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1955-11-08

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THE TRANSPORT OF CALCIUM AND
OTHER CATIONS IN SUBMERGED
AQUATIC PLANTS

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UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

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Benjamin Lowenhaupt

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THE TRANSPORT OF CALCIUM AND OTHER CATIONS
IN SUBMERGED AQUATIC PLANTS

Benjamin Lowenhaupt

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November 8, 1955

ABSTRACT

Photosynthetic bicarbonate assimilation and cation transport by aquatic plants are briefly reviewed. It is suggested that these two processes stand in a cause-and-effect relation to each other. A number of observations of cation transport not directly associated with photosynthesis are also reviewed, and are related to current concepts of active transport in the plant kingdom.

These characteristics of cation transport have been observed in many submerged aquatic plants:

a. Cations are transported through the leaves in the light but not in the dark.

b. Cations are accumulated at the ventral leaf face from the contiguous external medium and are excreted from the dorsal face into the contiguous external medium. Therefore accumulation and excretion can be studied separately.

A new theory of cation transport in bicarbonate-assimilating aquatics is presented. The theory entails the following propositions:

a.. Two active membranes pump cations through the leaf in the light. The membrane on the ventral leaf face pumps cations into the leaf; the one on the dorsal face pumps cations out of the leaf.

b. The cell plasma-membranes on the ventral and dorsal leaf surfaces form these two active membranes, respectively.

c. In the light--i.e., when the transport mechanism is functioning--the biochemical reactions of transport reach steady-state levels. These levels are determined by the rates of reaction between the constituents. Therefore one can alter the steady-state levels by suitably altering the conditions of one or more of these reactions.

d. In the dark--i.e., when the transport mechanism is not functioning--the biochemical constituents reach equilibrium levels. The equilibria can be altered by changing the environment of the leaf.

e. The reactions of transport supply links of a respiratory chain.

f. The reactions of transport synthesize a bicarbonate-accepting compound at the plasma membrane.

Evidence for each of these propositions is discussed.

The theory is considered applicable (with appropriate modification) to cation transport in a variety of cells.

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INTRODUCTION

Cation transport* through the leaves of submerged aquatic plants has mostly been thought about as an adjunct of photosynthetic bicarbonate assimilation. My discussion reverses this emphasis, giving primary consideration to transport itself. The transport of calcium has been singled out for special emphasis because data for it are more abundant.

The facts are interpreted to develop a theory of cation transport, a theory directly applicable to submerged aquatic plants and also pertinent (with appropriate modifications) to transport in a variety of cells. The theory, as it applies to most submerged aquatic seed plants, entails the following propositions, which are developed in this paper:

1. Cations journey through the leaf in the light; they are transported twice en route. The active membrane on the ventral (abaxial) surface pumps them from the contiguous external medium into the leaf cells in the light while the active membrane on the dorsal (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 ventral and dorsal 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.

* "Transport" or "active transport" in this paper refers to the metabolic pumping of material across an active membrane. Transport is considered to occur if the material is pumped, regardless of its relative chemical potentials on the uptake and release sides of the membrane.

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 reserves implement the action of a reducing agent which 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.

SECTION I: PHOTOSYNTHETIC BICARBONATE ASSIMILATION AND ITS RELATIONSHIP TO CATION TRANSPORT

A. Photosynthetic Bicarbonate Assimilation

The kinship between photosynthetic bicarbonate assimilation and cation transport in aquatics necessitates a review of the former for an understanding of the latter. Ruttner (1953) generalizes from his studies that green plants fall into two groups, physiologically different as regards the form in which they can accept carbon for photosynthesis. Members of one of these groups--i. e., many aquatic seed plants and algae--have been found able to utilize externally supplied bicarbonate ions in addition to carbon dioxide, while members of the other group--which includes the observed aquatic mosses and land plants--can obtain carbon for photosynthesis only from dissolved carbon dioxide (but not from bicarbonate ions external to the cells). Photosynthesis is blocked in the latter plants in any aqueous medium whose pH is too high for free carbon dioxide. Figure 1 indicates that this limiting alkalinity is about pH 9.*

Clarification of the existence of two physiologically different groups of plants and the ability of one of these to assimilate bicarbonate may be attributed primarily to three men, Ruttner, Arens, and Steemann Nielsen. (For a more complete review of the literature see "Österlind, 1949). Ruttner observed the effects of plants on the pH and electrical conductivity of bicarbonate solution. He showed that electrical conductivity rises with increasing pH of the solutions (Ruttner, 1948a). Comparative studies of a number of species of plants disclosed two types. (Ruttner, 1921, 1947). The observed seed plants and algae that normally grow submerged could raise the pH of tap water (Ruttner found this to be essentially calcium bicarbonate solution) in the light to about pH 11, (with a corresponding rise of conductivity). In contrast, the

* The hydration of carbon dioxide is not instantaneous and therefore one might expect deviations from equilibrium when there is biological activity in the medium. Carbonic anhydrase was found, however, in a number of submerged autotrophs, both bicarbonate users and nonusers, by Steemann Nielsen and Kristiansen (1949), and by Österlind (1950). The presence of the enzyme, these authors point out, can hardly be the key difference between the two types of plants because it occurs in both.

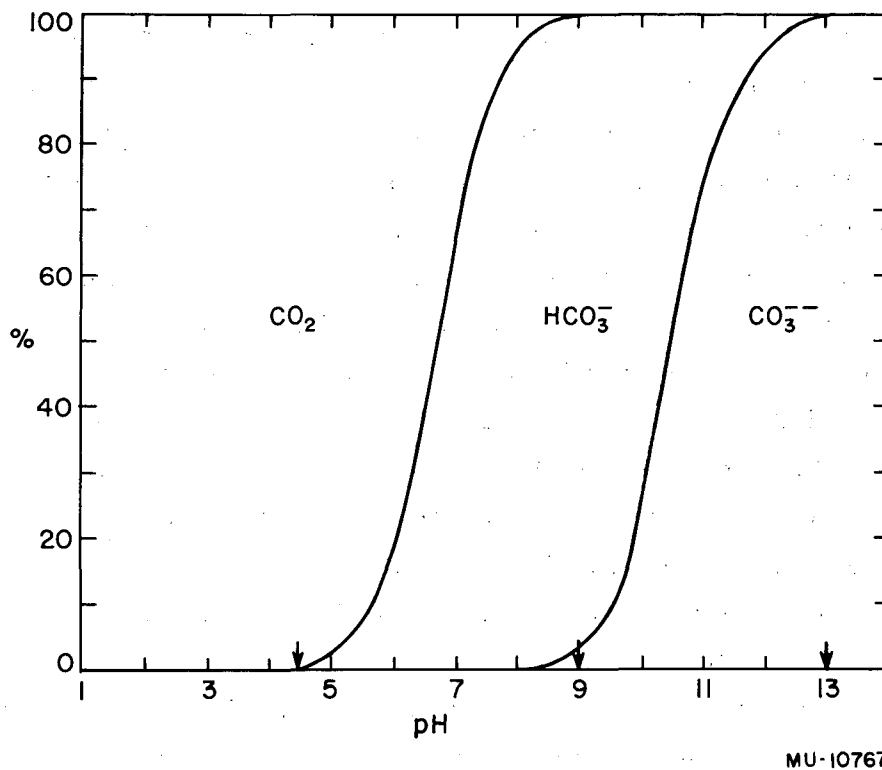


Fig. 1. (From Rabinowitch, 1945; after Faurholt) Percentage relation between CO_2 (H_2CO_3), HCO_3^- , and CO_3^{--} , and pH at 0°C , ionic strength 0. Arrows indicate (left) pH above which HCO_3^- first is found, (middle) pH above which concentration of free CO_2 becomes negligible, and (right) pH above which CO_3^{--} becomes predominant.

observed aquatic mosses and land plants raised the pH in the light only to about 9, with a drop in conductivity. Bubbling air free of carbon dioxide through the same medium without plants had effects similar to the second type.

