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POLAR TRANSPORT OF LABELLED RUBIDIUM IONS ACROSS THE LEAF OF POTAMOGETON LUCENS

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SUMMARY

Leaves of *Potamogeton lucens*, surrounded by a 1 mM rubidium solution, take up rubidium ions at their lower surface and release these ions into the upper solution.

Only small amounts of the ions taken up remain fixed within the leaf tissue, so that the greater part of the rubidium ions are transported across the leaf in one direction.

The above-mentioned polar transport depends on light and the presence of bicarbonate. However, it also occurs when rubidium bicarbonate is only applied to the lower surface of the leaves.

The results so far obtained are explained by the following simple hypothesis. Concentration gradients are developed owing to the bicarbonate assimilation in the light, causing the bicarbonate ions to be taken up and the hydroxyl ions formed to be released. These anions are accompanied by rubidium ions.

So, according to the hypothesis a completely inactive movement of cations as well as anions is involved. The polarity of the process is due to a difference in anion permeability between the lower and upper layers of the leaf tissue.

1. INTRODUCTION

A number of aquatics can assimilate bicarbonate ions in addition to normal carbon dioxide. Consequently, the pH increases to the value of 10 or even 11 when the leaves are allowed to assimilate in a restricted volume of a solution containing bicarbonate.

On the other hand, if a stream of nitrogen free from carbon dioxide is led through a solution containing bicarbonate as well as carbon dioxide, all of the gaseous carbon dioxide is removed.

This is also accompanied by a rise of the pH. However, the highest value reached in this way is approximately 9. At this value the solution will contain such a small amount of free carbon dioxide that any further removal of carbon dioxide appears to be practically negligible.

Similarly, some species of aquatics are unable to raise the value of the pH above 9. Apparently they assimilate only free carbon dioxide and are unable to use the bicarbonate ions for this purpose (RUTTNER 1948, STEEMANN NIELSEN 1960).

Additional evidence for bicarbonate assimilation was found by ARENS (1933). He found the exceptional increase of the pH value to occur only in the solution which was in contact with the upper side of the leaves.

It is unlikely that gaseous carbon dioxide can only penetrate the upper surface.

Further this release depends on the presence of bicarbonate and it suffices to apply the bicarbonate to the lower surface of the leaves.

As a result one can conclude that the rise of the pH is due to a release of some alkaline substance. Hardly any change of the pH is observed at the lower surface. This indicates that the bicarbonate ions are accompanied by an equivalent amount of cations in the process of absorption.

Under natural conditions there will be an abundance of calcium ions. Their release into the upper solution, together with the increase of the pH mentioned earlier, causes the formation of a precipitate, so commonly observed in aquatics.

The resulting polar transport of calcium and other cations was the starting point for our experiments, part of which will be discussed here. We have concentrated on the experiments with labelled rubidium, as they have corroborated and extended the findings known from literature.

Besides they were the starting point for experiments on exchange, the bicarbonate transport, the pH changes of the medium, and the evolution of oxygen, the results of which will be published in due time.

We hope in this article to draw the attention of the plant physiologist to the older papers mentioned above which, we think, have had too little attention.

2. MATERIAL AND METHODS

Single leaves were picked from plants of *Potamogeton lucens* one day before the actual experiment. The plants were grown in the open air in a large concrete basin measuring $2 \times 1 \times 0.8$ metres.

The plants started growing from the rhizomes in May and formed a dense vegetation filling the basin completely within the space of a month. At that time a slight precipitate of calcium carbonate on the upper surface of the leaves became clearly visible, indicating that bicarbonate assimilation and polar cation transport had made a start.

Depending on the weather conditions, the shoots started dying off in September or October. So, experimental material was available for about four months only. Attempts to extend this period by subjecting the rhizomes to a cold treatment or by growing plants in a greenhouse were not succesful until now.

