ROLE OF THE ROOT TIP IN DEVELOPMENT OF ENHANCED RUBIDIUM UPTAKE IN WASHED, EXCISED CORN ROOT TISSUE

By

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Major Department: Botany

"Low salt" excised corn root segments (DeKalb 805A) were used throughout this study. ^{86}Rb was used as a tracer for RbCl. When excised corn roots in which the apical 5 mm had been removed were washed in a dilute, well-aerated, $CaSO_4$ solution, the rate of Rb uptake increased rapidly with duration of washing. When root segments were washed in a water-saturated atmosphere, enhanced ion uptake did not develop during the first hour, and developed only slightly afterwards. Segments held in cold $CaSO_4$ solution, or in a solution of $CaSO_4$ and PEG-6000, or mannitol of sufficient strength to lower the osmotic potential to -8 bars, did not show enhancement. However, once removed from the stressing environment and placed in $CaSO_4$ solution at $30^{\circ}C$, these segments developed a capacity for a higher rate of ion uptake similar to controls. A study of the enhancement phenomenon in different portions of the primary root revealed that this response decreased with increasing distance from the tip, and at 65 mm from the tip it was absent. Segments taken 5 to 15 mm behind the tip developed the highest

rate of uptake. Ion uptake in these segments attained a maximum rate in 2 hours and remained constant thereafter.

When the terminal 5 mm of the root was left attached during aging, but removed immediately before absorption, the rate of Rb uptake was only 28 per cent of controls in which the tip was detached before washing. When decapitated root segments were aged in the culture solution in which seedlings had grown during the previous 24-hour period, the rate of uptake was 63 per cent of controls aged in fresh solution. Aging in concentrated culture solution was even more inhibitory to the enhancement response. When segments were incubated in fresh solution containing free-floating, freshly excised tips, the rate of uptake was 20 per cent less than in samples aged in solution containing no free excised tips.

Tray-grown root segments showed a lag of 20 minutes before any increase in uptake was apparent. This lag was shorter in solution-grown roots. The evidence presented in this study shows that the enhancement in the rate of Rb uptake with time by excised corn roots is strongly influenced by the root tip. Moreover, the results also indicate that this action is mediated by an unidentified substance which is synthesized by the root tip and translocated further back where it inhibits Rb absorption.

INTRODUCTION

The use of excised root tissue in mineral absorption studies is a common practice. There are several advantages of using isolated tissue or organs. The ease of handling the material makes it possible to establish better control over experimental procedures. Factors influencing the absorption of substances by the root system of plants are many. Moreover, the absorption by roots is also influenced by other processes taking place in the plant. For example, the rate of transpiration affects the rate at which water is absorbed by the roots. By using excised roots, the study of absorption is limited to those processes originating in the root itself, thus avoiding complicating effects from other organs of the plant.

The advantages of using "low salt" roots in experiments measuring rates of absorption are evident when one considers that in this tissue accumulation takes precedence over translocation out of the root. In using excised roots in uptake studies, it is desirable to reduce translocation to a minimum. This is further accomplished by short absorption periods. Epstein et al. (15) listed the advantages of short-term solute absorption periods by plant tissue. However, it is realized that difficulties are encountered when trying to relate results obtained in this manner to processes taking place in intact plants in nature. Pitman (37) cautioned against assuming that the uptake by low salt roots is the same as the uptake by plants in nature. Even though the soil in which a plant is growing may be poor

in nutrients, trace quantities of these elements are present, and plants are known to "mine" the medium in which they are growing.

Even though it has been known that different parts of the root respond differently to the accumulation and translocation of minerals, relatively little attention has been paid to the rate of uptake by different segments of the root. It is known that the root tip is morphologically, as well as physiologically, different from the more mature parts of the root. Moreover, it is known that hormone synthesis takes place in the root meristem, yet little work has been done to assess the role of the root tip in physiological processes taking place distal to the meristem. The root as an organ has been characterized as being less complex than the stem. While this may be true morphologically, physiologically the root is very complex. It was shown in a recent study by Leonard and Hanson (24) that the capacity of excised corn root tissue to absorb ions increased with time of incubation in a well-aerated $CaSO_4$ solution. This phenomenon was also observed in our laboratory in connection with studies dealing with recovery from physiological drought. In the present study, this increase in the rate of mineral uptake with time which we call the aging response ("washing" by Leonard and Hanson) was further investigated. The role of the root tip in the development of this response is specifically examined and its mediation by an endogenous inhibitor is proposed.

REVIEW OF LITERATURE

Enhancement of Solute Uptake by Disks of Storage Tissue

Enhanced uptake was first observed in disks of storage tissue during aging in aerated water, or $CaSO_4$ solution (3, 47). This increase in uptake was accompanied by an increase in the rate of respiration (47). Asprey (3) was the first to show clear evidence of enhanced ion absorption capacity by disks of potato and beets aged in an aerated solution. Parallel with this increase in ion absorption, there was an increase in respiration (26, 47). Laties (22) attributed the increased respiration rates in aged disks of storage tissue to the development of vigorous phosphorylation. Other workers have contended that the increase in respiration is due to increased protein synthesis (2). Ellis and MacDonald (11) have reported an increased rate of leucine incorporation into proteins in beet disks with time in aging solution. The same workers have reported also an increase in nucleic acid synthesis. In a later report, MacDonald *et al.* (29) showed that puromycin inhibited the development of Cl absorption capacity in aging disks of storage tissue. There seems to be a general increase in all aspects of metabolism with time. Anderson (1) stated that the aging response in storage tissue was due to the breaking of dormancy. Bryant and Ap Rees (8) summarized the aging phenomenon in slices of storage tissue as "...a reversal of changes that accompany dormancy."

Enhancement of Solute Uptake by Excised Stem and Leaf Tissue

More recent studies have shown that other plant tissues are also capable of increasing their capacity to absorb substances from the surrounding medium when incubated in well-aerated solutions of various salts or water. Bieleski (5) isolated vascular bundle tissue from celery petioles and aged it in $CaSO_4$ solution for 20 hours. After 4 to 10 hours of aging, the rate of phosphate uptake rose to 50 times that of fresh tissue. The sucrose accumulation rate was 3 to 8 times that of fresh controls. Bieleski suggested that the low affinity uptake mechanism of solute absorption was present in fresh tissue, while the high affinity mechanism developed during aging of the tissue. Hancock (18, 19) arrived at the same conclusion for 3-o-methylglucose (a non-metabolizable derivative of glucose) absorption by squash hypocotyls. The formation of the high affinity mechanism was not affected by light conditions, shaking, or the absence of $CaSO_4$ from the solution (19). A typical response curve obtained in these tests can be described as a lag of 2 hours, then a rapid increase in uptake up to 12 hours followed by a constant rate thereafter.

Palmer and Loughman (34) have reported that incubation of pea stem segments in potassium maleate buffer caused an increase in the rate of phosphate absorption. At the same time the rate of respiration decreased. Cotton and sunflower stem segments incubated in the same medium failed to show any increase in the rate of phosphate absorption. They showed a doubling in the rate of Rb absorption by pea stem segments after 18 hours of incubation. In a later study, Palmer and Blackman (35) reported that the enhancement in phosphate uptake which developed during washing could be prevented by the addition of

2,4-D to the washing solution. Leonard and Hanson (24) confirmed the inhibitory effects of 2,4-D and IAA on the development of enhanced phosphate uptake. However, they also reported that washing in the presence of 2,4,6-T, a substance which has little auxin activity, was even more inhibitory.

Rains (41) noted an increased absorption of K by bean stem slices aged for 20 hours in $CaSO_4$ solution at 30°C. He showed that this enhancement in K absorption was prevented if the tissue was incubated at 4°C. Addition of benzyladenine (an analog of kinetin) to the absorption medium inhibited the enhancement of K absorption. The same effects of benzyladenine have also been shown in aging disks of tobacco leaves (44). In a later report, Rains and Floyd (42) reported that the increased K absorption rate in aged bean stem slices was promoted by Ca. Aging of bean stem slices in the presence of cycloheximide prevented the enhancement of K uptake, thus indicating that inhibition of protein synthesis also prevented the increase in K uptake developed during aging. The process controlling the increased ion uptake in bean stem tissue appeared to be different from the process in storate tissue in as far as there was no increase in the rate of oxygen uptake. On the contrary, respiration decreased during the first 4 hours of aging (16).

Macklon and Higinbotham (30) reported a considerable increase in K and NO_3^- content in excised segments of pea epicotyls immersed in complete nutrient solution for 72 hours, as compared to intact, etiolated tissue. During this time, there was an increase in cell electropotential differences. Although the increase in ion uptake showed a lag of 6 to 8 hours, the difference in cell potential increased

rapidly. They considered the increase in potential difference a prerequisite for the rapid ion accumulation. Incubation of sufficient duration in water, prior to placing in solutions containing K, eliminated the lag in K uptake. Sacher (43) reported an increase in uptake of orotic acid, glucose, and phenylalanine from 5- to 50-fold in bean endocarp after aging the tissue in water for 24 hours. Auxin (NAA) largely prevented this enhancement in substrate uptake.

Enhancement of Solute Uptake by Excised Root Tissue

Enhanced solute uptake has also been reported for non-dormant, excised root tissue. Tanada (48) using segments of mung bean roots showed an enhancement in Rb uptake with time by samples incubated in solutions containing Ca. No increase in uptake was shown when Ca was absent. In the presence of Ca, the uptake of Rb was not immediately stimulated. There was a lag period of about 10 minutes before the rate of absorption gradually increased and then proceeded linearly at a rate much faster than the initial rate. In mung bean, the enhancement of Rb uptake was most pronounced in the 2.5 to 5.0 mm zone from the root tip. Tanada attributed the enhanced Rb capacity to effects of Ca on the availability of binding sites in the membrane-bound carrier. The role of Ca in ion transport by plant tissue has been emphasized by Epstein (13).

Cereals, particularly barley, have been favorite plants for mineral uptake studies. Pitman (39) reported that when excised barley roots were kept in 0.5 mM $CaSO_4$ solution for 2 to 4 hours, this tissue took up more K than either intact plants or freshly excised roots. He attributed the net increase in K uptake to an increased selectivity

of K over Na, and to increased K efflux from the tissue. However, no difference in tracer uptake was reported. In a recent study Pitman et al. (40) reported on the changes in electropotential difference between excised barley roots and the dilute $CaSO_4$ solution in which they were aging. After 6 to 8 hours of aging, the potential difference increased from 65 millivolts to 185 millivolts. As previously reported, there was no difference in tracer uptake between fresh and aged tissue. They suggested 2 possible hypotheses to explain this effect of aging on potential difference: (1) that cutting exposed plasmadesmata which are leaky initially, but which seal in time, and (2) that some internal factor, such as hormones, have a regulatory effect on cell potential; an influence which dissipates with time after excision. Leonard and Hanson (24) reported a 150 per cent increase in the rate of phosphate absorption by barley root segments after 3 hours of washing in aerated 0.2 mM $CaCl_2$ solution, and a 200 per cent increase in phosphate absorption by oat root tissue incubated for the same period of time under the same conditions.

There are several reports of increased rate of mineral absorption in excised corn root tissue. Brown and Cartwright (7), using apical segments of corn roots in which cells were not fully elongated, showed that increased Rb uptake was due to higher protein content in expanding cells. Handley et al. (20) using segments of corn root tissue taken 1.8 to 3.8 mm from the root tip,showed that aging increased the rate of Rb uptake over freshly excised tissue. When pre-incubated for 2 hours in $CaCl_2$, the rate of absorption remained constant. They attributed this phenomenon to a dual effect of Ca on Rb uptake: an initial inhibitory effect, followed by a stimulation with time. A

similar effect of Ca on the rate of K uptake in corn root tissue, but not in barley, was reported by Elzam and Hodges (12).

Laties and Budd (23) reported a 20-fold increase in Cl uptake by aged corn root steles over fresh steles, while the increase in aged cortex tissue was only 2.5 times that of freshly separated cortex. They attributed this difference in absorption between fresh and aged steles to a leaky condition of freshly isolated steles. That some stelar cells are normally leaky was proposed earlier by Crafts and Broyer (10). In a later study, Luttge and Laties (27) reported a 10-fold increase in K absorption by aged corn root steles. They concluded that this increase in uptake in aged steles was due to development of a high affinity mechanism of solute uptake (14). Yu and Kramer (49, 50) reported a higher content of Rb in excised corn root tissue than in intact roots after 23 hours of absorption. However, these workers were not able to confirm the early findings of Laties and co-workers on the increased capacity of uptake by aged, isolated steles over the cortex. In a recent study, Hall *et al*. (17) reported a greater increase in the rate of Cl uptake by isolated steles than by cortex of corn roots, after 24 hours of aging. However, the increase in uptake by isolated steles was much less when the seedlings were grown under sterile conditions.

Leonard and Hanson (24) have reported a 280 per cent increase in the rate of phosphate absorption by excised corn root tissue that had been aged in 0.2 mM $CaCl_2$ solution for 3 hours before absorption. This enhanced rate of uptake was prevented by anaerobiosis, low temperatures, metabolic inhibitors, and several plant hormones. An enhanced uptake capacity appeared to be general, since the rate of

absorption of several substances was also increased during 2 hours of washing in dilute $CaCl_2$ solution. The presence of Ca in the washing solution was not necessary since incubation in distilled water gave similar results. After a lag of 30 minutes, the tissue showed a constant rate of increase which continued for 4 hours before leveling off. Analysis of membrane-bound ATPase showed an increase with time in washing (25). Electron microscopy of subcellular structures of fresh and washed tissue showed no detectable changes due to washing.

