J. Membrane Biol. 70, 157–164 (1982)

The Journal of
Membrane Biology

Pump-Leak Sodium Fluxes in Low Salt Corn Roots

John M. Cheeseman
Department of Botany, University of Illinois, Urbana, IL 61801

Summary. The influx and efflux of sodium from 4-hr washed, low salt corn roots (*Zea mays* L.) has been studied for characterization of passive and active components. Initial Na^+ content of the roots is very low, 2.25 ± 0.4 μmol/g fresh weight. Na^+ influx in the presence of 0.2 mM Ca^{2+} and 0.002 to 20 mM K^+ is passive (a "leak") based upon Goldman-type models, being determined by Na^+ and cell potential (ψ). Na^+ was not transported by the K^+ carrier and influx was unaffected by 50 μM dicyclohexylcarbodiimide (DCCD). Permeability of the cells to Na^+ was of the same order as P_K.

Efflux of Na^+ was by an efficient and rapid active transport system (a "pump"), thus accounting for the failure of these roots to accumulate high levels of Na^+. In short-term loading and efflux experiments, internal Na^+ turnover had a half-time of about 5 min. Sodium efflux was unaffected by DCCD. Net H^+ flux was zero in the presence of DCCD regardless of sodium efflux, indicating absence of Na^+/H^+ antiport. Efflux of Na^+ was equally rapid into medium containing no Na^+ and only 0.002 mM K^+. K^+ influx accounted for less than 4% of Na^+ efflux, prompting the hypothesis that the Na^+ (or cation?) efflux pump is the second electrogenic system previously defined based upon electrophysiological measurements.

Key Words: Sodium · *Zea mays* · electrogenic pumps · active transport · roots · DCCD · pump-leak

Introduction

Many higher plant cells, especially those in glycophytes, are relatively rich in K^+ and poor in Na^+ despite growth in soil of the reverse ion status. This condition is apparently possible due to active K^+ influx and active Na^+ efflux, though the processes are not linked obligatorily through one ATPase as they are in animal cells [11, 17].

In previous reports [2–6] we have considered active and passive K^+ fluxes into corn roots, interpreting our data using passive models as the beginning step. The results showed that, in aerated low salt tissue, neither cell potential[1], ψ, *vs.* K^o nor influx *vs.* K^o were as expected for the passive case. However, inhibiting energy-linked transport with uncouplers or anoxia gave results over a wide range of K^o which did conform with such a model. The energy-linked component of ψ proved separable into two phases with a range of transition over the region where $\psi \simeq E_K$. Using Epstein's terminology [8] system I involved active K^+ transport against a thermodynamic gradient [5] possibly *via* a K^+-carrying H^+ extruding ATPase. Increased active K^+ influx as K^o increased correlated well with a decline in a DCCD sensitive, hyperpolarizing component of ψ [4–6]. System II K^+ influx largely represented passive movement down the electrical gradient. K^+ levels in this range appeared to inhibit the carrier activity of system I [3]. In this range ψ included a component resulting from activity of a second electrogenic system which did not have active K^+ carrying characteristics, which was insensitive to DCCD and other putative inhibitors of the plasmalemma ATPase, but which was still sensitive to anoxia and uncouplers.

In the first part of this report a similar approach is used to extend our understanding of Na^+ influx mechanisms in low salt corn root segments. The results show that Na^+ influx is passive even at low K^o and when Na^+ is present in large excess, i.e., Na^+ is not available to the K^+ carrier. The second part is a study of Na^+ efflux and the hypothesis that this might be directly related to system II electrogenic activity. Efflux was rapid and efficient: the halftime of exchange was approximately 5 min and 90% of the Na^+ label acquired in a 10 min loading period was exchangeable within 60 min. Efflux was insensitive to DCCD and to removal of K^+ and Na^+ from the medium; thus, it was not a cation exchange process.

Materials and Methods

Methods for raising corn seedlings [*Zea mays* L., (A619 × Oh43) × A632, Crow's Hybrid Corn Co., Milford, IL], cutting and washing root segments, and determination of cell potentials and influxes were as previously described with minor modifica-

[1] *Abbreviations:* ψ, ψ_D: total and diffusion cell potentials; ϕ_{Na}: Na^+ influx; Na^o, K^o: cation *concentrations* outside the cell; P_j: permeability coefficient for ion j; DCCD: dicyclohexylcarbodiimide.

