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Bicarbonate Assimilation by Fresh-Water Charophytes and Higher Plants: I. Membrane Transport of Bicarbonate Ions is not Proven

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Summary. Although it is generally believed that *Chara* and some fresh-water angiosperms transport bicarbonate ions inwards across their plasma membranes, there has been no direct demonstration of such transport in these plants. The (indirect) arguments for their transporting HCO_3^- are arguments against the inward diffusion of CO_2 at the observed rates. They rest on calculations of the equilibrium concentration of CO_2 or of the maximum rate at which CO_2 might be produced from HCO_3^- at the pH of the medium outside the cells. Since *Chara* acidifies the medium over about half the cell surface during C assimilation, these calculations have been based on questionable premises.

We propose a model for *Chara* in which the acidification is attributed to active efflux of H^+, and we calculate that both the equilibrium concentration of CO_2 and its rate of production outside the cell can be high enough to support the observed rates of C assimilation, without postulating transport of the species HCO_3^- or H_2CO_3.

Calculations are presented also for alternative models in which there is membrane transport of HCO_3^-. The first includes symport of H^+ with HCO_3^-, again dependent on active H^+ efflux. In the second, there is active electrogenic transport of HCO_3^-. In this case the low pH in the medium outside the cell is caused by the dissociation of H_2CO_3 produced by hydration of CO_2 which leaks from the cell cytoplasm.

All three models are consistent with the observations to date, but the first is more economical of postulates. It can also explain the apparent transport of HCO_3^- by fresh-water angiosperms such as *Egeria*.

It is generally accepted that some charophyte plants, notably *Chara,* and some fresh-water angiosperms, e.g., *Potamogeton* and *Egeria*, can transport HCO_3^- across their cell membranes during photosynthesis (Steemann-Nielsen, 1960; Raven, 1970). It has been shown that such transport must be thermodynamically uphill (Raven, 1970), but the reaction involved is still uncertain. Primary, electrogenic, active transport of HCO_3^- (e.g., by an ATPase) has been suggested (Hope, 1965; Lucas, 1977; Walker & Smith, 1977); but equally the mechanism might be the active efflux of H^+ combined with proton-bicarbonate symport (Walker & Smith, 1977) or with passive permeation of H_2CO_3 (Walker, 1980). These mechanisms have been invoked because it has generally been accepted that under alkaline conditions the permeant species CO_2 cannot be sufficiently concentrated, and cannot be produced sufficiently rapidly, to explain the observed rates of assimilation. However, recently Prins and Helder (1980) suggested that *Egeria* (*Elodea*) and *Potamogeton* do acquire CO_2 from alkaline media by acidifying the medium at the leaf surface.

Therefore, we examine again the evidence against CO_2 as the species transported, i.e., the arguments for HCO_3^- transport. We discuss *Chara* and draw the parallel between this plant and the fresh-water angiosperms. We do not consider HCO_3^- transport by microalgae or by marine macrophytes.

Chemical Background

Carbonic acid is a weak acid, which undergoes the following reactions of interest to us:

$$2H^+ + CO_3^= \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O \quad (1) \quad (2) \quad (3)$$

$$HCO_3^- \rightleftharpoons CO_2 + OH^- . \quad (4)$$

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Reaction (2) has a forward rate-constant of 5×10^7 m^3 mol^{-1} sec^{-1} (Bell, 1973) and (1) will have one of a similar order of magnitude; we can consider them to be so fast that only their equilibrium is important; their equilibrium constants are 3.98×10^{-8} mol m^{-3} and 0.172 mol m^{-3}, respectively. Reaction (3) has forward and backward rate constants of 20 sec^{-1} and 9.39×10^{-7} m^3 mol^{-1} sec^{-1}, while (4) has rate constants of 2×10^{-4} sec^{-1} and 8.5 m^3 mol^{-1} sec^{-1} (Gutknecht, Bisson & Tosteson, 1977). For the present purpose we will be concerned with the reaction composed of (2) and (3) in series, with (4) in parallel. This overall reaction has a pH-dependent time-constant given by:

$$t_c^{-1}=0.052+20/(1+0.172/[H^+]) +2\times 10^{-4}+8.5[OH^-]. \quad (5)$$

The values are:

pH: –	3	4	5	6	7	8	9	10
t_c(sec): –	0.06	0.14	0.87	5.96	15.5	16.2	7.28	1.11

The tabulated values illustrate the speeding of the reaction at low pH, caused by the increase in the equilibrium concentration of H_2CO_3, which will be the basis of this paper, and the speeding at high pH, where reaction (4) has a significant rate, which is not important in the present discussion.

In their study of permeation of these species through lipid bilayers, Gutknecht et al. (1977) showed that CO_2 was the permeant species, with a permeability coefficient of 3.5×10^{-3} m sec^{-1}. The permeability coefficient for H_2CO_3 was apparently too small to estimate, as was that of HCO_3^-.

The diffusion coefficients in aqueous solution are taken to be: CO_2, 1.8×10^{-9} m^2 sec^{-1}; H_2CO_3, 1.5×10^{-9} m^2 sec^{-1}; HCO_3^-, 1.15×10^{-9} m^2 sec^{-1}. The first value comes from International Critical Tables, the second is from it using Stokes' Law, and the third is calculated from the electrophoretic mobility (Glasstone & Lewis, 1960). This gives the diffusion coefficient of the single ion, which is appropriate for this problem, not that of a neutral salt.

