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Development and Characteristics of Sodium-selective Transport in Red Beet[1]

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ABSTRACT

Slices of storage tissue of red beet (*Beta vulgaris* L.) washed for only 1 day in distilled water readily absorb K^+ but lack a mechanism for rapid Na^+ uptake. A Na^+ transport mechanism develops if the tissue is washed for several days, and the tissue then excludes K^+ during Na^+ uptake.

Both the high affinity and low affinity absorption mechanisms show a development of Na^+ transport with washing, and, in contrast to barley roots, cation selectivity in beet is not affected by the presence of calcium ions.

K^+ and Na^+ do not appear to compete for binding sites at the surface of the cells, but, as in barley roots, the cations compete in some way for electrically balancing transported ions. Nevertheless, the development of Na^+ transport and the interaction of Na^+ with K^+ occur in the same way whether cation transport is balanced by Cl^- uptake or by H^+ or HCO_3^- transport.

Slices of storage tissue of red beet, when washed for several days in distilled water, show an unusual preference for the uptake of Na^+ rather than K^+ from solutions containing both ions, although the ions may be taken up at approximately equal rates when given separately (22). Thus the uptake of K^+ from KCl solution is severely inhibited by the addition of Na^+. (On the other hand, addition of KCl to an external solution of NaCl has little effect or may even increase the absorption of Na^+, probably because of the influence of the added Cl^-.) This phenomenon was described by Sutcliffe (22), who also showed that the selectivity for Na^+ over K^+ depends on the duration of washing of the tissue before the experiment, and on the temperature during the absorption period. It remains unclear what system of transport processes in the cell can account for these results.

The present investigation describes the development of a distinct Na^+ transport mechanism during the washing of red beet tissue and examines in more detail the interaction between Na^+ and K^+ transport in this material.

METHODS

Roots of red beet (*Beta vulgaris* L.), stored in moist vermiculite at 5 C, were cut into disks 10 mm in diameter and 0.7 mm thick. The tissue was washed in 100 times its volume of aerated distilled water for at least 1 day before use. The water was renewed two or three times during the 1st day, and once daily thereafter. Most of the experiments were performed in the autumn. The magnitude of the effects reported here varies somewhat with the time of year and other factors, but the general pattern of results is consistently reproducible.

Except for Figures 1 and 2 and Table IV, which are concerned with isotope influx *versus* concentration, all experiments measure net uptake of Na^+ or K^+ from an external concentration of 0.5 mM. At this concentration, which is low compared with that in a number of selectivity studies discussed below (11, 12, 22), the high affinity mechanism is more or less saturated while the low affinity mechanism is operating relatively slowly (Figs. 1, 2, and Ref. 9). However, there is no evidence of any difference in selectivity between the two mechanisms in beet (see below).

In net uptake experiments, 2 g of tissue were equilibrated in the uptake solution for 1 hr. The solution was then renewed, and uptake was measured over the 2nd hr by flame photometry of the external solution. The volume of the solution was chosen to give a change in concentration of the order of 10% during the uptake period. All net uptake results are means of duplicates, and the error is not more than 10% or ± 0.1 μeq/g·hr, whichever is greater.

RESULTS

Preparation of Na^+-selective or K^+-selective Tissue. Table I compares the uptake of K^+ and Na^+ by slices of red beet after the tissue has been washed for various periods of time. Net uptake was measured in a solution containing 0.5 meq/liter of each ion, together with tris buffer at pH 7.5. The effect of tris buffer is to promote cation uptake after only short periods of washing (20), when uptake from sulfate or chloride solutions is very small. The preference for K^+ uptake or Na^+ uptake depends on the time for which the tissue is washed after slicing the root, and also on the temperature during the washing treatment (Table I). Thus with constant conditions of uptake the selectivity varies from a 5-fold preference for K^+ (after 1 day of washing at 20 C) to an 8-fold preference for Na^+ (after 8 days of washing at 10 C). Sutcliffe (22) has previously observed a change in the preference for Na^+ with washing, but, since his tissue was washed for at least 3 days, a preference for K^+ was never observed.

It may be noted that an increase in temperature during pretreatment of the tissue (Table I) leads to a greater preference for K^+ uptake, whereas an increase in temperature during uptake has the opposite effect (22, and unpublished observations). In the course of the present work it was also observed that changing the pretreatment temperature for an hour or so before the uptake period has no effect. The pretreatment temperature thus affects the development of the tissue over a period of time, rather than exerting a temporary effect on the transport system.

