UNIVERSITY OF
CALIFORNIA

*Radiation
Laboratory*

ACTIVE CATION TRANSPORT IN SUBMERGED
AQUATIC PLANTS.

I. EFFECT OF LIGHT UPON THE ABSORPTION
AND EXCRETION OF CALCIUM BY
POTAMOGETON CRISPUS (L) LEAVES

BERKELEY, CALIFORNIA

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

0

UCRL-3460
Distribution:
Health and Biology

UNIVERSITY OF CALIFORNIA

Donner Laboratory of Biophysics and Medical Physics
Berkeley, California

Contract No. W-7405-eng-48

ACTIVE CATION TRANSPORT IN SUBMERGED AQUATIC PLANTS.
I. EFFECT OF LIGHT UPON THE ABSORPTION AND EXCRETION
OF CALCIUM BY POTAMOGETON CRISPUS (L) LEAVES

Benjamin Lowenhaupt

July 5, 1956

Printed for the U. S. Atomic Energy Commission

ERRATA

Please make the following corrections and additions to your copy of UCRL-3460.

<u>Page</u>	<u>Line</u>	<u>Change</u>
2	12, 13	Sentence should read: Movement is into the <u>abaxial</u> (morphological under) side and out of the <u>adaxial</u> (morphological upper) side.
5	1	... the external <u>medium</u> becomes...
5	6	... the <u>cyclical</u> repetition...
6	9	... hydrogen peroxide (5×10^{-2} <u>millimoles</u> total;...
7	12	There is a <u>contrast</u> , however,...
8	3d from bottom	4. There is <u>a</u> material, labile...
10	Fig. 1	For "ventral side up" write " <u>abaxial</u> side up." For "dorsal side up" write " <u>adaxial</u> side up."
11	legend	<u>Add:</u> Solid line: adaxial leaf faces in contact with the medium. Broken line: abaxial leaf faces in contact with the medium.

ACTIVE CATION TRANSPORT IN SUBMERGED AQUATIC PLANTS.
I. EFFECT OF LIGHT UPON THE ABSORPTION AND EXCRETION
OF CALCIUM BY POTAMOGETON CRISPUS (L) LEAVES

Benjamin Lowenhaupt

Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

July 5, 1956

Abstract

The dependence upon illumination of calcium transport in Potamogeton crispus leaves has been studied. Four experiments are reported.

Experiment 1 shows that calcium is moved through illuminated leaves, but not darkened leaves. Movement is into the (morphological under) side and out of the (morphological upper) side.

Experiment 2 shows that floated leaves accumulate calcium in the light when the abaxial faces touch the aqueous medium and, in contrast, they excrete it when the adaxial faces touch the medium. The calcium moves in the reverse directions when the light is turned off. The explanation is suggested that the reactions of calcium transport are driven by photosynthesis but assume equilibrium in the dark.

Experiment 3 shows (as would be expected) that the calcium content of submerged leaves is different in the light (when transport is functioning) from in the dark (when it is not).

Experiment 4 shows that the equilibrium calcium content, in the dark, can be altered. Darkened leaves hold more calcium after exposure to H_2O_2 than before; subsequently light releases calcium. The explanation is suggested that a material that is destroyed by H_2O_2 shifts the equilibrium of the transport reactions toward calcium release. Light apparently is necessary for synthesizing this material.

ACTIVE CATION TRANSPORT IN SUBMERGED AQUATIC PLANTS.
I. EFFECT OF LIGHT UPON THE ABSORPTION AND EXCRETION
OF CALCIUM BY POTAMOGETON CRISPUS (L) LEAVES

Benjamin Lowenhaupt

Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

July 5, 1956

Introduction

Active transport* in submerged aquatic plants has been little studied. This is unfortunate because several aspects of their structure and physiology are nearly made to order for transport studies. A series of experiments has been initiated that is designed to exploit effects of light under several conditions.

