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Uptake and Distribution of Sodium and Potassium by Corn Seedlings[1]

II. ION TRANSPORT WITHIN THE MESOCOTYL

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ABSTRACT

In this paper, uptake and distribution of sodium and potassium within the mesocotyl are considered in excised, 8-day-old corn (*Zea mays* L.) seedlings supplied with label via the transpiration stream. The stele and cortex were dissected following uptake and analyzed separately. At equal concentrations, sodium uptake by the stele was much more rapid than potassium uptake, and sodium was preferentially retained within the stele. Transport of sodium to the cortex halted when the supply of ions in the transpiration stream was interrupted. Potassium would not substitute for sodium in restoring this transport but neither did it compete with sodium for transport to the cortex. In the presence of continued sodium supply, transport was temperature sensitive.

By labeling first with ^{22}Na for 2 hours and subsequently with ^{24}Na for up to 21 hours, three sodium pools were identified within the stele. The first was rapidly transportable to the cortex. The second equilibrated rapidly with the first but was not itself directly available for transport. We postulate that these represent the stelar symplasm and apoplasm, respectively. A third pool was not transported and probably represents sequestration within the vacuoles of some cell type. Transport of label acquired during the initial 2 hours proceeded with a half-time of approximately 10 hours with 10 millimolar sodium present during the redistribution period, and with a half-time of approximately 30 hours at 1 millimolar sodium.

A working model is presented which explains these characteristics and supplies approachable questions for subsequent study.

In the previous paper, we considered the uptake of sodium and potassium by the mesocotyl of corn seedlings when the ions were supplied via the transpiration stream. We noted that in the mesocotyl region there was considerable discrimination of the uptake system(s) in favor of sodium over potassium, and that this flux of sodium could play an important role in the detoxification of the ascending transpiration stream, reducing sodium accumulation in the shoot (7). In this regard, the experiments extended those of Jacoby (4–6) to corn, and they also extended the results of Shone *et al.* (9) by showing that the sodium accumulation which they reported in basal regions of corn roots extended upward into the root-like mesocotyl. The results also indicated the possibilty that the mesocotyl could be used as a model or 'plastic-coated' root in studies aimed at elucidating the process of selective ion transport to the shoot.

In the course of the previously reported experiments, we discovered that we could easily separate the cortex and the stele of the mesocotyl by dissection and, thus, that we could analyze both the uptake of ions by the mesocotyl and their distribution within that organ. Separation of the stele and cortex of roots has been used previously as a technique for considering the ability of the stele itself to accumulate ions (1), though such measurements must include an unknown injury factor. More recently, this technique has been used to consider the distribution of enzymes and biochemical systems in corn roots and mesocotyls (3, 11, 12).

In this paper, we extend the results of the previous paper and consider outward redistribution and compartmentation of sodium within the mesocotyl, introducing the technique of labeling the uptake solution at different times with ^{22}Na and ^{24}Na.

MATERIALS AND METHODS

The methods of growing plants, performing uptake experiments and analyzing tissue were those reported in the previous paper (7) with the modification that following the uptake period the mesocotyl was ringed just below the coleoptile node and slit lengthwise with a sharp razor blade. This allowed the stele to be peeled from the cortex using the shoot as a handle. The stele was then cut from the shoot and the tissues were weighed into separate scintillation vials for drying, ashing, and counting.

Table I. *Sodium and Potassium Concentrations within the Tissues of the Mesocotyl in Corn Seedlings*

The seedlings were grown in the dark for 3 d on $CaCl_2$ followed by 7 d in the light on sodium-free modified sea water (equivalent to 0.1× sea water in all other ions) or on modified sea water containing sodium. Values followed by the same letter on the same line are not significantly different at the 5% level. The stele represented 23 ± 3% of the mesocotyl fresh weight.