The permeability of a lipid bilayer to CO_2 is thus seen to be the same as that of a layer of water about 0.5 μm thick; this is so high a permeability that its effect can be neglected in what follows (*cf.* Raven, 1970).

Cells in water are surrounded by unstirred layers of solution whose thickness may make them rate-limiting for the permeation of some solutes (Dainty, 1963). In a study of the transport of amines into *Chara*, Walker, Beilby and Smith (1979) found the unstirred layer to be about 30 μm thick when medium flowed axially at 10 mm sec^{-1}, and up to 150 μm thick at lower flow rates. Smith and Walker (1980*b*) have calculated unstirred layer thicknesses in photosynthetic experiments as follows:

40 μm, *Nitella*, well-stirred (Brown & Tregunna, 1967)

> 2 mm, *Chara*, stagnant (Lucas, 1975)

60 μm *Egeria*, well-stirred (Browse, Dromgoole & Brown, 1979).

In such unstirred layers the local pH may be very different from that in the bulk (stirred) medium, and chemical reactions may occur which affect apparent transport rates (Gutknecht & Tosteson, 1973). Here we reevaluate the role of reactions (1) to (4) in the transport of carbon species at high bulk pH.

The Evidence for Bicarbonate Transport

In the literature on HCO_3^- assimilation by macroscopic fresh-water plants, there are two arguments for the membrane transport of HCO_3^- that have been found convincing:

(i) that in alkaline media the equilibrium concentration of CO_2 is too low to support the observed rate of C assimilation. This is a strong argument only if the equilibrium concentration is too low to produce the observed rate even with no unstirred layer, since the production of CO_2 from HCO_3^- in the unstirred layer can "short circuit" its diffusion resistance (Gutknecht & Tosteson, 1973).

(ii) that in alkaline media the maximum rate of conversion of HCO_3^- to CO_2 is lower than the observed rate of C assimilation.

Both these arguments were used by Lucas (1975) in his study of C assimilation by *Chara*, and he was led to conclude that HCO_3^- must be transported. Similarly Browse et al. (1979) used the strong form of (i), together with (ii), and concluded that in *Egeria*, too, HCO_3^- is transported.

The calculations of equilibrium concentration of CO_2, and of its maximum rate of production by reactions (2) to (4), both directly involve the pH of the medium, since the concentration of HCO_3^- is a datum, and both the calculated quantities are proportional to the concentration of H_2CO_3. In the calculations mentioned the workers have used the pH of the bulk medium.

Local Deposition of $CaCO_3$, Local Alkalinity, and local Acidity

In the light, the *Chara* internodal cell is surrounded normally by acid and alkaline bands or patches of

unstirred medium, which alternate along its length (Spear, Barr & Barr, 1969; Lucas & Smith, 1973). Under natural conditions there is often a deposition of $CaCO_3$ on the cell wall in the alkaline bands (Arens, 1939). There are measurements indicating that the pH near the cell lies between 9.5 and 10.5 in the alkaline bands and between 6.0 and 7.5 in the acid bands (e.g., Lucas, 1977). However, these studies were all made with hemispherical pH electrodes of diameter 1.5 or 1.2 mm, so that they underestimate, perhaps seriously, the difference in pH between the cell surface and the bulk medium.

In *Chara*, acid/alkaline banding is associated with C assimilation in alkaline media, and the influx of C species occurs in the membrane zone under the acid bands (Arens, 1939; Lucas, 1976*a*). A large electric current circulates between neighboring acid and alkaline bands, positive charge flowing from acid to alkaline in the medium (Walker & Smith, 1977). The current densities are of the order of 0.1 A m^{-2}, equivalent to 1.0 μmol m^{-2} sec^{-1} of univalent ions. The transport process that operates in the membrane under the alkaline bands is passive uniport of H^+ or OH^- (Bisson & Walker, 1980*a*, *b*). The transport process producing the acid band is discussed below.

The best measurements of C assimilation by *Chara* are those of Lucas (1975); they were obtained by putting batches of 10 cells into unstirred medium in 20-ml test-tubes. With bulk pH values from 7.5 to 9.5, the saturated rate of assimilation was 0.5 μmol m^{-2} sec^{-1}, about half the saturated rate below 7.5 (1.2 μmol m^{-2} sec^{-1}). The buffering used in these experiments was not strong: 3 mM HCO_3^- was used at up to 1.4 units below its second pK_a. In more recent experiments HCO_3^- at only 0.5 mM was used (Lucas, 1977). Using thymol blue (Merck **9**:9142, range 8.0–9.6) as an indicator, we have observed acid and alkaline bands to occur in solutions containing 0.5–3.0 mM HCO_3^- at pH 8.7–9.2. Lucas (1975) also found that C assimilation was not affected by 5 mM TES[1] at up to 1.1 units above its pK_a (i.e., up to pH 8.6); our own experiments with thymol blue again suggest that acid/alkaline banding is not suppressed. There is no reason therefore to accept that the pH at the cell surface was the same as that in the bulk medium: yet the conclusion that there was an influx of HCO_3^- rather than of CO_2 rests entirely on this assumption, which was made in the calculations of maximum rate of CO_2 production (Lucas, 1975, pp. 342–5).

