Kinetic Models of Oxygen Evolution in Photosynthesis†

TED MAR AND GOVINDJEE‡

Department of Botany, and Division of Biophysics,
Department of Physiology and Biophysics,
University of Illinois, Urbana, Illinois

Oxygen production in photosynthesis occurs by a dark reaction of water molecules with a powerful oxidant produced by light absorbed in pigment system II. The production of the oxidant that reacts directly with water requires several steps. Recently, P. Joliot and co-workers (1969) and B. Kok and co-workers (1970, 1971) have proposed models that involve the accumulation of two or four positive charges before oxygen is evolved. In order to explain their experimental data, Joliot *et al.* additionally suggested a "flip flop" mechanism in the operation of the reaction center, and Kok *et al.* added "double hits" and "misses" in the same reaction center. Alternative models have been proposed here that equally well explain the data. In the first one, oxygen can be evolved from an accumulation of two positive charges as in Joliot's model; in contrast to Joliot *et al.*'s two charge "flip flop" model, this particular two charge hypothesis explains the existing experimental data. In the second one, an accumulation of four charges are needed for oxygen evolution as in Kok's model; this model, however, differs from the latter model because it does not require a "double hit" on the same reaction center, but in it, each oxygen evolving site has two bound reaction centers II. This alternate four charge hypothesis also explains the existing experimental data. From detailed mathematical analyses of all the models, we conclude that the earlier models are *not* unique, and the two models presented here should be considered as valid alternate models for oxygen evolution in green plants. The pitfall of considering a model unique, because it fits all data, is, therefore, emphasized here.

1. Introduction

In green plants, eight photons are needed to produce a molecule of oxygen from water (and to reduce a molecule of CO_2) (Emerson, 1958; Govindjee, Rabinowitch & Govindjee, 1968). The overall process requires the transfer

‡ Send reprint requests to Govindjee, Botany Department, University of Illinois, Urbana, Illinois 61801, U.S.A.

of four electrons, in two steps, from H_2O to CO_2 (see discussions in Rabino-witch & Govindjee, 1969). The oxygen evolution step requires the transfer of four electrons from water to an intersystem intermediate (A), and this requires four photons (Delerieu, 1968). How this is done remains a mystery. (For a recent review, see Cheniae, 1970). Allen & Franck (1955) showed that after a long dark period no oxygen was evolved if algae were illuminated by a single, short, bright flash of light. Oxygen, however, was evolved on sub-sequent flashes of light. These observations were confirmed by Whittingham & Brown (1958). Joliot (1965) found that after a long dark period there was a lag in the time course of oxygen evolution at low intensities. If a pre-illuminating flash was given, oxygen evolution started immediately on illumination. Joliot interpreted these and other results (not cited here) to mean that two quanta must be absorbed successively in the same photo-chemical center to produce an oxygen atom; oxygen atoms from neighboring units can then combine to give an oxygen molecule. Rosenberg (1969) has also measured the time course of oxygen evolution at low light intensities and from these studies has suggested that four photoacts on the same reaction center lead to the evolution of one oxygen molecule. Kok, Forbush & McGloin (1970) found that the difference in the area bounded by the time course curve of O_2 evolution taken directly after continuous pre-illumination (no deactivation) and that taken after a 10 min dark period was independent of intensity at low light levels. With the assumption of constant quantum efficiency, this result implies that photons are needed to fill a finite pool of intermediates before oxygen can be evolved. Renger (1970) has speculated on the mechanism of charge co-operation.

More recently, Joliot, Barbieri & Chabaud (1969) and Forbush, Kok & McGloin (1971) have reinvestigated the evolution of oxygen by a series of short saturating flashes of light. Joliot *et al.* (1969) found that the amount of oxygen given off per flash of light, as a function of the numbers of flashes given, showed oscillations with a period of four that damped after 15 flashes. (This will be referred to as damped-four cycle oscillations.) On the basis of these experiments, they proposed a new scheme for the mechanism of oxygen evolution in photosynthesis. The main features of this scheme are: (i) the reaction center II includes two electron donors (Z) and one electron acceptor (Q); (ii) transfer of two electrons from the same donor leads to the formation of one oxygen atom; (iii) the reaction center acts as a switch that connects alternately each donor to the acceptor; this last aspect is what we call a "*flip-flop*" mechanism. This switch works with an efficiency of 85% and is induced by each photoact. Kok *et al.* (1970) made similar experiments and suggested that oxygen evolution is a four quanta process that occurs in a sequence. To fit the damped-four cycle oscillations, Forbush *et al.*

(1971) proposed that after a long dark period all reaction centers do not deactivate to the ground state and that the damping is caused by "misses" (α) in the photochemical conversions at the trapping centers. Damping is also due to "double hits" (β), some reaction centers receiving two photons and performing two photochemical acts within the duration of the light flash. In this communication, we propose two new alternate models that will fit the published data of both Kok *et al.* (1970) and Joliot *et al.* (1969).

2. General Treatment

Any model for oxygen evolution must satisfy the following conditions. It must explain: (i) that oxygen evolved per flash, when the photosynthetic units are illuminated by a series of short (~ 10 μsec) saturating flashes of light spaced 300 msec apart, oscillates with a period of four and "damps out" after four to six periods (Joliot *et al.*, 1969; Forbush *et al.*, 1971); (ii) the differences in the patterns of curves for oxygen yield in a series of light flashes after various pretreatments with light (Kok *et al.*, 1970; Forbush *et al.*, 1971). After 25 flashes were used to attain steady state of oxygen evolution, chloroplasts were left in the dark for 5 min and then given a single flash or a sequence of two or three flashes or none at all. After the last treatment, chloroplasts were left in the dark for 30 min. The oxygen yield per flash, in a sequence of light flashes, was then measured and found to vary with different pretreatments. In addition, any model of oxygen evolution must also predict the time course of the rate of oxygen evolution obtained in continuous weak light following darkness or different numbers of flashes (Joliot *et al.*, 1969).

