*THE DUAL MECHANISMS OF ALKALI CATION ABSORPTION BY PLANT CELLS: THEIR PARALLEL OPERATION ACROSS THE PLASMALEMMA**

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When the rate of absorption of potassium and many other ions by plant tissue is measured as a function of the external concentration of the ions, a dual pattern of absorption becomes apparent. At low concentrations, an isotherm is obtained in which for many tissues the rate of absorption levels off at around 0.2 mM K, with a wide plateau from that value to about 1 mM. The relationship between the external concentration and the rate of absorption follows simple Michaelis-Menten kinetics.[1]

At higher concentrations (1–50 mM), the rate of absorption of K does not remain constant at the level of that plateau but rises again, the isotherm depicting the relation between external concentration and rate of uptake having several characteristic inflections.[2] Evidence for such a dual pattern of ion absorption has been shown for ions of many elements, and for many and diverse plant materials.[3]

The interpretation of these and other findings has been that there are two mechanisms of absorption of a given ion. The first of these (mechanism 1) operates even at low external concentrations, i.e., its affinity for the ions is high. The other (mechanism 2) has much less affinity for the ions and hence does not operate except at high concentrations, on the order of 1 mM and above. The two mechanisms differ not only in their relative affinities for the ions but in several other well-defined ways.[3]

One question these findings provoke concerns the locale of the two mechanisms. Earlier, good reasons were given for the conclusion that the mechanisms operating even at low concentrations (the type 1 mechanisms) reside in the plasmalemma.[3, 4] Apart from specific experimental evidence for this conclusion there is the consideration that the concentration of K and other ions in the soil solution is often very low, within the range where mechanism 1 is the only one functioning. Presumably, then, this mechanism must be in the membrane facing the medium, i.e., the plasmalemma.

As for the type 2 mechanisms, Laties and his collaborators in many papers[5–11] have advanced the claim that they lie in the tonoplast. This view necessitates the assumption that at those concentrations at which mechanism 2 comes into play (i.e., above 1 mM), mechanism 1 is not rate-limiting for entry into the cytoplasm. If it were, mechanism 1 would always be rate-limiting for the over-all process, as it would be supplying the ions to mechanism 2, and the rate of operation of mechanism 2, lying in series behind mechanism 1, could therefore not exceed the maximal rate of mechanism 1.

To meet this difficulty, Laties and co-workers assume that at concentrations above 1 mM, the plasmalemma becomes permeable to the ions and they enter

the cytoplasm by diffusion at rates higher than the maximal rate of entry via mechanism 1. This rapid diffusion across the plasmalemma causes mechanism 1 to be set aside as a rate-limiting step, according to these authors, and mechanism 2, assumed by them to lie in the tonoplast, then shows up as rate-limiting.

In the present paper we present evidence that even in the high range of concentrations the plasmalemma does not become diffusively permeable to K and Na, and that mechanisms 1 and 2 operate in parallel across the plasmalemma.

Materials and Methods.—Roots of barley, *Hordeum vulgare*, var. *Arivat*, were grown as described earlier.[12] The method essentially is to keep the seeds in aerated water which is renewed several times for 24 hr, followed by growth of the seedlings over an aerated 0.2 mM solution of $CaSO_4$ for 5 days in the dark.

On the day of the experiment, roots were excised and used in short-term experiments essentially as described before,[13] with minor modifications. Briefly, samples of excised roots weighing 1.00 gm (fresh wt.), kept in open-weave cheesecloth "tea bags," were suspended in the aerated experimental solutions at 30°C. All solutions contained KCl, NaCl, or both, and, in addition, 0.5 mM $CaSO_4$ (see ref. 12). The pH of the solutions, which were unbuffered, was about 5.5 and did not vary by more than 0.2 unit in any experiment. Potassium was labeled with ^{42}K or ^{86}Rb, and Na with ^{22}Na. Unless otherwise indicated, the absorption period was discontinued by procedure B of Epstein *et al.*,[13] i.e., the "outer space" fraction of the labeled ion was desorbed by exchange with unlabeled ion, still in the presence of 0.5 mM $CaSO_4$, in the cold. The roots were then rinsed with water, transferred to counting cups, and ashed at 500°C; the ash was taken up in water with a trace of detergent, dried under infrared lamps, and counted with a gas-flow Geiger-Müller counter.

