in agreement with ours.

Materials and Methods

The materials and methods used in this investigation were essentially the same as those described previously (5). Five-day-old corn seedlings (*Zea mays* L. var. Peoria) grown in $2.5 \times 10^{-4} \times \text{CaCl}_2$ in the dark at 26° were again used. Sections were cut from the primary root 0 to 1.8 and 1.8 to 3.8 mm from the tip. These are designated section 1 and section 2, respectively. Twenty segments were used for each determination (approx wt. 20 mg) with a solution volume of 100 ml. Na and Rb were determined by conventional tracer techniques. The 0.005 \times solutions of these ions used were labeled with approximately 0.03 μc per ml. K was determined using the Beckman flame photometer, Model DU with a photomultiplier. For this determination the root samples were sulfated with 2 drops of a 5% solution of H_2SO_4 in 95% ethyl alcohol. The alcohol was evaporated and the root samples then dissolved and partially oxidized with about 1.0 ml of 30% H_2O_2 under an infrared lamp before being ashed at 550° for 1 hour. The resulting ash was free of carbon particles and was taken up in 10.00 ml of 50% methyl alcohol, 0.01 \times in HCl. Flame standards were prepared in the same solution. Na 22 was obtained from the Nuclear Science and Engineering Corporation. Rb 86 was obtained from the Oak Ridge National Laboratory. The labeled 0.005 \times solutions of NaCl and RbCl were prepared by adding the appropriate quantity of the carrier-free radioisotope to 5.00 ml of 1.00 \times salt in a Teflon beaker. The mixture was evaporated to dryness, rewetted and again evaporated before dilution to 1 liter. This procedure removes HCl. The pH of the solutions was about 5.8 and did not change during the experimental periods. Recrystallized NaCl, glass redistilled water and polyethylene ware were used. Points on the graphs represent the averages of 3 determinations.

Results and Discussion

The initial K content of this tissue in both sections studied amounts to 80 to 100 meq per kg on fresh weight basis. Relatively large amounts of this K are lost to the medium when the sections are placed in pure water or in NaCl or RbCl solutions. Losses to H₂O at 1.0° are shown in table I. The

Table I. *Loss of Endogenous K to H₂O*

Time (hr)	K lost, meq/kg fr wt	
	Section 1	Section 2
1	7.3	5.8
2	11.9	14.6
3	14.4	22.2
5	22.8	29.3

loss of K to water takes place over an extended period of time. At the end of 5 hours roughly one-third of the K originally present has disappeared from the tissue. It appears certain therefore that the loss involves hydrolysis of K originally adsorbed upon cytoplasmic proteins. It is a puzzle why large amounts of K remain labile in the vacuolated section (1.8–3.8 mm) which almost certainly has the capacity for metabolic accumulation of this ion. The roots are grown in a medium essentially free of K consisting of 0.00025 N CaCl₂. The K found there must derive from the seed. One would expect such K to be rapidly accumulated into vacuoles and thus rendered relatively unavailable for hydrolysis or exchange. The presence of small amounts of Ca greatly reduce the loss of endogenous K to H₂O. In 0.0001 N CaCl₂ at 1.0° only about 10% of the original K was lost in 5 hours from section 1 (0–1.8 mm). This undoubtedly explains why large amounts of K are retained in the tissue during the period the roots are growing in CaCl₂.

The uptake of Na and loss of K in 0.005 N NaCl at 1.0° by the first section (0–1.8 mm) is shown in figure 1. K loss exceeds considerably the amount of Na taken up. This is to be expected since K is also lost to pure water. In NaCl the losses of endogenous K are larger and reflect both hydrolysis

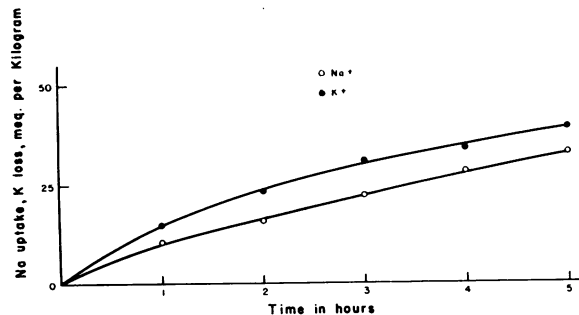


FIG. 1. Time course of Na uptake and K loss in 0.005 N NaCl. Section 1 (0–1.8 mm from root tip), 1.0°.

and exchange for Na. Similar results were obtained with this section at 26°; neither Na uptake nor K loss was appreciably affected by the temperature increase.

