A CO_2 Concentrating System in Leaves of Higher C_3-Plants Predicted by a Model Based on RuBP Carboxylase/Oxygenase Kinetics and $^{14}CO_2/^{12}CO_2$ Exchange

F. MÄCHLER, B. LEHNHERR, H. SCHNYDER[1] AND J. NÖSBERGER

Institut für Pflanzenbau, Eidgenössische Technische Hochschule, Universitätstrasse 2, CH-8092 Zürich, Switzerland

ABSTRACT

Mächler, F., Lehnherr, B., Schnyder, H. and Nösberger, J. 1985. A CO_2 concentrating system in leaves of higher C_3-plants predicted by a model based on RuBP carboxylase/oxygenase kinetics and $^{14}CO_2/^{12}CO_2$ exchange.—J. exp. Bot. 36: 1542–1550.

A model is presented which compares the ratio of the two activities of the enzyme ribulose bisphosphate carboxylase/oxygenase as determined *in vitro* with the ratio of photosynthesis to photorespiration in leaves as determined from differential $^{14}CO_2/^{12}CO_2$ uptake or from CO_2 compensation concentration. Discrepancies between measurements made *in vitro* and *in vivo* are attributed to the effect of a CO_2 concentrating system in the leaf cells. Interference from dark respiration is discussed. A CO_2 concentrating system is postulated which is efficient mainly at low temperature and low CO_2 concentration.

Key words—Photosynthesis, photorespiration, ribulose bisphosphate carboxylase/oxygenase.

Correspondence to. Institut für Pflanzenbau, Eidgenössische Technische Hochschule, Universitätstrasse 2, CH-8092, Zürich, Switzerland.

INTRODUCTION

Net uptake of CO_2 by illuminated leaves is the result of CO_2 fixation due to photosynthesis and of CO_2 release due to photorespiration and dark respiration. Photosynthetic CO_2 uptake and photorespiratory CO_2 release are related to the reactions catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (RuBPCO), whereby photosynthetic CO_2 uptake corresponds to the carboxylase activity and photorespiratory CO_2 release to the oxygenase activity of the enzyme.

Agreement between gas exchange of leaves and RuBPCO kinetics was found by Jordan and Ogren (1984) who studied the influence of temperature on the properties of RuBPCO *in vitro*. They compared the ratio of carboxylase to oxygenase activity of the enzyme with the CO_2 compensation point (Γ) of leaves and suggested that gas exchange of leaves is fully explained by RuBPCO kinetics. Discrepancy between gas exchange and RuBPCO kinetics

---

[1] Present address: Institut für Pflanzenbau, Universität Bonn, Katzenburgweg 5, D-5300 Bonn, F.R.G.

was found by Azcón-Bieto, Farquhar, and Caballero (1981), by Peisker, Tichá, and Čatský (1981), and by Fleck and di Marco (1984). These authors investigated the effect of leaf age on the oxygen dependence of Γ. Azcón-Bieto *et al.* (1981) attributed the deviation of their measurements from RuBPCO kinetics to dark respiration in the light. Peisker *et al.* (1981) found that the discrepancy of their results could only be related to dark respiration if the dark respiration was assumed to be partly inhibited in the light, and if the extent of inhibition decreased with age. Fleck and di Marco (1984) found a similar increase of the O_2 dependent and of the O_2 independent part of Γ with age which does not correspond with dark respiration effects as predicted by the model of Farquhar, von Caemmerer, and Berry (1980). Lehnherr, Mächler, and Nösberger (1985) compared differential $^{14}CO_2/^{12}CO_2$ uptake of leaves with RuBPCO kinetics and found higher ratios of photosynthesis to photorespiration than of carboxylase to oxygenase activity at low temperature, and a CO_2 dependence of this difference which could not be explained by dark respiration. A CO_2 concentrating mechanism in leaf cells was proposed. However, some uncertainties in the interpretation of the results arose from the underestimation of gross photosynthesis in the $^{14}CO_2/^{12}CO_2$ method due to refixation of photorespired CO_2 and from the unknown concentration of CO_2 in the chloroplast stroma. The present paper describes a model which eliminates these uncertainties.

