In addition to acting as the ultimate electron acceptor in photosynthesis, CO₂ (bicarbonate) plays another important role during the electron flow from water to the plastoquinone pool (photosystem II reactions). Bicarbonate depletion of isolated chloroplasts leads to a reversible increase in the half-time of decay of chlorophyll a fluorescence yield, after a brief saturating flash, from 600 msec to 2.6 msec [1]. This is due to the slowing down of the reoxidation of Q⁻ to Q by about fivefold (where Q is the primary electron acceptor of photosystem II) and it explains the severalfold decrease in the relaxation rate of the $S_n$ to $S_{n+1}$ states of the oxygen evolving system observed by A. Stemler, G. Babcock and Govindjee [2]. Recent chlorophyll a experiments of Govindjee, M. P. J. Pulles, R. Govindjee, H. J. van Gorkum and L. N. M. Duysens [3] have now provided an explanation of the five-to-tenfold decrease in the rate of electron flow under continuous saturating light. In bicarbonate-depleted chloroplasts, the chlorophyll a fluorescence decayed with a half-time of about 150 msec after the third and subsequent flashes, but appreciably faster after the first or the second flash of a series of brief saturating light flashes, given after a dark period. In control and bicarbonate-depleted chloroplasts resupplied with 10 to 20 mM bicarbonate, the decay was fast after all the flashes of the sequence. This was interpreted to indicate that the bicarbonate depletion causes a major block in the reoxidation of the secondary acceptor (R; in its doubly reduced state $R^{2−}$) by the plastoquinone pool. This conclusion was consistent with the measurements of the DCMU (3-(3, 4-dichlorophenyl)-1,1 dimethyl urea)-induced chlorophyll a fluorescence yield increase in hydroxylamine-treated chloroplasts, after a series of light flashes in the presence and absence of bicarbonate. If the bottleneck reaction of electron flow in Hill reaction, which is about 20 msec in normal chloroplasts, is increased to about 105 msec, a 25-fold reduction in the saturation rate of this reaction is predicted. It is suggested that the usual bottleneck reaction of the oxidation of plastoquinone ($\sim$20 msec) is replaced by a new bottleneck reaction of the oxidation of $R^{2−}$ by plastoquinone pool ($\sim$150 msec) in bicarbonate-depleted chloroplasts.

Finally, chlorophyll a fluorescence yield rise in the microsecond range (interpreted to be due to the electron donation by Z to $P_{680^{+}}$) and the decay of ESR signal II (due to the electron donation from H₂O side to Z²⁺) remain unchanged by bicarbonate depletion suggesting that bicarbonate action is not located on the oxidizing side of photosystem II [1].

**Introduction**

Fluorescence

Both the prompt and delayed emission from photosynthesizing plants and bacteria are due to deexcitation of the first singlet excited state of chlorophyll a (or bacteriochlorophyll); they both have almost identical emission spectra (see, e.g. LAVOREL [4]). The measured lifetime of the prompt fluorescence is almost linearly proportional to the quantum yield (see, e.g. [5]), and is in the nanosecond range (see, e.g. MAR et al. [6]. The quantum yield of chlorophyll (Chl) a fluorescence *in vivo* is
of the order of 0.03 and the yield of delayed emission is several orders of magnitude lower (see, e.g. Stacy et al. [7]).

The difference between prompt (to be referred from now on simply as fluorescence) and delayed emission (to be referred to as DLE) lies in the steps that lead to the production of the excited singlet state; for DLE, it is mainly by the recombination of the primary products of the so-called system II light reactions, and for fluorescence, it is either by direct absorption of a quantum, or by deexcitation of higher excited states.

Measurements of Chl a fluorescence have been of a great value in the investigations of photosynthesis (see reviews by Govindjee et al. [8]; Govindjee and Papageorgiou [9]; Goedheer [10]; Papageorgiou [11]; Lavorel [12]). The following discussion will rely mainly on the work done in our laboratory and citation to the work of other authors could be found in the cited reviews and our papers. These include measurements on the fluorescence excitation and emission spectra, fluorescence induction and polarization of fluorescence, providing information on the composition of pigment systems I and II [13—16], on the excitation energy transfer from various accessory pigments to Chl a, from one spectral form of Chl a to another [17—21]; among the different molecules of chlorophyll a [22—23] among different photosynthetic units of pigment system II [5—24], and from such units of system II to pigment system I [23, 25—27].