Recently, Barbieri, Delosme & Joliot (1970) have shown that the intensity of delayed light emission (DLE), measured 0·24 sec after each light flash, oscillates as a function of the number of light flashes given after a long dark period. These oscillations are advanced by one flash number as compared to those in the amount of oxygen produced per flash. These results seem to indicate that any model proposed to explain the oscillations of the oxygen yield per flash must also explain its correlation to the oscillations of the DLE. However, the decay of the intensity of DLE with time is known to have complicated kinetics. Comparison of the intensity of DLE at one specific time may be meaningless, as the change may be due to a change in the decay kinetics and not in the intensity at zero time. If one compares the intensity of DLE at another time, the oscillation of the intensity of DLE produced by a series of light flashes is found to be exactly in phase with the oscillation of the amount of oxygen produced (Zankel & Kok, 1970). In the present paper, we have not attempted to analyze the models of O_2 evolution in terms of the results on DLE.

Two general alternative models are possible. The first is that the evolution of an oxygen molecule is due to a two step mechanism in which the reaction center successively accumulates two positive "charges" to produce an atom of oxygen; two oxygen atoms then quickly combine to produce a molecule of oxygen. The second model proposes that the evolution of an oxygen molecule is the result of a four step mechanism in which the reaction centers must successively accumulate four positive charges before a molecule of oxygen is evolved.

(A) TWO STEP MECHANISM

The model of Joliot *et al.* (1969) for oxygen evolution involves a two step mechanism. This model is shown schematically in Fig. 1(a). Detailed calculations on this model show that the amount of oxygen given off by the chloroplasts exposed to the second and third flashes, the fourth and fifth
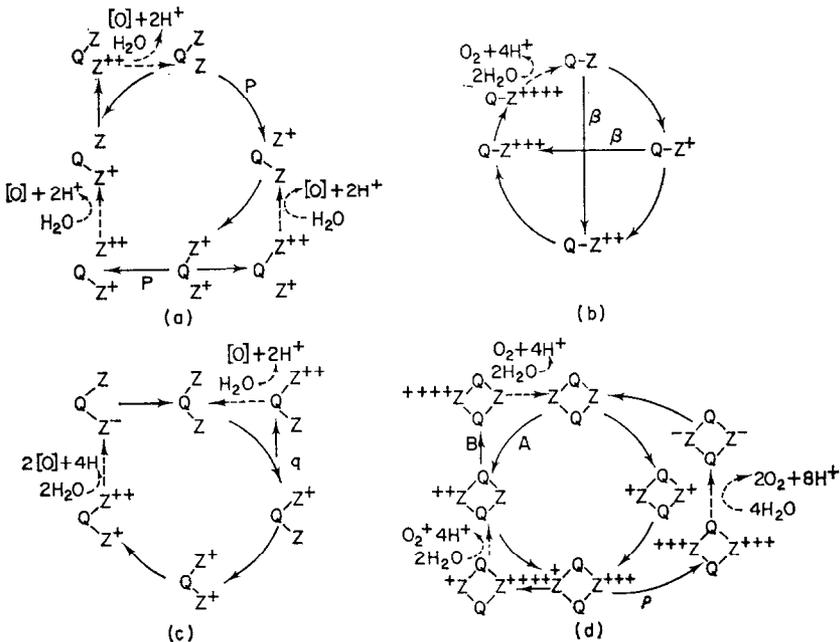

FIG. 1. Schematic diagram of four different models of oxygen evolution. Q is the primary electron acceptor; Z is the primary electron donor; solid arrows show light reactions; broken arrows show dark reactions. (a) Model A for oxygen evolution redrawn from Joliot *et al.* (1969); P is a probability constant. (b) Model B for oxygen evolution redrawn from Kok *et al.* (1970); β is the probability of "double hits". Kok's S_0 has been replaced by Q—Z and S_1 by Q—Z^+, etc. (c) Model C (see text); q is a probability constant. (d) Model D (see text); A, B, p are probability constants.

flashes and to every succeeding pair of flashes is equal to twice the amount of oxygen evolved per flash after the oscillations have damped out (Joliot et al., 1969). We refer to the latter state as the "steady state". This prediction does not agree with either their own or Forbush et al.'s (1970) experimental results. As shown in Figs 2(a) and 3(a), the sum of the amounts of the oxygen given off by chloroplasts exposed to the second and third flashes
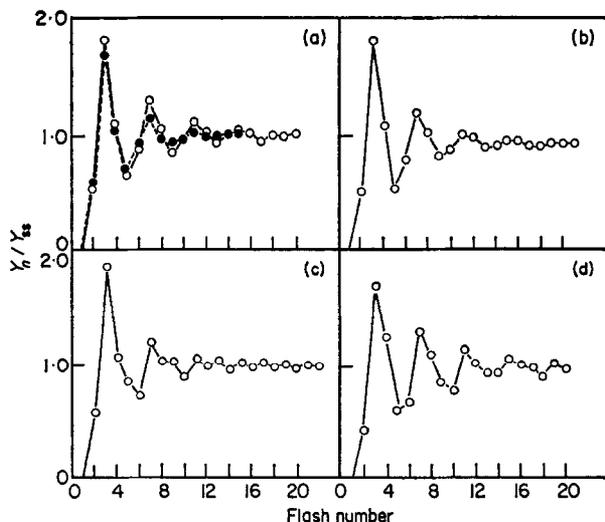

