THE ESSENTIAL ROLE OF CALCIUM IN SELECTIVE CATION TRANSPORT BY PLANT CELLS [1]

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The mechanisms whereby plant cells absorb inorganic ions from the external media are selective: they discriminate among different ions. Particularly intriguing is the discrimination between potassium and sodium ions. Most plant cells accumulate much higher concentrations of potassium than of sodium ions, even from media in which the sodium concentration greatly exceeds that of potassium.

Perhaps the best way to investigate these specificities is to study the manner in which different ions affect each other's absorption. In a previous paper (4) experiments were described demonstrating that in excised barley roots potassium and rubidium compete with each other in an identical absorption mechanism. At moderate concentrations, neither sodium nor lithium ions competed for the potassium-rubidium transport mechanism. The deduction was that there are specific ion binding sites on carrier molecules which effect the transport of the ions across cellular membranes. Potassium and rubidium ions are bound and transported by sites which fail to discriminate between them, hence these ions compete with each other for identical carrier sites. Sodium and lithium are not bound by the potassium-rubidium sites, hence do not compete with potassium and rubidium but are transported by separate sites.

In this paper experiments with barley roots are presented which show that calcium ions are essential for the integrity of the selective absorption mechanism referred to, and information is given about some quantitative features of the calcium effect.

MATERIALS & METHODS

The distilled water piped into the laboratory was passed through a mixed ion exchange column [2] to free it from heavy metal and other impurities. This purified water was stored in pyrex carboys. Glassware was rinsed with about 0.5 M HCl, followed by exhaustive rinsing with distilled water. Salts used were C.P. grade. Radioactive Rb^{86} and K^{42} were obtained from Oak Ridge National Laboratory, and Na^{22} from Nuclear Science & Engineering Corp., Pittsburgh 36, Pa. The stock solutions were labeled to the extent of about 0.002 μc per μmole.

PREPARATION OF ROOT MATERIAL. Seeds of barley, *Hordeum vulgare* L. var. Arivat, were allowed to germinate for 24 hours in 1 liter aerated water in the dark, at room temperature (about 20° C). The water was renewed once during this time, 19 hours after the start of the germination period. The germinating seeds were spread on a layer of cheesecloth which had been washed and rinsed with distilled water. The cheesecloth was supported by a stainless steel screen about one centimeter above the surface of 4 liters of 2×10^{-4} M $CaSO_4$ solution in a 4-liter pyrex beaker. A second cheesecloth was spread over the seeds. The corners of both cheesecloths dipped into the solution. An inverted watchglass, 17.5 cm in diameter, was placed on the beaker. The top cheesecloth was removed as the germinating seedlings pushed it up.

This assembly was placed in the dark, at room temperature. The solution was aerated continuously. Three days after the seeds were planted on the cheesecloth, the stainless steel screen with the seedlings was removed from the beaker, the roots were rinsed in two changes of water, and the assembly put on a beaker with fresh solution. The watchglass was not replaced. Two days after this, the roots were rinsed repeatedly in water, excised just below the screen, rinsed several more times, and suspended in about four liters of water.

ABSORPTION EXPERIMENTS. For the experiments proper, roots were gently blotted on dry cheesecloth which had been washed and rinsed with distilled water. One-gram samples were weighed out on a torsion balance and transferred to 100 ml water in 30×300 mm culture tubes. The tubes were placed in a waterbath maintained at 30° C, and the water in the tubes was aerated. To start the absorption period proper, each tube in turn was removed from the bath, the water was decanted, the roots were rinsed twice with about 100 ml water each time, and 100 ml of prepared experimental solution were then poured in. The tubes were replaced in the bath, and the solution was kept aerated.

[1] Received January 9, 1961.

[2] Bantam Barnstead Demineralizer, made by Barnstead Still and Sterilizer Co., Boston 31, Mass.

The absorption period was discontinued by decanting the experimental solution and rinsing the roots for 1 minute with a total of at least 500 ml water.

All salts of the experimental solutions were chlorides. The initial pH (unbuffered) was 4.9, and this changed by no more than 0.2 pH units during the experimental runs. The roots contained initially no detectable Rb, less than 1 μmole Na per gram fresh weight, and about 15 μmole K. These two values varied slightly from experiment to experiment, although all seed used came from the same batch.

