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ACTIVE CATION TRANSPORT IN SUBMERGED AQUATIC PLANTS

II. EFFECT OF AERATION UPON THE EQUILIBRIUM CONTENT OF POTAMOGETON CRISPUS (L) LEAVES

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Benjamin Lowenhaupt

October 15, 1956

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ABSTRACT

The effect of aeration upon the equilibrium calcium content of Potamogeton crispus leaves has been studied. Oxygen was found to release calcium from the leaves into the external medium.

The interpretation of this observation is that the reactions of calcium transport are links in a respiratory chain. The binding of calcium to the active membrane is accomplished by the metabolic reduction of a calcium carrier in the membrane. Its subsequent release is accomplished by the metabolic oxidation of this carrier, oxygen being the ultimate electron acceptor.

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INTRODUCTION

Cation transport can be observed in the leaves of submerged aquatic seed plants more advantageously than in other biological materials. The work reported here is part of a series of experiments initiated to exploit the advantages of these leaves. Two reports of this series have preceded this one. The first of these[1] surveys cation transport with special reference to its mechanism in submerged aquatic plants. The following generalizations are developed for most submerged aquatic seed plants:

1. Cations journey through the leaf in the light; they are transported twice en route. The active membrane on the abaxial surface pumps them from the contiguous external medium into the leaf cells in the light while the active membrane on the adaxial leaf surface pumps them out of the leaf into its contiguous external medium.

2. These two active membranes consist of the cell plasma-membranes on the abaxial and adaxial leaf surfaces respectively.

3. Action at the active membranes involves three steps in the light:

   a. cation attachment to a binding group;

   b. reorientation of the group toward the other surface of the active membrane,

   c. release of the cation.

   The energy needed for transport is provided by photosynthesis. Direct application of this energy is for the cation-release step.

4. In darkness, the cation transport mechanism is shut off. The biochemical constituents of this mechanism then reach resting levels. These levels are functions of the equilibria of the reactions between different constituents, therefore, one can alter the resting level of one constituent by controlling the supply of another.

5. In the light, when the transport mechanism is functioning, the levels of the biochemical constituents reach steady-state values. These levels generally different from the resting levels, are functions of the rates of the reactions between the constituents. Therefore, one can alter the steady-state levels by suitably altering the conditions of one or more of these reactions.

6. The transport mechanism at the active membrane supplies links of a respiratory chain. Cation attachment is an oxidation-reduction reaction. Organic researves implement the action of a reducing agent that reduces the carrier in the active membrane. As a result the carrier attaches a cation. Cation release is likewise an oxidation-reduction reaction. An oxidizing agent formed as a result of photosynthesis liberates the cation from the carrier.

7. The transport reactions synthesize a bicarbonate-accepting compound at the plasma membrane. Apparently, carbon from external bicarbonate ions is thus enabled to penetrate the plasma membrane and so reach the site of photosynthesis inside the cells.

The second report[2] presents data relating illumination to calcium transport through Potamogeton crispus leaves. The following conclusions are drawn:

a. Light powers calcium transport in Potamogeton crispus leaves.

b. 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.

c. The calcium content of darkened leaves is determined by the equilibria of the reactions of transport.

d. There is a material, labile in the presence of hydrogen peroxide, that reduces the equilibrium level of calcium in darkened leaves.

e. Light synthesizes this material.

This report presents data on the effects of aeration upon the equilibria in the Potamogeton crispus leaf for the reactions of calcium accumulation and excretion. The results reported here are some of the bases for generalization 6, above.

MATERIALS AND METHODS

The methods of growing and preparing the P. crispus leaves and of preparing and measuring the radiocalcium were described in a previous report,[2] Three experiments testing effects of aeration upon the amount of calcium in the leaves will be reported here.

The first experiment is a modification of the third experiment of the second report.[2] It resembles that experiment in that leaves were transferred from illumination to darkness while their calcium content was measured. It differs in that some of the leaves were supplied with nitrogen instead of air when the light was first turned off. A variation of this experiment was also run in which air was supplied to leaves during one dark period. Then, after a period in the light, the leaves were again put in the dark. This time nitrogen was supplied for the first hour, and air thereafter.