FIG. 2. (a) The amount of oxygen evolved per flash by illumination with a series of light flashes of saturating intensity after cells were kept five minutes in the dark. Dark time between each flash, 300 msec; Y_n, the amount of oxygen emitted by the nth flash of light; Y_{ss}, the oxygen emitted per flash after oscillations had damped out ("steady state"). O——O, isolated spinach chloroplasts; ●- - -●, green alga *Chlorella*. (Experimental data of Joliot et al., 1969.) (b) Predictions based on Kok's model (Kok et al., 1970; Forbush et al., 1971); S_0, concentration of Q—Z species, equals 0·25; S_1, concentration of Q—Z⁺ species, equals 0·75; α, "misses", equal(s) 0·15; β, "double hits", equal(s) 0·20. (c) Predictions based on model C (see text); γ, inefficiency index, is 0·100; q, a probability factor, is 0·388. (d) Predictions based on model D; A, B and p, probability constants, are 0·255, 0·918 and 0·204 respectively; γ, inefficiency index of the trap, is 0·020.

is greater than the sum when exposed to the fourth and fifth flashes. Furthermore, Kok et al. (1970) and Forbush et al. (1971) found that the sum of the amount of oxygen from the second and third flashes is larger than twice the amount of oxygen given off at the "steady state". Joliot et al. (1969) assume that after a long period of darkness all the trapping centers deactivate to one stable state having the lowest energy (the "ground state"). However, this assumption does not explain why the amount of

oxygen evolved per flash should be different if three flashes of light are given before the long period of darkness or if no light is given (see data of Forbush *et al.*, 1971). These difficulties can be overcome by removing the assumption that all trapping centers deactivate to one "ground" state. Joliot *et al.*'s (1969) model, in its present form, cannot explain all experimental results.

We have evolved a two quanta model (model C) that will fit the experimental results. The model is shown in Fig. 1(c). We assumed, as did Joliot *et al.* (1969), that there are two donors (Z) to one acceptor (Q). After a long period in the dark, there are two stable states $\left(Q\substack{\diagup Z \\ \diagdown Z}\right)$ and $\left(Q\substack{\diagup Z \\ \diagdown Z^-}\right)$.

After the absorption of light in the first flash, Z is oxidized, and Q is reduced, i.e. $\left(Q\substack{\diagup Z \\ \diagdown Z}\right)$ becomes $\left(Q^-\substack{\diagup Z \\ \diagdown Z^+}\right)$ and $\left(Q\substack{\diagup Z \\ \diagdown Z^-}\right)$ becomes $\left(Q^-\substack{\diagup Z \\ \diagdown Z}\right)$. After a short dark period (10^{-4} sec), Q^- becomes reoxidized to Q (via "A") and $\left(Q\substack{\diagup Z \\ \diagdown Z^+}\right)$ becomes $\left(Q\substack{\diagup Z \\ \diagdown Z^+}\right)$. We assume that after the second flash of light $\left(Q\substack{\diagup Z \\ \diagdown Z^+}\right)$ has a probability of q to change into $\left(Q^-\substack{\diagup Z \\ \diagdown Z^{++}}\right)$ and $1-q$ to change into $\left(Q^-\substack{\diagup Z^+ \\ \diagdown Z^+}\right)$. In the subsequent dark period $\left(Q\substack{\diagup Z \\ \diagdown Z^{++}_{\cdot}}\right)$ will transform back to $\left(Q\substack{\diagup Z \\ \diagdown Z}\right)$ by reacting with water, resulting in the evolution of an atom of oxygen.† (We could, however, suggest that [O] is merely a species that is at the same oxidation state as atomic oxygen, or as a 2-electron oxidation product requiring reaction in pairs to form a molecular oxygen, thus allowing for some as yet unidentified bound form.) Two oxygen atoms from neighboring units would combine to give one oxygen molecule. Hence, in this model, the probability q can be determined by the amount of oxygen given off after the second flash. After the third flash of light, $\left(Q\substack{\diagup Z^+ \\ \diagdown Z^+}\right)$

---

† It has been questioned as to whether the formation of free oxygen atoms is even possible! We know that many techniques have been used to make free oxygen atoms (see Young, Black & Slouger, 1968). The reason why they do not exist under normal conditions is that the atoms combine very rapidly to form oxygen molecules.

will become $\left(Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^{++}}{Z^+}\right)$. We assume, then, that this species with three charges acts in such a way that at each Z two oxygen atoms are evolved within the same unit; thus, a molecule of oxygen is produced.† Three of the four resultant electrons from water are used to reduce the oxidized Z; the fourth electron from water is assumed to be trapped by another Z. This trapped electron is assumed to be very stable and can only be deactivated by a quantum of light. Hence, $\left(Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^{++}}{Z}\right)$ becomes a very stable $\left(Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z^-}\right)$.

On the fourth flash of light, as in the first flash, $\left(Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z^-}\right)$ attains the initial

state $\left(Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z}\right)$. This cycle of reactions is repeated on the fifth flash of light.

