SODIUM AND POTASSIUM INFLUX INTO CITRUS LEAF SLICES

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Abstract

The influxes of sodium and potassium have been measured in slices of mature citrus leaves, using ^{22}Na and ^{42}K as tracers. External concentrations were 0·03–100 mM (sodium) and 0·1–100 mM (potassium). The sodium influx was always less than the potassium influx, for any given external concentration. In neither case was the influx increased by light. There was no effect on the influxes when chloride ions in the experimental solutions were replaced by sulphate ions.

The sodium influx versus concentration curve showed only a small departure from a single (enzyme-type) hyperbola: the influx was about 20% higher than expected from such a relationship at the lowest sodium concentrations (0·03 and 0·1 mM). Sodium influx was reduced by 20–25% at low temperature, under dark plus anaerobic conditions, or in the presence of 2,4-dinitrophenol (DNP).

The potassium influx versus concentration curve had two hyperbolic components, with the change in shape occurring when the potassium concentration was between 3 and 10 mM. Potassium influx was reduced by 55–60% at low temperature, and in the presence of DNP. The progressive addition of potassium to solutions containing 10 mM sodium reduced sodium influx by up to 33%. Potassium influx was much less sensitive to the presence of sodium.

The results are discussed in relation to previous studies of ion transport in leaf slices. Taking into account the possible artefacts produced by slicing, ways in which this approach may provide useful information about the ionic relations of intact leaves are suggested.

I. INTRODUCTION

Measurements of ^{36}Cl uptake into freshly cut slices of citrus leaves (Robinson and Smith 1970) showed that chloride influx rises with increasing external chloride concentrations, and that the shape of the influx versus concentration curve depends on the nature and concentration of the accompanying cations (sodium and potassium). There was no evidence for the two distinct chloride transport systems reported by other workers (Epstein 1966; Laties 1969). Under aerobic conditions the chloride influxes in light and darkness were similar. Under anaerobic conditions the influx was maintained in the light but was greatly reduced in the dark. It was suggested that chloride uptake is dependent on phosphorylation reactions.

The present paper describes measurements of sodium and potassium influxes under conditions similar to those used previously. The results are compared with the

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chloride influxes measured previously. The work as a whole is discussed with respect to the ionic relations of plant leaf tissue under normal conditions and under conditions of salinity stress.

II. Materials and Methods

Mature leaves were taken from the orange tree [*Citrus sinensis* (L.) cv. Valencia] which was used in the previous work (Robinson and Smith 1970). The studies of sodium and potassium uptake were carried out separately, using leaves from two different growth flushes. In each case the leaves were 4–5 months old. The techniques used were similar to those already described, except that the radioisotopes were ^{22}Na and ^{42}K (supplied as $^{22}NaCl$ and ^{42}KCl at high specific activity). For the measurement of cation influx, leaf slices were cut and rinsed in deionized water for approximately 1 hr. They were then aerated in the experimental solutions for 2 hr. This was done at controlled temperatures (normally 24°C) using a water-bath. The intensity of the light incident on the slices was approximately 30,000 ergs cm^{-2} sec^{-1} (from fluorescent tubes).

The slices were placed in aerated non-radioactive solutions for 30 min to remove ^{22}Na or ^{42}K from the free space (cut cells, cell walls, and intercellular spaces). Slices were then rinsed, blotted, and weighed and the ^{22}Na or ^{42}K content of the slices was determined as described previously (Robinson and Smith 1970). Results were calculated as μmoles of sodium or potassium absorbed per gram fresh weight per hour. These values represent influx and not net uptake of cations.

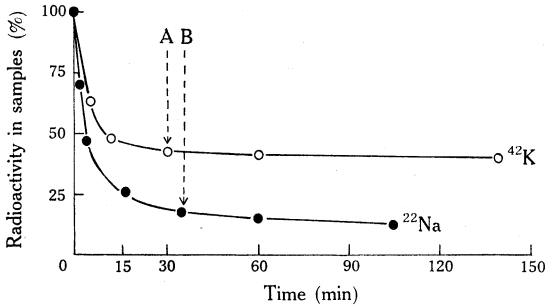


Fig. 1.—Efflux of ^{22}Na and ^{42}K from leaf slices into non-radioactive solutions, in light at 24°C. Radioactivity remaining in samples is expressed as a percentage of that in the samples after a 2-hr influx period in solutions labelled with ^{22}Na or ^{42}K. Values used to estimate rapidly exchanging potassium and sodium are shown (A and B).

