Short Communication

Transport of Potassium and Rubidium in Plant Roots

THE SIGNIFICANCE OF CALCIUM

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Plant cell membranes possess mechanisms by which ions are selectively absorbed from the external solution provided that Ca is present at an adequate concentration in the solution bathing the tissue (7). Certain closely related ions, however, can scarcely be distinguished by these mechanisms. Potassium and Rb are two such ions which in experiments on absorption behave virtually as isotopes of the same element, with little discrimination between them. Consequently, ^{86}Rb has often been used as a radioactive tracer for K in investigations concerning ion absorption by excised tissues of higher plants. Rubidium-86 is more convenient to use than ^{42}K as a label for K because the half-life of ^{86}Rb is 18.6 days, compared with 12.47 hr for ^{42}K.

The situation becomes more complex when the absorption and translocation of K in entire plants is studied. Potassium and Rb ions, after being absorbed, might be sequestered by cytoplasmic organelles while moving through the symplasm. Discrimination between the two ions might then occur. Nevertheless, Collander (4) on the basis of experiments in which plants grew in nutrient solutions containing K and Rb concluded that they absorbed these elements "somewhat as identical ions or as two isotopes of the same element." Menzel and Heald (13) in similar experiments found that there was a slight discrimination against Rb in absorption of K and Rb by several plants, but in flowers Rb was preferentially accumulated. Wallace (15) investigated the distribution of K and ^{86}Rb in bean plants and observed that there was only a small degree of discrimination between the two elements in various plant organs. Vakhmistrov and Zakharin (14) found that absorption of K by intact sunflower plants virtually coincided with the absorption of Rb. In the experiments of Burr and Tanimoto (3) the distribution of Rb in sugar cane plants so closely resembled that of K that Rb could be used as an indicator of K distribution.

The general conclusion has emerged from these and many other findings that "the mechanisms of transport for Rb and K are similar, the ion transporters that convey them being the same" (1). In contrast to this conclusion is that of Maas and Leggett (12), who in short term experiments with corn roots, *Zea mays*, DeKalb 441, found not only the magnitude but even the direction of K and Rb movement to differ. There was a net loss of potassium from the roots to a 0.1 mM solution of KCl labeled with ^{86}Rb, while radioassay for ^{86}Rb indicated a steady gain. We are currently concerned with the absorption and translocation of ions in corn roots. In this report we present results of experiments on the absorption and translocation of K by corn roots as measured by labeling with ^{42}K and ^{86}Rb and by chemical analysis of the roots, and the importance of Ca in experiments of this kind.

Experiments on absorption were done with 6-day-old roots of corn, *Z. mays*, DeKalb 805. The seedlings were grown as described earlier for barley (6). Apical segments 6 cm long were excised and used in short term experiments (9). For the experiments with double labeling, root samples weighing 0.50 g fresh weight were placed in aerated solutions containing 0.2 mM KCl and 0.5 mM CaSO$_4$ at 30 C. Potassium was double-labeled with ^{42}K and ^{86}Rb. The period of absorption was discontinued by exchanging the labeled ions from the "outer space" (9) with a solution of 5 mM unlabeled KCl and 0.5 mM CaSO$_4$ at 4 C for 30 min. The roots were then rinsed with water and directly radioassayed by Cerenkov radiation (11).

For the experiments on the effect of Ca, culture of the plants and excision of the roots were as described above. The roots to be used with addition of Ca (+Ca roots) were kept for 30 min in 0.5 mM CaSO$_4$ solution, while the −Ca roots were kept in deionized water during the same time. The experimental solutions, which were aerated, consisted of 0.1 mM KCl labeled with ^{86}Rb, with and without 0.5 mM CaSO$_4$, respectively. After the absorption period, the samples of both treatments were rinsed for 1 min with cold (4 C) 5 mM CaSO$_4$ solution and then placed in large volumes of identical, aerated solution for 30 min in order to desorb the exchangeably adsorbed fraction of the monovalent cations. They were finally rinsed with water for 1 min. The samples were wet-ashed with nitric acid, and the digests were assayed for K by means of atomic absorption spectrophotometry and for ^{86}Rb by the Cerenkov radiation technique (2, 11). For the chemical determination of the amounts of K absorbed during the experimental periods the initial K content of identical roots (19.2 μmoles/g) was subtracted from the values obtained for the experimental samples.