Radioactive Assay. The root samples were transferred to 1-inch × 5/16-inch nickel-plated planchets, dried, and ashed at 500° C. A little water and one drop detergent solution were added to the ash and the samples dried under infra-red lamps. The samples were counted with a thin-window (1.4 mg/cm²) Geiger-Mueller tube connected to a conventional scaler. Each sample was counted twice, to at least 3,000 counts each time. In most experiments, samples were counted to 10,000 counts each time. Samples of an appropriate dilution of the radioactive stock solution were pipetted into counting cups and after addition of a drop of detergent were dried under infra-red lamps.

Flame Photometric Assay. The root samples were transferred to 30-ml pyrex beakers, dried, and ashed at 500° C. The ash was taken up in 0.16 N HNO_3, and assayed in a Beckman Model DU spectrophotometer with photomultiplier and flame attachment. Standard curves were prepared from known solutions of the salts in 0.16 N HNO_3. Potassium was assayed at 766 mμ, sodium at 589 mμ, and rubidium at 780 mμ.

Table I

Effectiveness of Divalent Cations in Reversing Inhibition of Rb Absorption by Na Ions*

Na Conc, mM	Divalent cation	Rb Absorption μmole/g/hr	Rb Absorption % of control**	Rank of divalent cation
0	...	6.6	...	...
0	Ca	6.9	...	...
20	...	2.0	100	...
20	Mg	2.3	115	8
20	Ca	4.3	215	1
20	Sr	3.2	160	5
20	Ba	3.5	175	4
20	Mn	4.0	200	2
20	Co	2.8	140	6
20	Ni	2.6	130	7
20	Zn	3.7	185	3

* Concentration of Rb: 1 mM. Concentration of divalent cations: 0.05 mM; time: 60 minutes.

** The sample with Na present at 20 mM concentration, but without added divalent cation, is considered the control (100 %)

Consistency & Reliability of Results. For each experiment reported, one or several others were done with or without modifications. No results were obtained that were inconsistent with those reported.

Results

The effect of Na on absorption of Rb during a 60-minute period is shown in figure 1[3]. In the absence of Ca, Rb absorption from a 1 mM solution of RbCl was progressively diminished by increasing concentrations of NaCl. In the presence of Ca, the effect of Na was quite different, and this typical response to Na was shown in experiments both on Rb and K absorption (figs 1, 2, 3, 5). There always is a slight inhibition of absorption at 1 to 2 mM Na. Between 5 and 10 mM Na, absorption of K or Rb rises to almost the control (no Na) value, and at still higher Na concentrations declines slightly.

This marked indifference of K and Rb absorption to even high concentrations of Na, in the presence of Ca, is in conspicuous contrast to the pronounced inhibition of K absorption by Rb (fig 2), and of Rb absorption by K (fig 3). Both of these experiments lasted 60 minutes, and the K and Rb concentration was 1 mM. Calcium was present at a concentration of 0.05 mM.

In the previous experiments, the concentration of Ca was 0.05 mM. The effect of varying the Ca concentration over a wide range is shown in figure 4. The Rb concentration was 1 mM and that of Na 20 mM throughout. Calcium concentrations are plotted on a logarithmic scale, experimental points lying at full powers of ten and three times these values. At the Na concentration used (20 mM), Rb absorption from a 1 mM solution was reduced to 2.1 μmole/g/hour, in the absence of Ca, and there was no effect from Ca at 1×10^{-6} N. At higher Ca concentrations Ca became increasingly effective in reversing the inhibition due to Na, Rb absorption rising most steeply at about 1×10^{-4} N Ca, and leveling off at 3×10^{-4} to 1×10^{-3} N Ca. In a similar experiment with K as the ion absorbed, the Na concentration was 10 mM, and the absorption of K from a 5 mM solution was determined as a function of the Ca concentration over the same range. Assay in this experiment was by flame photometry. The response in terms of K absorption paralleled that in the present experiment on absorption of Rb.

Is calcium the only divalent cation capable of protecting the K-Rb absorption mechanism against the Na inhibition? Table I gives the results of an experiment in which the effectiveness of eight divalent cations was tested. The first two lines show results obtained without Na in the solution. In the absence

[3] Asterisks in the figures denote radioactively labeled ions.

of Na, Ca at 0.05 mM does not appreciably increase the amount of Rb absorbed. The severe inhibition of Rb absorption by Na (line 3) is reversed by Ca (line 5) and the other divalent cations tested, to varying degrees. Calcium is the most effective ion. The last column of table I ranks all ions tested in order of their effectiveness.