Since efflux of ions has been reported to take place readily (21, 31, 36, 38), some workers have attributed the increase in absorption to the loss of minerals during aging (39). However, Leonard and Hanson (24) reported net accumulation of K after the tissue was washed at 30°C, but not in freshly excised tissue. Handley et al. (20) showed no change in K content in corn root tissue after 6 hours of aging in solution.

The influence of microorganisms on the development of enhanced solute uptake by excised tissue has been reported by several workers. Palmer (33) and MacDonald (28) have shown that in storage tissue, non-sterile conditions reduced considerably the rate of uptake. However, Bowen and Rovira (6) reported that uptake of ^{32}P by tomato and clover tissue was higher when plants were grown in non-sterile conditions. Barber and Frankenburg (4) reported rates of uptake of phosphate and Rb by excised barley roots to be higher in plants grown in non-sterile conditions than in plants grown under sterile conditions. Leonard and Hanson (24) aged corn root segments in the presence of chloramphenicol and found no difference in the rate of phosphate uptake between control and treated samples during 8 hours of incubation.

MATERIAL AND METHODS

Plant Material

Solution-grown Roots

The tissue was obtained from the primary root of 4-day-old seedlings of Zea mays (DeKalb 805A) grown according to the method described by Smith et al. (46), but slightly modified. One hundred grams of dry seeds were placed in a 2-liter Erlenmeyer flask containing 1 liter of distilled water, shaken and the water drained. This washing procedure was repeated twice. The seeds were surface sterilized in 200 ml of 15 per cent Clorox (5 per cent sodium hypochlorite) to which a few drops of liquid detergent were added. After 5 minutes of gentle shaking, the detergent-Clorox solution was poured off and the seeds rinsed with distilled water 10 times. In the last 3 rinses the flask was allowed to overflow. The flask containing the seeds was filled to the 1800 ml mark with distilled water and the seeds were soaked 18 to 20 hours with vigorous aeration. Then the seeds were rinsed 3 times with distilled water, and the flask was filled to the 1800 ml mark with distilled water and placed in the incubator. After 2 to 3 more hours of soaking, the water was decanted, and the seeds rinsed with distilled water. At this stage most of the seeds showed the coleorhiza protruding through the seed coat. Seeds not showing the coleorhiza at the end of the soaking period were discarded. The remaining seeds were planted between 2 layers of boiled, coarse-grade

cheesecloth. The cheesecloth was supported by a stainless steel mesh and hung at the top of a 4-liter beaker filled with 0.2 mM $CaSO_4$ solution. The seeds were distributed among 4 beakers. A sintered glass aeration tube was placed inside a glass jacket and this assembly placed in each beaker for aeration purposes. The corners of the cheesecloth were placed to wick the solution, but the seeds were held a few mm above the aerated solution. The beakers were covered with a watch glass and placed in the incubator. After 24 hours the top cheesecloth was removed and discarded and the seeds washed thoroughly with distilled water. At this stage most of the roots were about 2 centimeters long and were extending into the solution. The seeds were placed over fresh 0.2 mM $CaSO_4$ solution in a clean beaker, the watch glass replaced, and the beakers placed back in the incubator. The third day the same procedure was followed, except that the watch glass was not replaced because of the length of the coleoptiles. The roots were harvested the fourth day. At this stage the roots were usually 12 to 15 cm long.

Tray-grown Roots

The seeds were sterilized as described above. After the tenth rinse with distilled water, the seeds were planted in Pyrex trays, with the embryo side in contact with several layers of white paper towel saturated with 0.2 mM $CaSO_4$ solution. The trays were covered with transparent food wrap in which holes were punched to allow gas exchange. The trays were placed in the incubator at 28°C and left undisturbed until harvest time. The roots were harvested the fourth day. At this stage the roots were usually 8 to 10 cm long.

Preparation of Samples

Root segments of either 3 cm or 1 cm, taken 5 mm from the root tip, were the experimental material. Immediately after excision, the segments were placed in a small fiberglass basket in a 2-liter beaker containing 0.5 mM $CaSO_4$. They were then agitated for 1 minute to remove material from damaged cells, removed, gently blotted, weighed to $\pm$ 0.1 mg, and placed in fiberglass bags for handling. Each bag of 10 or 15 root segments was prepared separately and as rapidly as possible, requiring 6 to 8 minutes.

Aging Procedure

The aging solution consisted of 4 liters of 0.5 mM $CaSO_4$ solution prepared in 4-liter beakers. The temperature of the aging solution was maintained at 30°C in a water bath. The solution was vigorously aerated throughout the aging process. Hereafter these are referred to as standard aging conditions. Seven or eight samples, each containing 10 or 15 root segments were aged in each beaker before discarding the solution.

Determination of K Content

Samples used for K content determination were rinsed 3 times in distilled water after aging, placed in crucibles and ashed at 500°C. The ashes were dissolved in distilled water and diluted to exactly 25 ml in a volumetric flask. The K content was determined by using a Flame Emission Spectrophotometer (Beckman B and DU).

Rubidium Absorption

Preparation of the Solution

The absorption solution consisted of 0.1 mM RbCl and 0.5 mM $CaCl_2$, prepared in a 2-liter volumetric flask. Enough ^{86}Rb was added to give a counting rate of approximately 10,000 cpm per µmole of RbCl. Four-hundred ml volumes were poured in 500 ml wide mouth Erlenmeyer flasks and placed in a water bath at 30°C. The flask contents were aerated vigorously throughout the absorption period. At the end of 10 minutes of absorption, the concentration of Rb in the solution had decreased less than 1 per cent.

Uptake Procedure

Thirty seconds before absorption started, each sample was removed from the aging solution, and swung around to remove excess water from the bag. At exactly zero time the sample was dropped in the absorption solution. After 10 minutes, the absorption of Rb was stopped by dropping the sample in cold (3°C) exchange solution, containing 5.0 mM KCl and 0.5 mM $CaCl_2$. After 3 rinses, the samples were submerged for 30 minutes in 4 liters of aerated exchange solution kept at 3°C. At the end of exchange the samples were rinsed 3 times in distilled water and the tissue placed in stainless steel planchets for determination of radioactivity.

Assay of Radioactivity

The planchets containing the samples were ashed at 500°C. After cooling, the ashes were dissolved in water, and a drop of detergent solution added to break the surface tension. The samples were evap-

orated to dryness under low heat on a hot plate. The activity of the samples was determined by a low-background counting system in which the sample detector was a G-M tube with window thickness of 150 $\mu g/cm^2$. The background averaged less than 2 cpm. Each sample was counted at least 2 times to 10,000 counts, or for 10 minutes, whichever came first. Each experiment was repeated at least 2 times with similar results. The data shown, however, are from single experiments.

EXPERIMENTS AND RESULTS

Development of Enhanced Rate of Rubidium Absorption

When 3-cm segments of corn root, in which the terminal 5 mm of the tip had been removed, were aged in well-aerated 0.5 mM $CaSO_4$ solution, the rate of Rb absorption increased rapidly (Figure 1) for several hours until it reached a maximum and then approached a constant value. In some experiments, however, after reaching a maximum, the rate of uptake declined. This was attributed to a depletion of food in the excised tissue. Figure 1 shows that after 8 hours of incubation, the rate of absorption of Rb was 13 times that of freshly excised tissue.

To test whether the development of the enhanced rate of absorption was due to excision, an experiment was designed in which one group of roots were excised and aged under standard conditions. In another group, intact roots were incubated in 0.5 mM $CaSO_4$ at 30°C, and then segments excised before determining the rate of absorption. At different periods of time after aging (incubation) started, a sample from each treatment was taken out and its rate of Rb uptake determined. The results of this experiment are shown in Figure 2. In both groups the capacity to absorb Rb was increased. However, the rate of absorption was greater in roots which had been excised. Excision amplified this response, but was not the cause of it. Six hours after transfer to fresh $CaSO_4$ solution, segments from roots

Figure 1. Development of enhanced Rb absorption in 3-centimeter excised, tipless corn root segments aged in $CaSO_4$ solution. The concentration of the aging solution was 0.5 mM.

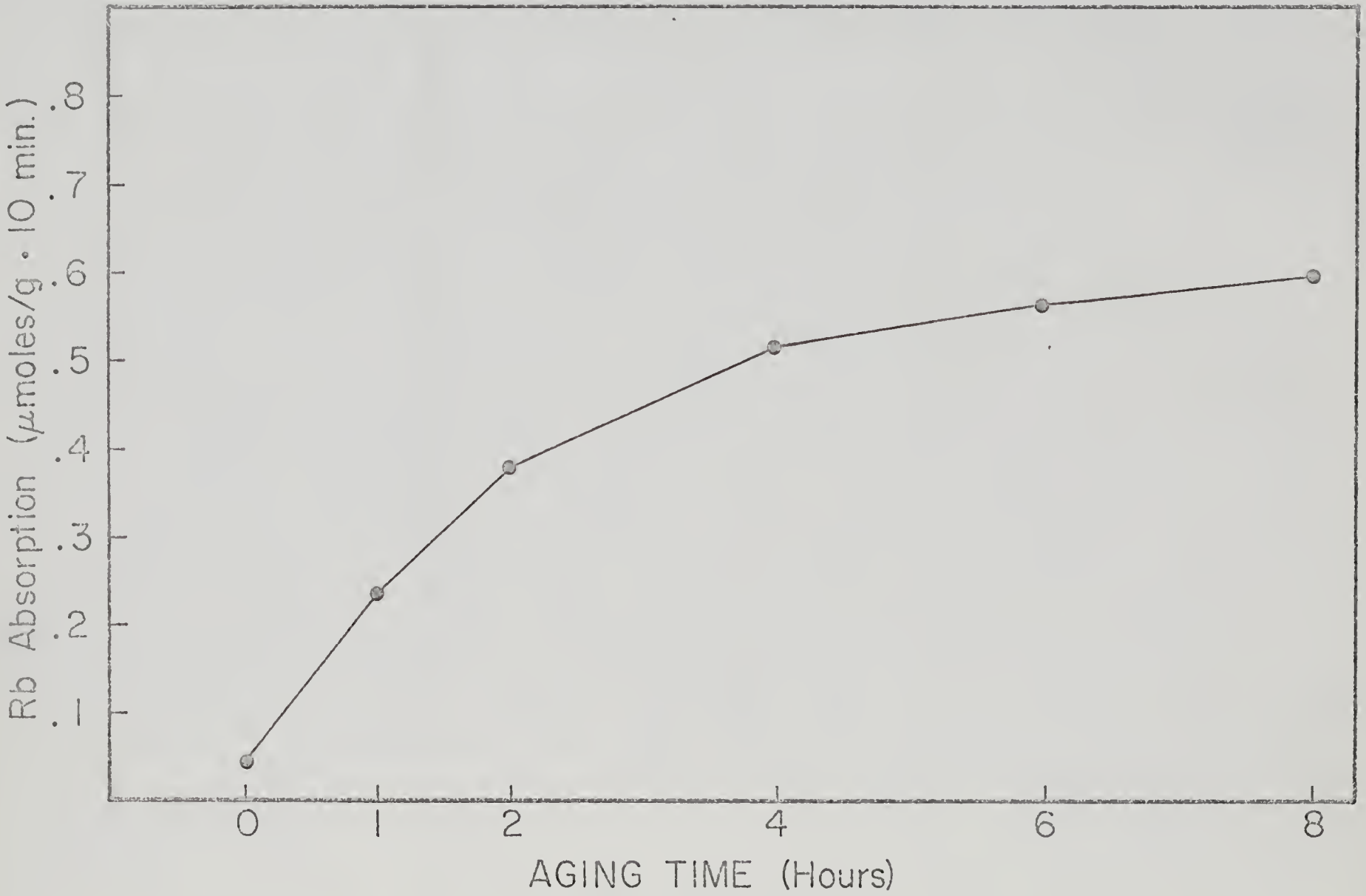

Rb Absorption (μmoles/g · 10 min.)
.1
.2
.3
.4
.5
.6
.7
.8
0
1
2
4
6
8
AGING TIME (Hours)

Figure 2. Development of enhanced Rb absorption in corn roots aged intact or aged as 3-centimeter excised, tipless segments. The aging solution consisted of 0.5 mM $CaSO_4$. Root segments were taken 5 to 35 mm from the tip.

Rb Absorption (μmoles/g · 10 min.)
.1
.2
.3
.4
.5
.6
0
1
2
3
4
5
6
TIME IN FRESH $CaSO_4$ (Hours)
Excised Roots
Intact Roots

which had been incubated intact were absorbing Rb at a rate 6 times that of samples which had been given zero aging time. After 6 hours of aging, excised segments were absorbing at a rate 9.5 times that of excised roots at zero aging time.

Effects of Environmental Stresses on the Subsequent Development of Enhanced Rate of Rubidium Uptake

Two of the most common environmental stresses encountered by plants in nature are low temperatures and drought.

Effects of Low Temperature

Three-centimeter excised root segments were aged for different periods of time in aging solution at either 3°C or 30°C before Rb absorption. Both solutions were well aerated. Figure 3 shows the results of this experiment. When excised roots were aged at 3°C, the enhancement of Rb uptake was largely prevented, and at the end of 4 hours of aging it was still only 17 per cent of samples aged at 30°C for the same period of time. This is consistent with the concept that mineral absorption by roots is metabolically dependent since low temperature is known to reduce metabolism. Figure 4 shows the results of an experiment in which root segments were pre-treated in cold $CaSO_4$ solution for 30 minutes before aging under standard conditions. Thirty minutes of cold treatment at 3°C did not prevent the subsequent development of enhanced Rb uptake. Cold-treated samples showed a slightly lower rate of uptake than control samples. This has been interpreted as being due to chilling injury.

Figure 3. Development of enhanced Rb absorption in 3-centimeter excised, tipless corn root segments aged at 3°C as compared to 30°C. The aging solution consisted of 0.5 mM $CaSO_4$. Root segments were taken 5 to 35 mm from the tip.

