

EXCHANGE AND POLAR TRANSPORT OF RUBIDIUM IONS ACROSS THE LEAVES OF POTAMOGETON

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SUMMARY

The polar transport of rubidium ions across *Potamogeton* leaves was studied in further detail by using labelled and unlabelled rubidium solutions. In the dark no net transport takes place, but there is a considerable exchange of rubidium ions across the leaf. On the other hand, in the light net transfer as well as a simultaneous exchange of the ions occur. A formula for the calculation of the rate of exchange in the presence of net transport was derived. Net transport and exchange may represent two independent parallel processes. If not, it must be concluded that the mechanism of this active transport is essentially different from the mechanism involved in the accumulation of ions into the vacuoles of plant cells.

1. INTRODUCTION

Leaves of *Potamogeton lucens* are able to transport cations upwards across the tissue, provided that assimilation is made possible by illuminating the leaves in the presence of bicarbonate. This phenomenon, discovered by ARENS (1933), has been the subject of investigations by ARISZ and his students in a spasmodic way ever since. The experimental difficulties involved were greatly reduced as perspex became available for the construction of the half-chambers which hold the upper and lower solutions and between which the leaf is mounted. The analytical procedure was facilitated considerably as radioactive isotopes were introduced. The basic results obtained in this way were discussed in our previous article (HELDER & BOERMA 1972). Labelled rubidium was used for reasons of convenience only. However, use of labelled ions renders it possible to study exchange phenomena in addition to net transport. This is an essential advantage, as we know that cations actively accumulated by plant tissue are not exchangeable to any appreciable extent. So, if the polar transport of cations across a *Potamogeton* leaf would resemble this active accumulation process, exchange of cations across this leaf would not be expected to occur.

2. MATERIAL AND METHODS

Essentially the same procedures were followed as in our previous experiments. Single leaves, picked from *Potamogeton* plants kept in a concrete tank in the open, were mounted between two perspex half-chambers each containing 10 ml of a 1 mM. rubidium bicarbonate solution. The vessels were constructed in such a way that 5 cm² of either side of the leaf was in contact with the exper-

imental solutions. The leaf was illuminated by an incandescent lamp, the light of which was filtered by a copper sulphate solution.

The only difference between the experiments reported here and the previous ones was in the use of both labelled and unlabelled rubidium bicarbonate solutions. So, for any further details the reader is referred to our previous article (HELDER & BOERMA 1972).

3. EXPERIMENTAL RESULTS

Exchange of rubidium ions can be studied most easily by putting the leaves into the dark. As there is no active transport under these conditions, it will not interfere with any exchange of the ions. This exchange can be made visible by applying a labelled rubidium solution to one side of the leaf and a similar unlabelled solution to the other one. Which side of the leaf is brought into contact with the labelled solution is of no importance. This is also apparent from the experimental results given by *table 1*.

From this experiment we may conclude that some 2.5 μeq rubidium ions have been transferred by 5 cm^2 of leaf tissue in both directions from one solution to the other within 60 minutes.

This amount represents a net transfer which is the result of a continuous exchange across the leaf, the rate of which is much higher than this net transfer. For, owing to the continuous exchange, the labelled and unlabelled rubidium, originally separated by the leaf, will become intermixed so that finally 5 μeq of labelled rubidium and 5 μeq of unlabelled rubidium will be found on either side of the leaf.

Table 1. Exchange of rubidium ions across a leaf of *Potamogeton lucens* in the dark. A fresh 1 mM. rubidium bicarbonate solution was administered to either side of a single leaf at the start of each of the eight successive experimental periods. Labelled rubidium was applied to either the lower side (A) or the upper one (B).

A	Rb*-content (μeq) at the start of each period	Rb*-content (μeq) at the end of period				Average value (μeq)	Change of Rb*-content (μeq)
		1	2	3	4		
Upper solution	0.00	2.48	2.36	2.38	2.40	2.40	+2.40
Lower solution	10.00	7.90	7.44	7.55	7.45	7.58	-2.42
Total	10.00	10.38	9.80	9.93	9.85	9.98	+0.02
B		5	6	7	8		
Upper solution	10.00	7.50	7.59	7.61	7.35	7.51	-2.49
Lower solution	0.00	2.63	2.58	2.53	2.64	2.59	+2.49
Total	10.00	10.31	10.17	10.14	9.99	10.10	+0.10

This state of equilibrium will be reached at a rate depending on the rate of exchange e . It will be approached exponentially according to

$$Rb^* = \frac{A}{2} (1 - e^{-\frac{2et}{A}})$$

where Rb^* represents the changing amount of labelled rubidium at that side of the leaf to which unlabelled rubidium was administered. The total amount of labelled and unlabelled rubidium present in each half chamber remained constant in dark experiments and is represented in the equation by A .

