PREFERENTIAL ABSORPTION OF POTASSIUM BY LEAF TISSUE OF THE MANGROVE, *AVICENNIA MARINA*: AN ASPECT OF HALOPHYTIC COMPETENCE IN COPING WITH SALT

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Summary

One aspect of the salt tolerance of the mangrove, *Avicennia marina*, was investigated: the preferential absorption of potassium by leaf tissue in the presence of high concentrations of salt (sodium chloride). The rate of absorption of potassium, over the concentration range 0·02 to 1·5 mM, follows the Michaelis–Menten relation, approaching the theoretical maximum, V_{max}, at 1·5 mM. The apparent Michaelis constant is 0·20 mM. At higher concentrations, up to 50 mM potassium, the rate of potassium absorption reaches values several times higher than the theoretical maximum calculated on the basis of the relation applying over the low range of concentrations, indicating the operation of a second mechanism of absorption. At both low (1 mM) and high (10 mM) concentrations of potassium, its absorption was little affected by sodium chloride concentrations up to 200 mM. At 10 mM potassium, sodium chloride at concentrations up to and including 500 mM (more than its concentration in sea-water) failed to interfere with the absorption of potassium.

I. INTRODUCTION

Among the inhibitory substances that higher plants may encounter in their chemical environment, none impairs or prevents the growth of crop plants on so large a scale as does salt. All irrigated crops are subject to or threatened by the effects of salinity. Even moderate concentrations of sodium chloride in soil solutions or irrigation waters, of the order of 25 mM (about 1500 p.p.m.), may be harmful to many species of crop plants.

Nevertheless, high concentrations of salt in the nutrient substrate are not necessarily inimical to the growth of plants. Plate 1, Figure 1, shows a group of trees growing in a solution 490 mM (29,000 p.p.m.) in respect to sodium chloride and containing many other salts in substantial concentrations. The trees are mangroves of the species *Avicennia marina*, and the solution is sea-water. The picture was taken on Bathurst Head, N. Qld. Extensive associations of mangroves occur along the north-eastern shores of Australia (Macnae 1966) and elsewhere in the tropics (Chapman 1960; Scholander *et al.* 1962, 1966; Biebl and Kinzel 1965).

Evidently, mangroves and other extremely halophytic plants possess a physiological competence most crop plants lack — the competence of coping with high

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levels of salt in their nutrient substrate. The cellular mechanisms responsible for fitting these plants for life in salt water, fatal to nearly all crop plants, are of great intrinsic interest. Furthermore, knowledge concerning this competence and its physiological and, ultimately, molecular basis is of great potential importance to human welfare. Irrigation agriculture in arid and semi-arid regions of the world, where soils and waters are saline, represents the greatest single potential for increasing world food production in the near future.

In the present work, recent findings and concepts based largely on experiments with conventional crop species have been applied to ion absorption by tissue of *A. marina*. The earlier findings (Epstein 1966) showed that potassium and sodium are absorbed by the cells of higher plants via dual mechanisms which differ markedly in their affinities for these two elements, and in other characteristics. Specifically, the kinetics of potassium absorption and the effect of sodium on this process have been investigated.

II. Experimental Methods and Materials

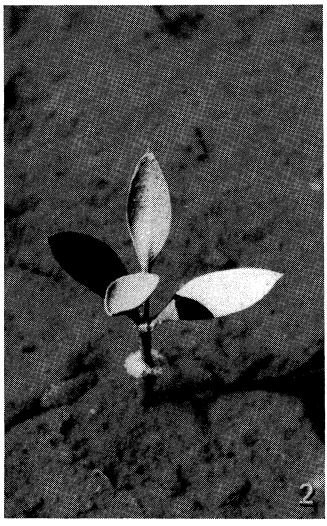
Seedlings of *A. marina* roughly 12 cm tall, with secondary and tertiary leaves, were collected from the tidal swamps near Bathurst Head, N. Qld. Plate 1, Figure 2, shows a seedling growing in a tidal swamp. At high tide it would be completely immersed in sea-water. Plate 1, Figure 3, is a close-up of a seedling. Collections were made about every 6 days. The seedlings were put in buckets containing sea-water diluted by adding an equal volume of tap water. The seedlings were kept on the main deck of the Scripps Institution of Oceanography physiological research vessel, the R. V. *Alpha Helix*, off Flinders Island, N. Qld.