The above model (C) is a two quanta model because it satisfies the criterion that O_2 can be evolved from the cooperation of two quanta, whereas in a four quanta model, O_2 *cannot* be evolved until four quanta are absorbed. In model C, the mechanism postulated for O_2 evolution is the one involving the cooperation of two quanta, i.e.

$$Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^{++}}{Z} \xrightarrow{H_2O\ [O]+2H} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z}; \quad 2[O] \dashrightarrow O_2.$$
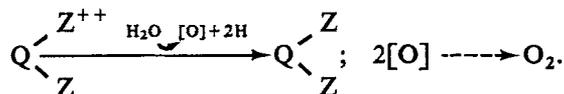
If more quanta are needed, the mechanism postulated is one of simultaneous occurrence of two chains of events, i.e.

$$Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z} \xrightarrow{h\nu} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^+}{Z} \xrightarrow{h\nu} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^+}{Z^+} \xrightarrow{h\nu} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z^{++}}{Z^+} \xrightarrow[\text{dark}]{H_2O\ [O]+2H} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z^+} \xrightarrow[\text{dark}]{H_2O\ [O]+2H} Q\diagup\diagdown\genfrac{}{}{0pt}{}{Z}{Z^-}.$$
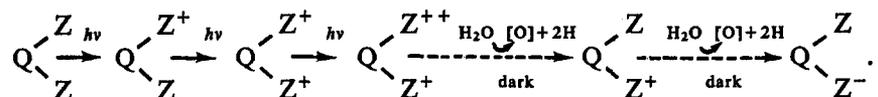
To match the experimental results with model C, we make the last assumption: the photochemical reaction centers are not 100% efficient but operate with an efficiency of $(1-\gamma)$; this is very similar to the idea of "misses" (Kok *et al.*, 1970; Forbush *et al.*, 1971). However, the reason why a photo-

---

† We note that it is difficult to visualize this step as a two charge step. We wish to emphasize that if there are two plus charges on a reaction center "Z", oxygen atoms can be formed; this does not preclude the evolution of a whole molecule of oxygen if the proper state of Z occurs within the same reaction center. However, we look at it as evolution of two oxygen atoms at the two Z's with the subsequent formation of an oxygen molecule, just as it occurs in the case when two atoms are formed in neighboring reaction centers.

chemical reaction is inefficient may not be due to a photon "missing" a particular unit. We do not expect a quantum to "miss" a reaction center, as the photosynthetic units are connected to one another; perhaps, Kok's "misses" are nothing but the inefficiency of the trapping center.

From the model in Fig. 1(c), one can derive the following recursion relations

$$[X_1]_{n+1} = (1-\gamma)[X_4]_n + \gamma[X_1]_n + (1-\gamma)q[X_2]_n, \tag{1a}$$

$$[X_2]_{n+1} = (1-\gamma)[X_1]_n + \gamma[X_2]_n, \tag{1b}$$

$$[X_3]_{n+1} = (1-\gamma)(1-q)[X_2]_n + \gamma[X_3]_n, \tag{1c}$$

and

$$[X_4]_{n+1} = (1-\gamma)[X_3]_n + \gamma[X_4]_n, \tag{1d}$$

where $[X_1]$ is the relative concentration of $\left(Q\begin{smallmatrix} Z \\ Z \end{smallmatrix}\right)$, $[X_2]$ of $\left(Q\begin{smallmatrix} Z^+ \\ Z \end{smallmatrix}\right)$,

$[X_3]$ of $\left(Q\begin{smallmatrix} Z^+ \\ Z^+ \end{smallmatrix}\right)$ and $[X_4]$ of $\left(Q\begin{smallmatrix} Z \\ Z^- \end{smallmatrix}\right)$, and n is the number of flashes.

The amount of oxygen evolved by the $(n+1)$th flash can be calculated from

$$2[O_2]_{n+1} = (1-\gamma)q[X_2]_n + 2(1-\gamma)[X_3]_n. \tag{2}$$

To calculate the amount of oxygen evolved per flash as a function of the number of flashes when cells are exposed to a series of flashes, we must first calculate the initial concentrations of the two stable states X_1 and X_4 after a long period of darkness, and the amount of oxygen evolved after a large number of flashes so that we can use that amount as the normalization constant. At "steady state", i.e. when oscillations are damped out and the amount of oxygen evolved per flash becomes constant, the concentrations of X_1, X_2, X_3, X_4 also remain constant. Hence by simple algebraic manipulation of the recursion relations, one can show that at the "steady state"

$$[X_1] = [X_2] = 1/D, \tag{3a}$$

$$[X_3] = [X_4] = (1-q)/D, \tag{3b}$$

where $D = 2(2-q)$, and $[X_1] + [X_2] + [X_3] + [X_4] = 1$.
The amount of oxygen evolved at "steady state" (ss) is then

$$2[O_2]_{ss} = (1-\gamma)/2. \tag{4}$$

To compare the calculated with the experimental results, $[O_2]_{ss}$ will be set to unity, and for all calculated $[O_2]_n$, the measured one must be divided by $[O_2]_{ss}$.

After "steady state" of oxygen yield has been reached and after the flashing light has been turned off, X_2 and X_3 deactivate back to X_1. Hence,

after a long dark period, the relative concentration of the X's are

$$1 - [X_1]_0 = [X_4]_0 = (1-q)/D, \tag{5a}$$

and

$$[X_2]_0 = [X_3]_0 = 0. \tag{5b}$$

After substituting the above values into the recursion relation, one can calculate the relative concentrations of X_1, X_2, X_3 and X_4. One can then calculate the amount of oxygen evolved per flash as a function of the number of flashes.