In the second experiment leaves were washed in HCl solution (pH 3.5) and transferred to 20 ml of 10^{-3} M $Ca^{45}Cl_2$ solution. (This experiment differs from the others in that leaves were not pretreated with radiocalcium solution. Accordingly, changes in radioactivity cannot be considered proportional to changes in calcium content.) After an hour's illumination, leaves and medium were divided into two equal portions, both of which were placed in the dark. Eight x 10^{-3} millimoles of K_2CO_3 were added to each portion and in one, the air was replaced with nitrogen. The other was left undisturbed. After an hour air was again supplied to the anoxic portion. Radioactivities and pH's of both media were followed throughout the experiment and changes in the radioactivity of the leaves were calculated from the observed radioactivities in the medium.

In the third experiment, leaf segments about 5 mm long were used. After several hours immersion in $Ca^{45}Cl_2$ solution (initial concentration 10^{-4} M) in darkness, these segments were clamped between glass cells filled with the bathing $Ca^{45}Cl_2$ solution. The placing of the leaf segments between the cells was done as quickly and in as dim light as possible. The apparatus was transferred to a metal box and humid air was bubbled into the solution in the upper cell through a capillary tube; the solution in the lower cell remained unaerated. Figure 1 diagrams the experimental setup inside the box. The inner walls of the box were sprayed with water and the box closed. Air was introduced in the dark for about 6 hr. At the end of this period, the two cells were separated as quickly as possible in a minimum amount of light. The radioactivities of measured aliquots from each were compared with that of the $Ca^{45}Cl_3$ solution before the experiment, the latter value being taken as 100%.

RESULTS

Leaves transferred from light to darkness gave up part of their calcium to the medium and regained it when illuminated. (Figures 2a and 2b.) This result is similar to those reported in the third experiment of the preceding report. When nitrogen instead of air was supplied to the darkened leaves, however, a different result was obtained. Then, instead of releasing calcium, the leaves accumulated it from the medium. When air was restored, calcium was released.

Leaves that had been in HCl solution removed about 40% of the calcium-45 radioactivity from a $Ca^{45}Cl_2$ solution within 1 hr and the acidity of this solution increased from a pH value slightly less than 7.0 to a value of 3.8. (Figure 3) When K_2CO_3 was added to counteract the acidity, the medium first became alkaline, then returned gradually to a value of pH 5.8. Radioactivity entered both experimental groups of leaves after the K_2CO_3 was added, but it entered more rapidly and completely into those deprived of oxygen. As a result, the anoxic leaves contained about 400 cps more radioactivity after an hour than the leaves in air. When air was restored to the oxygen-deficient leaves, about 150 cps of radioactivity moved out in about 1.5 hr. During the same time, radioactivity continued to enter the other set of leaves that had been in air throughout, so that final radioactivities of the two were the same.