0022-2631/82/0070-0157 $01.40

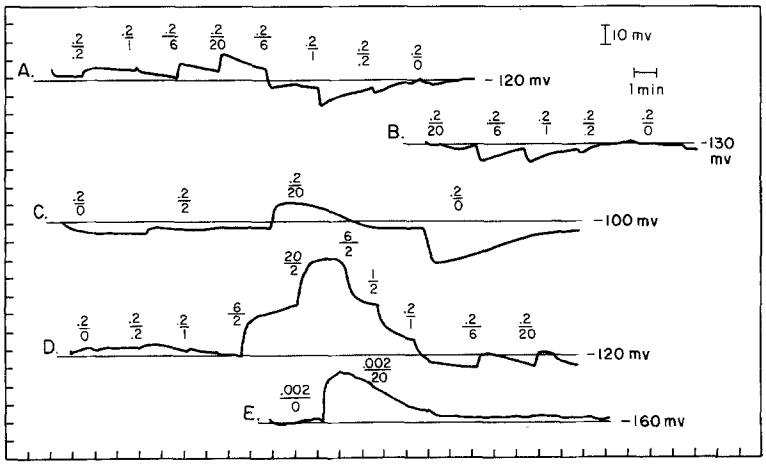

Fig. 1. The effect of changing Na^o and K^o on cell potential in low salt corn roots. Numerators denote K^o in (mM) and denominators, Na^o. Depolarization is upward. (*A–B*): Short term transients upon changing Na^o. (*C*): Longer term effects showing re-establishment of initial potential following each change in Na^o. (D): Comparison showing stable changes with changing K^o. (*E*): The extreme case: potential is re-established in 6 min despite a large excess of Na^+

tions given below [2, 3, 9]. Briefly, these were the 0.5 to 2.5 cm segments of primary roots of 3-day old corn. They were washed, after excision, for 4 hr at 30 °C in 0.2 mM $CaSO_4$ + 0.1 mM K_2SO_4 plus 0.2 mM MES buffer, solution pH adjusted to 6.0 with tris. Calcium and buffer were present in all other solutions as well; Na^+ and K^+ were adjusted to desired levels with sulfate salts. Tetramethylammonium was used as a chloride salt to adjust ionic strength without addition of mineral cations.

Washing and all subsequent operations for flux studies were done with 10 to 15 roots (0.2 to 0.25 g fresh weight) in small plastic boxes with slitted upper and lower surfaces (Tissue Tek III Unicassette, American Scientific Products). In these boxes, roots floated freely with unrestricted solution circulation. When boxes were placed horizontally in shallow, well-aerated solution, K^+ uptake rates were comparable to those determined previously with free-floating roots [3]. For short-term Na^+ influx experiments aimed at determining relative Na^+ permeability and the influence of Na^+ on K^+ influx, washed roots were rinsed well in unlabeled solution at appropriate Na^+ and K^+ levels for 10 min, then transferred to labeled uptake solution for 10 min. This pretreatment assured equilibration of cell walls and allowed establishment of steady cell potentials. Uptake solutions were labeled with both ^{22}Na and ^{42}K. Following uptake, roots were transferred to a large volume of ice cold 10 mM $CaCl_2$ for 5–7 min to exchange cell wall label. Preliminary experiments showed this treatment did not completely inhibit Na^+ efflux; thus, timing was selected to maximize removal of wall label (>97%) and minimize loss of internal label (<5%). Roots were then blotted, weighed, and counted by liquid scintillation using Amersham ACS scintillant. Double labeled samples were recounted after 7–10 days following ^{42}K decay.

For calculation of η_{Na}, total ionic concentrations were corrected to activity based upon Table 2.1 in Plonsey [18].

For long-term influx experiments, roots were transferred at time zero to labeled solution with no pre-rinse in unlabeled solution. The first 10 min of those experiments, therefore, were away from steady state with respect to ψ and cell wall conditions. For net uptake studies, and initial ion content determinations, cellular Na^+ and K^+ were determined by flame photometry (IL643) following $CaCl_2$ exchange (as above), drying and ashing at 450–500 °C. Ash was resuspended in 1 M Mg Acetate.

For efflux studies, washed tissue was equilibrated at the appropriate levels of Na^+ and K^+ for 55 min prior to transfer to labeled solution for 10 min. Efflux was measured as ^{22}Na remaining in roots following exchange in unlabeled solutions. These solutions were changed three times in the first 10 min and following 30 min. They never accumulated appreciable counts (<75 cpm/ml). At intervals, root samples were removed, exchanged in cold $CaCl_2$ as above, and analyzed. Because this cold wall exchange period was common to all samples, there is no cell wall component in the efflux curves.

Results

Figure 1 illustrates the response of ψ changes in Na^o and K^o in washed corn root segments. Increasing Na^o at constant K^o resulted in transient depolarization. Even when $Na^o >> K^o$ (Fig. 1 E), ψ recovered to the original level in 5 to 10 min. Decreasing Na^o produced the converse response (Fig. 1 *A–C*). The effect of Na^o is in marked contrast to the stable changes in ψ which accompany changes in K^o (Fig. 1 *D*).