	[Na^+] (mM)			
	0	45	60	75
	μmol/g fresh wt			
Stele				
Na^+	7.93a	174.2b	188.4b	535.4c
K^+	62.6a	25.0b	24.3b	76.8c
Cortex				
Na^+	3.38a	80.5b	95.4b	243.9c
K^+	117.2a	91.9b	100.8b	117.0a
Stele/mesocotyl				
Na^+	0.43	0.35	0.34	0.44
K^+	0.15	0.07	0.60	0.18

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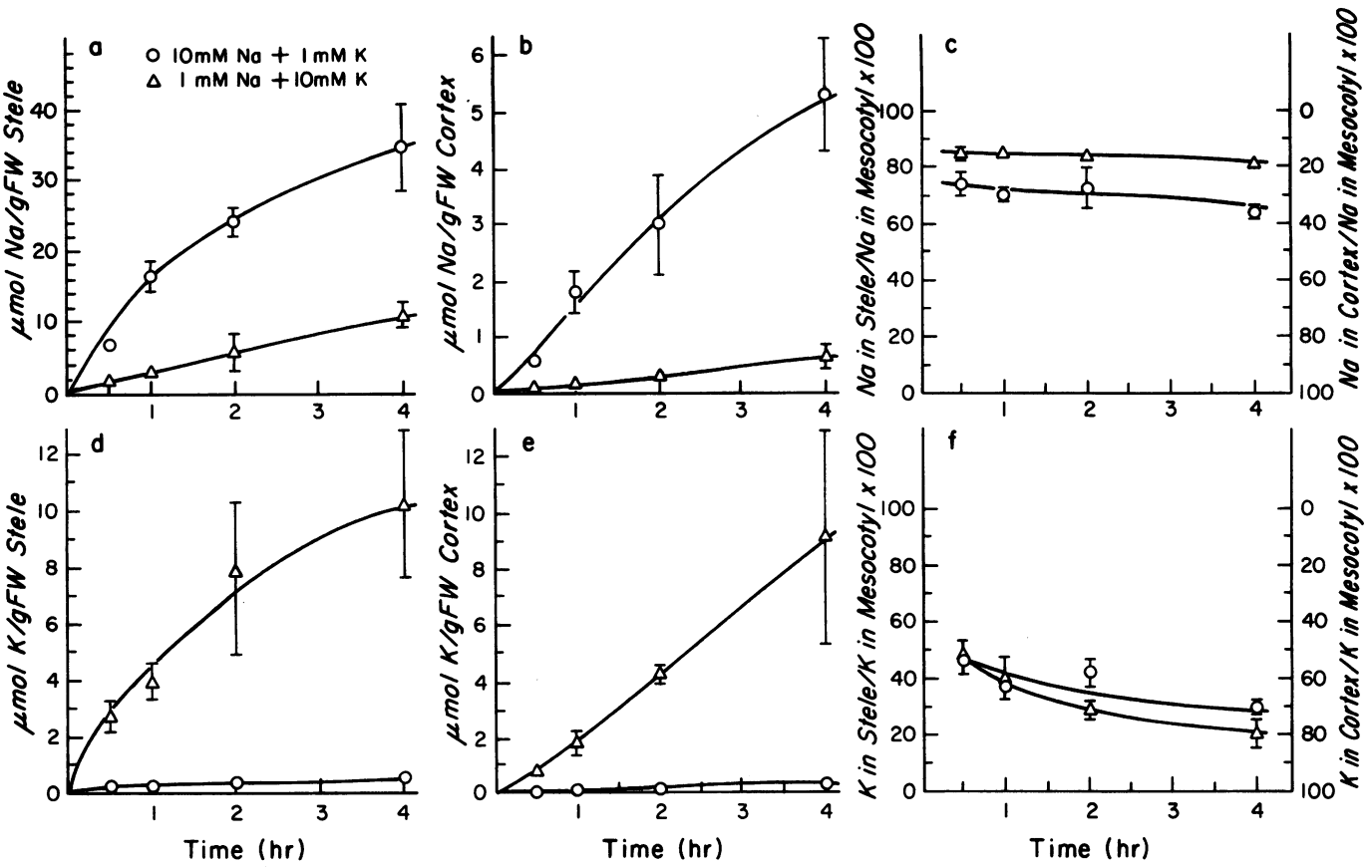

FIG. 1. Sodium and potassium accumulation within the mesocotyl of 8-d-old corn seedlings supplied with labels via the transpiration stream under conditions of constant $Na^+ + K^+$. a and d, Accumulation within the stele; b and e, accumulation within the cortex; c and f, distributions between stele and cortex. Each point represents mean ± SD of four determinations of four plants each, and the experiment is that illustrated in Figure 1 of reference 7. Lines were placed visually and do not represent any regression analysis.