These observations led Ruttner to suggest that the aquatic seed plants and algae, unlike the aquatic mosses and the land plants, assimilate bicarbonate in the light. He argued that, because free carbon dioxide is not available in the medium in significant amounts in strongly alkaline solutions, plants that cannot utilize bicarbonate from the medium cannot obtain carbon for photosynthesis. He suggested that, in contrast, plants that are able to utilize bicarbonate remove part of it from the medium, converting the remainder into carbonate and raising the pH above 9. In a later report he devoted special attention to a comparison between Elodea canadensis, an aquatic seed plant, and Fontinalis antipyretica, an aquatic moss (Ruttner 1948b). This comparison, under a variety of conditions more carefully controlled than previously, confirmed the contrast between the two. A noteworthy observation was that Fontinalis consumed carbon dioxide from its medium in the light at almost a constant rate while the free carbon dioxide supply lasted; a sudden cessation of removal occurred on exhaustion of free carbon dioxide. This can hardly be accounted for by pH effects. In contrast, photosynthesis in Elodea continued after the free carbon dioxide was used up, as bicarbonate was converted into carbonate.

Arens (1930, 1933, 1936a, 1936b) concluded that a number of cations are transported through certain aquatic leaves in the light, entering the ventral side in amounts equivalent to the bicarbonate that enters there and leaving the dorsal side along with hydroxyl or carbonate ions. Evidence for this conclusion was that the medium adjacent to the ventral leaf face displayed no change in pH in the light (if mixing was prevented), but eventually its ions were nearly all removed. In contrast, the medium adjacent to the dorsal leaf face was enriched in the light with cations equivalent to those removed from the medium on the ventral side, and its pH rose sharply. In the dark no ion movement through the leaves occurred. These facts indicated to Arens that bicarbonate ions are assimilated during photosynthesis. Stoichiometry between carbon dioxide uptake and cation uptake would be unintelligible, he argues, but he suggests that when bicarbonate is taken up some means for neutralizing the electrical charge must exist, and he interprets his results as indicating that cations move through the leaf to neutralize this charge.

Steemann Nielsen demonstrated the difference in physiology between several flowering aquatics, on the one hand, and Fontinalis. This he did by correlating growth and photosynthesis with the amounts of carbon dioxide (carbonic acid), bicarbonate, and carbonate in the medium as calculated from the known amounts of added solutes and the equilibrium constants for the interchange between carbon dioxide and the carbonic anions (Steemann Nielsen, 1944, 1946, 1947, 1952). He found growth and photosynthesis of both types of plants to be affected little by pH over a considerable range, provided free carbon dioxide was available. The rate of photosynthesis of Fontinalis, however, was determined by the available free carbon dioxide, whereas photosynthesis of Myriophyllum and other aquatic seed plants was determined by both carbon dioxide and bicarbonate. Carbonate he considers available to neither because it was not correlated with photosynthesis.

Steemann Nielsen's confirmation of Arens's conclusion regarding the movement of cations in the light was an important contribution. His techniques permitted precise control of the experiments and accurate quantitative analyses. He demonstrated decisively the equivalence of cation uptake and photosynthetic bicarbonate assimilation at the ventral faces of Potamogeton lucens leaves. Also he confirmed that cation excretion at the dorsal leaf face is equivalent to cation accumulation at the ventral face and that pH is raised on the dorsal side and remains unchanged on the ventral side. He found, however, that Potamogeton lucens leaves could assimilate bicarbonate not only from the ventral side but also from the dorsal side. (See also Gessner, 1937, for evidence leading to the same conclusion for Potamogeton perfoliatus.) Arens had been led to believe that in leaves of this sort bicarbonate entered the ventral side only. The entry of bicarbonate into both leaf faces appears to present an enigma because its entry through the dorsal face is accompanied by cation release, not uptake. If one accepts Arens's suggestion that cation entry neutralizes the electrical charge on the entering bicarbonate, one asks how bicarbonate can enter the dorsal side of the leaf where cations move out, not in. Steemann Nielsen (1951) suggests that the electrical charge of the bicarbonate entering both leaf faces is neutralized by cations which move in simultaneously, and that inside the leaf the bicarbonate is assimilated and hydroxyl ions are formed in its place. These hydroxyl ions are excreted out of the dorsal side of the leaf accompanied by equivalent cations.

The concept of active transport of anions in the leaf--hydroxyl ions or others--would appear to pose several theoretical contradictions. It suffices to state without discussing these that movement of cations across the leaves in the light has been observed repeatedly, whereas there is no evidence for a corresponding anion movement from one side of the leaf to the other.* Therefore postulation of anion transport to carry cations is much less plausible than cation transport to carry cations.

The occurrence of cation transport as a concomitant of bicarbonate assimilation is consistent with data from diverse sources. These will be considered below. Three experiments whose interpretation in terms of cation transport seems most reasonable are presented here, however:

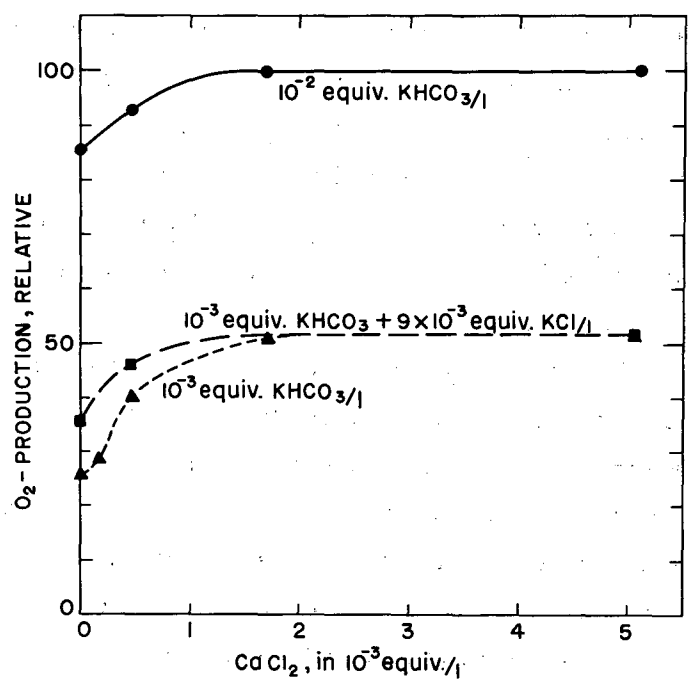
1. If cation transport be a requisite of bicarbonate utilization, one would expect the rate of this utilization to depend upon the supply of both bicarbonate and cations in the medium. Steemann Nielsen's own data substantiate this inference for he found that addition of calcium chloride to the medium increased the rate of bicarbonate assimilation by Myriophyllum. Figure 2 shows some of his results. He observed the assimilation of free carbon dioxide, on the other hand, to be independent of added salts.