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Table I. *Effect of Washing Pretreatments on Cation Selectivity of Red Beet Tissue*

External solution in all cases contained 0.5 meq/liter K_2SO_4 + 0.5 meq/liter Na_2SO_4 + 10 mM tris SO_4, pH 7.5. Uptake temperature: 20 C.

A. Pretreatment temperature 10 C.

Pretreatment Time in Distilled Water	Net Uptake	
	K^+	Na^+
days	*μeq g·hr*	
1	1.47	0.66
2	1.95	1.36
3	1.95	2.07
6	0.75	3.39
7	0.65	3.46
8	0.50	4.01

B. Pretreatment temperature 20 C.

Pretreatment Time in Distilled Water	Net Uptake	
	K^+	Na^+
days	*μeq g·hr*	
1	2.69	0.56
2	2.18	0.84
3	2.00	1.69

Table II. *K^+ and Na^+ Uptake by Na^+-selective Red Beet Tissue in Various Solutions*

Beet tissue was washed in distilled water at 10 C for 8 days. Uptake temperature: 20 C. Data are from same experiment as Table I.

External Solution	Net Uptake	
	K^+	Na^+
	μeq/g·hr	
0.5 mM KCl + 0.5 mM NaCl	−0.04	1.73
0.5 mM KCl + 0.5 mM NaCl + 0.5 mM $CaCl_2$	0.16	3.25
0.5 meq/liter K_2SO_4 + 0.5 meq/liter Na_2SO_4 + 10 mM tris-SO_4, pH 7.5	0.50	4.01

Factors Shown Not to Affect Selectivity in Beet. Studies of cation selectivity in other plants have implicated a number of factors in the control of selectivity. These include the ion content of the tissue (12), the pH at the cell surface (11), and the presence of Ca^{2+} (17). None of these factors appears to be involved in the selectivity changes observed in beet tissue. Changes in ion content during washing are very small compared with those inducing selectivity changes in barley roots (12). In a typical case, the storage tissue contained 77 μeq/g K^+ and 10 μeq/g Na^+ after washing for 1 day, and the content of each ion declined by 10 or 11% by day 6. A significant change in pH seems unlikely in solutions buffered with tris (Table I). Moreover, in the experiment of Table II, the addition of either tris buffer or $CaCl_2$ promoted cation uptake from a solution containing 0.5 mM KCl + 0.5 mM NaCl, without affecting the strong preference for Na^+ uptake in that material. The accompanying paper (14) also shows that concentrations of Na^+ as low as 0.1 meq/liter are inhibitory to K^+ uptake even in the presence of 1.0 mM $CaCl_2$.

The experiment of Table II also tests the possibility that Na^+ transport may be linked with the transport of Cl^-, or of HCO_3^- or H^+. It is known that, in the presence of Ca^{2+}, cation uptake is quantitatively balanced by Cl^- uptake (10), whereas in tris sulfate at pH 7.5, cation uptake is balanced by HCO_3^- or H^+ (6, 15, 21). Table II shows that Na^+ selectivity is maintained under both of these conditions and is therefore independent of the ion involved in electrical balance of the Na^+-K^+ movements. In addition, comparison of Table I with the results of Sutcliffe (22) shows that the development of selectivity during washing follows a similar course whether tested in tris solution with low concentrations (0.5 mM) of Na^+ and K^+, or in Cl^- solutions containing 10 mM of each cation.

Changes of cation selectivity are not confined to either the vascular tissue or the purely parenchymatous regions of the beet tissue. Slices cut so as to contain mostly vascular tissue and slices containing only parenchyma both show a marked increase in the ratio of Na^+ uptake to K^+ uptake when washed for several days at 10 C.

Development of Na^+ Transport Mechanism during Washing. Table III shows cation uptake in three representative experiments, one with K^+-selective and two with Na^+-selective material. Both types of tissue are able to absorb K^+ at a rapid rate when this ion is presented alone. In contrast, when Na^+ is provided alone, it is readily absorbed only by the Na^+-selective tissue. Thus the difference in selectivity seems to be related to a difference in the capacity for Na^+ transport, and not to any difference in K^+ transport.

Experiments with ^{22}Na (Fig. 1) show that differences in net uptake of Na^+ are due to differences in Na^+ influx. Further-

Table III. *Interaction between K^+ and Na^+ Uptake in Red Beet*

A. Potassium-selective tissue was prepared by washing in distilled water for 1 day at 20 C. Uptake solution contained 10 mM tris-SO_4, pH 7.5. Uptake was at 10 C.