THE MODEL

The specificity of the enzyme RuBPCO for the substrates CO_2 and O_2 is defined by the specificity factor (S) (Laing, Ogren, and Hageman, 1974; Jordan and Ogren, 1981)

$$S = \frac{v_c}{v_o} \cdot \frac{O}{C} = \frac{V_c K_o}{V_o K_c} \tag{1}$$

where v_c and v_o are the velocities of the carboxylation and oxygenation, V_c and V_o the maximal velocities of the two reactions, K_c and K_o the Michaelis constants for CO_2 and O_2, and C and O the concentrations of the substrates CO_2 and O_2.

Oxygenation by RuBPCO is related to the release of photorespired CO_2 in leaves. The CO_2 release is due to glycine decarboxylation in the photorespiratory glycolate pathway. Half a CO_2 molecule is produced per O_2 fixed (Ogren and Chollet, 1982). Thus the specificity factor of RuBPCO can be expressed in terms of CO_2 exchange of leaves.

$$S = \frac{P}{2(P-F)} \cdot \frac{O}{C_s} \tag{2}$$

where P is gross photosynthesis, F is net photosynthesis, and C_s is CO_2 concentration in the stroma. Oxygen concentration (O) is assumed to be the same in the stroma as in ambient air since it is known to penetrate well even across hydrophobic parts of leaves (Lendzian, 1982).

