

Sodium Absorption by Barley Roots: Role of the Dual Mechanisms of Alkali Cation Transport¹

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Summary. Radioactively labeled Na^+ absorbed by barley roots was sequestered in an intracellular compartment or compartments ("inner" spaces) in which it was only very slowly exchangeable with exogenous Na^+ . Absorption of this fraction proceeded at a constant rate for at least 1 hour.

When the rate of Na^+ absorption was examined over the range of concentrations, 0.005 to 50 mM, the isotherm depicting the relation showed dual kinetics as follows. Over the range, 0.005 to 0.2 mM, a single Michaelis-Menten term describes the relation between the concentration of Na^+ and the rate of its absorption. The mechanism of Na^+ absorption operating over this range of concentrations, mechanism 1 of alkali cation transport, is severely inhibited in the presence of Ca^{2+} and virtually rendered inoperative for Na^+ transport by the combined presence of Ca^{2+} and K^+ . The mechanism is equally effective in Na^+ transport whether Cl^- or F^- is the anion, but is somewhat inhibited when the anion is SO_4^{2-} .

Over the high range of concentrations, 0.5 to 50 mM Na^+ , a second, low-affinity mechanism of Na^+ absorption comes into play. In the presence of Ca^{2+} and K^+ , this mechanism 2 is the only one to transport Na^+ effectively, since Na^+ absorption via mechanism 1 is virtually abolished under these conditions.

Anaerobic conditions, low temperature, and the uncoupler, 2,4-dinitrophenol, inhibit Na^+ absorption both at low and high Na^+ concentrations.

Sodium is the predominant cation in the world's oceans and the preponderant soluble cation in many of the soils of those very large land areas lying in the arid and semi-arid regions of the earth. However, its absorption by plants and plant tissues has been studied much less than that of potassium, the only alkali metal known to be universally essential for all higher plants (7). In the present work, concepts and findings gained primarily from experiments on the absorption of potassium (and rubidium) have been applied to the study of sodium absorption by excised barley roots.

The previous conclusions referred to are 2-fold. 1) Calcium ions in the medium play a crucial, indeed an indispensable, role in selective ion transport by plant tissues. This is so for absorption of alkali cations (4, 5, 14, 16, 18, 19, 22, 23), halides (1, 3, 4, 16, 19, 23), and for other ions as well (25). The role of calcium in preventing or reversing damage to the cellular transport mechanisms caused by sodium is particularly striking (4, 5). The effects of calcium deprivation are profound: they

involve damage to normal respiratory patterns and other aspects of metabolism in addition to ion transport (12, 15). Neglect of this factor is doubtless responsible for many contradictory and perplexing statements in the literature. 2) The absorption of potassium and many other inorganic ions by plant tissues is effected by dual mechanisms which differ drastically, often in diametrically opposed fashion, in their properties and responses to the external ionic environment (8). This dual pattern characterizes the absorption of so many ions, by various tissues of so many plant species, as to lead to the claim that it may be well-nigh universal in mature tissues of higher plants (8).

Available evidence indicates that dual transport mechanisms operate in the absorption of sodium as they do in the case of other ions (2, 4, 5, 9, 13, 17, 20). However, with the exception of the experiments from this laboratory (4, 5, 9, 20), these investigations were done without regard to the essential role of calcium in membrane transport and without inclusion of calcium in the experimental solutions. It therefore became necessary to conduct a systematic series of experiments on the kinetics of sodium absorption, its selectivity, and the role of the associated anions and other factors, similar to the experiments in which the dual pattern of potassium transport has been delineated (9, 10).

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Material and Methods

The method for growing roots of barley, *Hordeum vulgare*, was as described before (5). Briefly, seeds are germinated for 24 hours in aerated water, in the dark. Seedlings are then grown 5 days over an aerated 0.2 mM solution of CaSO_4 , in the dark. On the day of the experiment, the roots are thoroughly rinsed in water, excised, repeatedly rinsed again, and suspended in 4 liters of water.