For an experiment, secondary and tertiary leaves of roughly the same size were cut off and rinsed with several changes of demineralized water. Rinsing had to be thorough because there was salt on the surface of the leaves, partly from contact with sea-water, and partly as a result of excretion from salt glands. The rinsing and all subsequent experimental operations were done in the air-conditioned chemical laboratory of the *Alpha Helix*, at 21°C.

Both sides of the lamina were cut from the midrib, which was discarded. The leaf halves were cut into rectangles roughly 3 by 1·5 cm and the pieces rinsed repeatedly with demineralized water. The leaf tissue was then put in a beaker, covered loosely with moist cheesecloth (previously washed and rinsed with demineralized water to remove sizing), and kept overnight.

On the day of the experiment, a stack of five pieces of tissue was fitted into a hand-microtome and narrow slices were cut for use in the absorption experiment. The technique was as described by Smith and Epstein (1964*a*) for corn leaves. As indicated there, use of wide slices results in low apparent rates of absorption on a unit weight basis because only those cells near the cut edges participate in absorption. When progressively narrower slices are used, the rate of absorption per unit weight of leaf tissue increases up to a point where further reduction in the width of the slices causes no further increase in the apparent rate of absorption. At this width all cells capable of absorption are bathed by the experimental solution and absorption is not

ABSORPTION OF POTASSIUM BY *A. MARINA*



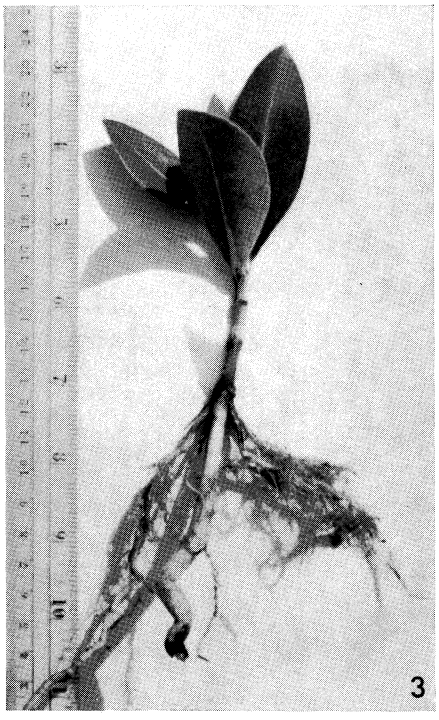


Fig. 1.—A group of mangroves of the species *Avicennia marina* growing in sea-water off the coast of North Queensland.

Fig. 2.—A seedling of *A. marina* growing in a tidal swamp near Bathurst Head, N. Qld. Foam line around the stem indicates water level at time picture was taken.

Fig. 3.—A seedling of *A. marina*.

limited by the length of the diffusion path. For corn leaves, the optimal width of the tissue slices was found to be 300 μ (Smith and Epstein 1964*a*). For leaf tissue of *A. marina*, the optimal width was near 400 μ, and slices 400 μ wide were consequently used in the experiments reported here.

Randomized samples of 50 slices, each sample weighing approximately 150 mg (fresh weight) were placed in cheesecloth "tea bags" [see Epstein, Schmid, and Rains (1963*b*) for details of this procedure]. The samples were suspended in an aerated solution of 0·50 mM $CaSO_4$ for 30 min. At time zero the samples were transferred to the experimental solutions containing potassium labelled with rubidium-86. The validity of this labelling procedure will be demonstrated below.

At the end of the absorption period (usually 60 min) the samples were rinsed briefly with a solution containing 0·50 mM $CaSO_4$ and 5 mM KCl, and then kept in identical aerated solution for 30 min for removal of that fraction of rubidium-86 loosely associated with the tissue in diffusible and readily exchangeable form (Epstein, Schmid, and Rains 1963*b*). The tissue was finally rinsed with water, lightly blotted, weighed, and then transferred to counting cups, dried under infrared lamps, and counted with a thin-window Geiger–Müller tube. Values obtained were converted to amounts of potassium absorbed by using the ratio of rubidium-86 radioactivity to potassium content of the experimental solution — the equivalent of specific activity if a potassium isotope had been used. Exceptions to the procedure outlined will be mentioned.