The second or third leaf from the top proved most suitable for these experiments. It is very active and sufficiently large and flat which renders it ideal for the experimental set-up described below. Younger leaves are often too small whereas older leaves are not flat enough. Moreover, the latter are often more or less perforated owing to the activity of snails.

After picking, the precipitate on the leaves was removed gently. Then the leaves were placed in aerated distilled water and kept in the dark overnight in order to get rid of any calcium carbonate left on the leaves.

A leaf was then mounted between two experimental absorption vessels with the aid of a mixture of vaseline and bees' wax to ensure that the set-up was water-tight. The details of the vessels, which were made of perspex, can be seen

in *fig. 1*. The leaf area exposed to the experimental solution is 5 cm^2 on either side of the leaf.

For reasons of convenience the leaf is placed in an upright position with the upper side facing the light source.

In a few instances the lower side happened to be directed towards the light source by mistake. This, however, did not have any influence on the results at all as the polarity is not induced by the direction of the light.

During the experiment itself 5 ml of a labelled $RbHCO_3$ 1 mM solution was added to either side of the leaf during regular one-hour intervals. At the end of each interval the solution was drained off, the vessels washed a few times, the liquid collected in a 50 ml volumetric flask and made up to volume.

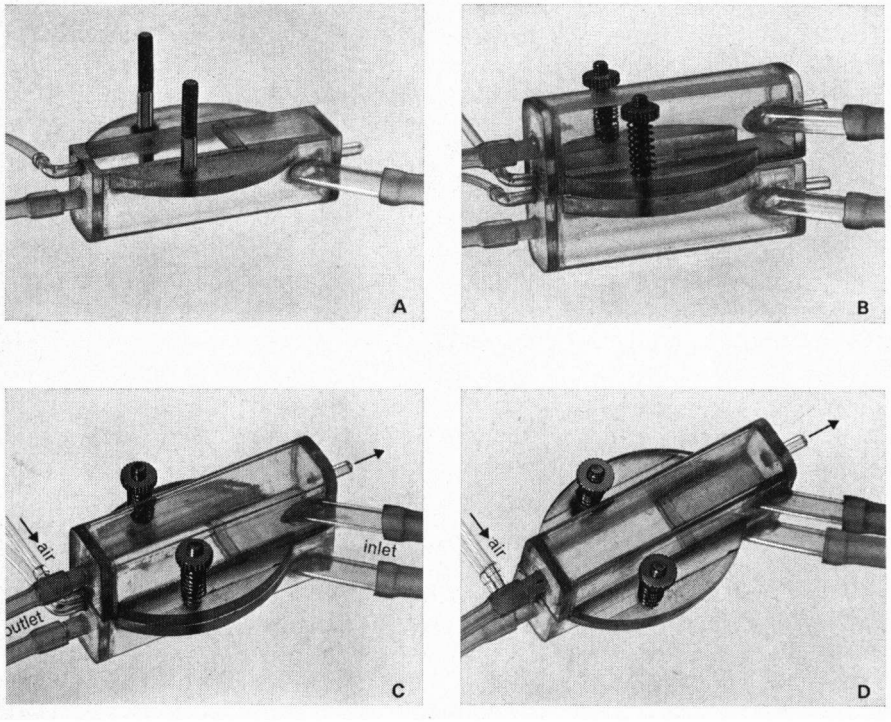

Fig. 1. Absorption vessels used for the study of ion transport across leaves of *Potamogeton*

A: The lower vessel. A leaf is put onto the opening so that its lower side may contact the solution administered afterwards. B. The upper vessel placed on the upper side of the leaf and screwed onto the lower vessel. The springs ensure a more even pressure of the vessels on the leaf. C: Solutions are added and renewed via the inlet and the outlet. For the purpose the set-up is used in an up-right position. D: The solution is stirred and aerated by an air stream.

The leaf area exposed to the experimental solution via the opening was the same i.e. 5 cm^2 in all experiments.

The other dimensions of the vessels varied, however, and so did their capacity. For reasons of clarity the bigger ones of 10 ml capacity are shown here.