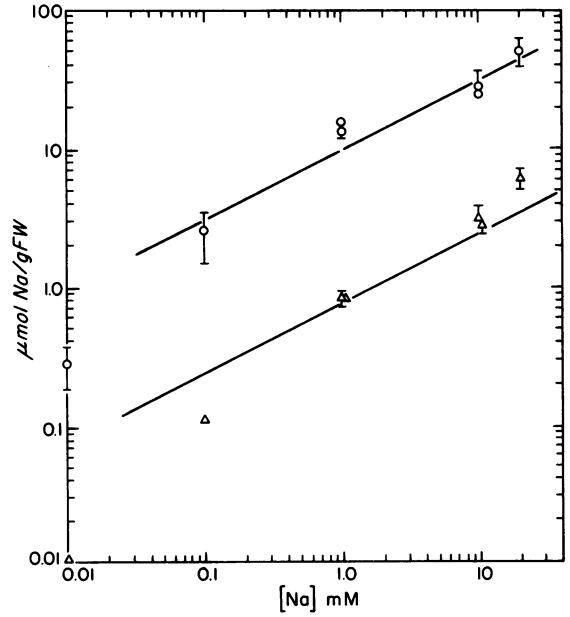

FIG. 2. Concentration dependence of sodium accumulation within the stele and transport to the cortex at 1 mM K^+. Solid lines have a slope of 0.5. Each point is mean ± SD of four determinations, each including four plants. Uptake time was 2 h.

In experiments double-labeled with ^{22}Na and ^{24}Na, analysis was similar to those involving ^{42}K, the ^{24}Na being allowed to decay prior to the second counting. ^{42}K and ^{24}Na were prepared by irradiation of K_2CO_3 and Na_2CO_3, respectively, in the central thimble of the TRIGA reactor, University of Illinois Nuclear Engineering Program. Following preparation, they were dissolved in distilled H_2O and neutralized with H_2SO_4.

RESULTS

In the previous paper, the mesocotyl was considered as no more than a 'black box' with no regard for its obvious morphological heterogeneity. This heterogeneity is manifest in ion distributions as well (Table I). In hydroponically grown plants, there was a disproportionately high concentration of sodium and low concentration of potassium within the stele. Sodium accumulation within the stele appeared to be at the expense of potassium content though the total content of the two ions was not constant. Sodium toxicity symptoms (tip burn and seedlings mortality) became noticeable in these plants above 45 mM NaCl in the growth medium (7) and the elevated ion contents at 75 mM probably reflect this toxicity. Within the cortex, potassium contents were less affected at intermediate salinities. These results corroborate the findings of Shone *et al.* (9) who showed by autoradiography that sodium accumulated within the stele of corn roots. The effect is likely to be more dramatic in a true root due to sodium efflux from the cortex (2).

We have reported (7) that sodium and potassium accumulation by the mesocotyl are linear over a 4-h period. During this time, the stele accumulated sodium more rapidly at first, especially at higher (10 mM) concentrations (Fig. 1). At lower concentrations, uptake was linear with time. Transport of sodium