In some experiments the solutions used for washing the slices contained NaCl or KCl at the same concentrations as in the radioactive solutions. For others tap-water was used. It was checked that this made no difference to the results. The washing-out procedure was carried out at room temperature (about 24°C). This allowed comparison with previous studies of cation uptake into leaf slices (Rains and Epstein 1967; Rains 1968). It was recognized that there would be some loss of ^{22}Na or ^{42}K from the cytoplasm during the washing-out period. Nevertheless when slices were washed at 2°C, in an attempt to reduce efflux from the cytoplasm, there was only a slight change in the values for ^{22}Na influx. The rates were about 10% higher than those obtained with the normal procedure.

Cation efflux was measured by quickly blotting slices labelled with ^{22}Na or ^{42}K, and then placing in successive 10-ml aliquots of non-radioactive solutions. 2-ml samples were then evaporated to dryness on planchets, and the ^{22}Na or ^{42}K washed out of the slices was counted as usual.

III. Results

(*a*) *General*

The standard solution used in the study of chloride uptake (Robinson and Smith 1970) contained 10 mM NaCl and 0·5 mM $CaSO_4$, and the chloride influx from this solution averaged 1·2 μmoles g^{-1} hr^{-1}. The mean sodium influx from

solutions of this composition, measured in six experiments, was 1·6 μmoles g^{-1} hr^{-1}. The standard error of the mean was 0·1 μmole g^{-1} hr^{-1}. For comparison, the potassium influx from solutions containing 10 mM KCl+0·5 mM $CaSO_4$ was 7·4±0·8 μmoles g^{-1} hr^{-1} (five experiments).