Corresponding data for the second section (1.8–3.8 mm) are presented in figure 2. These curves resemble greatly those obtained for the first section. Again in the absence of metabolic uptake (this being suppressed by lowering the temperature to 1.0°) the loss of K exceeds Na uptake. Figures 1 and 2 show that despite large anatomical differences existing between the tissues of the 2 sections their patterns of nonmetabolic uptake of Na and loss of K are virtually identical.

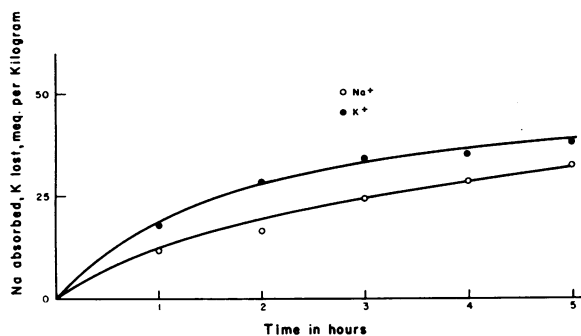


FIG. 2. Time course of Na uptake and K loss in 0.005 N NaCl. Section 2 (1.8–3.8 mm from root tip), 1.0°.

The relationship between Na absorption and K loss is significantly different in the second section when metabolic activity is allowed. This is apparent in figure 3 which depicts the course of Na uptake and K depletion by the second section at 1.0° and 26.0°. The loss of K is entirely independent of temperature in this range whereas absorption of Na is much greater at the higher temperature. Apparently the metabolic uptake of Na induced by raising the temperature is not related to the loading of the cytoplasmic proteins with Na and the consequent displacement of K from these sites. Foote and Hanson (4) have previously suggested that cytoplasmic sites freed of Ca and Mg by treatment with K-EDTA do not appear to be involved in transport of K to the vacuole. The presence of large amounts of labile endogenous K in section 2 also favors the idea that metabolic uptake does not proceed via an exchange diffusion step to deposition into the vacuoles. This problem is worthy of further study. It must be pointed out that the apparent independence of K loss found here may be illusory. It is entirely possible, for example, that some K displaced by Na may be metabolically reabsorbed i.e., the actual displacement of K may exceed the net loss.

The concavity displayed by the Na uptake curve of figure 3 deserves some comment. The rate of metabolic Na accumulation increases with time. It seems likely that this may be due to the effect of

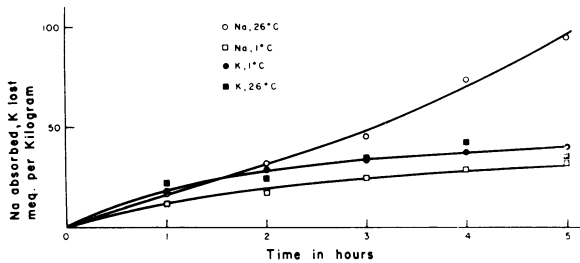


FIG. 3. Time course of Na uptake and K loss in 0.005 \times NaCl. Section 2 (1.8–3.8 mm from root tip).

Na itself upon the plasmalemma. Since these roots are grown in a dilute Ca solution we must suppose that initially the cell membrane is partially saturated with this ion and is therefore a reasonably stable structure presenting considerable resistance to Na entry. Upon exposure to pure NaCl solutions loss of stabilizing Ca may be presumed to occur with progressive loss of this resistance. As before noted the data of Waisel (18) suggested that penetration of the plasmalemma is rate limiting in Na uptake as do data to be presented here.

