Plant Physiol. (1986) 81, 742–747
0032-0889/86/81/0742/06/$01.00/0

Sodium Transport and Compartmentation in *Spergularia marina*[1]

PARTIAL CHARACTERIZATION OF A FUNCTIONAL SYMPLASM

Received for publication October 25, 1985 and in revised form March 6, 1986

DENNIS LAZOF* AND JOHN M. CHEESEMAN
Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

ABSTRACT

In this paper, a combination of tracer uptake, efflux, and pulse-chase techniques is applied to the problem of compartmentation of Na^+ ($^{24}Na^+$) in the roots of intact, midvegetative *Spergularia marina* (L.) Griseb. plants. An approach is presented for conducting useful compartmental analysis when it is known that the assumptions required for straightforward interpretations of influx and efflux studies are invalid. Linear rates of $^{24}Na^+$ accumulation in both roots and shoots were attained within at most a few minutes following the start of labeling. Shoot $^{24}Na^+$ contents equaled root contents within about 20 minutes. Analysis of root accumulation rates, and compartmental and pulse-chase efflux studies indicated that the unidirectional flux rates involved were at least an order of magnitude greater than linear rates of root and shoot accumulation. These rapid fluxes involved only a small portion of the total root Na^+ (about 1%). The results suggest the existence of a small symplastic compartment, distinct from the 'bulk cytoplasm,' rapidly exchanging with the medium, and responsible for delivery of Na^+ to the xylem. The physical identity of this compartment and its physiological significance are discussed with respect to precedents in the literature.

The ultimate significance of the study of root level ion transport and compartmentation in higher plants lies in the understanding of the dual role of roots as organs of ion absorption, with physiological needs of their own, and as organs responsible for nutrient resource partitioning within the whole plant. Analysis of the transport processes has been facilitated by the use of isotope techniques which allow increased resolution of ion movements with respect to both time and a large background of nontransported ions.

The theory and techniques of isotopic flux estimations, along with several limitations of those techniques, were carefully evaluated by Walker and Pitman (29). It was clear from their review, however, that the circumstances under which either influx or efflux studies can give unequivocal results are very limited. In influx studies, only if the period under consideration is short enough to preclude movement of the isotope across more than one transport barrier is isotope influx a valid estimator of unidirectional flux. Over longer uptake periods, direct interpretation of influx studies requires that the specific activity of the initial internal compartment can be determined, and in general, it cannot. In efflux analyses, the time periods involved are longer since the specific activity of the external solution can be kept very low. However, when efflux analysis is used as the sole analytical technique, a number of critical assumptions are made concerning the arrangement and interconnection of compartments. These assumptions may not always be valid. For example, MacRobbie (20) suggested that the basic assumption of a series arrangement of compartments may be invalid in *Nitella*. Pallaghy *et al.* (22) noted for corn that efflux may follow a pattern inconsistent with any exponentially decaying system. Davis and Higinbotham (10) dealt extensively with the complications arising if label could pass to the shoot as well as to the external medium during efflux. We have recently reported, based on computer simulations of efflux experiments, that even under the best of experimental conditions and with the compartments properly in series, it may be impossible to identify correctly either the number of compartments or their decay constants (4).

There has, to our knowledge, never been a consideration of the requirements for interpretable use of isotopes in transport studies using whole plants under conditions in which one could be assured that unidirectional influx was *not* being measured, and in which the arrangement of compartments was demonstrably *not* serial. In this paper we will present such a consideration, showing in particular that by combining influx and compartmental efflux techniques, each of which in itself is theoretically invalid under these circumstances, with pulse-chase studies, it is possible to characterize the compartmentation accounting for the observed ion fluxes and the partitioning between root and shoot. We will present results obtained using intact, vegetative *Spergularia marina* plants growing on 0.2 × sea water medium in solution culture. The particular virtues of this system have been discussed elsewhere in detail (5, 6). For the present purposes, we will restrict our consideration to sodium uptake, accumulation, and translocation because of its relatively straightforward transport characteristics.

MATERIALS AND METHODS

Spergularia marina (L.) Griseb. seeds were collected from plants growing in our growth chambers and were germinated, transferred to solution culture, and grown as described in detail previously (5, 6). Briefly, plants were selected for uniformity and were transferred to solution culture approximately 2 weeks after germination. The initial growth medium was Na^+-free, 0.1 × sea water containing (in mol m^{-3}): KNO_3, 0.75; $KHCO_3$, 0.265; $CaCl_2$, 1.05; $MgSO_4$, 3.2; $MgCl_2$, 2.3; $(NH_4)H_2PO_4$, 0.5; micronutrients and Fe. Salinization was accomplished in two steps, to 0.1 × sea water by addition of 45 mol m^{-3} NaCl on d 9 after transfer to solution culture, and to 0.2 × sea water by doubling all concentrations on d 10. Plants were grown until 17 d after transplanting in a growth chamber with a 15 h photoperiod. Temperatures were 25°C during the day and 16°C at night. Light intensity was 350 to 450 μmol m^{-2} s^{-1} provided by F96T12/D/SHO fluorescent tubes approximately 1.5 m above plant height. Chamber RH was uncontrolled and varied considerably over the period of these studies. All solutions were constantly aerated in all phases of the study.