A curious result (table I) was the ineffectiveness of the Mg ion in affording protection against the Na inhibition of Rb absorption. The value to which the Na inhibition reduced Rb absorption, viz. 2.0 μmole, was more than doubled in the presence of Ca, but Mg at the same concentration increased it by merely 15 %. No other ion, among those tested, was equally ineffective. Figure 5 shows the results of an experiment in which both Ca and Mg were studied in their relative effectiveness in counteracting the Na inhibition of K absorption. The roots absorbed from 1 mM solutions of K, for 60 minutes. At the lowest Na concentrations Mg had some effect, but at the higher concentrations of Na the effectiveness of Mg declined and at 20 mM Na, K absorption in the presence of Mg was not significantly higher than in absence of any divalent cation. Calcium largely protected against Na inhibition, as usual.

The effect of time was studied in an experiment in which roots absorbed Rb from a 1 mM solution in the presence, throughout, of 20 mM Na. In one set, Ca was present at 0.1 mM from the beginning. Another set had no calcium. A third was without Ca for the first 24 minutes of the absorption period, then Ca was added in 0.1 mM concentration. The results are shown in figure 6.

The foremost features in this experiment are as

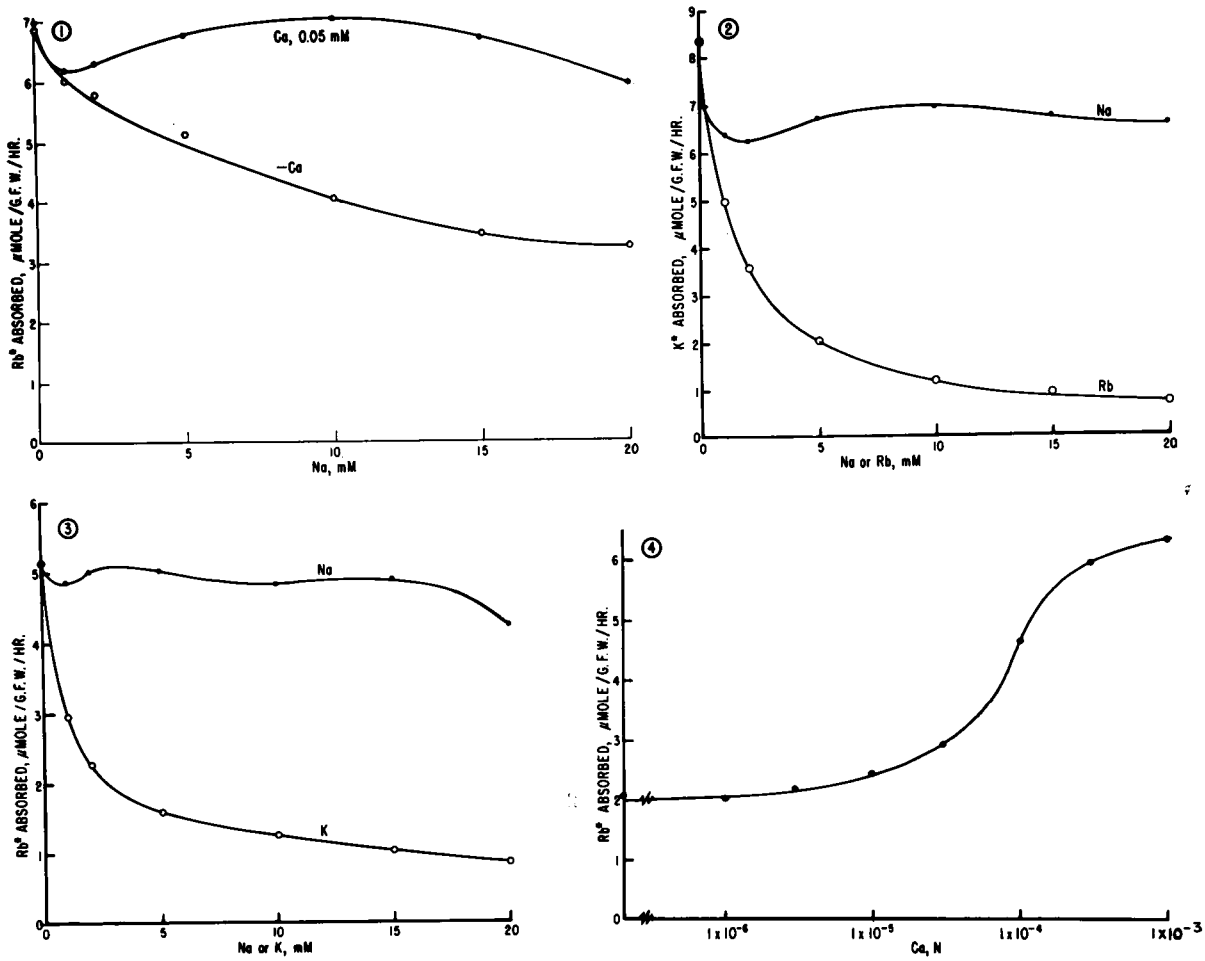

FIG. 1. Effect of Na on absorption of Rb in the absence and presence of Ca. Rb: 1 mM; time: 60 minutes.

