

## SHORT COMMUNICATIONS

### METABOLITE TRANSPORT IN C<sub>4</sub> PHOTOSYNTHESIS\*

By C. B. OSMOND†

Recent experiments which demonstrate the limited and complementary photosynthetic capacity of mesophyll and bundle sheath chloroplasts in the leaves of C<sub>4</sub> plants emphasize the role of structural organization in this photosynthetic process (Slack, Hatch, and Goodchild 1969; Edwards *et al.* 1970; Woo *et al.* 1970). The current interpretation of C<sub>4</sub> photosynthesis involves the cooperative activity of chloroplasts in both cell layers and demands the transport of photosynthetic metabolites between tissues during CO<sub>2</sub> fixation (Karpilov 1969; Downton 1970; Hatch and Slack 1970). This paper examines the rates of organic anion transport implied by this hypothesis, demonstrates the rapid appearance of label in bundle sheath cells during <sup>14</sup>CO<sub>2</sub> fixation, and discusses structural aspects of the system relevant to transport.

#### *Materials and Methods*

The distribution of label in tissues of *Atriplex spongiosa* leaves was examined by micro-autoradiography after 2 sec <sup>14</sup>CO<sub>2</sub> fixation. Leaves were taken from glasshouse seedlings and exposed to <sup>14</sup>CO<sub>2</sub> by a modification of methods described elsewhere (Osmond, Troughton, and Goodchild 1969). The leaf was exposed to 50 μCi of <sup>14</sup>CO<sub>2</sub> (48 Ci mole<sup>-1</sup>) injected with 10 ml of air into a 75-ml vessel. After the preset time of 2 sec had elapsed (measured by a Sodeco counter) the <sup>14</sup>CO<sub>2</sub> was removed by evacuation, the light source extinguished, and the leaf dropped into isopentane-8% methyl cyclohexanone at -100°C. All operations were completed within 0.2 sec. The frozen leaf tissue was dried at -40° in a Christ freeze-drier for 48 hr, then embedded in paraffin at 58°C under vacuum. 10 μm sections cut from the paraffin block were spread together with a few drops of diethyl ether on microscope slides coated with AR-10 stripping film and exposed for 2 weeks at -10°C. The autoradiograph and unstained sections were examined under interference phase, using a Nikon photomicroscope.

#### *Results and Discussion*

The geometry of the arrangement of mesophyll and bundle sheath cell layers in the leaves of C<sub>4</sub> plants is shown schematically in Figure 1 in transverse section. Although the dimensions vary between species, those used in Table 1 are representative of the two principal types: the centrifugal type (*Saccharum*, *Zea*), in which bundle sheath chloroplasts are arranged adjacent to the mesophyll layer, and the centripetal type (*Atriplex*, *Amaranthus*), in which the chloroplasts are

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† Department of Environmental Biology, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601.

adjacent to the vascular system. During steady-state photosynthesis, all the  $\text{CO}_2$  fixed passes through the  $\text{C}_4$  dicarboxylic acids malate and aspartate (Johnson and Hatch 1969; Osmond and Avadhani 1970), and these acids move from the mesophyll cells (where they are synthesized) to the bundle sheath [where they are metabolized to sugars (Björkman and Gauhl 1969; Slack, Hatch, and Goodchild 1969; Berry, Downton, and Tregunna 1970)]. The transport of malate or aspartate implied is quantitatively equal to the net  $\text{CO}_2$  flux in the leaf.

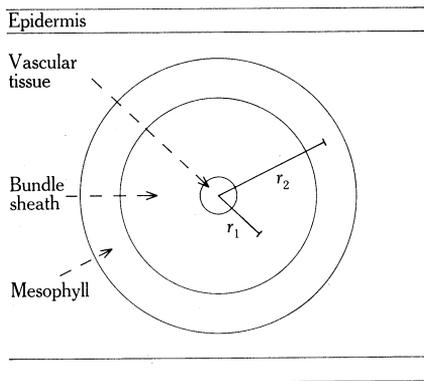


Fig. 1.—Geometry and arrangement of the photosynthetic tissues in leaves of  $\text{C}_4$  plants in transverse section.  $r_1$ ,  $r_2$ , are as defined in Table 1.

The flux ( $J$ ) of  $\text{C}_4$  acids across the mesophyll bundle sheath interface may be calculated from the estimated surface of this interface shown in Table 1. Tyree (1970) compared the permeability of cell membranes with estimates of the apparent permeability during diffusion in the plasmodesmata. Ions were estimated to move through plasmodesmata a thousand times more rapidly than across cell membranes.