[1] Supported by grant PCM 83-04417 from the National Science Foundation.

Unless otherwise noted, $^{24}Na^+$ was used to label the uptake media. The isotope was prepared locally by irradiation of Na_2CO_3 in the central thimble of the TRIGA receptor, University of Illinois Nuclear Engineering Program. Samples were neutralized with H_2SO_4 following irradiation. $^{22}Na^+$, when substituted, was obtained from New England Nuclear. The specific activity of the labeled solutions varied with the experiment, and depended in part upon the sample counts which were expected in each case. Precise and uniform labeling of the solutions was impractical because of the diversity of the experimental protocols and the short half-life of $^{24}Na^+$.

For uptake and pulse-chase experiments, plants were harvested and prepared for analysis as follows: plants were removed from the uptake solution and rinsed briefly in ice cold (<2°C) 20 mol m^{-3} $CaCl_2$, followed by two additional rinses in identical solution. For the sake of uniformity and to rule out any shoot contamination, a similar postharvest treatment was used in pulse-chase experiments when plants were removed from the unlabeled solution. The total rinse time was 3.5 min. Plants were placed directly into counting vials from the third rinse and the roots excised immediately. Root/shoot interfaces were then removed and the shoots placed into counting vials as well. All counting was done with an LKB 1282 Compugamma counter with internal decay correction and conversion of counts to μmol Na^+ based upon the specific activity of the uptake solution. Immediately following counting, samples were removed and dried overnight at 85°C and dry weights were measured on a Cahn microbalance to three significant digits. Conversion to fresh weight for comparison to other experiments was based on the average moisture content of 91.3 ± 0.06%. This value has been consistent for several hundred determinations over a period of 2 years. Data were analyzed using the BMDP Statistical Software Package (11).

The sufficiency of the 3.5 min $CaCl_2$ exchange period for removal of wall Na^+ while not allowing loss of internal label was critical to interpretation of the experimental results, especially in the pulse-chase experiments. That sufficiency was substantiated with a time-course experiment over the $CaCl_2$ rinsing period, following a 10 min pulse. There was no significant loss of label between 1 and 4 min of rinsing ($p > 0.05$). This corroborated earlier estimates based on methylene blue exchange (0.5 min) and efflux analyses at 25°C (Table II), which indicated wall exchange times of no more than 0.6 min. Therefore, we concluded that the 3.5 min period was sufficient to remove wall label, that the low temperature treatment effectively prevented transmembrane movement of label, and that no adverse effects of low temperature were notable in this period.

There were two separate series of compartmental efflux experiments. In the first, the plants were labeled with $^{22}Na^+$ to isotopic equilibrium by addition of the isotope to the growth medium from the beginning of salinization. The efflux analyses were performed with intact plants using standard techniques (10). The exchange solution was 0.2 × sea water at 25°C and the experiment was performed in the growth chamber.

The second series of efflux analyses involved a shorter labeling period of 2 h at 25°C in the chamber used for plant growth, followed by 0.5 h in a second chamber with identical lighting but maintained at 10°C. This period was used to cool the plants preceding efflux. The plants were transferred briefly to unlabeled medium (about 4 s) then placed in exchange vessels (10 ml syringes without plungers) with 10°C, unlabeled 0.2 × sea water medium, and aeration lines. For each solution change, the solution was collected by opening a stopcock valve and draining the solution into a 15 ml counting tube. The tube was also fitted with a Y-connector and a vacuum line to speed the draining process. The syringe was refilled from a plastic squirt bottle, being careful not to damage the roots. The total time required for solution draining and replacement was about 12 s. Samples were collected at 30 s intervals to 4.0 min, at 1 min intervals to 10 min, 2 min intervals to 20 min, at 25, 30, 35, 40, 50, 60, 75, 90, 105, and 120 min, and finally at 60 min intervals until 540 min (*cf.* 4; sampling protocol 3).

Nonlinear regression analyses of the efflux data were performed using the DISCRETE program of Provencher (25) as discussed previously (4).

RESULTS

The major and accepted use for short-term isotope influx studies is the quantification of unidirectional ion influx rates. Figure 1 illustrates the short term (45 s–20 min) time course of root accumulation and transport to the shoot of $^{24}Na^+$ in *S. marina*. Both the growth solution and the uptake solution were 0.2 × sea water and the plants, at 17 d after transfer to solution culture, were growing under "steady state" conditions. It has been shown elsewhere that transport of Na^+ under these conditions is not determined by transpiration (7). Consequently, this figure implies that an internal, "symplastic" compartment, which supplies the xylem, reaches a nonnegligible specific activity very quickly. As ions in this compartment may also be exchangeable with ions in the external medium, the results shown in Figure 1 imply that the use of isotopes to determine unidirectional influx rates in *S. marina* is, at best, not straightforward.