A working hypothesis (Fig. 1) is as follows. Functionally speaking, there is a tetrapartite arrangement. Two light harvesting assemblies I and II are connected to two reaction center complexes I and II which are in close proximity to each other. Two reaction center molecules are assumed to be “special pairs” of Chl a molecules but in different microenvironments (P700 and P680). The system II units are not only close to other system II units for excitation energy exchange, but also to other system I units to account for system II—system I transfer. The mechanism of energy transfer is not yet certain but there are indications that it is by Förster’s slow transfer method; other mechanisms have not yet been excluded (see [19—23, 28]). There is obviously energy exchange among system II units (see [29]) and from system II to system I units (see, e.g. review by Papageorgiou [11]).

The fast Chl a fluorescence changes in the 1 to 10 microsecond range have been used to monitor the recovery of the reaction center Chl a of system II (P680) from its oxidized to its reduced state (P680⁺ has been suggested to be a quencher of Chl a fluorescence) [30]. Chl a fluorescence has been used, for quite some time [31], as an indicator of the redox state of the primary electron acceptor of the system II (Q). When changes due to P680 are not significant (or not being monitored) reduction of Q leads to an increase in the Chl a fluorescence yield and its oxidation leads to a decrease in this yield; the decay of Chl a fluorescence yield in the 10 to 1,000 μsec after a saturating light flash,
and measured with weak flashes is used to monitor the reoxidation of $Q^-$ to $Q$ [31]. Equation (1) summarizes these events:

1 (a) $\text{P680}^+ + \text{hv} \rightarrow \text{P680}^\ast$ excitation of reaction center II
1 (b) $\text{P680}^\ast \cdot Q^- \rightarrow \text{P680}^+ \cdot Q^- \text{ charge separation; low fluorescence yield}$
1 (c) $Z \cdot \text{P680}^\ast \cdot Q^- \rightarrow Z^+ \text{P680} \cdot Q^- \text{ recovery of P680; high fluorescence yield}$
1 (d) $Z^+ \cdot \text{P680} \cdot Q^- \cdot R \rightarrow Z^+ \text{P680} Q^- \cdot R^- \text{ recovery of Q; low fluorescence yield}$

Chlorophyll $a$ fluorescence changes induced by the addition of DCMU (which is suggested to block electron flow from $Q^-$ to the next intermediate (R) in the electron transport chain) to chloroplasts already treated with hydroxylamine (which donates electrons to the oxidized form of the first electron donor, Z, to $\text{P680}^+$), and after they have been exposed to a series of flashes have been used to infer the existence of R between Q and the plastoquinone pool [32]. After odd number of flashes, DCMU induces high fluorescence yield, but after an even number of flashes, this yield is low. This can be understood by Eq. (2).

\[
\begin{align*}
\text{QR} & \xrightarrow{\text{hv}} Q^-R \rightarrow QR^- & \xrightarrow{\text{DCMU}} Q^-R & \quad 2\text{(a) (high yield)} \\
\text{QR} & \xrightarrow{\text{hv}} Q^-R \rightarrow QR^- & \xrightarrow{\text{hv}} Q^-R^- & \rightarrow QR^2^- & \xrightarrow{\text{POQ}^2^-} QR & \xrightarrow{\text{DCMU}} QR & \quad 2\text{(b) (low yield)}
\end{align*}
\]

The antagonistic effect of light absorbed in pigment system I and II on Chl $a$ fluorescence has supported the existence of two light reactions — two pigment system scheme of photosynthesis [31, 33]. Finally, when electron flow in system II is blocked by DCMU, long term Chl $a$ fluorescence can be used as an indicator of changes in chloroplast membranes [34, 35].