Experiment	External Solution (Ions at 0.5 mM)	Net Uptake	
		K^+	Na^+
		μeq/g·hr	
1	K^+	1.90	...
	Na^+	...	0.30
	$K^+ + Na^+$	2.00	−0.02

B. Sodium-selective tissue was prepared by washing in distilled water for 7 days at 10 C. Uptake was at 30 C. Experiment 2: same experiment as in Table I. Uptake solution contained 10 mM tris-SO_4, pH 7.5. Experiment 3: uptake solution contained 1.0 mM $CaCl_2$ in addition to tris-SO_4.

Experiment	External Solution (Ions at 0.5 mM)	Net Uptake	
		K^+	Na^+
		μeq/g·hr	
2	K^+	3.54	...
	Na^+	...	4.17
	$K^+ + Na^+$	0.65	3.46
3	K^+	1.78	...
	Na^+	...	2.25
	$K^+ + Na^+$	0.34	1.89

more, both the high affinity and the low affinity mechanisms have an increased capacity for sodium transport in Na+-selective tissue.

Effect of Na+ on K+ Transport. Table III shows that inhibition of K+ uptake by Na+ occurs only in the Na+-selective tissue: the presence of Na+ is not inhibitory in tissue which lacks the enhanced capacity for Na+ transport. In other words, the extent of inhibition appears to be determined by the rate of transport of the inhibitor (see "Discussion"), and not, as in classical competitive inhibition, by the concentration of the inhibitor.

The effect of Na+ on a 15-min influx of ^{42}K at various K+ concentrations is shown in Figure 2. Although the magnitude

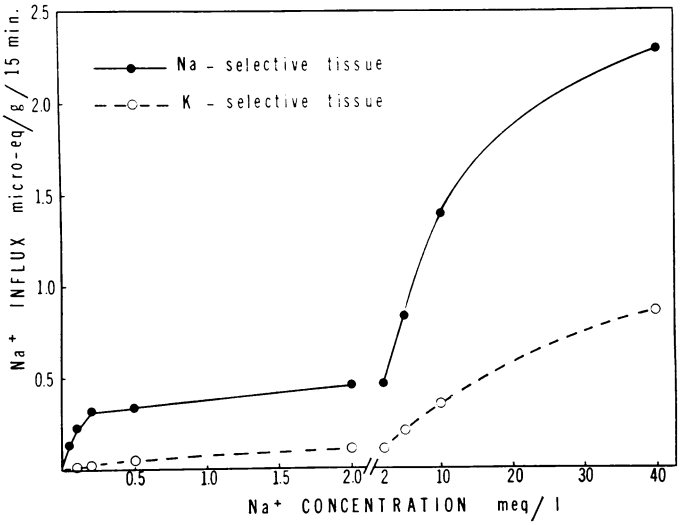

FIG. 1. Na+ influx *versus* concentration. Results of two experiments with red beet tissue pretreated to induce Na+-selective or K+-selective uptake. Na+-selective tissue was prepared by washing for 8 days in distilled water at 10 C before the experiment. K+-selective tissue was washed for 1 day at 20 C. In both cases Na+ was supplied as $^{22}NaCl$. Tris-SO_4 (10 mM, pH 7.5) was present throughout. The tissue was exposed to the isotope solution at 30 C for 15 min, then washed with 1.0 mM KCl + 1.0 mM $CaCl_2$ at 2 C for 1 hr, and ashed at 500 C.

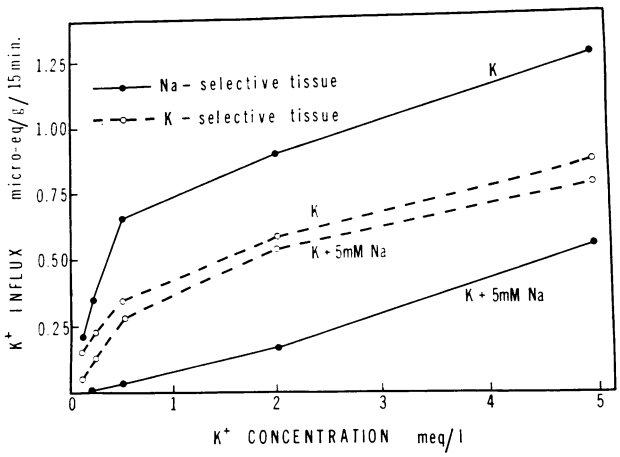

FIG. 2. Effect of Na+ on K+ influx *versus* concentration. Na+-selective tissue was prepared by washing for 1 day at 10 C followed by 6 days at 2 C. K+-selective tissue was washed for 1 day at 20 C. In both cases, K+ was supplied as ^{42}KCl and Na+ as NaCl. Tris-SO_4 (10 mM, pH 7.5) was present throughout. For other methods, see legend for Figure 1.