Figure 2 illustrates one indirect estimation of unidirectional influx. For each point in Figure 1 the average rate of total uptake, in μmol Na^+ (g fresh $weight_{root} \cdot h)^{-1}$, was calculated by dividing the total uptake by the elapsed time. These values were then analyzed as an exponentially decaying function of time and the y-intercept was taken as an estimate of the initial influx rate, ϕ. In Figure 2, ϕ was 398 μmol (g fresh $weight_{root} \cdot h)^{-1}$.

Extension of the uptake period to longer times may provide additional information useful in developing an integrated transport model for whole plants; Figure 3 illustrates such an experiment. The labels α to ϵ indicate characteristic features, which any valid model of root compartmentation must be able to explain. The y-intercept, α, is the extrapolation of the linear root accumulation rate to the beginning of the uptake period. The positive value shown here and in Figure 1 was a consistent feature of these influx experiments. The parameter α may also be used as a first estimate of the contents of the symplastic compartment. As the mean root Na^+ content of *S. marina* grown

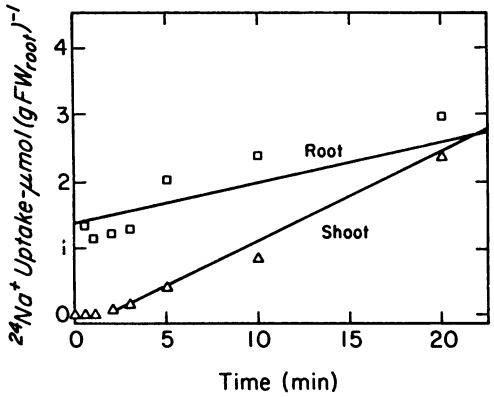

FIG. 1. Time course of $^{24}Na^+$ accumulation in the root (□) and translocation to the shoot (△) by midvegetative *S. marina* plants growing on 0.2 × sea water, 17 d after transfer to solution culture. Linear accumulation rates were, for roots, $y = 0.061 \times \text{time} + 1.38$, $r^2 = 0.935$; for translocation to the shoot, $y = 0.135 \times \text{time} - 0.231$, $r^2 = 0.990$ (time in min, $^{24}Na^+$ in μmol [g fresh $weight_{root}]^{-1}$). Polynomial analysis indicated the sufficiency of linear regression. Each point is the mean of 18 determinations; SE did not exceed the symbol sign.

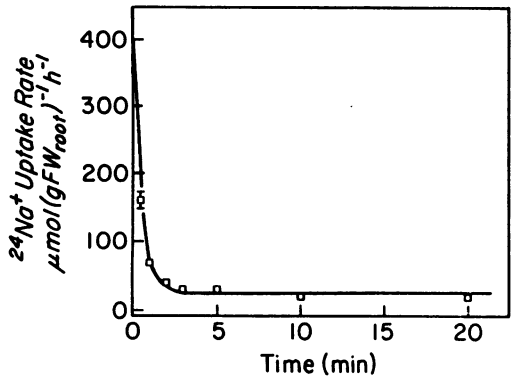

FIG. 2. The time dependence of $^{24}Na^+$ influx rates in *S. marina*. Rates were calculated as total uptake μmol Na^+ (g fresh weight$_{root}$)$^{-1}$ divided by the uptake time (h). The exponential decay curve was fit by derivative-free nonlinear regression (BMDP program AR; Ref. 11). The fit equation is $y = 375 \times \exp -(2.01 \times \text{time}) + 22.6$. The y-intercept (g fresh weight$_{root}$ h)$^{-1}$ is 398 ± 36 (asymptotic SD). Data were from the same experiment illustrated in Figure 1. Unrepresented SE bars are smaller than symbols.

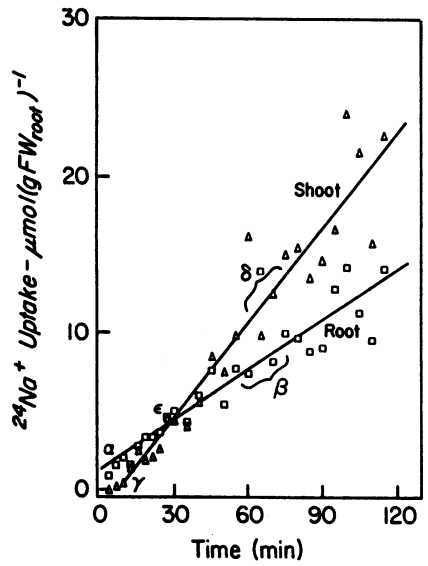

FIG. 3. Extended time course of $^{24}Na^+$ accumulation in the root (□) and translocation to the shoot (△) by midvegetative *S. marina* plants grown on 0.2 × sea water. Polynomial analysis indicated the sufficiency of the linear regression. For root accumulation the regression was $y = 0.105 \times \text{time} + 1.37$, $r^2 = 0.917$; for translocation to the shoot, $y = 0.200 \times \text{time} - 1.30$, $r^2 = 0.960$ (time in min, $^{24}Na^+$ in μmol [g fresh weight$_{root}$]$^{-1}$). Symbols represent individual, single plant samples.