The system II reaction: a summary,

This set of reactions, in terms of electron flow from $\text{H}_2\text{O}$ to PQ (plastoquinone pool) may be summarized by Eq. (3) (with approximate half times of reactions):

\[
\begin{align*}
\text{H}_2 & \xrightarrow{< 1 \text{ msec}} \text{M} & \xrightarrow{200 \text{ msec}} & \text{Z}_2 & \xrightarrow{35 \text{ msec}} & \text{Z}_1 & \xrightarrow{1 \text{ msec}} & \text{P680} & \xrightarrow{600 \text{ msec}} & \text{Q} & \xrightarrow{< 1 \text{ msec}} & \text{R} & \xrightarrow{< 1 \text{ msec}} & \text{PQ}
\end{align*}
\]

where M is identified with the charge accumulator complex (which after accumulating 4 positive equivalents reacts with 2 molecules of $\text{H}_2\text{O}$ to produce 1 molecule of $\text{O}_2$ and 4$H^+$). $\text{Z}_2$ with an electron donor to $\text{Z}_1$, $\text{Z}_1$ with an electron donor to P680, P680 with the primary electron donor and the energy trap, Q with the first stable electron acceptor, R with an electron donor to the plastoquinone pool (PQ).

R is suggested to operate by accepting two electrons, one at a time, from $Q^-$ in two steps and then donating both its electrons in one step to PQ as mentioned above. In all likelihood, Q and R are both quinone-type molecules, M may be a manganese-protein; the nature of $\text{Z}_1$ and Z$_2$ is not known at all but they may be also associated with manganese. To explain other results, several other unknown endogenous donors have been involved which can donate electrons to $Z_2^+$, $Z_1^+$, and P680$^+$. At low tem-
perature cyt b<sub>559</sub> acts as an endogenous donor on this side. Another electron acceptor, separate from Q and labeled W, has recently been suggested by VAN BEST and DUYSSENS [36] to exist, from µsec delayed light emission measurements. There is a possibility that this may be a phaeophytin molecule and serve as the real primary electron acceptor prior to Q.

2. The bicarbonate effect

In order to appreciate the use of Chl a fluorescence in understanding the site of bicarbonate effect in the electron flow in system II reactions, it is instructive to provide a brief review of this effect.

WARBURG and KRIPPAHLE [37] discovered that CO<sub>2</sub> was necessary for the Hill reaction (production of O<sub>2</sub> with quinone as an electron acceptor) in algae. WARBURG argued that this phenomenon and the uncertainties involved in the original <sup>18</sup>O experiments of RUBEN et al. [38] which had led to the belief that O<sub>2</sub> in photosynthesis originates in H<sub>2</sub>O must be abandoned; instead, he suggested that O<sub>2</sub> originated from CO<sub>2</sub>. The stimulation of Hill reaction by CO<sub>2</sub> (or bicarbonate anion) has been studied by STERN and VENNESLAND [39], IZAWA [40], GOOD [41], HEISE and GAFRON [42], BATRA and JAGENDORF [43], VENNESLAND et al. [44], and WEST and HILL [45], but no explanation of this phenomenon was obtained. STEMLER and GOVINDJEE [46—48] have shown that bicarbonate is involved in system II reactions (see [49]). However, STEMLER and RADEMER [50], showed that when NaH <sup>18</sup>CO<sub>3</sub> is injected into bicarbonate-depleted chloroplasts, all evolved oxygen is in <sup>32</sup>O<sub>2</sub>, not <sup>36</sup>O<sub>2</sub>. WYDRZYSKII and GOVINDJEE [51] showed that at least one site of bicarbonate effect was on the reducing side of system II.

JURSINIC et al. [1] demonstrated that absence of bicarbonate causes a fivefold reduction in the decay rate of the chlorophyll a fluorescence yield after a brief saturating flash of light: the half-time of this decay (which is due to the decay of Q<sup>-</sup> to Q, Q being the primary electron acceptor of system II) was increased from the normal 600 µsec to 2.6 msec (Fig. 2). This phenomenon explained the large increase in the relaxation of Sn to Sn+1 state observed earlier by STEMLER et al. [2].

Bicarbonate depletion also causes a reversible inactivation of about 50% of the reaction centers of pigment system II explaining the about twofold decrease in the rate of electron flow at low light intensities, and a twofold decrease in the amplitude of the electron spin resonance signal H<sub>e</sub> due to the oxidized Z (where Z is the first secondary electron donor to the reaction center chlorophyll a P<sub>680</sub>) [1].

The above-mentioned effects did not explain the five-to-tenfold reduction of the Hill reaction in saturating continuous light. The bottleneck reaction under steady-state conditions in the Hill reaction has an approximate half-time of 20 msec. Therefore, to explain the five-to-tenfold reduction in the steady-state saturation rate, we must suggest that the Hill reaction has been slowed down to yield a half-time of 100 to 200 msec. A 2.6 msec step could not be of much significance for the steady-state phenomenon. GOVINDJEE et al. [3] have now shown that the major block caused by the absence of bicarbonate is between the component R and the plastoquinone pool; and, this reaction is slowed down to a value in the 100—200 msec range.