For the experiments proper, the technique described earlier (11) was used. Samples of roots weighing 1.00 g (fr wt) were suspended in the aerated experimental solution, usually at 30°, which contained Na^+ in radioactively labeled form. Sodium was labeled with ^{22}Na . The pH, initially 5.7, varied by no more than 0.2 unit during the experimental runs. The absorption period, usually of 20 minutes except when time was a variable, was discontinued by procedure B of Epstein et al. (11), i.e., the fraction of the labeled Na^+ ions associated with the tissue in diffusible and readily exchangeable form was removed by immersion of the sample in a solution containing unlabeled Na^+ at 5 mM, as well as 0.5 mM Ca^{2+} . Reference 11 should be consulted for details and discussion of the technique.

Results

In the technique used the tissue is transferred, after the period of absorption of labeled substrate ion, to an unlabeled solution of the same ion for removal of that fraction of labeled ions superficially associated with the cell wall. The implication is that another fraction is transported by cellular processes into "inner" spaces of the cells where they are not readily subject to exchange. That this is so for Na^+ was shown in an experiment in which intact seedlings absorbed labeled Na^+ for 4 hours from a 0.1 mM solution also containing 0.5 mM Ca^{2+} . At the end of this period the roots had attained an accumulation ratio of 150 to 1. The roots were excised, briefly rinsed, and 1.00 g samples suspended in unlabeled solution of identical composition. In 2 hours the roots lost only 5% of the labeled Na^+ they contained. Repetition of the experiment with 10 mM labeled Na^+ during the absorption period and 10 mM unlabeled Na^+ during the desorption period gave the same result; only 5% of the labeled Na^+ was lost in 2 hours.

Figure 1 brings out 2 points. First, at 0.1 mM NaCl , absorption of the non-exchangeable ("inner" space) fraction of Na^+ proceeds at a steady rate for an appreciable period, and second, Ca^{2+} at 0.5 mM causes a marked reduction in the rate of Na^+ absorption. Repetition of this experiment at 10 mM NaCl gave similar results, except that the rate

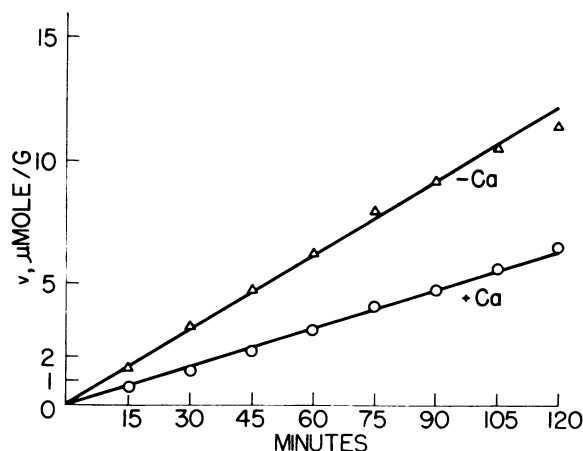


FIG. 1. Absorption of Na^+ as a function of time in the presence and absence of Ca^{2+} . NaCl : 0.1 mM; CaCl_2 , when added: 0.5 mM.

of Na^+ absorption remained constant for only 1 hour, and the inhibitory effect of Ca^{2+} was smaller.

The effect of Ca^{2+} and of the Na^+ concentration on the rate of Na^+ absorption was examined in the experiment shown in figure 2. Both in the absence and the presence of Ca^{2+} at 0.5 mM, the absorption isotherm strictly conforms to the Michaelis-Menten relationship. The maximal rates calculated for non-limiting concentrations of Na^+ differed by only about 10% in the 2 runs, but the apparent affinity of the transport mechanism for Na^+ was drastically lowered by the presence of Ca^{2+} , the Michaelis constant being 0.06 mM in the absence of Ca^{2+} and 0.32 mM at 0.5 mM Ca^{2+} . When in addition to 0.5 mM Ca^{2+} , K^+ was present at 1.0 mM, absorption of Na^+ over the range of concentrations shown