on 0.2 × sea water was about 100 μmol (g fresh weight)$^{-1}$, α clearly represents a very small portion of the total root Na^+ content. Further characteristics of this Na^+ pool will be considered below. The nonzero intercept was also consistent with the high initial influx rate suggested by Figure 2. Should the linear rate, β, represent unidirectional influx, α should be zero.

The linear root accumulation rate, β, is a complex parameter representing (a) exchange of Na^+ between nongrowing regions and the medium, and (b) uptake related to growth. Previous studies have shown that net Na^+ accumulation by nongrowing regions is negligible in *S. marina* and that growth related root accumulation may be as much as 1 μmol Na^+ (g fresh weight$_{root}$ h)$^{-1}$ in plants at this age and under these culture conditions (9).

The x-axis intercept of the shoot accumulation rate, γ, represents the 'lag' period during which upward transport approached the eventual linear rate, δ. In *S. marina* and for Na^+, this period was very short and often not statistically different from zero. The linear rate, δ, has been compared elsewhere to the net rate of Na^+ transport to the shoot (9). The rates were nearly identical, indicating that the 'nonnegligible' specific activity of the transporting component discussed above in fact equaled that of the external solution within a few minutes at most, as indicated by γ (Fig. 3).

Finally, ϵ represents the 'cross over time' or the period required for 50% of the total label to be in the shoot. If the postulated symplastic Na^+ pool represents the intersection of transport pathways for upward transport, movement to the nonsymplastic root compartments, and efflux to the medium, the value of ϵ will depend upon the various exchange constants as well as on the size of the symplastic pool.

The values of α, β, γ, δ, and ϵ for four separate influx time course experiments are summarized in Table I.

Apart from suggesting the existence of a small Na^+ pool with a rapid turnover rate, influx experiments can give no further information with respect to root compartmentation (29). Such information may be obtainable by compartmental efflux analysis. Application of that technique, however, is also straightforward only in very restricted circumstances, not including those which might involve either more than three compartments or compartments which are not functionally in series (4).

Table IIa summarizes the results of initial analyses in which plants were labeled to isotopic equilibrium. Three components were resolved with decay half-times, $t_{1/2}$, which were somewhat less than the values typically reported for higher plant roots. This difference might be expected given that the mean diameter of the roots (200 μm) was much less than that of more traditionally studied species (5). These results were questioned for several reasons: (a) even at 4.2 min, the $t_{1/2}$ of the first internal component was long by comparison to the value which would be expected based upon Figures 1 to 3; (b) the first internal component accounted for 38% of the total Na^+ in the roots rather than about 1% as would be expected based on the values of α and of total root Na^+ content. If it is assumed that the 'cytoplasmic' Na^+ concentration was not greater than the 'vacuolar' concentration (using the usual compartment identification in three component analyses), this also implies that the cytoplasmic volume was at least 38% of the total root volume. In addition to being an unusually high value for mature root tissue, this was also inconsistent with transmission electron microscopical observations (D Lazof, unpublished data); (c) the apparent Na^+ content of the roots was more than 60% higher than the mean values of 80 to 100 μmol (g fresh weight)$^{-1}$ reported previously (5, 9); and (d) following 7 d exposure to $^{22}Na^+$, the plants were abnormal in appearance. Shoots were well below average size and the root:shoot ratios were well above average (data not shown). The overall unhealthy appearance of the plants, by comparison to nonlabeled plants, was also noted independently by several visitors to the laboratory who were previously unfamiliar with the species.

Table I. *Summary of Estimates of Characteristic Uptake Parameters, α to ε, from Four, Two to Eight Hour Influx Time Courses using Intact S. marina Plants, 17 Days after Transfer to Solution Culture*

Values are based on linear regression of root and shoot accumulation of $^{22}Na^+$ or $^{24}Na^+$ from labeled 0.2 × sea water medium. Parameters are defined as in Figure 3.

