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Sodium and Potassium Absorption by Bean Stem Tissue

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Abstract. **The effect of various periods of pretreatment in $CaSO_4$ solutions (aging) on the absorption of Na and K by bean stem slices was investigated. Freshly sliced tissue absorbed Na over the entire range of concentrations studied (0.02–50 mM). Potassium absorption by fresh tissue was nil at concentrations below 0.5 mM but at higher concentrations was similar to that of Na. When tissue was aged by aerating slices for 20 hr in 0.5 mM $CaSO_4$, K absorption was substantial over the entire range (0.01–50 mM), with evidence of a dual mechanism of absorption, whereas Na absorption was nil at concentrations below 0.2 mM. The formation of K-absorbing capacity with aging, and the loss of Na-absorbing capacity at low concentrations, were temperature-dependent and did not result from significant changes in rates of efflux of either ion. The absorption of Na by fresh tissue and K by aged tissue was sensitive to antimetabolites, with K uptake the more sensitive. Benzyladenine, an analog of kinetin, suppressed the formation of K-absorbing capability in aged tissue but did not prevent the loss of Na-absorbing capacity. Possible mechanisms for this alteration in ion-specificity of transport mechanisms are discussed.**

When stem segments or thin discs of various types of storage and tuberous tissues are washed for a time in aerated solutions the characteristics of ion absorption by the tissues change (10, 12, 16, 18, 19, 21, 22, 27, 30, 32). Both rate of ion absorption and respiration usually increase as a function of washing time (aging) (10, 11, 16, 17, 27)—though not always. For example, an increase in PO_4 uptake with time by epicotyl segments of *Pisum* was not accompanied by an increase in respiration (22). The same investigators found that PO_4 uptake increased only slightly when hypocotyl segments of *Gossypium* and *Helianthus* were aged in solutions before the absorption period. The stem segments of the 3 plant species showed little increase in Rb absorption when aged for 18 hr in distilled water (22).

The literature is replete with reports on studies of aging tissue and enhanced ion absorption (12, 16, 21, 27). There have been many suggestions as to the causes of the observed increase in capacity for ion absorption as tissue ages.

It has been suggested that slicing the tissue would expose interior cells to a higher O_2 concentration and increase metabolic activity (32). Such is also thought to be the case for stelar tissue freshly removed from corn roots (13). Recent evidence, however, throws doubt on this interpretation (17).

It has also been proposed that washing thin slices or whole tissues for a period removes ions from the cells and permits ion absorption to proceed in the ion-depleted tissue (30). This has been questioned by MacDonald *et al.* (18, 19).

Laties and co-workers (10, 11, 12) considered the possibility that release of a volatile inhibitor from slices might enhance metabolic activity, but this, too, remains problematical (17).

Reorganization of endoplasmic reticulum (ER) was demonstrated when slices of beet root tissue were aged (7). The ER, disorganized after the slicing operation, reorganized with time into a normal-appearing structure. The reorganization was correlated with an observed decrease in Na and K efflux from the tissue. It has also been noted that the incorporation of various compounds into lipid-membranous fractions increases in aging potato slices (34).

Protein synthesis and content have been observed to change in aging tissues (1). Enhanced ion uptake has been suggested to be indirectly connected with protein synthesis (20). Any such connection could be very important to studies of changes that develop in ion specificities when bean stem slices are aged.

An investigation of Na and K absorption by slices of stem tissue of brittle wax bean (*Phaseolus vulgaris* L.) indicated a specific Na-absorption mechanism that had little affinity for K (24). The capacity to absorb Na was due to cells closely associated with xylem tissue.

It was noted, however, that Na uptake was variable and correlated with the period that slices were left in an aerated $CaSO_4$ solution prior to absorption. Since stem is considered to be a storage-type tissue and aging phenomena have been observed in stem segments (22), an investigation was made of

the effect of aging on the ion-absorption characteristics of bean stem slices.

Materials and Methods

Brittle wax bean plants were grown in nutrient solution in the greenhouse for approximately 3 weeks. The 4 l of nutrient solution contained 0.4 mM KNO_3, 0.8 mM $Ca(NO_3)_2$, 0.4 mM NaH_2PO_4, 0.2 mM $MgSO_4$, 0.025 mM Fe-EDTA, and 2 ml micronutrient stock solution (9).

Preparation of tissue for the experiments was as described previously (23, 24, 29) except for minor alterations. The methods are briefly discussed below.