FIG. 2. Effect of Na and of Rb on absorption of K in the presence of 0.05 mM Ca throughout. K: 1 mM; time: 60 minutes.

FIG. 3. Effect of Na and of K on absorption of Rb in the presence of 0.05 mM Ca throughout. Rb: 1 mM; time: 60 minutes.

FIG. 4. Effect of Ca concentration in reversing the inhibition of Rb absorption by Na. Rb: 1 mM; Na: 20 mM; time: 60 minutes.

follows: In the presence of Ca, Rb absorption was a strictly linear function of time, extrapolating to zero at zero time. Without Ca, the rate of absorption declined all along. Upon addition of Ca to the solutions lacking Ca there was no immediate effect on the rate of Rb absorption, but after about an 8-minute lag the rate of Rb absorption increased. The recovery was not complete, however, in that these roots did not absorb Rb as fast as did those that had Ca present from the beginning of the absorption period, nor was the rate of absorption constant.

In the previous experiments it has been shown that in the presence of Ca at concentrations of 0.05 to 0.1 mM, Na over a wide range of concentrations fails to compete with either K or Rb in the absorption process (figs 1, 2, 3, 5), while K and Rb do mutually compete (figs 2, 3). What is the converse effect, that of K or Rb upon the absorption of Na? Figure 7 shows the results of an experiment in which Na absorption from a 1 mM solution was studied as affected by increasing concentrations of K, in the absence and presence of Ca at 0.1 mM concentration. The absorption period was 60 minutes.

In the absence of Ca, Na absorption progressively declines with increasing K concentration, much as Rb absorption and K absorption are decreased by progressively higher concentrations of Na, without added Ca (figs 1 & 5). In the presence of Ca, on the other hand, there is a sharp drop in Na absorption at 1 mM K. At higher K concentrations, Na uptake decreases much more gradually, and virtually levels off at the highest K concentrations tested. These features are brought out even more clearly in the experiment shown in figure 8, identical with the previous one in every respect except that the Na concentration was 5 mM.