Previous studies of cation transport in aquatics have all been in conjunction with photosynthesis studies. Two names are notable in this connection, Arens and Steemann Nielsen. Arens (1930) (1933) (1936a) (1936b) began his investigations to ascertain why many aquatic leaves become encrusted with lime on the adaxial surface. He demonstrated that leaves that become thus encrusted can photosynthesize bicarbonate (in addition to carbon dioxide) from the external medium and that movement of cations through the leaf from the abaxial (morphological lower) to the adaxial (morphological upper) side is associated with this photosynthesis. He found precise equivalence between the bicarbonate assimilated at the abaxial surface and the cations transported. Steemann Nielsen (1944) (1946) (1947) (1951) (1952) refined Arens's experiments so that there can now be no question of the reality of cation transport or of the stoichiometry between bicarbonate assimilation at the abaxial surface and transport. Ruttner (1953) has reviewed the evidence for bicarbonate assimilation in submerged aquatics, and Österlind (1949) has summarized the history of the study of photosynthesis in aquatics.

The relationship between photosynthetic assimilation of bicarbonate and cation transport affords little doubt that light is linked to transport through photosynthesis. All previous efforts to explain this link have invoked an anion

* Transport or active transport refers to the metabolic pumping of material across an active membrane.

pump, whereas cations were assumed to be carried electrochemically. The emphasis on anions probably was the result of the experimental emphasis on photosynthesis. This made observations of cation movement seem secondary to uptake of the bicarbonate anion. I have rejected this view, however, partly because there is no evidence for anion transport through the leaf. Instead I have suggested that there is cation transport, powered by photosynthesis, and that associated biochemical reactions entail a carboxylation that uses bicarbonate ions. It is impossible to present this theory fully here, but it has been considered in another paper (Lowenhaupt, 1956). The data presented here are some of the observations from which the theory was inferred.

Material and Methods

Potamogeton crispus plants were grown in an artificial pond. For each experiment healthy-looking, full-grown leaves were brought into the laboratory.

Calcium-45, as obtained from the Atomic Energy Commission, 15 to 20 mc/g, was purified by precipitating out added zinc and copper as the sulfides and then precipitating the calcium-45 by adding oxalate. The calcium-45 oxalate was dried, weighed, ashed, and dissolved in dilute hydrochloric acid. The excess acid was evaporated and the residue redissolved in water. This yielded a calcium chloride solution approximately neutral and of known concentration.

The experiments required quantitative comparisons for calcium; they were made by radioactivity measurements. This involved placing measured aliquots of solution into a counting cup, adding water to spread the solution, evaporating the water, and estimating the radioactivity with a Geiger-Müller counter. If radioactivity were proportional to the amount of calcium, i.e., if specific radioactivity did not change, the amount of the element in an unknown sample might be calculated by the ratio of its radioactivity to that of a standard sample.* Changes in specific radioactivity did occur, however, because the leaves contained nonradioactive calcium, some of which was excreted during transport while radioactive calcium from the medium was being absorbed. Experience has shown, though, that the specific activity of

* Autoabsorption can be ignored because the mass of material in the counting cups was small.

the external medium becomes constant after a few hours, provided the leaves are illuminated, and thereafter any changes in radioactivity may be considered proportional to changes in calcium content. Evidence for this includes

- (a) the constancy of radioactivity of the medium under uniform conditions, and
- (b) the cyclical repetition of radioactivity changes, corresponding with cyclical changes in the environment.

Both of these have been observed frequently. Accordingly leaves were pre-treated to bring specific radioactivity to constancy throughout the system before experimental procedures were instituted. Radioactivity of the medium, not of the leaves, was measured. The radioactivity of the leaves was calculated by subtracting radioactivity of the medium from the total radioactivity.

In the experiments whose results are summarized in Fig. 1, leaves were floated on an approximately neutral solution of calcium-45 bicarbonate, some leaves abaxial face up, some adaxial face up. Drops of this solution were placed on top of the leaves and the dish covered with a watch glass and left in the light for about 1.5 hours. Radioactivity per unit volume of the drops on top of the leaves was compared with that of the solution underneath, the latter being taken as 100%. Check experiments were run in the dark with the same leaves after they had been in darkness several hours prior to placing the drops on top.

In the experiments whose results are summarized in Fig. 2, leaves that had been in darkness for some time were floated with minimal exposure to light on calcium-45 chloride solution (2 ml; 6×10^{-4} M), either with all the abaxial faces touching the solution or with all the adaxial faces touching. Thus only one side of any set of leaves had the potentiality of transport, the other side being dry. Darkness was continued for about 2 hours and after this the periods of light and darkness were alternated, as indicated in Fig. 2. At intervals throughout the experiment radioactivity of the medium was determined. Occasionally leaves were turned over, as indicated in the figure.