The device of labelling potassium with rubidium-86 was prompted by the remote location where the work was done, which made it impracticable to use potassium-42 on account of the half-life of this potassium isotope of 12·4 hr. There is considerable previous evidence to the effect that the transport mechanisms of the cell membranes of many plant species hardly distinguish between potassium and rubidium (Collander 1941; Epstein 1961; Epstein, Rains, and Elzam 1963*a*; Smith and Epstein 1964*b*). This is fortunate, because it has made it possible to use rubidium-86 for labelling potassium in short-term absorption experiments with plant tissues (Kahn and Hanson 1957; Jackson and Adams 1963; Elzam 1966; Rains and Epstein 1967).

It was nevertheless deemed wise to check the validity of this procedure for leaf tissue of *A. marina*. Experiments were therefore done on absorption of potassium labelled with rubidium-86 to determine whether its absorption as measured by radioassay for rubidium-86 adequately coincided with the value obtained by chemical analysis for potassium. For reasons which will be apparent from the body of the paper these methodological experiments were done both at a low (0·50 mM) and a high (50 mM) concentration of potassium.

For the experiment at 50 mM, triplicate samples of tissue were kept in the experimental solution (50 mM KCl labelled with rubidium-86 and 0·50 mM $CaSO_4$) for 15 and 75 min, respectively, followed by a 30-min exposure to a 0·50 mM solution of $CaSO_4$. The radioactivity of the samples was determined as described above. The samples were then ashed at 500°C, taken up in acidified water, and made to 50 ml. Potassium was determined by emission flame spectrophotometry in a Beckman DU-2 instrument with flame attachment. The difference between the 75-min and 15-min values was taken to represent the hourly rate of absorption.

For the experiment at 0·50 mM, the same method could not be used. At this low concentration, the difference between the potassium content of the tissue before and after a 1-hr period of absorption would be too small for accurate determination. For this reason absorption was measured by the disappearance of rubidium-86 and of potassium from the solution. A relatively large amount of tissue (300 slices instead of the usual 50) was exposed to a small volume of aerated solution (13 ml) 0·50 mM with respect to KCl labelled with rubidium-86, and 0·50 mM with respect to $CaSO_4$. After 15 min, and again after 75 min, 2-ml samples of solution were withdrawn, made to 4 ml, and both the radioactivity and potassium concentration of the samples were determined. The difference between the 15-min and 75-min values was taken to be the hourly rate of absorption.

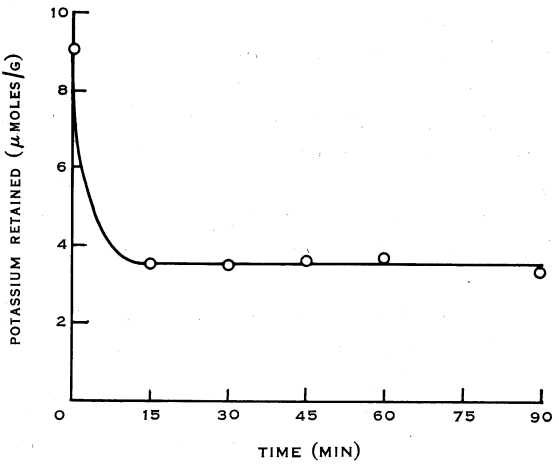


Fig. 1.—Retention of previously absorbed labelled potassium as a function of time. Tissue exposed first to a solution containing labelled KCl at a concentration of 10 mM for 60 min and then transferred to an unlabelled 5 mM KCl solution for varying periods of time. Calcium (as $CaSO_4$) 0·5 mM throughout.

In the experiment done at 50 mM potassium, results obtained by the radioassay and the chemical method differed by less than 2%. In the experiment at 0·50 mM, the difference was 14·3% of the mean, the value for absorption obtained by chemical analysis being the greater.