If after the "steady state" of oxygen yield per flash is reached, the flashing light is turned off for 5 min, whereupon one flash is given and the chloroplasts are then allowed to sit in the dark for 30 min, the initial concentrations of the stable states $[X_1]$ and $[X_4]$ are found to change. Immediately preceding the single flash, the concentration of X_1 and X_4 is $[X_1]_0$ and $[X_4]_0$. On applying the single flash, most of the $[X_4]_0$ will change into $[X_1]_0$ and in the dark will not decay back to $[X_4]_0$ because $[X_1]_0$ is stable. The other states that $[X_1]_0$ will change into, following a single flash, will deactivate back to $[X_1]_0$. Hence, one can show that following a single flash

$$1 - [X_1]_0 = [X_4]_0 = \gamma(1-q)/D, \tag{6a}$$

and

$$[X_2]_0 = [X_3]_0 = 0. \tag{6b}$$

Using the above argument, with two flashes given, the initial conditions would be

$$1 - [X_1]_0 = [X_4]_0 = \gamma^2(1-q)/D, \tag{7a}$$

and

$$[X_2]_0 = [X_3]_0 = 0. \tag{7b}$$

If three flashes are given, the initial conditions would be

$$1 - [X_1]_0 = [X_4]_0 = (1-\gamma)^3(1-q)\left[1 - \frac{(1-q)}{D} + \gamma^3\frac{(1-q)}{D}\right], \tag{8a}$$

and

$$[X_2]_0 = [X_3]_0 = 0. \tag{8b}$$

Using these initial conditions to calculate the amount of oxygen evolved per flash, one can easily see that the oxygen yield per flash would be different for each initial condition.

(B) FOUR STEP MECHANISM

In the four step mechanism, four charges must be accumulated before a molecule of oxygen can be evolved. In the linear four step mechanism of

Kok and co-workers, there exist four intermediate states S_0, S_1, S_2 and S_3 (Fig. 1(b)). Schematically, we interpret their S_0 to be (Q—Z), S_1 to be (Q—Z$^+$), S_2 to be (Q—Z^{++}) and S_3 to be (Q—Z^{+++}). After a quantum of light is absorbed, S_0 goes to S_1, S_1 to S_2, S_2 to S_3, and S_3 undergoes a dark reaction with water molecules which transforms S_3 back to S_0, and in this process, an oxygen molecule is evolved. If after a long period of darkness the only existing state is S_1, then the amount of oxygen per flash, as a function of the number of flashes, will oscillate with a period of four and will not damp out. As noted in the introduction, the perturbations of this cyclic reaction are due to the possibility that not every S state will move to the next on applying a flash of light ("misses") and also that intermediate states can have double excitations ("double hits") (Kok *et al.*, 1970; Forbush *et al.*, 1971). These authors assume that within the time of a flash of light used in their experiments, only S_0 and S_1 can use two photons to change into S_2 and S_3, respectively. To explain why oxygen produced on the third flash should be greater than oxygen produced on the fourth flash, they made the further assumption that the intermediate states S_2 and S_3 "relax" back to the S_1 state in the dark and that S_1 is an "infinitely" stable state. This assumption also explains the differences in the "flash yield sequences" observed after various pretreatments with light.

We present an alternate four step model for oxygen evolution (model D) in which two reaction centers, rather than one, are needed to evolve an oxygen molecule. There is some evidence that two reaction centers may act cooperatively (Stiehl & Witt, 1969). Furthermore, there is no "double excitation" within one reaction center within the time of the flash. This model is shown in Fig. 1(d). We assume that after a long dark period, most of the "double" reaction center is in the state $\left(Z \underset{\diagdown Q \diagup}{\overset{\diagup Q \diagdown}{}} Z \right)$. (This should have been written as $\left(\begin{matrix} Z \diagup Q \diagdown Z \\ Z \diagdown Q \diagup Z \end{matrix} \right)$, but we use the simpler form.) After the absorption of one quantum of light in each reaction center of the twin, (i.e. a total of two quanta), this state has a probability A to become $\left(Z^{++} \underset{\diagdown Q^- \diagup}{\overset{\diagup Q^- \diagdown}{}} Z \right)$ and $1-A$ to become $\left(Z^+ \underset{\diagdown Q^- \diagup}{\overset{\diagup Q^- \diagdown}{}} Z^+ \right)$. After a dark period (300 msec), followed by the absorption of the next two quanta of light, both states change into $\left(Z^+ \underset{\diagdown Q \diagup}{\overset{\diagup Q \diagdown}{}} Z^{+++} \right)$. State $\left(Z^{++} \underset{\diagdown Q \diagup}{\overset{\diagup Q \diagdown}{}} Z \right)$ has also a prob-

ability B to change into $\left(Z^{++++} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z\right)$; in this reaction, with two mole-

cules of water, an oxygen molecule is evolved. State $\left(Z^{+} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{+++}\right)$, with

two more quanta, undergoes two alternative light reactions. One reaction

changes it to $\left(Z^{++} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{++++}\right)$ which, with two molecules of water,

becomes $\left(Z^{++} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z\right)$ after giving off an oxygen molecule in dark. The

other reaction changes $\left(Z^{+} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{+++}\right)$, with a probability of ρ, to

$\left(Z^{+++} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{+++}\right)$, which is then postulated to undergo a dark reaction

with four molecules of water to evolve two molecules of oxygen and a stable

state $\left(Z^{-} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{-}\right)$. This state will not deactivate in the dark. With two

more quanta of light, the double reaction center $\left(Z^{-} \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z^{-}\right)$ changes

back to the initial state $\left(Z \begin{smallmatrix} Q \\ \\ Q \end{smallmatrix} Z\right)$. As in Kok's model, we assume that the

photochemical reaction centers are not 100% efficient but operate with an
efficiency of $(1-\gamma)$. From Fig. 1(d), the following recursion relations can
be derived

$$[X_1]_{n+1} = (1-\gamma)B[X_4]_n + (1-\gamma)[X_5]_n + \gamma[X_1]_n, \tag{9a}$$

$$[X_2]_{n+1} = (1-\gamma)(1-A)[X_1]_n + \gamma[X_2]_n, \tag{9b}$$

$$[X_3]_{n+1} = (1-\gamma)[X_2]_n + (1-\gamma)(1-B)[X_4]_n + \gamma[X_3]_n, \tag{9c}$$

$$[X_4]_{n+1} = (1-\gamma)A[X_1]_n + (1-\gamma)(1-\rho)[X_3]_n + \gamma[X_4]_n, \tag{9d}$$

and

$$[X_5]_{n+1} = (1-\gamma)\rho[X_3]_n + \gamma[X_5]_n, \tag{9e}$$

where $[X_1]$ is the relative concentration of the twin (or double) reaction

center $\left(Z \overset{\diagup Q \diagdown}{\underset{\diagdown Q \diagup}{}} Z \right)$, $[X_2]_n$ of $\left(Z^+ \overset{\diagup Q \diagdown}{\underset{\diagdown Q \diagup}{}} Z^+ \right)$, $[X_3]_n$ of $\left(Z^+ \overset{\diagup Q \diagdown}{\underset{\diagdown Q \diagup}{}} Z^{+++} \right)$,

