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ION AND WATER TRANSPORT IN LIMONIUM

II. SHORT-CIRCUIT ANALYSIS

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SUMMARY

Radioisotope fluxes of Na^+, K^+, Cs^+, Rb^+, Cl^-, Br^- and I^- over the salt glands of *Limonium vulgare* have been measured under short-circuit conditions. All these ions are actively transported out of the parenchyma by the gland cells.

The transglandular short-circuit current has been measured in NaCl solution, and is equal to the total net ion current. In choline chloride solution the short-circuit current is increased, and subsequent increases in Na^+ or K^+ concentration in the medium reduce the current to zero. In Cl^--free media, however, there is virtually no short-circuit current. A simple model is proposed in which the ion transfers are coupled.

INTRODUCTION

In a previous paper the driving forces on Na^+, K^+ and Cl^- ions were determined and all these species appear to be outwardly transported by the gland cells. In this paper the analysis is extended by the use of short-circuit techniques to include Cs^+, Rb^+, Br^- and I^-. The short-circuit technique as developed by USSING[1] has been little used in plant physiology owing to the difficulty of obtaining any tissue or preparation with the right geometry; the elegant experiment of BLOUNT AND LEVEDAHL[2] in which the vacuolar compartment of Halicystis was perfused and shorted is perhaps indicative of what can be done with such giant coenocytes. Glandular tissue provides a promising material, and NEMCEK, SIGLER AND KLEINZELLER[3] have measured transglandular potentials and currents in Nepenthes, illustrating that it is essential to consider two transport systems in series *i.e.* ion transport into the parenchyma cells, and subsequent transport from these by the gland cells. In Nepenthes the separate ion transfers seem to be electrogenic and independent, as indicated by the fact that in solutions containing no Cl^- or no Na^+, the short-circuit currents are manifestly present but of opposite polarity.

METHODS

Leaf discs were pretreated overnight in 100 mM solutions of alkali halides in aerated plastic vials, and after removal of the lower cuticle mounted between perspex chambers of cross sectional area 1.76 cm^2. These were connected to calomel electrodes *via* agar-filled polythene bridges. The potential-recording bridges were close to each side of the leaf disc and were connected directly to a high-impedance electrometer. In some experiments the current injection was manually controlled, being adjusted every 2 min, whilst in others the potential-controlling circuit of Fig. 1 was used with which the transglandular potential can be clamped at any desired

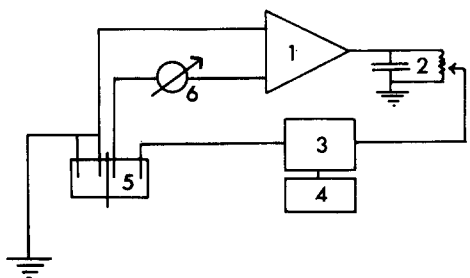

Fig. 1. The potential-controlling circuit 1. Differential amplifier. 2. Feedback control. 3. Microamp recorder. 4. Electronic microcoulometer. 5. Half chambers. 6. Series potentiometer.

value. In the efflux experiments, *i.e.* transport of an ion from the parenchymatic side to the outside, the discs were treated in a radioisotope solution and mounted in the short-circuit chambers with labelled solution on the parenchymatic side and unlabelled solution on the other. The unlabelled solution was then sampled for the appearance of radioactivity. In the influx experiments the discs were pretreated in an unlabelled solution and mounted between the chambers with unlabelled solution on the parenchymatic side and labelled solution on the outside; after the experiment, the discs were removed, lightly rinsed, and stuck to a planchette with vaseline for direct radioassay. All the radioactivity crossing the gland cells is trapped in the parenchyma zone through which diffusion is slow. Experiments lasted 0.5–1 h. Eight leaf discs all from a single plant were used, thus representing a clone. The chambers were mechanically stirred and the whole apparatus Faraday-caged. The isotopes used were ^{22}Na, ^{42}K, ^{137}Cs, ^{82}Rb, ^{36}Cl, ^{82}Br and ^{131}I, supplied by the Radiochemical Centre, Great Britain.

Short-circuit studies were finally made in solutions of potassium or sodium benzene sulphonate, or in choline chloride. Solutions containing for example, Na^+ and Cl^- in varying proportions were also prepared from mixtures of these two salts.

RESULTS

The partial ion fluxes are summarised in Table I, where they are presented as ion currents, flowing over the time span of the experiment. These are compared with the average recorded short-circuit currents in each group. From the results it can be seen that all the ions concerned are actively transported by the gland cells, and the

TABLE I

SHORT-CIRCUIT ION CURRENTS IN LIMONIUM

The current is highly variable, and depends to some extent on leaf age. All values are expressed in μA.