In our experiments A amounted to $10 \mu\text{eq}$ rubidium, t was one hour, and Rb^* was determined at the end of the experimental period. It follows from the data of *table 1* that the rate of exchange was of the order of $3.5 \mu\text{eq Rb}$ per hour. This, in turn, corresponds to a half time of 1 hour for the isotopic equilibration between the upper and lower solution. For that reason experimental periods of half an hour were often preferred to the longer periods used in studies on net active transport across the leaf.

It seems appropriate to point out here that these results about exchange do not produce much evidence about the underlying mechanism of the exchange-process. E.g., holes in the tissue, however small, will allow diffusion across the leaf. This will lead to an exchange between both solutions through simple mixing.

In order to avoid this mixing as much as possible, all leaves used in our experiments were checked for small visible holes. In addition they were put into the light for one or two hours with labelled solutions administered to both sides of the leaf in order to find out whether they were capable of active transport across the tissue. Another advantage of this procedure was that the leaf tissue itself had become more or less saturated with rubidium, so that during the experiment proper accumulation did not interfere with the movement of ions across the tissue to any appreciable extent.

In *table 2* the results of an experiment are given in which the leaf remained in the light throughout the experiment, after being checked in the way just indicated.

Under these conditions active transport occurred. This is clearly demonstrated by figures obtained in the fourth period. It amounted to $1.7 \mu\text{eq}$ rubidium per 90 minutes, i.e. $1.1 \mu\text{eq Rb/h}$. This corresponds to what is normally found in healthy leaves kept under the same conditions as in these experiments.

However, if unlabelled rubidium had been administered to the upper side of the leaf, the increase of the labelled rubidium content of the upper solution was much greater than could be accounted for by active transport alone. Clearly, exchange of unlabelled rubidium for labelled rubidium had taken place in addition to active transfer.

This is also apparent from the third period, in which labelled rubidium was applied to the upper side only. Owing to exchange some $1.6 \mu\text{eq}$ labelled

rubidium was found in the lower solution at the end of the experimental period, in spite of the fact that active transport of rubidium ions from the lower to the upper solution must have taken place in this period.

From the data obtained in the first and last period we see that about 3.4 μeq labelled rubidium had been transferred from the lower to the upper solution owing to the combined effect of active transport and exchange.

Table 2. Active transport and exchange of rubidium ions across the leaf of *Potamogeton*.

At the start of each experimental period 10 μeq rubidium bicarbonate was administered to either side of the leaf. Labelled and unlabelled solutions were used as indicated.

At the end of each period the labelled rubidium content was determined. By subtracting from these values the amount administered at the start of the period (0 or 10 μeq Rb^*) the change of the Rb^* content was obtained.

	Treatment in successive 90 min. experimental periods				
	1	2	3	4	5
Upper solution	Rb^0	Rb^0	Rb^*	Rb^*	Rb^0
Lower solution	Rb^*	Rb^0	Rb^0	Rb^*	Rb^*
	Change of Rb^* -content (μeq) of the solutions				
	1	2	3	4	5
Upper solution	+3.07	+0.12	-1.81	+1.69	+3.49
Lower solution	-3.33	+0.05	+1.64	-1.68	-3.45

As 1.6 μeq Rb was transferred by active transport (fourth period) it follows that an amount of 1.67 μeq rubidium must have been transferred as the result of some exchange mechanism. Consequently, an equal amount of rubidium must have been transferred from the upper solution to the lower. This is confirmed by the results obtained in the fourth period, where 1.64 μeq rubidium was found in the lower solution.

In these calculations it has been assumed tacitly that the rubidium content of the leaf tissue does not change, so that the changes in the solutions can not be attributed to a net gain or loss of the rubidium by the leaf tissue.

It can be seen from the data obtained in the second period that this assumption is justified. There is only a slight release of rubidium previously absorbed by the tissue. The amounts are hardly significant and, at any rate, small as compared to the amounts involved in the transfer processes.

The amounts transferred by exchange, i.e. 1.67 and 1.64 μeq , mentioned above, do not represent the rate of exchange. They are merely the net result of the transfer processes. However, as active transport goes along with exchange phenomena, the calculation of the rate of exchange from the experimental data is much more complicated here than it was for the results of the dark experiments. For that reason the calculation will be postponed till after the mathematical treatment of this problem in the general discussion.