The plants were harvested and the stem tissue collected and separated into upper and lower hypocotyl. Bean stem tissue grown in this manner contained approximately 25 μmoles K per gram fresh weight and 1.2 to 2.5 μmoles Na. The tissue was placed between 2 pieces of Styrofoam and fitted securely in a hand microtome for slicing (29). The tissue was sliced transversely to give slices 400 μ thick. Slices were randomly sampled and placed in small cheesecloth bags (5).

Samples comprising approximately 40 slices were suspended for various periods in an aerated solution of 0.5 mM $CaSO_4$ in a constant-temperature bath. They were then suspended in a solution containing specified concentrations of Na or K. The Na was labeled with ^{22}Na, and K with ^{86}Rb or ^{42}K. Although the use of ^{86}Rb as a tracer for K has been questioned in uptake studies involving marine algae (37) there was little qualitative or quantitative difference in results when ^{86}Rb or ^{42}K was used to label K solutions. In uptake studies with higher green plants use of ^{86}Rb to label K appears to be a valid procedure and is well documented (23, 26). Since relatively little is known about the absorption characteristics of stem tissue, however, it seemed advisable to test the validity of such a procedure.

The bean stem slices were exposed to a solution containing 0.1 mM K labeled with ^{86}Rb. At the end of the absorption period, samples were rinsed for 30 min in 0.5 mM $CaSO_4$. The samples were blotted, weighed, and ashed. The ash was put into solution, and aliquots were taken for determination of K content by flame spectrophotometry and by radioactive analysis for ^{86}Rb. Since amounts of K absorbed as determined by those 2 procedures did not differ significantly, ^{86}Rb was considered valid as a radioactive label of K and was used in all subsequent experiments.

The absorption periods were terminated by suspending the samples for 30 min in solutions containing 1.0 mM KCl or NaCl and 0.5 mM $CaSO_4$. This desorption procedure was carried out to remove any freely exchangeable ions (5).

Experiments on the effect of aging on K and Na absorption were carried out by placing stem slices in 0.5 mM solutions of $CaSO_4$ for various periods. Then the samples were removed and placed for 1 hr in labeled solutions containing various concentrations of either KCl or NaCl and 0.5 mM $CaSO_4$. A desorption period followed as indicated above.

When the net uptake of K or Na was investigated as a function of time, the slices were exposed for various periods to a labeled solution containing 0.1 mM KCl or NaCl and 0.5 mM $CaSO_4$. Then the samples were removed and exchangeable ions desorbed as described. The absorption is therefore a net accumulation over a selected period.

All solutions contained 0.5 mM $CaSO_4$ to make the conditions optimum for ion transport (25).

At the end of the desorption period the samples were rinsed in water, blotted, weighed, and ashed at 500° in 2.5 cm metal planchets. The ash was wetted and spread with a detergent solution. The samples were dried under infrared lamps. Radioactivity was determined in a gas-flow counter, and the amounts of K and Na absorbed are expressed as μmoles per gram fresh weight.

Bacterial Contamination. Bacterial populations increase considerably in aerated solutions containing plant tissue, and the possibility has been raised that bacteria affect the aging process (14). Therefore, the problem of bacterial contamination was attacked by various procedures.

Bean plants were grown in a low-bacteria environment. The seeds were sterilized, germinated and grown on a sterilized nutrient agar in a lighted glove box, previously treated with U.V. Pieces of stem grown in this manner had approximately 10^3 bacteria per gram fresh weight *versus* 10^7 to 10^8 bacteria in the control.

The stem tissue was collected and sliced, and experiments were carried out in the glove box with sterile procedures and equipment. The experiments indicated that there was no difference between the low-bacteria tissue and the control when absorption experiments were carried out even though the bacterial count differed by several orders of magnitude. Since this was a very laborious procedure and difficult to replicate quantitatively, a different procedure was utilized.

The antibiotic, chloramphenicol, is quite effective in minimizing bacterial contamination (14). A concentration of 50 μg/ml kept bacterial counts below 10^2 per ml of solution, in contrast to 10^5 per ml in the control solution. With chloramphenicol added, the count of the organisms associated with the tissue was also quite low. A series of experiments demonstrated that neither the bacteria nor chloramphenicol had any significant effect on absorption by fresh or aged stem tissue. Consequently, it was not considered necessary to regulate the bacterial population in this study. In experiments with barley roots it has been demonstrated that bacteria have little influence on K absorption (3). Even so, the aging solutions containing 0.5 mM $CaSO_4$ were renewed frequently during the 18- to 24-hr aging period so as to maintain a low bacterial count.