2. If bicarbonate be bound by a product of the transport reactions, whereas carbon dioxide be not, one would expect the bicarbonate to be incorporated into a different molecule from the carbon dioxide. Also, one would expect this incorporation to occur at a different place in the cell--bicarbonate at the active membrane and carbon dioxide at the site of photosynthesis.

Both these inferences are substantiated by observations by Benson, Kawaguchi, Hayes, and Calvin (1952) and by Ouellet and Benson (1952) on Scenedesmus quadricauda.** They report that there are two acceptors for

* Aren's and Steemann Nielsen's observations that the uptake of bicarbonate ions on the ventral leaf face balances the cation transport would seem to preclude anion transport from one face to the other. Furthermore, I, myself, have observed that while phosphate can enter both leaf faces, neither it nor iodide is transported from one side of the leaf to the other.

** Osterlind (1950, 1951a, 1952a, 1952b) has demonstrated the ability of this species to assimilate both carbon dioxide and bicarbonate.



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Fig. 2. (After Steemann Nielsen) Effect of calcium chloride concentration on rate of photosynthesis (measured by oxygen production) of Myriophyllum; pH of medium = 8.4.

carboxylation. The one that predominates when the medium is acid--i. e., when carbon dioxide is bound--leads to formation of phosphoglycerate. The other, which predominates when the medium is alkaline--i. e., when bicarbonate is bound--leads to formation of malate. The limiting carbon concentration when phosphoglycerate is formed is higher than when malate is formed. Apparently this is because the carbon from carbon dioxide must migrate into the cell to be bound at the site of photosynthesis, and with low external carbon dioxide supply the rate of this migration becomes limiting. But in contrast, one infers, bicarbonate is incorporated at the active membrane. Evidence considered below places this membrane as the outermost layer of the cytoplasm, adjacent to the external medium. Therefore migration of bicarbonate into the cell is not necessary and cannot become the rate-limiting factor.

3. If the uptake of bicarbonate be accomplished by a biochemical mechanism different from the mechanism for carbon dioxide uptake, one might find that biochemical poisons affect the two differently. This inference is substantiated by observations by Osterlind (1952a) on the effect of cyanide on the photosynthesis of Scenedesmus quadricauda. He found that when carbon dioxide is assimilated, cyanide is a competitive inhibitor, effective only at relatively high concentrations. This appears to be an effect of cyanide on photosynthesis itself. But when bicarbonate supplies the carbon for photosynthesis, cyanide is a noncompetitive inhibitor, effective at much lower concentrations. It would seem that this effect of cyanide is to inhibit transport and thereby to block photosynthesis indirectly. Lundegardh's view (1950b, 1951, 1952) that cyanide inhibits transport in plants by blocking the cytochrome system seems to be supported by substantial evidence. Therefore one infers that photosynthetic bicarbonate assimilation requires the respiration of the cytochrome system because the assimilation depends upon transport. Further evidence linking calcium transport with aerobic respiration is considered below.

B. Comparison of Cation Transport in Submerged Aquatic Plants with Cation Transport in Roots

The lines of thought on the mechanism of active transport in cells are diverse, and accordingly it is not practical to survey them all. Among plants the structures and functions involved are less varied than among animals. Probably

for this reason the thinking among plant physiologists is more homogeneous than among students of different aspects of animal physiology. Nonetheless, theories that have been developed for plants appear pertinent for animals as well. Therefore it has been deemed advisable to discuss active transport in plants and to leave extension of the concepts into the animal kingdom largely to the imagination of the reader. Conway (1953b) has considered the similarity between transport by yeast cells and by the stomach parietal cells.

The cation transport concomitant with photosynthetic bicarbonate assimilation has many similarities to transport in roots. Evidence is discussed (below) that aeration has a role in the former, and it would seem that the role of aeration for roots may be similar. Likewise formation of malic acid through bicarbonate uptake appears to be common to the cation-pumping mechanisms in green bicarbonate-assimilating cells and in roots. Evidence that malic acid is formed by Scenedesmus has been considered. That roots do likewise is indicated by observations of Poel (1953). He supplied $C^{14}O_2$ to barley roots and found that in a few minutes there was C^{14} in a number of organic acids (including malic). Oxygen deficiency (which inhibits transport in roots) inhibited C^{14} fixation. His observation is particularly significant that withholding minerals from the external medium of the roots before but not during the C^{14} uptake period strikingly increased the malic acid radioactivity. Apparently when ions are not available for transport, a backlog of the reactants of transport is formed, so that when ions again become available the transport reactions are more rapid for a time. The increase in radioactivity of malic acid in roots resulting from previous mineral starvation thus appears to substantiate the incorporation of carbon into malic acid as a concomitant of ion accumulation.

The observations of Jacobson (1955) and of Jacobson and Ordin (1954) seem also to support this view. They found a correlation in roots between the amount of bicarbonate absorbed, the amount of malate formed, and the cations transported in excess of anions. (See also Overstreet, Ruben, and Broyer, 1940, for further evidence that cation transport enhances bicarbonate assimilation.) The failure to obtain precise equivalence between bicarbonate uptake and cation transport in roots contrasts with the easy observation of precise equivalence for aquatics. This contrast is explained by the reduction of the malate in the photosynthetic cells as it is formed, whereas the roots respire it.

The resemblance between transport in roots and in bicarbonate-assimilating aquatics does not exist in the dark because in the latter the transport does not occur in the dark. But transport in roots is inhibited by cyanide. Accordingly the cyanide-resistant "ground" respiration of roots might be considered to correspond with the dark respiration of aquatics. This inference is supported by Osterlind's observation (1952a) that the dark respiration of Scenedesmus is cyanide-resistant, as is the ground respiration* of roots (recall that photosynthetic bicarbonate assimilation by Scenedesmus is cyanide-sensitive, page 8).

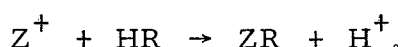
C. Theories of Cation Transport

The biochemical mechanism proposed by Lundegardh (1950a, 1954) for the independent movement of anions and cations is an attempt to explain salt accumulation by plants. However, his proposals seem to account for only some of the observed phenomena. He has observed that hydrogen ions are generated in root respiration and, in the absence of other cations bindable from the medium, these hydrogen ions concentrate on the root surface. If other cations exist in the medium they replace the hydrogen from the root surface. This replacement Lundegardh interprets as ion exchange--anions anchored to the root surface reversibly binding hydrogen ions and other cations in equilibrium with the medium. He further suggests that the hydrogen ions generated by root respiration migrate to the root surface along the cytoplasmic micelles, while other cations tend to exchange inward with them on these colloidal surfaces. Thus cations other than hydrogen ions tend to migrate into the root without intrinsic use of metabolic energy.