It is very likely that these transient effects result in part from the replacement of K^+ on cell wall exchange sites with Na^+, with a resulting transient increase in K^o in the vicinity of the cell membranes. However, the relative importance of this effect in the overall response is not easily determined. In order to prevent such changes an alternative cation is required, the protocol being first to establish a stable potential in the presence of the alternative cation, and then to replace it with Na^+ at the same ionic strength. As might be expected, such efforts were hampered by unavailability of a totally innocuous cation. The results using Ca^{2+}, Mg^{2+} and tetramethylammonium (TMA^+) were equivocal, consistent with the hypothesis that we do not fully understand the complexities of root cell membrane potentials, and uninformative enough to preclude further consideration here. Clarification of the wall involvement in the transients must therefore await perfection of techniques to measure short-term changes in ψ in protoplasts. This, however, does

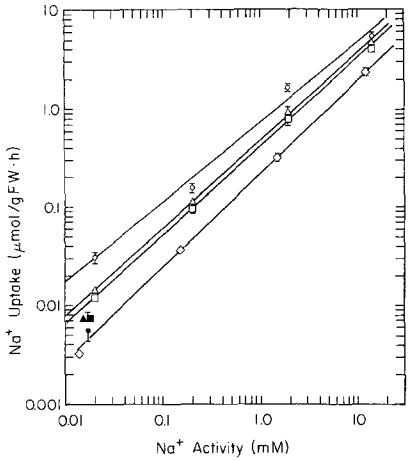

Fig. 2. Na^+ influx *vs.* external Na^+ *activity* for four levels of K^+. The log-log plot is used to emphasize agreement with the Goldman model. Corresponding steady-state potentials are given in Table 1. Solid lines are fluxes predicted using Eq. (1). Each point is mean ±SD of four replicates. $K^o = 0.002$ mM (○), 0.02 mM (△), 0.2 mM (□), 20 mM (◇)

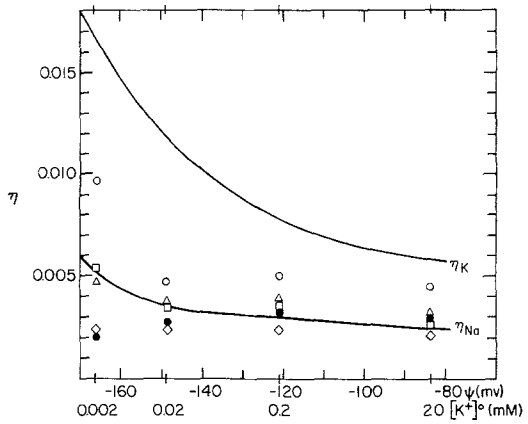

Fig. 3. η_{Na} and η_K *vs.* ψ. η_{Na} was determined from fluxes shown in Fig. 2 η_K from ref. [3]. Abscissa is labeled to show steady-state ψ at each level of K^+. $Na^o = 0.02$ mM (○); 0.2 mM (△); 2 mM (□), 20 mM (◇). Closed symbols – 20 mM TMA^+ in addition to Na^+. As Na^+ and K^+ activity depend on total concentrations, and as these curves are not used in modeling, η's are plotted with respect to concentration for clarity

nothing to negate a very useful consequence of the return to the original potential: by allowing ψ to stabilize before addition of label for flux measurements, the effects of changing Na^o at constant ψ, and of changing ψ (by varying K^o) at constant Na^o, could be studied.

Figure 2 shows Na^+ influx as a function of external Na^+ activity at four levels of K^o, and thus at four values of ψ. Use of activity rather than concentration is appropriate here to consider the linear relationship expected using models based upon Goldman theory [3]. When plotted on a semi-logarithmic basis the pattern is similar to that shown by Rains and Epstein [19] in barley roots with Ca^{2+} and K^+ present in the medium. Even in the case of very low K^o, there is no indication of dual phase Na^o uptake.

It has been suggested earlier that in barley [19] and corn [1] roots, discrimination between Na^+ and K^+ influx occurs due to the difference in the affinity of the active K^+ carrier for the two ions, K_m being approximately 0.02 for K^+ and 0.3 for Na^+ (determined in the absence of K^+). Exception to this has been taken, however. Jeschke [14] noted that this simple difference would not account for the magnitude of selectivity which occurs. In the present study, should the affinity model hold, the excess of Na^+ over K^+ should have been sufficient for carrier mediated Na^+ influx to occur and to indicate dual-isotherm Na^+ influx kinetics (i.e., Fig. 2, $K^o = 0.002$ mM). Alternately, it should have been possible to demonstrate excess Na^+ influx (over a passive value) when carrier mediated transport was maximal. That is, when $K^o = 0.2$ mM and carrier mediated transport was nearly maximal, addition of 20 mM Na^o should have resulted in significant (2.5 to 3 μmol/g fresh weight·hr) carrier mediated Na^+ influx. The linearity in Fig. 2 at $K^o = 0.2$ mM implies this flux was absent. The interaction of Na^+ and the active K^+ influx system are considered further below.