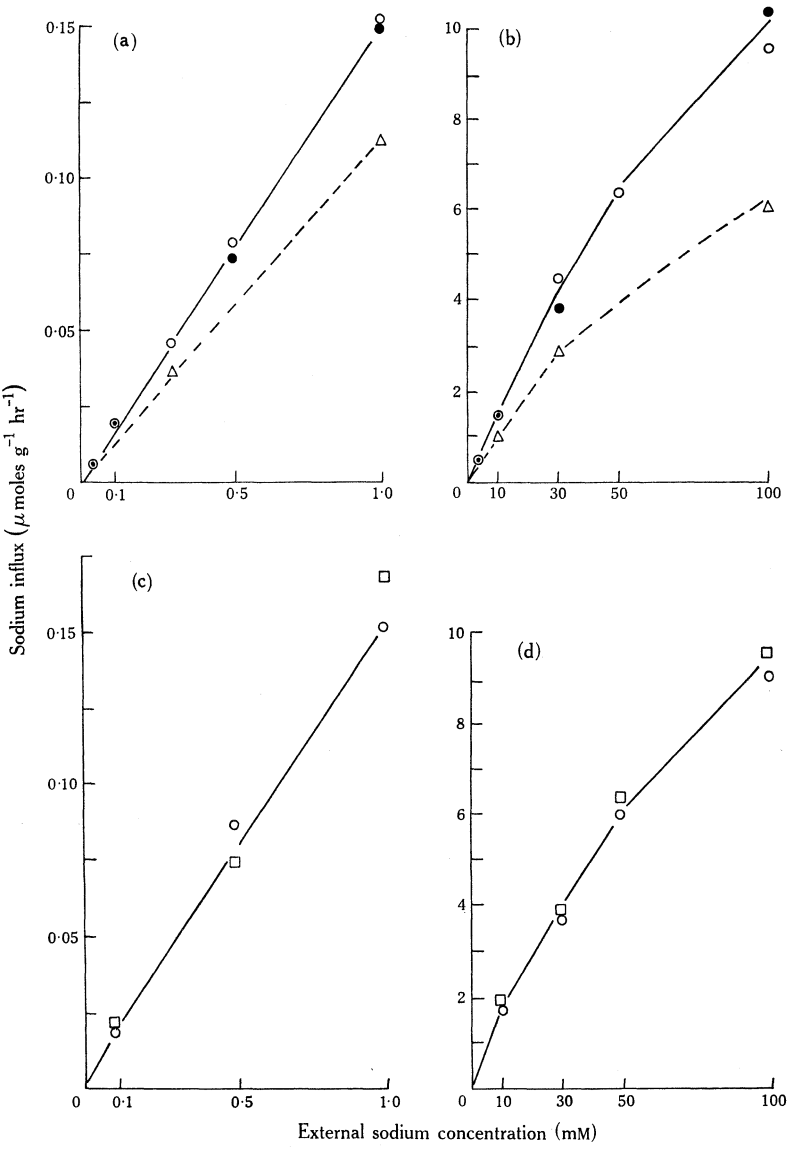


Fig. 2.—(*a*) and (*b*). Sodium influx from solutions containing 0·5 mM $CaSO_4$ and 0·03–100 mM NaCl. Conditions: light, 24°C (○); dark, 24°C (●); light, 6°C (△). The low (*a*) and high (*b*) concentrations are plotted separately. (*c*) and (*d*). Sodium influx from solutions containing 0·5 mM $CaSO_4$ and 0·1–100 mM sodium either as NaCl (○) or Na_2SO_4 (□). Conditions: light, 24°C. The low (*c*) and high (*d*) concentrations are plotted separately.

When measuring cation uptake it is important that uptake into the free space should not mask uptake into the cells. To check that 30 min was sufficient to remove ^{22}Na and ^{42}K from the free space the efflux of these ions was followed over an extended period. As shown in Figure 1, the rate of loss of both ^{22}Na and ^{42}K had slowed down considerably after 30 min. If all the radioactivity lost in this time came from the free space then, using the known specific activities of the radioactive bathing solutions and the data in Figure 1, the amounts of rapidly exchanging sodium and potassium are 12 μmoles g^{-1} and 15·5 μmoles g^{-1} respectively. It would be expected that the amounts of sodium and potassium absorbed into the free space would be similar. However, the two experiments shown in Figure 1 were not carried out simultaneously and there may have been differences in the efficiency with which the slices were blotted at the beginning of the washing-out period. The values for the free space quoted above would be overestimates if there were large effluxes of sodium and potassium from the cytoplasm.

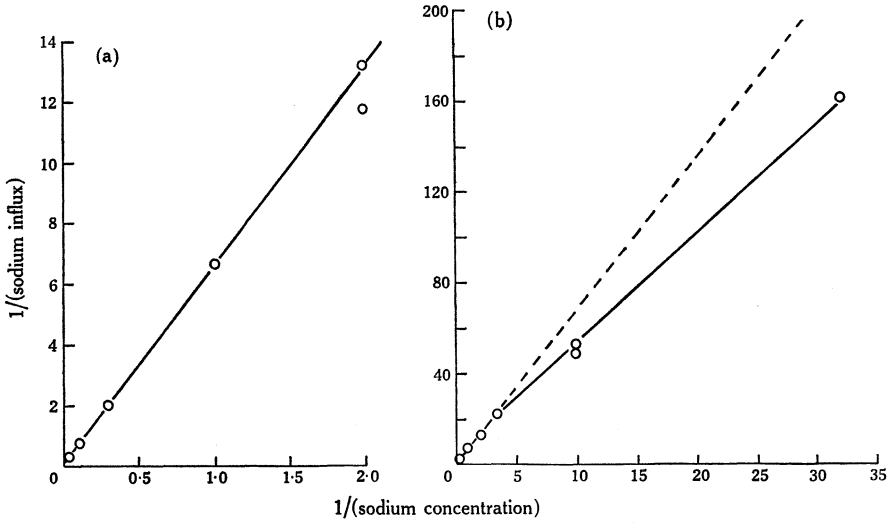


Fig. 3.—Double reciprocal plots [1/(sodium influx) against 1/(sodium concentration)] using data from Figure 2. External concentrations are 0·5–30 mM (*a*) and 0·03–3 mM (*b*). The dashed line (*b*) shows the continuation of the line in (*a*).