* Respiration can be measured either by carbon dioxide evolution or by oxygen uptake. Lundegardh has shown (1949) that cation accumulation by roots is not correlated with carbon dioxide evolution. But this is to be expected if one accepts the thesis that cation accumulation by roots entails incorporation of bicarbonate ions into malic acid. On the other hand a relation between oxygen consumption and cation accumulation in roots has been reported by Ulrich (1942) and others.

Various objections have been raised in the past against Lundegardh's suggestions. The chief weakness in these objections, however, is that they are negative; they offer no alternative interpretations of his observations. This paper does offer alternative interpretations, which are consistent with the theory developed here.

Overstreet and Jacobson (1952) have suggested that an ion carrier produced during metabolism forms a chelated complex with cations from the external medium. They suggest, in agreement with Lundegardh, that cations are bound in exchange for equivalent hydrogen ions,



But ZR is not a salt, according to Overstreet and Jacobson; it is a chelated complex. This complex subsequently undergoes chemical alteration, according to their view, to release the cation into the internal medium.

In the ideas expressed by Overstreet and Jacobson there is a minimum of inference. Their ideas are less than a total theory of ion transport because they do not describe the process. Furthermore, it is difficult to find in their paper any statements that are subject to experimental test. However, the formation of the carrier complex ZR by exchange of hydrogen from HR, the reactive radical, seems to conflict with experimental results described below; an oxidation-reduction reaction is indicated instead.

An oxidation-reduction mechanism for transporting cations has been suggested by a series of observations by Conway and his associates (Conway 1949, 1951, 1953, 1954, 1955; Conway and Brady 1950; Conway, Brady, and Carton 1950; Conway and Downey 1950a, 1950b). Yeast cells can transport potassium ions to their interior during fermentation, substituting hydrogen ions in the medium so that the pH of the medium may fall as low as 1.4. Potassium accumulation cannot be an exchange of potassium ions for hydrogen ions of organic acid produced inside the cells. This had been postulated previously by Conway and O'Malley (1944), but a variety of arguments can be assembled against it. Conway himself presents some of these arguments (1953). He has concluded that, instead, reduction of a metal catalyst is responsible for the production of hydrogen ions at the external face of the active membrane, a reduction entailing acceptance of electrons but not of equivalent hydrogen ions.

Conway offers the explanation that the subsequent oxidation of the catalyst does not entail reuptake of the released hydrogen ions because potassium, combined with the reduced catalyst, is carried to the interior of the active membrane, where the oxidation releases it. Supporting this suggestion is evidence that the acidifying reaction (i.e., formation of a negative electrical charge) is confined to a peripheral region of the yeast cell, whereas the reaction entailing the development of alkalinity is more central.

The importance of the work with yeast cannot be overemphasized. This paper lends support to the basic idea of a redox pump. However, it suggests important modifications of certain aspects of Conway's ideas.

Differences between transport in aquatic plants and roots, on the one hand, and in yeast, on the other, appear to include a concomitant assimilation of bicarbonate from the medium in the former, apparently to form malic acid, whereas in the latter this malic acid synthesis seems not to be involved. As a result of the uptake of bicarbonate from the medium, it would seem, cation transport in leaves and roots does not cause the hydrogen ion excretion which is so striking in yeast.

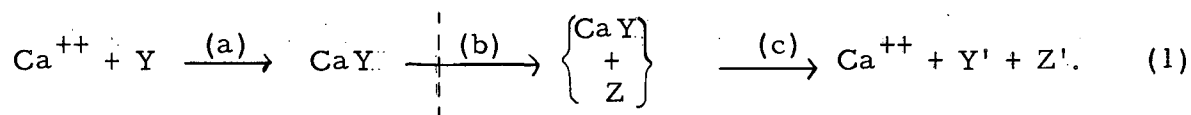
SECTION II: CATION TRANSPORT IN BICARBONATE-ASSIMILATING AQUATICS IN RELATION TO PHOTOSYNTHESIS AND TO RESPIRATION AT THE CELL SURFACE

Section I constitutes background necessary to understand the theory of cation transport, which is developed throughout the remaining pages. One premise of the theory has not yet been presented, however. This is that a reaction occurs during transport between the transported cation and a carrier in the active membrane.

There are two aspects to this proposition, both generally accepted by physiologists. The first is that there is a reaction between the transported ion and a constituent of the active membrane. This has verification in observations by Epstein and Hagen (1951) and Epstein (1953), who studied the effects on ion absorption by roots of varying ion concentrations in the medium. Plots of ion uptake vs concentration in the medium gave the expected curve rising

toward a maximum uptake rate. However, there was a straight-line relation between the reciprocal of the uptake velocity and the reciprocal of the ion concentration. This method of plotting has become usual for enzyme kinetic studies, but, according to Epstein and Hagen, it can be expected to give a straight line whenever the reaction mechanism involves combination of a material with an agent (present in small, essentially constant amount) to form a union that subsequently breaks down, but as the result of which some physical or chemical change occurs. Thus the straight-line relation indicates that the studied ion forms a bond with a carrier in the active membrane and then is released, transport being the result.* The second aspect, implied by the word carrier, is that bonding and release do not occur at the same place in the cell, but on opposite sides of the active membrane. Experimental verification of this aspect is hardly necessary because it would seem to follow from first principles.

This premise, applied to calcium** ion, is represented by



Equation (1) indicates that a carrier (Y) combines with calcium ion to form CaY. CaY then reorients in the active membrane (indicated by the dotted line) and is acted upon by another substance (Z) to liberate the calcium ion and yield Y' and Z'.

* Nielsen's reexamination (1954) of the kinetic arguments of Epstein and Hagen, although challenging details of their deductions, seems to cast no doubt upon the conclusion that the carrier is bonded to the ion as a step in transport.

** Calcium transport through bicarbonate-assimilating aquatic leaves can be studied more easily than can the transport of any other cation. This is because the leaves die when deprived of calcium, whereas they function for days in media to which no other cation has been added. Therefore data for calcium transport are more abundant and reliable than for the transport of other cations. Most of the inferences developed in the following pages regarding transport are based upon calcium data and accordingly it has been deemed advisable to likewise restrict the implications of the corresponding equations to calcium.

A. The Biochemistry of Cation Transport
In Bicarbonate-Assimilating Aquatic Leaves, Light Effects Excluded

It would seem that the direct relation of light to transport in aquatic leaves is due to an aspect of the transport mechanism in them different from the mechanism in other transport organs. Differences can be understood only after fundamental likenesses are comprehended, and accordingly attention is directed first toward aspects of transport not directly related to light.

Statement 1. Oxidation implements cation release from the active membrane.

Cation release (Reaction c, Eq. (1)) depends on the presence of oxygen, according to the results of several experiments for calcium ion with Potamogeton crispus leaves (Lowenhaupt, 1954). Briefly, these results are as follows:

(a) During transport the calcium content of leaves assumes a steady-state level. Transport ceases when the light is turned off, and the calcium content then shifts to the equilibrium level. This level, under suitable conditions, is higher when the medium is aerated than when it is not.

(b) Aeration of one side of a leaf in the dark causes a shift of calcium toward this side. The direction of shift toward the aerated medium is independent of the dorsal-ventral orientation of the leaf.