Gross photosynthesis and net photosynthesis can be estimated by measuring differential $^{14}CO_2/^{12}CO_2$ uptake immediately after exposing a leaf to $^{14}CO_2$ (Ludwig and Canvin, 1971). In previous experiments we exposed leaves to $^{14}CO_2$ for 20 s, killed them, and determined fixed ^{14}C. $^{12}CO_2$ uptake was measured by IRGA. Discrimination against $^{14}CO_2$ was accounted for (Lehnherr *et al.*, 1985). However, the short time uptake of $^{14}CO_2$ underestimates gross photosynthesis since the specific activity of $^{14}CO_2$ in the stroma is decreased due to photorespired CO_2 released by the mitochondria (Peisker, 1980). The decrease of specific activity of $^{14}CO_2$ within the leaf is due to the super-position of two CO_2-gas streams which are assumed to be independent (Fig. 1). One of them is labelled with $^{14}CO_2$ at a defined specific activity and diffuses from ambient air to the chloroplast stroma. The other CO_2-stream is unlabelled. It is formed in the mitochondria of the leaf cells due to photorespiration and diffuses partly to the ambient air, and partly to the chloroplast stroma. The CO_2 concentrations in ambient air (C_a), in the intercellular gas space (C_i), in the cytosol of the leaf cells (C_c), and in the stroma (C_s) are sums of the two CO_2 components with different origins as shown in Fig. 1. The two CO_2 components compete for the active sites of the RuBPCO with the same affinity. Discrimination against
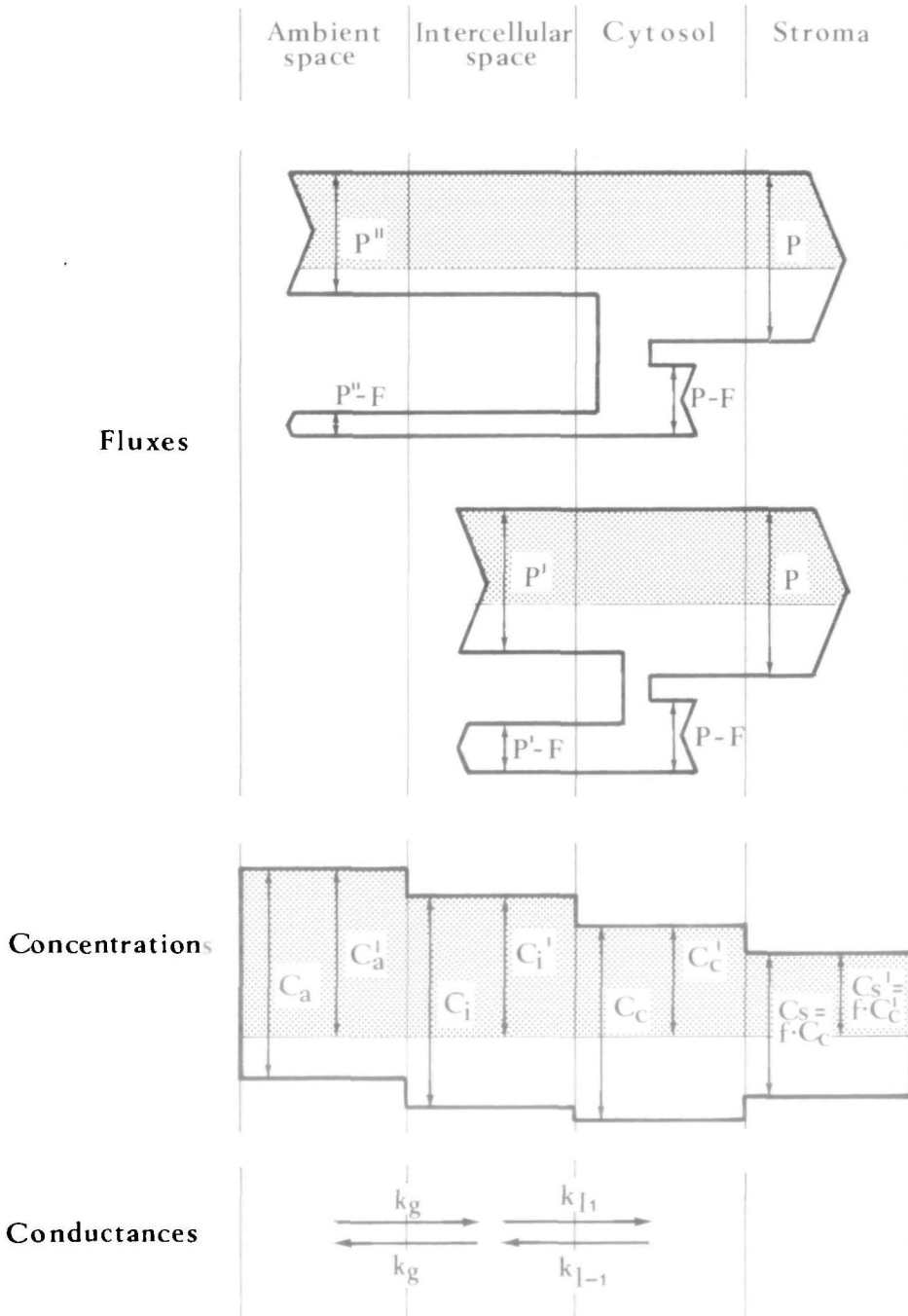
FIG. 1. Scheme of fluxes, concentrations, and conductances of $^{12}CO_2$ (bright areas) and $^{14}CO_2$ (shaded areas) during short time uptake by a leaf. *Fluxes of CO_2: F*: Net photosynthesis. P: Gross photosynthesis. P': Gross uptake of intercellular CO_2. P'': Gross uptake of ambient CO_2. *Concentrations of CO_2: C_a*: Total CO_2 in ambient space. C_a': ^{14}C-labelled component in ambient space. C_i: Total CO_2 in intercellular space. C_i': ^{14}C-labelled component in intercellular space. C_c: Total CO_2 in cytosol. C_c': ^{14}C-labelled component in cytosol. C_s: Total CO_2 in stroma. C_s': ^{14}C-labelled component in stroma. *Conductances of CO_2 and concentration ratio*: k_g: Between ambient and intercellular space, gas phase. k_{l_1}: From intercellular space to cytosol, liquid phase. $k_{1_{-1}}$: From cytosol to intercellular space, liquid phase. f: Ratio of concentrations in stroma and cytosol.