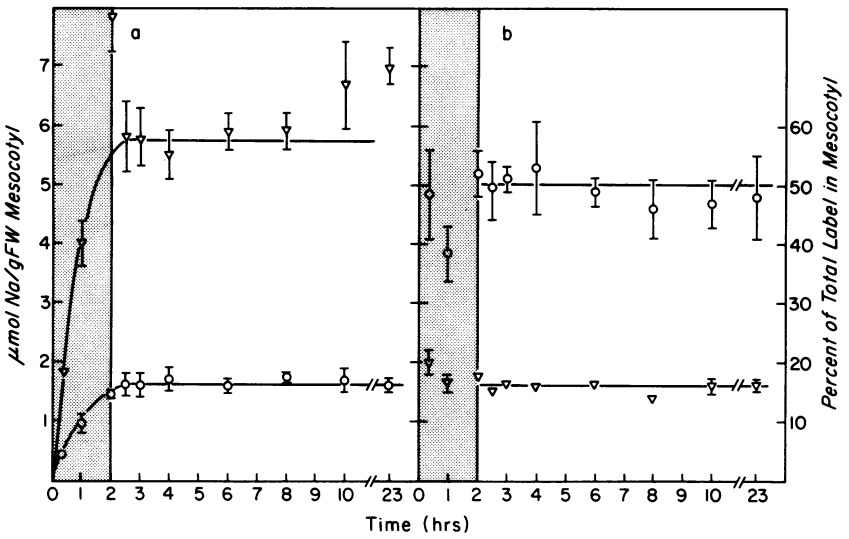

FIG. 3. Mesocotyl sodium accumulation (a) and retention (b) in plants supplied with ^{22}Na-labeled solutions at 1 or 10 mM in the presence of 10 mM K^+. Each point is mean ± SD of four determinations, four plants each. Label was supplied only during the first 2 h, and plants were harvested at times shown. (○), 1 mM $^{22}Na^+$ in first period, 10 mM Na^+ after 2 h; (▽), 10 mM $^{22}Na^+$ in first period, 1 mM Na^+ after 2 h.

Table II. *^{22}Na Distribution in Mesocotyl 2 Hours after the End of a 2-Hour Labeling Period at 10 mM Na plus 1 mM K*

Temperatures are of the solution: low temperature was achieved by use of an ice bath around the vials, higher temperature by augmenting light with an incandescent spot light through an IR reflector. Figures are means ± SD of four, four plant samples.

Efflux Treatment	Stele Na^+/Mesocotyl
	%
None	69 ± 2
10 Na^+/1 K^+ (25°C)	49 ± 1
10 K^+ (25°C)	63 ± 2
10 Na^+/1 K^+ (2°C)	59 ± 1
10 K^+ (2°C)	60 ± 2
10 Na^+/1 K^+ (35°C)	46 ± 4
10 K^+ (35°C)	63 ± 3
10 Na^+/1 K^+ (dark)	54 ± 2
10 K^+ (dark)	61 ± 2

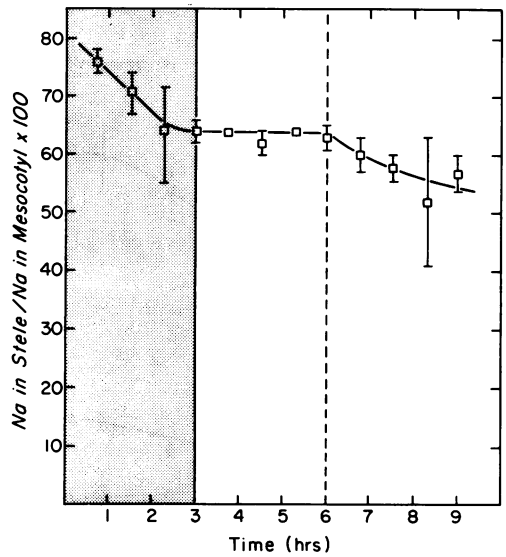

FIG. 4. Sodium distribution in a three part experiment involving ^{22}Na only. Sodium concentration was 10 mM (^{22}Na), 0 to 3 h; 0 mM, 3 to 6 h; 10 mM (unlabeled), 6 to 9 h.

to the cortex was nearly constant with time at both concentrations (Fig. 1b). The net effect was a gradual decrease in the portion of the total sodium retained within the stele, this decrease being more pronounced at the higher sodium level (Fig. 1c). Qualitatively, potassium accumulation was similar, but total uptake of potassium was much slower than sodium uptake. Redistribution to the cortex was faster for potassium (Fig. 1f) and, by the end of 4 h, similar levels were found in the cortex and stele (Fig. 1, d and e). At equal sodium and potassium concentrations in the medium, accumulation of labeled sodium within the stele was more than 3 times the accumulation of potassium.

Figure 2 shows the concentration dependence of sodium accumulation. Like accumulation within the mesocotyl as a whole, stelar uptake was proportional to the square root of the concentration. This implies that the putative active transport step (7) occurs within the stele.

Once accumulated by the mesocotyl, downward transport of label to the medium was not measurable after 8 h (data not shown). Redistribution from the mesocotyl to the shoot was also absent: Figure 3b shows that the proportion of the total accumulated sodium found in the mesocotyl was constant for 21 h after labeling for 2 h, despite variability in the initial labeling (Fig. 3a; Ref. 7).