Parameter	Units	Mean ± SE (n = 4)
α	μmol	1.9 ± 0.3
β	μmol (g fresh wt$_{root}$ h)$^{-1}$	4.4 ± 0.9
γ	min	4.4 ± 4.5
δ	μmol (g fresh wt$_{root}$ h)$^{-1}$	12.9 ± 2.9
ϵ	min	21.6 ± 7.4

Table II. *Estimates of Root Compartmental Na^+ Contents and Transport Half-Times for Intact S. marina Plants Grown on 0.2 × Seawater Medium*

(a) Plants were labeled to isotopic equilibrium (see "Materials and Methods"); during efflux, plants and solutions were at 25°C. (b) Plants were labeled for a total of 2.5 h and efflux was performed at 10°C. Data summarized for 5 replicates resolvable to 3 compartments in (a) and for 2 replicates resolvable to 4 compartments in (b). Q_0 values (Na content at start of efflux) in (b) are shown for the two faster decaying compartments, which would have reached a relative specific activity of > 0.95 in the uptake period. Data are means ± SE.

Q_0^1	$t_{1/2}$	Q_0^2	$t_{1/2}$	Q_0^3	$t_{1/2}$	Q_0^4	$t_{1/2}$
μmol Na^+/g fresh wt	*min*	*μmol Na^+/g fresh wt*	*min*	*μmol Na^+/g fresh wt*	*min*	*μmol Na^+/g fresh wt*	*min*
a. 97.0 ± 8.8	0.57 ± 0.10	60.8 ± 15.9	4.2 ± 0.8	100 ± 13	701 ± 158		
b. 36.5 ± 0.2	0.19 ± 0.01	0.88 ± 0.17	3.3 ± 1.2		38 ± 9		18,300 ± 12,600

Therefore, the efflux analyses were repeated with two major differences in technique. First, the plants were labeled for a total time of only 2.5 h. Second, during efflux, the solution and the chamber were at 10°C. The reduced temperature was used to slow the exchange rates, resulting in greater separation of the decay constants, without completely stopping exchange.

The results are summarized in Table IIb. In four replicate trials, nonlinear regression analysis indicated the presence of at least four components twice. The other two analyses indicated at least three components. Given the statistical limitations of efflux analyses (4) and the demonstration of rapid radial symplastic transport (Figs. 1 and 3), we concluded that a four component system should be seriously considered.

A more detailed characterization of the small, rapidly exchanging symplastic compartment is clearly critical to acceptance of such an hypothesis. Therefore, a thrid series of experiments was designed to establish more firmly its size and to characterize its translocating function. Figure 4 shows the results of a pulse-chase study in which plants were labeled with $^{24}Na^+$ in 0.2 × sea water for 10 or 30 min, then transferred to unlabeled medium for varying periods. The time course of efflux from the roots was determined by harvesting and analysis of the plants, rather than by collection and counting of the solution. In this way, redistribution within the plants could also be considered. Nonlinear regressions (——, Fig. 4) gave initial flux estimates, with the wall component removed, of 230 and 130 μmol Na^+ (g fresh $weight_{root})^{-1}$ h^{-1}, and total apparent rapid losses of 1.21 and 0.93 μmol Na^+ (g fresh $weight_{root})^{-1}$ for the 10 and 30 min pulses, respectively.

There were no apparent increases in label in the shoot in these experiments, but the noise levels were high enough to obscure small changes. A second pulse-chase experiment was, therefore, designed for increased resolution of such changes and for a statistically more accurate estimation of total rapid loss from the roots. Shorter pulses (2 and 10 min) were used to minimize the background of label in the roots or shoots against which any movement of label during the chase would have to be measured. As the rapid initial loss was complete within 2 to 3 min (Fig. 4), labeling was compared at the end of the uptake period and following a 4 min chase (Table III).

Consistent with the results shown in Figure 2, the average total uptake rate decreased with time. Between 2 and 10 min, the average total uptake rate was 11.5 μmol Na^+ (g fresh $weight_{root}$ $h)^{-1}$ which is within the range of the rates summarized in Table I. During the chase period, label was lost from the roots both to the medium and to the shoots. Table III indicates that the symplastic compartment contained approximately 1.1 μmol Na^+ (g fresh $weight_{root})^{-1}$. Loss to the medium increased only 20% with the longer pulse length. Transport to the shoots during the chase period was much smaller than the loss to the medium and was the same for both pulse lengths. The increase in the loss of label from the roots to the medium with the longer pulse must reflect the label accumulated by root compartments other than the symplasm under these conditions. This additional accumulation (50% higher) does not result in a distinguishable increase in delivery to the xylem during efflux.