In the experiments on ion exchange in which roots containing labeled K were transferred to solutions containing unlabeled KCl, the 0.1 mM unlabeled KCl solution was renewed at intervals to prevent any reabsorption of label that might have left the tissue by exchange. For the 5 mM KCl solution this was not necessary, since reabsorption of label from this solution would be negligible.

For the experiments on exudation, seedlings of barley and corn, *Zea mays*, var. *De Kalb 805*, were grown in the greenhouse in dilute nutrient solution ($^1/_{10}$ the concentration of that used by Johnson *et al.*, ref. 14) containing 0.60 mM K. Barley was grown for 7 days, and corn for 7–10 days. For the experiments proper, roots were cut off below the seed, the cut ends fitted into collection tubes similar to those described by Moore *et al.*,[15] and the roots placed in aerated experimental solutions at room temperature, about 22°C. Exudate was collected and analyzed for K by flame photometry.

All experimental points in the figures represent the mean values from duplicate samples. The values in the tables represent concentrations in exudate of single roots.

Results.—Mutual interference between K and Na: Figure 1 shows the results of an experiment on the rate of absorption of Na by barley roots as a function of increasing K concentrations. The concentration of Na was 5 mM throughout. In the absence of K, Na was taken up at a rate of 11.1 μmoles/gm/hr. In the presence of 0.5 mM K, this rate was sharply reduced, by 6.0 μmoles/gm/hr, with little further reduction at higher K concentrations. The total (control) rate of absorption of Na consists of two contributions, one (*1*) from a mechanism exceedingly sensitive to K, the other (*2*) from a mechanism resistant to it (see *Discussion*).

Figure 2 presents the results of an experiment which complements the one shown in Figure 1. In the present experiment, the concentration of K, labeled with ^{42}K, was 10 mM throughout, and that of Na ranged from nil to 50 mM. With no Na present, the rate of K absorption was 13.4 μmoles/gm/hr. Sodium

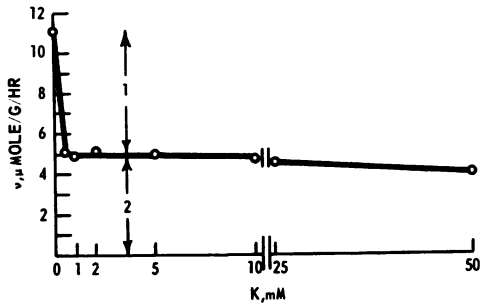

FIG. 1.—Rate of absorption of Na by barley roots as a function of the concentration of KCl. Concentration of NaCl, 5.0 mM.

at 20 mM reduced this to 10.1 μmoles/gm/hr, with no further reduction of the rate of K absorption at 50 mM Na. Thus, the total (control) rate of absorption of K also consists of two contributions, one (*2*) from a mechanism sensitive to Na, the other (*1*) from a mechanism very resistant to it.

Ion exchange: Before time zero of the experiment shown in Figure 3, the roots were exposed for one hour to a solution of 0.1 mM KCl (the K being labeled with ^{86}Rb) and 10 mM NaCl. As always, all solutions also contained 0.5 mM $CaSO_4$. The samples were then rinsed briefly with a 0.5 mM solution of $CaSO_4$ and transferred, at time zero of Figure 3, to unlabeled solutions as shown (all solutions containing 0.5 mM $CaSO_4$). The unlabeled solutions were at the same temperature as the labeled solutions used initially, viz. 30°C. The results were identical for the solutions containing 0.1 and 5 mM KCl: there was no appreciable loss of label to either. Repetition of the experiment with ^{42}K label gave the same results.

Exudation: Exudate was collected from excised roots of barley and corn as described under *Materials and Methods.* The solutions from which the roots were absorbing during these experiments contained KCl at either 0.1 or 5 mM, and 0.5 mM $CaSO_4$. Representative data are given in Table 1 for barley and in Table 2 for corn. At both 0.1 and 5 mM K in the medium, exudate contained K at concentrations which were multiples of its concentration in the medium.