Knowing the values of Na^+ influxes, ψ and Na^o, the relative apparent permeability coefficient of Na^+, η_{Na} [3] can be calculated using Eq. (1):

$$\eta_{Na} = -\frac{\phi_{Na}(1-\xi)}{Na^o \psi} \tag{1}$$

where ϕ_{Na} is the rate of Na^+ influx (μmol/g fresh weight·hr), and $\xi \equiv \exp(F\psi/RT)$ (Ref. 2). η_{Na} is proportional to $F/P_{Na}/RT$ but incorporates the unknown surface area to root weight factor. These values of η_{Na} are shown in Fig. 3. Note that in this figure, concentrations rather than activities are shown for clarity of presentation. Activity was, however, used in Eq (1). η_{Na} and η_K [3] are both dependent either on ψ, ionic strength, or membrane surface change. This effect is probably different from the transients shown in Fig. 1. η_{Na} thus appears to be approximately one-half of η_K.

The hypothesis that Na^+ enters roots passively was also supported by measurements of Na^+ influx under anoxia (Table 1). η_{Na} estimated by this method was somewhat higher, possibly reflecting the elimination of active Na^+ efflux (*see* below). Thus we conclude only that P_{Na} and P_K are of

Table 1. Relative sodium permeability, η_{Na}, as determined by influx under anoxia[a].

ψ (mV)	K^o (mM)	ϕ_{Na} at Na^o (μmol/g fresh wt·hr)			η_{Na}
		0.02	0.2	2.0	
−99	0.002	0.014 ±0.002	0.170±0.075	–	0.0077
−97	0.2	0.013 ±0.002	0.144±0.017	–	0.0069
−50	20	0.0047±0.0004	0.048±0.003	0.469±0.031	0.0058

[a] Concentration to activity corrections were incorporated into η_{Na} calculations. ψ values were reported previously [4]. $n=4$.

Table 2. $^{42}K^+$ influx measured simultaneously with ^{22}Na influx of Fig. 2, (μmol/g fresh wt·hr) 10 min uptake after 10 min equilibrium[a]

K^o (mM)	Na^o (mM)						% ↓ ϕ_K
	0	0.02	0.2	2	$P(F)$	20	
0.002	0.195±0.026	0.216±0.051	0.154±0.013	0.196±0.041	0.088	0.089±0.009	54
0.02	1.43 ±0.28	1.82 ±0.21	1.34 ±0.41	1.50 ±0.43	0.21	0.96 ±0.13	33
0.2	6.64 ±0.57	5.95 ±1.27	5.11 ±1.03	4.95 ±0.95	0.054	3.94 ±0.49	41

[a] Means of 5 replicates, ±SD $P(F)$ is significance level in one-way analysis of variance of all conditions except $Na^o=20$ mM. Last column is reduction of K^+ influx at $Na^o=20$ mM from Na^+-free level.

Table 3. Net Na^+ uptake obtained by flame photometry of ashed samples[a]

	Time (hr)		
	0	1	2
Na content	2.25±0.40	3.08±0.22	3.98±0.52
Uptake/hr	–	0.82	0.90

[a] Loading solution was 0.2 mM K^+, 20 mM, 0.2 mM Ca^{2+}, 0.2 mM MES-Tris, pH 6.0. Na^+ content is μmol/g fresh wt $n=4$.

the same order, a result similar to that of Pierce and Higinbotham who estimated the ratio P_{Na}/P_K to be 0.7 and1.3 in two experiments with oat coleoptiles [16].

Finally, we considered the interaction of sodium and the active K^+ transport system by considering the effect of external Na^+ on K^+ influx. These experiments were performed simultaneously with the Na^+ influx experiments shown in Fig. 2. Table 2 shows the effect of Na^+ at three levels of K^o. In this range, K^+ influx is almost entirely active [3]. At external concentrations of Na^+ up to 2 mM, there was no effect on K^+ influx ($P(F)>0.05$ in one way analysis of variance). This strengthens the conclusion of Jeschke [14] that discrimination against Na^+ is not just a matter of K_m differences. At $Na^o=20$ mM, however, K^+ influx was reduced 30–55% from the rate in sodium-free solution. Figure 2 clearly shows that the lost K^+ influx is not replaced by active Na^+ influx. TMA^+ at 20 mM also reduced K^+ influx, by 34% at 0.02 mM K^o and by 47% at 0.2 mM K^o. These reductions are of the same magnitude as the reduction (by 39% from V_{max}) of active influx by K^+ at the same level [3], suggesting a nonspecific effect of monovalent cations on a carrier control or binding site. Thus we have shown that Na^+ influx is passive over four orders of magnitude, controlled by the electrical driving forces, and independent of K^+ influx, and sodium exclusion, a characteristic of corn, cannot be attributed to absence of uptake. Other possible mechanisms are "outgrowing" the Na^+ influx, i.e., addition of new water faster than addition of Na^+, and active Na^+ efflux. We therefore investigated the latter in this nongrowing tissue.