$[X_4]_n$ of $\left(Z^{++} \overset{\diagup Q \diagdown}{\underset{\diagdown Q \diagup}{}} Z \right)$ and $[X_5]_n$ of $\left(Z^- \overset{\diagup Q \diagdown}{\underset{\diagdown Q \diagup}{}} Z^- \right)$ following the nth flash.
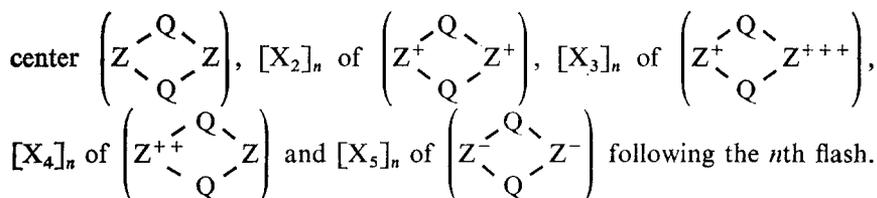
The amount of oxygen evolved by the $(n+1)$th flash is calculated from the equation

$$[O_2]_{n+1} = (1-\gamma)B[X_4]_n + (1-\gamma)(1-\rho)[X_3]_n. \tag{10}$$

To calculate the amount of oxygen evolved per flash when it becomes constant after a large number of flashes ("steady state"), we use the recursion relations of equation (9) and the fact that $[X_i]_{n+1} = [X_i]_n$, where $i = 1, 2, 3, 4$. The calculated concentration of X_4 and X_3 at "steady state" can then be substituted into equation (10) to obtain the amount of oxygen evolved at "steady state".

Again, by calculations similar to those used for model C, the initial concentrations of X_i after a long dark period following a "steady state", can be found. Similarly, we calculate the initial concentration of $[X_i]$ after a long dark time following one, two or three flashes of light which were given after 5 min of a dark period that, in turn, followed "steady state" conditions. From these initial concentrations and their substitution into the recursion relations, we obtain the amount of oxygen evolved per flash in a series of flashes following different light pretreatment.

3. Comparison with Experimental Results

(A) COMPARISON WITH JOLIOT ET AL.'S EXPERIMENTAL DATA

The experimental results of Joliot et al. (1969) are shown in Fig. 2(a). (For a brief description, see Section 1, Introduction.) The predicted results of the three models (B, C and D) correspond fairly well with the experimental data. (Model A was rejected on the grounds discussed in section 2 (A).) Using Kok's model, with a probability of 0·15 for "misses" (α) and 0·20 for the probability of "double hits" (β), we obtain a good correlation with the experimental results (Fig. 2(b)). Using model C, with γ (the probability of loss in the reaction center) of 0·10 and q $\left(\text{the probability that} \left(Q \overset{\diagup Z^+}{\underset{\diagdown Z}{}} \right) \right)$

will go to $\left(Q \overset{\diagup Z^{++}}{\underset{\diagdown Z}{}} \right) \right)$ of 0·388, we predicted Joliot et al.'s results (Fig. 2(c)).

Joliot et al.'s results are again predicted (Fig. 2(d)) by using model D, with $A = 0.255$, $B = 0.918$, $\gamma = 0.20$ and $\rho = 0.204$. We have tried model D with a zero value for both ρ and γ. The numerical results are identical to that of Joliot et al.'s "flip-flop" model. In all the cases, the calculated results agree well with the experimental results in that they show a damped oscillation with a period of four, no oxygen output in the first flash, and maximum oxygen output in the third flash. The match with experimental results of flash numbers greater than five, however, is only fair for models C and D. This may result from "errors" with each flash which cause slight variations in the probabilities of γ and q in model C and in parameters in A, B and γ in model D.

(B) COMPARISON WITH KOK ET AL.'S AND FORBUSH ET AL.'S EXPERIMENTAL DATA

The experimental data of Forbush et al. (1971) are shown in Fig. 3(a). (For a brief description see the Introduction.) Again, the predicted values from the three models (B, C and D) fit fairly well with the experimental results. Figure 3(b) shows Forbush et al.'s predictions from their model,
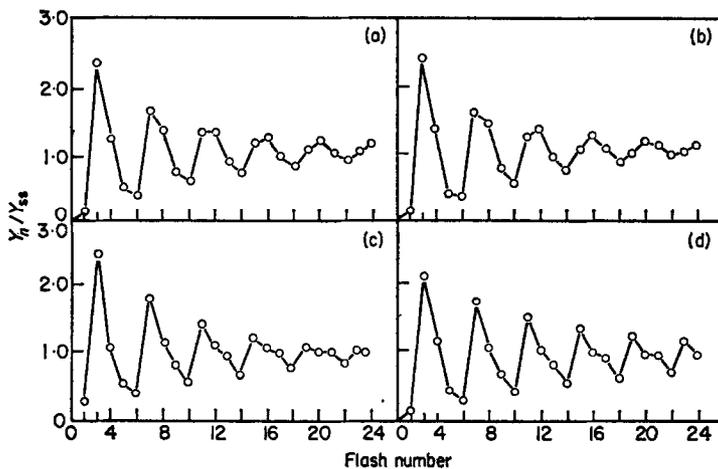