Absence of bicarbonate in broken chloroplasts isolated from spinach led to (a) the elimination of the oscillations, with a period of two, in chlorophyll a fluorescence yield after a series of saturating light flashes followed by injection of 5 µM
DCMU and 1 mM hydroxylamine [32]; (b) a slow decay of fluorescence yield after illumination with 4 sec continuous saturating blue light: At one second after this light was turned off, the variable fluorescence yield was several fold higher in the absence than in the presence of bicarbonate; (c) a large increase in fluorescence yield, measured after 150 msec of the cessation of the third and the following light flashes, but not after the first and second flashes; and (d) a large difference in the fluorescence yield after the third minus the second flash—this difference had an approximate half-time of 150 msec. Addition of 10 to 20 mM bicarbonate to bicarbonate-depleted chloroplasts, at about pH 7.0, restored the conditions prevailing in the untreated controls. The above results have been explained by proposing that the major block in electron flow is between the component R and the plastocyanin pool to yield a rate limiting step in the range of 100 to 200 msec. This then is the explanation of Warburg phenomena in saturating light.

**Slow fluorescence decay after 4 sec saturating light**

Jursinic et al. [1] measured the Chl a fluorescence decay after a brief saturating flash and showed that absence of bicarbonate caused a fivefold slowing down of the decay rate of \( \Phi^- \) to \( Q \) in the 0.05 to 2 msec range. This range was not enough to uncover changes in the longer time region needed to explain the steady-state effects. Fig. 3 shows the decay of chlorophyll a fluorescence up to 3 secs. Chloroplasts with
5 μM DCMU, bicarbonate-depleted samples with 2 mM ferricyanide, bicarbonate-depleted samples resupplied with 10 or 20 mM NaHCO₃ and 2 mM ferricyanide, and control chloroplasts with 2 mM ferricyanide were exposed to a saturating pulse (4 sec) of blue light. The decay of fluorescence yield was measured after the cessation of illumination with weak flashes. The minus HCO₃⁻ samples showed a decay curve intermediate between DCMU and control or plus HCO₃⁻ samples. At about 1 sec after saturating light was turned off, the yield of the “variable fluorescence” was still 50% of that in the DCMU case, whereas in control chloroplasts, there was no “variable” fluorescence; the ratio of the yield of variable fluorescence in minus HCO₃⁻/plus HCO₃⁻ was in the range of 10—15 in several experiments. This experiment shows that bicarbonate-depleted chloroplasts are blocked in a high fluorescence state even up to 1 sec suggesting that certain reactions are slowed down beyond the msec region as suggested earlier. In order to find the site of this slow reaction, we looked at the reactions associated with the component “R” which is between Q and the PQ pool [3].

**Oscillations in chlorophyll a fluorescence due to “R”**

If the absence of HCO₃⁻ blocks or slows down the QR²⁻ to QR⁻ reaction (see Eq. 2(b)), then QR²⁻ would accumulate and the following reaction would occur upon DCMU injection: QR²⁻ → DCMU Q⁻ R⁻, and the fluorescence will be high as Q⁻ is produced; this could, however, decay at long times. Subsequent flash could produce Q⁻ R²⁻ and the fluorescence will remain high. (This point is further tested in the next section.)

\* Plus HCO₃⁻, bicarbonate-depleted chloroplasts resupplied with 20 mM HCO₃⁻. 
Fig. 4 shows our results (see Govindjee et al. [3]) on DCMU-induced chlorophyll a fluorescence rise under the above conditions. The open-circled curve shows oscillations in control chloroplasts confirming the data of Velthuys and Amesz [32]; here, 1 mM hydroxylamine was used to block oscillations on the water side as well as to act as an electron donor. The minus HCO$_3^-$ sample (solid squares) showed a complete absence of oscillations in three experiments we performed. Addition of 20 mM HCO$_3^-$ was enough to restore the oscillations (open squares). Thus, these data are consistent with a block in the QR$^2^-$ to QR reaction. In order to further locate the site of HCO$_3^-$ action more precisely, we measured long term fluorescence (100 to 500 msec) after a sequence of saturating flashes as described below.