Discussion.—The experiment on absorption of Na shown in Figure 1 was done at 5 mM Na. This concentration of Na is well within the range where mechanism 2 of alkali cation absorption operates, as shown earlier for absorption of Na by

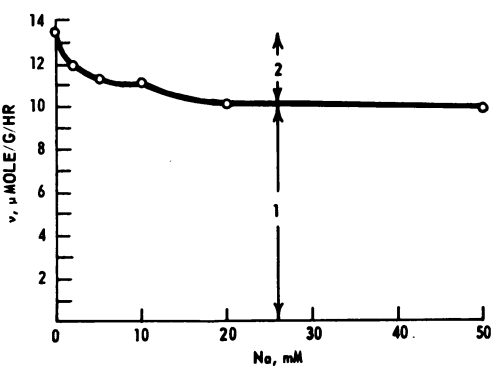

FIG. 2.—Rate of absorption of K by barley roots as a function of the concentration of NaCl. Concentration of KCl, 10.0 mM.

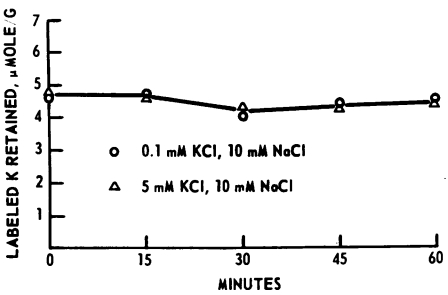

FIG. 3.—Retention of labeled K by barley roots kept in unlabeled KCl solutions. Before zero time the roots had absorbed labeled K for 1 hr from a 0.10 mM solution of KCl also containing 10 mM NaCl.

roots of both barley[2, 16–18] and two species of *Agropyron*,[19] and for absorption of K by many species.[3] It has also been shown that K severely inhibits Na absorption via mechanism 1,[17–19] a finding consistent with the earlier demonstrations that mechanism 1 of alkali cation transport is highly selective for K, with little affinity for Na.[1, 12, 20] As a result, mechanism 1, in the presence of K at about 0.5 mM or higher, is virtually preempted by K, and Na transport via this mechanism is all but abolished under these conditions.

The results shown in Figure 1 for absorption of Na from 5 mM NaCl reflect the operation of two clearly distinguishable mechanisms of which one, contributing fraction 1, is eliminated by 0.5 mM K, while the other, which furnishes fraction 2, is unaffected by the presence of K. The former thus has the earmarks of mechanism 1, as demonstrated in the references cited above. To test the identification of fraction 1 of this experiment with the operation of mechanism 1, an experiment was done on the rate of Na absorption over the concentration range 0.01–0.30 mM. This experiment yielded a calculated maximal rate of Na absorption of 5.6 μmoles/gm/hr, in good agreement with fraction 1 of Figure 1.

The experiment of Figure 1 was repeated at 10 mM Na. Fraction 1 did not significantly differ in the two runs; its value when obtained at 10 mM Na was 104 per cent of that found at 5 mM Na. Fraction 2, on the other hand, was 30 per cent higher at 10 than at 5 mM Na. This provides a further test for the association of fractions 1 and 2 with mechanisms 1 and 2, respectively: mechanism 1, already operating at its maximal rate at 5 mM Na, can operate no faster at 10 mM. The increase in the over-all rate caused by raising the concentration of Na must be due entirely to mechanism 2.

According to these results, mechanism 1 contributes to the observed rate of Na absorption in the range of high concentrations. Laties and his collaborators claim that mechanism 1 in this range makes no contribution to the observed rate of absorption, being diffusively by-passed as a rate-limiting step. Their view is that absorption in the high range reflects solely the kinetics of mechanism 2. The present experiments show that the observed rate of absorption of Na in the high

TABLE 1. *Exudation by barley roots.*

Concentration of KCl in medium, mM	Concentration of K in exudate, mM	Duration of experiment, hr
0.1	17.9	19.0
5.0	32.0	27.0
5.0	41.5	19.0

TABLE 2. *Exudation by corn roots.*

Concentration of KCl in medium, mM	Concentration of K in exudate, mM	Duration of experiment, hr
0.1	8.4	6.0
5.0	11.4	2.3
5.0	17.4	6.0

range represents the sum of the rates of mechanisms 1 and 2, implying that the two operate in parallel.