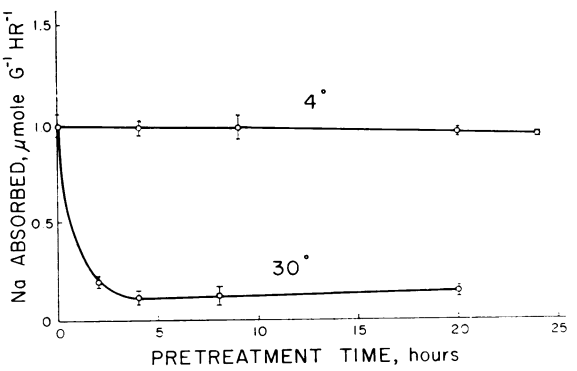

FIG. 1. Rate of Na absorption as a function of pretreatment time (abscissa) in a $CaSO_4$ solution. Pretreatment (aging) solution: 0.5 mM $CaSO_4$ at 30° or 4°. Absorption solution: NaCl, 0.1 mM; $CaSO_4$, 0.5 mM; pH 5.8 ± 0.2; temp 30°; absorption time 1 hr. Samples desorbed as described in text. All treatments replicated; circles represent the means of 2 values indicated by the short horizontal lines. Horizontal lines not drawn where the distance between them would have been less than the diameter of the circle.

Results

The results presented in Fig. 1 are from an experiment on the absorption of Na as a function of pretreatment time in 0.5 mM $CaSO_4$ at either 4° or 30°. Absorption of Na from a solution containing 0.1 mM Na, labeled with ^{22}Na, and 0.5 mM $CaSO_4$ was at 30°. The rate of Na absorption decreased as a function of aging time when the aging solution was maintained at 30°. When aging was done at 4°, there was hardly any decline in Na absorption.

The results presented in Fig. 2 are quite different. In the experiment shown here, the procedure was the same as described for Fig. 1 except that the monovalent cation studied was K. After various periods in 0.5 mM $CaSO_4$, the samples were exposed for 1 hr at 30° to a solution containing 0.1 mM K, radioactively labeled with ^{86}Rb, and 0.5 mM $CaSO_4$. The absorption of K increased with aging up to about 20 hr. The initiation of K absorption varied from experiment to experiment. The absorption of K after 4 hr of pretreatment ranged from 0.05 to 0.4 μmole g^{-1} hr^{-1}. The maximal uptake of K found for the 4 hr pretreatment period is shown in this Fig.; however, it was generally less than 0.4 μmole g^{-1} hr^{-1} as can be seen in Fig. 4. When the aging was carried out at 4° and subsequent absorption was studied at 30°, K absorption remained low even after 24 hr of aging, never exceeding that obtained with freshly sliced tissue.

The data seem to indicate that ion-absorption mechanisms are changed by aging in $CaSO_4$ solution, the change being temperature-dependent.

Figure 3 and 4 present results of experiments on net uptake of Na and K by slices of bean stem. The tissues were exposed continuously for up to 24 hr to solutions containing 0.1 mM Na or 0.1 mM K and 0.5 mM $CaSO_4$. At various times, samples were removed, desorbed, and analyzed for radioactivity. As shown in Fig. 3, the rate of Na absorption slowly declined for the first 3 hr and then became nil. After 8 hr of absorption (as shown in this Fig.) or even 24 hr of absorption (as demonstrated in other experiments) amounts absorbed did not change significantly from the value reached after 3 hr of uptake.

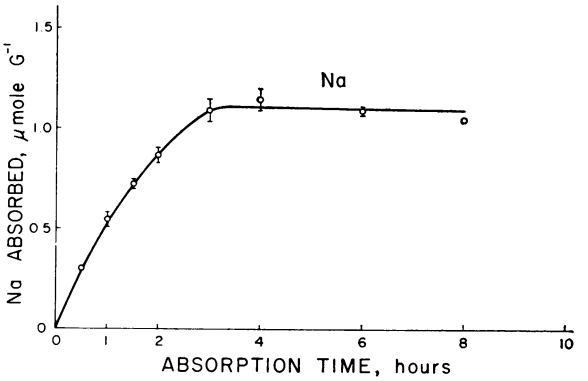

FIG. 3. Absorption of Na as a function of time. NaCl, 0.1 mM; $CaSO_4$, 0.5 mM; temp 30°. Other conditions as for Fig. 1.