III. Results

(*a*) *Exchangeability of Absorbed Potassium*

In Figure 1 are shown the results of an experiment demonstrating that leaf tissue of *A. marina* resembles barley roots and corn leaf tissue in transporting potassium into "inner" spaces where it is not readily subject to exchange with exogenous potassium. The tissue was first kept in an aerated, 10 mM solution of labelled potassium for 1 hr. It was then rinsed with a desorbing solution containing 5 mM KCl and 0·50 mM $CaSO_4$, and kept in an identical aerated solution. At intervals, samples were removed, rinsed with water, and assayed for labelled potassium. Figure 1

shows that there was a rapid loss of an exchangeable fraction of labelled potassium, but after 15 min no further loss of labelled potassium was detected. In all subsequent experiments the period of absorption of labelled potassium was followed by a 30-min exposure to unlabelled desorbing solution to remove the exchangeable labelled potassium, leaving for final assay the "inner" space fraction only (cf. Epstein, Schmid, and Rains 1963*b*).

(*b*) *Time Course of Potassium Absorption*

It was found that at 1·0 mM potassium, the ion was absorbed at a steady rate for 90 min [Fig. 2(*a*)]. At 10 mM, the initial rapid rate quickly fell off, whereupon a steady rate was maintained for the remainder of the 120-min experimental period [Fig. 2(*b*)].

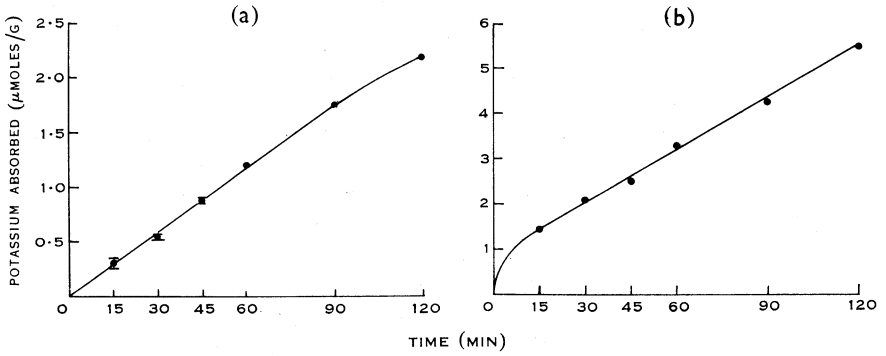


Fig. 2.—Absorption of labelled potassium as a function of time. (*a*) Potassium (as KCl) 1·0 mM; calcium (as $CaSO_4$) 0·5 mM. (*b*) Potassium (as KCl) 10 mM; calcium (as $CaSO_4$) 0·5 mM. At the end of the absorption period all samples were exposed to a solution containing 10 mM unlabelled KCl and 0·5 mM $CaSO_4$ for 30 min. Solid circles represent the means of two replicates indicated by short horizontal lines. Where horizontal lines are not shown the distance between them would have been less than the diameter of the circle.

(*c*) *Effect of Potassium Concentration on Potassium Absorption*

Figure 3(*a*) shows an absorption isotherm representing the results of an experiment in which the potassium chloride concentration was varied from 0·1 to 1·5 mM. Values for potassium absorbed approached a plateau of 1·48 μmoles/g/hr (the dashed line). Half that value was reached at a potassium concentration of 0·20 mM (the apparent Michaelis constant).

If the potassium absorption mechanism characterized in this experiment were the only one operating in this tissue, then even much higher concentrations of potassium should not cause the values for potassium absorbed to rise above the theoretical maximum, V_{max}, asymptotically approached in this experiment (the dashed line). Actually, at higher concentrations, amounts of potassium absorbed were greatly in excess of the value corresponding to the plateau of Figure 3(*a*). In the experiment shown in Figure 3(*b*), the potassium chloride concentration was

varied from 0·20 to 50 mM. To accommodate this range, the concentration scale is broken between 1 and 2 mM. The levelling-off of the amounts of potassium absorbed, documented in Figure 3(*a*), was again apparent at the same concentrations as before,