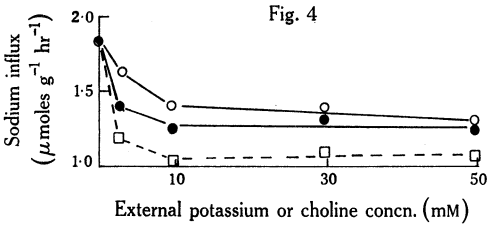
(*b*) *Sodium Influx*

Figures 2(*a*) and 2(*b*) show the uptake of sodium from solutions containing sodium at concentrations from 0·03–100 mM. There was no apparent difference between influx into slices in the light and slices kept in darkness. When slices were kept at 6°C the influx was reduced (by about 25% with concentrations up to 30 mM).

Figures 2(*c*) and 2(*d*) show that replacing chloride with sulphate had no effect on sodium uptake. Figures 2(*a*) and 2(*c*) suggest that sodium influx increases almost linearly with increasing sodium concentrations up to 1 mM, after which the influx

starts to saturate, producing a single hyperbolic absorption isotherm. However, the influx from solutions containing 0·03 and 0·1 mM sodium was somewhat higher than expected from such a relationship. A double reciprocal (Lineweaver–Burk) plot of 1/(influx) against 1/(sodium concentration) over the range 0·3–100 mM produced a single straight line. Figure 3(*a*) shows the plot for the range 0·5–30 mM, using data shown in Figures 2(*a*) and 2(*c*). Figure 3(*b*) shows that at the lowest concentrations (0·03 and 0·1 mM) the sodium influx departed by about 20% from this line. This departure is much smaller than that found in studies with other types of plant tissue (Epstein 1966; Laties 1969; see also below for further discussion).

Interactions between sodium and potassium ions are shown in Figure 4. The addition of potassium at 10, 30, or 100 mM to the basic experimental solution produced similar reductions in sodium influx. A qualitatively similar effect was observed when choline chloride was added to the solution (Fig. 4).



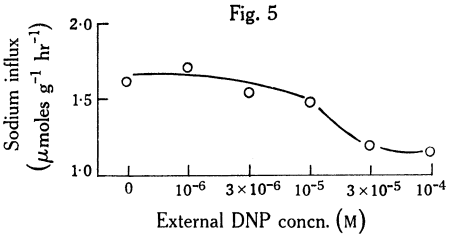


Fig. 4.—Effects of increasing potassium or choline concentrations on sodium influx, in light at 24°C. All solutions contained 10 mM NaCl+0·5 mM $CaSO_4$. Potassium was added as KCl (○) or K_2SO_4 (●). Choline was added as choline chloride (□).

Fig. 5.—Effects of DNP on sodium influx, in light at 24°C. The basic solution contained 10 mM NaCl+0·5 mM $CaSO_4$.

A comparison of sodium influx under aerobic and anaerobic conditions is shown in the following tabulation:

Conditions	Sodium Influx (μmoles g^{-1} hr^{-1})	
	Sample A	Sample B
Light, air bubbled	1·0	1·1
Dark, air bubbled	1·1	1·0
Light, nitrogen bubbled	1·1	1·1
Dark, nitrogen bubbled	0·8	0·9

This experiment was carried out with duplicate samples of slices (A and B). The solutions contained 10 mM NaCl+0·5 mM $CaSO_4$.

Anaerobic conditions did not apparently reduce sodium influx in light, and in the dark there was only a slight decrease.

A decrease in sodium influx was produced by adding 2,4-dinitrophenol to the solution, as shown in Figure 5. As with the dark-plus-nitrogen treatment (above) the decrease was quite small (about 25%).

(c) *Potassium Influx*

Figures 6(*a*) and 6(*b*) show the kinetics of potassium uptake, from solutions containing 0·1–100 mM KCl. As with sodium, there was no distinguishable effect