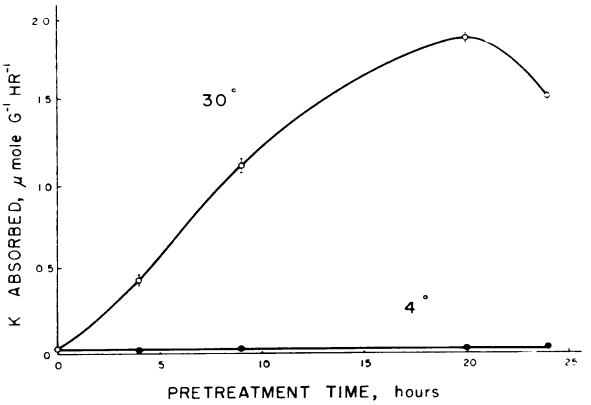

FIG. 2. Rate of K absorption as a function of pretreatment time (abscissa) in a $CaSO_4$ solution. Pretreatment (aging) solution: 0.5 mM $CaSO_4$ at 30° or 4°. Absorption solution: KCl, 0.1 mM; $CaSO_4$, 0.5 mM; pH 5.8 ± 0.2; temp 30°. Other conditions as for Fig. 1.

The absorption of K was quite different, as illustrated in Fig. 4. The absorption rate increased exponentially up to 16 hr and then began to decrease somewhat. This decrease would represent the decrease in hourly rate of K absorption after 20 hr of aging as shown in Fig. 2.

There is a possibility that the decreasing absorption of K or Na might be due to an increase in the efflux of these ions. The idea was tested although

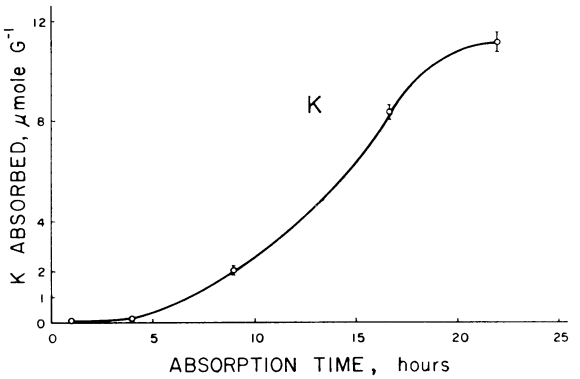

FIG. 4. Absorption of K as a function of time. KCl, 0.1 mM; $CaSO_4$, 0.5 mM; temp 30°. Other conditions as for Fig. 1.

it seemed unlikely since all previous results were obtained from samples exposed to solutions of unlabeled ions after the absorption period. The results of the tests are presented in Fig. 5 and 6.

Figure 5 represents the results of an experiment on leakage of Na ions from freshly sliced tissue. The tissue was exposed for 1 hr to a solution containing 0.1 mM Na labeled with ^{22}Na, and 0.5 mM $CaSO_4$, and the samples were then desorbed. The samples representing zero desorption time were rinsed in demineralized water for 30 sec, while the remainder of the samples were exposed for up to 1 hr to a solution containing 1 mM Na (nonradioactive) and 0.5 mM $CaSO_4$. The results indicate a negligible removal of previously accumulated Na, as measured by ^{22}Na. The initial content of Na in stem slices varied from 1.2 to 2.5 μmole g^{-1}. The absorption of Na amounts to 1.2 μmole g^{-1}, therefore isotopic dilution would be 2 to 3 fold and if efflux was substantial, exchange of previously absorbed, labeled Na should be measurable.

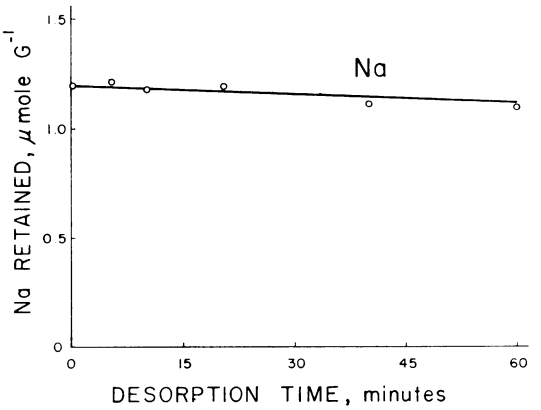

FIG. 5. Sodium retained by fresh slices of bean stem tissue as a function of time. NaCl, 0.1 mM; $CaSO_4$, 0.5 mM during 1 hr absorption period. Point at zero time represents values for samples rinsed 30 sec in water only. NaCl, 1.0 mM; $CaSO_4$, 0.5 mM during desorption period. Other conditions as for Fig. 1, except that no replications were run.

