# Estimation of the Transport and Carboxylation Components of the Intracellular Limitation to Leaf Photosynthesis

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## ABSTRACT

A model is presented which enables gas exchange data to be used to partition the intracellular resistance to leaf photosynthesis into carboxylation and transport components. A basic assumption is that the over-all kinetics of the carboxylation reaction fit the Michaelis-Menten equation.

The model was tested for cotton (*Gossypium hirsutum* L., var. Deltapine Smoothleaf), where photorespiration was suppressed by using gas mixtures containing less than 1.5% oxygen. It was concluded that the transport resistance formed the major component of the intracellular resistance for the plants studied. However, in some cases the major intracellular factor limiting photosynthesis, at an ambient $CO_2$ concentration of 600 ng cm$^{-3}$, was the carboxylation system, which was close to saturation.

In recent years the use of resistance analogues to describe leaf photosynthesis has led to important advances in the definition and evaluation of rate-limiting processes (*e.g.*, 4, 7, 11). In particular it has permitted the role of stomata, in limiting $CO_2$ entry to the leaf, to be clearly identified. This has meant that it is now possible to determine the degree to which any factor which causes a change in photosynthesis acts on the stomata, altering the $CO_2$ supply, as distinct from the degree to which it affects processes within the mesophyll cells themselves.

The usual method for making this separation is based on the equation

$$P = \frac{c_a}{r_g + r_i} = \frac{c_a - c_w}{r_g} = \frac{c_w}{r_i} \tag{1}$$

where $P$ is the rate of photosynthesis, $c_a$ and $c_w$ are the $CO_2$ concentrations in the ambient air and at the mesophyll cell wall, respectively, and $r_g$ and $r_i$ are the total "resistances" to $CO_2$ uptake in the gas phase and in the liquid phase, respectively. The gas phase resistance includes both the boundary layer and stomatal components, whereas the liquid phase or intracellular resistance includes components associated both with transfer of $CO_2$ within the cells and with the carboxylation reactions. The intracellular resistance is only meaningful under those conditions where photosynthesis is proportional to the $CO_2$ concentration at the cell wall. Details of the experiments necessary to evaluate $r_g$ and $r_i$ can be found elsewhere (12, 18, 20).

It is of considerable interest to attempt to extend the analogues further in order to subdivide the intracellular resistance itself. One can make the initial separation of $r_i$ into transport ($r_t$) and

carboxylation ($r_e$) resistances associated with the transfer of $CO_2$ within the cells and with carboxylation, respectively. These resistances are obtained from an expansion of the third term in equation 1

$$P = \frac{c_w}{r_i} = \frac{c_w - c_i}{r_t} = \frac{c_i}{r_e} \tag{2}$$

where $c_i$ is the $CO_2$ concentration at the carboxylation site. A discussion of the validity and application of equations 1 and 2 may be found in Jarvis (11).

Chartier (4) attempted to separate the diffusion and carboxylation components of $r_i$ by a mathematical analysis of experimental gas exchange data. He derived an equation giving the dependence of photosynthetic rate on $c_a$ and the incident photosynthetically active radiation. The model was based on Rabinowitch's model (16) for the carboxylation mechanism, and Gaastra's application of resistance analogues to the uptake of $CO_2$ (7). It has subsequently been refined to include the effects of photorespiration (5, 6). This model generally ascribes a major portion of $r_i$ to the diffusion component, at least for Calvin-cycle plants (6, 14). However, the apparent diffusion resistance would be better called a transport resistance, since it is probably a function of one or more enzyme catalyzed steps (9, 11). Also, the partitioning of $r_i$ on the basis of this model may be inaccurate since it is assumed that, for the range of conditions used, photosynthesis is linearly dependent on the light intensity at all $CO_2$ concentrations, and that simultaneously it is linearly dependent on the $CO_2$ concentration at all light intensities. This may be a serious oversimplification. Jarvis (11) and Raven (17) have reviewed this and a variety of other methods which have been used for the separation of $r_t$ and $r_e$.

In this paper we present an alternative model for partitioning $r_i$, using data from curves relating net photosynthesis to $CO_2$ concentration at the cell wall. The model enables partitioning of $r_i$ into transport and carboxylation components, and it may be extended to allow a description of the photosynthetic limitation, which is valid at any $CO_2$ concentration.