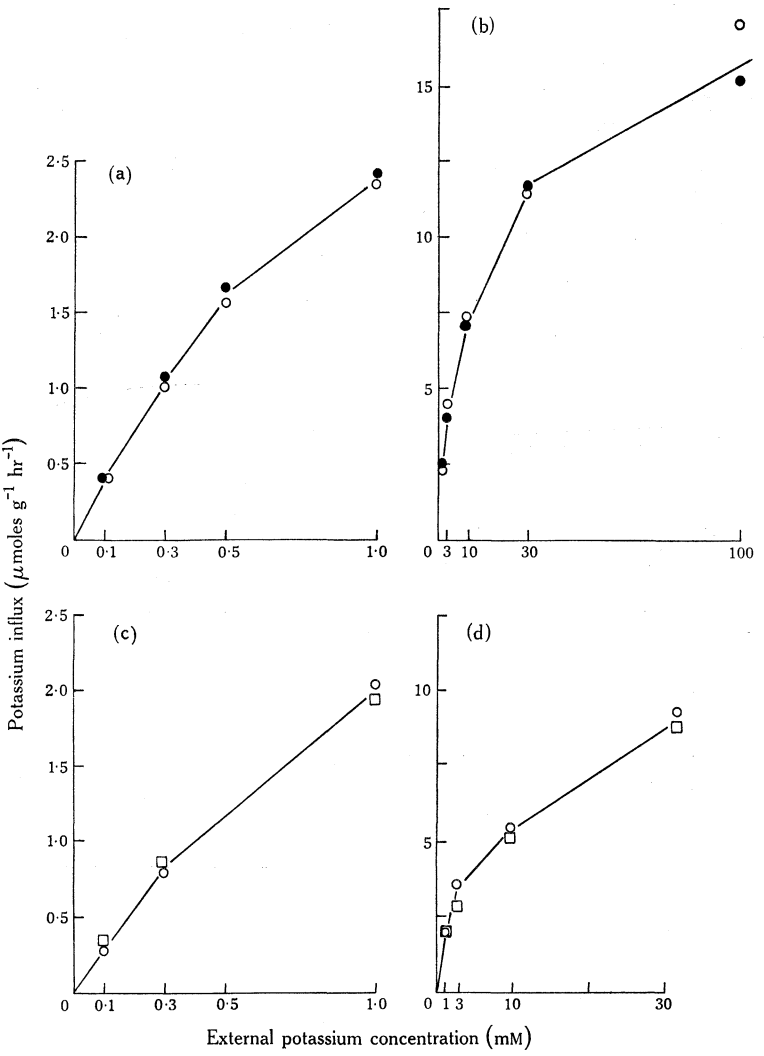


Fig. 6.—(*a*) and (*b*) Potassium influx from solutions containing 0·5 mM $CaSO_4$ and 0·1–100 mM KCl. Conditions: light, 24°C (○); dark, 24°C (●). The low (*a*) and high (*b*) concentration ranges are plotted separately. (*c*) and (*d*) Potassium influx from solutions containing 0·5 mM $CaSO_4$ and 0·1–100 mM potassium as KCl (○) or K_2SO_4 (□). Conditions: light, 24°C. The low (*c*) and high (*d*) concentration ranges are plotted separately.

of light on the influx, and the influx was likewise unaffected when chloride was replaced by sulphate. This is shown in Figures 6(*c*) and 6(*d*).

Figures 7(*a*) and 7(*b*) show the double reciprocal plot for potassium influx, using the data from Figures 6(*a*) and 6(*b*). In this case there is a marked change in shape when the external concentration is between 3 and 10 mM. A similar curve

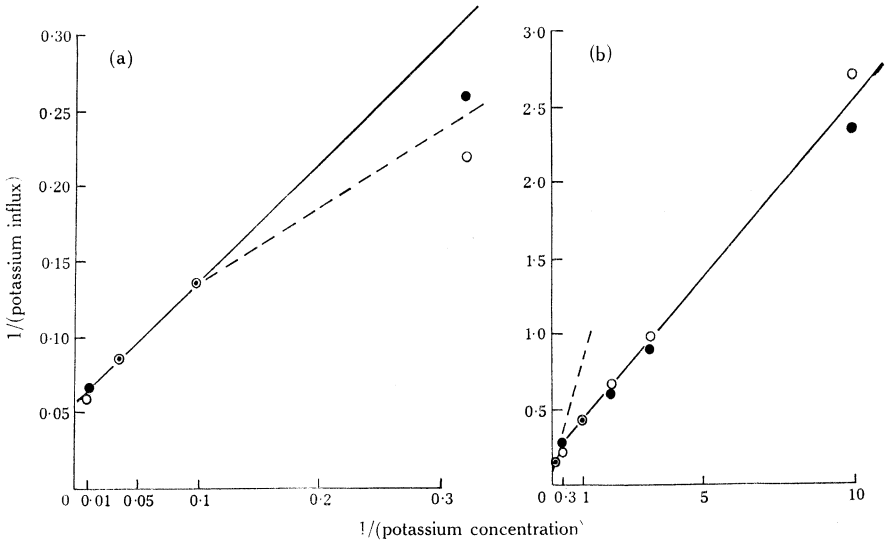


Fig. 7.—Double reciprocal plots [1/(potassium influx) against 1/(potassium concentration)] using data from Figures 6(*a*) and 6(*b*). The dashed line in (*b*) is the continuation of the line shown in (*a*) and vice versa.

may be drawn for the data shown in Figures 6(*c*) and 6(*d*), although the actual influx values are somewhat lower.