$^{14}CO_2$ has to be accounted for according to O'Leary (1981) and is not considered in the present model. The fixation rates of the two CO_2 components depend on their concentrations in the stroma:

$$k \cdot C_s = P \tag{3a}$$
$$k \cdot C'_s = \varphi \tag{3b}$$
$$k \cdot (C_s - C'_s) = P - \varphi \tag{3c}$$

where φ is the initial rate of $^{14}CO_2$ uptake and k is a rate factor which is affected by the concentration of total CO_2 (C_s) and is the same for both gas components C'_s and $C_s - C'_s$. Gross photosynthesis P can now be related to φ by

$$P = \frac{k \cdot C_s}{k \cdot C'_s} \cdot \varphi = \frac{C_s}{C'_s} \cdot \varphi = \frac{C_c}{C'_c} \cdot \varphi \tag{4}$$

C_s/C'_s can be substituted by C_c/C'_c if it is assumed that the ratio of the two gas components is the same in the cytosol of the leaf cells as in the stroma.

P is gross photosynthesis and consequently the rate of uptake of cytosolic and stromal CO_2. Auxiliary magnitudes can be introduced describing the gross rates of uptake of intercellular and ambient CO_2 (P' and P'', Fig. 1). In analogy to equation 4 they can be expressed by

$$P' = \frac{C_i}{C'_i} \cdot \varphi \tag{5a}$$

$$P'' = \frac{C_a}{C'_a} \cdot \varphi \tag{5b}$$

P can now be expressed in terms of P' and P''

$$P = \frac{C_c \, C'_i}{C'_c \, C_i} \cdot P' = \frac{C_c \, C'_a}{C'_c \, C_a} \cdot P''. \tag{6}$$

P in equation 2 can be substituted by (6). C_a and C'_a can be measured. C_c, C'_c, C_i and C'_i can be expressed in terms of parameters which can be determined by applying Fick's first law to data from $^{14}CO_2/^{12}CO_2$ experiments, and in terms of liquid phase conductance for CO_2 between intercellular space and cytosol of the cells (see appendix). CO_2 translocation between intercellular space and cytosol may be by diffusion or by an active process. The conductance from the intercellular space to the cytosol (k_{1_1}) is the same as the conductance in the opposite direction ($k_{1_{-1}}$) if translocation is purely by diffusion. However, k_{1_1} is greater than $k_{1_{-1}}$ if an active process is involved.

CO_2 translocation between cytosol and stroma may be by diffusion or by an active process. The ratio of the concentrations in the two compartments can be characterized by

$$f = \frac{C_s}{C_c} \tag{7}$$

f is smaller than one if this translocation step is by diffusion, and can be greater than one if it is by an active process. C_s in equation 2 can be substituted by $f \cdot C_c$.

Transformations as described in the appendix result in

$$S \cdot \frac{k_{1_1}}{k_{1_{-1}}} \cdot f = \frac{P''}{2(P'' - F)} \cdot \frac{O}{C_a} = \frac{P'}{2(P' - F)} \cdot \frac{O}{C_i} \tag{8}$$

P'' can be determined from the initial rate of $^{14}CO_2$ uptake if the decrease in specific activity of ambient CO_2 due to photorespiration is known. P' and C_i can be determined if the gas phase resistance to CO_2 diffusion is known from transpiration measurements (Lehnherr *et al.*, 1985).

Equation 8 shows that substitution of the unknown parameters P and C_s in equation 2 by either P'' and C_a or P' and C_i enables the determination of S times the factor $f \cdot k_{1_1}/k_{1_{-1}}$. The factor $f \cdot k_{1_1}/k_{1_{-1}}$ can be determined if S is known from *in vitro* experiments. The factor $f \cdot k_{1_1}/k_{1_{-1}}$ depends on the transport processes of CO_2 in the liquid phase. The factor is smaller than one if transport occurs purely by diffusion ($k_{1_1}/k_{1_{-1}} = 1$; $f < 1$). However, the factor can be greater than one if an active process is involved either in the translocation of CO_2 from the intercellular space to the cytosol ($k_{1_1}/k_{1_{-1}} > 1$) or in the translocation from the cytosol to the stroma ($f \geq 1$). Equation 8 shows that the factor is independent of the gas phase conductance and of the liquid phase conductance between intercellular space and cytosol, as long as no active processes are involved.