[1] TES – N-tris[hydroxymethyl]methyl-2-aminoethanesulphonate, pK_a 7.5.

Models for C Assimilation in Alkaline Media

I. External Production of CO_2

In this model the electric current emerging from the cell membrane under the acid band is taken to be produced by the efflux of H^+. We assume that HCO_3^- does not permeate; and for simplicity ignore the possible permeation of H_2CO_3, although a high permeability for this species would "improve" the model. We assume that there is no diffusion barrier for CO_2 between the membrane and the site of the assimilation. Since an acid band will be modelled, we make the simplifying assumption that the species OH^- and $CO_3^=$ can be ignored; it turns out that reaction (4) can also be ignored, since interest centers on reaction rates at pH values of 7 and below, where its rate is negligible.

The movement of charged species in the medium is determined by gradients of concentration and of electrical potential difference. For simplicity again we will ignore the electric potential gradient, which is of the order of 1–2 V m^{-1} (Walker & Smith, 1977), and calculate the effects of the concentration gradients and chemical reactions (this procedure was also used by Ferier & Lucas, 1979).

The diffusion and chemical reaction of each species of solute means that each concentration obeys Poisson's equation in the steady state:

$$D_i \cdot \nabla^2 c_i + Q_i = 0 \tag{6}$$

where D_i is the diffusion coefficient of the species i, ∇ is the operator $(d/dx + d/dy + d/dz)$, c_i is the concentration of the species i and Q_i is its rate of production per unit volume. This equation is derived from the law of conservation of matter together with Fick's law of diffusion. As a further simplification we consider a model of an acid band surrounding a *Chara* cell with concentration gradients in the radial direction only (Fig. 1), so that in cylindrical coordinates ∇^2 becomes $\{(d/dr)^2 + (1/r)\cdot(d/dr)\}$; we can then represent the model by the set of equations:

$$D_C \cdot \nabla^2[CO_2] + R_3 = 0; \tag{7}$$

$$D_A \cdot \nabla^2[H_2CO_3] - R_3 + R_2 = 0; \tag{8}$$

$$D_B \cdot \nabla^2[HCO_3^-] - R_2 = 0; \tag{9}$$

$$D_H \cdot \nabla^2[H^+] - R_2 = 0; \tag{10}$$

$$[H_2CO_3] = [H^+]\cdot[HCO_3^-]K_2; \tag{11}$$

$$R_3 = k_3 \cdot [H_2CO_3] - k'_3[CO_2]\cdot[H_2O] \tag{12}$$

where R_2 and R_3 are the net rates of the reactions (2) and (3), respectively, k_3 and k'_3 are the forward and backward rate-constants for reaction (3), K_2 is

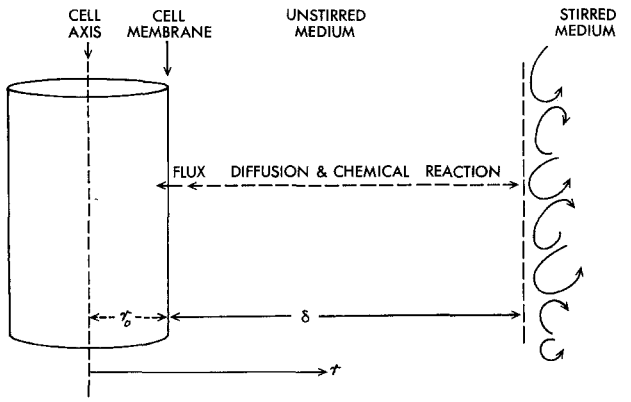

Fig. 1. Diagram of space in which calculations of diffusion and chemical reaction are carried out, showing boundaries at $r=r_o$, where flux or concentration of each species is given, and at $r=r_o+\delta$, where concentration of each species is given

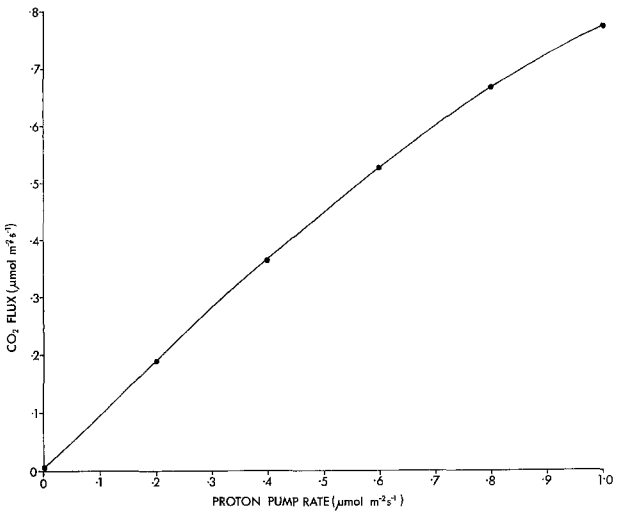

Fig. 2. Calculated CO_2 flux into cell against rate of proton pump, for external conversion model *I*. Conditions: bulk pH, 9; bulk $[HCO_3^-]$, 1 mM; δ, 1 mm

the equilibrium constant for reaction (2), and r is the radial coordinate measured from the axis of the cell. The labels *A*, *B*, *C* and *H* refer, respectively, to H_2CO_3, HCO_3^-, CO_2 and H^+. It can be seen that reaction (2) is assumed to be very near equilibrium at all values of r, so that Eq. (11) is satisfied, and R_2 can have any value required by the other parameters: the rate of reaction (3) is specifically calculated in Eq. (12).