In experiments on the lateral transfer of K across corn roots into their vessels we used a novel technique which enables us to pursue the kinetics of translocation into the exudate in short term experiments (Läuchli and Epstein, in preparation). Briefly, we used corn seedlings which were germinated in the dark for 4 days and then grown for 4 days in a growth chamber on 1/10 concentration Johnson nutrient solution (10); Fe-EDTA, however, was supplied at 4/10 concentration. The experimental solution contained 0.5 mM KCl, the K being double-labeled with ^{42}K and ^{86}Rb, and 0.5 mM CaSO$_4$. The roots of an intact plant were first exposed to this solution for 3 hr. A root was then excised, and a length of polyethylene tubing was attached to it. The root then exuded for 1 hr, after which all but a 2-mm sleeve of the tubing was cut off. Serial collections of exudate were then obtained by inserting 25-μl microcapillary tubes into the upper end of the polyethylene sleeve and replacing them as they were filled. The tubes with the exudate were directly radioassayed (11).

Figure 1 shows that the absorption of K by corn roots was linear with time up to at least 90 min. The rate of absorption as
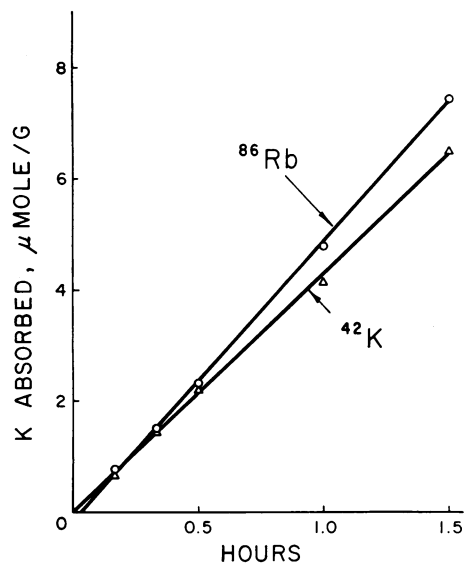

Fig. 1. Time course of K absorption by excised corn roots. Absorption solution: 0.2 mM KCl + 0.5 mM CaSO₄, 30 C, aerated. Potassium double-labeled with ^{42}K and ^{86}Rb. Experimental data given are mean values of duplicate samples.
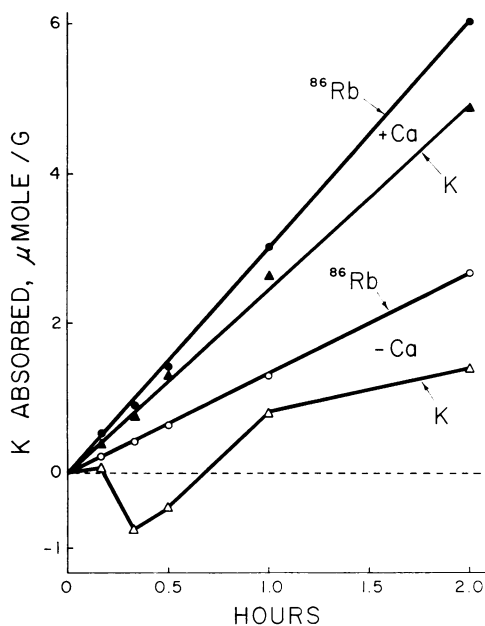

Fig. 2. Absorption of K by excised corn roots as a function of time in the presence (+Ca roots) and absence (−Ca roots) of 0.5 mM CaSO₄. Pretreatment solutions: 0.5 mM CaSO₄ (+Ca roots) and deionized water (−Ca roots), 30 C, aerated, for 30 min. Absorption solutions: 0.1 mM KCl + 0.5 mM CaSO₄ (+Ca roots) and 0.1 mM KCl (−Ca roots), 30 C, aerated. Potassium labeled with ^{86}Rb. Experimental data given are mean values of duplicate samples.