TABLE 1. *Influence of temperature and CO_2 concentration on the concentrating factor* $f \cdot k_{l_i}/k_{l_{-i}}$ *and on parameters which are needed for its calculation. From data by Lehnherr et al.* (*1985*)

	Temperature (°C)					
	10	20	30	10	20	30
O_2 (mole fraction)	0·21	0·21	0·21	1·0	1·0	1·0
C_a (mole fractions · 10^6)	319	316	309	1471	1464	1459
k_g (nmol m^{-2} s^{-1})	277	261	255	186	198	165
C_i (mole fraction · 10^6)	262	241	225	1390	1366	1327
F (μmol m^{-2} s^{-1})	15·8	19·7	21·4	14·9	19·5	22·0
P' (μmol m^{-2} s^{-1})	17·7	23·8	28·8	18·1	24·2	28·4
P'' (μmol m^{-2} s^{-1})	17·3	22·7	26·3	17·9	23·8	27·6
$S \cdot f \cdot k_{l_i}/k_{l_{-i}}$ (for mole fractions of O_2 and CO_2)	3699	2494	1810	2032	1877	1670
$S \cdot f \cdot k_{l_i}/k_{l_{-i}}$ (for dissolved O_2 and CO_2)	118	88	71	65	66	66
S (in vitro, for dissolved O_2 and CO_2)	78	78	78	78	78	78
$f \cdot k_{l_i}/k_{l_{-i}}$	1·51	1·13	0·91	0·83	0·85	0·84

At the CO_2 compensation point the CO_2 concentration in the stroma can be determined by

$$C_s = \frac{k_{l_i}}{k_{l_{-i}}} \cdot f \cdot \Gamma \tag{9}$$

as shown in the appendix.

CALCULATIONS FROM EXPERIMENTAL DATA

The concentrating factor $f \cdot k_{l_i}/k_{l_{-i}}$ was calculated from data by Lehnherr *et al.* (1985) for various temperatures and CO_2 concentrations (Table 1). The factor is smaller than one at high CO_2 concentration, independent of whether temperature is 10 °C or 30 °C, and at air level CO_2 concentration if temperature is high (30 °C). A concentrating factor smaller than one is to be expected in the absence of a CO_2 concentrating mechanism when $k_{l_i}/k_{l_{-i}} = 1$ (same conductance from intercellular space to cytosol as vice versa) and $f < 1$ (decrease in CO_2 concentration between cytosol and chloroplast stroma due to diffusion resistance). At air level CO_2 concentration, the concentrating factor increases as temperature is decreased reaching a value of 1·51 at 10 °C. This means either that the conductance from the intercellular space to the cytosol (k_{l_i}) is 1·82 (1·51/0·83) times higher than the conductance in the opposite direction ($k_{l_{-i}}$) or that the CO_2 concentration in the chloroplast stroma is 1·51 times higher than in the cytosol. The two possible sites for a CO_2 concentrating mechanism, plasmalemma and chloroplast envelope, cannot be distinguished.

DISCUSSION

The model presented in this paper does not consider dark respiration. However, effects of dark respiration on gas exchange of leaves can be distinguished from effects of a CO_2 concentrating system. The CO_2 dependence of the ratio of CO_2 uptake to CO_2 release is linear in the presence of dark respiration. This is apparent in the linear relationship between O_2 concentration and Γ as predicted by the model of Farquhar *et al.* (1980) and as shown by Azcón-Bieto *et al.* (1981). The CO_2 dependence of the ratio of CO_2 uptake to CO_2 release is

not linear in the presence of a CO_2 concentrating system with saturation kinetics. A nonlinear CO_2 response of the ratio of CO_2 uptake to CO_2 release is shown by Lehnherr *et al.* (1985) if the temperature is low. The ratio of CO_2 uptake to CO_2 release is decreased by dark respiration as compared with the ratio of carboxylase to oxygenase *in vitro*, whereas it is increased in the presence of a CO_2 concentrating system. Although dark respiration and the CO_2 concentrating system may occur in parallel, dark respiration may predominate at high temperatures and the CO_2 concentrating system at lower ones.