TABLE 1  
ESTIMATION OF  $\text{C}_4$  ACID FLUX ( $J$ ) AND APPARENT DIFFUSION COEFFICIENT ( $D'$ ) BETWEEN MESOPHYLL AND BUNDLE SHEATH CELLS DURING  $\text{C}_4$  PHOTOSYNTHESIS

Parameter	<i>Zea</i> (centrifugal)	<i>Atriplex</i> (centripetal)
Net photosynthesis ( $\mu\text{moles cm}^{-3} \text{sec}^{-1}$ )	0.08	0.065
Area of mesophyll–bundle sheath interface ( $\text{cm}^2 \text{cm}^{-3}$ )	236	50.7
Flux, $J$ ( $\mu\text{moles cm}^{-2} \text{sec}^{-1}$ )	$3.4 \times 10^{-4}$	$12.6 \times 10^{-4}$
Mean mesophyll radius, $r_2$ ( $\mu\text{m}$ )	60	80
Mean bundle sheath radius, $r_1$ ( $\mu\text{m}$ )	45	25
Concn. of $\text{C}_4$ acids in photosynthetic pool, $C_m$ ( $\mu\text{moles cm}^{-3}$ )	30	75
Apparent diffusion coefficient, $D'$ ( $\text{cm}^2 \text{sec}^{-1}$ )	$2 \times 10^{-8}/\alpha$	$16 \times 10^{-8}/\alpha$

However, the thickened cell walls of the bundle sheath are undoubtedly additional barriers to the free movement of ions in solution and the effective transport path is probably restricted to the plasmodesmata connecting the two cell layers (Laetsch 1969). If  $\alpha$  is the fraction of the interface occupied by plasmodesmata, then the flux across the plasmodesmata,  $J_{p1}$ , is given by  $J/\alpha$ .

A flux calculated in this way is not strictly relevant when considering rates of transport in other systems and the apparent diffusion coefficient through the plasmodesmata,  $D'$ , is a more useful measurement for comparison.  $D'$  may be estimated using the equation

$$D' = (J/\alpha)[r_2 \ln(r_2/r_1)/C_m],$$

modified from that used by Pitman (1965*a*) when considering the movement of ions across the root cortex, a system analogous to that considered here. The radii  $r_2$  and  $r_1$  are the outer and inner radii specified in Figure 1 and correspond to the median distance between mesophyll and bundle sheath chloroplasts.  $C_m$  is the concentration of  $C_4$  acids in the photosynthetic pool in mesophyll cells. The concentration of  $C_4$  acids in the bundle sheath is assumed to be zero.

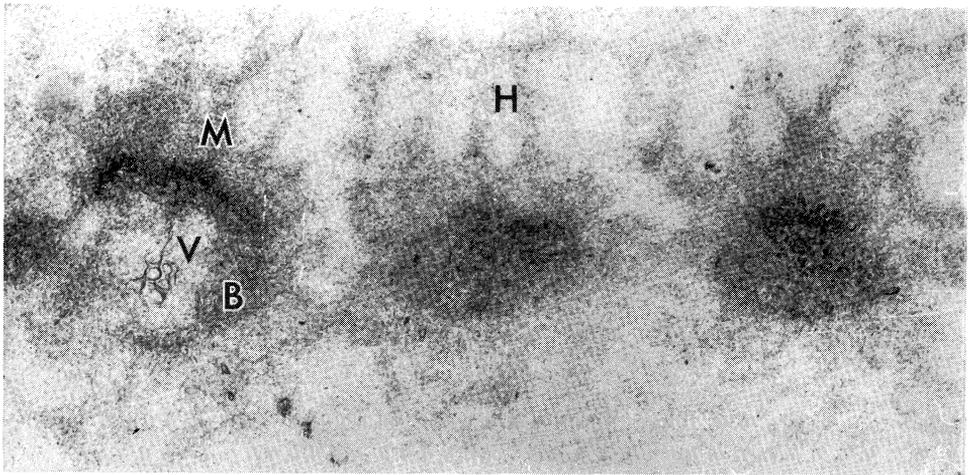


Fig. 2.—Autoradiograph of a transverse section of *A. spongiosa* leaf tissue after 2 sec photosynthesis in  $^{14}\text{CO}_2$ . 10  $\mu\text{m}$  section exposed for 2 weeks. *M*, mesophyll cells; *B*, bundle sheath; *V*, vascular bundle; *H*, hypodermis.