4. DISCUSSION

Rubidium bicarbonate was administered to both the upper and the lower side of the leaf. In the experiments discussed here the amounts were equal for both sides, i.e. 10 ml of a 10 mM. rubidium bicarbonate solution, which corresponds to an amount of 10 μ eq rubidium ions. However, for the sake of clearness this amount will be marked by I_0 and S_0 according to whether the lower or the upper solution is involved.

Owing to active transport the rubidium content of the lower solution I_t will decrease steadily, whereas the reverse applies to the content of the upper solution, S_t .

Exchange of rubidium ions across the leaf became apparent from experiments in which only one of the two solutions was labelled. However, as a result of the exchange mechanism labelled as well as unlabelled rubidium will be found in both solutions in the course of time. The amounts will be indicated by I_t^0 , I_t^* , S_t^0 and S_t^* , so that we have $I_t = I_t^0 + I_t^*$ and $S_t = S_t^0 + S_t^*$.

For our present discussion it is immaterial which solution administered to the leaf was labelled. If the lower solution is chosen for the purpose we have:

$$I_0 = I_0^* = S_0 = S_0^0 = 10 \mu\text{eq and } S_0^* = I_0^0 = 0 \mu\text{eq.}$$

Seeing that active net transport of ions goes along with an exchange of these ions, we must conclude that there exists an upward flux and a downward flux and that the rate of the former one i surpasses the rate e of the latter by an amount equaling the rate of net transport a (fig. 1A). Moreover, the leaf tissue itself proved to consume such a small amount of the ions taken up under the pertaining conditions prevailing in the experiments that it will be neglected here. This simply means that the sum of the amounts of labelled rubidium ions found in both solutions remained constant, i.e. 10 μ eq in these experiments.

This also applies to the amount of unlabelled rubidium ions. This is an important aspect of the experimental results because it considerably facilitates further mathematical treatment.

For this treatment the following simple relations between the various quantities, dealt with so far, have proved to be essential:

$$a = i - e \tag{1}$$

$$I_t = I_t^0 + I_t^* = I_0^* - at \tag{2}$$

$$S_t = S_t^0 + S_t^* = S_0^0 + at \tag{3}$$

$$I_0^* = I_t^* + S_t^* \tag{4}$$

We now wish to express the rate of change of the amount of labelled rubidium in the upper solution S_t^* in terms of the time. We already know that for $t = 0$ the labelled rubidium content amounts to $S_t^* = S_0^* = 0$.

At any moment during the experimental period S_t^* will be increased by the upward flux carrying labelled rubidium ion from the lower solution into the upper solution, whereas S_t^* will decrease simultaneously by the opposite downward flux.

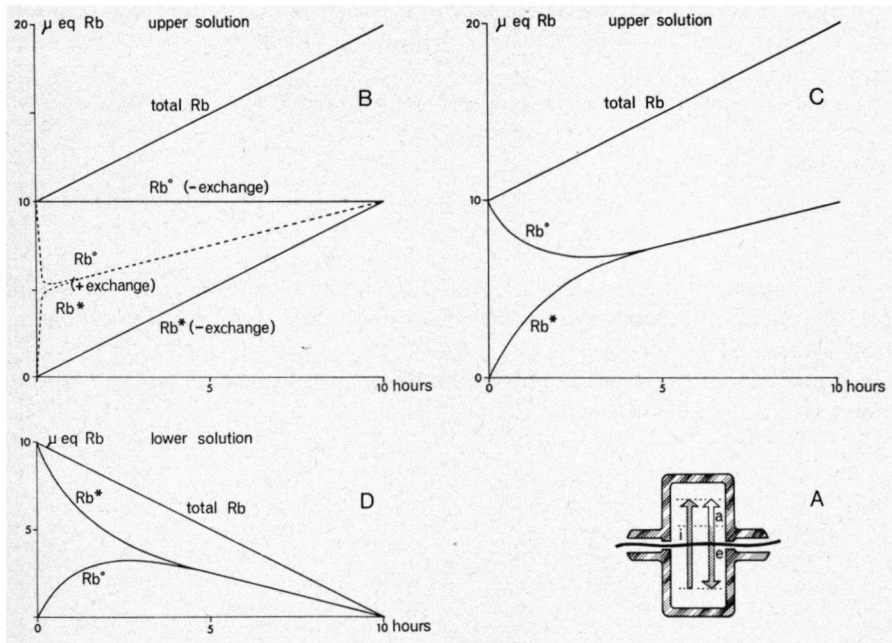


Fig. 1. The theoretical course of the rubidium content of the bicarbonate solutions contacting the upper and the lower surface of a *Potamogeton* leaf, which is kept in the light to induce active polar transport.