Results were similar when K was the cation investigated (Fig. 6). The experimental conditions were the same as described for Fig. 5 except that the slices were aged for 20 hr prior to absorption and the monovalent cation was K. Desorption periods of up to 2 hr resulted in no appreciable loss of previously accumulated K, as measured by ^{86}Rb.

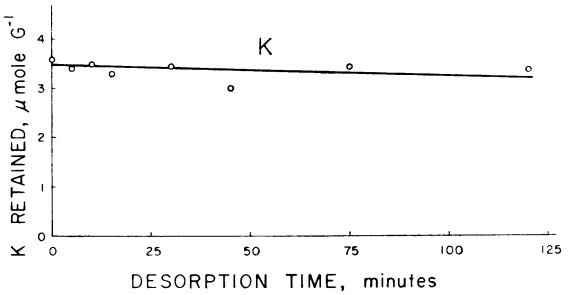

FIG. 6. Potassium retained by aging slices of bean stem tissue as a function of time. KCl, 0.1 mM; $CaSO_4$, 0.5 mM during 1 hr absorption period. KCl, 1.0 mM; $CaSO_4$, 0.5 mM during desorption period. Aging time 20 hr. Other conditions as for Fig. 5 except that no replications were run.

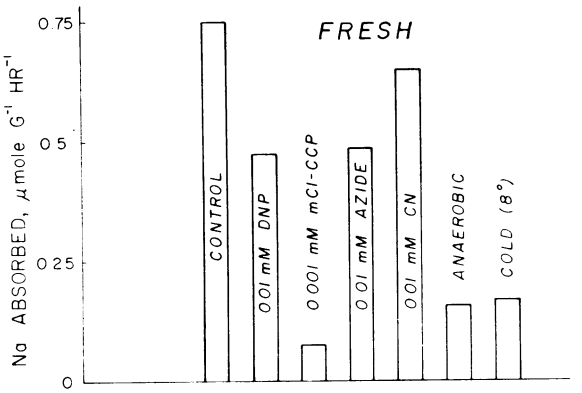

FIG. 7. Rate of Na absorption by fresh stem slices in the presence of antimetabolites. NaCl, 0.1 mM; $CaSO_4$, 0.5 mM; inhibitor concentrations as indicated on figure; pH 5.6 ± 0.2. Absorption period, 1 hr; NaCl, 1.0 mM and $CaSO_4$, 0.5 mM during desorption period; no replications.

One other experiment was carried out to test the leakiness of stem slices. Freshly sliced stem pieces were analyzed chemically for K and Na. In addition, slices were exposed for 24 hr to a 0.5 mM $CaSO_4$ solution, which was periodically renewed, and K and Na contents were determined. The Na and K contents of aged slices did not differ significantly from those of fresh slices.

Figure 7 and 8 present results of experiments on the effects of metabolic inhibitors on Na and K absorption by fresh and aged stem slices. Figure 7 shows the results of an experiment on the absorption of Na as influenced by various antimetabolites. The

FIG. 8. Rate of K absorption by aged (20 hr) stem slices in the presence of antimetabolites. Inhibitors present only during absorption period (1 hr). KCl, 0.1 mM; $CaSO_4$, 0.5 mM. Other conditions as for Fig. 7, except KCl in desorption solutions.

slices were freshly cut and exposed to solutions containing 0.1 mM Na and 0.5 mM $CaSO_4$. The solutions were treated with various inhibitors and maintained at 30° except for the cold treatment. The addition of 0.01 mM DNP[1] or azide inhibited Na absorption approximately 40 %. The addition of 0.01 mM CN inhibited uptake only by 15 %. Anaerobic conditions, low temperature, and 0.001 mM *m*Cl-CCP (6) inhibited uptake by 80 to 90 %. Although these agents varied considerably in relative effectiveness, the data suggest a metabolically-mediated, Na-transport system in fresh slices of bean stem.

The data in Fig. 8 are from an experiment similar to that of Fig. 7 except that the ion studied was K and the tissues were aged for 20 hr prior to the absorption period. The absorption of K by aged bean slices was sensitive to all the metabolic inhibitors except CN, which had no effect. When the inhibition of Na absorption was studied the inhibitors were added as the K salts if counter cations were required. It has been shown that K has no influence on Na uptake by fresh tissue (24). The same applies to the use of Na salts of inhibitors in the studies on K uptake.