(c) Hydrogen ions reversibly release calcium from the leaves and as a result leaves withdraw calcium from a calcium chloride solution after they have been immersed in dilute hydrochloric acid. The amount of calcium re-absorbed is greater in the dark if nitrogen, rather than air, is supplied to the medium.

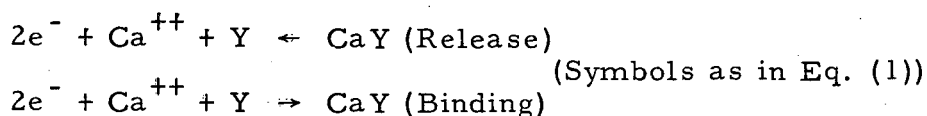
Only one interpretation of the effect of aeration is considered here.* This is that oxygen alters the equilibrium of the calcium-release reaction because it is the electron sump for a respiratory chain of which calcium release is a link. It would seem, according to this view, that oxygen alters the balance of an oxygenation reaction at the end of this chain; this balance in turn alters a dependent oxidation which in turn alters another, etc., until the

* Admittedly, other interpretations can be suggested. I have examined a number of these in my thesis. No other seems nearly so plausible as the one presented. Furthermore, this one leads to a mechanism of cation transport consistent with many data, whereas the others seem to lead to contradictions.

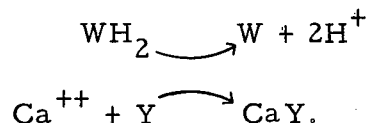
calcium-liberation reaction in its turn is affected. The oxygen effect would be expected to become diluted with each link in this balance sequence. This apparently is what happens, because the oxygen effect is small whereas, in contrast, corresponding phosphate effects are striking (unpublished data).

Oxidation often means loss of hydrogen, but not necessarily here. Passing of electrons from CaY (symbols refer to Eq. (1)) down the respiratory chain constitutes oxidation of CaY. Electrical neutrality at the active membrane appears to be maintained by releasing, not hydrogen ions, but other cations--calcium ions, for example.

Cation binding and release appear to be a cyclic process.* If this is correct, and if cation release is an oxidation, binding would seem to be a reduction:



Each (binding and release) would be a half reaction. Binding would require an electron donor, i.e., a reducing agent. The symbol WH_2 may be assigned to this agent,** which would be the material that drives cation binding. The reaction for calcium binding thus would be written



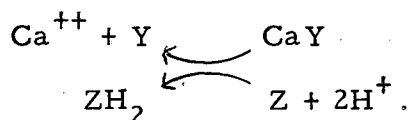
(The pairs of curved arrows are used to indicate the mutual dependence of the

* . Proof of this is not available. However, evidence of Rothstein and Meier (1952) indicates that the cation-binding material in yeast is a metaphosphate. (There is some controversy regarding the interpretation of these observations--Rosenberg and Wilbrandt, 1952; Rothstein and Larrabee, 1953--but it seems sure, at least, that the material must be a phosphate.) Such a material seems hardly likely to be continuously synthesized, but would seem more reasonably to be a permanent structure of the active membrane. (This cyclic reutilization of the cation binding group is inferred also by Conway and Downey, 1950b, who suggest that the trace of azide which suffices to inhibit potassium transport in yeast would seem inadequate if there were continuous synthesis of this group.) Further substantiation of the cyclic pattern is derived below.

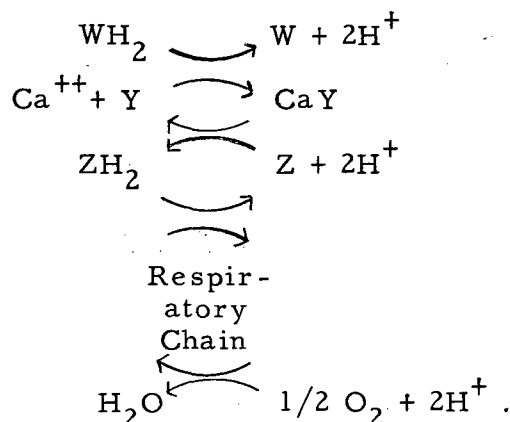
** Two hydrogens are indicated in WH_2 and ZH_2 (rather than any other number) because this number simplifies balancing the reactions for calcium binding and release.

chemical changes; thus the formation of CaY is coupled to the formation of W and 2H^+ from WH_2 .)

The electron acceptor for cation release would be Z , defined in Eq. (1) as the agent that implements cation (calcium) release. The release reaction for calcium would be



This discussion leads to a concept of transport as part of an electron-passing chain in which WH_2 shifts its electrons to Y , Y to Z , and Z ultimately to oxygen. This mechanism is depicted below.

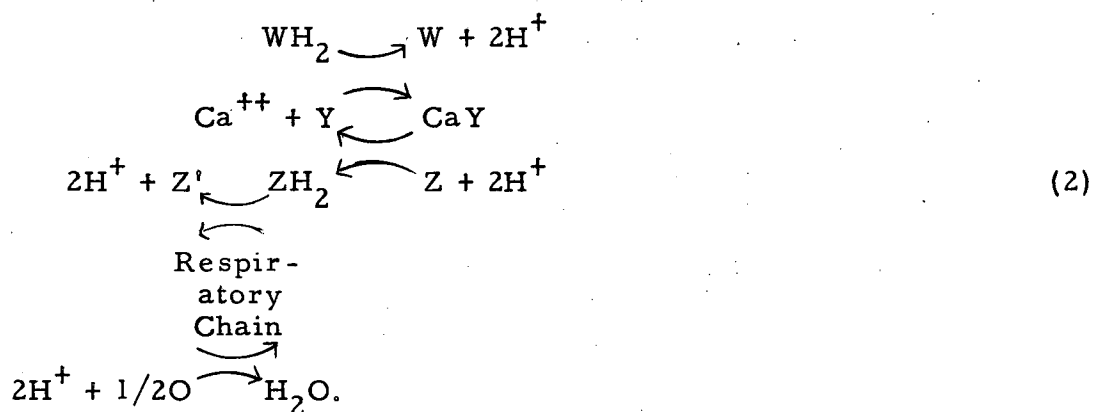


This chain would occupy a place in the total picture postulated in Eq. (1). One modification is indicated, however; Z would not necessarily be regenerated by the oxidation of ZH_2 . Three reasons for indicating that the product of oxidation of ZH_2 may not be Z may be suggested:

- (a) Light generates Z (the argument for this appears below) but Z is consumed in the dark.
- (b) Transport entails synthesis of a bicarbonate acceptor. The transformations of Z might contain the bicarbonate-accepting step, which would then synthesize a compound different from Z .

(c) The suggestion that calcium release entails breaking a high-energy bond is consistent with effects of exposing Potamogeton crispus leaves to dilute hydrogen peroxide in the dark (Lowenhaupt, 1954). The initial effect is to release calcium from the leaves and this constitutes further evidence that calcium release is an oxidation. But subsequently the peroxide is dissipated and then the leaves contain excessive calcium, which can be released by light. Peroxide, it would seem, destroys the high-energy group for calcium release whereas light seems to synthesize this group. (Unpublished studies of the effects of phosphate support the same conclusion.)