## THEORY

In the steady state, under conditions of $CO_2$ limitation, equation 2 gives the relative magnitude of intracellular transport and carboxylation resistances to photosynthesis as the ratio of $(c_w - c_i)$ to $c_i$. In Figure 1, the over-all photosynthetic response curve $P = f(c_w)$ represents the sum of the transport limited curve $P = f(c_w - c_i)$ and the carboxylation limited curve $P = f(c_i)$. By making assumptions as to these relationships one can deduce the component curves from an experimental curve relating net photosynthesis to $c_w$.

One major assumption we have made is that the over-all kinetics of the carboxylation system fit the Michaelis-Menten equa-
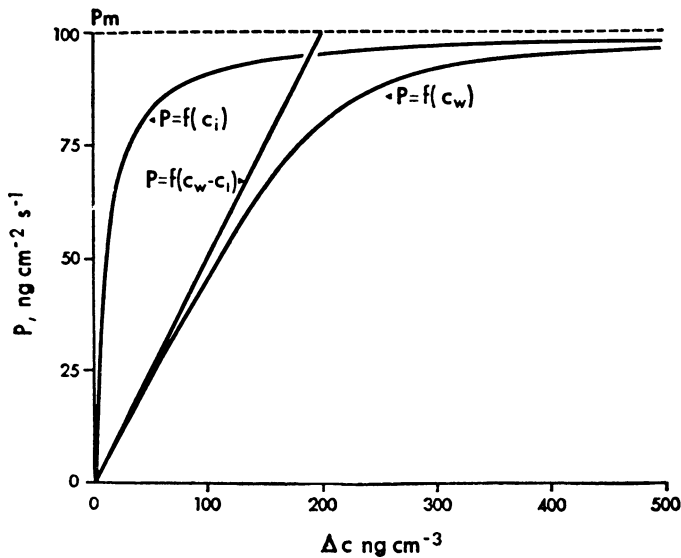
FIG. 1. A graphical representation of the analysis presented, where the overall response curve $P = f(c_w)$ is the sum along the $\Delta c$ axis of the transport limited curve $P = f(c_w - c_i)$ and the carboxylation limited curve $P = f(c_i)$.

with respect to $CO_2$ as substrate. Another assumption is that the leaf may be regarded as a simple linear system, where the transport resistance is independent of the $CO_2$ concentration. As a further simplification we shall treat the case where photorespiration is not detectable, and the compensation point is zero. Making these assumptions, $r_t$ and $r_e$ may be derived from the experimentally obtained parameters $P_m$ (the maximum photosynthetic rate at $CO_2$ and light saturation), $K_m^{app}$ (the value of $c_w$ which gives half the maximum photosynthetic rate) and $r_i$ (the reciprocal of the initial slope of a curve relating net photosynthesis to $c_w$).

Assuming that the over-all kinetics of the carboxylation system, are Michaelis-Menten, the photosynthetic rate is given by

$$P = \frac{P_m c_i}{k' + c_i} \qquad (3)$$

where $k'$ is the Michaelis constant for the carboxylation system. Also, from equation 2

$$P = \frac{c_w - c_i}{r_t} \qquad (4)$$

where $r_t$ is defined as the transport resistance in the liquid phase between the cell wall and the carboxylation site. Eliminating $c_i$ from equations 3 and 4 and rearranging gives

$$c_w = \frac{-P^2 r_t + P(k' + P_m r_t)}{(P_m - P)} \qquad (5)$$

The experimental parameter $K_m^{app}$ may be expressed in terms of equation 5 by substituting $c_w = K_m^{app}$ and $P = P_m/2$ in equation 5 and rearranging

$$K_m^{app} = \frac{P_m r_t}{2} + k' \qquad (6)$$

The $r_i$ may be expressed in terms of the same parameters, since a corollary of equation 2 is that $r_e$ may be defined as the reciprocal of the initial slope of a curve relating $P$ to $c_i$. Therefore, from equation 3

$$\frac{dc_i}{dP} = \frac{P_m k'}{(P_m - P)^2} \qquad (7)$$

taking the limit, as $P$ tends to zero,

$$r_e = \frac{k'}{P_m} \qquad (8)$$

It also follows from equation 2 that $r_i$ is the sum of $r_e$ and $r_t$, which, on substitution of $r_e$ from equation 8, gives

$$r_i = \frac{k'}{P_m} + r_t \qquad (9)$$

It is now possible to express $r_t$ in terms of experimentally obtainable parameters by elimination of $k'$ from equations 6 and 9 and rearranging

$$r_t = 2r_i - \frac{2K_m^{app}}{P_m} \qquad (10)$$

It is only necessary to know two of $r_i$, $r_e$, and $r_t$, since $r_i$ is the sum of the other two resistances.