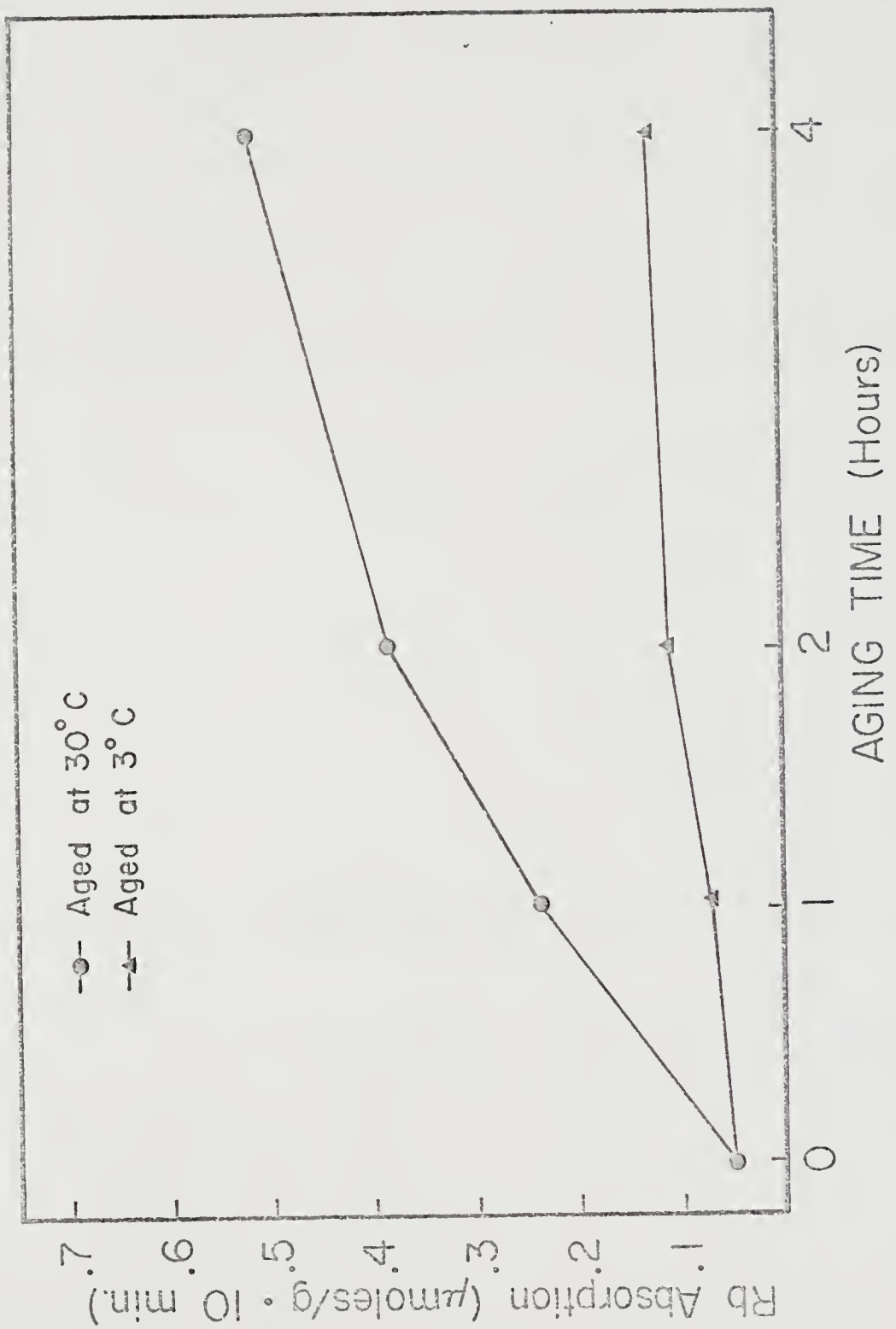
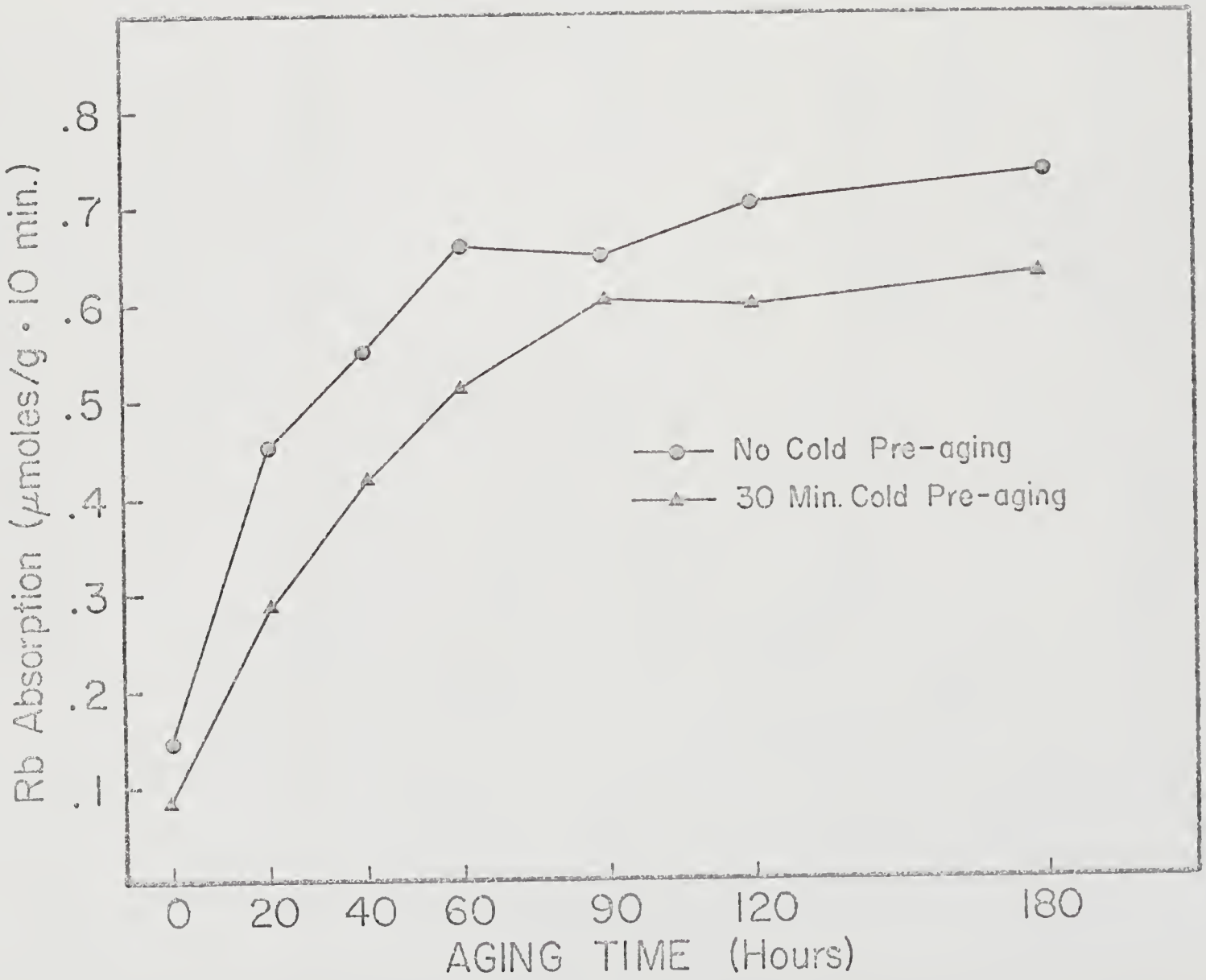

Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
0
1
2
4
AGING TIME (Hours)
Aged at 30°C
Aged at 3°C

Figure 4. Effect of a 30-minute cold pre-aging treatment on the subsequent development of enhanced Rb absorption. The cold solution consisted of 0.5 mM $CaSO_4$. The aging solution consisted of 0.5 mM $CaSO_4$. The root segments were taken 5 to 35 mm from the tip.


Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
.8
0
20
40
60
90
120
180
AGING TIME (Hours)
No Cold Pre-aging
30 Min. Cold Pre-aging

Effects of Water Stress

Drought conditions were simulated in the laboratory in two different ways: by desiccation and by incubation in solutions of low osmotic potential.

Excised root segments were suspended over 500 ml of 5.0 M NaCl in 2-liter flasks where they remained for various time periods. Control samples were suspended over distilled water. The flasks had earlier been sealed with Parafilm, and placed in a water bath at 30°C for 24 hours before the experiment started to allow water vapor equilibration between air and solution. For treatment, the samples were suspended just above the solution. The time lapse between opening and re-sealing the flask was no more than a few seconds, thus minimizing changes in humidity inside the flask. The results are presented in Table 1. Roots held over 5.0 M NaCl showed a lower rate of Rb absorption than controls held over distilled water. Samples held over NaCl solution lost as much as 52.9 per cent of their fresh weight in 6 hours, while control samples lost less than 5 per cent of their fresh weight. A lower rate of Rb uptake by control samples after 1 hour over distilled water was frequently observed, but the cause of it could not be assessed. Two significant results are shown in this experiment: (1) desiccation reduced the rate of Rb absorption, and (2) control samples over distilled water did not show the rapid increase in uptake (aging response) shown by roots incubated in aerated $CaSO_4$ solution. This shows that the "aging" response is not elicited by time alone. The loss of weight by control samples was not considered to be stressing to the tissue.

Two different osmotica were used to simulate drought: Polyethelene

Table 1. Effects of desiccation on the rate of absorption of Rb by excised corn root tissue. (Absorption by freshly excised root segments was 0.108 μmoles/g./10 min.)

	Treatment			
	Distilled water		5M NaCl	
Time (hours)	% loss of wt.	Rb Absorption (μmol./g· 10 min.)	% loss of wt.	Rb Absorption (μmol./g · 10 min.)
1	2.4	0.050	11.9	0.023
2	0	0.161	22.5	0.038
4	3.1	0.141	40.0	0.041
6	4.7	0.119	52.9	0.024

glycol, with an average molecular weight of 6000 (PEG-6000), and mannitol. PEG-6000 does not plasmolyse the tissue, while mannitol is known to cause plasmolysis (32). Excised root segments, 3 cm long, were aged for various time periods in 0.5 mM $CaSO_4$, or in $CaSO_4$ containing 212 g/l of PEG-6000, or 55 g/l mannitol. The concentration of the osmoticum was adjusted so that the osmotic potential of the solution was approximately -8 bars. The results with both osmotica are similar, but only the results with PEG-6000 are shown in Figure 5. Incubation of root segments in a solution of low osmotic potential prevented the increase in rate of Rb uptake, while samples aged in $CaSO_4$ solution without osmoticum showed a rapid increase in the rate of Rb uptake with time.

In the next experiment, samples were incubated for 30 minutes in 0.5 mM $CaSO_4$, or $CaSO_4$ plus mannitol or PEG-6000 as described in the previous experiment. After this stressing period, the samples were rinsed 3 times with 0.5 mM $CaSO_4$ to remove the osmoticum, and were transferred to the aging solution under standard conditions for different periods of time before determining the rate of Rb uptake. The results are presented in Figure 6. The most significant feature is the fact that water stress did not prevent the subsequent development of enhanced Rb uptake. At the end of 2 hours of aging, the Rb uptake by mannitol-treated segments was 3 times the initial rate of stressed samples. However, this rate was only about 50 per cent of that of the controls. PEG-treated samples showed a greater enhancement than mannitol-treated samples. This difference is attributed to damage due to plasmolysis by mannitol. The difference in uptake by PEG-treated samples and control indicates that there was a certain amount of tissue damage

Figure 5. Effect of osmotic stress during aging on the development of enhanced Rb absorption. The concentration of $CaSO_4$ in stress and control solution was 0.5 mM. The root segments used were taken 5 to 35 mm from the tip.