Table IV. *Effect of Na+ on Influx of K+ at Different K+ Concentrations*

For details see Figure 2.

Material	K+ Concn	K+ Influx		
		−Na+	+5 mM Na+	Difference
	mM	*μeq/g·15 min*		
K+-selective	0.1	0.16	0.06	0.10
	40.0	1.69	1.60	0.09
Na+-selective	0.5	0.66	0.04	0.62
	40.0	2.46	2.00	0.46

of the Na+ effect is very different in Na+-selective and K+-selective tissue, the pattern of inhibition in the two cases shows some common features. In particular, the absolute magnitude of the reduction in K+ influx is almost constant throughout a wide range of K+ concentrations. This is also seen in Table IV, which presents data from the same experiment as Figure 2 but includes measurements at 40 meq/liter K+. In the case of the K+-selective material, for example, the Na+ effect is essentially the same at 40 meq/liter K+ as at 0.1 meq/liter K+, although the external concentration of K+ is increased 400-fold, and the influx of K+ is increased 10-fold. These results are not readily explained in terms of Michaelis-Menten kinetics. However, they are understandable in the light of previous results, since the extent of inhibition of K+ uptake is determined by the rate of Na+ uptake (see "Discussion"), and Na+ uptake is relatively insensitive to the concentration of K+ (Table III, and Ref. 22).

The apparent Michaelis constant (*Km*) for transport of Na+ and K+ provide further evidence that these ions do not compete for transport sites at the outer surface of the cell membrane. In Na+-selective tissue, with 1.0 mM $CaCl_2$ in the external solution, the mean value of *Km* for K+ in three experiments was 0.28 ± 0.13 mM, while the mean *Km* for Na+ was 0.4 ± 0.06 mM. Thus there appear to be no significant differences between the affinities of the two ions for the transport system. Yet, when the ions are present in equal concentrations, the rate of Na+ uptake (Table II) is several times that of K+ uptake.

DISCUSSION

Selectivity of High Affinity and Low Affinity Transport Mechanisms in Beet. Epstein *et al.* (4) and Rains and Epstein (17) have shown that in barley roots of low salt status (*cf.* 12) the high affinity and low affinity mechanisms differ in selectivity. In the presence of Ca^{2+}, the high affinity mechanism transports K+ and excludes Na+ when both ions are present, while the low affinity mechanism shows a preference for Na+. Rains (16) has shown that this pattern of selectivity does not extend to bean stem tissue, since in fresh slices of this material the high affinity mechanism transports only Na+, whereas in aged slices it transports only K+. It is thought that in bean stem, Na+ and K+ are transported by different mechanisms which develop at different times. The present results with red beet are different again, since Na+-K+ selectivity does not seem to relate specifically to either the high affinity or the low affinity mechanism, nor does it depend on the presence of Ca^{2+}. Both mechanisms show changes in Na+ transport (Fig. 1), and the selectivity at 0.5 mM (present results) is similar to that at 10 mM (22).

Developmental Changes in Ion Transport. The development of Na+ transport in red beet during washing (see "Results") is

comparable with the development of Cl^- transport in the same material. It is known that freshly cut tissue is deficient in Cl^- transport (3, 20), although such tissue has the ability to absorb organic anions supplied in the external solution (3) and to transport HCO_3^- or H^+ (20). The ability to take up Cl^- increases steadily for several days after slicing the tissue and thus parallels the development of Na^+ transport, although the present results (*e.g.*, Table II) indicate that Na^+ transport is not dependent in any way on the transport of Cl^-. The asymmetry of the interaction between Na^+ and K^+ (Table III) also seems to have a parallel in the interaction of Cl^- with HCO_3^- or H^+ (6), since Cl^- transport can inhibit the transport of these ions without itself being significantly affected by them.

Changes in Na^+-K^+ selectivity observed by Pitman (11) and Pitman *et al.* (12) in barley roots appear to be somewhat different in nature from those induced by washing in beet tissue. In barley, with an external cation concentration of 10 mM, differences between Na^+ and K^+ uptake are revealed only when the two ions are absorbed simultaneously. When offered separately, the ions are absorbed at comparable rates (12). This is true for Na^+-selective beet, which can absorb either Na^+ or K^+, but not in the case of K^+-selective beet, which lacks the capacity for rapid Na^+ transport (Table III). In beet, as in bean stem (16), it appears that Na^+ and K^+ are transported by distinct mechanisms which develop at different times.