The relative errors in estimation of $r_e$ and $r_t$ resulting from measurement errors in the parameters required for their calculation, are given by the following equations

$$\frac{\Delta r_e}{r_e} = \frac{2}{P_m r_e} \delta K_m^{app} - \frac{2K_m^{app}}{(P_m)^2 r_e} \delta P_m - \frac{1}{r_e} \delta r_i \qquad (11)$$

and

$$\frac{\Delta r_t}{r_t} = \frac{2K_m^{app}}{(P_m)^2 r_t} \delta P_m - \frac{2}{P_m r_t} \delta K_m^{app} - \frac{2}{r_t} \delta r_i \qquad (12)$$

where $\Delta r_e/r_e$ and $\Delta r_t/r_t$ are the relative errors in $r_e$ and $r_t$, respectively, and $\delta K_m^{app}$, $\delta P_m$, and $\delta r_i$ are the errors in estimation of $K_m^{app}$, $P_m$, and $r_i$, respectively.

There is another method available for the estimation of $r_t$ and $r_e$ which is more amenable to statistical analysis, and that is to use an appropriate transformation to convert a normal photosynthetic response curve into a linear form. Equation 5 may be rewritten in the following form

$$\frac{c_w(P_m - P)}{P} = -r_t P + (P_m r_t + k') \qquad (13)$$

Therefore, if the proposed model fits the experimental data, a plot of $c_w(P_m - P)/P$ against $P$ should be a straight line of slope $-r_t$ and intercept $(P_m r_t + k')$ as shown in Figure 2. Hence, $r_t$ may be obtained from the slope, and $k'$ and $r_e$ may then be calculated from the intercept on the $c_w(P_m - P)/P$ axis. Not only is this a good method for estimating the component resistances, but also it provides a good test of the model.

From these estimates of the photosynthetic limitation by transport and carboxylation processes at limiting $c_w$, it is possible to determine their relative importance at all values of $c_w$. As was suggested above, this ratio could be given as the ratio of the concentration drops across the two components, that is $(c_w - c_i)/c_i$. However, for measurements off the initial linear portion of the $P/c_w$ curve, such an approach is not very useful. A more general description of the relative limitation by the two processes is given by the ratio of the change in $(c_w - c_i)$ to the change in $c_i$ for any given small change in $c_w$ or $P$. This ratio can be obtained from equations 4 and 7

$$\frac{d(c_w - c_i)}{dc_i} = \frac{r_t}{k'} \frac{(P_m - P)^2}{P_m} \qquad (14)$$

At low values of $P$ (or $c_w$) this reduces to the expected ratio of resistances, $r_t/r_e$.

The model presented here can be tested using air with low $O_2$ concentrations, in which photorespiration is suppressed (2, 19). If the inhibition so achieved is incomplete, the residual respiratory component is reflected in a nonzero $CO_2$ compensation point, and a failure of the $P/c_w$ curve to pass through the origin. If this component is small, an approximate correction for not passing through the origin may be achieved by replacing $c_w$ in equations 13 and 14 by $(c_w - \Gamma)$, where $\Gamma$ is the $CO_2$ compensation concentration (ng cm$^{-3}$). It can be shown that, for low respiration rates, application of this simple correction produces similar results to those obtained on the basis of more realistic models for the internal pathways for $CO_2$ exchange.