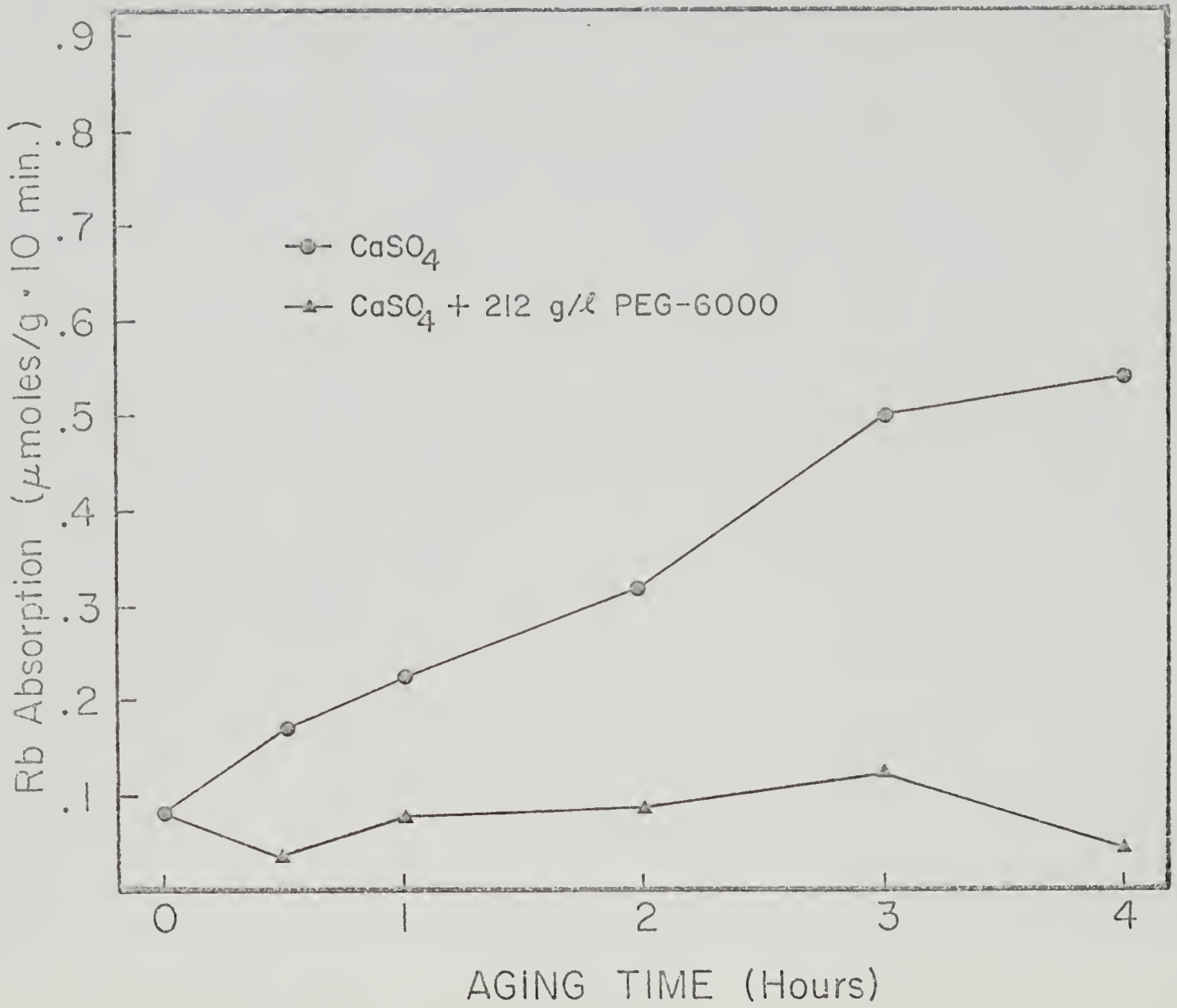
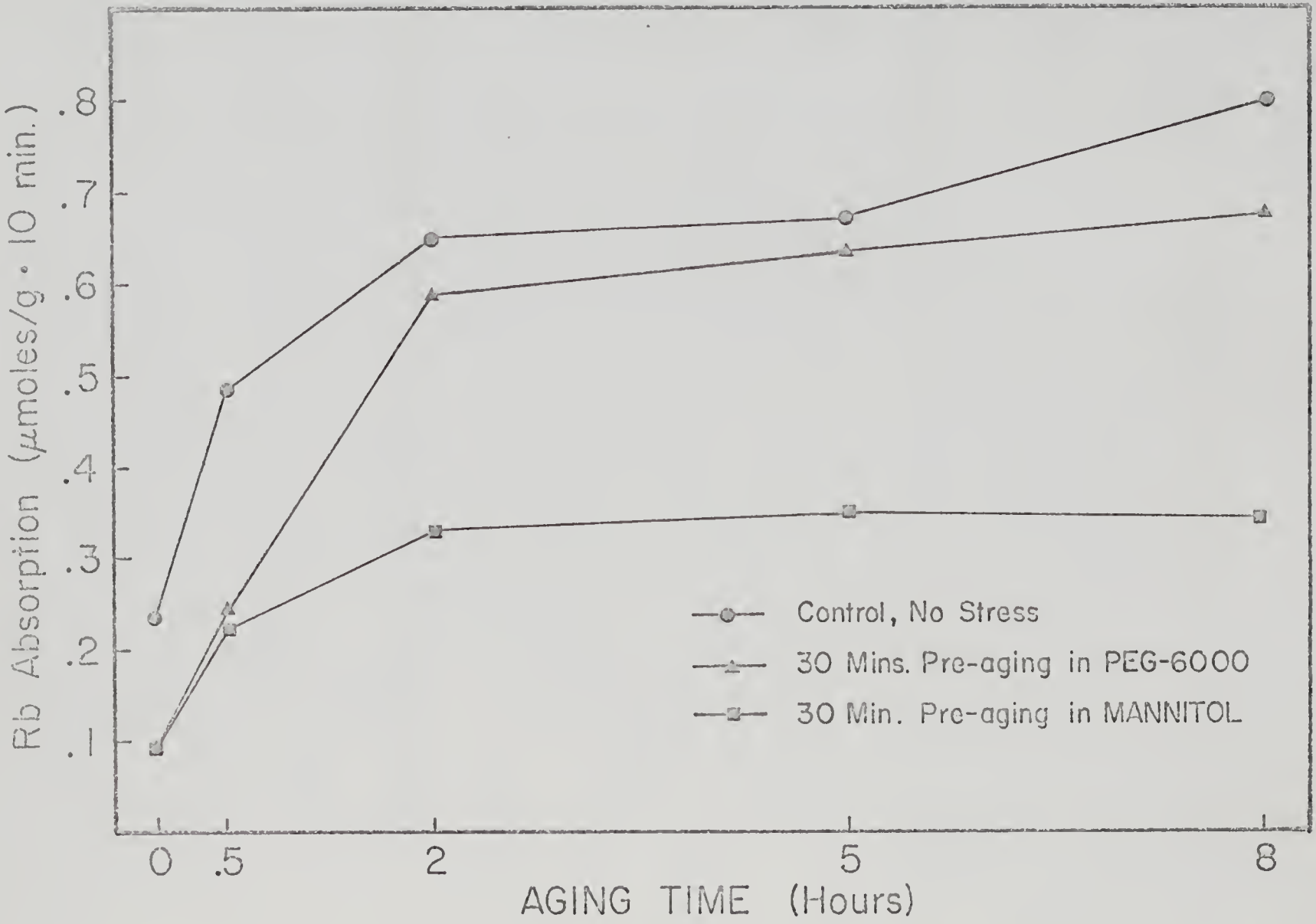

Rb Absorption (μmoles/g • 10 min.)
.9
.8
.7
.6
.5
.4
.3
.2
.1
CaSO4
CaSO4 + 212 g/ℓ PEG-6000
0
1
2
3
4
AGING TIME (Hours)

Figure 6. Effects of a 30-minute period of osmotic stress on the subsequent development of enhanced Rb absorption. $CaSO_4$ was present throughout at 0.5 mM. The root segments were taken 5 to 35 mm from the tip.

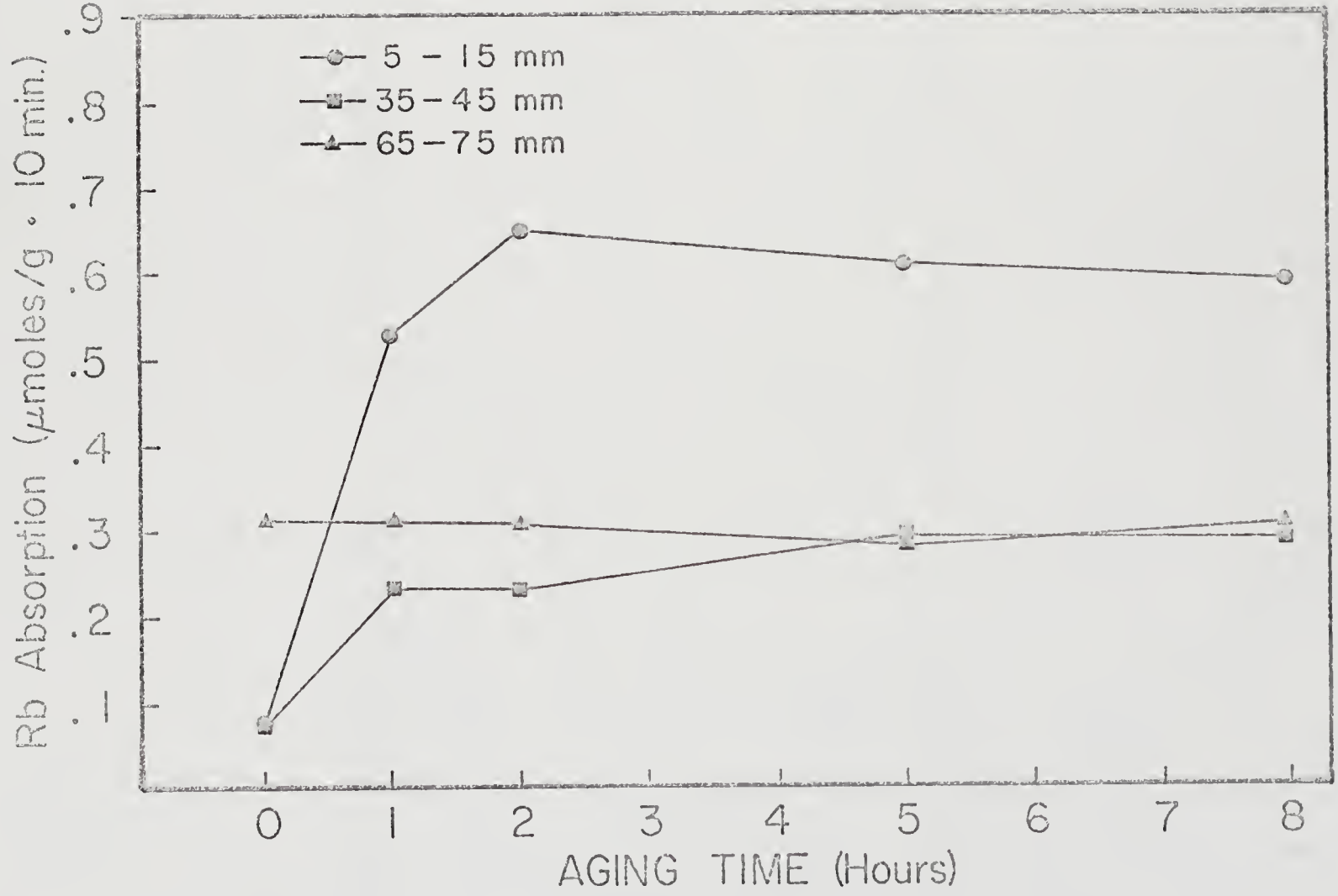

Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
.8
0
.5
2
5
8
AGING TIME (Hours)
Control, No Stress
30 Mins. Pre-aging in PEG-6000
30 Min. Pre-aging in MANNITOL

by this osmoticum. It is not known whether this damage was due to direct toxicity or due to damage to membranes or other subcellular structures.

Development of Enhanced Rubidium Absorption along the Apical 75 mm of the Primary Root of Corn

This experiment was conducted to determine to what extent the enhanced capacity of Rb uptake developed at different distances from the apex of the primary root of corn. Due to the presence of lateral root primordia near the base of the root, this study was limited to the apical 75 mm of the root. One cm segments were taken 5-15, 35-45, and 65-75 mm behind the root tip. Each sample contained 15 root segments with an average weight of 120 mg. These segments were aged in 0.5 mM $CaSO_4$ for periods up to 9 hours before measuring the rate of Rb uptake. The results of this experiment are presented in Figure 7. Segments taken 5 to 15 mm behind the root tip showed the highest increase in the capacity to absorb Rb. The maximum rate was reached in 2 hours, and remained constant thereafter. The basal segments had the highest initial rate of absorption (0.31 μmoles/g·10 min.), and this rate was not affected by the aging treatment. The middle segments, which initially absorbed at the same rate as the apical segments, increased their rate of uptake to the level of the basal segments, but did not go any higher. This experiment was repeated 3 times, with similar results being obtained. On the basis of these results, all subsequent experiments utilized only the apical segment, 5 to 15 mm behind the root apex.

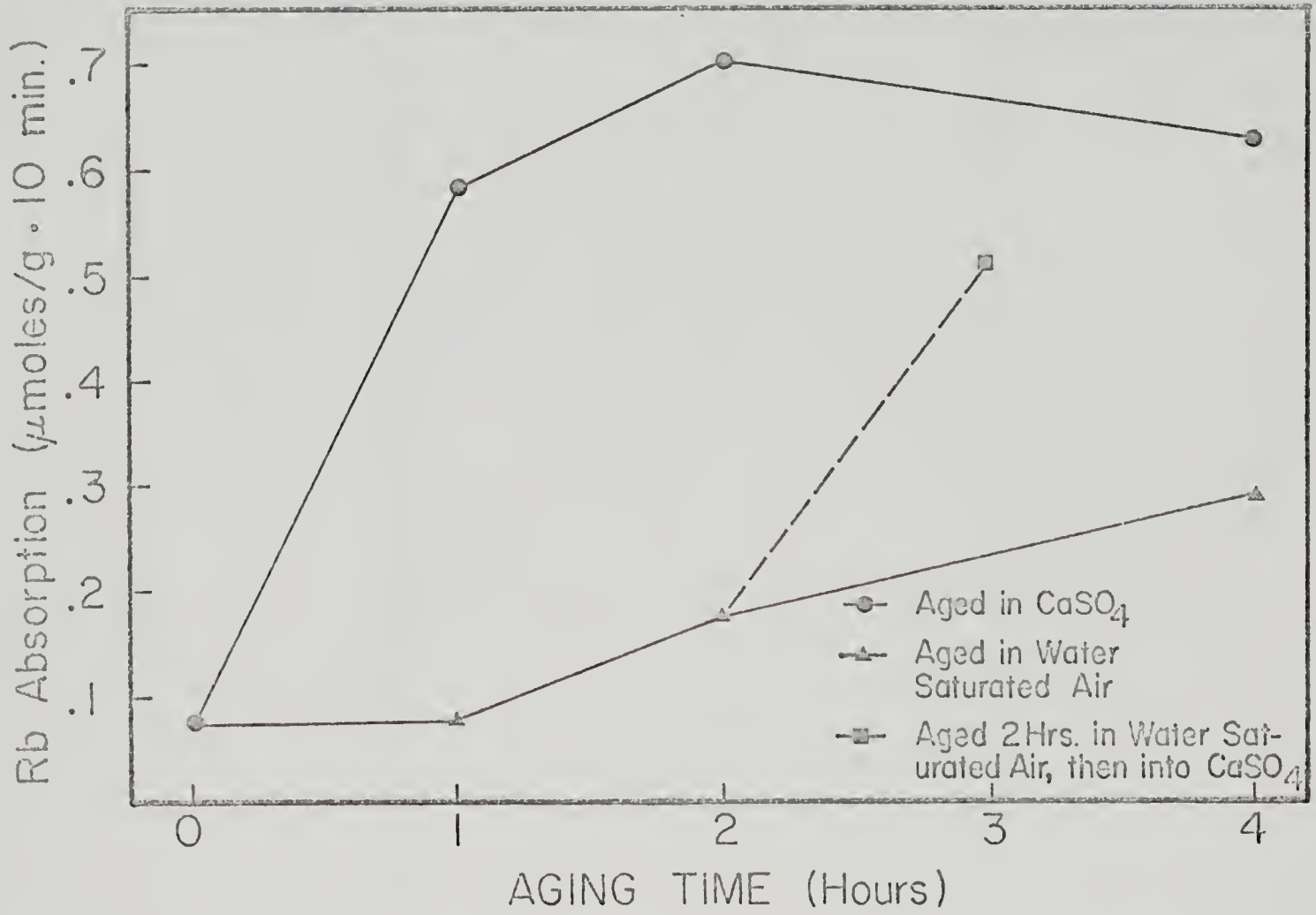
Figure 7. Development of enhanced Rb absorption by one-centimeter excised segments taken from three different positions along the primary root of corn. The aging solution consisted of 0.5 mM $CaSO_4$.