The set of equations (7) to (12) can be simplified by elimination to give a set of three differential equations in which the unknowns are $[CO_2]$, $[H_2CO_3]$ and $[HCO_3^-]$. Corresponding finite-difference equations were set up, giving the values of these three variables at equal intervals of r. These equations, though nonlinear, were found to be soluble by Gaussian iteration. The solutions were generally satisfactory after 1500 to 4000 iterations, using 25, 50, or 100 steps in r. The stopping criterion was that the calculated CO_2 flux into the cell changed by less than 0.1% in the last 100 iterations. The boundary conditions imposed were:

(*a*) at the surface of the cell ($r=0.5$ mm):
either $[H^+]=$constant, or $\phi_H=$constant,

$$\phi_C = V_m \cdot [CO_2]/(K_M - [CO_2]); \quad (13)$$
$$\phi_A = 0;$$
$$\phi_B = 0;$$

where ϕ_i is the flux of species i, and K_M and V_m are the Michaelis-Menten parameters for photosynthetic fixation of CO_2, taken to be 20 μM and 1.0 μmol m^{-2} sec^{-1} (*see* Smith & Walker, 1980*b*);

(*b*) at the boundary of the unstirred with the stirred medium ($r=0.5$ mm$+\delta$):

$[HCO_3^-]=$constant;
$[H^+]=$constant;
$[H_2CO_3]=$value satisfying equilibrium for Eq. (2);
$[CO_2]=$value satisfying equilibrium for Eq. (3).

In Fig. 2, the calculated CO_2 influx (fixation rate) is shown as a function of H^+ efflux, for an unstirred layer 1 mm thick, a bulk pH of 9, and a bulk $[HCO_3^-]$ of 1 mM. If the ratio ϕ_C/ϕ_H is regarded as measuring the efficiency of the proton pumping in producing CO_2 influx, it can be seen that in the case displayed the efficiency is rather high, falling only as the V_m for fixation is approached. Under these conditions, which resemble those used experimentally (Lucas, 1975), the calculation indicates a sufficient rate of dehydration of H_2CO_3 in the unstirred medium near the membrane to produce C assimilation rates of the magnitudes actually observed — on the assumption that the low pH in the medium is the result of rapid proton efflux. A high efficiency, approaching 1.0, can be obtained because the diffusion resistance of the unstirred layer retains the CO_2 that is produced near the cell surface, and the H^+ that flow out from the cell. Figure 3 shows the radial concentration profiles for this case, and the profile of the rate of reaction (3). It can be seen that the reaction occurs within the 50 μm nearest the membrane, most within the first 20 μm, and that there is a rapid drop in pH in the same region. There is also a peak in the CO_2 concentration, and an inward concentration gradient for HCO_3^-, which, of course, supplies the C input for reactions (2) and (3) and thus for assimilation.

If the conditions are modified to include a representation of the cell wall (10 μm thick, with all diffusion coefficients reduced by a factor of 5), the results

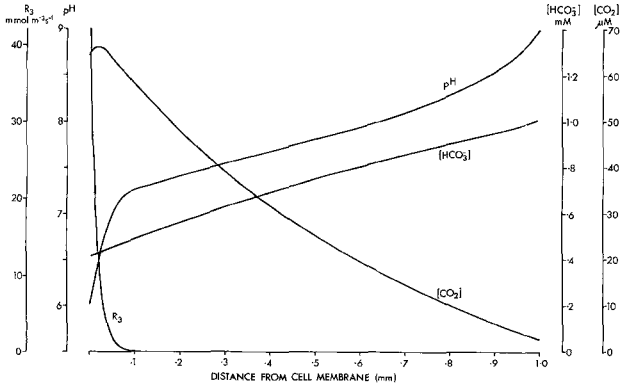

Fig. 3. Calculated profiles of concentration, pH, and rate of reaction (3) against radial distance from cell membrane ($r-r_o$), for external conversion model *I*. Conditions: bulk pH, 9; bulk [HCO_3], 1 mM; δ, 1 mm; H^+ pump rate, 1 μmol m^{-2} sec^{-1}