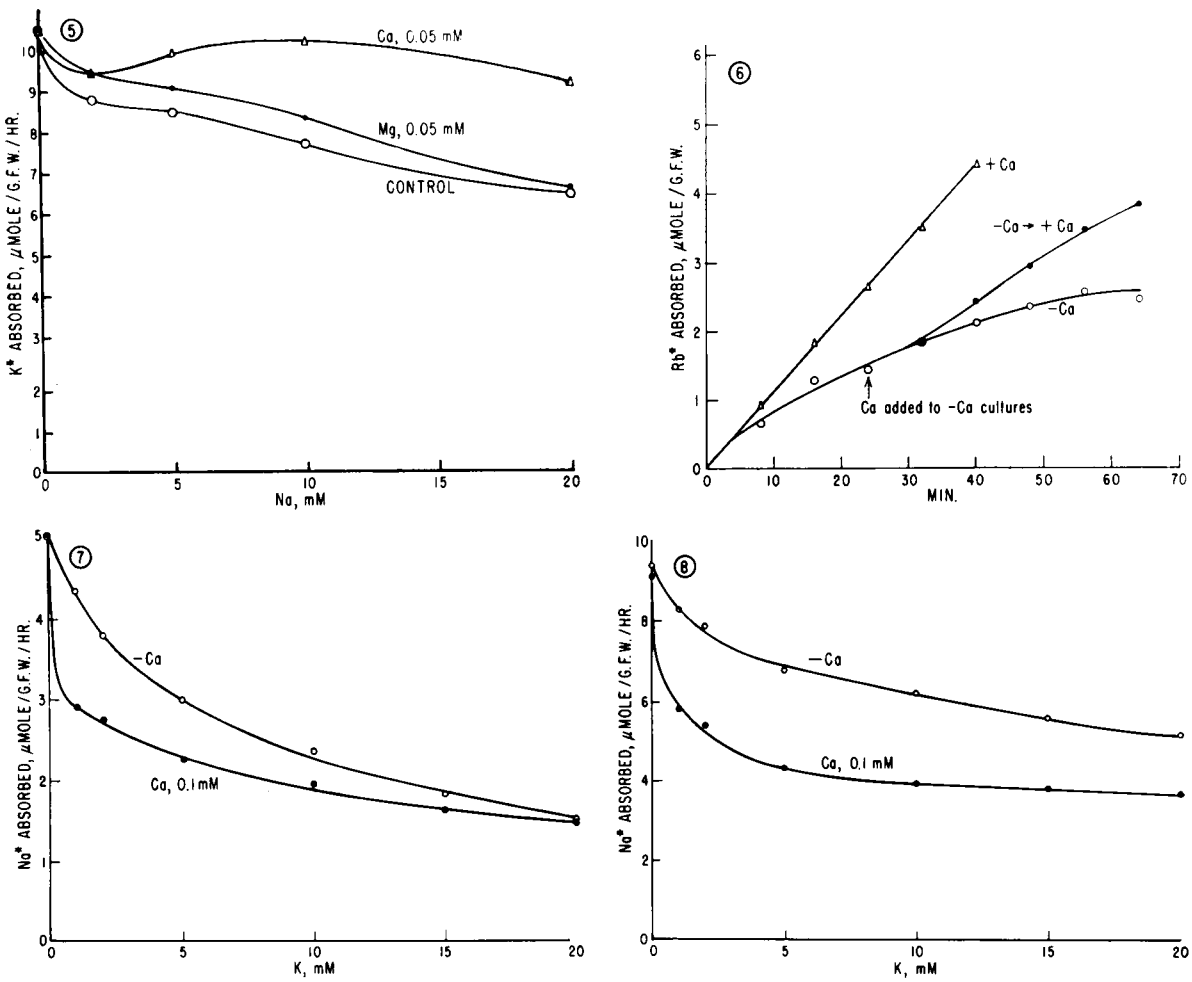

FIG. 5. Effectiveness of Ca and Mg in reversing the inhibition of K absorption by Na. K: 1 mM; time: 60 minutes.

FIG. 6. Time course of Rb absorption in the presence of Na, and the effect of Ca. Rb: 1 mM; Na: 20 mM; Ca where present: 0.1 mM.

FIG. 7. Effect of K on absorption of Na in the absence and presence of Ca. Na: 1 mM; time: 60 minutes.

FIG. 8. Effect of K on absorption of Na in the absence and presence of Ca. Na: 5 mM; time: 60 minutes.

TABLE II

ABSORPTION OF Rb & Na AS A FUNCTION OF Na CONCENTRATION IN ABSENCE & PRESENCE OF Ca*

Na Conc mM	Rb Absorbed, μmole		Na Absorbed, μmole	
	−Ca	+Ca	−Ca	+Ca
0	6.5	6.6	...	...
5	5.0	6.4	5.4	3.9
10	4.3	6.6	8.7	5.7
20	3.5	6.8	13.5	8.0

* Concentration of Rb: 1 mM throughout. Calcium, where present: 0.1 mM. Absorption period: 60 minutes. Experiment done in duplicate, one set with Rb* for radioactive assay, the other with Rb for Na assay.

In table II the results of an experiment are presented in which the absorption of Rb and Na was measured both in the absence and presence of Ca at 0.1 mM. The Rb concentration was 1 mM; Na ranged from zero to 20 mM. In stabilizing the K-Rb absorption mechanism against inhibition by Na, Ca appreciably reduces the absorption of Na (cf. figs 7 & 8).

DISCUSSION

The results confirm and extend previous conclusions (4): K and Rb ions are mutually competitive, Na does not compete in at least one K-Rb absorption mechanism. In the present work Ca is shown to be essential for the maintenance of the integrity of this selective ion transport mechanism.

In particular, Ca prevents Na from competing with K and Rb at the higher Na concentrations. In the earlier experiments, Na at low concentration (up to 10 mM) did not compete with Rb, but it did compete at higher concentrations. It is shown here that without Ca, the selectivity between K and Rb on the one hand and Na on the other breaks down even at low concentrations of Na. Evidently enough endogenous Ca was present in the earlier experiments to permit demonstration of the relative indifference of the K-Rb transport mechanism to Na. In the present experiments another variety of barley was used, the volumes of experimental solution were larger, and the roots were much more exhaustively rinsed before the absorption period. These factors made for a more thorough removal of diffusible Ca from the tissue, and permitted demonstration of the essentiality of Ca in the selective ion transport mechanism.