5 - 15 mm
35 - 45 mm
65 - 75 mm
Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
.8
.9
0
1
2
3
4
5
6
7
8
AGING TIME (Hours)

Effects of Aging Excised Root Tissue in $CaSO_4$ Solution and in Water Saturated Atmosphere on the Development of Enhanced Rb Uptake

The results of Table 1 show that segments placed over distilled water developed only a small increase in their capacity for Rb uptake with time. This was further investigated. One-centimeter segments were aged in a water saturated environment or in aerated 0.5 mM $CaSO_4$ under standard conditions. Experimental samples were placed in 2-liter Erlenmeyer flasks containing 500 ml of distilled water. The flasks were sealed with Parafilm and placed in a water bath at 30°C, 24 hours before the experiment started, as described before. One sample was placed in each flask. To insure that the tissue did not suffer any significant loss of water, the fiberglass bag containing the tissue was covered with a piece of damp cheesecloth. In the flask, the edges of the cheesecloth touched the water, but the bag containing the roots remained several centimeters above the water. After hanging the sample inside, the mouth of the flask was sealed immediately with a new piece of Parafilm. The oxygen in the flask was calculated to be 300 times that consumed by respiration of the tissue during a 4-hour period. The results of this experiment are shown in Figure 8. Samples aged in $CaSO_4$ solution showed the usual enhancement of Rb uptake with time, reaching the maximum rate in 2 hours. However, little enhancement of the absorption capacity occurred in experimental samples held over distilled water. After 1 hour, the rate of uptake had not changed. After 2 hours, the rate of uptake was only 15 per cent of controls. A sample taken out after 2 hours of aging over distilled water and immersed in aerated $CaSO_4$ solution for 1 hour showed an increase in the rate of absorption similar to controls aged in $CaSO_4$ solution

Figure 8. Development of enhanced Rb absorption in humid air as compared to $CaSO_4$ solution. The root segments were 1 centimeter long, taken 5 to 15 mm from the root tip.


Rb Absorption (μmoles/g • 10 min.)
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
4
AGING TIME (Hours)
Aged in $CaSO_4$
Aged in Water Saturated Air
Aged 2 Hrs. in Water Saturated Air, then into $CaSO_4$

throughout the aging period. Changes in weight in experimental samples were less than 1 per cent and in some cases the tissue gained weight.

The Effect of Time of Excision of the Root Tip on the Development of Enhanced Rb Uptake

Apical root segments were aged with and without the root tip attached, but the root tips were excised in all cases before Rb absorption. Figure 9 shows the results of this experiment. The presence of the root tip during aging largely prevented the enhancement of Rb absorption. Segments aged without the tip rapidly increased their rate of uptake, most of it taking place within the first hour of aging. At the end of the first hour of aging the rate of uptake by experimental samples was only 10 per cent of controls, and at the end of 8 hours of aging the rate was still only 24 per cent of controls.

In order to determine further the role of the root tip in preventing the enhancement of Rb uptake, another experiment was designed in which the roots were aged under 4 different experimental conditions. (1) Roots excised and aged with the terminal 5 mm of the root intact during aging but removed immediately before absorption. (2) Roots treated as in 1, except that the terminal 5 mm of the root tip was removed before aging. (3) Root tip (5 mm) removed, the seedling incubated in 0.5 mM $CaSO_4$, and root segments excised just prior to Rb absorption. (4) Intact seedlings incubated in a culture solution in which seedlings had grown for 24 hours before the experiment, and samples excised before Rb absorption. At different time periods a sample from each group was taken out and the rate of Rb uptake determined. The results are shown in Figure 10. As before, excised segments

Figure 9. Effect of time of excision of root tips on the development of enhanced Rb absorption. Root segments were taken 5 to 15 mm from the tip. The aging solution consisted of 0.5 mM $CaSO_4$. In experimental samples, the root tip was removed after the aging period.

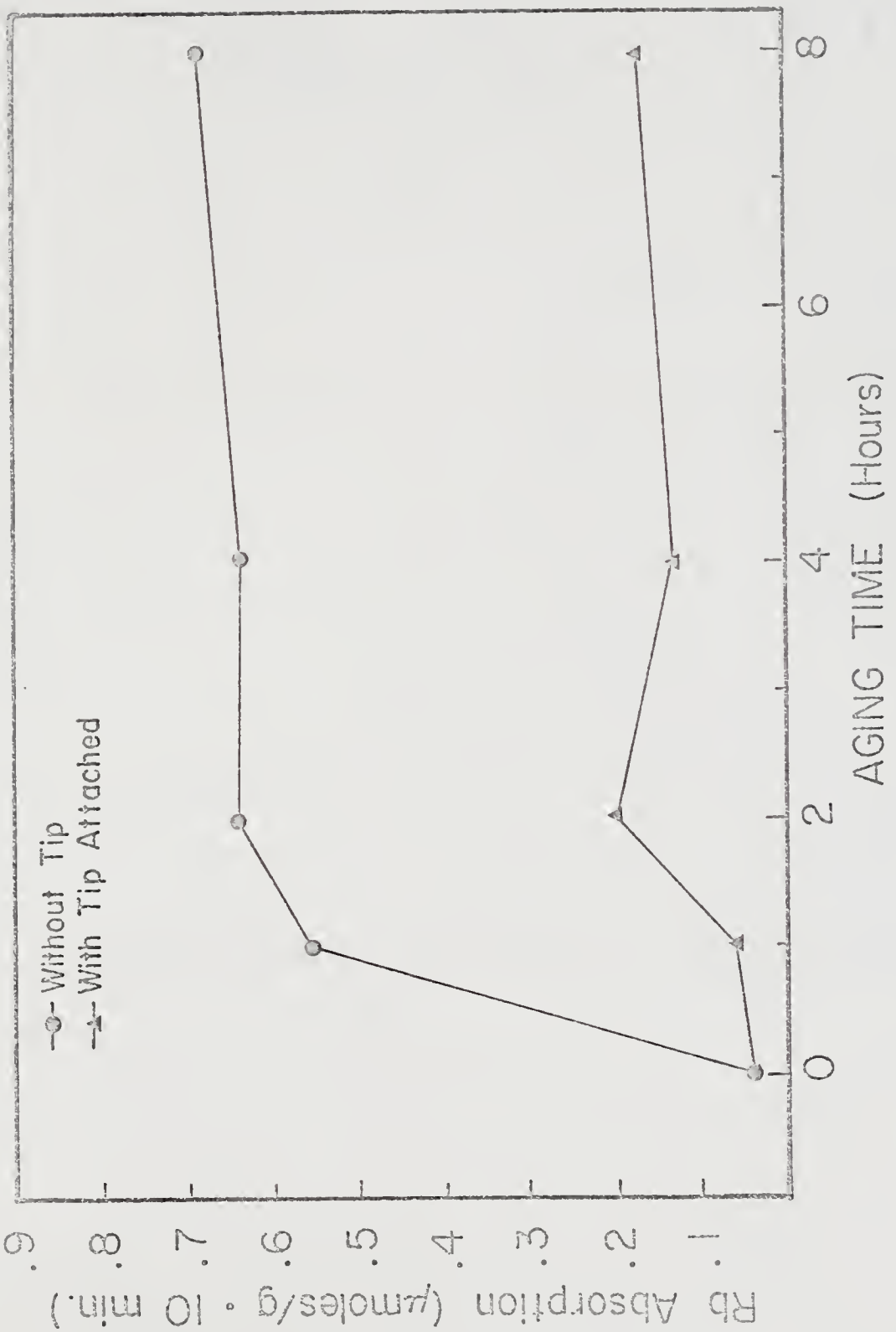
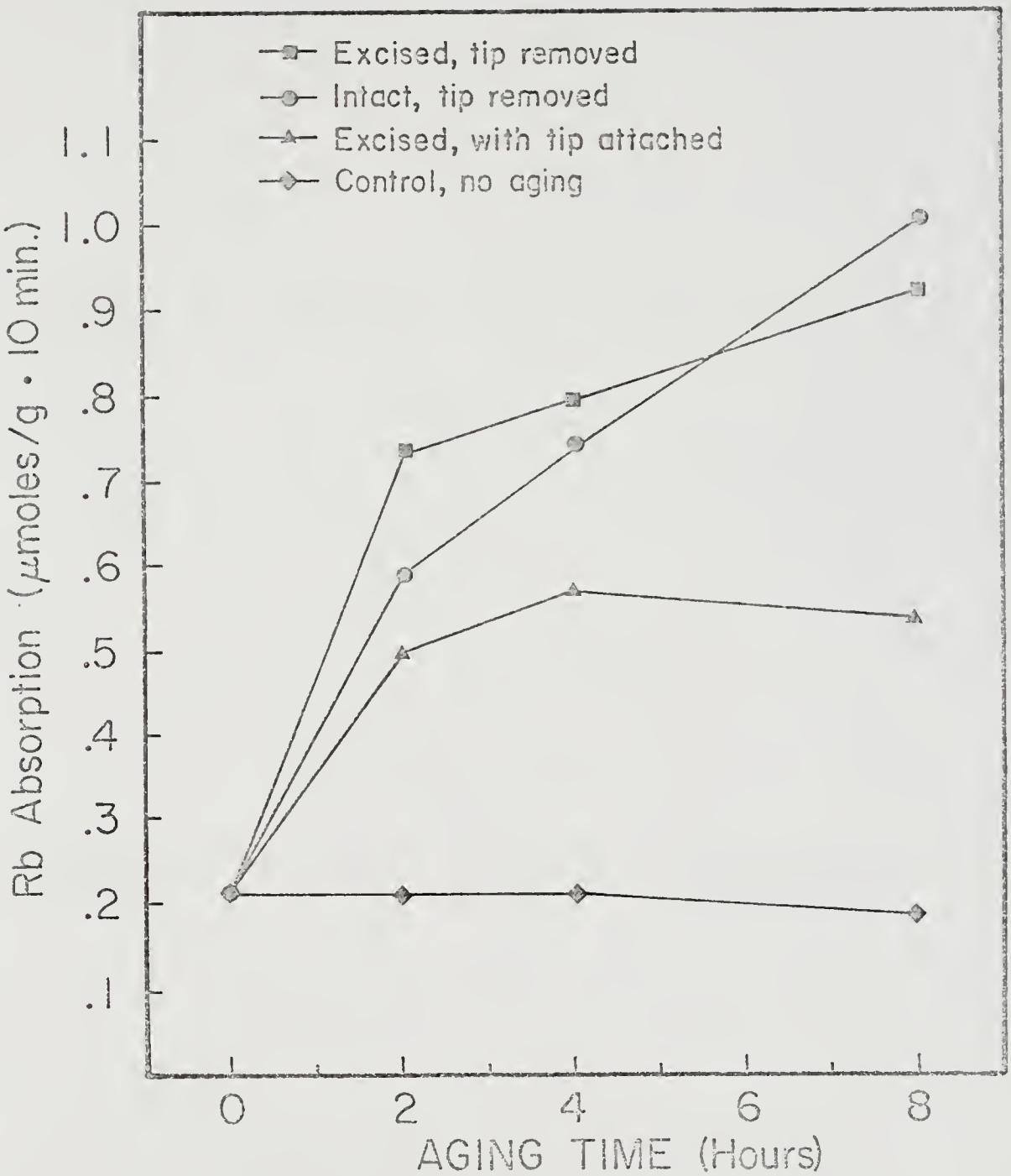

Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
.8
.9
0
2
4
6
8
AGING TIME (Hours)
Without Tip
With Tip Attached

Figure 10. Effect of time of the upper and the lower excision of 1-centimeter root segments on the development of enhanced Rb absorption. The root segments were taken 5 to 15 mm from the tip. In cases where the tissue was aged with the tip attached, the tip was removed after the aging period.