Figure 8 shows that when increasing concentrations of sodium ions were added to the basic solution (10 mM KCl+0·5 mM $CaSO_4$) there was an effect on potassium

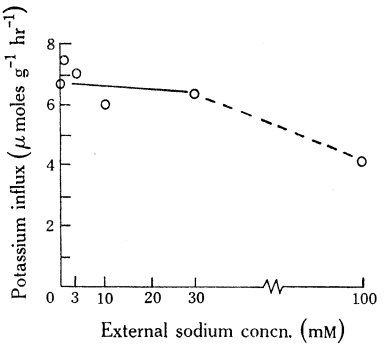


Fig. 8.—Effects of increasing sodium concentrations on potassium influx, in light at 24°C. All solutions contained 10 mM KCl+0·5 mM $CaSO_4$. Sodium was added as NaCl.

influx only with the highest sodium concentration (100 mM). This may have been due to the low water potential of the external solution, or the high ratio of monovalent to divalent cations, rather than the high sodium concentration. Potassium influx

was reduced by about 55% when the temperature was reduced to 2°C and by about 60% in the presence of 10^{-4}M DNP, as is shown in the following tabulation:

Conditions	Potassium Influx (μmoles g^{-1} hr^{-1})	
	Sample A	Sample B
Experiment 1		
Light, 24°C	9·9	9·6
Light, 2°C	4·2	4·5
Experiment 2		
Light, 24°C	8·3	—
$+5\times10^{-6}$M DNP	8·1	—
$+2\times10^{-5}$M DNP	5·4	—
$+1\times10^{-4}$M DNP	3·3	—

The first experiment was carried out with duplicate samples. All experiments contained 10 mM KCl+0·5 mM $CaSO_4$.

IV. Discussion

(*a*) *Leaf Slices as Experimental Material*

The main advantage of leaf slices as experimental material is that access of bathing solutions to the leaf cells is not limited by the cuticle. The main disadvantage is that damage caused by the slicing procedure may affect ion transport in the remaining intact cells. Van Steveninck and Jackman (1967) and Jackman and Van Steveninck (1967) have shown that there are biochemical and ultrastructural changes following slicing of beetroot tissues. Such changes may also occur in leaf cells, and this may make difficult, or even invalidate, attempts to extrapolate results to the intact leaf (see below). Nevertheless, there is no doubt that metabolically controlled ion transport occurs in fresh leaf slices, and it has been suggested that the mechanisms are analogous to those in roots and other plant material (Smith and Epstein 1964*a*, 1964*b*; Rains and Epstein 1967; Rains 1968). To what extent these mechanisms are affected by metabolic changes associated with "wounding" or "aging" as found in storage tissues, remains to be seen.

(*b*) *Ionic Relations of Citrus Leaf Slices*

The sodium absorption isotherm shown in Figure 2 resembles the chloride absorption isotherm for citrus leaf slices (Robinson and Smith 1970). Immediately after the series of experiments on sodium uptake, the chloride influx was again measured using $Na^{36}Cl$ and the values were found to be very similar to those reported previously. Comparing the sodium and chloride influxes, it is seen that with NaCl concentrations of less than 10 mM the sodium influx in light or darkness is the same as the chloride influx, but at higher concentrations the sodium influx is the greater. Representative values are shown below:

NaCl Concn. (mM)	Ion Influx (μmoles g^{-1} hr^{-1})	
	Sodium	Chloride
1	0·15	0·15
10	1·5	1·2
100	10	5

However, there does not seem to be a direct coupling between sodium and chloride uptake even at low concentrations of NaCl, as in the absence of chloride the sodium influx was unchanged. Furthermore, at low temperature, in the presence of DNP, or under dark and anaerobic conditions sodium influx was affected only slightly, whereas chloride influx was drastically reduced under all these conditions (Robinson and Smith 1970).