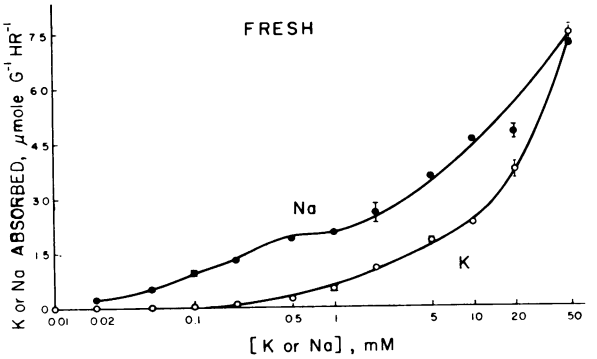

FIG. 9. Rate of Na (top curve) and K (bottom curve) absorption by fresh stem slices as a function of Na and K concentration, respectively. NaCl or KCl, 0.02 to 50 mM; $CaSO_4$, 0.5 mM. Absorption period 1 hr, temp 30°. Desorption as described before. Other conditions as for Fig. 1.

There are several reports on changes in ion-absorption characteristics when the relationship between ion uptake and ion concentrations is studied as a function of increasing tissue age (12, 13, 16, 21). Experiments were designed to test this relationship.

The absorption of Na and K was studied as a function of increasing concentrations of these 2 cations. Figure 9 presents results of experiments with freshly sliced tissues. Absorption of K was nil from solutions ranging in K concentration from 0.02 to 0.2 mM. Above 0.5 mM K, the rate of K absorption increased exponentially up to a K concentration of 50 mM. In contrast, absorption of Na was substantial over the entire range of Na concentrations.

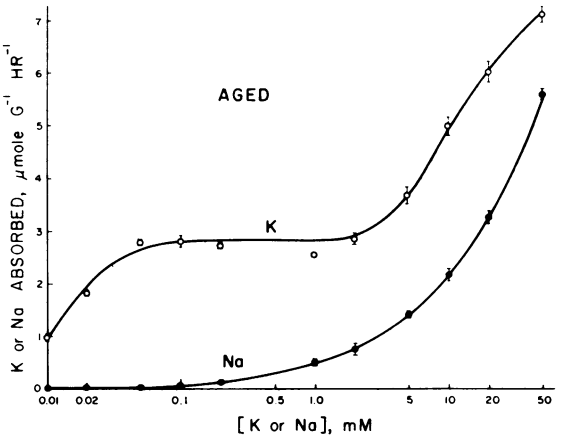

FIG. 10. Rate of Na (bottom curve) and K (top curve) absorption by aged stem slices as a function of Na and K concentration, respectively. NaCl or KCl, 0.01 to 50 mM; $CaSO_4$, 0.5 mM. Aging period 20 hr, absorption period 1 hr, temp 30°. Desorption as described before. Other conditions as for Fig. 1.

Experiments with aged slices gave quite different results (Fig. 10). When Na and K absorption by aged slices was studied over a similar range of concconcentrations, the absorption of Na was nil up to 0.2 mM Na, but then the uptake increased markedly with increasing concentrations of Na. The Na-uptake curve for aged tissue was very similar to the K-uptake curve for fresh tissue.

Potassium absorption increased with increasing K concentrations from 0.01 to 0.05 mM and then leveled off, with no further increase of K absorption between 0.05 and 2 mM K. At K concentrations greater than 2 mM, the uptake of K again increased up to at least 50 mM. The K uptake curve is very similar to those described as representing dual mechanisms of ion absorption (2, 4, 25, 35).

[1] Abbreviations: DNP, 2,4 dinitrophenol; *m*Cl-CCP, carbonyl cyanide *m*-chlorophenylhydrazone; BA, benzyladenine.

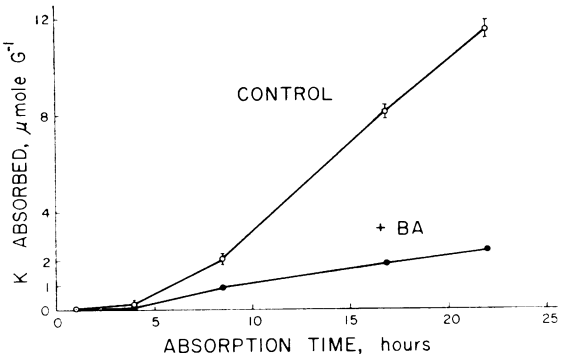

FIG. 11. Absorption of K as a function of time in the presence or absence of 5 μM benzyladenine (BA). KCl, 0.1 mM; $CaSO_4$, 0.5 mM, temp 30°, pH 5.7. Desorption as described for K uptake experiments. Samples replicated and presented as for Fig. 1.