Ion	*Efflux*	*Influx*	*Net flux*	*Short-circuit current*
Na^+	3.03 ± 0.74	−0.33 ± 0.03	2.67 ± 0.23	−1.86 ± 0.39
K^+	2.83 ± 1.17	−0.38 ± 0.09	2.45 ± 1.18	0.06 ± 0.09
Rb^+	14.51 ± 2.33	−2.21 ± 1.24	12.30 ± 2.64	0.48 ± 0.26
Cs^+	15.35 ± 2.62	−3.94 ± 1.12	11.41 ± 2.85	0.58 ± 0.14
Cl^-	−5.72 ± 0.63	0.82 ± 0.50	−4.90 ± 0.80	−1.93 ± 0.19
Br^-	−7.07 ± 1.79	2.44 ± 1.26	−4.63 ± 2.19	−0.14 ± 0.23
I^-	−4.81 ± 1.48	0.84 ± 0.24	−3.97 ± 1.50	0.03 ± 0.06

net flux represents the apparent magnitude of the process. The mean influx is never more than an order of magnitude less than the active flux, and possibly represents diffusion over the whole cuticular area. In the experiments with choline chloride, low salt status discs were mounted in the perspex chambers with the salt at 100 mM concentration. After the establishment of a constant short-circuit current (1–2 h)

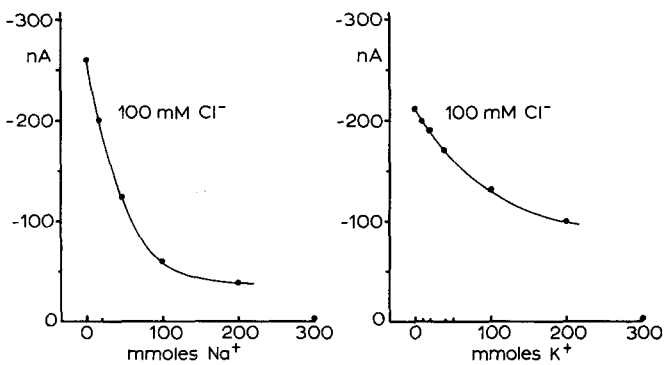

Fig. 2. Left: Effect of independently raising the Na^+ concentration in the presence of 100 mM choline chloride on the short-circuit current. Right: Effect of independently raising the K^+ concentration in the presence of 100 mM choline chloride on the short-circuit current.

the solutions were replaced at 0.5-h intervals by solutions containing progressively higher concentrations of sodium (seven discs) or potassium (six discs), and the resultant currents recorded. A typical experiment is shown in Fig. 2, where the reduction of the current by added sodium or potassium is plotted against its concentration. The current returns approximately to its initial value on replacement of the solution by pure choline chloride, although this may take 10 h due to the fact that the alkali ion has to be pumped out of the intracellular pools. In sodium or potassium benzene sulphonate solution there was no clearly measurable current; in nine experiments it never rose above 0.05 μA. A comparison of short-circuit currents in 100 mM choline chloride, NaCl and sodium benzene sulphonate is made in Table II.

TABLE II

COMPARISON OF SHORT-CIRCUIT CURRENTS IN Cl^-- OR Na^+-FREE MEDIA

	100 mM NaCl	*100 mM sodium benzene sulphonate*	*100 mM choline chloride*
μA (% of NaCl)	100	1.5 ($n = 7$)	189 ($n = 8$)

DISCUSSION

The short-circuit current flowing in an experiment is due to the combined transport of anion and cation; consequently it is of interest to compare the observable current with that calculated from the active fluxes. If agreement is not to be found, then transport of metabolic ions is possibly indicated. In 100 mM NaCl the average net Cl^- current is $-4.90\ \mu$A and this represents an outward transport of the ion; the short-circuit current was $-1.93\ \mu$A. The average net Na^+ current in NaCl was $2.67\ \mu$A, the short-circuit current here being $-1.86\ \mu$A. There is no significant difference between the average short-circuit current and the sum of the Na^+ and Cl^- net currents at the $P = 0.05$ level, and thus the current is accounted for. The active sodium efflux here represents about 50% of the active Cl^- efflux in 100 mM NaCl.

As the other halide ions have similar physiological properties to Cl^- their transport was studied in 100 mM sodium salt solutions. It can be seen that both Br^- and I^- are outwardly transported to a similar extent, and in fact there is no reason to believe that they are not transferred by the Cl^- pump. Both Rb^+ and Cs^+ are outwardly transported like K^+ and Na^+ but the ion current in these experiments was rather high, 11–12 μA. The concentration of these two ions in sea water is quite low (less than $10^{-3} \times [K^+]$) and it would seem unusual for a separate mechanism to exist for their transport; the existence of a separate high-affinity carrier for these ions must remain an open question at present.

In Nepenthes it would appear that the transport of cation and anion are separate electrogenic mechanisms, but the evidence here points towards a coupling of the transports in Limonium. The small size of the short-circuit current in Cl^--free media may well be due to electrical pick-up from surrounding cells, especially as the glands appear to possess numerous plasmodesmatal connections; it is in no way comparable to the 2–3 μA measured by radiosodium or radiopotassium transport. By comparison, the short-circuit current in choline chloride is augmented, and is then progressively reduced by higher concentrations of alkali ions in an asymptotic manner, as indicated in Fig. 2, thus resembling a process which kinetically saturates a transport site; the actual ion concentration at the transport site may not, however, be that in the bathing medium as the glands probably transfer salts from an internal cellular compartment the exact ionic composition of which may be regulated by the plasma membrane. The abrupt decrease of the current to near zero at 300 mM Na^+ or 300 mM K^+ represents a discontinuity in the curves, and may be due to a reversible osmotic effect.

A simple explanation of these results is that the gland cells possess an electrogenic mechanism for Cl^- transfer which has an affinity for Na^+ or K^+, and as the concentration of these is raised, the process approaches neutrality. If such a mechanism exists, it may underlie other reported examples of coupling between cation and anion transfers in plant cells[4,5].

ACKNOWLEDGEMENTS

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