The resulting electron-passing mechanism is thus as follows:

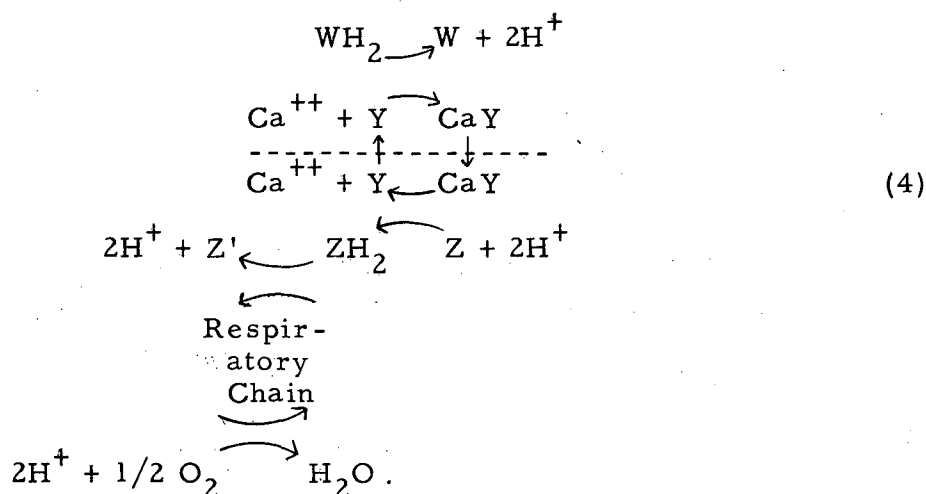


Two concepts proposed in this discussion may be introduced into the basic equation, Eq. (1) (p. 18). First, the suggestion that calcium binding and release is a cyclic process may modify Eq. (1) as follows:



Equation (3) results from modifying Eq. (1) to indicate that Y and Y' are the same material and accordingly remain a permanent structure of the active membrane.

The second concept, that calcium binding and calcium release are links in a respiratory chain (indicated in Eq. (2)), may be combined with Eq. (3) to give Eq. (4),



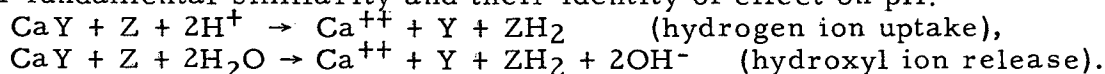
Equation (4) is a modification of Eq. (3) that indicates that calcium transport affords links in a respiratory chain. It is the graphic postulation that release is an oxidation and binding a reduction.

Statement 2. Y is reduced in binding a cation, oxidized in releasing it.

In Statement 1 the interpretation was presented that oxygen implements cation release by permitting the oxidation of Y. It was admitted, however, that other interpretations of the experimental results were feasible. Statement 2 supports the elected interpretation by showing that its implications are consistent with diverse facts.

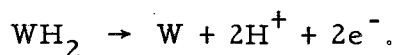
Reference to Eq. (4) discloses several biochemical implications of its mechanism of cation transport, implications some of which have been studied. It indicates that cation release is accompanied by equivalent pH rise of the medium. This is because Z is reduced, thus binding hydrogen ion* from the medium, whereas the material oxidized releases the transported cation rather than hydrogen ion. This rise in pH does occur on the dorsal leaf face of transporting aquatic leaves, exactly as indicated by theory.

* The fact that Eq. (4) indicates hydrogen ion uptake from the medium on the cation release face during transport might appear to pose an enigma because the medium on this face often becomes alkaline, i. e., hydrogen ion activity is suppressed. It should be understood, however, that the basic equation cannot express all possible mechanisms, and so one possibility is chosen, others whose results are the same being implied alternatives. The reduction of Z is represented as hydrogenation. However, equally logically, this reduction could be represented as a release of hydroxyl ions. Comparison of the calcium-release reaction, as implemented by these two manners of reducing Z, demonstrates their fundamental similarity and their identity of effect on pH:

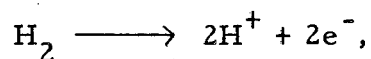


Thus removal of hydrogen ions from an alkaline medium is seen to pose no fundamental problem for Eq. (4).

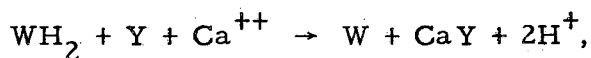
That cation uptake is accompanied by equivalent hydrogen ion release into the medium is also indicated. This is because WH_2 is oxidized in the uptake reactions, and although Y is reduced it does not take up W's hydrogen, but combines with the transported cation instead. This pH decline does occur for yeast. Likewise for Potamogeton crispus a pH decline has been observed under suitable conditions (Lowenhaupt, 1954). However, bicarbonate uptake, which apparently is concomitant with cation transport in the Potamogeton leaf, would seem also to entail pH adjustment. It appears from the results of Arens and of Steemann Nielsen that when transport and bicarbonate uptake are in a steady state their pH effects nullify each other. Evidence for hydrogen ion release when cations are bound by roots is found in Lundegardh's observation (1940). Furthermore, Lundegardh reports that roots in distilled water or in dilute acid solution behave like hydrogen electrodes.* This suggests that in the absence of bindable cations the reducing agent for binding piles up. The reducing power would be expressed by the half reaction



The analogy to hydrogen as a reducing agent,



is thus apparent, and the strength of the reducing power in roots may be gauged by the resemblance to hydrogen at 10^{-3} atmosphere. Removal of cations from the medium would appear to block the reaction because the complete reaction,



requires calcium (or a cation other than hydrogen ion) as part of the oxidizing agent. It would seem from Lundegardh's results that this reducing power can be reversed in strongly acid solutions in which roots do not bind cations.

* Attention is directed to the parallel between the electrical potentials reported by Lundegardh at the surfaces of roots and the potentials reported by Conway and Downey (1950a, 1950b) at the surfaces of yeast cells.

Thus it appears that Eq. (4) is confirmed, because it predicts the transport effects as they have been observed.

One assumption in the development of Eq. (4), i.e., that calcium binding and release constitute a cyclic process, was not proved. However, reconsideration of this assumption in the light of subsequent ideas seems to confirm it. Evidence that calcium binding results from the reduction of Y has been presented. Likewise evidence has been offered that release results from the oxidation of CaY. Arguments for these two were independently derived, the former from effects of transport on pH and hydrogen potential, the latter from effects of oxygen on calcium release. Where oxidation follows reduction a cyclic reaction is the natural inference.

B. The Structures of Transport--Location and Function

Metabolic poisons can block transport even when the cells are not killed. Dead cells never transport even when the enzymes are still active. Therefore it seems that two factors are necessary for transport, the biochemical reactions and the structural integrity of the living system. Certain aspects of the reactions have already been considered. In this section the structures of transport are discussed in more detail.

Statement 3. The plasma membranes at the external cytoplasmic surface of each leaf face are active membranes. Binding cations to these is a step in cation transport.

A calcium-binding compound exists on certain areas on the two external faces of Elodea leaves. This was demonstrated by Arens (1938a) by immersing the leaves in sodium oleate or ammonium oxalate solutions and observing the precipitated calcium salt under the microscope. Mature leaves attached calcium in patterns on the leaf surface distinct for different cells. Table I summarizes the patterns for the major cell types. Immature cells did not bind calcium, but dead cells, killed by boiling in alcohol or mineral acid solutions, could still bind calcium in the same pattern as living cells.* That

* Cells killed by the parasite (Table I) contrast with experimentally killed cells in that the former do not bind calcium. Decay of the calcium-binding agent in the parasitized cells is a possible explanation of this difference.