**Fluorescence yield after a series of flashes**

If DCMU and hydroxylamine were not injected and chlorophyll a fluorescence yield was measured 150 msec after each flash (1 through 20), the yield was independent of flash number with and without 2 mM ferricyanide (Figs. 5A and 5B). However, in minus bicarbonate samples, the yield after the second flash was only slightly higher than after the first flash. But, the yields after the third and subsequent flashes were high whether ferricyanide was present or not. Data for minus bicarbonate samples was interpreted by the following hypothesis. After the first flash, the following reactions occur: \( QR^{hv} \rightarrow Q^-R \) and \( Q^- \) decays to Q with a half-time of 2.6 msec (see [1]). After two flashes \( QR^{hv} Q^-R \rightarrow QR^-^{hv} Q^-R^- \rightarrow QR^2^- \) reactions occur and the fluorescence has decayed away with a half-time of 2.6 msec (last step in the above scheme). However, after the third flash:

\[ QR^-^{hv} Q^-R \rightarrow QR^-^{hv} Q^-R^- \rightarrow QR^2^- \rightarrow QR^2^- Q^-R^2^- \]

reactions occur and the fluorescence decays with a long half-time because of the block beyond this step, \( i.e. \) from \( QR^2^- \rightarrow Q^-R \). Subsequent flashes, given after 30 msec, produce

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*Fig. 5. Chlorophyll a fluorescence intensity 160 msec after the last of a series of 3 μsec saturating flashes, spaced at 30 msec, as a function of the number of flashes. Addition as indicated. Concentrations: Bicarbonate, 20 mM; Chl, 20 μg ml$^{-1}$ of spinach chloroplast suspension. Ferricyanide, 20 mM. (After Govindjee et al., 1976.).*
high fluorescence as the system is blocked, in this time scale, in the $\text{Q}^{-}\text{R}^{3-}$ step and no further reaction can occur. Thus, these experiments show that the major block is between R and the PQ pool.

Fig. 6A and 6B show the fluorescence decay after the third flash minus that after the second flash. It is clearly shown that the fluorescence decay is slow in minus-

![Figure 6](image)

*Fig. 6. Chlorophyll $a$ fluorescence intensity after the third minus that after the second flash, as a function of time. Additions as indicated; see legend of Fig. 5. (After Govindjee *et al.* [3].)*

![Figure 7](image)

*Fig. 7. Rise in chlorophyll $a$ fluorescence yield during and after an excitation flash with and without 10 mM bicarbonate, normalized at $\tau = 3$ μsec. A trace of excitation flash intensity as a function of time is also shown. Excitation light flashes were provided by a General Radio Strobotac 1538—A through two Corning C. S. 4—96 filters. An EMI 9558 B photomultiplier protected by neutral density filters, a C. S. 2—64 and a Schott RG—8 filter combination was used. *Lactuca sativa* chloroplasts treated as described by Wydrzynski and Govindjee [51]. Similar results were obtained with *Zea mays* chloroplasts. (After Jursinic *et al.* [1].)*
bicarbonate samples; in plus-bicarbonate and control samples, there is no significant decay in the 100—500 msec range—the fluorescence yield is very low. At about 150 msec after the flash, the yield of fluorescence is about ten times higher in minus-compared to plus-bicarbonate samples.

It is evident from the above experiments that after the third flash, the block is a major one and Q\(^-\)R\(^{2-}\) decays with a half-time in the range of 100—200 msec. It was not possible to measure the exact time of this reaction here. However, unpublished experiments of R. Khanna, Govindjee, U. Siggreen, and G. Rengele on absorption changes at 265 nm, made in long flashes, show that the decay of this change is 20 msec in control and plus-bicarbonate samples, whereas it is 100—150 msec in the minus-bicarbonate samples. In control chloroplasts, this decay is a measure of plastquinone reoxidation, and in minus-bicarbonate samples, this decay is a measure of R\(^{2-}\) reoxidation as both “R” and PQ are quinones (M. P. J. Pulles, personal communication).

**Fluorescence rise in microsecond region**

Chlorophyll a fluorescence yield rise in the microsecond range (interpreted to be due to the electron donation by Z to P680\(^+\)) remained unchanged by bicarbonate depletion (see Fig. 7) suggesting that bicarbonate action is not located on the oxidizing side of photosystem II [1].