The results shown in Fig. 11 are from an experiment on the absorption of K as a function of time in the presence or absence of 5 μmolar benzyladenine (BA), an analogue of kinetin (15). Potassium was present at a concentration of 0.1 mM, and Ca as $CaSO_4$, at 0.5 mM. The experimental design was similar to the experiment shown in Fig. 4. It is apparent that the BA treatment suppresses the aging effect, as reflected in a marked inhibition of the increase in K absorption. In some preliminary experiments there was a small effect on K uptake by previously aged tissue treated with BA during the absorption period. This indicated that BA only slightly inhibits ion transport *per se* but severely depresses the generation of K absorption capacity.

When the absorption of Na was studied it was found that BA present during the aging period did not prevent the decrease in subsequent Na uptake shown before (*cf.* Fig. 1).

Similar experiments were also carried out with kinetin and virtually the same results were obtained.

Discussion

A Na-specific absorption mechanism has been indicated in the stem tissue of bean (8, 24). The tissue contained substantial K even though K absorption at low concentrations was nil, which suggests that a mechanism for K absorption must also be present. Amounts of cations absorbed changed when pretreatment periods varied. It seemed quite possible that bean stem slices were capable of undergoing the phenomenon of aging (22). There are many reports that the absorption of ions increases as a function of washing time or aging (12, 27). This is true for storage and tuberous tissue (16, 18, 21, 30, 31, 32), stem segments (22), and leaf tissue (33).

The absorption of Na and K (present at a low concentration, 0.1 mM) was altered by aging thin slices of bean stems. Aging changed the species of ion absorbed, and the change was temperature-sensitive (Fig. 1 and 2). Sodium is the ion absorbed at low concentrations by fresh tissue, but such Na absorption decreases rapidly until after 4 hr, little Na is taken up and K is absorbed instead. This whole process can be prevented by keeping the tissue cold which indicates a sensitivity to temperature that is commonly observed in the aging phenomenon (16, 18).

The temperature sensitivity of the aging phenomenon has been suggested to represent the formation of latent ion-absorbing capacity dependent upon metabolic activity (18). The disappearance of ion-absorbing capacity upon aging, and the prevention of this effect by cold, however, is novel and could represent a unique aging response (Fig. 1).

The loss of this Na-absorbing capacity when bean stem slices are aged does not appear to be due to an increase in Na efflux, which could be reflected by lower net accumulation (30, 31), but seems to be due to a cessation of Na absorption. The data seem to indicate that the capacity of aged tissue to absorb Na at this concentration (0.1 mM Na) becomes nil, with no further flux into or out of the tissue. The lack of Na movement out of the tissue was demonstrated by the maintenance of the Na content of the tissue even when bean slices were exposed for 24 hr to a 0.5 mM $CaSO_4$ solution and by the negligible exchange of Na, as measured by ^{22}Na (Fig. 3).

The absorption of K, in contrast, increases markedly when tissue is aged, with little movement out of the tissue. The result is a net accumulation of K, as demonstrated by the nonexchangeability of previously accumulated K (Fig. 6), the increase in K content of the tissue as shown by chemical analysis, and the maintenance of a constant K content when fresh tissue is incubated for 24 hr in a $CaSO_4$ solution.

Ion absorption has been found to be mediated by at least 2 distinct mechanisms when uptake is studied over a wide range of concentrations (2). This is true for barley roots (4), corn roots (35), mangrove leaves (26), and storage tissue (21). In fact this dual pattern seems to be universal in plant cells (2). These mechanisms are described comprehensively in some recent papers (2, 12).

It has been observed that the relation between external concentration and ion absorption may be altered by the aging of tissue (13, 21). One of the more common observations is that the type 1 absorption mechanisms, those operating at low concentrations (below 1 mM), are present in both aged and fresh tissue. The absorption processes mediated at higher concentrations (> 1 mM), *via* the type 2 mechanisms, are usually activated by washing storage-type tissues for a period (13, 21), although they are demonstrable at once in fibrous roots (4, 25, 36).