Of the label lost from the roots, the shoots received approximately 10%. If these two losses (to the shoot or medium) represent alternative movements and can be considered equivalent to a parallel electrical circuit, the ratio of the losses approximates

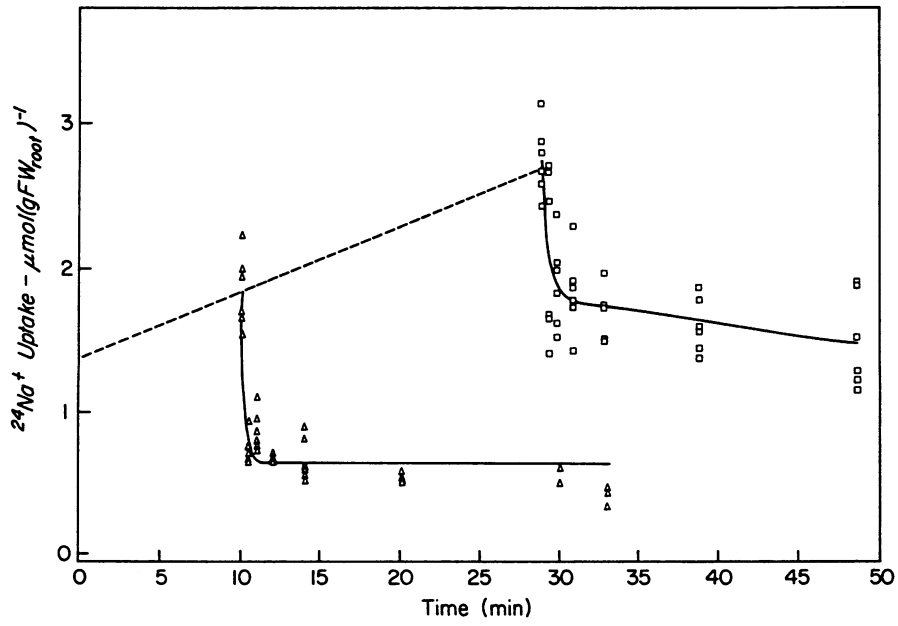

FIG. 4. Time courses of loss of label from roots of intact *S. marina* plants in pulse-chase studies with 10 min (△) and 30 min (□) pulses. Plants were grown and labeled in 0.2 × sea water. Solid lines were fit using derivative-free nonlinear regression (BMDP program AR, Ref. 10) using the maximum number of parameters which resulted in meaningful convergence. For the 10 min pulse, $y = 1.21 \exp -(0.992 \times \text{time}) + 637$; for the 30 min pulse, $y = 0.935 \exp -(2.23 \times \text{time}) + 1810 \exp -(0.012 \times \text{time})$; contents in μmol Na^+ (g fresh $weight_{root})^{-1}$. Dashed line indicating influx based on root contents without chase is included for visual comparison to Figure 3. Symbols represent individual, single plant samples.

Table III. *Results of Pulse-Chase $^{24}Na^+$ Uptake Experiment using Intact S. marina Plants Grown on 0.2 × Sea Water*

Contents of roots and shoots and their sums are presented following 2 and 10 min pulses, with or without a subsequent 4 min chase in unlabeled 0.2 × seawater. Δ represents the change in label content during the chase period. Data are means ± SE ($n = 24$).

	Contents (μmol Na^+ (g fresh wt_{root})$^{-1}$)		
	Root	Shoot	Total
2 Min pulse	1.34 ± 0.03	0.11 ± 0.01	1.46 ± 0.04
+ 4 Min chase	0.22 ± 0.01	0.23 ± 0.01	0.45 ± 0.02
Δ	−1.13 ± 0.04	+0.12 ± 0.04	−1.01 ± 0.04
10 Min pulse	2.01 ± 0.04	0.98 ± 0.03	2.99 ± 0.05
+ 4 Min chase	0.67 ± 0.02	1.10 ± 0.03	1.77 ± 0.04
Δ	−1.34 ± 0.05	+0.13 ± 0.04	−1.22 ± 0.06

the ratio of the exchange constants associated with the transport events, and in this case, that ratio would be about 0.1 ('xylem' to 'medium'). This estimate is probably somewhat higher than the actual value as movement of label already in the root xylem vessels at the end of the pulse is included. This explanation is also consistent with reports in other systems and for other ions that loss to the xylem was pronounced only early in the efflux period and involved a relatively small fraction of the root pool (e.g. 2, 9).

DISCUSSION

In this paper, we have considered the transport of Na^+ in midvegetative *Spergularia marina* growing at steady state on 0.2 × sea water. Our major concern has been the interpretation of the results of isotope studies under conditions in which we could be assured that the required assumptions for straightforward interpretation were invalid.

The results of these studies included several points which have not been widely reported in other systems. First, the estimated unidirectional flux rates were very high. The estimate shown in Figure 2, 398 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ h^{-1}, was more than 20 times the rate estimated from the linear rates of root and shoot accumulation. As there was no net flux to nongrowing regions (9), and as net uptake associated with growth was negligible in short times (9), the unidirectional efflux rate to the growth medium would have to be high as well, differing from influx only by the amount of transport to the shoot. From pulse-chase experiments (Fig. 4), unidirectional efflux was estimated as 130 to 230 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ h^{-1}. From Table IIa, the initial calculated rate of efflux from roots labeled to isotopic equilibrium was about 600 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ h^{-1}, neglecting efflux from the wall. At this point, the actual numbers are less important than the concept that such high fluxes might actually be occurring while the roots maintain a steady state condition involving a substantial electrochemical disequilibrium for Na^+ (6).