Excised, tip removed
Intact, tip removed
Excised, with tip attached
Control, no aging
Rb Absorption (μmoles/g • 10 min.)
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
2
4
6
8
AGING TIME (Hours)

aged with the tip showed lower rates of absorption than segments whose tip was excised before aging. Rates of uptake into segments from seedlings incubated without the root tip were close to those of excised, tipless segments. Moreover, in identical experiments in which the aging time was extended over a 24-hour period, segments from seedlings aged without the root tip showed a higher rate of Rb uptake than excised, tipless segments 12 hours after aging started.

The enhancement of the rate of Rb uptake with time in aging solution under standard conditions was followed over a 24-hour period in intact roots, excised roots with intact tip, and in excised, tipless roots. The purpose of this experiment was to determine whether the rate of Rb uptake in intact seedlings declined with time after reaching a maximum. At different times during the aging period, a sample from each treatment was taken and the rate of Rb absorption determined. Figure 11 shows the results of this experiment. Root segments from intact seedlings showed an enhancement in uptake, but after 12 hours the rate declined. Excised roots did not show this decline. Samples in which the root tip was left attached, whether the root remained attached to the seedling during aging or not, showed a lower enhancement than excised, tipless roots.

Effects of Aging in the Presence of Excised Root Tips and in Culture Solution

One-centimeter segments were aged in 400 ml volumes of test solution for 2 hours before determining the rate of Rb uptake. The roots were treated in 5 different ways as described in Figure 12. Each sample was duplicated and the average of the 2 is given. Variation between replicate samples is indicated by vertical lines. Only a

Figure 11. Effect of time of the upper and the lower excision of 1-centimeter root segments on the long-term development of enhanced Rb absorption. The root segments were taken 5 to 15 mm from the tip. In cases where the roots were aged with the tip attached, the tip was removed after the aging period.

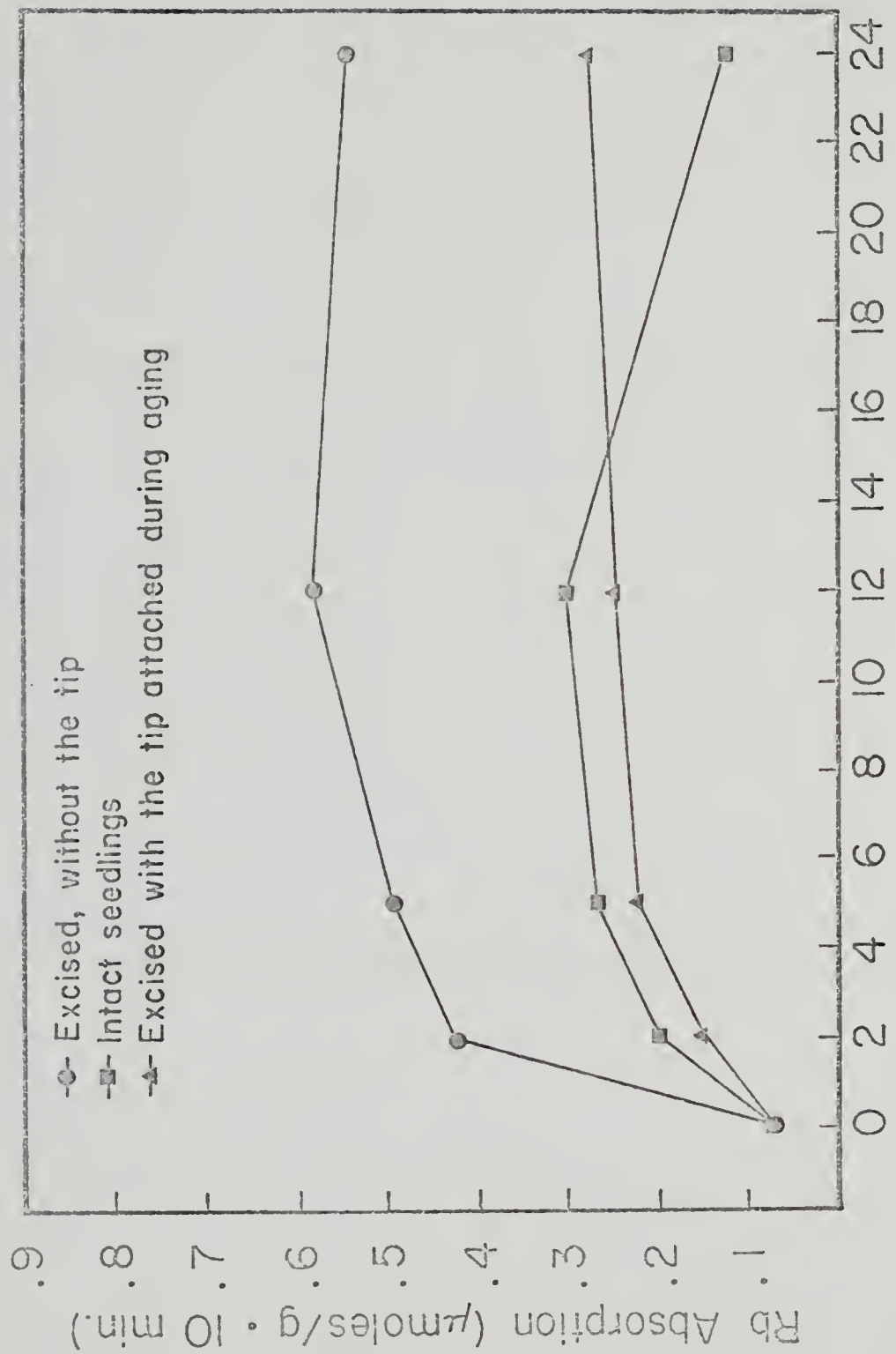
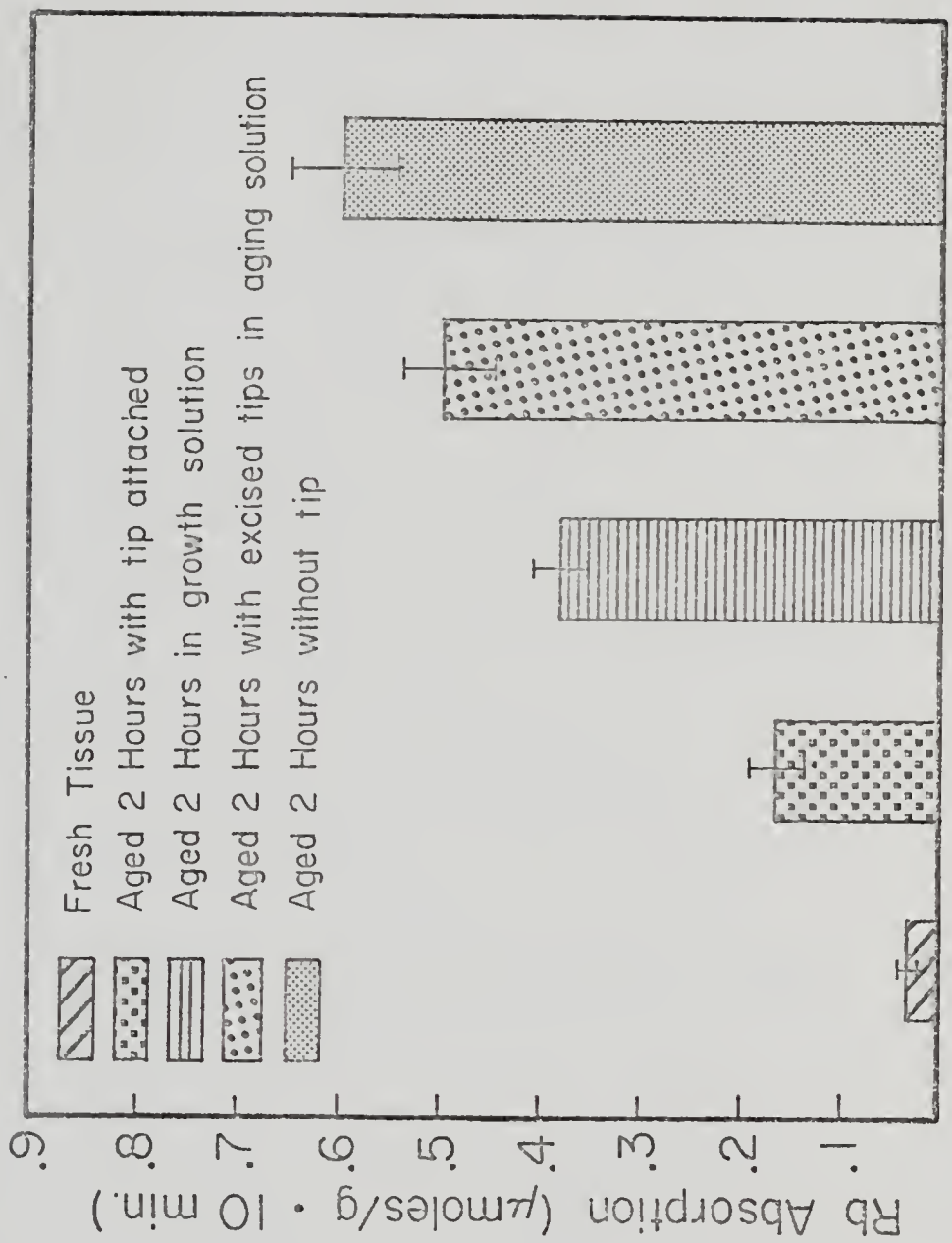

Excised, without the tip
Intact seedlings
Excised with the tip attached during aging
Rb Absorption (μmoles/g • 10 min.)
AGING TIME (Hours)
0 2 4 6 8 10 12 14 16 18 20 22 24
1 2 3 4 5 6 7 8 9

Figure 12. Effect of the presence of root tips, excised or attached, in the aging solution; and the effect of old culture solution used as the aging medium on the development of enhanced Rb absorption. All samples were aged 2 hours. In samples aged with the tip attached, the tip was removed after the 2-hour aging period.

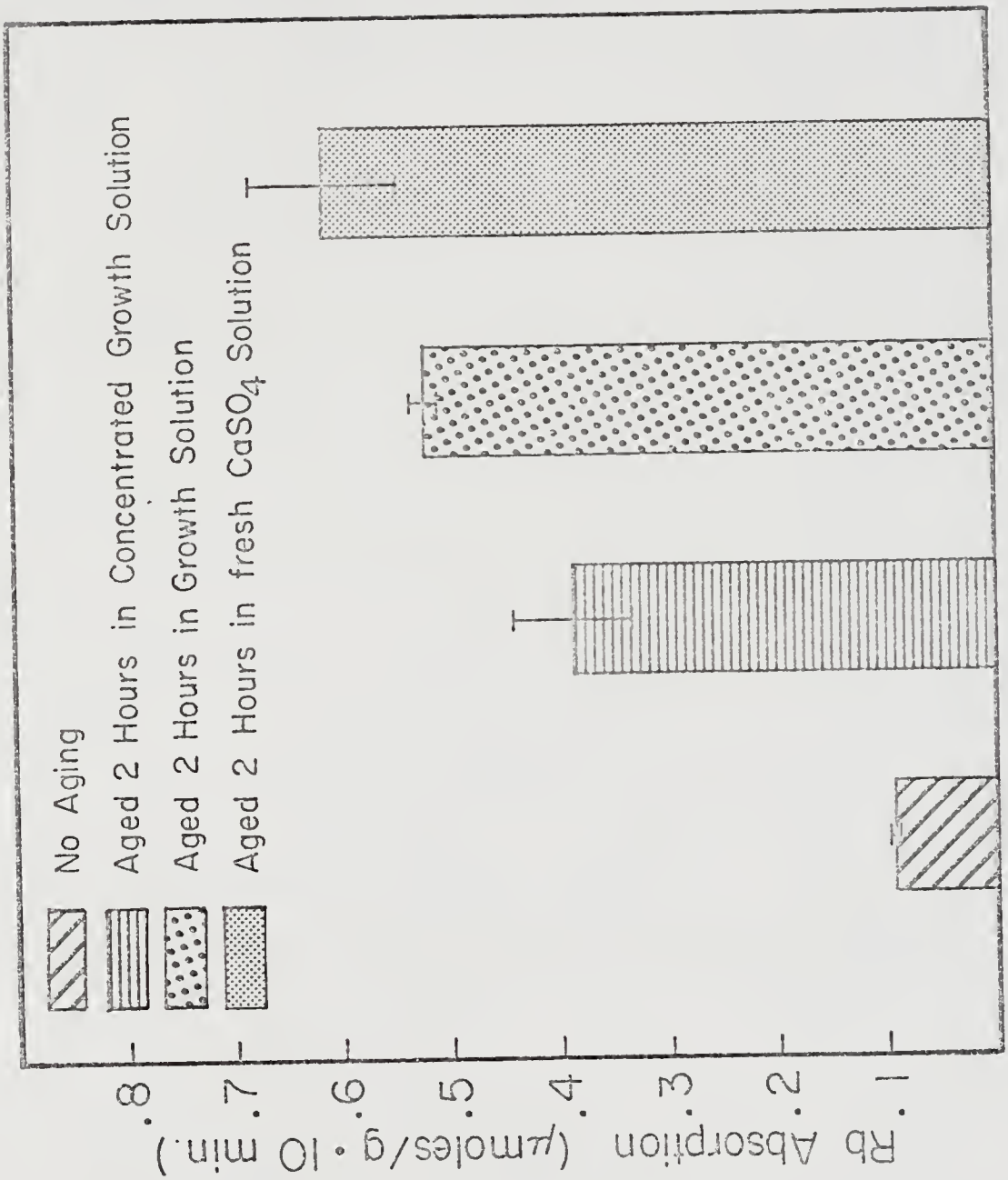

Rb Absorption (μmoles/g • 10 min.)
.9
.8
.7
.6
.5
.4
.3
.2
.1
Fresh Tissue
Aged 2 Hours with tip attached
Aged 2 Hours in growth solution
Aged 2 Hours with excised tips in aging solution
Aged 2 Hours without tip

slight enhancement of uptake was obtained with segments aged with the tip intact: about one fourth of that obtained with samples aged without tips. Aging of tipless root segments in growth solution was also inhibitory to the enhanced Rb uptake. Their rate of uptake was two-thirds of controls without the tip aged in fresh $CaSO_4$ solution. The enhancement of Rb uptake was also less pronounced in samples aged with root tips floating in the aging solution.