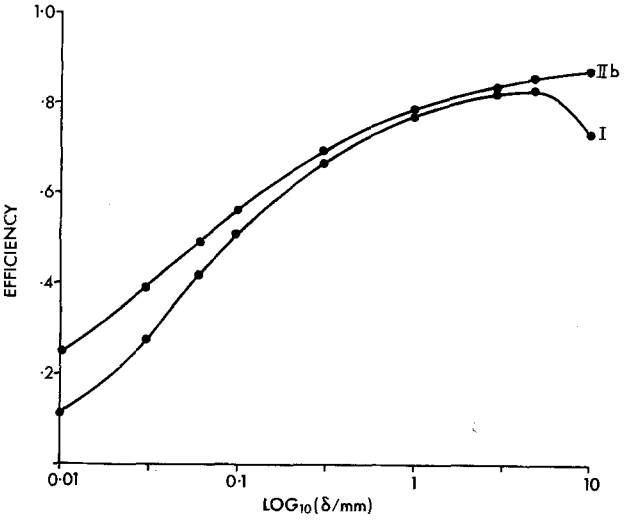

Fig. 4. Calculated efficiency (ϕ_C/ϕ_H) for external conversion model *I*, and ϕ_C/ϕ_B for bicarbonate pump model *IIb* plotted against δ. Conditions: bulk pH, 9; pump rate (H^+ or HCO_3^-], 1 μmol m^{-2} sec^{-1}

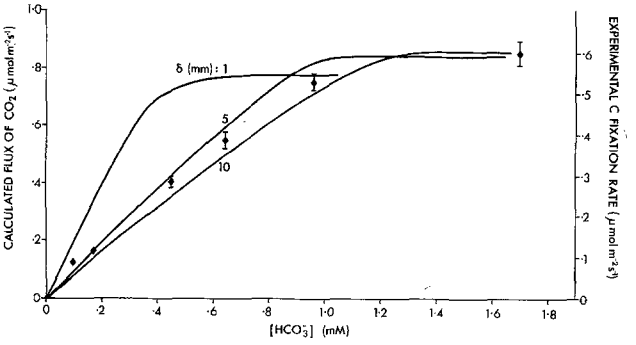

Fig. 5. Calculated dependence of ϕ_C on [HCO_3^-] for various values of δ, shown as solid lines, and referred to left-hand scale, with data of Lucas (1975) for rate of photosynthetic fixation of C at pH 9, referred to right-hand scale. (*See* text for explanation of relationship of scales.) Model: external conversion (*I*). Conditions: bulk pH, 9; pump rate = 1 μmol m^{-2} sec^{-1}

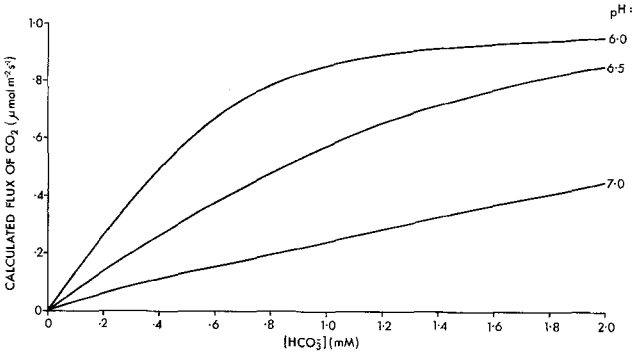

Fig. 6. Calculated dependence of ϕ_C on [HCO_3^-] for various values of surface pH. Model: external conversion (*I*). Conditions: bulk pH, 9; δ, 1 mm; pump rate, determined by condition of fixed surface pH

are very similar to those shown in Fig. 3. The drop in pH is about 10 μm closer to the wall, and for the same H^+ efflux the pH near the membrane is reduced (from 5.9 to 5.65). Subsequent calculations are determined without the model wall.

The thickness of the unstirred layer is less critical than might be supposed, although it does affect the efficiency. Figure 4 shows the efficiency as a function of thickness for the bulk conditions already used and a pump rate (1.0 μmol m^{-2} sec^{-1}) equal to V_m: at $\delta = 100$ μm, the efficiency is above 0.5, and at 30 μm above 0.25. Greater efficiency could be obtained at the expense of fixation rate by reducing the pump rate – the optimum H^+ pump rate would be a matter of the plant's "strategy", an inviting topic for future study.

Without a knowledge of the strategy actually adopted by *Chara* we cannot hope to model the real situation at all closely. It is interesting, however, that the simple assumption of a constant pump rate gives a dependence of assimilation rate on HCO_3^- concentration that resembles that found experimentally (Fig. 5). This figure shows that an unstirred layer about 5 mm thick gives a curve of the same shape as the experimental one, with a "knee" at about the same [HCO_3^-]. The matching of the saturated rates has no significance, since in the real cell this depends on the fraction of the surface covered by acid band. Using the boundary condition that the pH next to the membrane is constant at 6, gives a result not very different (Fig. 6). If the pH near the surface is assumed to be higher, the model still works

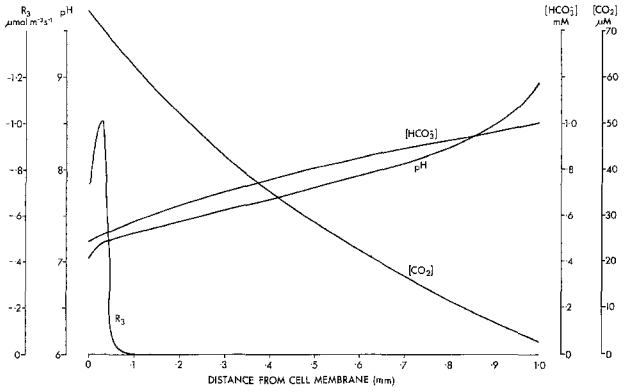

Fig. 7. Calculated profiles of concentration of carbon species, pH and rate of reaction (3) against radial distance from cell membrane ($r-r_o$), for bicarbonate symport model (*IIa*). Conditions: bulk pH, 9; bulk [HCO_3^-], 1 mM; δ, 1 mm; H^+ pump rate, 1 μmol m^{-2} sec^{-1}; symport $V_m=1$ μmol m^{-2} sec^{-1}; symport K_m, 10 μM for HCO_3^-; symport assumed saturated for H^+. Note that R_3 is here plotted negative upwards