The experiment shown in Figure 2 is the converse of the one shown in Figure 1. In the present experiment, the concentration of K was 10 mM, and that of Na varied from nil to 50 mM. Two mechanisms contribute to the rate of K absorption. One of these mechanisms is rendered inoperative for K transport in the presence of Na, identifying it as mechanism 2.[1, 16] The characteristic inflection expected for a K concentration of 10 mM is apparent in the upper part of the graph, confirming the identification of fraction 2 as due to the operation of mechanism 2 (see Fig. 1 of ref. 2, and ref. 16). The other mechanism of K absorption, contributing fraction 1, resists interference by Na—the hallmark of mechanism 1. To test the identification of fraction 1 of this experiment with the operation of mechanism 1, an experiment was done on the rate of absorption of K labeled with ^{42}K, over the concentration range 0.005–0.20 mM, in the presence of 10 mM Na. This experiment yielded a calculated maximal rate of K absorption of 9.9 μmoles/gm/hr, in excellent agreement with fraction 1 of Figure 2. At 10 mM K, therefore, the observed rate of K absorption represents the sum of the rates of uptake via mechanisms 1 and 2, in confirmation of the earlier conclusions from this laboratory,[1, 2, 16] and of the experiment shown in Figure 1, dealing with Na.

The next experiment, shown in Figure 3, dealt with the fate of previously absorbed labeled K when the tissue was subsequently exposed to solutions of 0.1 or 5 mM unlabeled K. Throughout the experiment, i.e., during both the initial period when the tissue was in 0.1 mM labeled K and the period shown in Figure 3, when it was in unlabeled K solutions, Na was present at 10 mM. It was included because Na strongly inhibits K and Rb transport via mechanism 2.[1, 16]

During the period of prelabeling, ^{86}Rb label would enter the cytoplasm first, and if mechanism 2 lay in the tonoplast, would largely remain confined in the cytoplasm, because its transport into the vacuole via mechanism 2 would be minimized through competition by Na. This cytoplasmic ^{86}Rb would be lost by exchange with unlabeled K during the period shown in Figure 3 in that run in which the concentration of unlabeled K was 5 mM, if the plasmalemma were diffusively permeable at that concentration. Such loss of label did not occur at 5 mM K any more than at 0.1 mM. The findings fail to demonstrate that the plasmalemma becomes diffusively permeable in the presence of K in the high range of concentrations. Venrick and Smith[21] have shown similar results even for much more heavily preloaded mature segments of corn roots. There was no loss of labeled Rb when the roots were subsequently put into unlabeled 5.0 mM K solutions for periods ranging up to four hours.

The experiments on exudation (Tables 1 and 2) consistently showed that exudate contained K at concentrations much higher than those of the external solu-

tions, whether the latter were in the low (0.1 mM) or high (5 mM) range of concentrations. This demonstrates the operation of accumulatory mechanisms at both concentrations. Working with corn roots, Anderson and Reilly[22] have similarly shown concentration factors of 3 in exudate for both K and Cl, when the external concentration of KCl was 10.0 mM. Time and again, barley exudate concentrations of K in our experiments reached levels of about 30 or 40 mM. In some experiments they were even higher. According to the views of Laties concerning lateral transfer of ions into the conducting elements, this implies K concentrations at least equally high in the cytoplasm of the cortical cells. However, if at concentrations above 1 mM the plasmalemma were diffusively permeable, no such concentrations could build up in the cytoplasm or the xylem exudate. The ions would diffuse out of the cytoplasm of the cortical cells across the plasmalemma—a distance of 75–100 Å—rather than into the xylem vessels through the symplast, a distance about 4 orders of magnitude greater.