In analyzing the results, a convention adopted earlier (4) will be used. The ion whose absorption is discussed at the moment will be called the substrate ion, and the ion whose effect on the absorption of the substrate ion is being tested will be the interfering ion. As a matter of fact, both the substrate and the interfering ions are absorbed, and the terminology is used for convenience only.

The results shown (figs 2 & 3) are consistent with the previous interpretation (4) that K and Rb are transported by identical carrier sites and compete with each other for these sites. With K as the substrate ion, Rb at increasing concentrations progressively diminishes the absorption of K indicating the progressive displacement of K ions by Rb ions from the available carrier sites (fig 2). Competition for identical sites is mutual, by definition, and figure 3 shows that K in quite similar manner reduces the absorption of Rb. The identity of the K and Rb transport mechanisms is further brought out by the fact that the absorption of both is affected in similar manner by Na ions, in the presence of Ca (figs 1, 2, 3, 5). One cannot tell, without reading the legends, whether a given graph depicts the effect of Na on K or Rb absorption.

The K-Rb carrier mechanism is highly indifferent to even a 20-fold excess of sodium in the solution, but only in the presence of Ca (figs 1, 5). Evidently under these conditions Na, over a wide range of concentrations, fails to compete for the K-Rb carrier sites. This difference between Na on the one hand, and K-Rb on the other, is strictly a phenomenon of cation selectivity, distinct from any anion effect. The concentration of Cl ions accompanying the interfering cation increased progressively in precisely the same manner, whether Rb or Na was the interfering cation (fig 2). The sharp contrast between the effects of these two chlorides on K absorption was therefore due entirely to the respective cations. The same considerations hold for the findings shown in figure 3.

Calcium has been shown by Viets (14) and others to accelerate the absorption of various ions by plant tissues, including the absorption of K (8, 11, 14) and Rb (3) by barley roots, the absorption of Rb by mung bean roots (13) and the absorption of K by corn and soybean roots (10). Is the Ca effect described in the present paper identical with this earlier effect of Ca and other divalent and trivalent cations on ion absorption? The answer to this question is no, for several reasons.

I. The effect described heretofore of accelerated ion absorption is produced not only by Ca ions but by several other divalent cations as well, including Mg ions. In all but one of Viets's (14) experiments dealing with the comparison of various divalent cations, Mg was second only to Ca in accelerating ion absorption by barley roots. Tanada (13) also found that results obtained with Mg were essentially similar to those obtained with Ca in experiments on the influence of these ions on the absorption of Rb and H_2PO_4 by mung bean roots. In the present experiments, Mg was the least effective among the divalent ions tested (table I, fig 5).

II. The Ca effect described in the present paper approaches a maximum at Ca concentrations on the order of 3×10^{-4} to 1×10^{-3} N, and is about half maximal at 1×10^{-4} N Ca (fig 4). The acceleration

of ion absorption described by Viets (14) and others (3, 11, 13) was caused by Ca at much higher concentrations, saturating Ca concentrations being usually on the order of 1×10^{-2} N or even higher.

III. The previous reports referred to experiments in which the experimental solutions contained two salts: the salt of the substrate ion whose absorption was studied, and a salt of Ca or some other divalent or trivalent cation. Under similar conditions, i.e., in the absence of Na, Ca at concentrations of 0.05 mM or 0.1 mM did not accelerate the absorption of Rb significantly (table I, 1st 2 lines; table II, 1st line; figs 1, 5). Only when the absorption of K or Rb was inhibited by Na did Ca at these concentrations increase their absorption.

It is concluded that the Ca effect described here differs from that discussed by Viets and others. Their experiments showed that divalent cations caused an acceleration of ion absorption per se. The present experiments describe a reversal, by Ca and certain other divalent cations, of the inhibition of K-Rb transport by Na. For this reversal, Ca is effective at much lower concentrations than those required for straightforward acceleration of ion absorption, and Mg is largely ineffective.