The validity of the diffusion resistance concept is usually a presupposition when RuBPCO kinetics are applied to photosynthetic gas exchange (Hall, 1971; Peisker, 1976; Tenhunen, Yocum, and Gates, 1976; Farquhar *et al.*, 1980). Tenhunen, Weber, Yocum, and Gates (1979) applied RuBPCO kinetics and the diffusion resistance concept to data on photosynthetic gas exchange as influenced by CO_2 and O_2 from Ku and Edwards (1977). They calculated Michaelis constants for stromal CO_2 (K_c) of 0·4 mmol m^{-3}. This is extremely low and does not compare with K_c of purified enzyme (16 mmol m^{-3}) (Mächler, Keys, and Cornelius, 1980). However, underestimation of K_c *in vivo* seems to be inevitable if Michaelis–Menten kinetics are applied to data on the CO_2 dependence of photosynthesis since RuBP can be limiting and the activation state of RuBPCO decreases as CO_2 concentration is increased (Mächler, 1981; Schnyder, Mächler, and Nösberger, 1984). Saturating RuBP and a constant RuBPCO activity would be a prerequisite for the application of Michaelis–Menten kinetics. Underestimation of K_c in the Michaelis–Menten equation results in an underestimation of the CO_2 concentration in the stroma and, therefore, in an overestimation of the intracellular diffusion resistance. The presence of a CO_2 concentrating system with saturation kinetics would further decrease K_c as calculated from CO_2 concentration curves of photosynthesis. Although Michaelis–Menten kinetics are obviously not applicable directly to photosynthetic gas exchange, it seems that the specificity factor of RuBPCO is unchangeable (Ogren and Chollet, 1982) and that it is therefore the same *in vivo* as *in vitro*. The comparison of the ratio of carboxylase to oxygenase activity *in vitro* with the ratio of photosynthesis to photorespiration *in vivo* is the basis for our postulation of a CO_2 concentrating system in higher C_3-plants.

Active transport of inorganic carbon in the HCO_3^--form has been shown in aquatic plants (Badger, Kaplan, and Berry, 1980; Badger and Andrews, 1982; Spalding and Ogren, 1982). Inorganic carbon uptake in land plants has been investigated using isolated cells and protoplasts. They preferentially take up CO_2 by diffusion (Espie and Colman, 1982), although at least some uptake of HCO_3^- could be shown by Volokita, Kaplan, and Reinhold (1981). $^{14}CO_2/^{12}CO_2$ experiments with leaves by Lehnherr *et al.* (1985) showed that the suggested CO_2 concentrating system is only active at low temperature, and that its efficiency is much lower than in aquatic plants. A concentrating effect could only be shown at low CO_2 concentrations. It is suggested that protoplast experiments performed at low temperature and low CO_2 concentration may show a clear concentrating effect.

A decrease in the efficiency of a CO_2 concentrating mechanism with increasing temperature is surprising since it is certainly based on enzymatic processes. However, a concentrating effect is also dependent on the permeability of the membranes, and decreases as the permeability is increased. The permeability of the membranes is expected to increase with temperature. It is suggested that the temperature dependence of the CO_2 concentrating effect is related to the permeability of the membranes.

The discrepancy between the specificity factor of RuBPCO *in vitro* and the ratio of photosynthesis to photorespiration could be explained alternatively by incomplete metabolization of glycolate via the glycolate pathway at low temperature and low CO_2 concentration.

APPENDIX

Derivation of equation 8

P in equation 2 can be substituted by (6). C_a in equation 2 can be substituted by $f \cdot C_c$ according to (7). Equation 2 becomes

$$S = \frac{\dfrac{C_c C_i'}{C_c' C_i} P'}{\left(\dfrac{C_c C_i'}{C_c' C_i} P' - F\right) C_c} \cdot \frac{O}{2f} = \frac{C_i' P'}{C_c C_i' P' - C_c' C_i F} \cdot \frac{O}{2f} \tag{10a}$$

$$S = \frac{\dfrac{C_c C_a'}{C_c' C_a} P''}{\left(\dfrac{C_c C_a'}{C_c' C_a} P'' - F\right) C_c} \cdot \frac{O}{2f} = \frac{C_a' P''}{C_c C_a' P'' - C_c' C_a F} \cdot \frac{O}{2f} \tag{10b}$$

C_i and C_i' can be determined if gas phase conductance (k_g) is known by applying Fick's first law according to Jarvis (1971).