Second, the rapid fluxes involved only a very small portion of the total Na^+ in the roots. The contents of the small Na^+ pool were estimated as 1.9 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ by influx (α in Table I), 0.9 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ by efflux (Q_0^2, Table IIb), and 1.1 μmol Na^+ (g fresh $weight_{root}$)$^{-1}$ by pulse-chase analysis (Table III). Statistical analysis of compartmental efflux data at 10°C suggested that these pools represented an internal compartment other than those which have usually been identified as cytoplasm and vacuole. This possibility was also noted, but discounted, in a study of K^+, Na^+, and Cl^- transport in *Allium* by Macklon (18).

A third significant result was the rapid appearance of $^{24}Na^+$ in the shoots. The steady state rate of transport was established quickly (Fig. 1), and was constant with time up to 2 h (Fig. 3). We have previously shown similar data extending to 8 h (6, 8). Constant rates of uptake and transport to the shoots, and rapid crossover times were also found for Na^+ under low salinity conditions (7). Again, we have found similar results for K^+ uptake, but with slightly longer lags (γ) and crossover times (ϵ) (D Lazof, JM Cheeseman, unpublished data). On the other hand, crossover times and lags on the order of hours to days have been reported for other systems (14, 15, 24), the most obvious difference between those studies and this one probably being that the other studies used seedlings which were severely depleted of nutrients. In similar experiments using 14 d old *Zea mays* grown on complete nutrient solutions, we have found that up to 15% of the $^{42}K^+$ and 40% of the $^{22}Na^+$ in the plants was beyond the roots (i.e. in the mesocotyl or leaves) at the end of a 30 min uptake period (JM Cheeseman, unpublished data). With an external medium of 0.1 × sea water, the total uptake of the two ions was similar. Rush and Epstein (26) reported substantial movement of $^{22}Na^+$, $^{86}Rb^+$, and $^{36}Cl^-$ to the shoots of *Lycopersicon cheesmanii* and *Lycopersicon esculentum* on complete medium in a similar period. Our results, therefore, are not a peculiarity of this experimental system. Furthermore, as more than 80% of the total Na^+ in these plants was in the shoots (9) and as the distribution is similar for other nutrients, it seems likely that partitioning would be continuous during ion acquisition.

At this point, we are not able to identify the postulated 'small compartment' as a particular physical entity. This problem is not unknown in the transport literature, however, and has been addressed in several cases. Pitman (23) suggested that the 'cytoplasmic phase' identified in beet roots by compartmental efflux analysis may be a small portion of the bulk cytoplasm through which ions passed to the vacuole. Dodd *et al.* (12) suggested a separate serial phase in the cytoplasm of *Chaetomorpha* bounded by chloroplasts and described 'vacuole-like vesicles' near the tonoplast, possibly functioning in transport. There have been numerous reports of light enhanced ion uptake and transport independent of cellular ATP levels (*e.g.* 19). Lüttge and Osmond (17) studied light dependent Cl^- transport in *Atriplex* bladder cells and noted the difficulty of explaining the dependence of transport in one cell on electron transport in another. They suggested the possible involvement of vesicles moving in the cytoplasm.

Vesicular transport was also suggested by MacRobbie (21) to explain the appearance of two rates of Na^+ and K^+ transport to the *Nitella* vacuole, the faster rate being independent of the cytoplasmic specific activity. Recently, endocytosis involving coated vesicles has been reported by Tanchak *et al.* (28) in soybean protoplasts. The rapidity of the endocytosis recommends this process as another which might explain rapid ion fluxes such as those shown here. Arisz (1) suggested the possibility of a special (but "unknown") role for the ER in symplastic transport. The ER has often been noted to be in association with plasmodesmata (e.g. 16), where it gives rise to the desmotubule during cell plate formation (13). MacRobbie (21) mentioned the ER as possibly being involved in the *Nitella* transport problem discussed above. Chapman *et al.* (3) described a peripheral reticulum associated with the mitochondria and chloroplasts in C_4 plants and hypothesized its involvement in metabolite movement between the two organelles. Direct involvement of ER in ion movement across the root was postulated by Stelzer *et al.* (27), who demonstrated Cl^- compartmentation within the ER and plasmodesmata by AgCl precipitation. We are currently investigating some of these possibilities.

Finally, though we have presented evidence supporting the existence of a small compartment with unusual transport properties, its necessity and significance to the overall problem of ion

compartmentation and partitioning are not immediately obvious. These issues can be approached by simulation modeling as suggested by Walker and Pitman (29). We will present results of such an analysis in a subsequent paper (D Lazof, JM Cheeseman, in preparation).

Acknowledgment—The authors acknowledge with gratitude the technical assistance of Linda K. Wickens.