We turn now to the effect of Ca upon Na uptake and K loss. Figure 4 depicts the effect of increasing

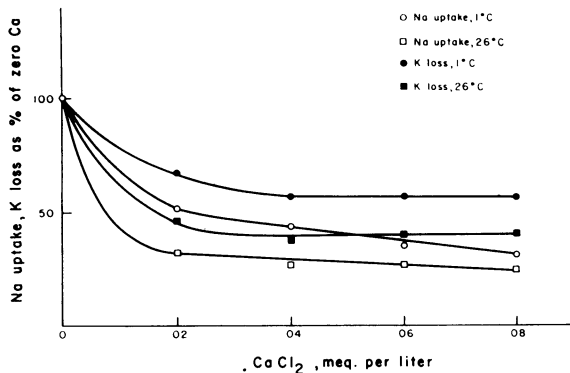


FIG. 4. Effect of Ca upon the 5 hour Na uptake and K loss in 0.005 \times NaCl. Section 1 (0–1.8 mm from root tip).

concentrations of Ca upon the Na absorbed and K lost by the first section, in which no metabolic accumulation occurs. The experimental period was 5 hours. Uptake and loss are expressed as percent of those occurring in the absence of added Ca. In this section at both temperatures, increasing concentrations of Ca have similar inhibitory effects upon Na and K loss. It is especially to be noted that the greatest part of the inhibition is achieved at a relatively low level of Ca. Concentrations of Ca above 0.03 meq per liter ($3.0 \times 10^{-5} \times$) have virtually no further effect upon the loss of K. Although considerable exchangeable K is present it apparently can not be removed from the tissue in the presence of Ca. Data such as this we believe

cast doubt upon the common practice of estimating the nonmetabolic (free space) uptake by washing tissue after an absorption period in cold CaCl_2 . Such washing probably removes completely ions adsorbed upon the cell walls. However, the results here suggest strongly that non-metabolic penetration of the cytoplasm may account for a considerable part of the total uptake (fig 3). It is evident from the data of figure 4 that this portion of the non-metabolic uptake would not be completely removed by washing with CaCl_2 . At concentrations above 0.03 meq per liter Ca causes a further albeit small reduction in Na uptake. This is probably due to competition between Na and Ca for access to the already saturated barrier membrane. Two further characteristics should be noted: At each temperature the effect of Ca is proportionately greater upon Na uptake than upon K loss and the effect upon both is greater at the higher temperature. The first characteristic may be related to changes in membrane pore size induced by Ca. Although the existence of pores in the plasmalemma may be considered unproved since no such structures have yet been actually observed, the assumption of their existence helps to explain the passive diffusion of nonlipid soluble substances through natural membranes. Measurements of equivalent pore radius based upon the osmotic pressure developed across membranes in the presence of diffusible solutes have been made by Solomon and others (15). Values for a number of different animal membranes (red cell, squid axon, etc.) ranged from 3.5 to 6.5 \AA . Whittembury, Sugino, and Solomon (19) have shown that the measured pore radius of *Necturus* kidney cells becomes significantly larger when Ca is removed from the medium. Their work indicates that the Ca effect upon pore size occurs at Ca concentrations below 1.0 mM. The sizes of the hydrated radii of Na and K are given by Jenny and Reitemeier (11) as 7.90 \AA and 5.32 \AA , respectively. It is thus possible that Ca may greatly restrict the passage of Na while affecting the diffusion of K to a lesser extent. The very large inhibitory effect of Ca upon Li uptake reported by Jacobson et al. (10) could be ascribed to this effect. The hydrated radius of the Li ion is 10.03 \AA (11). The virtual elimination by Ca of Na interference with K uptake reported by Epstein (3) could also be explained on this basis.

The temperature effect upon Ca inhibition of Na uptake and K loss noted with this section indicates that metabolism is concerned to some extent with the incorporation of Ca into the structure of the outer cell membrane. Since the effect is relatively small we may perhaps speculate that the effect of metabolism is indirect, possibly having to do with production of binding sites. Foote and Hanson (4) report increased Ca binding at the expense of ATP in corn shoot mitochondria. In our work with Sr uptake (8) we have found that the barrier to ion entry is a labile structure subject to injury or dissolution if not maintained continuously by processes de-