$$C_i = C_a - \frac{F}{k_g} \tag{11a}$$

$$C_i' = C_a' - \frac{\varphi}{k_g} \tag{11b}$$

F and φ can be expressed in terms of C_c, C_c', and liquid phase conductances k_l and k_{1-l}.

$$F = C_i k_{l_1} - C_c k_{1_{-1}} = \left(C_a - \frac{F}{k_g}\right) k_{l_1} - C_c k_{1_{-1}} \tag{12a}$$

$$\varphi = P' \frac{C_i'}{C_i} = C_i' k_{l_1} - C_c' k_{1_{-1}} \tag{12b}$$

$$\varphi = P'' \frac{C_a'}{C_a} = \left(C_a' - \frac{\varphi}{k_g}\right) k_{l_1} - C_c' k_{1_{-1}} \tag{12c}$$

k_{l_1} and $k_{1_{-1}}$ are not purely diffusion parameters, but also rate factors of chemical reactions which depend on CO_2 concentration. C_c can now be calculated from equation 12a and C_c' either from (12b) or from (12c) and substituted in equations 10a and 10b.

$$S = \frac{C_i' P'}{C_i' P' \left(\dfrac{C_i k_{l_1} - F}{k_{1_{-1}}}\right) - C_i F \left(\dfrac{C_i' k_{l_1} - P' \dfrac{C_i'}{C_i}}{k_{1_{-1}}}\right)} \cdot \frac{O}{2f} \tag{13a}$$

$$S = \frac{C_a' P''}{C_a' P'' \left(\dfrac{\left(C_a - \dfrac{F}{k_g}\right) k_{l_1} - F}{k_{1_{-1}}}\right) - C_a F \left(\dfrac{\left(C_a' - \dfrac{C_a' P''}{C_a k_g}\right) k_{l_1} - \dfrac{C_a'}{C_a} P''}{k_{1_{-1}}}\right)} \cdot \frac{O}{2f} \cdot \tag{13b}$$

Transformation of equation 13a and 13b results in equation 8.

Derivation of equation 9

At the CO_2 compensation point the fluxes of CO_2 from the intercellular space to the cytosol and vice versa are the same

$$C_i k_{l_i} = C_c k_{l_{-i}}. \tag{14}$$

The CO_2 concentration in the intercellular space is the same as in ambient air. Substitution of C_c by C_s/f results in equation 9.

ACKNOWLEDGEMENT

LITERATURE CITED

AZCÓN-BIETO, J., FARQUHAR, G. D., and CABALLERO, A., 1981. Effects of temperature, oxygen concentration, leaf age and seasonal variations on the CO_2 compensation point of *Lolium perenne* L. *Planta*, **152,** 497-504.

BADGER, M. R., and ANDREWS, T. J., 1982. Photosynthesis and inorganic carbon usage by the marine cyanobacterium *Synechococcus* sp. *Plant Physiology,* **70,** 517-23.

——KAPLAN, A., and BERRY, J. A., 1980. Internal inorganic carbon pool of *Chlamydomonas reinhardtii.* Ibid. **66,** 407-13.

ESPIE, G. S., and COLMAN, B., 1982. Photosynthesis and inorganic carbon transport in isolated *Asparagus* mesophyll cells. Ibid. **70,** 649-54.

FARQUHAR, G. D., VON CAEMMERER, S., and BERRY, J. A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta,* **149,** 78-90.

FLECK, I., and DI MARCO, G., 1984. Ribulose-1,5-bisphosphate carboxylase activity, net photosynthetic rate and CO_2 compensation concentration in detached barley leaves senescing in the dark. *Photosynthetica,* **18,** 248-54.

HALL, A. E., 1971. A model of leaf photosynthesis and respiration. *Carnegie Institution of Washington, Yearbook,* **70,** 530-40.

JARVIS, P. G., 1971. The estimation of resistances to carbon dioxide transfer. In *Plant photosynthetic production. Manual of methods.* Eds. Z. Šesták, J. Čatský, and P. G. Jarvis. Dr. W. Junk N. V. Publishers, The Hague. Pp. 566-631.

JORDAN, D. B., and OGREN, W. L., 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. *Nature,* **291,** 513-15.