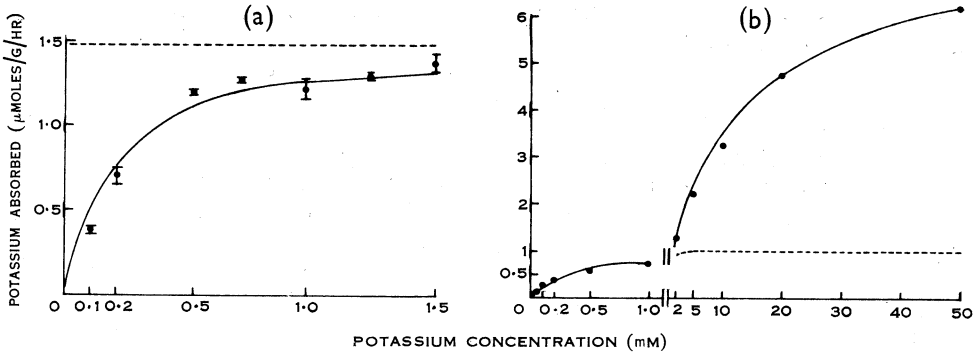


Fig. 3.—Absorption of labelled potassium as a function of its concentration. (*a*) Potassium (as KCl) 0·1–1·5 mM; calcium (as $CaSO_4$) 0·5 mM. Circles represent experimental data; the line is a plot of the Michaelis–Menten equation. V_{max} 1·48 μmole/g/hr; K_m 0·20 mM. (*b*) Potassium (as KCl) 0·02–50 mM; calcium (as $CaSO_4$) 0·5 mM. Plot of potassium concentration broken between 1 and 2 mM and horizontal scale changed. Dashed line represents calculated V_{max} for the high-affinity mechanism (1·05 μmole/g/hr). Other conditions and conventions as in Figure 2.

i.e. at about 1 mM. At this concentration, the observed rate of absorption of potassium was 80% of the maximal rate calculated on the basis of the relation applying over the low range of concentrations [see the dashed line of Figure 3(*b*), and the legend to the figure]. However, at 2 mM and beyond, amounts absorbed far exceeded this

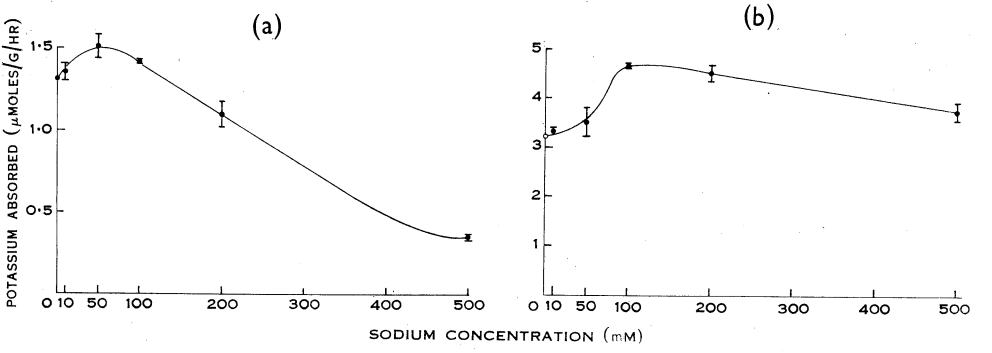


Fig. 4.—Absorption of labelled potassium as a function of the concentration of NaCl. (*a*) Potassium (as KCl) 1·0 mM; calcium (as $CaSO_4$) 0·5 mM. Concentration of NaCl 0–500 mM. (*b*) Potassium (as KCl) 10 mM; calcium (as $CaSO_4$) 10 mM. Concentration of NaCl 0–500 mM. Other conditions and conventions as in Figure 2.

value and at 50 mM potassium reached a level over six times as high. In this experiment, and that shown in Figure 4(*b*), the 60-min values for amounts of potassium absorbed were taken to represent the rates of absorption. At the high, but not the low

concentrations, this somewhat overestimates the steady-state rate [cf. Figs. 2(*a*) and 2(*b*)]. Apart from this quantitative difference, the responses at the high concentrations measured in this way were the same as when the difference between the 15-min and 75-min values was taken as the rate of absorption.