## MATERIALS AND METHODS

**Plant Material.** Two sets of experiments were used to test the model; the first was done in 1969, and the second in 1971. Cotton plants, *Gossypium hirsutum* L. var. Deltapine Smoothleaf, were used throughout. All plants were grown in modified Hoagland's solution, in a controlled environment cabinet. The photoperiod was 12.5 hr, at 100 w m$^{-2}$ (400–700 nm) for the 1969 experiments, and at approximately 90 w m$^{-2}$ for the 1971 experiments. The light was provided by fluorescent tubes supplemented by incandescent lights. For all experiments the day/night temperature regime was 30 C/25 C. The range of relative humidities in the cabinets was 20 to 35% in 1969, and 60 to 85% in 1971. The plants were used between 4 and 6 weeks after germination. Though measurements were usually conducted on the youngest fully expanded leaf, no attempt was made to select identical leaf material.

**Gas Exchange Measurements.** Photosynthetic response curves relating net photosynthesis to the cell wall $CO_2$ concentration were determined using the methods described by Lake and Slatyer (12), where the measured air was blown through the leaf. The $CO_2$ concentration at the cell wall was taken as the arithmetic mean of the concentrations in the ingoing and outgoing airstreams. Special emphasis was placed on obtaining accurate values for the maximum photosynthetic rate $(P_m)$, using $c_w$ values up to 1500 ng cm$^{-3}$ in 1969, and 1200 ng cm$^{-3}$ in 1971. In the 1971 experiments care was taken to ensure that readings were taken only after the steady state had been reached, since it was observed that following changes of $c_w$ there was often a lag of up to 2.5 hr before a new constant photosynthetic rate was achieved, though there was often a pseudo-steady state after 10 min. In all cases the measurements were made in gases containing less than 1.5% $O_2$. The vapor pressure of the ingoing airstream was maintained at about 15 mm Hg, to prevent tissue desiccation. Measurements in 1969 were made at a leaf temperature of 28.5 C ± 0.5, whereas in 1971 the leaf temperature was maintained at 25.0 C ± 0.5. Absolute light saturation for the 1969 plants was about 250 w m$^{-2}$, whereas for the 1971 plants it was about 150 w m$^{-2}$. The intensities used were all above light saturation.

## RESULTS

The results of several experiments are given in Figure 3 and in Table I. The stomatal resistances of plants comparable to those used in the experiments were between 1.5 sec cm$^{-1}$ and 2.5 sec cm$^{-1}$ over the range of light intensities used with a $CO_2$ concentration of 600 ng cm$^{-3}$ in the external air.

Figure 3 gives an indication of how well the plots of $(c_w - \Gamma)$ $(P_m - P)/P$ against $P$ fit straight lines. The 95% confidence limits for the slopes of the regression lines are given in Table I, and in all cases except for one they are within 10% of $r_t$.
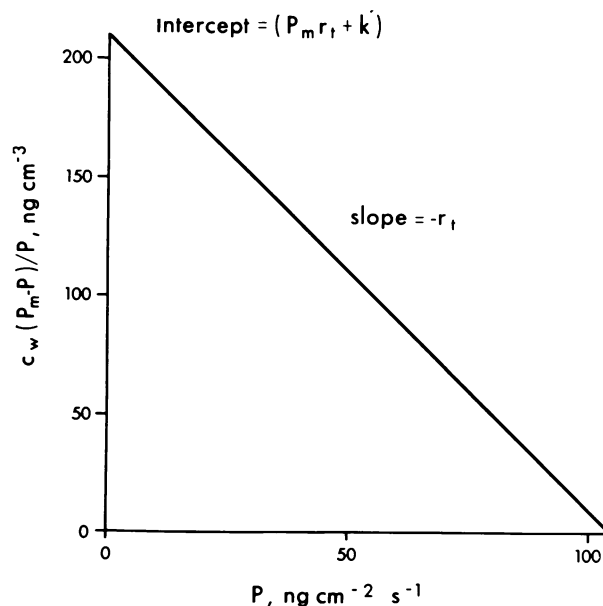
For the 1969 experiments the value of $c_w$, which gave 95%

saturation of the photosynthetic rate, was in all cases between 650 ng cm$^{-3}$ and 850 ng cm$^{-3}$. Therefore, photosynthetic measurements in nitrogen at ambient $CO_2$ concentration (600 ng cm$^{-3}$) would fall on the linear portion of a $P/c_w$ curve, even with open stomata. For the 1971 experiments, however, the value of $c_w$ which gave 95% saturation of the photosynthetic rate was between 290 ng cm$^{-3}$ and 400 ng cm$^{-3}$, and similar photosynthetic measurements made with open stomata at 600 ng cm$^{-3}$ $CO_2$ would be off the $CO_2$-limited portion of the $P/c_w$ curve.