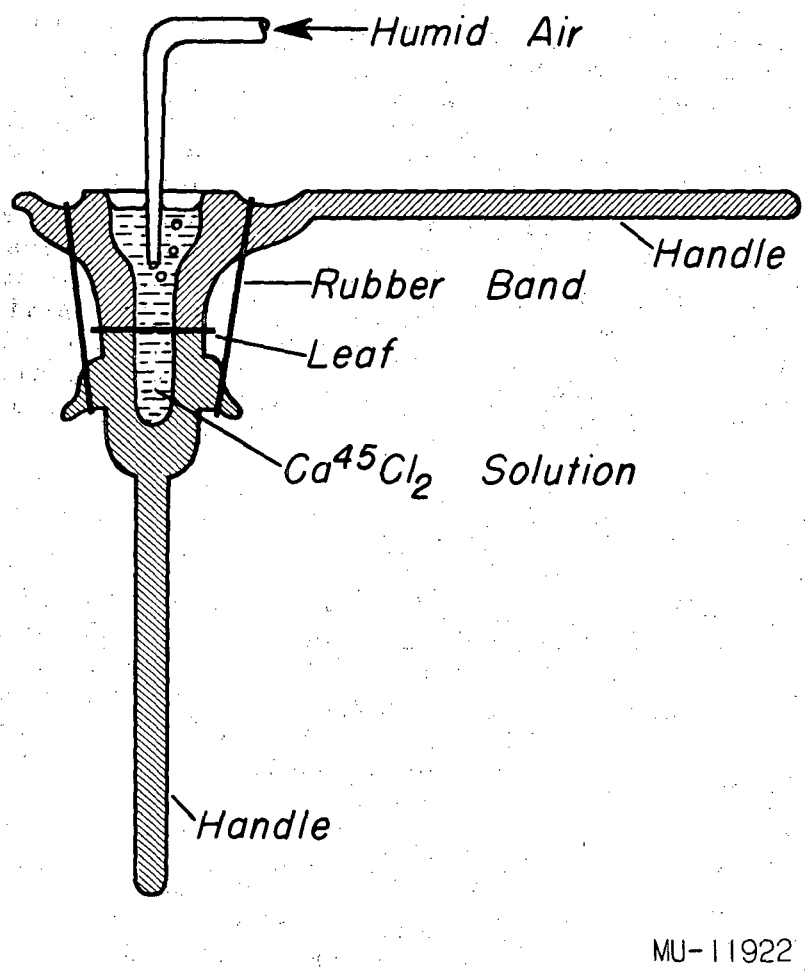
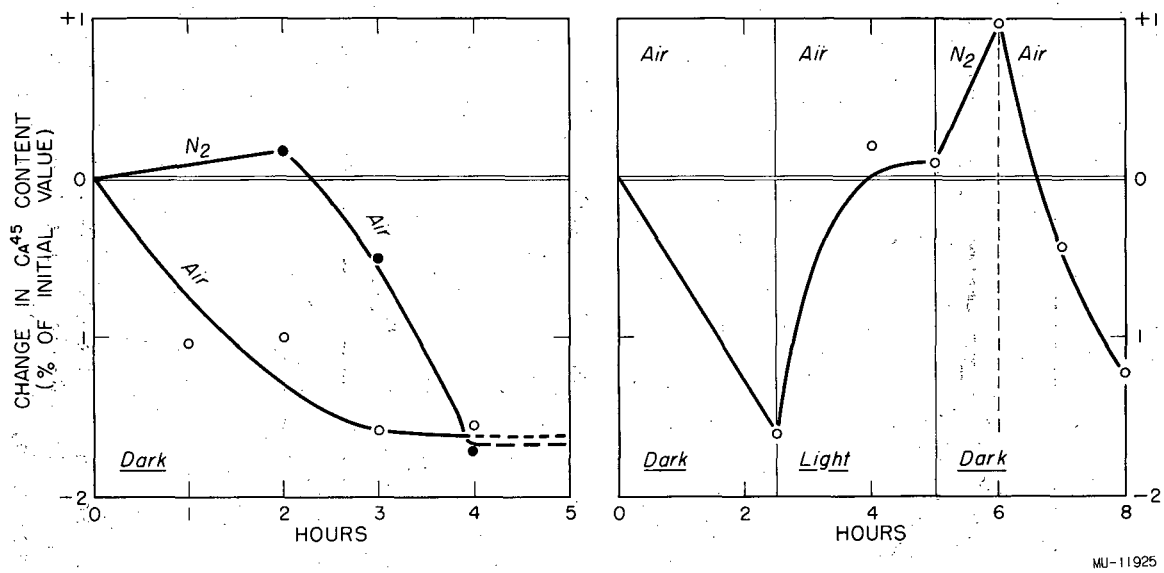


Fig. 1. Diagram of the setup for the third experiment. (Twice actual size)



Figs. 2a and 2b. Effect of air vs. nitrogen on calcium content of leaves submerged in 10^{-4} M $Ca^{45}Cl_2$ solution, as indicated by radioactivity determinations (leaves transferred from light to darkness at the beginning of both experiments).