**Fluorescence spectra at room and low temperatures**

Fluorescence spectra at 77 K show three major bands at 685 (F685), 695 (F695) and 730 nm (F730). F685 and F695 originate mainly in pigment system II and F730 in pigment system I (see, e.g. Gasanov and Govindjee [16]. If there is a change in excitation energy transfer from pigment system II to I, it is reflected in a change in the ratio of F685 + F695 to F730. The ratio of F685 + F695/F730 was the same in bicarbonate depleted and depleted chloroplasts resupplied with 10 or 20 mM HCO\(_3\)\(^-\). Fig. 8B (after T. Wydrzynski and Govindjee, unpublished observations) shows the ratio of emission spectra of plus to minus HCO\(_3\)\(^-\) samples showing the absence of change in excitation energy transfer from pigment system II to I. The same results were obtained at room temperature (see Fig. 8A) where F685 is mostly from system II and emission in the 715 nm region has a relatively stronger contribution from pigment system I.

3. **Concluding remarks**

Our experiments have now established that the bottleneck reaction in minus-bicarbonate sample is about 150 msec and is due to slow reduction of PQ by R\(^{2-}\). These data explain, for the first time, the inhibitory effect of the absence of HCO\(_3\)\(^-\) on the steady-state Hill reaction in saturating continuous light. Thus, measurements on Chlorophyll a fluorescence have yielded information on the major site of action of bicarbonate on the electron flow in system II reactions. In addition, fluorescence experiments have also shown that bicarbonate does not affect electron flow from Z to
Fig. 8. (A). Emission spectra at room temperature under anaerobic conditions (N₂ atmosphere). •, minus HCO₃⁻; O, plus 10 mM HCO₃⁻. Curves normalized at 685 nm; average of two spectra for each treatment; λ excitation, 435 nm with 24 nm slits, and Corning 4–96 filter; observation, 3.3 nm slit width; Corning C. S. 2–58 filter before the monochromator. Photomultiplier, EMI 9558B; [Chl], 10 μg ml⁻¹ of *Zea mays* chloroplast suspension. Assay medium, 0.05 M phosphate buffer, pH 6.8; 0.25 M NaCl; 0.04 M Na acetate. Same results were obtained with *Lactuca sativa* chloroplasts. (After T. Wydrzynski and Govindjee, unpublished observations of 1973.)

(B). Ratio of relative fluorescence yield at 77 K between plus HCO₃⁻ and minus HCO₃⁻ samples as a function of wavelength of observation. Conditions same as in (A) above; samples were frozen in dark under N₂ atmosphere. (After Wydrzynski and Govindjee, unpublished observations)

P680, and that it does not change the spillover of excitation energy from pigment system II to I. The slowing down of electron flow from Q⁻ to R may only be a consequence of block between R and PQ. In brief, we may write system II reaction as follows:

\[
\text{hv} \\
H₂O \rightarrow Z \rightarrow \text{P680} \rightarrow Q \rightarrow R \xrightarrow{\text{HCO}_₃⁻} \text{PQ} \xrightarrow{(\text{HCO}_₃⁻)} O₂ \xrightarrow{(\text{HCO}_₃⁻)}
\]  

\[
(4)
\]
References

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ОПРЕДЕЛЕНИЕ МЕСТА ДЕЙСТВИЯ СО₂ ВО II-ОЙ ПИГМЕНТНОЙ СИСТЕМЕ ФОТОСИНТЕЗА С ПОМОЩЬЮ ФЛЮОРЕНСЦЕНЦИИ ХЛОРОФИЛЛА-α

Кратковременное (600 μсек — 2,6 мсек) освещение хлоропластов при низком содержании СО₂ время жизни флюоресценции хлорофилла-α увеличивается по сравнению с не обработанной пробы. Это объясняется тем, что реакция Q⁻ в Q приближительно в пять раз уменьшается после обработки. Кроме этого ингибируется реакция вторичного акцептора электрона (R²⁻) при лишении CO₂.

По нашему мнению действие CO₂ объясняется не только влиянием на окислительную сторону II пигментной системы, так как после удаления CO₂ выход флюоресценции хлорофилла-α увеличивается в микросекундной области времени (Z⁻ + P680⁺ — — — Z⁺ + P680) и сигнал ЭПР Пх (перенос энергии от H₂O на Z⁺) не изменяется.