Table I gives the estimated value of $k'$ and the ratio of $r_t/r_e$ for each experiment. However, a problem with the model, as applied to the present data, is that there is considerable uncertainty in estimates of $k'$ and hence $r_e$. This leads to wide confidence limits for the ratio $r_t/r_e$, though in most cases this ratio was greater than 10. Table I also gives the relative contributions of intracellular transport and carboxylation limitations to photosynthesis at ambient $CO_2$ concentrations (calculated from equation 14), with $r_g$ assumed to be either 2.0 sec cm$^{-1}$ (stomata open), or 10.0 sec cm$^{-1}$ (stomata partially closed). Notwithstanding the uncertainty in the data, for the 1971 plants with open stomata the major intracellular limitation to photosynthesis at ambient $CO_2$ concentration was due to the carboxylation step in spite of $r_t$ being much larger than $r_e$. However, as the stomata close the effective value of $c_w$ decreases leading to an increase in the significance of the intracellular transport component. In the case of the 1969 experiments, however, even in the open stomata situation, the transport limitation was more important than that due to carboxylation.

## DISCUSSION

For the cotton plants used in the present experiments, it was found that the transport resistance was the major component of $r_i$. It is of interest that a similar result was also obtained by Chartier *et al.* (6) from their model using *Phaseolus vulgaris*.

As with any mathematical model, the realism of the results obtained is limited both by the quality of the experimental data, and by the validity of the assumptions used in its construction. In the present model the two key assumptions, regarding the kinetics of the carboxylation step and the linear behavior of the $CO_2$ uptake system, are both simplifications of the real situation.
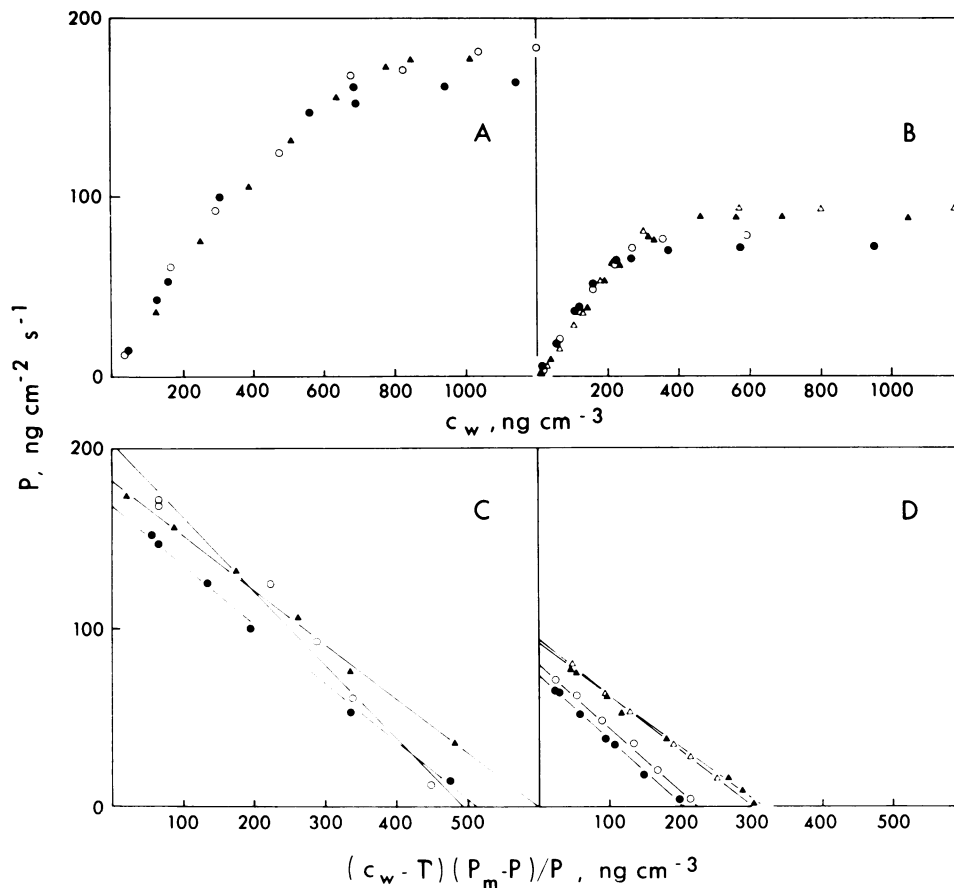
FIG. 3. Actual data from the 1969 experiments (A) and the 1971 experiments (B), with their transformations in C and D, respectively. Different symbols within a pair of boxes (*e.g.*, A and C) represent different plants.