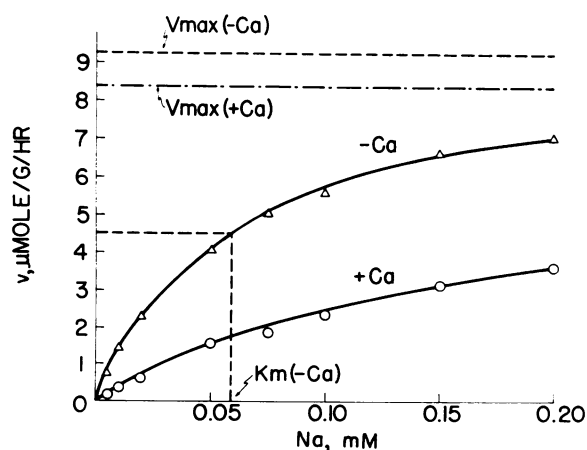


FIG. 2. Rate, v , of absorption of Na^+ as a function of NaCl concentration in the presence and absence of $\text{CaCl}_2 \cdot \text{Na}^+$: 0.005 mM to 0.2 mM; Ca^{2+} when added: 0.5 mM. Circles and triangles represent experimental data; the lines are plots of the Michaelis-Menten equation. -Ca: K_m , 0.06 mM; V_{max} , 9.2 $\mu\text{moles/g/hr}$. +Ca: K_m , 0.32 mM; V_{max} , 8.4 $\mu\text{moles/g/hr}$.

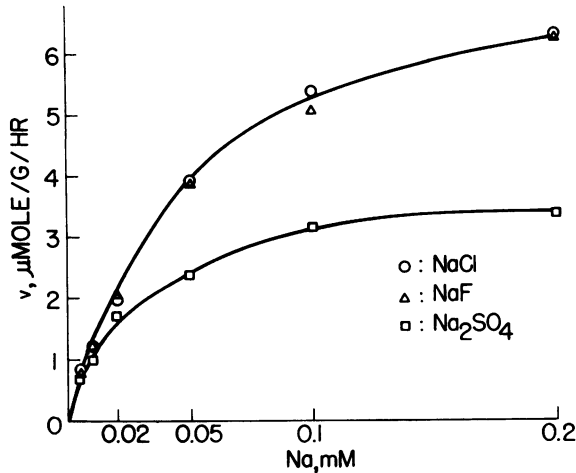


FIG. 3. Rate, v , of absorption of Na^+ as a function of Na^+ concentration and concomitant anion. No Ca^{2+} added.

in figure 2, i.e., up to 0.2 mM, was virtually abolished (see below, and fig 6).

The effect of the anion on the rate of Na^+ absorption over this same range of concentrations is shown in figure 3. With SO_4^{2-} as the anion, the rate of Na^+ absorption was lower than with Cl^- . On the other hand, substitution of F^- for Cl^- as the anion was without effect on the rate of Na^+ absorption.

In figure 4 are shown the results of an experiment in which the rate of Na^+ absorption was determined as a function of the Na^+ concentration over a very wide range of concentrations of NaCl , from 0.005 mM to 50 mM. No Ca^{2+} was added. Up to 0.2 mM Na^+ , the isotherm depicting the relation between the concentration of Na^+ and its rate of absorption shows saturation kinetics, virtually leveling off at 0.2 mM (also cf. the $-\text{Ca}^{2+}$ run in fig 2). However, at higher concentrations the rate of Na^+ absorption greatly exceeds the rate calculated on the basis of the relation applying over the low range of concentrations (the dashed line). Similar dual kinetics are observed when

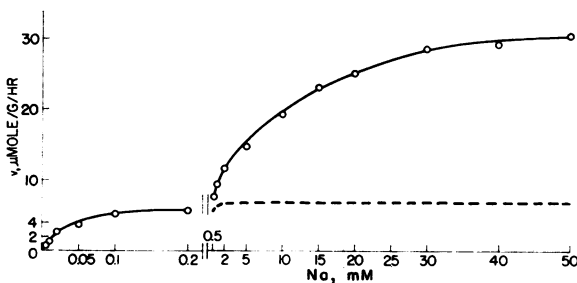


FIG. 4. Rate, v , of absorption of Na^+ as a function of NaCl concentration (0.005–50 mM). No Ca^{2+} added. Concentration scale broken between 0.2 and 0.5 mM. Dashed line represents calculated maximal velocity for the high-affinity mechanism 1 (7.2 $\mu\text{mole/g/hr}$).

the anion is SO_4^{2-} (fig 5), but the actual rates of Na^+ absorption are lower in this case than with Cl^- as the anion.