In slices of bean stems the relation between ion

uptake and concentration shows significant differences from that described above. In freshly sliced tissue, both the type 1 and type 2 mechanisms operate in Na transport whereas only the high-concentration (type 2) mechanism is involved in K transport (Fig. 9). When the tissue is aged the situation is reversed, K absorption now appearing to be mediated by 2 distinct mechanisms over the entire range of concentrations studied. Sodium transport, in contrast, is mediated by the type 2 mechanism operating only at high concentrations. This difference might be related to the special function intact bean stem tissue (fresh tissue) has in regulating the translocation of Na *via* a Na-specific absorption mechanism which removes Na from the transpiration stream (8, 24). Tissue with a Na-regulating function may respond quite differently to aging.

The data indicate that the characteristics of ion absorption change considerably when tissue is aged. It appears that the selectivity of the mechanisms for ion transport can be altered by washing in a solution of $CaSO_4$. Two interpretations of the observed changes are discussed below.

It could be assumed that the absorption mechanisms remain the same in fresh and aged tissue, and only ion specificity changes with aging. An alternative is that the activation of a K-absorbing mechanism is independent of the disappearance of the capacity to absorb Na. Of these 2 speculations the latter would seem to be supported by data presented here.

The absorption of Na virtually ceases after approximately 2 hr of aging. Potassium absorption, however, shows a characteristic "lag-phase" (31) and generally does not begin until after at least 4 hr of aging (Fig. 4). This difference of 2 hr between the end of Na absorption and the beginning of K absorption might be explained by assuming that a change in the ion specificity of the mechanism requires considerable reorganization of the site. A time dependence of breakdown and reorganization of membranous structures has been noted in aging beet root slices (7).

However, the observations would seem to be more easily explained by assuming that the mechanisms of Na and K uptake are not the same and that this is the reason for the observed difference between the kinetics of the change in ion specificity. Although the variation in the kinetics of the initiation of K absorption will not permit any definite conclusions (*cf.* Fig. 2 and 4), K absorption by freshly sliced tissue was always nil.

The difference in rates of Na absorption and K absorption between fresh and aged tissue suggests that the mechanisms for Na and K uptake are not identical. The Na-absorption mechanism does not appear to be as sensitive to metabolic inhibitors as the K-absorption mechanism. In fresh tissue the percentage inhibition of Na uptake by DNP, azide and anaerobiosis is considerably less than that of K uptake by aged tissue (*cf.* Fig. 7 and 8). Experiments in our laboratory (R. A. Floyd, unpublished data) indicate that the rate of respiration of fresh tissue is 30 % less than that of tissue aged for up to 10 hr in $CaSO_4$. This evidence along with other respiration studies (12, 18, 27) would imply a change in metabolic activity when fresh tissue is aged. This might explain the lower sensitivity of Na uptake by fresh tissue to metabolic inhibitors. The possibility that changes in permeability of aging tissue might result in a more rapid penetration of these inhibitors, resulting in greater inhibition, is not supported by anaerobiosis studies on Na and K uptake.

The effect of benzyladenine (BA), an analog of kinetin, on the aging phenomenon is interesting (Fig. 11). The action of kinetin and its analogs on plant tissue has not yet been completely defined, although much information has been collected on the physiological responses of plants to this hormone (15). Of particular interest is its effect on respiration (33), its ability to regulate the structure of cellular membranes (28), and its inhibition of enhanced PO_4 uptake in aging tobacco leaf discs (33).

Kinetin has been shown to delay degeneration of cellular fine structure and might be instrumental in delaying a breakdown of ER. The degradation and reorganization of ER has been correlated with the regulation of ion fluxes in aging beet root slices (7).

If the membrane structure of fresh tissue is responsible for Na transport and must be reorganized for K transport, BA might act by preventing or regulating this restructuring.

It is likely that the mechanisms for Na and K transport are not the same, and the effects of BA support this view. The cessation of Na absorption when tissue is aged is not influenced by BA, while the increase, with age, in the capacity to absorb K is largely prevented by BA. These 2 observations are not consistent with the idea of one mechanism changing in ion specificity with age, but instead support the contention that the mechanisms for Na and K uptake are different and independent.

The above discussion is very speculative. The ideas can be tested, however, by studying what changes in the ultrastructure of these slices are affected by BA and aging. It might also be instructive to determine whether there are changes in the membranous lipid fractions as aging proceeds and ion specificities change (34).

It is expected that the system described will lend itself to further studies of the changes in the ion-specificity of absorption processes which could lead to a better understanding of the biochemical and structural aspects of selective ion transport.

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