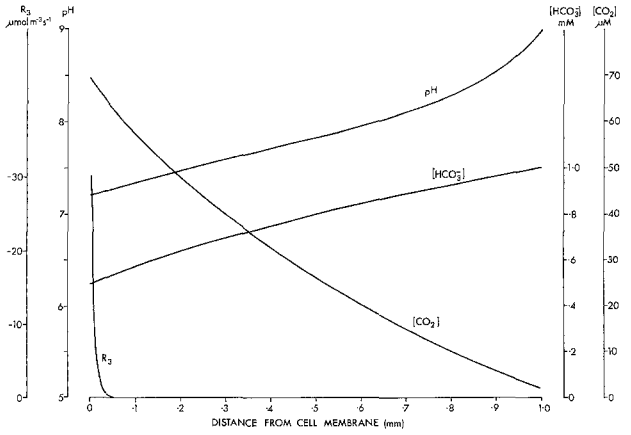

Fig. 8. Calculated profiles of concentration of carbon species, pH and rate of reaction (3) against radial distance from cell membrane ($r-r_o$), for bicarbonate pump model *IIb*. Conditions: bulk pH, 9; bulk [HCO_3^-], 1 mM; δ, 1 mm; HCO_3^- pump rate, 1 μmol m^{-2} sec^{-1}. Note that R_3 is here plotted negative upwards

with reasonable efficiency. Indeed the pH next to the membrane can be fixed at 7, with only the effect that [HCO_3^-] must be raised to restore the value of the CO_2 influx (Fig. 6).

II. Membrane Transport of HCO_3^-

The simplest viable models are symport with a proton or protons, and primary, electrogenic transport. We will consider these in turn.

(a) Proton Symport. In this model too the electric current crossing the membrane is taken to be an efflux of protons, which re-enter the cell with HCO_3^- in neutral symport. The addition of symport to the mechanism of model *I*, which must operate in any case, gives a range of possible models, depending on the kinetic characteristics assumed for the symport. Figure 7 shows a concentration profile for one such model, which can be compared with that in Fig. 3; the drop in pH near the cell surface is much reduced.

(b) Electrogenic Active Transport. In this model the electric current is produced by the transport system itself, and no proton efflux is assumed. It is assumed that in the cytoplasm the conversion of HCO_3^- to CO_2 is very rapid (carbonate dehydratase is supposed to be present), and in the steady state the concentration of CO_2 in the cytoplasm will be that at which the influx of HCO_3^- is equal to the rate of fixation given by Eq. (13) together with the rate of its diffusive efflux. The calculations, by the same method as before, give, for example, the concentration profiles of Fig. 8. As suggested previously (Walker & Smith, 1977), the hydration of the escaping CO_2 results in a low pH in the unstirred layer, without the rapid drop in pH near the membrane, characteristic of model *I*. As expected, a thin unstirred layer results in greater loss of CO_2 and in lower efficiency (defined as the ratio of fixation rate to HCO_3^- pump rate); *see* Fig. 4. A realistic model, as before, requires further information, here the relationship between ϕ_B and [HCO_3^-]; at present there is no evidence on which to base a guess at this relation (Smith & Walker, 1980*b*).

Discussion

In spite of much excellent experimentation in the past, there are few data which can be applied directly to distinguishing one of the present models from the others. This reflects perhaps some difficulty in appreciating the crucial role of the unstirred layer in C assimilation. Experiments do at least tell us that C is rapidly assimilated from media containing little free CO_2 and that this is accompanied by the circulation of electric current from parts of the cell or leaf surface that acidify the medium to parts that make it more alkaline. These features, and their quantitative expression, can be reproduced by each of the models discussed here.

It should be possible in principle to distinguish model *I* from the others by determining the pH profile near the cell membrane. The measurements so far published do not contain this information, since the electrodes used were so large[2] (Lucas & Smith, 1973;

[2] In the first reference a diameter of 1.5 mm is quoted; in the second is quoted a Microelectrodes Inc. type number, whose diameter is given by the makers as 1.2 mm.

Ferrier & Lucas, 1979) that the drop in pH (Fig. 3) would have been entirely missed. If the large (0.6 mm radius) electrode did not distort the diffusion gradients, it is easy to calculate that the average pH seen by its surface when touching the cell surface would be 7.6 for the profile of pH in Fig. 3. This bears no relation to the actual pH of 5.9 at the cell surface. The concentration profiles presumably are distorted by the electrode, and the net result will be difficult to predict – meanwhile we cannot reject the external conversion model (*I*) on the grounds that the drop in pH near the surface has not been observed.