(*d*) *Effect of Sodium on Potassium Absorption*

In Figure 4(*a*) are shown the results of an experiment on the effect of sodium chloride on potassium absorption. The concentration of potassium was 1·0 mM, and that of sodium varied from 0 to 500 mM (the concentration of sodium chloride in sea-water is 490 mM). Sodium chloride concentrations up to and even more than 100 times the concentration of potassium failed to diminish the rate of potassium absorption. Indeed, there was some acceleration. At still higher concentrations of sodium chloride, the rate of potassium absorption declined, and reached 27% of the control at 500 mM sodium chloride.

Figure 4(*b*) shows the findings from a similar experiment, but the potassium concentration in this case was 10 mM (the potassium concentration of sea-water is 12 mM). The calcium concentration was 10 mM (as in sea-water). At all sodium chloride concentrations added, the tissue absorbed more potassium than it did in the absence of sodium chloride.

IV. Discussion

(*a*) *Exchangeability of Absorbed Potassium*

After a period of absorption of labelled potassium, the tissue contains at least two sharply distinguishable fractions of labelled potassium: one readily exchangeable, the other one very much less so [Fig. 1; Section III(*a*)]. This state of affairs has been described for many ions (Epstein 1960; Briggs, Hope, and Robertson 1961). Those ions not easily exchangeable are in the "inner" space of the tissue (Epstein 1960; Epstein, Schmid, and Rains 1963*b*). It is absorption of this fraction that was studied in the present work.

(*b*) *Dual Mechanisms of Potassium Absorption*

Absorption of potassium by leaf tissue of *A. marina* exhibits a basic pattern which it shares with absorption of this and other ions by tissues of many species (Epstein 1966). The pattern is a dual one, reflecting the operations of two distinct mechanisms of absorption. At relatively low concentrations, a mechanism operates which, in *A. marina*, virtually reaches its maximal rate at an external potassium concentration of 1·5 mM. The rate of absorption via this mechanism, mechanism 1, is a function of the external potassium concentration according to the Michaelis–Menten equation, with an apparent Michaelis constant of 0·20 mM. This is about 10 times higher than the Michaelis constant for potassium absorption by most other tissues for which reliable data exist (Epstein 1966).

At much higher concentrations, far more potassium is absorbed than can be accounted for on the basis of the maximal rate calculated for mechanism 1. The

implication is that at these high concentrations, a second mechanism of absorption becomes operative. This mechanism 2 has a much lower affinity for potassium, so that it does not measurably contribute to potassium absorption at concentrations of about 1·5 mM and below. Mechanism 2 of alkali cation absorption has been characterized in several other tissues in considerable detail (Epstein, Rains, and Elzam 1963*a*; Epstein and Rains 1965; Rains and Epstein 1965, 1967; Elzam 1966; Torii and Laties 1966).

(*c*) *Discrimination between Potassium and Sodium*

At 1 mM potassium and 0·50 mM calcium, absorption of potassium was not diminished by even large excess concentrations of sodium [Fig. 4(*a*); Section III(*d*)]. Only when the concentration ratio, Na/K, in the solution substantially exceeded 100 to 1 did sodium interfere with potassium absorption. This high selectivity of mechanism 1 for potassium *vis-à-vis* sodium has been shown for other tissues (Epstein 1961; Epstein, Rains, and Elzam 1963*a*; Smith and Epstein 1964*b*; Elzam 1966).

Figure 4(*b*) [Section III(*d*)] also shows that potassium absorption was little affected by sodium. In this experiment, the concentration of potassium was 10 mM (*v.* 12 mM in sea-water), the concentration of calcium was 10 mM (about the same as in sea-water), and the concentration of sodium chloride ranged from 0 to 500 mM (just beyond the concentration of sodium chloride in sea-water, which is 490 mM). At the potassium concentration used, mechanism 2 substantially contributes to potassium absorption [cf. Fig. 3(*b*), Section III(*c*)]. It thus appears that mechanism 2 in leaf tissue of *A. marina* resembles mechanism 1 in its selective preference for potassium and resistance to interference by sodium. In this regard, potassium absorption via mechanism 2 of *A. marina* sharply differs from that in barley where it is severely and competitively inhibited by sodium (Epstein, Rains, and Elzam 1963*a*; Rains and Epstein 1967), and transports sodium in preference to potassium (Rains and Epstein 1967). In other species examined so far (see above), only the high-affinity mechanism 1 of potassium absorption is indifferent to sodium; in *A. marina*, both mechanisms 1 and 2 show this preferential affinity for potassium.