LITERATURE CITED

1. ARISZ WH 1960 Symplasmatischer Salztransport in *Vallisneria* Blättern. Protoplasma 52: 309–343
2. BANGE GGJ 1977 A lag phase in vacuolar Rb^+ accumulation during the initial stage of Rb^+ uptake by roots of low salt barley plants. Acta Bot Néerl 26: 53–62
3. CHAPMAN EA, JM BAIN, DW GOVE 1975 Mitochondria and chloroplast peripheral reticulum in C4 plants. Aust J Plant Physiol 2: 207–223
4. CHEESEMAN JM 1986 Compartmental efflux analysis: an evaluation of the technique and its limitations. Plant Physiol 80: 1006–1011
5. CHEESEMAN JM, P BLOEBAUM, C ENKOJI, LK WICKENS 1985 Salinity tolerance in *Spergularia marina*. Can J Bot 63: 1762–1768
6. CHEESEMAN JM, P BLOEBAUM, LK WICKENS 1985 Short term $^{22}Na^+$ and $^{42}K^+$ uptake in intact, mid-vegetative *Spergularia marina*. Physiol Plant 65: 460–466
7. CHEESEMAN JM, LK WICKENS 1986 Control of Na^+ and K^+ transport in *Spergularia marina*. I. Transpiration effects. Physiol Plant. In press
8. CHEESEMAN JM, LK WICKENS 1986 Control of Na^+ and K^+ transport in *Spergularia marina*. II. Effects of plant size, tissue ion contents and root-shoot ratio at moderate salinity. Physiol Plant. In press
9. CHEESEMAN JM, LK WICKENS 1986 Control of Na^+ and K^+ transport in *Spergularia marina*. III. Relationship between ion uptake and growth at moderate salinity. Physiol Plant. In press
10. DAVIS RF, N HIGINBOTHAM 1976 Electrochemical gradients and K^+ and Cl^- fluxes in excised corn roots. Plant Physiol 57: 129–136
11. DIXON WJ chief ed 1981 BMDP Statistical Software. University of California Press, Berkeley, CA
12. DODD WA, MG PITMAN, KR WEST 1966 Sodium and potassium transport in the marine alga *Chaetomorpha darwinii*. Aust J Biol Sci 19: 341–354
13. HEPLER PK 1982 Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. Protoplasma 111: 121–133
14. HOOYMANS JJM 1976 Competition between vacuolar accumulation and upward translocation of K^+ ions in barley plants. Z Pflanzenphysiol 79: 182–186
15. JESCHKE WD 1982 Shoot-dependent regulation of sodium and potassium fluxes in roots of whole barley seedlings. J Exp Bot 33: 601–618
16. LÓPEZ-SÁEZ JF, G GIMÉNEZ-MARTÍN, MC RISUEÑO 1966 Fine structure of the plasmodesm. Protoplasma 61: 81–84
17. LÜTTGE U, CB OSMOND 1970 Ion absorption in *Atriplex* leaf tissue. III. Site of metabolic control of light dependent chloride secretion to epidermal bladders. Aust J Biol Sci 23: 17–25
18. MACKLON AES 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
19. MACROBBIE EAC 1965 The nature of the coupling between light energy and active ion transport in *Nitella translucens*. Biochim Biophys Acta 94: 64–73
20. MACROBBIE EAC 1969 Ion fluxes to the vacuole of *Nitella translucens*. J Exp Bot 20: 236–256
21. MACROBBIE EAC 1970 The active transport of ions in plant cells. Rev Biophys 3: 251–294
22. PALLAGHY CK, U LÜTTGE, K VON WILLERT 1970 Cytoplasmic compartmentation and parallel pathways of ion uptake in plant root cells. Z Pflanzenphysiol 62: 51–57
23. PITMAN MG 1963 The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. Aust J Biol Sci 16: 647–668
24. PITMAN MG 1972 Uptake and transport of ions in barley seedlings. III. Correlation between transport to the shoot and relative growth rate. Aust J Biol Sci 25: 905–919
25. PROVENCHER SW 1976 A Fourier method for the analysis of exponential decay curves. Biophys J 16: 27–41
26. RUSH DW, E EPSTEIN 1981 Comparative studies on the sodium, potassium, and chloride relations of a wild halophytic and a domestic salt-sensitive tomato species. Plant Physiol 68: 1308–1313
27. STELZER R, A LÄUCHLI, D KRAMER 1975 Interzelluläre Transportwege des Chlorids in Wurzeln intakter Gerstepflanzen. Cytobiologie 10: 449–457
28. TANCHAK MA, LR GRIFFING, BG MERSEY, LC FOWKE 1984 Endocytosis of cationized ferritin by coated vesicles of soybean protoplasts. Planta 162: 481–486
29. WALKER NA, MG PITMAN 1976 Measurement of fluxes across membranes. *In* U Lüttge, MG Pitman, eds, Transport in Plants (Encyclopedia of Plant Physiology), Vol 2, pt A. Springer, Berlin, pp 93–126