Nature of Interaction between Na^+ and K^+ Uptake. The effect of Na^+ on K^+ uptake in beet is not readily explained by competition for a single transport site at the exterior of the cell. In the first place, the uptake mechanisms for the two ions develop separately. Secondly, the rates of uptake of the two ions are not related in a simple way to their relative concentrations, nor to their apparent affinities for the transport mechanism. Thirdly, the presence of Na^+ outside the cell is not inhibitory unless Na^+ is absorbed.

Although the initial binding of each cation appears to take place independently, there is evidence that the transport processes interact at the cell membrane (14), since in Na^+-selective material, Na^+ inhibits potassium influx at the cell membrane, and with no detectable time lag. This is also suggested in Figure 2, where the K^+ influx for a 15-min period is reduced almost to zero in some cases.

The interaction between Na^+ and K^+ uptake seems to be related to the need for electrical balance of the transported ion. It was shown by Sutcliffe (22) that total cation uptake by red beet from a mixture of 10 mM KCl + 10 mM NaCl was about equal to K^+ uptake from 20 mM KCl or Na^+ uptake from 20 mM NaCl. In other words, the interaction between Na^+ and K^+ was such that the sum of the uptake rates tended to remain constant, an increase in the rate of uptake of one ion being balanced by a decrease in the rate of uptake of the other. Since the total cation uptake from Cl^- solutions is determined by the rate of Cl^- uptake (10), it appears that the Na^+ and K^+ transport systems compete in some way for the electrically balancing ion. Similarly, the data of Tables III and IV and Figure 2 are readily interpreted on the basis that the maximal rate of transport of the electrically balancing ions sets a ceiling which limits the total cation uptake. Similar results have been obtained with barley roots by Pitman *et al.* (12).

There are a number of ways in which electrical balance across the cell membrane may be achieved. These include (*a*) the dependence of K^+ uptake on the membrane potential; (*b*) direct coupling of cation and anion transport, *e.g.*, movement on the same carrier; and (*c*) coupling of cation influx to H^+ efflux.

Although the present results do not clearly distinguish between these possibilities, they do show that Na^+-K^+ selectivity in beet is independent of the ionic species involved in electrical balance of the cations. In the presence of tris sulfate buffer at high pH, cation uptake is balanced by HCO_3^- or H^+ (6, 15, 21), whereas in Cl^- solution, and especially in the presence of $CaCl_2$, cation uptake is balanced by Cl^- uptake (10). Moreover, the transport of HCO_3^- or H^+ does not seem to involve the same mechanism as that responsible for Cl^- uptake, since the latter develops only after washing the tissue for some days (3, 20), whereas uptake in tris solutions commences after only a brief washing period (20). The similar development of Na^+-K^+ selectivity whether uptake is measured in Cl^- or in tris solutions suggests, therefore, that cation transport is not directly coupled to anion transport and has the same characteristics regardless of the electrically balancing transport mechanism. This is difficult to reconcile with the available data on membrane potentials in beet (13), since K^+ uptake in the absence of Cl^- transport is accompanied by large changes of potential, while KCl uptake shows no correlation with potential. This apparent contradiction must await further investigation.

"Carrier Competition." The cation interactions observed in the present investigation may be compared with those observed previously by Bange *et al.* (2) in barley roots. These authors concluded that it is impossible to explain their data in terms of Michaelis-Menten kinetics, no matter how many carriers are postulated. It was found that the extent of inhibition depends on the rate of uptake of the inhibiting ion, rather than on its external concentration. The extent of inhibition in the system of Bange *et al.* never approaches 100%, and the apparent Michaelis constant of the inhibited ion does not increase beyond a certain point, regardless of the concentration of the inhibitor. All of these results could be predicted, however, in terms of carrier competition (1, 2), *i.e.*, competition between the transport systems at a stage subsequent to the initial binding of the ions. This could mean competition for an energy source, for example, or for an enzyme required by several transport systems.

The present data and those of Pitman *et al.* (12) suggest that the results of Bange *et al.* (2) may be attributed to a limita- of the uptake rate by electrically balancing ions. Nevertheless, the model of carrier competition developed by Bange (1) may still be applicable if the mechanism of electrical balance involves coupling of cation influx to H^+ efflux (7, 8, 18, 19). The Na^+-K^+ interaction may then be due to competition between the Na^+ and K^+ carriers for cytoplasmic H^+ to be transported out of the cell. The Na^+-K^+ selectivity might be determined by the relative affinities of the Na^+ and K^+ transport mechanisms for cytoplasmic H^+. Cytoplasmic pH changes appear to be involved in the control of organic acid synthesis during salt uptake (5). The possible control of cation uptake also by the internal pH, although not at present supported by membrane potential data in beet, warrants further investigation.

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