Net influx and efflux were studied at 0.2 mM K^o and 20 mM Na^o. This required no treatment to remove K^+ from walls as would be required at lower levels, and Na^+ influx was rapid enough to substantially alter internal Na^+ content in a short period in the absence of efflux. Also initial tissue Na^+ levels (Table 3) were low, so the net increase driving force was very large.

Table 3 shows that net influx over 2 hr was considerably lower than suggested by short-term tracer fluxes. This was confirmed by trager influx

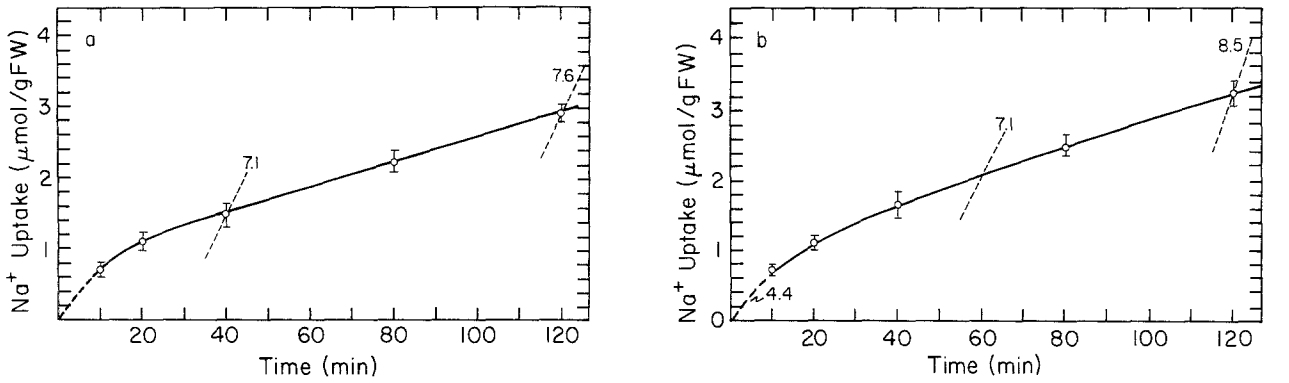

Fig. 4. Na^+ influx *vs.* time in control (*a*) and DCCD-treated roots (*b*). DCCD (50 μM; 0.5% ethanol) added at $t=0$. Dashed lines indicate 10-min influx rates at the times shown, and numbers are rates (μmol/g fresh wt·hr). Each point is the mean ±SD of four replicates

over a 2-hr period (Fig. 4*a*). Over this period, excepting the first 10 min after addition of Na^+, K^+ influx was linear, and the long and short-term tracer influx rates were equal (data not shown). In the case with Na^+, the flattening of the curve represents an increasing efflux rate rather than a decreasing influx rate as shown by the shortterm influx rates in Fig. 4*a*. Turnover of total cellular Na^+ was rapid: the slope of the line in Fig. 4*a* for the second hour is very close to the net influx rate calculated in Table 3, and after 2 hr, 73% of the internal Na^+ was labeled. This implies a much faster turnover than calculated by Davis and Jaworski [7], or that a large portion of the total Na^+ is cytoplasmic in low salt roots.

Figure 4*b* shows the effects of DCCD on short and long-term tracer uptake. DCCD reduces K^+ influx to a passive level under these conditions while only slightly reducing ATP levels [6]. There

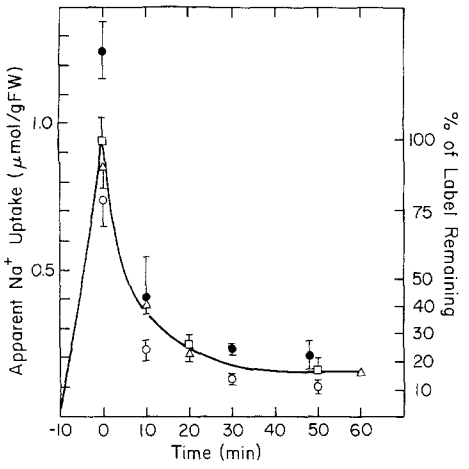

Fig. 5. Efflux of Na^+ from roots labeled in 0.2 mM K^+, plus 20 mM Na^+ for 10 min, in absence (closed symbols) or presence (open) of 50 μM DCCD. Efflux was into unlabeled solution ± DCCD. (○, ●) 0.2 mM K^+, 20 mM Na^+; (▲) 0.2 mM K^+; (□) 0.002 mM K^+