The same wide range of concentrations of Na_2SO_4 (0.005–50 mM Na^+) was explored in the experiment shown in figure 6, but in contrast to the previous 2 experiments the present one was done in the presence of both Ca^{2+} (0.5 mM) and K^+ (1.0 mM). Under these conditions, Na^+ absorption over the low range of concentrations (below 0.2 mM) was virtually eliminated. Results of a similar ex-

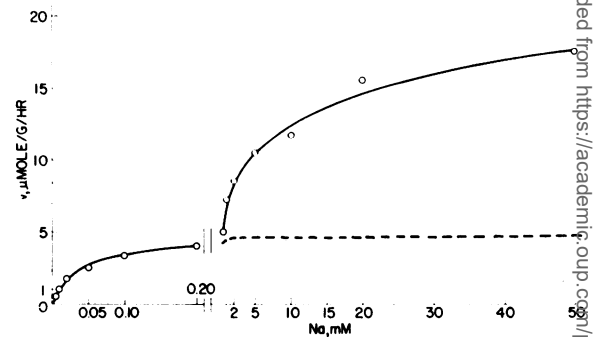


FIG. 5. Rate, v , of absorption of Na^+ from the SO_4^{2-} salt as a function of Na_2SO_4 concentration. No Ca^{2+} added. Na^+ : 0.005 to 50 mM. Concentration scale broken between 0.2 and 0.5 mM. Dashed line represents calculated maximal velocity for the high-affinity mechanism 1 (4.8 $\mu\text{mole/g/hr}$).

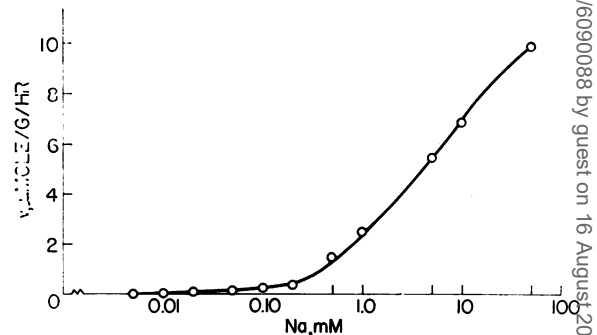


FIG. 6. Rate, v , of absorption of Na^+ , plotted arithmetically, as a function of Na_2SO_4 concentration. Na^+ concentration: 0.005 to 50 mM, plotted logarithmically. K^+ as K_2SO_4 : 1.0 mM; Ca^{2+} as CaSO_4 : 0.5 mM.

periment with Cl^- instead of SO_4^{2-} as the anion have been shown earlier (20). With SO_4^{2-} as the anion, rates of Na^+ absorption were lower than with Cl^- as the anion, as observed before (cf. figs 4 and 5).

Table I shows the results of various inhibitory treatments on the rate of Na^+ absorption at several concentrations of Na^+ , both with and without Ca^{2+} and K^+ .

Table I. *Effect of Various Metabolic Inhibitors on Na⁺ Absorption by Barley Roots*

Treatment	Concentration of ions present	% of Control
N ₂	0.1 mM Na ⁺	50
N ₂	10 mM Na ⁺ + 1 mM K ⁺ + 0.5 mM Ca ²⁺	35
0.1 mM DNP	0.1 mM Na ⁺	16
0.1 mM DNP	5 mM Na ⁺ + 1 mM K ⁺ + 0.5 mM Ca ²⁺	18
0.1 mM Azide	0.1 mM Na ⁺	34
0.1 mM Azide	5 mM Na ⁺ + 1 mM K ⁺ + 0.5 mM Ca ²⁺	52
Q ₁₀ (20–30°)	0.1 mM Na ⁺	Q ₁₀
Q ₁₀ (20–30°)	10 mM Na ⁺ + 1 mM K ⁺ + 0.5 mM Ca ²⁺	1.8
		2.3

Discussion

The work reported here leads to the following principal conclusions. The absorption of Na⁺ by barley root tissue is mediated by 2 distinct mechanisms. Of these 2 the first, which operates at low concentrations, is severely reduced in effectiveness for Na⁺ transport when Ca²⁺, and especially, when both Ca²⁺ and K⁺ are present. Under these conditions, which are the normal, physiological ones, absorption of Na⁺ proceeds almost wholly via the second, low-affinity mechanism which does not come into play except at fairly high concentrations of Na⁺. On the basis of considerations given below it is concluded that the same dual carrier mechanisms which effect the absorption of K⁺ (6, 8, 10) also mediate the transport of Na⁺, selection between these closely related alkali cations being accomplished through the differential affinities of these mechanisms for the 2 elements.