It was possible, therefore, to consider the problem of compartmentation of sodium within the stele without the complication of loss of total label. In the first experiments, plants were labeled for 2 h with 1 mM K^+ plus 10 mM $^{22}Na^+$ solution, followed by a 2-h period of unlabeled redistribution. During this period, the composition of the uptake solution was either unchanged except for label, or was sodium-free, 10 mM K^+ solution. The results are shown in Table II along with the effects of temperature and darkness. Removal of sodium markedly reduced redistribution in all cases. Low temperature had similar effects. Elevated temperature (35°C) increased the rate of transendodermal transport in the presence of sodium. Darkness reduced transport to the

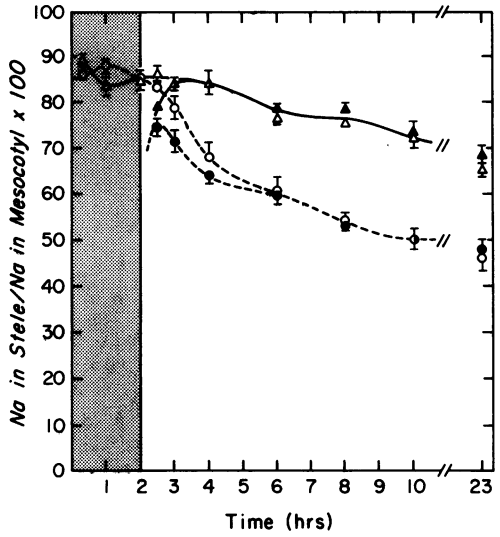

FIG. 5. Sodium distribution in mesocotyls of 8-d-old corn seedlings labeled with ^{22}Na (shaded section) and/or ^{24}Na at 10 mM K^+. Each point is mean ± SD of four determinations, four plants each. (Δ, ▲), 1 mM Na^+, both periods; (○, ●), 1 mM Na^+ in first period, 10 mM Na^+ in second period. (●, ▲), ^{24}Na values.

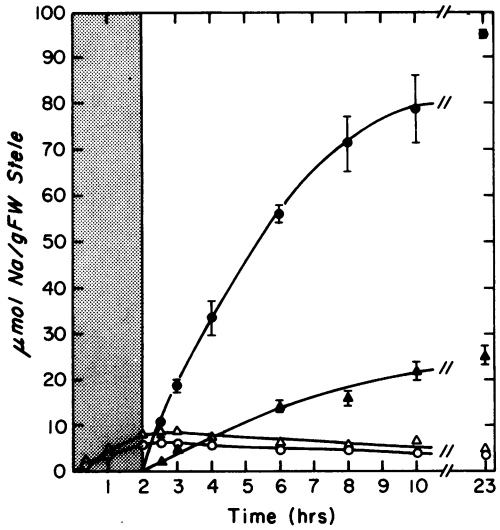

FIG. 6. Total sodium uptake by steles calculated from label contents and specific activities of uptake solutions. Data are from same experiment shown in Figure 4. (Δ, ▲), 1 mM Na^+, both periods; (○, ●), 1 mM Na^+ followed by 10 mM Na^+.

cortex, probably due to the reduction of ion delivery rates with reduced transpiration (7). Redistribution of label resumed when sodium (unlabeled) was resupplied. The labeled stelar sodium pool remained transportable for at least 3 h (Fig. 4).

The nature of compartmentation within the stele was explored further in double-labeling experiments. Excised plants were supplied with ^{22}Na-labeled solution for 2 h during which three harvests established the pattern of redistribution. Thereafter, the solution was replaced with one labeled with ^{24}Na. Figure 5 shows the results of two such experiments. In the first, the sodium concentration was 1 mM in both periods. In the second, 1 mM Na^+ was replaced by 10 mM Na^+ in the second period (potassium was constant at 10 mM). One h after the change of labels, the patterns of redistribution of label to the cortex were indistinguishable at 1 mM sodium and remained so for 21 h though the total ^{24}Na label in the stele increased to more than 4 times the ^{22}Na level (Fig. 6). When total sodium concentration was raised to 10