Effects of Concentrated Growth Solution on the Development of Enhanced Rb Uptake

In the previous experiment it was shown that the solution in which seedlings had been growing for 24 hours was inhibitory to the development of enhanced rate of Rb uptake. In the following experiment, this growth solution was concentrated by reduction to one-half of its original volume by lyophilization, to determine if concentrating this medium would permit even less enhancement of Rb uptake by excised, tipless corn root segments. Since the concentration of $CaSO_4$ in the growth solution was 0.2 mM, and that of the aging solution was 0.5 mM, a reduction in the volume of the growth solution by one-half would not increase the $CaSO_4$ concentration above that of the aging solution. The samples were aged for 2 hours before determining the rate of Rb uptake. One sample was aged in growth solution, a second sample was aged in concentrated growth solution. A third sample was aged in fresh 0.5 mM $CaSO_4$. A fourth sample was used to determine uptake by freshly excised tissue. Each sample was duplicated, and the average of the 2 is given in Figure 13. Variation between replicate samples is indicated by vertical lines. The enhancement in uptake by root tissue incubated in growth solution was again less than in control

Figure 13. Effect of two different concentrations of old culture solution used as the aging solution on the development of enhanced Rb absorption. Samples were aged 2 hours. The old culture solution was concentrated from 1 liter to 500 ml by lyophilization.

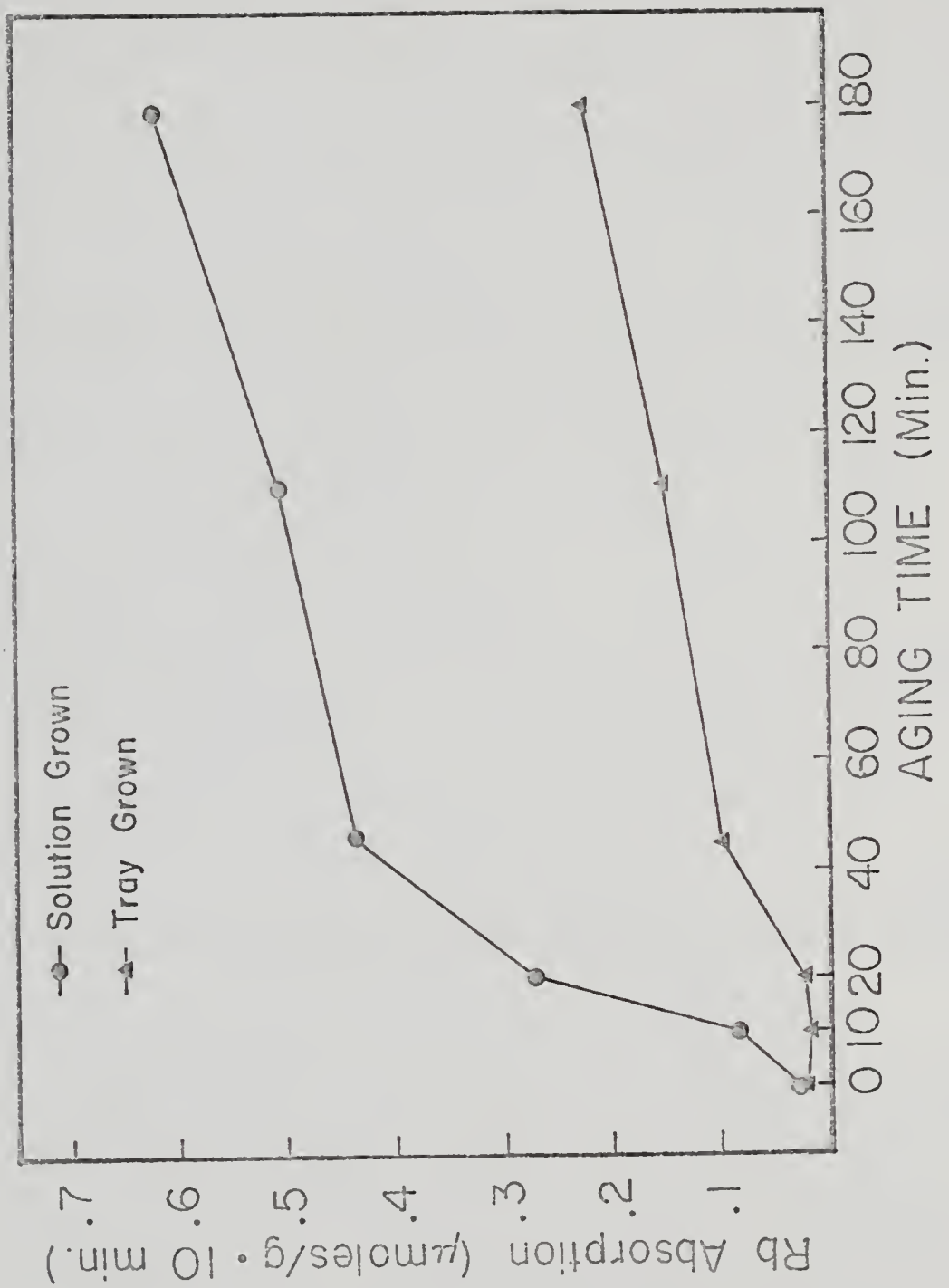

No Aging
Aged 2 Hours in Concentrated Growth Solution
Aged 2 Hours in Growth Solution
Aged 2 Hours in fresh $CaSO_4$ Solution
Rb Absorption (μmoles/g • 10 min.)
.8
.7
.6
.5
.4
.3
.2
.1

samples aged in fresh $CaSO_4$ solution. The prevention of the enhancement of Rb uptake, however, was more pronounced in tissue aged in concentrated growth solution than in tissue aged in normal strength growth solution.

Induction and Development of Enhanced Rb Absorption by Tray-grown and Solution-grown Roots

The effects of aging on Rb uptake in one-centimeter root segments from tray-grown versus solution-grown seedlings are shown in Figure 14. Freshly excised root tissue of both solution-grown and tray-grown seedlings showed initially the same rate of absorption (0.020 μmoles/g . 10 min.). Tray-grown roots showed a 20-minute lag period before an increase in the rate of absorption became apparent. An even longer lag has been reported for phosphate absorption (24). Solution-grown roots always showed an increase in the rate of uptake within 10 minutes after aging began. However, this increased rate of Rb uptake after 10 minutes was not always as pronounced as that shown in Figure 14. After 3 hours of aging, tray-grown tissue was absorbing at a rate 13 times that of freshly excised tissue, whereas solution-grown tissue was absorbing at a rate 23 times that of freshly excised tissue. The difference in absorption between tray and solution grown tissue with time in aging, presented in Figure 14, was not always as great as shown here, however, solution-grown roots consistently showed a higher rate of absorption with time in aging solution than tray-grown tissue.

Figure 14. Development of enhanced Rb absorption in root segments from tray-grown versus solution-grown seedlings. The segments used were taken 5 to 15 mm from the root tip.


Rb Absorption (μmoles/g • 10 min.)
.1
.2
.3
.4
.5
.6
.7
0 10 20 40 60 80 100 120 140 160 180
AGING TIME (Min.)
Solution Grown
Tray Grown

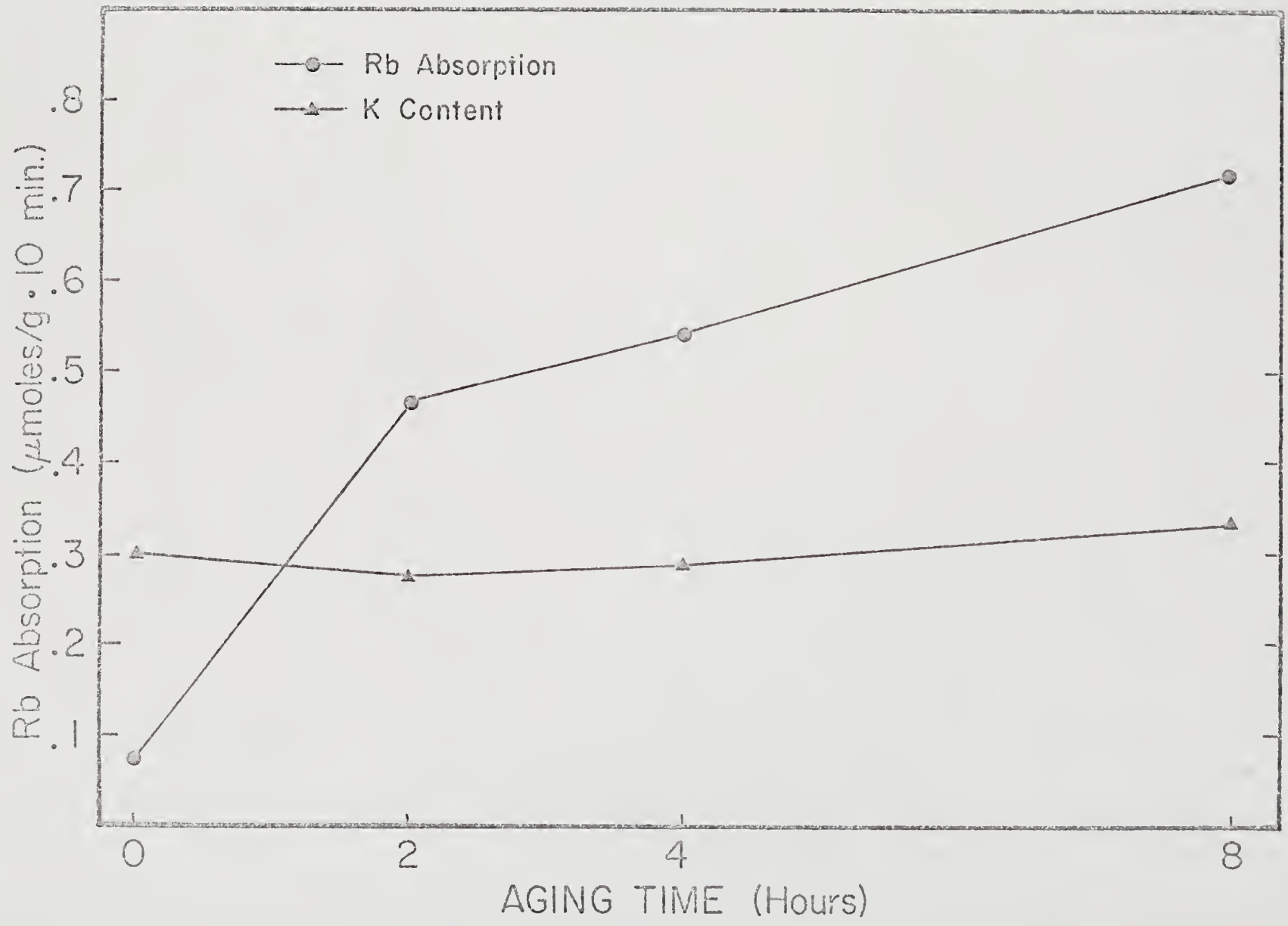
Rb Uptake and K Efflux from Excised Corn Root Segments

The following experiment was carried out to determine the extent of K loss from corn root segments and the degree to which this loss is influenced by aging. Excised root segments were divided into 2 groups. Both groups were aged under standard conditions. Two samples were taken out at each sampling during aging. One sample was used to determine the rate of Rb absorption, and the other sample was used to determine the K content. The K in the roots would necessarily have to come from the seed since K was not supplied at any time during the growth of the seedlings. The results are presented in Figure 15. The data show that the K content of the tissue remained constant during 8 hours of aging. The enhanced Rb uptake with time was not accompanied by an increased efflux of K from the tissue. Although K efflux might be significant in tissue with high salt content, tissue with low salt content such as that used in these experiments did not lose significant quantities of K during aging in $CaSO_4$ solution.

Elongation of Excised Root Segments with Time in $CaSO_4$ Solution

To determine whether further elongation occurred after excision and during aging, the change in length of 1-cm root segments, taken 5 to 15 mm behind the root tip was checked. These segments were accurately cut with the aid of a stereoscopic microscope. After 4 hours of aging in $CaSO_4$ solution under standard conditions, the increase in length was less than 1 mm. Thus it can be assumed that most of the cells in the segments had completed their elongation, or conditions for further cell elongation were lacking. Therefore, the enhancement

Figure 15. Development of enhanced Rb absorption compared to changes in K content during aging. The segments were 1 centimeter long, taken 5 to 15 mm from the root tip.


Rb Absorption
K Content
Rb Absorption (μmoles/g • 10 min.)
K Content (mg/g fresh wt.)
AGING TIME (Hours)
0
2
4
8
.1
.2
.3
.4
.5
.6
.7
.8
.4
.8
1.2
1.6
2.0

in the rate of Rb uptake of such proportions as shown in this study could not be attributed to cell growth.

DISCUSSION

There have been scattered reports in the literature (20, 24, 48) of a time-dependent enhanced capacity for mineral uptake by excised roots. Many of these reports have been limited to isolated observations made in the course of other studies relating to mineral absorption. Leonard and Hanson were the first to publish the results of experiments aimed at determining the causes of this increase in uptake. At the same time this phenomenon was being observed in our laboratory. It is of particular interest since so much work has been published on the subject of mineral absorption using excised roots of grasses. A clear understanding of this phenomenon is needed to relate uptake by excised tissue to intact plants. In this study an attempt has been made to determine the nature of this enhancement, as well as the extent of its development along the primary root. An effort has also been made to determine the cause or causes which induce this enhancement and the locus in the plant where it originates.