assayed with ^{42}K was 4.4 μmoles/g·hr; it was 5.1 μmoles/g·hr when measured by means of the ^{86}Rb label. That is, the rate of absorption of K was overestimated by 16% when ^{86}Rb was used as a tracer. Epstein et al. (8) studied the absorption by barley roots of K labeled with ^{42}K and Rb labeled with ^{86}Rb and also found only slight differences in the rates of absorption. They concluded that both ions are absorbed via a common carrier mechanism, with similar affinities. The conclusion appears to be valid for corn roots, also.
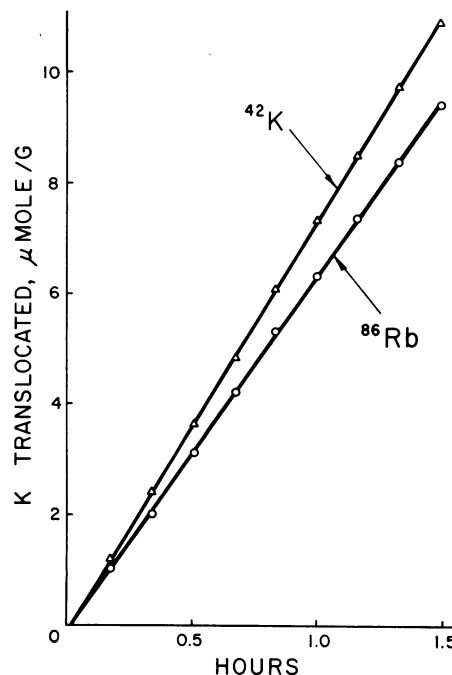

Fig. 3. Translocation of K across corn roots into the xylem exudate as a function of time. Absorption solution: 0.5 mM KCl + 0.5 mM CaSO₄, 30 C, aerated. Potassium double-labeled with ^{42}K and ^{86}Rb. Experimental data given are values for a single root.

Figure 2 shows the results of an experiment on the effect of Ca. In the absence of Ca there was an initial loss of K from the roots, as determined by chemical analysis. This loss was not apparent from the ^{86}Rb assay. In both these features, our results are in essential agreement with those of Maas and Leggett (12). However, when the experiment was done in the presence of 0.5 mM CaSO₄, the pattern of K absorption changed dramatically. The rate of absorption of K, assayed with ^{86}Rb, was much higher than in the absence of Ca, and there was no initial loss of K. Instead, absorption of K, determined by chemical analysis, was linear with time. There is thus no discrepancy between the present results and those of Maas and Leggett (12). The ^{86}Rb labeling procedure and chemical analysis give markedly different results only when Ca is omitted and normal functioning of cellular membranes is thereby impaired.

Radioassay by means of ^{86}Rb labeling overestimated the absorption of K by 20%, which agrees well with the 16% overestimation from ^{86}Rb labeling compared with ^{42}K labeling in the double label experiment shown in Figure 1.

Elzam and Hodges (5) observed that Ca inhibited K absorption by corn roots during an initial period of about 30 min. For longer periods of absorption, however, the effect of Ca changed to an enhancement of K absorption. We did not encounter this change of Ca effect with time. But the roots of the variety of corn used in our experiments showed much higher rates of absorption than those of Elzam and Hodges (5), and hence our results are comparable with those obtained earlier for barley roots which also absorb K at high rates and feature a virtually instantaneous response to Ca (6).

Figure 3 shows the translocation of K into the xylem exudate as measured with our new technique. Translocation proceeded at a constant rate for at least 90 min. The rate of translocation of K was 7.4 μmoles/g·hr (^{42}K assay) and 6.5 μmoles/g·hr (^{86}Rb), indicating that the use of ^{86}Rb to label K tends to underestimate the rate of translocation somewhat. The average rate for six roots was 14% lower for ^{86}Rb than for ^{42}K. That is, use of ^{86}Rb as a tracer underestimated the rate of K translocation to about the

same extent as it overestimated the rate of K absorption by the root tissue. These findings therefore suggest that absorption by the root tissue and transfer across the root into the conducting elements of the xylem are not identical processes.

Our experiments thus bear out our previous conclusion that ^{86}Rb may be used for labeling K in experiments on absorption of K provided that the roots are exposed to an adequate concentration of Ca and impairment of membranes is thereby prevented. In the presence of Ca, the discrepancy between ^{86}Rb labeling of K, on the one hand, and ^{42}K labeling or chemical assay, on the other, amounts to no more than certain "isotope effects" encountered with isotopes of elements with low atomic numbers. The experiments also strengthen the generally accepted view that Ca is essential for maintenance of the functional integrity of root cell membranes and especially the plasmalemma, which is the seat of the ion absorption mechanisms (7, 16). Omission of Ca causes membrane and transport phenomena to suffer various abnormalities and does so within minutes.

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