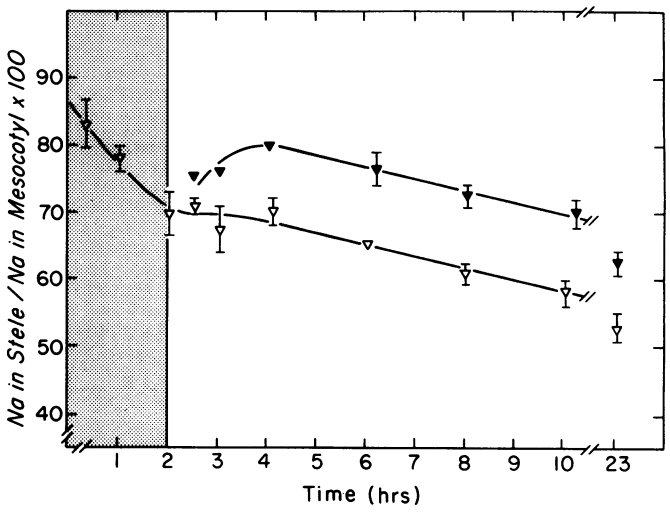

FIG. 7. Sodium distribution in mesocotyls of 8-d-old seedlings labeled first with 10 mM $^{22}Na^+$ solution followed by 1 mM $^{24}Na^+$. The reverse of the experiment represented by triangles in Figure 4. (▲), ^{24}Na; (Δ), ^{22}Na.

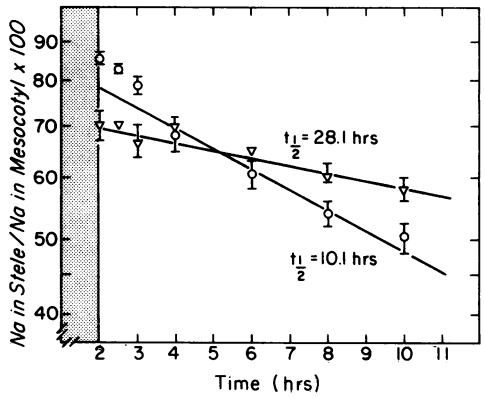

FIG. 8. Semi log plot of ^{22}Na redistribution kinetics in second period (2–10 h) of the experiment shown in Figure 4. Redistribution half times were calculated from slopes of such exponential transformations.

Table III. *Half Times of Redistribution of Sodium Label under Different Conditions of First and Second Period Sodium Concentrations at 10 mM Potassium*

Times were determined from linear regressions following exponential transformation (such as shown in Fig. 8) of data in the 2- to 10-h redistribution period. Last line refers to experiment shown in Figure 4.

[Na^+]			$t_{1/2}$
0–2 h		2–10 h	
			h
1		1	30.8
1		10	10.1
10		1	28.1
10		10	6.8
0–3 h	3–6 h	6–9 h	
10	0	10	∞ 15.1

mM in the second period, ^{22}Na label redistributed more rapidly to the cortex, and after 3 h, the relative distributions of the labels were indistinguishable (Fig. 5). Figure 6 again indicates this must involve rapid mixing of pool as, by 30 min, accumulation of