For obvious reasons calcium was omitted from all liquids used, although there can be little doubt that it would have been of some benefit to the condition of the plant material.

Changes in Rb* concentrations were determined by simple scintillation counting. From this data the simultaneous uptake and release of Rb* could be calculated as well as the net accumulation within the leaf tissue. The latter results could be checked by analysing the leaf at the end of the experiment. For that purpose the leaf was extracted with hot dilute nitric acid. From this check we learned that all activity measurements had to be done very accurately which made them tedious in spite of their simplicity.

As a light source a 150 W incandescent lamp with an internal reflecting surface was used. The light was passed through a 5 cm layer of a copper sulphate solution the strength of which was adjusted so that most of the infrared radiation was absorbed.

Light intensity was adjusted by inserting simple neutral glass filters in a holder. The maximum intensity measured by a Braun meter at the place of the absorption vessels amounted to 3000 lux of a greenish-bluish light.

3. EXPERIMENTAL RESULTS

In a number of experiments the behaviour of the rubidium ions was studied under constant conditions of temperature (20 °C) and light (3000 lux) during a 9 hours period. On the hour a fresh solution was added and the old solution was collected at the end of each hour and analysed.

As 5 ml of a 1 mM Rb* HCO_3 solution was applied to both the upper and the lower sides of the leaf, exactly 5 μeq Rb* was available for absorption on either side of the leaf. In all instances the concentration of the lower solution decreased, whereas the one of the upper solution increased. This decrease and increase will be referred to as uptake and release, respectively.

As is illustrated by *fig. 2* the uptake was high at the start of the experiment, increased a little during the second hour, and decreased steadily, though very slightly, during the remaining experimental period.

The release figures show a similar picture. During the first two hours the uptake figures exceeded the release figures, indicating that a small amount of rubidium was retained by the leaf tissue.

From the third hour onwards no significant differences occurred. During this period the uptake as well as the release figures represent the polar transport across the leaf tissue.

At the start of each hour the concentrations on both sides of the leaves were equal. However, because of polar transport the concentration of the upper solution exceeds the concentration of the lower one, so that transport takes place against a concentration gradient.

In some preliminary experiments, solutions of rubidium chloride were also tested. Here no polar transport was observed and the net amounts accumulated within the leaves were smaller than in the case of bicarbonate solutions.