In the experiment whose results are summarized in Fig. 3 leafy shoots, from which the growing tip and attached immature leaves had been removed, were submerged in 10 milliliters of calcium-45 chloride solution (10^{-4} M).

The vessel was covered with a watch glass and the whole illuminated. At approximately 2-hour intervals the solution was replaced by 10 ml of fresh radiocalcium solution to load the leaves with radioactivity. After three applications they were left illuminated overnight. The next day periods of darkness and illumination were alternated as indicated in Fig. 3. Changes in radioactivity of the medium were followed throughout.

The results indicated in Fig. 4 were obtained in an experiment similar to that of Fig. 3. It differed, however, in that during the second dark cycle, at the time indicated, hydrogen peroxide (5×10^{-2} milimols total; concentration in medium 5×10^{-3} M) was added to the medium.

Results

Figure 1 summarizes the results when drops of $\text{Ca}^{45}(\text{HCO}_3)_2$ solution were laid on top of floated leaves. Calcium concentration is lowered in the solution that lies on the abaxial faces of illuminated leaves and is raised in the solution that lies on the adaxial faces. In darkness the solutions on the two surfaces of the leaves do not differ significantly from each other in concentration.

Figure 2 indicates the absorption and excretion of calcium by floated leaves. In the light, calcium moved from the medium into the abaxial leaf faces, and it moved out of the adaxial faces. In the dark, on the other hand, calcium movement tended in the opposite direction.

Effects of alternating illumination and darkness on submerged leaves are indicated in Fig. 3. In the light, leaves held more calcium than in the dark.

Results of the experiment shown in Fig. 4 were similar to those of Fig. 3 until the application of hydrogen peroxide. (A) Then calcium moved out of the leaves for about half an hour. (B) After that it moved into the leaves until nearly all of it was removed from the medium. (C) Illumination then caused calcium to move out of the leaves, into the medium, as indicated at D.

Discussion

Arens (loc. cit.) and Steemann Nielsen (loc. cit.) have previously observed the movement of cations through Potamogeton and similar leaves. Results shown in Fig. 1, obtained with calcium-45, substantiate several important conclusions reached by Arens and Steemann Nielsen, using different methods, for a number of cations.

From the results shown in Fig. 1 it may be concluded that:

- (a) Calcium is accumulated into the abaxial leaf face in the light from its contiguous medium and is excreted from the adaxial face;
- (b) In darkness, calcium movement does not occur.

Of the results shown in Fig. 2, those for the light are consistent with the above conclusions. There is a contrast, however, between the dark results of Fig. 1 and 2; in the former case (Fig. 1) calcium movement in the dark did not occur, whereas in the latter the movement reversed the direction in the light. This difference is explained by the experimental differences. The dark results presented in Fig. 1 were obtained for leaves from which light was excluded, both for some time before the experiment and during it. But the results for Fig. 2 were obtained by alternating light and dark periods.

One concludes from the latter results that darkness creates no inhibitor for the reactions of transport, but rather that transport stops in the dark for lack of power. As a result (one infers), the reactions of transport all assume their equilibrium levels. This interpretation is consistent with the dark results of Fig. 1 because in those experiments equilibrium would have been attained during the pretreatment in the dark.

The results summarized in Fig. 3 contrast the equilibrium level in the dark with the steady-state level of calcium in illuminated leaves. Notable in this experiment is the shift back and forth from steady state to equilibrium when the light is turned off and on. The exact levels for these have, however, been found to depend upon experimental conditions, so that the size of this shift and even its direction (i. e. whether the leaves in the dark hold more or less calcium than illuminated leaves) can be controlled by the treatment.

The results shown in Fig. 4 demonstrate that treatment may change the equilibrium (dark) level of calcium. The change from light to darkness shifted the calcium content from the steady-state level to the equilibrium level, about

as it did in the experiment of Fig. 3. But adding hydrogen peroxide introduced another factor. The immediate effect was to release calcium. In a subsequent report I plan to present evidence that oxygen is necessary for the chemical reactions releasing calcium from the carrier molecule in the active membrane. The initial effect of hydrogen peroxide is consistent with this suggestion. But the leaves decompose hydrogen peroxide. Apparently when the hydrogen peroxide was gone, in the experiment of Fig. 4, a new equilibrium was established in which nearly all the calcium was contained in the leaves. Injurious treatments, including both hyperoxia and anoxia, have been observed to increase the calcium-holding capacity of the leaves. It would seem, therefore, that calcium release from the leaves employs a labile biochemical material. The ability of light to release calcium from leaves injured by hydrogen peroxide would indicate, further, that photosynthesis powers calcium transport by means of this labile calcium-release material.