Accurate estimates of the amount of malate and aspartate involved in photosynthesis in  $C_4$  plants may be obtained from the radioactivity in these acids during steady-state photosynthesis in  $^{14}\text{CO}_2$  of known specific activity. Johnson (unpublished data) found 0.6  $\mu\text{moles g}^{-1}$  of photosynthetic malate in *Saccharum*, and *A. spongiosa* leaves contain 1.5  $\mu\text{moles g}^{-1}$  of photosynthetic malate + aspartate (Osmond, unpublished data). The accuracy of these estimates may be judged by the fact that, when multiplied by the decay constant for the loss of  $^{14}\text{C}$  from the  $C_4$  acids in a pulse-chase experiment, they provide estimates of net photosynthesis equal to 50–80% of that measured by gas-exchange methods. However, *A. spongiosa* leaves contain 10–15  $\mu\text{moles g}^{-1}$  of these acids, most of which is presumably vacuolar (Osmond and Avadhani 1968). Thus the photosynthetic  $C_4$  acids are at least confined to the cytoplasmic 10% of mesophyll cells, occupying 20% of tissue volume. With these allowances, values for  $C_m$  of 30 and 75  $\mu\text{moles cm}^{-3}$  are obtained for *Saccharum* (used in *Zea* calculation) and *Atriplex* respectively.

Values for  $D'$  thus depend on the value of  $\alpha$ . Tyree (1970) estimates that plasmodesmata may occupy 1–10% of adjoining cell wall area in a range of tissues. No good estimates of plasmodesmata frequency are available for the mesophyll–bundle sheath interface at present. If we assume that  $\alpha$  is of the order  $10^{-1}$ – $10^{-2}$ , the values obtained for  $D'$  in *Zea* and *Atriplex* overlap and are of the same order as those found for inorganic anion movement through cell wall free space in plants (Pitman 1965b). Apparent diffusion coefficients of this magnitude would lead to the rapid appearance of label in bundle sheath cells during photosynthesis. Solutions for the equation describing the distribution of heat in a cylinder heated at the surface (Carslaw and Jaeger 1959) may be used to predict the distribution of  $^{14}\text{C}$  in the photosynthetic cylinder. Taking  $D' = 16 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$  as a probable maximum value in *Atriplex* ( $\alpha = 10^{-2}$ ), after 2 sec photosynthesis one would expect to find 40% of the  $^{14}\text{C}$  in the bundle sheath cells.

Figure 2 shows the distribution of radioactivity in an *A. spongiosa* leaf after 2 sec photosynthesis in  $^{14}\text{CO}_2$ , as revealed by microautoradiography. The cytoplasm of mesophyll cells and the hypodermal cells is clearly labelled. However, a large proportion of the label is associated with the bundle sheath cells. Quantitative interpretation of the silver grain distribution is difficult because of the higher chloroplast and cytoplasmic density in bundle sheath cells. (The bundle sheath contains 80% of leaf chlorophyll in *A. spongiosa*.) In spite of this, the autoradiograph shows significant fixation of  $^{14}\text{C}$  in the mesophyll cells and transport to the bundle sheath within 2 sec, features not evident in the autoradiographs obtained after long-term  $^{14}\text{CO}_2$  fixation and prolonged handling of the tissue (Pristupka 1964; Moss and Rassmussen 1969). It also suggests that the values for  $D'$  obtained above are reasonable estimates of the *in vivo* process. After 2 sec photosynthesis 80–90% of the  $^{14}\text{C}$  fixed by *A. spongiosa* is in  $\text{C}_4$  acids. The radiograph thus suggests the presence of a sizeable pool of  $\text{C}_4$  acids in the bundle sheath, which should be taken into account in more accurate estimates of  $D'$ .

These calculations suggest that the rapid movement of  $\text{C}_4$  acids between chloroplasts in different cells may be accommodated by diffusive processes in the symplasm and it may be unnecessary to postulate a special mechanism. They emphasize the role of the plasmodesmata and the importance of plasmodesmata density. Slack, Hatch, and Goodchild (1969) drew attention to the role of the peripheral reticulum of the chloroplasts but there is no evidence of the direct plastid connections proposed. Plastid surface area may limit overall transport. The peripheral reticulum increases this surface but does not show any association with the plasmodesmata. It may be significant that the reticulum is particularly well developed in dicotyledons such as *Amaranthus* and *Gomphrena* and may be associated with higher apparent diffusion coefficients (Table 1). The reticulum in *Gomphrena* seems to be very labile (Pyliotis, unpublished data) and is reminiscent of the labile stroma associated with spinach chloroplasts (Honda, Hongladarom, and Laties 1966).

The rapid movement of metabolite anions across two plastid membranes and through 10–70  $\mu\text{m}$  of cytoplasm during  $\text{C}_4$  photosynthesis represents an interesting and challenging example of symplastic transport. The complex system is accessible to flux and electrical measurements and requires much further ultrastructural study before the nature of the transport processes can be specified. Further development

of the C<sub>4</sub> dicarboxylic pathway of photosynthesis will hinge on a better understanding of these processes.

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