Fig. 2a (left) contrasts the calcium content of two lots of leaves, one supplied with air throughout the experiment, the other supplied with nitrogen for 2 hr.

Fig. 2b (right) shows effects of a succession of treatments on a single lot of leaves.

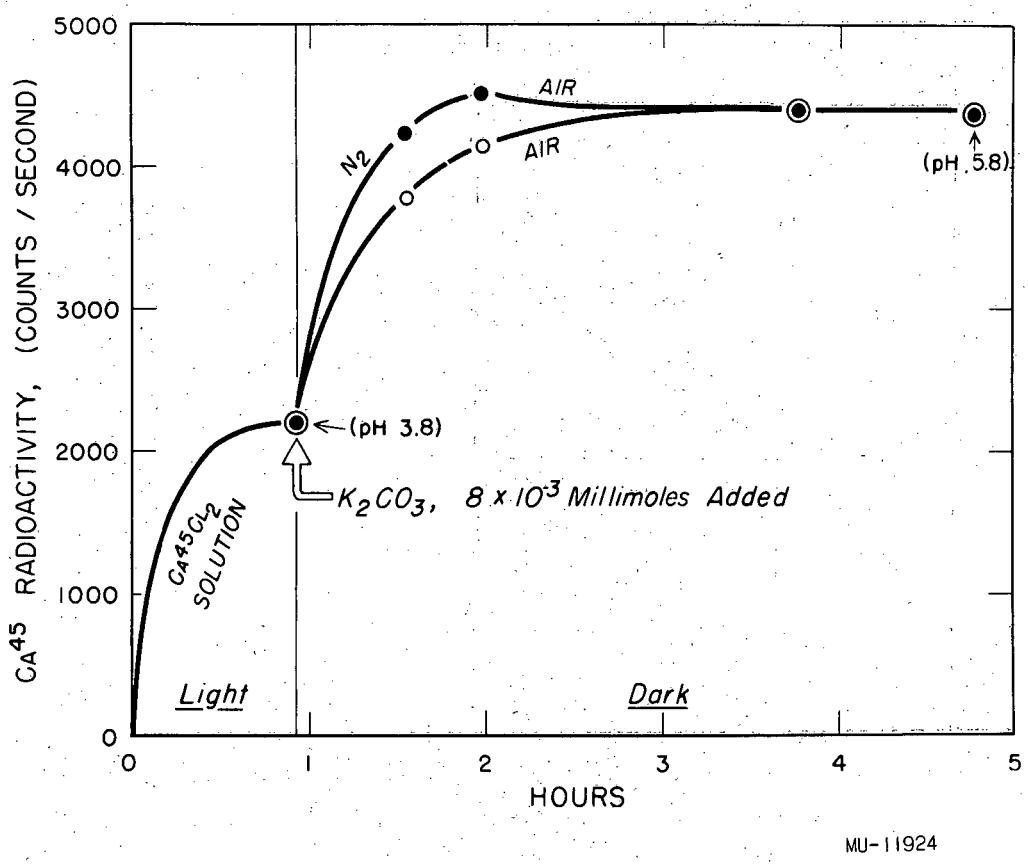


Fig. 3. Effect of aeration on calcium-45 radioactivity uptake by leaves. (These leaves, washed first in pH 3.5 HCl solution, and than in water, were placed in 10^{-3} M $Ca^{45}Cl_2$ solution, the pH of which fell from about 7 to 3.8.)

There was a shift of calcium across leaves aerated from one side. (Table I). This is in contrast to the unchanging calcium concentration on the two sides of dialysis tubing or aluminum foil (Table II). The shift when a leaf was used was toward the aerated medium regardless of whether this was in the abaxial-to-adaxial direction or vice versa. The amount of calcium removed from the medium on the adaxial side of the leaf is small (4% of the initial calcium in the medium); it is significant, however. This smallness is because of the difficulty of controlling conditions during an experiment of several hours so that there is no net loss or gain of calcium from the leaves. Small relative changes in the leaf calcium cause large changes in the medium because the calcium concentration in the leaves is greater than in the medium. Actually, however, the difference in calcium content between the aerated and unaerated media is more important than the absolute gain or loss from either medium.