Summary and Conclusions

In experiments on the relation between illumination and calcium transport in Potamogeton crispus leaves, the following observations were made:

1. Calcium was accumulated into the abaxial surface of illuminated leaves and was excreted from the adaxial surface.
2. Calcium transport did not occur in the dark.
3. The calcium content of illuminated leaves differed from that of darkened leaves.
4. Exposure to hydrogen peroxide increased the calcium-holding capacity of darkened leaves.
5. Light then released calcium from these leaves.

The following explanations are suggested for these observations:

1. Light powers calcium transport in Potamogeton crispus leaves.
2. The calcium content of illuminated leaves assumes a steady-state level that is determined by the rates of the reactions for calcium entry into the leaves and release therefrom.
3. The calcium content of darkened leaves is determined by the equilibria of the reactions of transport.
4. There is material, labile in the presence of hydrogen peroxide, that reduces the equilibrium level of calcium in darkened leaves.
5. Light synthesizes this material.

Literature Cited

1. Arens, K. (1930). Zur Kenntniss der Karbonatassimilation der Wasserpflanzen; *Planta* 10, 814-816.
2. ----- (1933) Physiologisch polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen. I; *Planta* 20, 621-658.
3. ----- (1936a) Physiologisch polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen. II. Die $\text{Ca}(\text{HCO}_3)_2$ Assimilation; *Jahrb. wiss. Botan.* 83, 513-560.
4. ----- (1936b) Photosynthese von Wasserpflanzen in Kaliumbikarbonatlosungen; *Jahrb. wiss. Botan.* 83, 561-566.
5. Lowenhaupt, B. (1956) The Transport of Calcium and Other Cations in Submerged Aquatic Plants. *Biol. Rev.* (in press: Vol. 31, No. 4); also UCRL-3247, Nov. 1955.
6. Österlind, S. (1949). Growth Conditions of the Alga *Scenedesmus Quadricauda* with Special Reference to the Inorganic Carbon Sources; *Symbolae Botan. Upsalienses* X:3, 1-141.
7. Ruttner, F. (1953) Die Kohlenstoffquellen für die Kohlensäureassimilation submerser Wasserpflanzen; *Scientia (Milan)* 88, 20-27.
8. Steemann Nielsen, E. (1944). Dependence of Freshwater Plants on Quantity of Carbon Dioxide and Hydrogen Ion Concentration Illustrated Through Experimental Investigations; *Dansk. botan. Arkiv* 11, No. 8, 1-25.
9. ----- (1946). Carbon Sources in the Photosynthesis of Aquatic Plants; *Nature (London)* 158, 594-596.
10. ----- (1947). Photosynthesis of Aquatic Plants with Special Reference to the Carbon Sources; *Dansk. botan. Ark.* 12, No 8, 1-71.
11. ----- (1951). Passive and Active Ion Transport during Photosynthesis in Water Plants; *Physiol. Plantarum* 4, 189-198.
12. ----- (1952). Experimental Carbon Dioxide Curves in Photosynthesis; *Physiol. Plantarum* 5, 145-159.

MU-11460

Fig. 1. Effect of light vs darkness on the relative calcium concentration of drops of $\text{Ca}^{45}(\text{HCO}_3)_2$ solution on top of floated leaves, as measured by radioactivity determinations.

MJ-11461

Fig. 2. Effect of light vs darkness on the calcium content of leaves floated on $\text{Ca}^{45}\text{Cl}_2$ solution, as indicated by radioactivity determinations.

MU-11462

Fig. 3. Effect of light vs darkness on calcium content of leaves submerged in $\text{Ca}^{45}\text{Cl}_2$ solution, 10^{-4} M, as indicated by radioactivity determinations.

MU-11740

Fig. 4. Effect of light and H_2O_2 on calcium content of submerged leaves, as indicated by radioactivity determinations. (Experimental conditions were similar to those of Fig. 3 except that H_2O_2 was used.)