FIG. 3. (a) The amount of oxygen evolved per flash as a function of the number of flashes; a series of light flashes of saturating intensity were used to expose chloroplasts after 40 min of darkness following continuous illumination. The dark time between flashes was 1 sec (experimental data of Forbush et al., 1971). (b) Predictions based on Kok's model (Kok et al., 1970; Forbush et al., 1971); the symbols in this and the following parts (c) and (d) have the same meaning as in Fig. 2; $[S_0]_0 = 0.25$, $[S_1]_0 = 0.75$, $\alpha = 0.10$ and $p^{1-2} = 0.05$. (c) Predictions based on model C; $\gamma = 0.050$ and $q = 0.158$. (d) Predictions based on model D; $A = 0.102$, $B = 0.918$, $\gamma = 0.020$ and $\rho = 0.408$.
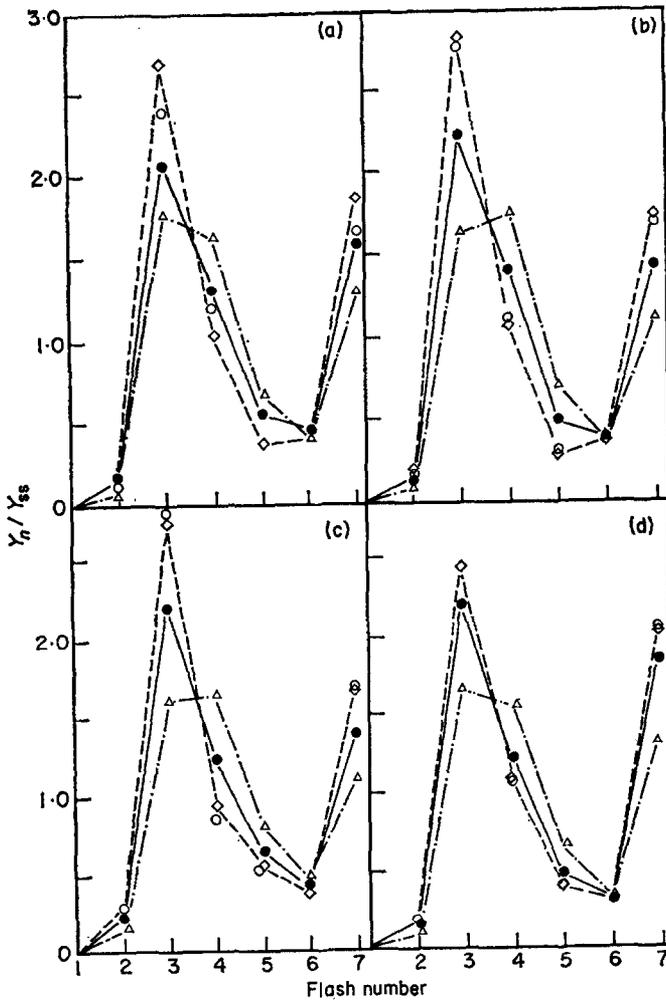
FIG. 4. (a) The amount of oxygen evolved per flash as a function of the number of flashes in a series; observations were made after various light pretreatments. Twenty-five flashes were used to attain "steady state", followed by five minutes of darkness. Then either none (●), one (◇), two (○) or three (△) flashes of light were given in a sequence spaced 1 sec apart. ₁This was followed by 30 min of darkness before a series of flashes were given (experimental data of Forbush *et al.*, 1971). (b) Predictions based on Kok's model (Kok *et al.*, 1970; Forbush *et al.*, 1971); the symbols in this and the following parts (c) and (d) have the same meaning as in Fig. 2; $\alpha^{0-3} = 0.12$, $\beta^{01} = 0.05$. (c) Predictions based on model C; $\gamma = 0.100$, $q = 0.116$. (d) Predictions based on model D; $A = 0.101$, $B = 0.909$, $\gamma = 0.010$ and $p = 0.404$.

assuming 0·10 for α, and 0·05 for β. Figure 3(c) shows the predicted values as calculated with model C, assuming 0·050 for γ, and 0·158 for q. In model D, A is assumed to be 0·102, B 0·918, γ 0·020 and ρ 0·408 (Fig. 3(d)). Again, the correspondence with experimental results is excellent; for flashes greater than five, the agreement with models C and D is only fair. However, slight variations in the probability values explain the discrepancy.

(C) COMPARISON WITH FLASH YIELD SEQUENCES AFTER VARIOUS LIGHT TREATMENTS

The experimental results of Kok *et al.* (1970) and Forbush *et al.* (1971) are shown in Fig. 4(a). (For a description of the experiment see section 2, General Treatment.) The predicted results from Forbush *et al.*'s (1971) model, with 0·12 for α and 0·05 for β, are shown in Fig. 4(b); Fig. 4(c) depicts the predicted results from model C, with 0·10 for γ and 0·166 for q. With A equal to 0·101, B to 0·909, γ to 0·010 and ρ to 0·404, predictions from model D are shown in Fig. 4(d). All the theoretical curves match well the experiment curves with the exception of the oxygen yield in the third flash after a two flash pretreatment. (In the experimental case, the oxygen yield of the third flash after two flash pretreatment is lower than that of one flash pretreatment.) In all the theoretical cases, the oxygen yields per flash for one or two flash pretreatments are very similar to each other.