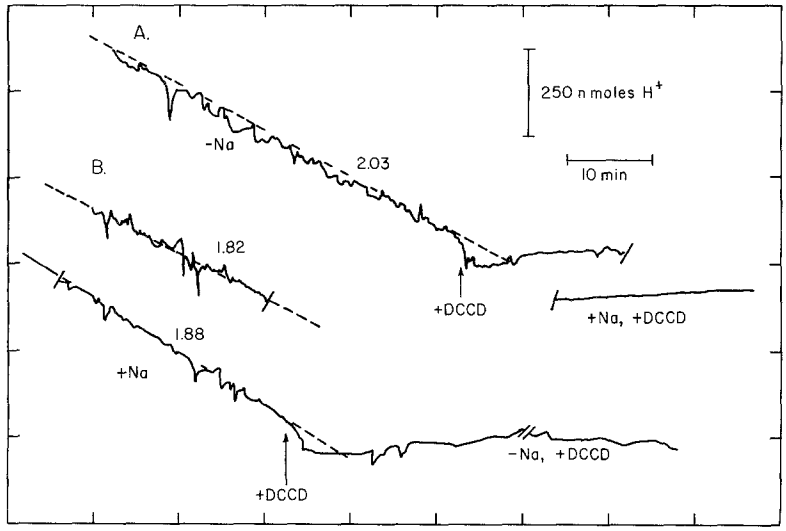

Fig. 6. Net H^+ efflux in 0.2 mM K^o. (*A*) 20 mM Na^+ added after DCCD (50 μM) had eliminated net H^+ efflux. (*B*): Na^+ added prior to DCCD and roots subsequently transferred to Na^+-free solution. Numbers are net H^+ efflux rates (μmol/g·hr) determined from slopes. For these experiments, buffer was reduced to 0.05 mM MES-tris

Table 4. $^{22}Na^+$ efflux and $^{42}K^+$ influx following 10 min labeling in 0.2 mM K^+, 20 mM Na^+ medium[a]

Efflux time (min)	^{22}Na content (μmol/g fresh wt)	% loss	^{42}K uptake (control) μmol/g fresh wt)	^{42}K uptake (plus Na) (μmol/g fresh wt)	Difference %
0	0.85±0.07	–	–	–	–
10	0.38±0.03	55	0.020±0.002	0.039±0.006	4.0
20	0.21±0.02	75	0.039±0.004	0.046±0.007	1.1
60	0.15±0.02	83	0.075±0.001	0.090±0.002	2.1

[a] DCCD (50 μM) was present at all stages of Na^+ loading. Control K^+ influx was measured in absence of Na^+ treatment. Difference is percent of effluxed Na^+ accounted for by excess K^+ uptake over control values.

was no effect on either Na^+ flux, implying no effect on the efflux system.

Short-term efflux was considered more directly by following loss of label after brief (10 min) loading with ^{22}Na. These experiments were done with the efflux models of Jeschke [14] and Ratner and Jacoby [21] in mind. Consequently, the effect of DCCD, K^o, and Na^o and the net H^+ fluxes were considered. The results are shown in Fig. 5. Noting (*see* Materials and Methods) that this efflux curve has no wall component, the loss of cellular Na^+ is rapid: $t_{1/2}$ is approximately 5 min. Efflux is not eliminated by DCCD and does not represent a Na^+ for Na^+ or Na^+ for K^+ exchange. Using DCCD to eliminate active K^+ influx, K^+ uptake during the efflux period accounted for less than 4% of the Na^+ removed (Table 4). Efflux was as rapid into 0.002 mM K^o solution as into 0.2 mM K^+ plus 20 mM Na^+, indicating no K^+-stimulated component of the efflux. Na^+ efflux was also not in exchange for H^+ as indicated in Fig. 6. DCCD eliminated net H^+ efflux in the presence or absence of Na^+. Addition of Na^+ after DCCD did not produce net H^+ influx indicative of such an exchange (Fig. 6*A*) nor did removal of Na^+ (Fig. 6*B*).