Table I. *Summary of Results*

The values of $r_i$, $r_t$, $r_t/r_e$, and $d(c_w - c_i)/dc_i$ are calculated from the data presented in Figure 3.

| | Date of Measurement | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | 1969 | | | 1971 | | | |
| | 1[1] | 2 | 3 | 1 | 2 | 3 | 4 |
| $P_m$ (ng cm$^{-2}$ sec$^{-1}$) | 184 | 177 | 164 | 78 | 71.7 | 93 | 88 |
| $\Gamma$ (ng cm$^{-3}$) | 0 | 0 | 0 | 4 | 3 | 10 | 7 |
| $r_i$[2] (sec cm$^{-1}$) | 2.77 ± 0.20 | 3.40 ± 0.20 | 3.10 ± 0.15 | 3.08 ± 0.14 | 2.90 ± 0.08 | 3.25 ± 0.15 | 3.70 ± 0.12 |
| $r_t$[2] (sec cm$^{-1}$) | 2.44 ± 0.50 | 3.30 ± 0.29 | 3.03 ± 0.26 | 2.76 ± 0.22 | 2.76 ± 0.18 | 3.24 ± 0.27 | 3.55 ± 0.18 |
| $k'$ (ng cm$^{-3}$) | <102 | <59 | <39 | <20 | <13 | <10 | <11 |
| $r_t/r_e$ | >4.4 | >10 | >13 | >11 | >16 | >30 | >28 |
| $c_w$ which gives $P = 0.95\ P_m$ (ng cm$^{-3}$) | 840 | 720 | 660 | 300 | 290 | 360 | 390 |
| $d(c_w - c_i)/dc_i$ (from equation 14) for | | | | | | | |
| $r_g = 2.0$ sec cm$^{-1}$ | >0.7 | >1.6 | >1.2 | >0.001 | >0.006 | >0.019 | >0.015 |
| $r_g = 1.0$ sec cm$^{-1}$ | >2.5 | >5.4 | >6.6 | >2.0 | >1.9 | >8.0 | >7.0 |

[1] Experiment number.

[2] Value and 95% confidence limits.

However, the linear form of the experimental plot of $(c_w - \Gamma)(P_m - P)/P$ against $P$ (see Table I and also Fig. 3), provides substantial though indirect justification for their use.

It has often been assumed that the carboxylation step obeys Michaelis-Menten kinetics (4, 13, 16), at least for the purposes of photosynthetic models. A feature of the experimental curves is the rapid saturation of the photosynthetic rate as $c_w$ is increased (Fig. 3). This behavior is difficult to explain on mechanistic models (16). One model which could lead to rapid saturation assumes carboxylation equilibria involving two or more $CO_2$ molecules, for which there is little evidence. Rapid saturation could also be obtained if the carboxylation step were inhibited

by $CO_2$ (substrate inhibition). Such a mechanism would produce an approximately linear plot of $c_w(P_m - P)/P$ against $P$, which would only deviate from linearity at high photosynthetic rates. Though isolated ribulose diphosphate carboxylase appears to be inhibited by very high substrate concentrations (1), there is no good evidence for a corresponding photosynthetic decline in the intact system. In the present experiments there was no significant decrease in $P$ up to a $c_w$ of 1200 ng cm$^{-3}$, nor was there any obvious deviation from linearity in the transformed plots at high photosynthetic rates. Of the available models, the present one, in which the rapid saturation is due to the super-imposition of a transport limitation on a rectangular hyperbolic model for the carboxylation step, gives the best fit to the experimental data.