Table I

Pattern of calcium oleate precipitation on various cells of <u>Elodea</u> leaves (After Arens)						
	Vein	Flank Cell	Lamina Cell	Edge Cell	Tooth	Dead ^a
Topside	free	unilateral	cap-shaped	entirely covered	tip	free
Underside	free	entirely covered	entirely covered	entirely covered	tip	free

^a This refers to cells that have been attacked by an animal parasite (protozoan?). The parasite penetrates the cell, kills the inside, and ultimately empties the entire cell.

calcium in the membrane was responsible for the precipitate was indicated by leaves boiled to disintegration. In them the precipitate was formed by disrupted cells without cell content, and even isolated external membranes from the top of the leaf formed typical cap-shaped deposits.

That this binding is a step in transport is indicated by the fact that it does not occur on immature cells that do not transport (Arens, 1936a) or on cells whose location above or below a vein precludes transport. Therefore calcium binding seems not to be performed by inert constituents of the cell wall; the binding appears to be part of transport.

That this calcium binding is part of calcium transport and, further, that the transport of potassium occurs at the same loci, is indicated by other observations by Arens (1938b). He illuminated Elodea leaves in calcium and potassium bicarbonate solutions containing manganous ions and observed the resulting brown precipitates under the microscope. (Manganous ions react with hydroxyl ions and oxygen to form a brown precipitate, probably MnO_2 . Manganous ion thus is a cytochemical locator for alkali.) He found that the sites of the brown precipitate and of the bound calcium were similar at the dorsal leaf faces. This locates the excretion of calcium and potassium

at the areas where the calcium-binding molecules occur on the dorsal face. That the corresponding areas on the ventral leaf face excrete no alkali is expected from other observations (discussed above) demonstrating that ions are moved into the ventral side in the light, not out. Therefore absence of the precipitate on the ventral leaf face is compatible with the view that calcium binding on this face also is a step in transport. (Potamogeton fluitans, one may note, showed similar brown areas on the upper cells of the leaves. But the locations of the calcium-binding molecules on the faces of these cells have not been ascertained.)

Consideration of the physiological role of cation transport, i. e., to make external bicarbonate available, also indicates that it must occur at the leaf face. It seems that the surface membrane of the cytoplasm impedes the penetration of bicarbonate ions and that therefore the transport reactions are necessary to make the bicarbonate's carbon available in the cytoplasm where photosynthesis occurs. Capture of bicarbonate from the medium would be possible only for reactions at the cytoplasmic surface.

Plasma membranes at the leaf surface fulfill the active membrane requirements. They are cytoplasm and so can participate in metabolic processes; they are the external surface of the protoplast; and they are not freely permeable to ions. (Barrier to ion permeation is an obvious essential of the active membrane.) Their thickness, of the order of a single layer of molecules (Danielli and Davson, 1943), seems to correspond to the thickness of the active membrane. This is because the bound calcium must orient from one side of the active membrane to the other, and for this to occur the thickness of the active membrane would seem not to exceed the length of one molecule. It seems, therefore, that the active membrane is in the same location as the external plasma membranes and it has the same properties and dimensions. Apparently these plasma membranes are the active membrane.

Two active membranes apparently act in series, the one on the ventral leaf face pumping cations into the leaf in the light and the one on the dorsal face pumping them out. Evidence for this, in the case of calcium, is found in one of my own experiments, in which Potamogeton crispus leaves were floated on solutions containing calcium ions and the effect of light vs darkness on the entrance and exit of calcium was observed (Lowenhaupt, 1954). Water

rolls off these leaves, so that they can be floated with only one face in contact with the solution, and the other face dry. It was found that when the ventral face was in contact with the solution, light pumped calcium into the leaf, whereas darkness allowed escape. With the dorsal face in contact with the medium, light pumped calcium out of the leaf, whereas darkness allowed it to enter.

The total thickness of all active membranes, it would seem, constitutes only a minute fraction of the conducting path through the leaf. The remainder of the path appears to be through the cytoplasm of successive cells. Evidence that vacuolar fluid does not conduct calcium in Elodea leaves is found in the observation of Mazia (1938) that injury such as slight plasmolysis permits calcium to enter the vacuoles and there to be precipitated as calcium oxalate. Calcium ion is thus shown to be insoluble in the vacuolar fluid which, accordingly, is unsuitable as a medium for calcium ion migration.

Elimination of the vacuolar fluid leaves the cytoplasm as the logical material in which cations must be distributed between the ventral active membrane and the dorsal one. Cytoplasmic streaming suggests itself as a method of hastening this distribution.

The presence of cations in the cytoplasm, not bound to an active membrane, does not mean they are free in solution. The negative charge on the cytoplasmic micelles (Frey-Wyssling, 1953) may be expected to attract cations; Jenny and Overstreet (1939) suggest that ions thus electrostatically attracted may migrate by exchange along the colloidal surfaces more rapidly than they could diffuse if the colloid were not present. Especially suggesting that ion exchange may facilitate cation migration is the fact that the ions must cross cell boundaries in their journey through the leaf.* (The leaves are thicker than a single layer of cells.)

* No active membranes are postulated at the cell boundaries inside the leaf because Arens observed no precipitated calcium on these boundaries after leaves were immersed in sodium oleate or ammonium oxalate solution. This was true for living and killed leaves alike.

C. The Relation of Light to Cation Transport in Bicarbonate-Assimilating Leaves

The role of light in cation transport in bicarbonate-assimilating leaves, not considered previously, is discussed here.

Statement 4. Photosynthesis and transport are mutually dependent.

That there is a close relation between photosynthesis and cation transport in aquatic leaves seems self-evident. Cation transport occurs at the leaf surface, however, whereas the photochemical reaction of photosynthesis takes place at the chloroplasts, within the cells. Therefore one step for transport and bicarbonate utilization would seem to be synthesis at the chloroplasts of an energy-containing coupling agent which streams outward to the active membrane.

Evidence that the reactions of transport are accompanied by synthesis of a bicarbonate acceptor at the active membrane has been discussed. Thus bicarbonate utilization in photosynthesis seems to occur in two mutually dependent aspects spatially separated from each other, i.e., one at the site of photosynthesis, where an energy-containing coupling agent is produced, and one at the active membrane, where bicarbonate carbon is brought into the cell. The combination of bicarbonate utilization in photosynthesis and cation transport might be outlined as follows:

1. Synthesis of an energy-rich coupling agent at the site of photosynthesis;
2. Migration of this agent to the active membrane;
3. Use of its energy in (a) Cation Transport, (b) Bicarbonate uptake;
4. Migration of the products of Step 3 to the site of photosynthesis (except the cation, which migrates from the uptake side in the leaf to the excretion side);
5. Use of these products (except the cation of course) in photosynthesis (this includes synthesis of more coupling agent).