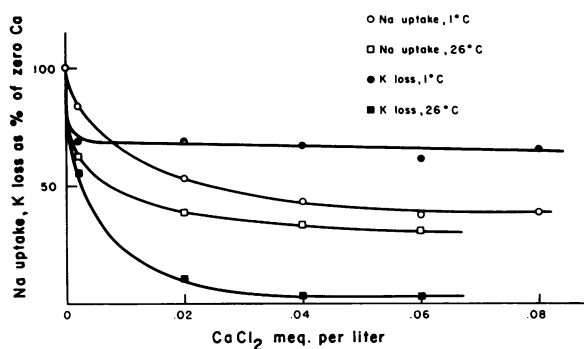


FIG. 5. Effect of Ca upon the 5 hour Na uptake and K loss in 0.005 N NaCl. Section 2 (1.8-3.8 mm from root tip).

pendent upon aerobic metabolism. It is therefore to be expected that incorporation of Ca into the membrane and hence the effect of Ca in reducing its permeability would be greater at the higher temperature.

Similar data for the second segment are presented in figure 5. The effects of Ca upon Na uptake at the 2 temperatures resemble closely those found for the first segment. That is, inhibition of metabolic Na accumulation closely parallels the blockage of nonmetabolic penetration. This is in agreement with Waisel's suggestion that penetration of the plasmalemma constitutes the rate limiting step in metabolic Na uptake. These data however do not tell us whether or not Ca may also exert an effect upon the metabolic phase of Na uptake. Is the Na which does penetrate the plasmalemma more or less efficiently sequestered in the presence of Ca? To answer this question the following experiment was performed: Root segments of section 2 were allowed to absorb labeled Na at 26.0° from 0.005 N NaCl + 0.001 N CaCl₂ and from 0.001 N pure NaCl. Approximately equal amounts of Na are absorbed in 3 hours from these solutions. After 3 hours the root segments were analyzed for total absorbed Na by counting in a sealed planchet and then immediately transferred to unlabeled 0.005 N NaCl at 1.0° for a further 3 hours, after which the Na remaining was determined. The final determination represents metabolically absorbed Na. The results are shown in table II.

It is apparent from these data that the metabolic

mechanism for Na absorption is very efficient. On the average more than 80 % of the total Na absorbed is found in the difficulty exchangeable fraction. Ca appears to be without significant effect upon the metabolic phase of Na accumulation by this tissue.

Another experiment was done which supports this conclusion. In this experiment 3 samples of the tissue (section 2) were allowed to absorb labeled Na from 0.005 N NaCl for 3 hours in the cold (1.0°). After measuring the amount of Na taken up they were transferred to (1) cold (1.0°) 0.005 N NaCl, unlabeled, (2) cold NaCl + 1.0 meq per liter CaCl₂ and (3) warm (26.0°) NaCl + CaCl₂ for 3 hours. 70 % of the absorbed Na was lost to the cold pure NaCl and 20 and 15 % to the other 2 elutants, respectively. Metabolism thus appears to play only a small role in the inhibitory effect of Ca upon loss of Na. The small effect observed is explicable in terms of better maintenance of the membrane in actively metabolizing tissue. The temperature effect upon K loss on the other hand is quite striking in the second segment (fig 5). At 1.0° a concentration of Ca as small as 0.002 meq per liter produces the maximum effect, a reduction of about 30 % in the K lost in 5 hours. This concentration is about one-tenth that required for maximum effect upon the first section. The reason for this is not known. It may be related to changes in the plasmalemma with cell development or to the relative lengths of the diffusion paths Ca must traverse in the 2 tissues. At 26.0° loss of K by the second segment is virtually abolished when the Ca concentration approaches 0.03 meq per liter. Such a large effect cannot be ascribed to better maintenance of the barrier membrane in actively metabolizing tissue, particularly in view of the small temperature effect found for the retention of Na in the presence of Ca. It is much more likely that Ca promotes the rapid metabolic reabsorption of K displaced by Na (and by hydrolysis). This appears to be a manifestation of the Viets effect. That 2 separate effects of Ca are involved is also suggested by the fact that about 15 times the Ca concentration required for maximum effect in blocking K loss at 1.0° is required for maximum effect at 26°. We interpret this to mean that the site of the Viets effect lies deeper in the cell, perhaps at the tonoplast. The experiments dealing with Rb uptake support this conclusion.