When the experimental solutions contained KCl, the potassium influx was much greater than the values for chloride influx reported previously (Robinson and Smith 1970) and again found subsequently (Smith, unpublished results). Comparable values are as follows:

KCl Concn. (mM)	Ion Influx (μmoles g^{-1} hr^{-1})	
	Potassium	Chloride
1	2·5	0·25*
10	7·5	2*
100	16	9*

* Smith, unpublished results.

As with sodium, removal of chloride had no effect on potassium influx. There was a large residual potassium influx at low temperature, or in the presence of DNP. Rates of potassium uptake into *Citrus* leaf slices were very similar to those in leaf slices from the mangrove *Avicennia* (Rains and Epstein 1967). No other comparable data are available from plants grown under natural conditions. Much higher rates have been found in maize leaf slices (Smith and Epstein 1964*b*; Rains 1968) but these experiments were carried out with plants grown in nutrient solutions low in potassium. Rains (1968) found that potassium influx from solutions containing 0·01–0·2 mM KCl plus 0·5 mM $CaSO_4$ was about 40% lower in the dark than in light. In light, potassium influx was decreased by about 50% by DNP, and by about 60% under anaerobic conditions, but influx was almost completely inhibited in the dark under these conditions. Rains interpreted the results in terms of an energy-dependent potassium transport system. The results obtained with *Citrus* suggest that there is considerable diffusion of sodium and potassium ions into the cells. The reduction of cation influx under certain conditions (i.e. at low temperature, and in the presence of DNP) may reflect a direct link between a component of cation influx (especially potassium influx) and cellular metabolism. Alternatively, this may be due to indirect effects on membrane properties. Different effects on sodium and potassium influx could be explained by the differential permeability of the plasmalemma to these ions. The interactions between sodium and potassium influx in the presence of both ionic species (Fig. 4) could be due to changes in the transmembrane electrical potential of the cells. There is no need to postulate any competition between ions for a hypothetical membrane carrier molecule.

The finding that cation influx is unaffected by replacement of chloride by sulphate is in agreement with the results of Smith and Epstein (1964*a*) and Rains (1968). This is of particular interest, as it has been shown that the chloride influx can be greatly affected by the levels of potassium and sodium in solution (Robinson and Smith 1970). Chloride influx is higher from a solution containing KCl than from

a solution containing the same concentration of NaCl (see also the tables in the text, above). Furthermore, chloride influx is increased when the external chloride is kept constant and the concentrations of sodium or potassium are increased (by adding Na_2SO_4 or K_2SO_4). Further experiments will be needed to establish whether chloride uptake proceeds in the absence of sodium and potassium, in other words whether there is a basal level of chloride uptake which is increased by the addition of monovalent cations.

The imbalances between the influxes of cations and of chloride must of course be accounted for. The most likely explanation is that excess influx of cations is balanced by efflux of cations which might include active efflux of sodium or hydrogen ions. (This is under investigation at present.)

The double reciprocal plots (Figs. 3 and 7) have been included to allow further comparison with the work of Smith and Epstein (1964*b*), Rains and Epstein (1967), and Rains (1968). It should be stressed that all of this work has been carried out with fresh leaf slices, using very similar experimental conditions. In the case of sodium, the slight deviation from a single hyperbolic function has been noted above. There is certainly no saturation of the sodium influx at sodium concentrations of 0·05–1 mM (the diagnostic criterion of Epstein's ion transport "System 1": Smith and Epstein 1964*b*; Rains and Epstein 1967; Rains 1968; see also Laties 1969). In the case of potassium, there is similarly no saturation of the influx at such low concentrations. The change in slope of the double reciprocal plot occurs where the external concentration is between 3 and 10 mM. If, following the approach of Epstein or Laties, Figure 7(*b*) were taken as representing an enzyme-like "System 1" then the apparent Michaelis constant for this system would be about 1·3 mM. This is much greater than the value of 0·03 mM for maize leaf slices (Rains 1968) and is also greater than the value of 0·2 mM for *Avicennia* (Rains and Epstein 1967). Although it is tempting to consider that the shape of an ion absorption isotherm defines properties of basic nutritional and even ecological significance, there is evidence that the isotherm is affected by prior conditions of growth (e.g. high salt or low salt conditions: see Pitman 1967; Pitman, Courtice, and Lee 1968) and experimental conditions, including interactions between the various ion species in solution (see Robinson and Smith 1970). At the present time the usefulness of the shape of an isotherm as an indicator of a specific ion transport mechanism at a specific membrane must remain in doubt.