DISCUSSION

Oxygen has long been known to be necessary for active transport in a number of biological systems. These experiments are an attempt to determine where oxygen fits into the transport mechanism.

The first experiment contrasted the equilibrium calcium content of both aerated and unaerated leaves in the dark with the steady-state content in the light. (That there is equilibrium in the dark and a steady-state condition in the light was demonstrated in the preceding report.[2]) The evidence shows that the equilibrium calcium level is reduced by aeration, i.e. that calcium release is implemented thereby. The second experiment utilized the fact that the calcium level in the leaves is a function of the acidity of the medium. The results of this and other experiments indicate that at a pH below 3.8 in the medium, calcium is extracted from the leaves. Neutralization of the acid permits calcium uptake to an equilibrium. The fact that this equilibrium was higher under anoxic conditions indicates that oxygen favors calcium release. The third experiment differs from the other two in that the leaves probably were not injured. Evidence for this is the fact that their net calcium content remained approximately unchanged during the experimental period, whereas injury from anoxia has been observed to cause a gradual release of calcium into the medium. In this experiment, one side of the leaves was aerated, and it seems that sufficient oxygen moved through the leaves to maintain the other side. Nevertheless, an oxygen gradient across the leaf must have existed and the shift of calcium into the leaf on the unaerated side and out of the aerated side is evidence that calcium release is favored by oxygen. Although the pH's of the aerated and unaerated media were not measured, it seems unlikely that pH effects could have caused the calcium shift, because the unaerated medium would hardly be more alkaline than the aerated one. One would expect the reverse, if there were any difference, because carbonic acid might accumulate in the unaerated medium, whereas it would be swept out of the aerated solution.

One is led to consider how oxygen may function in calcium release. Admittedly a number of speculations can be raised, but most of them seem implausable. The simplest suggestion, and therefore the one to be preferred, is that a respiratory chain links oxygen with a metabolite, part of this chain

Table I

Effect of unilateral aeration on relative calcium content of medium on either leaf face as indicated by radioactivity determinations. (Leaves that had been in darkness overnight were kept in the dark during the experiment. The medium at the outset on both leaf faces was 10^{-4} M $Ca^{45}Cl_2$ solution, having a relative radioactivity of 100%.)

Leaf orientation	A Relative radioactivity, aerated solution (%)	B Relative radioactivity, unaerated solution (%)	Difference (A - B)
	127	99	28
Dorsal	121	107	14
	107	94	13
Side	116	90	26
	106	89	17
Aerated	107	97	10
	120	87	33
	108	106	2
	Mean = 114.0	Mean = 96.1	Mean = 17.9
	Range = 106 to 127	Range = 87 to 107	Range = 2 to 33
Ventral	136	80	56
	115	88	27
Side	104	99	5
	94	59	35
Aerated	107	96	11
	Mean = 111.2	Mean = 84.4	Mean = 26.8
	Range = 94 to 136	Range = 59 to 99	Range = 5 to 56
	Mean of all = 112.9	Mean of all = 91.6	Mean of all = 21.3
	Range of all = 94 to 136	Range of all = 59 to 107	Range of all = 2 to 56