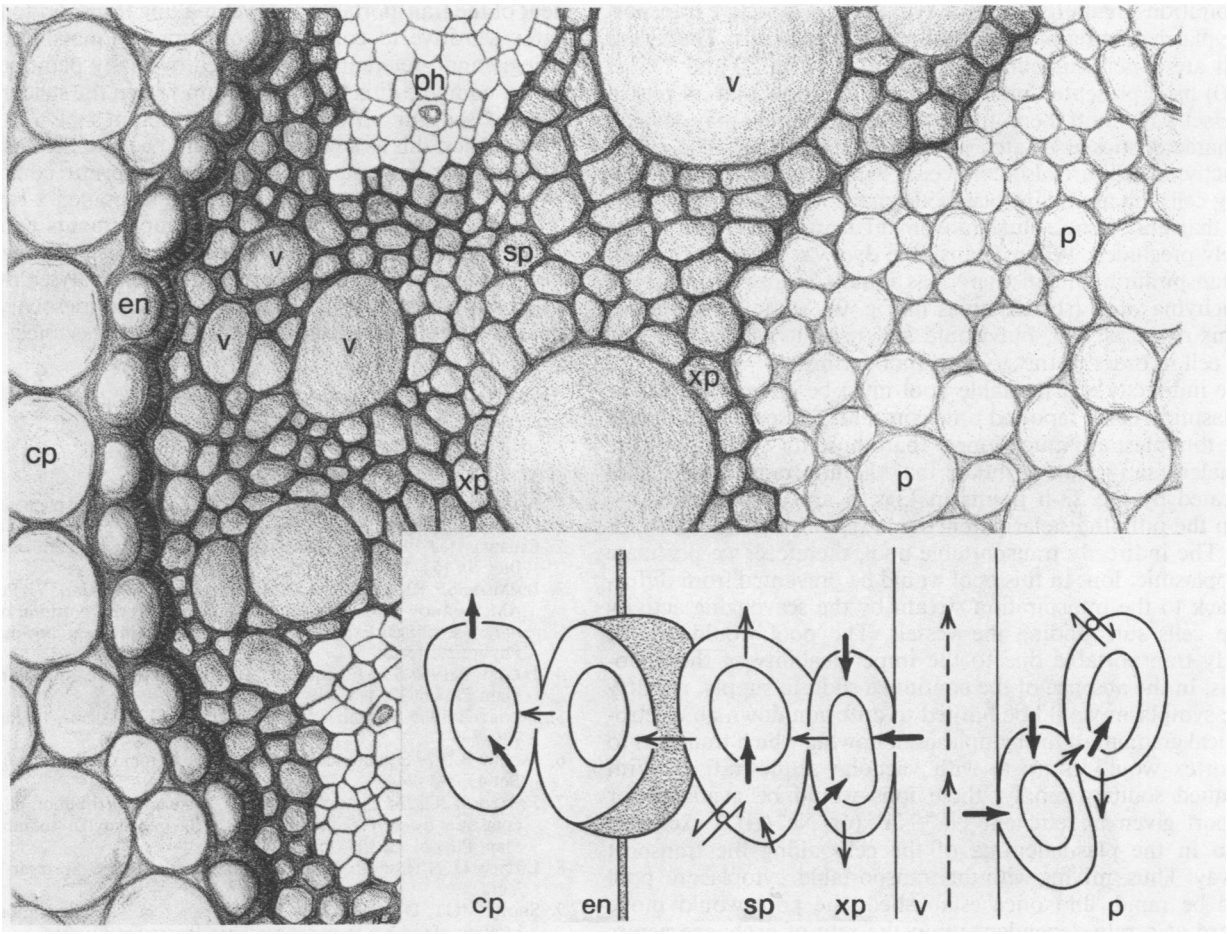

FIG. 9. Cross-section of 8-d-old corn mesocotyl and working model to explain sodium transport characteristics discussed in this paper. Heavy arrows represented postulated active transport events. Single arrows represent passive movement across membranes (→)or through plasmodesmata (→). Na^+/Na^+ (or Na^+/H^+?) exchange is indicated by circle with arrows. (xp), Xylem parenchyma; (sp), nonspecific stelar (parenchyma) cell; (en), endodermis; (cp), cortical parenchyma; (p), pith, (ph), phloem.

Note that not all stelar cells lie on a shortest distance path between xylem vessels and endodermis; both distance and number of cells which must be traversed is variable. Thus, pith cells in particular are a probable location for vacuolar immobilization of sodium. Note also that 'passage cells', notable in the endodermis of roots, are absent. The more heavily shaded, thick-walled portions of the stele are those stained when Evans blue solution is transpired. The stain is localized in the walls rather than in the cells. The path of uptake from the transpiration stream is unclear. We feel it is premature, however, to decide this flux represents a leak from the xylem.

^{24}Na-labeled sodium equaled that of ^{22}Na-labeled sodium.

Similar experiments were performed with 10 mM Na^+ present in the first period as shown in Figure 7. Upon replacement with 1 mM ^{24}Na-labeled solution, redistribution of ^{22}Na quickly slowed to a rate paralleling the redistribution of ^{24}Na. The latter showed kinetics indistinguishable from those shown in Figure 5 (△, ▲).

The redistributions of label shown in Figures 5 and 7 during the second period had exponential decay kinetics (Fig. 8). Table III summarizes the analysis of redistribution rates under the different conditions.

DISCUSSION

In this report, we have extended the results of the previous paper from consideration of uptake by the mesocotyl to consideration of uptake by the stele and redistribution through the endodermis of the cortex. The analyses make use of the fact that within the time frame of these experiments there was no transfer of label from the mesocotyl to the shoot or to the medium.