(*c*) *Extrapolation to the Intact Leaf*

Under natural conditions the main input of ions into leaves is from the xylem sap, which forms the natural bathing solution for the leaf cells. The ionic concentrations in the individual cells depend on the balance between influx of ions from this bathing solution and efflux into the solution. In the case of phloem cells there may be transport of ions out of the leaf. Otherwise, the only ways in which ions are lost from the leaf are by leaching (which may be considerable: see Tukey 1970) or, in certain cases, by active extrusion through salt-glands.

It was hoped that experiments with leaf slices could provide useful information about one of these processes, namely, influx of ions into leaf cells. As mentioned above, the main difficulty is that slicing may radically alter ion fluxes into and out of the cells. Nevertheless, there is some evidence that cation influx may not be

greatly affected. Van Steveninck (1962, 1964) showed that potassium influx in beet disks did not change significantly for many hours after the disks were sliced. Changes in net potassium movement during aging were due to reduction in potassium efflux. Furthermore, the fact that in *Citrus* potassium influx is much greater than sodium influx (from solutions of equivalent concentration) shows that the cell membranes retain specific permeability properties after slicing.

If the influxes in the slices are similar to those in intact leaves, then many of the results in this and the previous work (Robinson and Smith 1970) are of considerable interest (for example, the findings that there is no effect of light on ion influx, that influx does not saturate at low external concentrations, and that potassium is absorbed in preference to sodium). Further aspects, including effects of aging on ion fluxes, are at present under investigation. Measurements of intracellular ion concentrations and ion efflux will make possible calculations of rates of exchange of ions between cells and the bathing solution. This is of particular importance with regard to salinity problems. The absorption isotherms for chloride (Robinson and Smith 1970) and sodium [Figs. 2(*a*)–2(*d*)] suggest that any increase in the NaCl level outside the cells causes an increased influx of NaCl into the cells. (An increase in the ratio of Na/K in the xylem sap would compound this effect). However, it is not yet possible to say whether net accumulation of NaCl in the cells is reduced by simultaneous efflux of ions into the bathing solution.

Finally, it should be stressed that we are at present completely ignorant of the concentrations of ions outside the cells of the intact leaf. Until techniques for measuring these concentrations are devised it will not be possible to say which of the experimental concentrations used in this work are the most realistic.

V. Acknowledgments

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VI. References

Epstein, E. (1966).—*Nature, Lond.* **212**, 1324.
Jackman, M. E., and Van Steveninck, R. F. M. (1967).—*Aust. J. biol. Sci.* **20**, 1063.
Laties, G. G. (1969).—*A. Rev. Pl. Physiol.* **20**, 89.
Pitman, M. G. (1967).—*Nature, Lond.* **216**, 1343.
Pitman, M. G., Courtice, A. C., and Lee, B. (1968).—*Aust. J. biol. Sci.* **21**, 871.
Rains, D. W. (1968).—*Pl. Physiol., Lancaster* **43**, 394.
Rains, D. W., and Epstein, E. (1967).—*Aust. J. biol. Sci.* **20**, 847.
Robinson, J. B., and Smith, F. A. (1970).—*Aust. J. biol. Sci.* **23**, 953.
Smith, R. C., and Epstein, E. (1964*a*).—*Pl. Physiol., Lancaster* **39**, 338.
Smith, R. C., and Epstein, E. (1964*b*).—*Pl. Physiol., Lancaster* **39**, 992.
Tukey, H. B. (1970).—*A. Rev. Pl. Physiol.* **21**, 305.
Van Steveninck, R. F. M. (1962).—*Physiologia Pl.* **15**, 211.
Van Steveninck, R. F. M. (1964).—*Physiologia Pl.* **17**, 757.
Van Steveninck, R. F. M., and Jackman, M. E. (1967).—*Aust. J. biol. Sci.* **20**, 749.