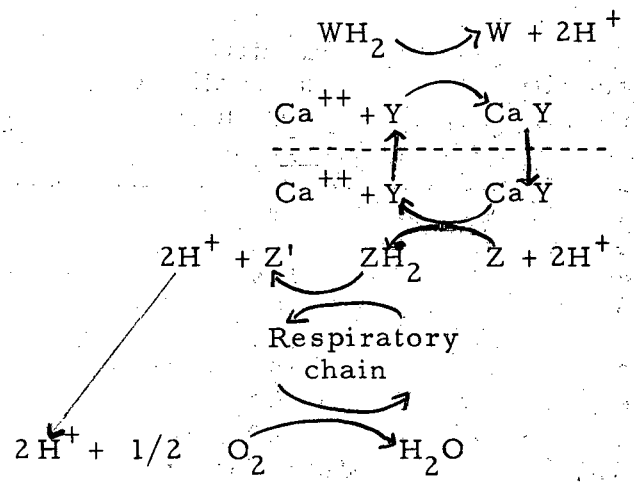
(Statistical analysis indicates significance of all differences, A - B, A - 100%, and 100% - B, for both leaf orientations. Thanks are due Professor Lucien Le Cam for this analysis.)

Table II

Effect of unilateral aeration on relative calcium content of medium on either face of inert membranes in the dark as indicated by radioactivity determinations. (Experimental conditions were similar to those of Table I except that inert membrane material was used instead of leaf.)

Membrane Material	A Relative radioactivity, aerated solution	B Relative radioactivity, unaerated solution	Difference (A - B)
	101	97	4
	105	105	0
Dialysis	104	104	0
	106	102	4
	97	101	-4
	100	102	-2
Tubing	96	97	-1
	104	97	7
	Mean = 101.6	Mean = 100.6	Mean = +1.0
	Range= 96 to 106	Range= 97 to 105	Range= -4 to+7
	106	103	3
	101	99	2
Aluminum	102	107	-5
	101	100	1
	104	99	5
Foil	102	96	6
	99	94	5
	--	107	-
	Mean = 102.1	Mean = 100.6	Mean = +2.4
	Range= 99 to 106	Range= 94 to 107	Range= -5 to+6
	Mean of all = 101.9	Mean of all = 100.6	Mean of all = +1.7
	Range of all = 96 to 106	Range of all = 94 to 107	Range of all = -5 to +7

being the calcium transport mechanism. According to this view, one step in transport occurs when a metabolite reduces the calcium carrier in the active membrane and, as a result, the carrier binds calcium. Oxidation of the carrier, it would seem, must release the calcium on the other side of the active membrane as a subsequent step in transport. These considerations (and others) have led to the following suggested biochemical mechanism:[1]



where WH_2 is the metabolite (in reduced form) that reduces the calcium carrier, W is this metabolite after it has been oxidized, Y is the calcium carrier, Z is the oxidizing agent necessary for calcium release from Y (effects of illumination indicate that Z is formed as a result of photosynthesis), ZH_2 is the product of Z formed when Z oxidizes CaY, Z' is the oxidation product of ZH_2, and the dotted line represents the active membrane. The kinship between this mechanism and the redox pump suggested by Conway[3] is apparent.

SUMMARY AND CONCLUSIONS

Previous reports have presented:

a. A theory of cation transport in submerged aquatic seed plants and

b. Data supporting parts of this theory.

A summary of the conclusions from these reports is presented in the introduction of this paper.

This report presents data supporting another aspect of the theory. *Potamogeton crispus* leaves were found to contain, under suitable conditions, more calcium if anoxic than if aerated. Three methods of demonstrating this phenomenon are presented.

The conclusion is suggested that the reactions of calcium transport are links in a respiratory chain between a metabolite and oxygen.

ACKNOWLEDGMENT

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BIBLIOGRAPHY

1. B. Lowenhaupt, "The Transport of Calcium and Other Cations in Submerged Aquatic Plants," Biol. Rev. (in press) 31, No. 4, (1956); also published as UCRL-3247, November 1955.

2. B. Lowenhaupt, Active Cation Transport in Submerged Aquatic Plants. 1. Effect of Light Upon the Absorption and Excretion of Calcium by *Potamogeton crispus* (L) Leaves, UCRL-3460, August 1956.

3. E. J. Conway "A Redox Pump for the Biological Performance of Osmotic Work, and Its Relation to the Kinetics of Free Ion Diffusion Across Membranes," Intern. Rev. Cytol. 2, 419-445 (1953).