Suggestive experimental support for this view is found in a reinterpretation of some observations of Osterling (1951b, 1952b). He found in Scenedesmus quadricauda that bicarbonate-assimilating ability depends upon culture conditions. Cells from certain of his observed conditions were able to assimilate

bicarbonate only after a period in the light. This can be interpreted in terms of separation of bicarbonate uptake at the active membrane and the photochemical reaction at the chloroplasts; these two reactions depend on each other, so that if the raw material of both is removed in the dark, light can start neither until the other begins; photosynthesis is blocked temporarily. Osterlind reports that the lag in bicarbonate assimilation does not occur if carbon dioxide is in the medium, and is terminated abruptly if carbon dioxide is introduced, regardless of pH.* Thus it appears that carbon dioxide can prime bicarbonate assimilation because the utilization of carbon dioxide is independent of transport.

Statement 5. Photosynthesis releases cations from the active membrane.

The first step in cation transport through the leaf is cation binding to the face of the leaf. Binding must be a dynamic process. Once bound, the cation must be oriented and released as indicated in Eq. (1) (page 18). This is transport.

Light implements the transport of cations. The action of light might be localized in Equation (1) in three ways:

- a. It could implement the cation-binding reactions.
- b. It could implement the cation-release reactions.
- c. Both binding and release might be implemented by light.

Two experiments that have been discussed above indicate that light releases calcium from the active membrane. First: Light released calcium from leaves that had been induced to hold an abnormally large amount of calcium by hydrogen peroxide treatment (p. 22). Second: Light released calcium from the dorsal leaf face of floated leaves into the medium (p. 27).

* Introduction of free carbon dioxide into strongly alkaline media apparently was possible in Osterlind's experiments because carbon dioxide hydration requires time.

The manner whereby light releases cations was suggested in the preceding step. It would seem that an energy-containing agent, created by photosynthesis, migrates to the active membrane and there causes cation release.*

D. The Role of Cation Transport in Plant Metabolism

Cation transport is not an isolated process but is coupled to other aspects of metabolism. Thus in roots, excessive hydrogen ion concentration prevents the binding of other cations, thereby preventing transport, and as a result the roots die (Lundegardh, 1940). In yeast, likewise, many observations relate metabolism and cation transport. Rothstein (1955) has observed effects of a number of cations from the external medium on both the rates and the pathways of metabolism. He cites evidence that much of the machinery of glycolysis occurs at the cell surface and that its functioning depends upon the entrance of cations from the medium**. Once inside the cells, these cations no longer can

* Before accepting the conclusion that light powers cation release one must also consider the alternate possibility that it powers cation binding directly and thereby makes release possible by supplying bound cations upon which the release reactions can act. Conflicting with this alternate suggestion, however, is the observation that a variety of injurious treatments (immersion in hydrogen peroxide solution, exposure to excess oxygen, anoxia prolonged several hours) increase the calcium-holding capacity of *Potamogeton crispus* leaves. The effect of some of these treatments (hydrogen peroxide and oxygen) can be reversed by light under suitable conditions. Therefore it appears that release, not binding, requires coupling to an energy-yielding metabolic system, and that this energy can be uncoupled by any of the listed injurious treatments, but that if injury is not too extensive light can restore the energy. Of course the binding reaction does require a reducing agent and therefore must depend upon light indirectly (since all reducing power in photosynthetic autotrophs stems from light). It appears, however, that a reserve of this reducing agent exists which can power binding in the dark.

** Cation transport in yeast seems to be more complicated than in the photosynthetic, bicarbonate-assimilating aquatics. Rothstein believes that at least three mechanisms can transport cations in yeast. One is concerned with the uptake of K^+ (and to a smaller extent, other monovalent cations) in exchange for H^+ from the cells. The second is concerned with the outward transport of Na^+ in exchange for K^+ . The third is responsible for the uptake of bivalent cations, when they are present, complexed with phosphate. There is no evidence, however, for more than one kind of cation transport in bicarbonate-assimilating aquatics.

alter this respiration. Furthermore, not all cations are suitable. Uranyl ion is not. It cannot penetrate the cell surface, but it competes with other cations that are suitable for the cation-binding groups, at the cell surface. This competition would seem to explain the toxicity of uranyl ion for yeast. (See Rothstein and Larrabee, 1948.)

Calcium transport appears to have a special biochemical position in many cells (including the bicarbonate-assimilating aquatics); the cells die when calcium is absent even though other cations are available. Distilled water appears to block the surface respiration of cells by blocking calcium transport, because roots have been observed to die in distilled water but to be preserved in dilute calcium solutions (True, 1914, 1922). This must result from a need for calcium transport into the cell, because calcium is abundant within the cells, and even where calcium is available from the seed (Burström, 1952) or from other roots of the same plant (True) it must be present also in the external medium of every root. (See also Libbert, 1953, Kersting, 1938, and Burström, 1953, for discussions of the need for calcium in the external medium of roots.) Oxalate ion--which competes with living cells for calcium at their surface (Arens, 1938a)--has been observed by Schimper (1890) and by Blagoveshchensky and Kologrivova (1945) to be toxic to plants. Yet oxalate is a normal constituent of many cells (Olsen, 1939). I have observed several photosynthetic leaves that assimilate bicarbonate and that likewise can live only if calcium is in the external medium. Nitella flexilis--which transports calcium (Arens, 1939)--if kept in distilled water loses its ability to respond to electrical and other stimuli, but it can be restored to its normal condition by addition of small amounts of calcium to the external medium (Osterhout and Hill, 1933). Further evidence for a role of calcium in the active membrane for the metabolism of Elodea cells is found in an experiment by Weber (1932). Elodea leaves were dipped into acetic acid solution and transferred to distilled water, and the cells were observed after some time. The effect of this treatment coincided with Arens's observations on calcium binding to the cell surface. (Refer to page 26 for a summary of Arens's observations.) Cells whose surface Arens found to bind calcium were killed, whereas cells that did not bind calcium were intact. Cells that bound calcium over only a small area were injured but not killed. It would seem that the acid solution competitively removed the physiological cations (including calcium ions) from the active membrane and that this destroyed the cells, whereas other cells not dependent upon the active membrane survived.

The biochemistry of cation transport in animal cells seems to occupy a place in metabolism similar to its place in plants even though examples demonstrating this place are not so numerous. The need for calcium of nerves and muscles is well known, and this need appears to be similar to the need of Nitella cells. Likewise toxicity of uranium for the cells of the kidney tubule seems similar to its toxicity for yeast. Uranium attaches at the tubule cell surface and thus blocks glucose metabolism, and death of the tubule cell is the outcome (Dounce, 1949). The prolongation of the glucose tolerance curve of animals poisoned with uranium (Dygert, LaBelle, Laskin, Pozzani, Roberts, Rothermel, Rothstein, Spiegl, Sprague, and Stokinger, 1949) suggests that glucose utilization in cells other than those of the kidney tubules may also be blocked.

Thus in the cases cited, cation transport and the associated respiration at the active membrane would seem to be essential for normal cell metabolism and vital integrity.

ACKNOWLEDGMENT

Heartfelt thanks are tendered to Professors J. P. Bennett, Roy Overstreet, and M. A. Joslyn for generous assistance without which this work could not have been accomplished.

This work was done under the auspices of the U. S. Atomic Energy Commission.

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