Mechanism 1. Over the low range of concentrations, up to 0.2 mM, the absorption of K⁺ has been shown to be mediated by a high-affinity mechanism, mechanism 1, with a Michaelis constant of about 0.02 mM (8, 10). Absorption of Na⁺ over this same range of concentrations is shown in figures 2, 3 and the left side of figures 4 and 5 to exhibit similar kinetics. In addition to this similarity, the following evidence shows that absorption of Na⁺ at these concentrations is via the same mechanism which transports K⁺ at low K⁺ concentrations, i.e., mechanism 1 of alkali cation transport.

Absorption of Na⁺ over this low concentration range is virtually abolished in the presence of Ca²⁺ at 0.5 mM and K⁺ at 1.0 mM (fig 6, and fig 1 of ref 20), indicating a common mechanism with much higher affinity for K⁺ than for Na⁺, so that under these conditions it is virtually preempted by K⁺ to the exclusion of Na⁺ (20). If this conclusion is correct, it follows that absorption of K⁺ by this mechanism should be highly indifferent to Na⁺, and this is so (5, 6, 10). Mechanism 1 can be rendered somewhat effective for Na⁺ absorption only by imposition of the physiologically abnormal conditions of the absence of both Ca²⁺ and K⁺.

Like K⁺ absorption via mechanism 1 of alkali cation transport (6, 10), Na⁺ absorption via this mechanism does not depend on the presence of an absorbable anion. The results were the same whether the anion was Cl⁻, which is readily absorbed (3), or F⁻, which is not (24). With SO₄²⁻ as the anion, there was this difference between K⁺ and Na⁺ absorption by this mechanism: K⁺ absorption was almost totally unaffected by substitution of SO₄²⁻ for Cl⁻ as the anion (6, 10; also cf. 17, 23), whereas Na⁺ absorption was appreciably slowed down thereby, at least over the 0.05 to 0.2 mM range of Na⁺ concentrations (fig 3).

Mechanism 2. At concentrations above 0.2 mM Na⁺, a second mechanism of Na⁺ absorption comes into play (fig 4 and 5), as is the case in K⁺ absorption (6, 10). Since in this range of concentrations, Na⁺ severely and competitively inhibits the absorption of K⁺ (10, 21), absorption of Na⁺ at these concentrations is considered to be mediated by the same mechanism which effects the absorption of K⁺ at these concentrations, i.e., mechanism 2 of alkali cation transport (10, 21). But while mechanism 1 is highly selective for K⁺ and can be rendered somewhat effective for Na⁺ transport only as discussed above, mechanism 2 shows appreciably higher affinity for Na⁺ than for K⁺ (10, 21). Further common characteristics of Na⁺ and K⁺ absorption at high concentrations will be discussed in the succeeding paper (21).

With Ca²⁺ present at 0.5 mM and K⁺ at 1.0 mM, mechanism 1 is unavailable for Na⁺ transport, and absorption of Na⁺ then does not occur except at those high concentrations where the low-affinity mechanism 2 comes into play (fig 6 and refs 9, 20, and 21). If the findings of the present research are of general validity, and this seems likely (8), the work thus furnishes a rationale for the almost universal prevalence of K⁺ over Na⁺ in plant cells.

No definite explanation can as yet be offered for the interaction by which Ca²⁺ renders mechanism 1 highly selective for K⁺ vis-a-vis Na⁺ ions. The most attractive hypothesis is that the configuration of the active site of the carrier is such in the presence of Ca²⁺ as to make for a prefer-

ential "fit" for K^+ (and Rb^+) ions, whereas in the absence of Ca^{2+} the geometry is loosened to the extent where other ions, sodium included, are also admitted. Because of the far-reaching effects which omission of Ca^{2+} has on various other phases of metabolism (12, 15) additional functions of Ca^{2+} are almost certainly involved.

Interpretation of the effects of inhibitory conditions (table I) is rendered difficult because absorption of K^+ , added to deny mechanism 1 to Na^+ , is itself inhibited by these agents. It is in any event clear that absorption of Na^+ is severely inhibited by the various antimetabolic conditions that were imposed.

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