A: diagram of the experimental set-up. To either side of the leaf 10 ml of a 1 mM. $RbHCO_3$ solution is administered. Rb^* is present only in the lower solution. The subsequent changes in the distribution of Rb^0 and Rb^* are due to net transport from the lower solution to the upper one and to exchange between the solutions across the leaf. The rate of net transport a is taken to be of the order of $1 \mu eq/h$. It depends on the rates of the two opposite fluxes i and e .

B: Change of Rb-content of the upper solution if there is no exchange or a very fast one, respectively.

Total Rb will increase steadily until all Rb has been transferred from the lower solutions into the upper one.

Assuming no exchange ($e = 0, i = a$), Rb^0 -content will remain constant ($10 \mu eq$) throughout the experiment, whereas the Rb^* -content will increase until the lower solution has become depleted.

Assuming a very fast exchange (e very large as compared to a), mixing of the two solutions will be completed within a few minutes. Thereafter, both the Rb^0 -content and the Rb^* -content will increase at a constant rate ($0.5 a$) until the lower solution becomes exhausted (broken curves).

C: Course of Rb-, Rb^0 - and Rb^* -content of the upper solution starting from rate figures as found in actual experiments ($a = 1, e = 4$).

D: Course of Rb-, Rb^0 - and Rb^* -content of the lower solution corresponding to the data for the upper solution (C).

The extent of the increase is determined by the flux-rate i and the composition of the lower solution, in particular to the ratio of labelled ions I_t^* to the total rubidium ions I_t .

Similarly, the rate of decrease of S_t^* is determined by e and the ratio of S_t^* to S_t .

Therefore we have

$$\frac{dS_t^*}{dt} = i \frac{I_t^*}{I_t} - e \frac{S_t^*}{S_t} \quad (5)$$

In this equation the variables I_t^* , I_t and S_t can be substituted with the aid of (4), (2), and (3), respectively. In this way a linear first-order differential equation is obtained.

In order to get the solution into a simplified form the quantities S_t , I_t and a were introduced again by using (3), (2) and (1).

We obtained:

$$S_t^* = \frac{1}{2} S_t - \frac{I_t \frac{i}{a}}{S_t \frac{e}{a}} \quad (6)$$

The solution is given graphically in *fig. 1*. In addition this figure shows the time course of S_t^0 , I_t^0 and I_t^* . The equations for these variables can be derived from (6) by simple algebraic manipulations.

A similar set of four equations can be produced for an experiment in which the treatment is reversed, i.e. in which labelled rubidium is administered to the upper side and unlabelled rubidium to the lower side. Only the symbols for labelled and unlabelled have to be interchanged in the expressions given here.

This mathematical treatment starts from two assumptions, both of which may reduce its usefulness. Firstly, it is assumed that the rate of net transport across the leaf remains constant until all rubidium has been moved from the lower solution into the upper. In fact, this process slows down from the beginning of an experimental period. However, in many experiments this slowing down proved to be small enough during the first few hours to justify the use of the formula.

Another simplification pertains to the neglect of the rubidium present within the tissue. The amount of tissue rubidium involved in the polar transport is small and for that reason we do not consider the neglect to be a serious objection against the analysis of the experimental results discussed here.

If formula (6) is applied to the data obtained in the third and fourth period of the experiment of *table 2* the following figures were obtained: rate of net transport $a = 1.1 \mu\text{eq Rb/h}$ and rate of exchange $e = 1.9 \mu\text{eq Rb/h}$.

It can be seen that the rate of exchange is quite impressive as compared to the rate of net polar transport. This strongly suggests that the movement of the

cations across the tissue of *Potamogeton* leaves is a passive one.

This conclusion has received strong support by some recent measurements of the electric potential difference across the leaf tissue.

In contrast to a statement by STEEMANN-NIELSEN (1960) we found that, although there is no potential difference between the two solutions on either side of the leaf at the start of the experimental period, the potential of the upper solution gradually became negative with respect to the lower solution.

Unfortunately, these measurements were made when the activity of the leaves had already been reduced because of the weather conditions. More experimental evidence to check these preliminary results will be collected as soon as the appropriate plant material is available.

Apart from the lack of the convincing experimental evidence just mentioned one objection should be raised against the hypothesis of a purely passive movement of cations. The small value for the rate of net transfer of rubidium ions as compared to the exchange-rate of these ions suggests that although the greater part of the ions may move along adsorption tracks, a small fraction may move along quite different tracks. So, it remains conceivable that the rubidium ions involved in the bicarbonate relations of the leaf tissue are subjected to an active transport mechanism, which is masked by the passive exchange-mechanism.

However, recent experiments on the interaction between calcium and rubidium ions with respect to polar transport and exchange have not furnished much evidence in support of this possibility.

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