(*d*) *Adaptive Significance*

Of all those essential nutrient elements other than hydrogen present in the substrate in the form of cations, potassium is the one required by plants in largest amount (Epstein 1965). In many environments, the membrane mechanisms effecting its absorption and concentration within the cells must have the competence to deal with two conditions that often obtain: (1) Where external potassium concentrations are low, these mechanisms must have a sufficiently high affinity for potassium to be able to absorb it and build up internal concentrations much higher than those prevailing in the substrate. (2) In the presence of high concentrations of a chemically closely related ion, sodium, the potassium-absorbing mechanisms must effect a sufficiently sharp preferential selection of potassium to keep the internal cellular milieu from being swamped by sodium.

In root tissue of barley, mechanism 1 of alkali cation transport possesses both characteristics mentioned above (Epstein, Rains, and Elzam 1963*a*). Its affinity

for potassium is high ($K_m = 0{\cdot}02$ mM), as required in tissue confronting a solution, the soil solution, in which the concentration of potassium may be in the micromolar range (Reisenauer 1966). Potassium absorption via mechanism 1 in barley root tissue is not inhibited by even high concentrations of sodium (Epstein 1961) — an essential feature for a species which frequently encounters high levels of sodium in the soils where it grows.

Mechanism 1 of alkali cation absorption in leaf tissue of *A. marina* does not have as high an affinity for potassium as does mechanism 1 in barley roots (Epstein, Rains, and Elzam 1963*a*) and other tissues (Epstein 1966). Indeed, there would seem to be no need for an extremely high affinity. The seedlings grow in sea-water, with a 12 mM concentration of potassium. Thus, although the composition of the solution to which the mesophyll cells of the leaves are normally exposed is unknown, the concentration of potassium in this solution is not likely be be low. On the other hand, the concentration of sodium in sea-water is 490 mM, and unlike the situation in barley, mechanism 2 as well as mechanism 1 of potassium transport in leaf tissue of *A. marina* resists interference by sodium.

Preferential absorption of potassium (which probably occurs in the roots as well as in the leaves) is only one aspect of the physiological competence of *A. marina* in coping with salt. There are at least two more.

One is the ability of the tissue to tolerate, without injury, high internal levels of salt. Like many other halophytes (Collander 1941; Takeda 1954; Black 1960; Scholander *et al.* 1962, 1966; Binet 1963; Greenway and Rogers 1963; Biebl and Kinzel 1965; Rickard 1965; Elzam 1966), shoot tissue of *A. marina* does not exclude salt but absorbs it in substantial amounts. On a dry basis, leaf tissue of seedlings used in this work contained 1·0% potassium, 4·1% sodium, and 7·5% chloride. The concentrations of the ions expressed on the basis of the water content of the leaves were 30 mM potassium, 210 mM sodium, and 245 mM chloride. The effect of the preferential absorption of potassium discussed in this paper, therefore, is not to exclude sodium, but rather to raise the concentration ratio, K/Na, from the value in sea-water (about 1/40) to roughly 1/7 within the tissue. The tissue thus contains quite a high concentration of sodium chloride, about 1·8 m-mole/gram dry weight, far more than conventional crop plants can tolerate without injury.

Finally, leaves of *A. marina* are equipped with salt glands by means of which salt is excreted onto the surface of the leaves, eventually to be removed by the action of wind and water (Scholander *et al.* 1962, 1966).

V. Acknowledgments

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During the period of this research, D. W. Rains was National Science Foundation Post-doctoral Fellow, Scripps Institution of Oceanography, University of California, San Diego, LaJolla, California, and E. Epstein was Senior Fulbright Research Scholar while on sabbatical from the Department of Soils and Plant Nutrition, University of California, Davis, California.

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