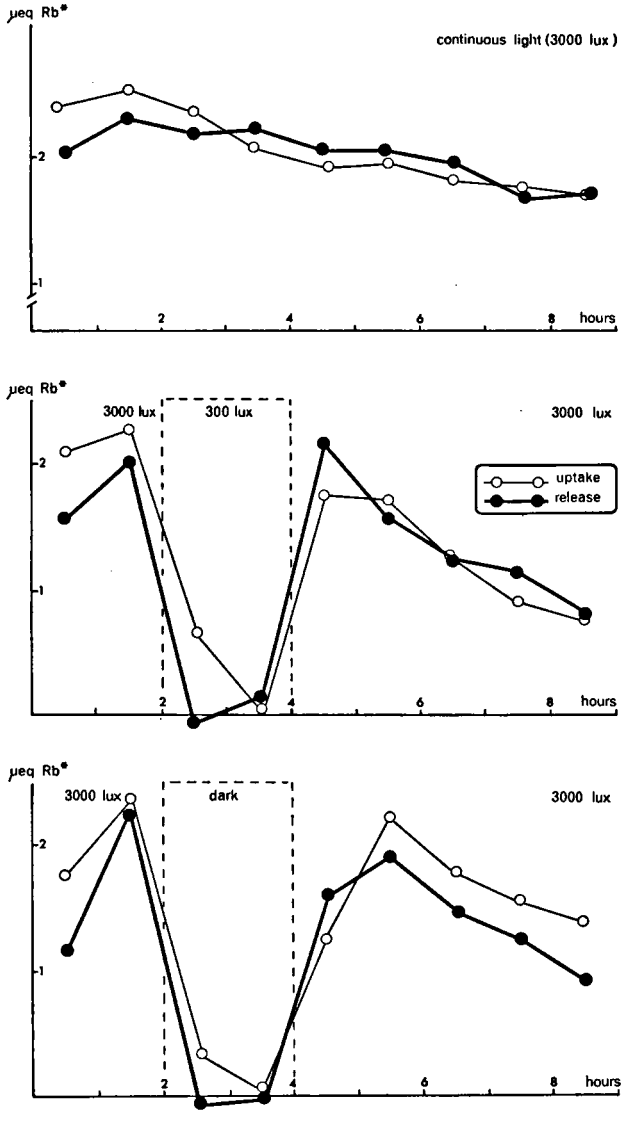

Fig. 2. The influence of light on the course of the rubidium transport across a leaf of *Potamogeton lucens.*

A 1 mM labelled rubidium bicarbonate solution was added to both sides of a leaf, using the set up shown in fig. 1. The solutions were renewed each hour.

Uptake refers to the drop of the rubidium concentration of the lower solution, whereas release refers to the observed increase of the upper solution.

The differences between the release and uptake values represent the rate of rubidium accumulation within the leaf tissue.

The figure is based on the results of three selected experiments with three different leaves, showing about the same rate of transport during the first two hours of the experiment.

The effect of bicarbonate is undoubtedly related to assimilation in the light. This is also apparent from the other experiments represented in *fig. 2*, in which the light intensity was reduced during the third and fourth hour of the experiment.

If the leaf was placed in the dark, polar transport came to an immediate stop. The uptake from the lower solution, however, continued for a while, although at a much reduced level, and came to a stand-still not before the end of the first hour. Consequently, the rubidium content of the leaf must have increased in the dark.

If, however, the light was turned on again, both uptake and release started afresh, but now the release surpassed the uptake during the first hour in the light. As a result the Rb*-content of the leaf decreased during this part of the experiment.

This increase of uptake and release was continued during the second hour after a treatment in complete darkness. The level reached was almost equal to the level in the first two hours of the experiment, before the light was switched off.

During the remaining hours of the experiment a gradual slowing down, similar to that found under conditions of continuous light, could be observed.

In a number of experiments distilled water was administered to the upper side of the leaf instead of a rubidium bicarbonate solution. The results concerning the effect of light and darkness were virtually the same as may be illustrated by the following experiment.

In this experiment the liquids were renewed every two hours. During the second two-hours period the leaf was kept in the dark.

The following figures for the release into water at the upper side and uptake from the rubidium solution at the lower side were obtained:

Period	0–2 h. light	2–4 h. dark	4–6 h. light	6–8 h. light
Release	2.1	0.7	1.3	3.5 μ eq Rb*
Uptake	2.7	1.2	1.4	3.6 μ eq Rb*

These figures show that a dark treatment reduced the polar transport as it did in the experiments of *fig. 2*. The induction period after the dark treatment is even more pronounced, whereas the high rate at which the transport is finally reached, is most striking.

In other experiments the upper side of the leaf was left in contact with the rubidium solution, but at the lower side the solution was changed for water. In contrast with the previous experiments polar transport stopped altogether.

In one experiment, in which the rate of transport was of the order of 3 meq Rb*/h, a slight amount of Rb* previously absorbed by the leaf was given off to water (0.3 m eq), whereas there was a very slight uptake (0.1 m eq) from the upper solution. The amount can hardly be considered significant.

We repeated these experiments by using a rubidium sulphate solution instead of distilled water. The results were quite similar to those discussed, indicating that the effects obtained were mainly due to the absence of bicarbonate.

Polar transport was resumed when the water or the rubidium sulphate solution present at the lower side was replaced by the normal rubidium bicarbonate solution. However, in many instances the results suggested that the water and sulphate treatments had been somewhat harmful to the leaf. We concluded that the resistance against such treatments varied from leaf to leaf, depending on their internal conditions.