If there is no observation already made that can distinguish between the models discussed here, we fall back on the requirement for economy of postulate, for the time being. The external conversion model requires less "hardware" than the bicarbonate transport models, needing only a proton pump, for which there is already rather good evidence (Spanswick, 1972, 1974; Walker & Smith, 1975). Model *I* does require that this pump run at up to 1.0 μmol m^{-2} sec^{-1}, which is a higher rate than has been postulated before, but this does not seem a very important objection, since all the models require a pump (either H^+ out or HCO_3^- in) that can run at that rate. The economy of postulate in model *I* is connected with an economy in the demand on the evolutionary process, since the proton pump is probably of ancient origin (Raven & Smith, 1976).

The external conversion model also provides neat explanations for two kinds of observation that are otherwise hard to explain without *ad hoc* arguments.

The Effect of Buffers and $CO_3^=$

Lucas (1975, 1977) has shown that buffers tend to inhibit C assimilation at high pH; this is true of TES, TAPS, Tris, tricine and borate. Smith and Walker (1980*a*) found methylamine and CAPS, whose pK_a values are similar, to be equally effective in inhibiting C assimilation at high pH. The transport model requires specific reactions to be postulated at the transport site for a wide variety of structures. If C assimilation at high pH depends on the formation of a narrow acid region near the cell membrane, as the dehydration model implies, the inhibition by buffers is explained independently of their chemical structure. The inhibition by $CO_3^=$ (Lucas, 1977) is explained in the same way: by removal of H^+ from the region near the membrane. Both models can account for Lucas' observations (1975, 1977) that the inhibition by buffers is greater when HCO_3^- is low (e.g., 0.5 mM compared with 3.0 mM). According to the transport model this is due to increased competition at the HCO_3^- transport site. The alternative explanation is that with a low HCO_3^- concentration the H^+ concentration at the cell membrane needed for a constant reaction rate is greater, i.e., the pH must be lower, and there is increased sensitivity to buffering.

The Effect of Low Ca^{++} and of High K^+

In a study of the resting electrical properties of the *Chara* membrane, Hope and Walker (1961) found that the PD was reduced to the level determined by diffusion of K^+ and Na^+ if the cells were treated to remove Ca^{++} and measured in media without Ca^{++}. It is now clear that the membrane PD is normally more negative than this level (e.g., Spanswick, Stolarek & Williams, 1967); this hyperpolarization is ascribed to the operation of a proton efflux pump. The inference is that the pump is inhibited by removal of Ca^{++}, although this has not been systematically studied.

The effect of raising the (usually low) concentration of K^+ in the medium is to produce a transition to the depolarized state in which, it is reasonable to postulate, the pump does not run (e.g., Walker, 1980).

Both of the above treatments inhibit HCO_3^- assimilation (Lucas, 1976*b*) and the explanation suggested for model *I* is simply that any treatment which inhibits the proton pump will inhibit C assimilation. The alternatives are to postulate two electrogenic pumps in *Chara*, one for H^+ and one for HCO_3^-, both sensitive to low Ca^{++} and to high K^+, or to postulate an HCO_3^- pump that is for no clear reason inhibited by depolarization.

Conclusions

It seems possible at the moment to suggest that in *Chara* there is a specific mechanism for C assimilation at high pH that does not involve the membrane transport of HCO_3^-. Instead, H^+ is circulated between two different regions on the surface of the photosynthetic organ; the regions of H^+ re-entry are at high pH (10.5) and are put out of action for C uptake, while the regions of H^+ exit are at a sufficiently low local pH (6–6.5) to be supplied with CO_2 by the external dehydration of H_2CO_3. Whether this process is significantly assisted by either H_2CO_3 permeation or proton/bicarbonate symport is not known. This suggestion offers rational interpretations for the effects of buffers, of high K^+ and of low Ca^{++}, and makes a testable prediction as to the pH profile near

the cell membrane. A similar mechanism could operate in such fresh-water angiosperms as *Egeria* and *Potamogeton* in which C assimilation at high pH is associated with the development of differences in pH and electric potential between the lower and upper surfaces of the leaf (e.g., Helder, 1975; Helder & Zanstra, 1977; Prins & Helder, 1980). In marine macrophytes the phenomena appear to be rather different, and we have not attempted so far to extend the present thesis to C assimilation by these organisms; nor do we here consider the question of scale involved in asking whether the micro-algae transport HCO_3^- across their membranes by this mechanism.

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References

Arens, K. 1939. Physiologische Multipolarität der Zelle von *Nitella* während der Photosynthese. *Protoplasma* **33**:295

Bell, R.P. 1973. The Proton in Chemistry. (2nd Ed.) Chapman & Hall, London

Bisson, M.A., Walker, N.A. 1980*a*. Membrane p.d. and OH^- transport in *Chara* as functions of pH. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 597–598. Elsevier/North-Holland, Amsterdam

Bisson, M.A., Walker, N.A. 1980*b*. The *Chara* plasmalemma at high pH. Electrical measurements show rapid specific passive uniport of H^+ or OH^-. *J. Membrane Biol.* **56**:1

Brown, D.L., Tregunna, E.B. 1967. Inhibition of respiration during photosynthesis by some algae. *Can. J. Bot.* **45**:1135

Browse, J.A., Dromgoole, F.I., Brown, J.M.A. 1979. Photosynthesis in the aquatic macrophyte *Egeria densa*. III. Gas exchange studies. *Aust. J. Plant Physiol.* **6**:499