The dependence on sodium concentration of uptake by the stele and transport to the cortex (Fig. 2) strengthen our previous conclusion that diffusion of label in the apoplast was not responsible for the apparently excessive uptake of sodium at low transpiration rates (7). In such a case, the cortical sodium levels should have been proportionately much higher at low delivery rates, whereas approximately 80% of the label was in the stele in all experiments at concentrations of 10 mM and lower (Fig. 2).

Two other findings of this study are particularly noteworthy. First, transfer of sodium from a labeled pool in the stele to the cortex required continued uptake of sodium from the xylem (Fig. 4; Table II). When that uptake was interrupted, transfer stopped but the label remained in a transportable pool (Fig. 4). Second, the rate of transfer to the cortex was determined by the stelar uptake rate and not by the level of sodium already present within the stele (Figs. 5–8; Table III). Though full explanation of these findings is not possible at this point, we can suggest a working model for the transport of sodium from the xylem vessels to the mesocotyl (or root) cortex. This model, summarized in Figure 9, is a basis for future studies.

The endodermal walls of the mesocotyl are heavily lignified (Fig. 9) and the endodermis lacks passage cells to the cortex (J. M. Cheeseman, unpublished observations). Transfer of sodium to the cortex must therefore be symplastic, and the readily transportable pool is probably cytoplasmic.

It is reasonable to suggest that the initial uptake from the

transpiration stream to the stelar symplasm is by active transport at the plasmalemma of the xylem parenchyma cells. The xylem vessels are closely surrounded by such cells (Fig. 9) and Yeo *et al.* (10) have presented micrographs which show that all pits in the vessel walls abutt them. In some cases, these cells may develop the characteristics associated with transfer cells (10). The xylem parenchyma cells are also connected with each other and neighboring cells via abundant plasmodesmata. We must note, however, that apoplastic solute movement from the vessels is not entirely precluded. When Evans blue dye was supplied through the transpiration stream there was rapid staining of the stelar parenchyma walls (shaded areas in Fig. 9). Sodium may move via this route as well, but would still need to be absorbed by some cell in order to traverse the endodermis.

The indirectly transportable pool must be either vacuolar or apoplasmic. As the reported time constants for sodium exchange at the tonoplast are much longer than those for exchange at the plasmalemma (8), we postulate that the nontransportable pool (indicated by the 23-h points in Figs. 5 and 7) is vacuolar—within the pith, the stelar parenchyma region ('sp' in Fig. 9), or both. The indirectly transportable pool, therefore, we postulate as apoplasmic. Ions in this pool would be prevented from diffusing back to the transpiration stream by the scavenging activity of the cells surrounding the vessels. The pool would not be directly transportable due to the impermeability of the endodermis. In the absence of the continued sodium supply, re-entry to the symplasm would be limited to diffusion down an electrochemical gradient to the cytoplasm, following which transport to the cortex would compete with vacuolar sequestration. With continued sodium supply, these ions would be available for transport given an efficient Na^+/Na^+ (or Na^+/H^+?) exchange system in the plasmalemma of the cells along the transport pathway. Thus, mixing with the transportable, cytoplasmic pool would be rapid, and once established, the pool would move outward at a rate dependent upon the rate of exchange across the plasmalemma, *i.e.* with a half-time proportional to the cytoplasmic concentration. Given the characteristic sodium efflux pump of plasmalemma of many plant cells, it is not unreasonable to include a similar pump in cells within the stele. This would tend to speed up mixing and keep the overall cytoplasmic sodium levels low. The combined result of these activities would be a diffusion gradient toward the endodermis encouraging movement of the transportable pool yet making transport to the cortex highly sensitive to continued ion supply. Potassium, which is strongly and preferentially absorbed by many plant cells would neither compete directly with sodium within the stele, nor would it 'chase' sodium outward, thus the situation shown in Table II.

We suggest this as a working model to be modified as the result of further experiments. In particular, two prime considerations for immediate attention are much more detailed x-ray analysis to determine which cell types and compartments contain high or low sodium levels (compared with other cells, not with potassium) and more extensive compartmental analyses over much longer periods of time using directly perfused mesocotyls and/or isolated steles. These experiments will be the subject of future papers.

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