The assumption that the transport resistance is independent of $c_w$, which implies that the $CO_2$ uptake system has the characteristics of a plane sink separated from the cell wall by a constant diffusion resistance, though clearly a simplification, is probably justified in terms of the accuracy of the present model. Nonhomogeneous distribution of light through the mesophyll is not a problem, since the model is only used at saturating light intensities. Our transport resistance includes all of $r_i$ not in the carboxylation resistance, and thus may include diffusion, active transport, and enzymic components.

An important restriction to the model, particularly in terms of its experimental application, is that the model does not allow for photorespiration. For plants with the C-4 dicarboxylic acid pathway of photosynthesis, which do not exhibit photorespiration (10), this is not a problem, but in plants with C-3 photosynthesis, meaningful data may only be obtained if photorespiration is suppressed by the use of less than 1.5$^{c}_{c}$ oxygen in the experimental gas mixtures (2, 19). In the present experiments, even under zero oxygen there was sometimes a small positive compensation point. This was attributed to a residual dark respiration component. The correction employed here to allow for this residual respiration is approximate and is only useful if the rate of respiration, $R$ (as estimated by extrapolation of the $P_i c_w$ curve to $c_w = 0$) is a small fraction of $P_m$. Under these conditions the results presented are not significantly different from those obtained using models where the respiratory flux is assumed either to go direct to the photosynthetic site (achieved by adding $R$ to $P$ and $P_m$ in equation 3), or to be released into the intercellular spaces (in which case $R$ is added to $P$ and $P_m$ in both equation 3 and equation 4). The elimination of photorespiration by measurement under nitrogen will normally lead to smaller estimates of $r_i$ than will measurements made in air (e.g., 12).

The use of the present model requires very accurate gas exchange measurements over a wide range of $c_w$ values. However, stomatal closure frequently occurs as $CO_2$ concentrations are increased above 660 ng cm$^{-3}$ and may prevent accurate values of $P_m$ from being obtained. With the cotton plants used in these experiments, this problem was minimized by the use of a through-flow rather than a diffusive technique for $CO_2$ exchange measurements. For other plant material a diffusive measurement may be satisfactory. The differences in the maximum photosynthetic rates of the two sets of plants may be attributable partly to differences in growth conditions and partly to the different measurement temperatures used (J. Downton and R. O. Slatyer, unpublished data).

For the 1971 experiment, the estimates of the photosynthetic rates at 660 ng cm$^{-3}$ $CO_2$ concentrations in the external air ($P_{amb}$), are all close to saturation, at least for the open stomata situation. Under these conditions equation 14 gives the contribution of transport limitation to photosynthesis as less than one tenth of that due to carboxylation. However, as the stomata close, the transport limitation becomes increasingly important, as mentioned earlier. Several authors have attempted to get an indication of the relative importance of carboxylation and transport limi-

tations to photosynthesis from correlations between photosynthetic rate and the activity of extracted carboxylases. The most popular measure of photosynthetic rate has been $P_{amb}$ (e.g., 3, 15, 21, 22). In some cases, as in the 1971 experiments, photosynthesis may have been close to $CO_2$ saturation; thus a good correlation with enzyme activity might have been expected. However, in the 1971 experiments and in reference 3, photosynthesis was definitely $CO_2$-limited and thus $P$ might be expected to be more directly related to $r_i$ than to $P_m$. In spite of these differences, all these papers demonstrated a correlation between $P_{amb}$ and the activity of the extracted carboxylation enzymes. In addition, J. Downton and R. O. Slatyer (unpublished data) have shown good correlations between $r_i$, $P_m$ and carboxylase activity for cotton grown at various temperatures. On the basis of the present model, $P_m$ might be expected to depend entirely on the carboxylation enzymes, while the over-all $r_i$ would be dependent mainly on the transport resistance. However, the evidence quoted above indicates that $r_i$ may often be correlated both with $P_m$ and with the activity of the carboxylation enzymes. This could be interpreted in terms of a factor common to both transport and carboxylation steps, or else that the activities of both systems are regulated together, even under a wide variety of conditions. The more attractive hypothesis is that a common factor may be involved. Such a role has been proposed for the enzyme carbonic anhydrase (8, 9).

In conclusion, the model provides a valuable method for obtaining information concerning the rate-limiting processes of photosynthesis. It could also be used as a tool for studying intracellular lesions in the photosynthetic apparatus under various conditions.

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