The effect of Ca upon the 5-hour uptake of Rb from 0.005 N RbCl and upon the accompanying loss

Table II. Effect of Ca upon Na Retention

Absorption medium	Total Na* absorbed	Na found* after elution	Na retained %
0.005 N NaCl	11.3	9.3	82.3
+ 0.001 N CaCl ₂	10.6	8.7	82.1
0.001 N NaCl	9.7	7.9	81.4
	8.7	6.9	79.3

* meq/kg, fresh weight.

of K from the first segment is shown in figure 6. This experiment was done at 26.0° but represents nonmetabolic uptake since no metabolic absorption takes place in this segment (0–1.8 mm) of the root tip. The curves generally resemble those obtained for Na uptake by this section (fig 4). There are, however, 2 significant differences: Ca exerts an appreciably smaller effect upon Rb uptake than upon Na uptake in this range of concentration and the effects of Ca upon Rb uptake and K loss are more nearly equal, the inhibition of K loss being slightly greater than that of Rb uptake. Na uptake is inhibited considerably more than K loss in this section (fig 4). This is in agreement with the suggestion made above that the inhibitory effect of Ca, i.e., its effect upon the rate at which a given ion can penetrate the plasmalemma should depend upon the position of the ion in question in the lyotropic sequence. The hydrated radius of Rb is 5.09 \AA , slightly less than that of K, 5.32 \AA (11). The data of figure 6 do not support Waisel's (18) conclusion

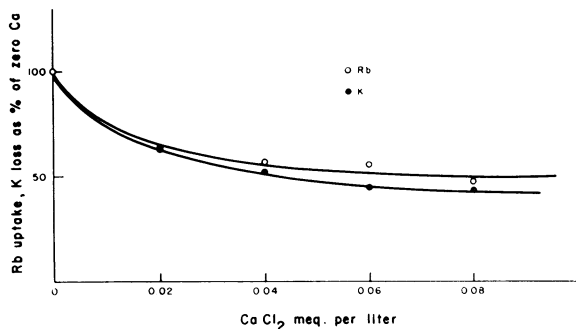


FIG. 6. Effect of Ca upon the 5 hour uptake of Rb and loss of endogenous K in $0.005 \times \text{RbCl}$. Section 1 (0–1.8 mm from root tip), 26.0° .

that Ca actually increases the permeability of the cell membrane to Rb. Rather, in this tissue the effects of Ca upon Rb uptake are similar in kind but not so severe as those upon Na uptake.

The effects of Ca upon Rb uptake by the second section at 26.0° are shown in figure 7. Here the 2 postulated effects of Ca upon ion uptake can be clearly seen. At very low concentrations of Ca a fairly well marked inhibition of Rb uptake is observed. A concentration of approximately $0.002 \text{ meq per liter}$ ($2 \times 10^{-6} \times$) is apparently sufficient to saturate the membrane and thus hinder Rb penetration. Note that this concentration is quite close to that causing maximum inhibition of K loss at 1.0° (fig 5). As the concentration of Ca is raised above $0.002 \text{ meq per liter}$ metabolic uptake is stimulated more and more. At a Ca level of $1.0 \text{ meq per liter}$, Rb uptake is slightly greater than that taking place in the absence of Ca. Since the 2 effects are complementary upon K retention the loss of K becomes almost nil at a very low Ca concentration. To the best of our knowledge Ca is the only inhibitor of ion uptake that shows the type of concentration dependence

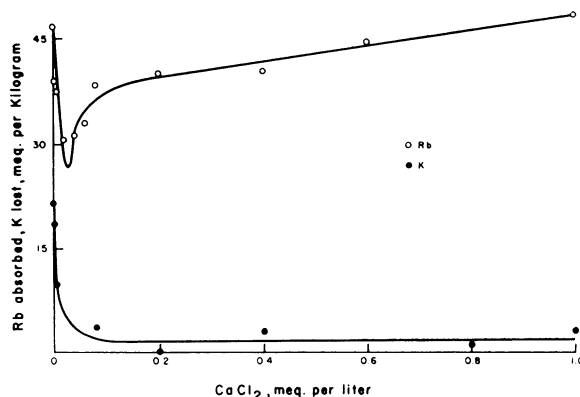


FIG. 7. Effect of Ca upon the 5 hour uptake of Rb and loss of endogenous K in 0.005 RbCl . Section 2 (1.8–3.8 mm from root tip), 26.0° .