In the presence of Na at 20 mM, the rate of Rb absorption steadily declined if no Ca was present, but remained constant in the presence of 0.1 mM Ca (fig 6). The findings suggest a progressive derangement of the K-Rb transport mechanism by Na, in the absence of Ca. Recovery from this Na- induced impairment upon addition of Ca was neither immediate nor complete. It took more than 8 minutes after addition of the Ca before the rate of Rb absorption increased, and even then it remained lower and less steady than the rate of absorption by those roots that had Ca present from the beginning. The lag in the recovery after addition of Ca was slightly shorter in another experiment similar to that shown in figure 6, being a little less than 7 minutes. Such a lag is not surprising in view of the fact that the initial penetration of divalent cations into the tissue is fairly slow, about 75% equilibration being reached in 8 minutes (5).

Inasmuch as Na, in the presence of Ca, failed to compete with K-Rb over a wide range of concentrations, experiments were done to examine the reciprocal effect, viz., the influence of K-Rb on the absorption of Na. Absorption of Na was depressed by K more severely in the presence of Ca than in its absence, but comparison is invited of this K effect on Na absorption (figs 7 & 8) with the straightforward competitive situation, as when K competes with Rb (fig 3), or Rb with K (fig 2). The latter responses are consistent with the previous interpretation (4) that these two ions, K and Rb, displace each other from identical carrier sites. As the ratio of interfering to substrate ions is progressively raised, complete displacement of the substrate ions by the interfering ones is asymptotically approached.

Clearly, such a simple competitive situation does not prevail when K interferes with the absorption of Na, in the presence of Ca (figs 7 & 8). At low K concentrations there is a pronounced inhibition of Na absorption, but at the higher concentrations of K the response of Na absorption to added increments of K becomes very slight, especially when the Na concentration is 5 mM (fig 8). This is interpreted as indicating the existence of at least two Na carrier sites. The operation of one of these is inhibited by K, the other one is quite indifferent to K and accounts for the absorption of Na at the higher concentrations of K.

A double mechanism is suggested for K-Rb absorption as well. Figure 2 shows that at low concentrations, Na interfered somewhat with K absorption, indicating a Na-sensitive K transport mechanism, but the mechanism effecting K absorption at the higher Na concentrations was quite indifferent to Na. The same phenomenon is apparent to a lesser extent in the other experiments on Na interference with K-Rb absorption, in the presence of Ca. It is more pronounced when the K-Rb concentration is higher (see 4, fig 1).

In these experiments it has been shown that from solutions containing both K (or Rb) and Na, the tissue absorbs relatively more K (or Rb) and relatively less Na in the presence of Ca than in its absence. It might be tempting to advance the explanation that Ca minimizes Na interference with K-Rb transport by excluding Na, thus preempting carrier sites for K-Rb transport by preventing Na from having access to them. However, the facts do not support the idea of a simple one-to-one-relationship. Table II shows, as did the experiments presented in figures 1, 2, 3, and 5, that Ca reversed the progressive inhibition of the K-Rb transport by ever larger concentrations of Na. Nevertheless, absorption of Na, though less than in the absence of Ca, increased with increasing Na concentrations even in the presence of Ca, and at the highest Na concentration, 20 mM, exceeded the absorption of Rb. At this concentration Na, in the absence of Ca, caused a drop in Rb uptake of 3 μmoles, from 6.5 to 3.5 μmoles, while 13.5 μmoles Na were absorbed. In the presence of Ca, Rb absorption was essentially unaffected, and 8.0 μmoles Na were absorbed at the highest Na concentration.

The following hypothesis is consistent with the facts. Two species of carrier sites transport K-Rb. One of these, A, with a relatively low affinity for K-Rb ions, is inhibited by Na (fig 2), and more severely so, the higher the K-Rb concentration (4, fig 1). This latter feature is due to the fact that this low-affinity site accounts for a greater proportion of the total K-Rb transport at the higher K-Rb concentrations. The inhibition by Na of K-Rb transport by this site is mitigated but not completely reversed by Ca (figs 1, 5). The other site, B, has a higher affinity for K and, in the presence but not in the ab-

sence of Ca, is largely unaffected by Na. This is the site responsible for the lack of inhibition of K-Rb transport at the higher Na concentrations when Ca is present (figs 1, 2, 3, 5).

Two carrier sites are indicated for the transport of Na as well. In the absence of Ca, Na is transported by a site, C, with relatively low affinity for the ion. When Ca is present this site is largely preempted by K (figs 7, 8, over the lower range of K concentrations). Another part of the Na transport is through a site, D, with high affinity for Na ions and largely indifferent to K, in the presence of Ca (figs 7, 8, at the higher concentrations of K).