Discussion

In this report we have considered both Na^+ influx and efflux in 4-hr washed, low salt corn roots. Influx was considered from the viewpoint of the dual isotherm kinetic theory, and of Goldman theory for passive membrane fluxes. The results show that the active K^+ influx system is unavailable for Na^+ influx, even at a 10,000-fold excess of Na^+. Interaction of Na^+ with K^+ influx appears to be a nonspecific cation effect. Modeled on the basis of Goldman theory, Na^+ influx appears strictly passive (a "leak") over four orders of magnitude at rates determined by Na^o and ψ. The sodium permeability of corn root cell membranes was of the same order as potassium permeability. Long term (2 hr) net influx of Na^+ was much lower than indicated by short term (10 min) tracer studies, and an efficient Na^+ efflux mechanism ("pump") was shown. Following 10 min of ^{22}Na uptake, label was lost by efflux with a half time of about 5 min. Unlike active K^+ influx and H^+ efflux, Na^+ efflux was not eliminated by DCCD and did not represent exchange of Na^+ for Na^+ K^+, or H^+.

The mechanism of Na^+ influx has previously [1, 14, 19] been analyzed either using the dual isotherm perspective or the Teorell-Ussing flux ratio method [15, 16]. Dual isotherm analysis indicated that Na^+ influx in barley roots in the presence of both Ca^{2+} and K^+ was by system II alone [17]. This is fully consistent with the present results as we have previously shown that system II K^+ influx is in fact passive movement down an electrical gradient. That Na^+ influx may be passive has been suggested based upon the Teorell-Ussing criterion, primarily because the flux is thermodynamically downhill [14, 16]. This criterion is much more likely to point to the need for an active system, but cannot conclude a passive one. For this the present approach is a stronger one. Occasional reports of active Na^+ influx based on the Teorell-Ussing criterion [15, 22] can probably be explained on the basis of abnormally low cell potentials, the roots having been excised and split just before the measurements of ψ.

Active efflux of Na^+ has been studied in corn [7, 13] and extensively in barley [14, 21]. Both Jeschke [14] and Ratner and Jacoby [21] studied Na^+ efflux in barley roots and found distinct stimulation of efflux by K^+ added to the medium. No K^+ stimulation was found in corn [13]. Davis and Jaworski [7] used the compartmental analysis technique in corn roots loaded in the presence of K^+ and Ca^{2+}. Flux rate analysis showed Na^+ efflux

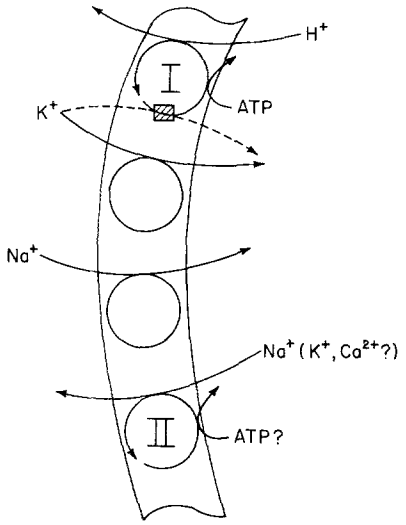

Fig. 7. Schematic model of electrogenic K^+ influx and Na^+ efflux systems at the plasmalemma of corn root cells under low and high K^o conditions. The model has been modified from reference [3] to show passive Na^+ entry (a uniport) and active, electrogenic Na^+ efflux

to be active. In those experiments Davis and Jaworski were concerned with long-term fluxes in preloaded tissue and with the effects of ouabain on those fluxes. They reported a cytoplasmic $t_{1/2}$ somewhat slower than that indicated by the pulse-chase approach used here (nearly 20 min *vs.* 5 min in this case). Unfortunately, neither efflux nor ouabain effects are directly comparable to the present study as the fluxes and effects were determined on freshly excised tissue. It is known that excision inhibits several active fluxes [9] and ψ in corn roots and the effects on active Na^+ efflux are yet to be determined.

In conclusion, we will postulate that the active Na^+ efflux system is electrogenic and possibly is the electrogenic system II reported previously [4, 6]. We start by arguing that Na^+ must stimulate some electrogenic system. More important than the fact that ψ repolarizes upon addition of Na^o (Fig. 1) is that ψ does not become or remain depolarized. ψ represents a balance between electrogenic components and passive components (ψ_D), and ψ_D exists in the normally respiring cell, i.e., the passive ion conductances of the membrane do not become zero when the electrogenic system(s) is operating [6]. We have now shown that P_{Na} is not negligible with respect to P_K. It follows that ψ_D is reduced by high Na^o and the maintenance of ψ at the Na^+-free level (e.g., Fig. 1*E*) thus must represent stimulation of some electrogenic system.