shown in figure 7. We believe that the concentrations of Ca producing either an overall stimulation or an overall depression of Rb absorption are dependent upon the absorption period chosen. The data of figure 8 suggest that although the depressant effect is manifested very quickly the stimulatory effect requires a rather longer period of time to become apparent possibly because diffusion of Ca to sites of active Rb accumulation is involved. Figure 8 depicts the course of Rb uptake and K loss over an 8-hour period at 26.0° by the second section without added Ca and with $1.0 \text{ meq per liter Ca}$. At this Ca level the inhibitory effect predominates during the first hour or so and this is reflected in a markedly lower initial rate of Rb uptake. Following this, the rate quickens and eventually exceeds considerably that obtaining in the absence of Ca. This is in agreement with the results obtained by Tanada (16) who also found that some time is needed for Ca to penetrate the cell to sites of active absorption.

Whereas the time curve of Na uptake in the absence of Ca is slightly concave (fig 3) that for Rb uptake is convex. This is in harmony with the suggestion that the resistance to penetration of the

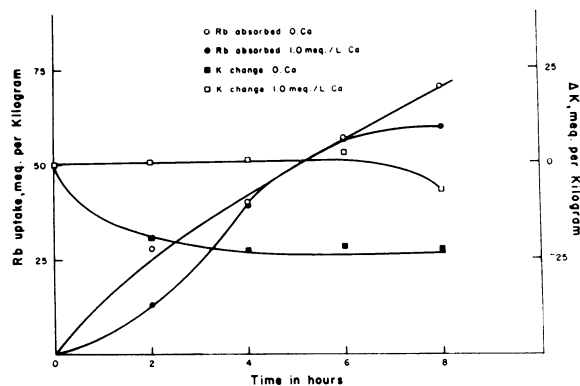


FIG. 8. Time course of Rb uptake and K loss in $0.005 \times \text{RbCl}$. Section 2 (1.8–3.8 mm from root tip), 26.0° .

plasmalemma depends upon the size of the hydrated ion. Rb apparently encounters little resistance compared with that encountered by Na.

After 6 hours the rate of Rb uptake in the presence of Ca drops sharply (fig 8). This slackening after a relatively long absorption period may be due to depletion of substrate or other causes unknown. It is apparently accompanied by some loss of K. In the absence of added Ca, accumulation of Rb proceeds for a longer period. Ca stimulates the respiration of this tissue (7) so that earlier depletion of substrate is to be expected. Also, K and Rb are very probably transported by identical mechanisms (2, 3). In the presence of Ca and Rb, therefore, we must suppose that the amount of cations transported by this mechanism is represented by the sum of Rb absorbed and K retained over that which leaks out in Ca-free Rb solutions. If this is the case an earlier slackening of Rb uptake in the presence of Ca is perhaps to be expected. It is clear, however, that this explanation will require further verification.

The delay involved in Ca stimulation of Rb uptake can be eliminated by pretreatment of the tissue with CaCl_2 . This is shown in figure 9. To obtain

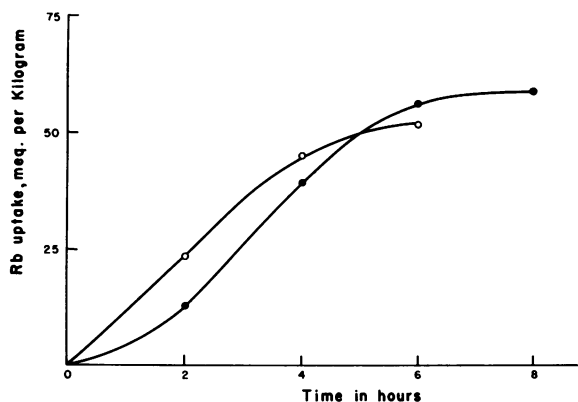


FIG. 9. Effect of Ca pretreatment upon subsequent Rb uptake in 0.005 N RbCl + 1.0 meq/liter CaCl_2 , Section 2 (1.8–3.8 mm from root tip), 26.0° . O = Tissue pretreated for 2 hours in 1.0 meq per liter CaCl_2 at 26.0° . ● = No pretreatment.

these data the segments (1.8–3.8 mm) were placed for 2 hours at 26.0° in a solution containing 1.0 meq per liter CaCl_2 and subsequently transferred to 0.005 N RbCl with the same concentration of Ca. The results support the hypothesis that Ca must penetrate to sites deeper inside the cell than the plasmalemma before its stimulatory effect upon Rb uptake is manifested. Note also that the slackening of Rb uptake occurs 6 hours after the sections are exposed to Ca rather than after exposure to Rb. Quite evidently Ca initiates or greatly stimulates some process which entails an increased rate of Rb uptake but which is either completed in 6 hours or ceases because of substrate depletion or other causes.