A careful consideration of all the data suggests that the K-Rb transporting site A may be identical with the Na transporting site C, and to that extent there exists indeed a mechanism common to K-Rb and Na transport. This site has a much higher affinity for K than for Na when Ca is present, hence the sharp drop in Na absorption at even low K concentrations, in the presence of Ca (figs 7, 8).

The significance of these findings lies in the fact that plant cells under normal physiological conditions absorb K and Na *in the presence of Ca.* Only the presence of sufficient Ca of endogenous origin permitted the earlier demonstration (4) of distinct transport sites for K-Rb and Na, respectively. When the precautions described here are taken, this selectivity breaks down if Ca is omitted from the solutions.

This conclusion probably applies to plant cells generally. The red alga, *Porphyra perforata,* differs in its K and Na transport from barley roots in that it accumulates K and extrudes Na, but here, too, Ca is essential in maintaining the integrity of the distinct K and Na transport mechanisms (2). Jacobson et al (9) have shown that the roots of plants of several species absorb more K and less Na in the presence of Ca than in its absence. A general impairment of ion transport through removal of Ca has been described by Hanson (6) and Helder (7), but their studies did not deal with the selective feature of K-Rb vs. Na transport described here.

Certain other divalent cations besides Ca, notably Mn, can partially reverse the inhibition of K-Rb absorption by Na (table I), yet Ca probably is the ion chiefly responsible for the maintenance of ion selectivity under normal, physiological conditions. Physiological concentrations of such divalent micronutrient cations as Mn and Zn are on the order of micromoles per liter, but in this range of concentrations even Ca was without much effect (fig 4). Manganese and zinc, when tested at a concentration of 5 μM (1×10^{-5} N), were about as effective as Ca at that concentration (cf. fig 4). Only Mg, among the divalent cations tested, is present in natural substrates at concentrations of the same order of magnitude as Ca ions, and it was quite ineffective in reversing the inhibition, by Na, of K-Rb absorption (table I, fig 5).

The present findings strengthen the earlier conclusion (4) that in barley roots and probably quite generally in plant cells, there are carrier sites involved in the transport of K-Rb which are distinct from others that transport Na ions. The opposite view, that there is a single common mechanism responsible for the absorption of these cations, has been discussed in the literature (1, 9, 12). Particularly relevant are the conclusions of Conway and Duggan (1) on ion absorption by yeast because they used the same kinetic treatment (4) that had led to the finding of separate mechanisms of K and Na absorption in barley roots. They concluded that in yeast, absorption of K and Na is by one and the same carrier mechanism. These experiments with yeast were done at high concentrations of the monovalent cations (up to 100 mM Na), in the absence of divalent cations. It is thus quite possible that different results would have been obtained under more nearly physiological conditions, which include the presence of Ca or other divalent cations.

The present results cannot be explained by a scheme of simple mutual competition by K-Rb and Na for a single common carrier site. The operation of at least three sites is demonstrated of which one is highly selective for K-Rb and another for Na. The present findings confirm the validity of the "enzyme kinetic" analysis of absorption rates which initially led to the conclusion of separate K-Rb and Na transport sites (4).

Summary

Excised barley roots, rinsed thoroughly free of diffusible Ca, absorbed ions from solutions containing KCl or RbCl or NaCl, or combinations of these, in the presence or absence of Ca or other divalent cations, for periods of 60 minutes in most experiments.

In the absence of Ca, Na interferes with K absorption, and K with Na absorption, at all concentration ratios examined. In the presence of Ca, Na interferes slightly with K absorption at low Na concentrations (1–2 mM), but at progressively higher concentrations of Na there is little or no further inhibition of K absorption.

In the presence of Ca, K inhibits absorption of Na at low K concentrations, but as the K concentrations are raised inhibition of Na absorption per added increment of K becomes very slight.

Potassium and rubidium are mutually competitive in the presence of Ca.

Calcium is maximally effective in maintaining the integrity of the selective absorption mechanisms at a concentration of about 1×10^{-3} N. Other divalent cations, notably Mn, are also active, but Mg is largely ineffective.

When Ca is added to solutions from which roots absorb Rb in the presence of Na, the impairment of Rb absorption by the Na present is only partially reversed, and only after a time lag of some minutes.

It is concluded that transport of K-Rb is through two carrier sites, one inhibited by Na, the other largely indifferent to this ion. Transport of Na also in-

volves two carrier sites, one of which is largely indifferent to K-Rb. Calcium is essential to the integrity of the selective ion transport mechanisms.

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