The mechanism of increased mineral uptake in corn root tissue does not seem to be the same as that operating in slices of storage tissue, the time dependence curves are different. In storage tissue, a lag time of 24 hours has been reported before an increase in uptake was apparent (47). In excised, solution-grown corn roots an increase in uptake was evident after 10 minutes of aging. In tray-grown roots, a lag of 20 minutes was required before increased uptake could be detected. Leonard and Hanson reported a 30-minute lag in excised corn

roots (24). In corn root tissue the increase in uptake reached a maximum in 2 to 4 hours, while in potato tuber slices the rate of phosphate uptake was still increasing after 30 hours of aging (26). The increase in uptake by storage tissue is accompanied by an increase in respiration, typical of tissue breaking dormancy (26). In corn root tissue the rate of respiration remains constant (24).

The enhanced rate of Rb uptake was not considered to be due to microbial contamination. Even though no attempt was made at determining the bacterial count in the aging solution, there is evidence which indicates that microorganisms were not responsible for the enhancement. If microorganisms were responsible for increased uptake, the rate would continue to rise as the bacterial population continues to increase. However, the rate of Rb uptake reached a maximum in 2 hours and remained constant thereafter. Further evidence published by others (24) showed that incubation of corn roots in the presence of chloramphenicol at bacteriostatic concentrations did not have any effect on the development of enhanced mineral uptake capacity.

The response of Rb uptake to aging could not be attributed to increased K efflux from the tissue. The K content of excised roots remained constant during 8 hours of aging in $CaSO_4$ solution under standard conditions. Similar findings have been reported for phosphate uptake by Leonard and Hanson (24), and for Rb uptake by Handley *et al.* (20).

Whatever the cause, or causes, it appears that the increase in uptake is dependent on the metabolism of the cell. This is indicated by the fact that when excised roots were aged at low temperature at which metabolism is minimal, the enhancement of Rb uptake was reduced

(Figure 3), or prevented (24). However, when the tissue was returned to 30°C the enhancement proceeded rapidly (Figure 4). Further evidence which indicates that this process is metabolically dependent is given by results from water-stressed tissue. In tissue under water stress, the enhancement of Rb uptake was prevented. Excision does not appear to be the cause of this phenomenon since roots from intact seedlings when transferred to fresh $CaSO_4$ solution showed a similar enhancement, although not quite as high as when roots were excised before aging. Leonard and Hanson did not observe any difference in the rate of uptake by samples in which the roots were cut into several segments from samples in which the root was not subdivided.

The potential for enhancement was not manifested along the entire length of the primary root of corn, but rather it was limited to the portion of the root near the root apex (Figure 7). Canning and Kramer (9) have shown that in corn root the ability to accumulate minerals is highest at a distance of 20 to 25 mm from the apex and decreases toward the base of the root. Smith (45) has shown that exudation volume as well as Rb output was higher in apical segments than in basal segments. It is of no surprise then that enhancement of Rb uptake also varies along the length of the root. There are certain peculiarities in the way in which different segments of the corn root respond to the aging effect that are worthy of comment. Freshly excised root segments taken 5 to 15 mm behind the tip had a rate of uptake of only 25 per cent of freshly excised segments taken 65 to 75 mm from the tip. At the end of 2 hours of incubation, however, the apical segments had increased their uptake dramatically, while basal segments continued to absorb at the initial rate. Intermediate segments taken 35 to 45 mm

from the tip initially absorbed at the same rate as apical segments, but their highest rate of uptake after 5 hours of aging was essentially the same as that of basal segments. It appears that the ability to respond to aging decreased with distance from the apex. Moreover, the rate of Rb absorption appears to be permanently set at a lower level once the tissue has reached some particular stage of development. This difference in response could not be attributed to cell elongation after excision since elongation of the segments was less than 10 per cent during the time that the rate of Rb uptake reached a maximum.

The increase in uptake by segments from intact seedlings when transferred to fresh $CaSO_4$ solution suggested that the enhanced rate of Rb uptake may be due to the leaching of an inhibitor which builds up in the culture solution with time. It was possible to test this hypothesis by aging root segments in an atmosphere in which leaching could not take place. The results shown in Figure 9 lend support to this hypothesis. Root segments aged above water showed a much lower rate of Rb absorption than segments aged in $CaSO_4$ solution. However, when tissue held in the air was immersed in $CaSO_4$ solution for 1 hour, enhancement similar to that of samples aged in $CaSO_4$ solution occurred. The lower rate of absorption by samples aged in air can hardly be attributed to desiccation since the tissue did not lose more than 5 per cent of the fresh weight. The oxygen supply was calculated to be 300 times that required by the tissue for the longest period of time that a sample was kept in the flask.

If enhancement of the rate of Rb absorption by corn roots is due to leaching out of an inhibitor, it should be possible to identify

the source of the inhibitor. It would have to be synthesized in situ or it must come from the shoot or root tip. If the inhibitor is synthesized in the region where it exerts its regulatory influence, tipless segments and tipless seedlings would not have increased their uptake with time in the aging solution, since the synthesis of this inhibitor presumably should continue after excision of the roots. Also the fact that the response to aging decreased with distance from the root apex suggests that the control of enhanced absorption may come from the root tip (support for this hypothesis is shown in Figure 10). When root segments were aged with the tip intact, but removed before absorption, the enhancement of Rb uptake was much less than when the tips were removed prior to aging. Further evidence was shown in Figure 11. When the terminal 5 mm of the primary root of intact corn seedlings was removed and the decapitated roots of the otherwise intact plants were incubated in $CaSO_4$ solution, the uptake was higher than when excised roots were aged with tips intact.

If an endogenous inhibitor leaks from the root tip the rate of Rb uptake by root segments from intact seedlings, as well as by excised roots with intact tips, should decrease with time, if left in the aging solution long enough for the concentration of the inhibitor to build up. The results in Figure 12 confirm this expectation for intact seedlings, but not for excised roots with intact tips. The decrease in uptake by root segments from intact seedlings after 12 hours of incubation in $CaSO_4$ solution could hardly be attributed to the lack of food in the tissue since the roots were not excised until after the aging period. Moreover, roots from seedlings left in the solution where they had grown for 24 hours before the experiment

showed no enhancement in the rate of Rb uptake (Figure 10). Tipless root segments aged for 2 hours in fresh $CaSO_4$ solution absorbed less Rb in 10 minutes when the tips were present in the solution than when the tips were absent (Figure 13). However, if the tips remained attached during aging, the enhanced rate of Rb uptake was much less than when the tips were floating in the solution. This difference probably resulted from dilution of the inhibitor or discontinuity of the supply when the tip was severed. Low penetration of the membrane by the inhibitor may have also contributed to this difference. In intact tissue, the inhibitor probably is translocated from the tip to other parts of the root in the symplastic continuum.

More evidence in support of the presence of an inhibitor is shown in Figures 13 and 14. Incubation of excised, tipless root segments in the culture solution in which the seedlings had been grown for 24 hours prior to the experiment was inhibitory to the development of enhanced Rb uptake (Figure 13). Root tissue aged in this solution for 2 hours absorbed only 66 per cent as much as samples aged for the same period of time in fresh solution. Concentrating the growth solution further inhibited the rate of Rb uptake by excised, tipless root tissue (Figure 14).

The mechanism by which this inhibitor regulates the rate of mineral uptake is not yet known. Elucidation of the mechanism will have to await future research, possibly until the identity of the inhibitor is known. Several possibilities are suggested here. First, it may be that the increase in uptake is brought about by an increase in the membrane-bound carrier, the synthesis of which is held in check by the inhibitor. Another possibility is that the inhibitor itself

combines with the carrier in the membrane, rendering it inoperative. The inhibitor-carrier association may be weak so that leaching of the inhibitor takes place readily when the roots are immersed in fresh solution. The first possibility is supported by the findings of Leonard and Hanson, who reported that an increase in protein content accompanied the enhancement of phosphate uptake in corn root tissue. This mechanism would require a lag period before the increase in the rate of uptake is apparent. Leonard and Hanson have reported a lag period of 30 minutes before the rate of phosphate uptake increased. A 20-minute lag is reported here for Rb absorption by tray-grown roots (Figure 15), but not by solution-grown roots. It may be significant that most of the tissue used by Leonard and Hanson was tray-grown. In our laboratory, solution-grown roots showed an increase in uptake within 10 minutes after aging started. It would seem that this short period of time would be adequate for leaching out of a water-soluble compound (the hypothetical inhibitor) whereas it might not be long enough for appreciable protein synthesis. One difficulty in interpreting these data is that an increase in total protein content may not necessarily mean an increase in the carrier. This will be difficult to show since the identity of the carrier is not yet known. The second suggestion receives some support from the experiments with solution-grown tissue by the fact that the lag period was 10 minutes or less. As soon as the inhibitor-carrier association is broken, the carrier is free to bind with a Rb ion, thus an immediate increase in the rate of uptake. Still another possibility is that the inhibitor may act by interfering with the delivery of energy required for active transport. Leonard and Hanson have shown an increase in membrane-bound ATPase activity with aging in corn root tissue.

Leonard and Hanson attributed the increase in phosphate uptake to an increase in protein synthesis brought about by submersion of the root tissue. As evidence for this they showed that freshly excised solution-grown roots absorbed at a much higher rate than tray-grown roots. They showed this difference in the absorption of several ions, including Rb. However, this difference in freshly excised tissue between tray and solution grown roots could not be verified in our laboratory for Rb absorption. It could very well be that this difference was due to differences in handling the tissue, or perhaps to the genetic differences in the corn strains. The difference in Rb uptake with time in $CaSO_4$ solution between tray-grown and solution-grown tissue (Figure 15) may have been due to the fact that roots grown in trays had been under the influence of higher concentrations of the inhibitor during the growth period. In solution-grown roots, the $CaSO_4$ solution was changed every 24 hours, and the seedlings were rinsed with distilled water each time that the solution was changed. Tray-grown roots were left undisturbed during the entire growth period. Tray-grown roots may not be able to overcome the effects of the inhibitor to the extent that solution-grown roots do. Conditions in the tray, however, resemble more closely the natural environment than growing the seedlings in solution.

It is not known whether both mechanisms of ion uptake reported by Epstein *et al.* (14) are influenced by this inhibitor. The Rb concentration of the absorption solution used in these experiments (0.1 mM) was in the range of the high affinity mechanism. Thus it appears that at least the high affinity mechanism is involved in the aging effect.

The significance of this response of corn roots is viewed in

regard to its possible survival value to the plant. A mechanism of this kind, whereby the rate of mineral uptake is increased rapidly during short periods of high water content in the soil when the maximum amount of dissolved nutrients are available, would be of a definite advantage to the plant.

SUMMARY AND CONCLUSIONS

The rate of mineral uptake by excised corn root tissue has been shown to increase following aging in a solution of $CaSO_4$. This increased capacity for solute uptake was prevented by conditions which lowered the metabolism of the tissue. However, once the tissue was returned to normal metabolic conditions, the enhancement of Rb uptake was restored. Excision does not appear to cause this enhancement, since roots from intact seedlings when transferred to fresh $CaSO_4$ solution showed a similar response, although somewhat smaller. However, the rate of Rb uptake began declining in tissue from intact seedlings 10 hours after being transferred to fresh $CaSO_4$ solution. It appears that the Rb absorption capacity in corn root tissue is limited by the presence of an inhibitor, and the increased rate of uptake is caused by the leaching of this substance when the tissue is immersed in aerated $CaSO_4$ solution. This conclusion is strengthened by the fact that enhanced capacity for Rb absorption is prevented or greatly reduced when excised roots were aged in humid air. However, when roots aged in air were subsequently submerged in aerated $CaSO_4$ solution, the capacity for Rb uptake developed similar to that developed in samples aged in solution without pre-aging in the air. The idea that a substance inhibitory to Rb uptake leaches from the roots is also supported by the fact that the $CaSO_4$ solution in which roots had been growing partially prevented the enhancement of Rb uptake during aging. Concentrating the solution where seedlings had grown further reduced the response to aging.

The experimental data obtained in this investigation have been interpreted to indicate that some water-soluble substance(s), originating in the root tip prevents the enhancement response of tissues farther back in the root. This hypothesis is specifically supported by the following: (1) The aging response decreased as the distance from the tip increased; (2) at a distance of 65 to 75 mm from the tip there was no response at all; (3) aging the segments with the tip attached largely prevented the response; (4) aging tipless roots which had been left attached to the plant showed a response similar to that of excised, tipless roots.

The response to aging could not be attributed to Rb absorption by microorganisms, or to increased efflux of K from the tissue. The data presented in this investigation strongly suggest that the enhanced rate of Rb absorption with time by excised corn root tissue was not due to submersion itself, but to the leaching out of a water-soluble endogenous inhibitor which occurs when the tissue is submerged.

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Rolando T. Parrondo was born in Camaguey, Cuba, on December 23, 1937. He attended high school at Colegio Pinson, in Cuba. In 1961, he came to the United States to attend Andrew Junior College, in Cuthbert, Georgia. In 1962, he attended Auburn University, in Auburn, Alabama, and later transferred to George Peabody College for Teachers, in Nashville, Tennessee, where he received a B.S. degree in Biology. Before returning to the University for graduate studies, he taught biology and chemistry at Englewood High School in Jacksonville, Florida. In 1968, he entered graduate school at the University of Florida. In 1969, he received an M.S. degree in Botany. From 1969, to the present time, he has pursued work toward the degree of Doctor of Philosophy. While a graduate student he was a teaching assistant in general botany, general biology, and plant physiology. He is a member of the Botanical Society of America, and the American Society of Plant Physiologists.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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