—————— 1984. The CO_2/O_2 specificity of ribulose 1,5,-bisphosphate carboxylase/oxygenase. Dependence on ribulose bisphosphate concentration, pH and temperature. *Planta,* **161,** 308-13.

KU, S. B., and EDWARDS, G. E., 1977. Oxygen inhibition of photosynthesis. II. Kinetic characteristics as affected by temperature. *Plant Physiology,* **59,** 991-9.

LAING, W. A., OGREN, W. L., and HAGEMAN, R. H., 1974. Regulation of soybean net photosynthetic CO_2 fixation by interaction of CO_2, O_2, and ribulose 1,5-bisphosphate carboxylase. Ibid. **54,** 678-85.

LEHNHERR, B., MÄCHLER, F., and NÖSBERGER, J., 1985. Influence of temperature on the ratio of ribulose bisphosphate carboxylase to oxygenase activities and on the ratio of photosynthesis to photorespiration of leaves. *Journal of Experimental Botany,* **36,** 1117-25.

LENDZIAN, K. J., 1982. Gas permeabilities of plant cuticles. *Planta,* **155,** 310-15.

LUDWIG, L. J., and CANVIN, D. T., 1971. The rate of photorespiration during photosynthesis and the relationship of the substrate of light respiration to the products of photosynthesis in sunflower leaves. *Plant Physiology,* **48,** 712-19.

MÄCHLER, F., 1981. Influence of temperature on activation state of RuBP-carboxylase in intact leaves of white clover. In *Proceedings of the fifth international photosynthesis congress, Volume 4.* Ed. G. Akoyunoglou. Balaban International Science Service. Philadelphia. Pp. 63-7.

——KEYS, A. J., and CORNELIUS, M. J., 1980. Activation of ribulose bisphosphate carboxylase purified from wheat leaves. *Journal of Experimental Botany,* **31,** 7-14.

OGREN, W. L., and CHOLLET, R., 1982. Photorespiration. In *Photosynthesis. II. Development, carbon metabolism, and plant productivity.* Ed. Govindjee Academic Press, New York, London. Pp. 191-230.

O'LEARY, M. H., 1981 Carbon isotope fractionation in plants. A review. *Phytochemistry*, **20**, 553-67.

PEISKER, M., 1976. Ein Modell der Sauerstoffabhängigkeit des photosynthetischen CO$_2$-Gaswechsels von C$_3$-Pflanzen. *Kulturpflanze*, **24**, 221-35.

——1980. Differential short-time uptake of $^{14}CO_2$ and $^{12}CO_2$ as a method for determining photorespiration. A theoretical approach. *Photosynthetica*, **14**, 406-12.

——TICHÁ, I., and ČATSKÝ, J., 1981. Ontogenetic changes in the internal limitations to bean-leaf photosynthesis. 7. Interpretation of the linear correlation between CO$_2$ compensation concentration and CO$_2$ evolution in darkness. Ibid. **15**, 161-8.

SCHNYDER, H., MÄCHLER, F., and NÖSBERGER, J., 1984. Influence of temperature and O$_2$ concentration on photosynthesis and light activation of ribulose bisphosphate carboxylase oxygenase in intact leaves of white clover (*Trifolium repens* L.). *Journal of Experimental Botany*, **35**, 147-56.

SPALDING, M. H., and OGREN, W. L., 1982. Photosynthesis is required for induction of the CO$_2$-concentrating system in *Chlamydomonas reinhardii*. *FEBS Letters*, **145**, 41-4.

TENHUNEN, J. D., WEBER, J. A., YOCUM, C. S., and GATES, D. M., 1979. Solubility of gases and the temperature dependency of whole leaf affinities for carbon dioxide and oxygen. An alternative perspective. *Plant Physiology*, **63**, 916-23.

——YOCUM, C. S., and GATES, D. M., 1976. Development of a photosynthesis model with an emphasis on ecological applications. I. Theory. *Oecologia*, **26**, 89-100.

VOLOKITA, M., KAPLAN, A., and REINHOLD, L., 1981. Evidence for mediated HCO$_3^-$ transport in isolated mesophyll protoplasts. *Plant Physiology*, **67**, 1119-23.