Secondly, we have shown that active Na^+ efflux occurs, that it is insensitive to DCCD (as is the electrogenic system II), and that it does not represent neutral exchange for Na^+, K^+ or H^+. It is probable that the system is not specific for Na^+ as the electrogenic system II operates in the absence of added Na^+ [6]. One experiment suggests that K^+ is also a substrate: when roots were loaded for 10 min in 20 mM K^o plus 20 mM Na^o and then effluxed, 32% of the $^{42}K^+$ as well as 90% of the $^{22}Na^+$ was lost in 50 min. Such loss of K^+ did not occur when roots were loaded at 0.2 mM K^o. Studies by Higinbotham et al. [12] indicate that active Ca^{2+} efflux also occurs and an ATP-dependent Ca^{2+} pump associated with the plasmalemma has been isolated [10]. Though no data are available suggesting involvement of the same pump for Ca^{2+} and Na^+, it may for the moment be considered a possibility, as well. Therefore we again modify our previously published model of the plasma membrane transport systems, as shown in Fig. 7, incorporating an active Na^+ or cation efflux as the second electrogenic system.

This work was supported by National Science Foundation grants PCM 76-80886, PCM 80-11138, and United States Department of Energy contract EY-76-S-02-0790.

References

1. Bange, G.G.J. 1959. Interactions in the potassium and sodium absorption by intact maize seedlings. *Plant Soil* **11**:17–29
2. Cheeseman, J.M., Hanson, J.B. 1979. Mathematical analysis of the dependence of cell potential on external potassium in corn roots. *Plant Physiol.* **63**:1–4
3. Cheeseman, J.M., Hanson, J.B. 1979. Energy-linked potassium influx as related to cell potential in corn roots. *Plant Physiol.* **64**:842–845
4. Cheeseman, J.M., Hanson, J.B. 1980. Characterization of two electrogenic systems in corn roots. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 409–410. Elsevier/North-Holland Biomedical Press, Amsterdam
5. Cheeseman, J.M., Hanson, J.B. 1980. Does active K^+ influx to roots occur? *Plant Sci. Lett.* **18**:81–84
6. Cheeseman, J.M., LaFayette, P.R., Gronewald, J.W., Hanson, J.B. 1980. Effect of ATPase inhibitors on cell potential and K^+ influx in corn roots. *Plant Physiol.* **65**:1139–1145
7. Davis, R.T., Jaworski, A.Z. 1979. Effects of ouabain and low temperature on the sodium efflux pump in excised corn roots. *Plant Physiol.* **63**:940–946
8. Epstein, E. 1972. Mineral Nutrition of Plants. Principles and Perspectives. John Wiley & Sons, New York
9. Gronewald, J.W., Cheeseman, J.M., Hanson, J.B. 1979. Comparison of the responses of corn root tissue to fusicoccin and washing. *Plant Physiol.* **63**:255–259
10. Gross, J., Marmé, D. 1978. ATP-dependent Ca^{2+} uptake into plant membrane vesicles. *Proc. Natl. Acad. Sci. USA* **75**:1232–1236
11. Higinbotham, N. 1973. Mineral absorption processes in plants. *Bot. Rev.* **39**:15–69

12. Higinbotham, N., Etherton, B., Foster, R.J. 1967. Mineral ion contents and cell transmembrane electropotentials of pea and oat seedlings tissue. *Plant Physiol.* **42**:37–46
13. Jacoby, B., Ratner, A. 1974. Mechanism of sodium exclusion in bean and corn plants – a reevaluation. *In:* Plant Analysis and Fertilizer Problems. J. Wehrman, editor. pp. 175–184 German Society of Plant Nutrition, Hannover
14. Jeschke, W.D. 1977. K^+-Na^+ exchange and selectivity in barley root cells: Effects of Na^+ on the Na^+ fluxes. *J. Exp. Bot.* **28**:1289–1306
15. Macklon, A.E.S. 1975. Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. I. Potassium, sodium and chloride. *Planta* **122**:109–130
16. Pierce, W.S., Higinbotham, N. 1970. Compartments and fluxes of K^+, Na^+ and Cl^- in *Avena* coleoptile cells. *Plant Physiol.* **46**:666–673
17. Pitman M.G. 1976. Ion uptake by plant roots. *In:* Transport in Plants. II, B. Encylopedia of Plant Physiology. U. Luttge and M.G. Pitman, editors pp. 95–128 Springer, Berlin
18. Plonsey, R. 1969. Bioelectric Phenomena. McGraw-Hill, New York
19. Rains, D.W., Epstein, E. 1967. Sodium absorption by barley roots: The role of the dual mechanisms of alkali cation transport. *Plant Physiol.* **42**:314–318
20. Rains, D.W., Epstein, E. 1967. Sodium absorption by barley roots: Its mediation by mechanism 2 of alkali cation transport. *Plant Physiol.* **42**:319–323
21. Ratner, A., Jacoby, B. 1976. Effect of K^+, its counter anion, and pH on sodium efflux from barley root tips. *J. Exp. Bot.* **27**:843–852
22. Shepherd, N.H., Bowling, D.J.F. 1973. Active accumulation of sodium by roots of five aquatic species. *New Phytol.* **72**:1075–1080

Received 22 February 1982; revised 18 May 1982