Dainty, J. 1963. The polar permeability of plant cell membranes to water. *Protoplasma* **57**:220

Ferrier, J.M., Lucas, W.J. 1979. Plasmalemma transport of OH^- in *Chara corallina*. II. Further analysis of the diffusion system associated with OH^- efflux. *J. Exp. Bot.* **30**:705

Glasstone, S., Lewis, D. 1960. Elements of Physical Chemistry. Macmillan, London

Gutknecht, J., Bisson, M.A., Tosteson, D.C. 1977. Diffusion of carbon dioxide acrose lipid bilayer membranes. *J. Gen Physiol.* **69**:779

Gutknecht, J., Tosteson, D.C. 1973. Diffusion of weak acids across lipid bilayer membranes: Effects of chemical reactions in the unstirred layers. *Science* **182**:1258

Helder, R.J. 1975. Polar potassium transport and electrical potential difference across the leaf of *Potamogeton lucens* L. *Proc. Kon. Ned. Akad. Wet* **C78**:189

Helder, R.J., Zanstra, P.E. 1977. Changes of the pH at the upper and lower surface of bicarbonate-assimilating leaves of *Potamogeton lucens* L. *Proc. Kon. Ned. Akad. Wet.* **C80**:421

Hope, A.B. 1965. Ionic relations of cells of *Chara australis*. X. Effects of bicarbonate ions on electrical properties. *Aust. J. Biol. Sci.* **18**:789

Hope, A.B., Walker, N.A. 1961. Ionic relations of *Chara australis*. IV. Membrane potential differences and resistances. *Aust. J. Biol. Sci.* **14**:26

Lucas, W.J. 1975. Photosynthetic fixation of ^{14}C by internodal cells of *Chara corallina*. *J. Exp. Bot.* **27**:32

Lucas, W.J. 1976*a*. Plasmalemma transport of HCO_3^- and OH^- in *Chara corallina:* Non-antiporter systems. *J. Exp. Bot.* **27**:19

Lucas, W.J. 1976*b*. The influence of Ca^{++} and K^+ on $H^{14}CO_3^-$ influx in internodal cells of *Chara corallina*. *J. Exp. Bot.* **27**:32

Lucas, W.J. 1977. Analogue inhibition of the active HCO_3^- transport site in the characean plasma membrane. *J. Exp. Bot.* **28**:1321

Lucas, W.J., Smith, F.A. 1973. The formation of acid and alkaline regions at the surface of *Chara corallina* cells. *J. Exp. Bot.* **24**:1

Prins, H.B.A., Helder, R.J. 1980. Photosynthetic use of HCO_3^- by *Elodea* and *Potamogeton,* pH changes induced by HCO_3^-, CO_2, K^+ and H^+/OH^- transport. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 625–626. Elsevier/North-Holland, Amsterdam

Raven, J.A. 1970. Exogenous inorganic carbon sources in plant photosynthesis. *Biol. Revs.* **45**:167

Raven, J.A., Smith, F.A. 1976. The evolution of chemiosmotic energy coupling. *J. Theor. Biol.* **57**:301

Smith, F.A., Walker, N.A. 1980*a*. Effects of ammonia and methylamine on Cl^- transport and on the pH changes and circulating electric currents associated with HCO_3^- assimilation in *Chara corallina*. *J. Exp. Bot.* **31** (*in press*)

Smith, F.A., Walker, N.A. 1980*b*. Photosynthesis by aquatic plants: Effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^-, and to carbon isotopic discrimination. *New Phytol.* (*in press*)

Spanswick, R.M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* **288**:73

Spanswick, R.M. 1974. Evidence for an electrogenic pump in *Nitella translucens*. II. Control of the light-stimulated component of the membrane potential. *Biochim. Biophys. Acta* **332**:387

Spanswick, R.M., Stolarek, J., Williams, E.J. 1967. The membrane potential in *Nitella translucens*. *J. Exp. Bot.* **18**:1

Spear, D.J., Barr, J.K., Barr, C.E. 1969. Localisation of hydrogen ion and chloride ion fluxes in *Nitella*. *J. Gen. Physiol.* **54**:379

Steemann-Nielsen, E. 1960. Uptake of CO_2 by the plant. *In:* Handbuch der Pflanzenphysiologie V/1. W. Ruhland, editor. pp. 70–84. Springer-Verlag, Berlin

Walker, N.A. 1980. The transport system of charophyte and chlorophyte giant algae and their integration into modes of behavior. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 287–300. Elsevier/North-Holland, Amsterdam

Walker, N.A., Beilby, M.J., Smith, F.A. 1979. Amine uniport at the plasmalemma of charophyte cells. I. Current-voltage curves, saturation kinetics, and effects of unstirred layers. *J. Membrane Biol.* **49**:21

Walker, N.A., Smith, F.A. 1975. Intracellular pH in *Chara corallina* measured by DMO distribution. *Plant Sci. Lett.* **4**:125

Walker, N.A., Smith, F.A. 1977. Circulating electric currents between acid and alkaline zones associated with HCO_3^- assimilation in *Chara*. *J. Exp. Bot.* **28**:1190

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