4. DISCUSSION

The findings concerning the polar transport of rubidium ions corroborate the older results of ARENS (1933) on the behaviour of cations in *Potamogeton* leaves. However, from a quantitative point of view more details have become available.

The transport could take place against a concentration gradient and no significant increase of the rate of transport could be obtained by changing the upper rubidium solution for water. It shows that the rate of transport is highly independent of the prevailing rubidium concentration gradient. It can be concluded, as there seems to be no electrical potential difference across the leaf tissue (STEEMANN NIELSEN 1960), that the polar transport represents an active process. However, this statement does not exclude the possibility that, on further analysis, the cation transport will prove to be coupled to an active transport of anions.

Clearly, light is the primary source of energy for active cation transport dealt with in this paper. But, if the light was switched on and off, the release of rubidium at the upper leaf surface responded more rapidly to the light conditions than did the uptake at the lower surface.

The simplest conclusion at which one can arrive from this evidence is that the release represents the truly active part of the transport across the leaf. In other words, the transport depends on active secretion by the upper cell layers of the leaf tissue.

This idea has been advocated by LOWENHAUPT (1956), who relates this secretion activity with the well-known sodium pump, assumed to be present in other, mainly animal, tissues. On the other hand, STEEMANN NIELSEN (1960) claims that it is the hydroxyl ions formed as a result of bicarbonate assimilation which are secreted actively.

According to this view the secretion lowers the rubidium content of the leaf which in turn induces the uptake at the lower surface of the leaf.

However, one can also explain the different responses of the release and uptake processes by assuming that the uptake depends on an energy source which becomes depleted in the dark more slowly than the source for the release process.

The polar cation transport depends on the availability of bicarbonate in the

lower solution. As light is also required, there is little doubt that the assimilation of bicarbonate represents the crucial active process responsible for the phenomena observed.

One can imagine that the assimilation is the only active part of the transport processes discussed here. We think it also leads to the simplest explanation for the phenomena observed. That was the reason for accepting this idea as a part of the following hypothesis.

The consumption of bicarbonate creates a concentration gradient for both the bicarbonate and the hydroxyl ions between leaf tissue and the surrounding solution.

If the lower cell layers of the leaf are rather more permeable to bicarbonate ions and the upper cell layers more permeable to hydroxyl ions, the gradients will cause the bicarbonate ions to enter at the lower surface of the leaf, the hydroxyl ions to be released into the upper solution.

The anions moved in this way will be accompanied by equivalent amounts of cations. This leads to a transport of the cations from the lower solution across the tissue to the upper solution.

It might be argued that the assumed differences in permeability of the leaf tissue to bicarbonate and hydroxyl ions are somewhat unlikely. Also the difference between the response of the uptake and that of the release to changes in light conditions, discussed before, are somewhat at variance with our hypothesis.

Still, we believe that much more convincing experimental evidence is needed to discount the simple explanation given here.

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REFERENCES

ARENS, K. (1933): Physiologisch polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen I. *Planta* **20**: 621–658.

— (1936a): Physiologisch polarisierter Massenaustausch bei submersen Wasserpflanzen II. Die $Ca(HCO_3)_2$-Assimilation. *Jahrb. wiss. Bot.* **83**: 513–560.

— (1936b): Photosynthese von Wasserpflanzen in Kaliumbikarbonatlösungen. *Jahrb. wiss. Bot.* **83**: 561–566.

LOWENHAUPT, B. (1956): The transport of calcium and other cations in submerged aquatic plants. *Biol. Review* **31**: 371–395.

RUTTNER, F. (1948): Zur Frage der Karbonat-Assimilation der Wasserpflanzen. Eine vergleichende Untersuchung. I: Die beiden Haupttypen der Kohlenstoffaufnahme. *Österr. bot. Z.* **94**: 265–294.

STEEMANN NIELSEN, E. (1960): Uptake of CO_2 by the plant. In: *Encyclopedia of Plant Physiology* **V/1**: 70–84. Berlin. Springer-Verlag.