Summary

The effects of Ca upon metabolic and nonmetabolic uptake of Na and Rb by segments of the primary root of *Zea mays* were investigated. The results obtained support the hypothesis that Ca exerts 2 antagonistic effects. The first effect is inhibitory and results from a modification of the permeability of the outer cell membrane. This effect reduces the nonmetabolic penetration rates of K, Na and Rb. The second effect is stimulatory and results from an involvement of Ca with metabolic uptake mechanisms. The site of this effect apparently lies deeper in the cell than the plasmalemma. Only the first effect was demonstrated for Na uptake. Both effects are apparent in the effect of Ca upon Rb uptake and K depletion.

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Colony Formation by Isolated *Convolvulus* Cells Plated on Defined Media^{1, 2}

Elizabeth D. Earle³ and John G. Torrey

The Biological Laboratories, Harvard University, Cambridge, Massachusetts

In recent years the cultivation of small clumps of vegetative cells and even single cells from higher plants has been achieved in several laboratories. Muir, Hildebrandt and Riker (14, 15) established the first plant tissue culture clones (callus tissues of single cell origin) by placing single cells on filter paper over nurse callus cultures. Torrey (22) was able to demonstrate division of single isolated pea root callus cells set around a callus piece on yeast-extract medium. Jones et al. (11) showed that single tobacco cells could divide and survive for long periods in a microchamber containing liquid medium in which callus tissue had previously been grown (conditioned medium).

Suspension cultures of plant cells in agitated liquid medium [see (20) for review] are useful for large-scale quantitative experiments with cell populations, but the fate of individual cells cannot be followed in such cultures. A plating technique developed by Bergmann (1) made it possible both to handle many cells at a time and obtain single cell clones. In 1 experiment, Bergmann found that 40% of plated *Phaseolus* cells divided at least once, and 13% formed clones of more than 32 cells. The effectiveness of Bergmann's technique of plating filtered cell suspensions in agar medium has been confirmed using cells of *Convolvulus arvensis* (24), *Haplopappus gracilis* and carrot (3, 8), and tobacco (9).

The media used in experiments with isolated cells have almost always been complex rather than defined, containing coconut milk or yeast extract or having been conditioned by the presence of large numbers of cells. Isolated single cells have shown quite exacting nutritional requirements. Kato and Takeuchi (12) found that 1 carrot cell alone divided only on conditioned yeast-extract medium while many single cells together on the same plate could divide on unconditioned yeast-extract medium. In a recent paper, Blakely and Steward (3) reported that free carrot cells divided much more frequently when the cells were plated in conditioned coconut milk media and when carrot root explants were placed on the medium near the cells.

Defined media have usually proved inadequate for supporting division in inocula as large as several hundred plated cells. Bergmann (1) and Torrey and Reinert (24) reported that cells plated on media lacking coconut milk or yeast extract were capable of a few divisions but not of continued growth. In their experiments no attempts were made to replace the complex components of the media with vitamins, hormones, or amino acids; however, Gibbs and Dougall (9) also failed to obtain clones from tobacco cells plated on a defined medium which supported growth of tobacco callus.

The only report of sustained division of small numbers of single plant cells on a defined medium is that of Reinert (17). When friable crown-gall callus from *Vitis vinifera* was spread on agar medium containing only salts, glucose, and thiamin, 40% to 70% of the single cells present divided. Some eventually produced clones. Larger cell masses were also present on the agar in this experiment. It seems significant that success was achieved with crown-gall cells, which have greater synthetic capacities and less elaborate nutritional requirements than normal cells (5).

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³ Present address: Department of Biology, Princeton University, Princeton, New Jersey.