Although the present conclusion concerning the location of the two types of transport mechanisms is diametrically opposed to that of Laties, evidence which he and his collaborators have presented frequently supports the present conclusion. For example, in their experiments (Fig. 1 of ref. 7), excised corn roots kept in 10 mM Cl solution produced an exudate containing about 35 mM Cl, in good agreement with our own results for K and those of Anderson and Reilly[22] for both K and Cl. Other such instances could be mentioned, but it is not the purpose of this report to present an analysis of Laties' work. Various aspects of it are open to question in view of independent evidence presented by several authors.[22–29]

In the present investigation, three different and independent kinds of experiments were performed: experiments on mutual effects of K and Na, experiments on ion exchange, and experiments on delivery of ions to the conducting elements of the stele as determined by analyses of exudate. All three lead to the same conclusion: the plasmalemma does not become diffusively permeable to alkali cations at concentrations above 1 mM, and mechanisms 1 and 2 operate in parallel. Since mechanism 1 on all the evidence resides in the plasmalemma, parallel operation of both mechanisms implies that the plasmalemma is the locale of mechanism 2 also. It is possible, as pointed out earlier,[1] that the two mechanisms may deliver ions into separate "inner spaces" or compartments—perhaps one into the aqueous phase of the cytoplasm, the other via the endoplasmic reticulum into the vacuole. This, however, is conjecture.

Summary.—Experiments on mutual interactions of K and Na in their absorption by barley roots, experiments on ion exchange in barley roots, and experiments on exudation from barley and corn roots lead to the conclusion that the dual mechanisms of alkali cation absorption which have been demonstrated in these and many other plant tissues operate in parallel across the plasmalemma.

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[1] Epstein, E., D. W. Rains, and O. E. Elzam, these PROCEEDINGS, **49**, 684 (1963).

[2] Epstein, E., and D. W. Rains, these PROCEEDINGS, **53**, 1320 (1965).

[3] Epstein, E., *Nature*, **212**, 1324 (1966).

[4] Epstein, E., in *Plant Biochemistry*, ed. J. Bonner and J. E. Varner (New York: Academic Press, 1965), p. 438.
[5] Torii, K., and G. G. Laties, *Plant Physiol.*, **41**, 863 (1966).
[6] Torii, K., and G. G. Laties, *Plant Cell Physiol. (Tokyo)*, **7**, 395 (1966).
[7] Lüttge, U., and G. G. Laties, *Plant Physiol.*, **41**, 1531 (1966).
[8] *Ibid.*, **42**, 181 (1967).
[9] Lüttge, U., and G. G. Laties, *Planta*, **74**, 173 (1967).
[10] Laties, G. G., *Austral. J. Science*, **30**, 193 (1967).
[11] Osmond, C. B., and G. G. Laties, *Plant Physiol.*, **43**, 747 (1968).
[12] Epstein, E., *Plant Physiol.*, **36**, 437 (1961).
[13] Epstein, E., W. E. Schmid, and D. W. Rains, *Plant Cell Physiol. (Tokyo)*, **4**, 79 (1963).
[14] Johnson, C. M., P. R. Stout, T. C. Broyer, and A. B. Carlton, *Plant and Soil*, **8**, 337 (1957).
[15] Moore, D. P., B. J. Mason, and E. V. Maas, *Plant Physiol.*, **40**, 641 (1965).
[16] Rains, D. W., and E. Epstein, *Plant Physiol.*, **42**, 319 (1967).
[17] *Ibid.*, p. 314.
[18] Rains, D. W., and E. Epstein, *Science*, **148**, 1611 (1965).
[19] Elzam, O. E., "Absorption of sodium, potassium, and chloride by two species of *Agropyron* differing in salt tolerance," doctoral dissertation, University of California, Davis (1966).
[20] Smith, R. C., and E. Epstein, *Plant Physiol.*, **39**, 992 (1964).
[21] Venrick, D. M., and R. C. Smith, *Bull. Torrey Bot. Club*, **94**, 501 (1967).
[22] Anderson, W. P., and E. J. Reilly, *J. Exptl. Bot.*, **19**, 19 (1968).
[23] Hiatt, A. J., *Plant Physiol.*, **42**, 294 (1967).
[24] Lüttge, U., and K. Bauer, *Planta*, **78**, 310 (1968).
[25] Krichbaum, R., U. Lüttge, and J. Weigl, *Ber. Deutsch. Bot. Ges.*, **80**, 167 (1967).
[26] Yu, G. H., and P. J. Kramer, *Plant Physiol.*, **42**, 985 (1967).
[27] Yu, G. H., and P. J. Kramer, manuscript in preparation.
[28] Leggett, J. E., *Ann. Rev. Plant Physiol.*, **19**, 333 (1968).
[29] LaHaye, P. A., manuscript in preparation.