(D) THE KINETICS OF OXYGEN PRODUCTION IN LOW LIGHT INTENSITIES AFTER DIFFERENT NUMBERS OF SATURATING FLASHES

Joliot *et al.* (1970) and Forbush *et al.* (1971) calculated the time course (kinetics) of oxygen production at low light intensities in cells exposed to different numbers of "saturating" flashes. The numerical values were obtained from the recursion relations based on their models. We will show that these kinetic data can be theoretically calculated from the experimental flash yield data.

Let N_1 be the number of units that have received no photon from the weak continuous light (to be abbreviated as WCL), N_2 one photon from WCL, N_3 one photon from WCL, but in which Q^- has been reoxidized to Q, N_4 two photons (the second one) from WCL, and N_5 the second photon from WCL, but in which Q^- has been reoxidized to Q.

Then

$$N_1 \xrightarrow{kI} N_2 \dashrightarrow{k_1} N_3 \xrightarrow{kI} N_4 \dashrightarrow{k_1} N_5 \xrightarrow{kI} N_6 \dashrightarrow \ldots,$$
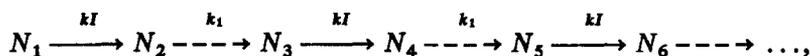
where I is the intensity of light, k the rate of conversion of N_1 into N_2 and N_3 to N_4, etc., at unit intensity of light, and k_1 the dark rate in which Q^-

goes to Q (via A). Hence

$$\frac{dN_1}{dt} = -kIN_1,$$

$$\frac{dN_2}{dt} = kIN_1 - k_1 N_2,$$

$$\frac{dN_3}{dt} = k_1 N_2 - kIN_3, \tag{11}$$

and

$$\frac{dN_i}{dt} = kIN_{i-1} - k_1 N_i.$$

In general, the solution for the above set of differential equations can be found. The solution for N_{2i+1}, assuming $k_1 \gg kI$ is

$$N_{2i+1}(t) = N_1^{\circ} \frac{(k_1)^i (kI)^i t^i}{(k_1 - kI)^i i!} e^{-kIt}, \tag{12}$$

where $i = 0, 1, 2, 3, \ldots$, and N_1° is the total number of units.

In weak continuous light, without any preilluminating flash, the amount of oxygen evolved at a given time depends upon the amount of oxygen the ith units will evolve and the number of ith units. The amount of oxygen evolved by the $2i$th unit is exactly the amount of oxygen that will be evolved after the ith flash in a series of flashes (Yi). Since the reaction between the stored charges and water is a dark reaction, we will designate k_2 as the rate of this reaction. Hence, the kinetics of oxygen production at low light intensities should follow

$$[O_2](t) = k_2 \sum_{i=0}^{\infty} \left(\frac{Yi}{Y_{ss}} \right) N_{2i+1}, \tag{13}$$

where $i = 0, 1, 2, 3, 4, \ldots$ and $[O_2](t)$ is the amount of oxygen evolved, normalized at "steady state".

After the nth preillumination flash, the amount of oxygen that the N_ith number of units will evolve will be equal to $Y(i+n)$. Hence

$$[O_2]_n(t) = k_2 \sum_{i=0}^{\infty} \left(\frac{Y(i+n)}{Y_{ss}} \right) N_{2i+1}. \tag{14}$$

These equations were numerically evaluated. The results are shown in Fig. 5. The values of k_1 was 10^4 as this is the experimental value obtained by Kok *et al.* (1970). The value of kI was 2·5, chosen for the best fit of the experimental data. Since k_2 and N_1° were arbitrary constants that would not change the shape of the transient curve, they were designated as one. Figure 5(a) shows the theoretical curves (solid lines) obtained from equations

(13) and (14) and the experimental flash yield data of Joliot. They are compared here with the time course curves after one, two and three flashes, obtained by Joliot *et al.* (1969). Our calculated curves agree well with Joliot *et al.*'s experimental data. Figure 5(b) shows the predicted curves for oxygen
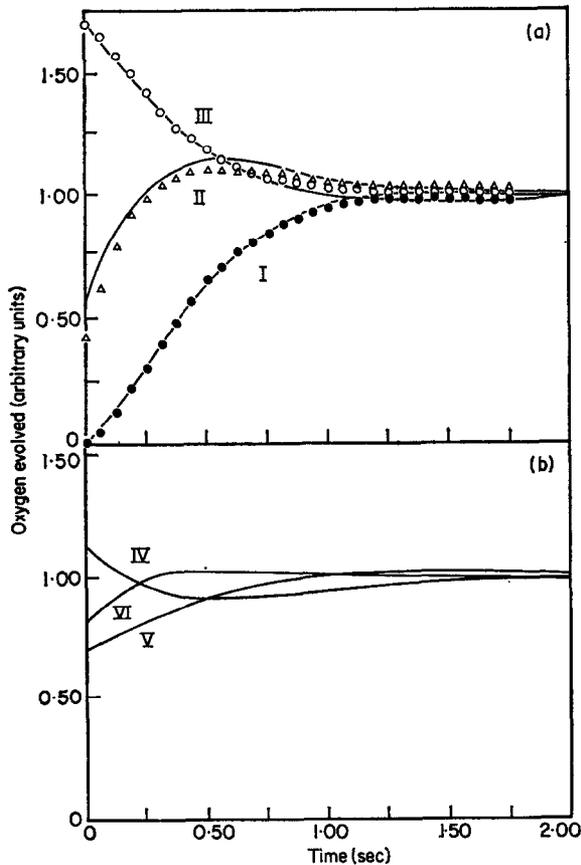

FIG. 5. (a) The kinetics of oxygen evolution of *Chlorella* illuminated by weak modulated light ($\lambda = 684$ nm; intensity, 700 erg/cm² sec; modulation frequency, 25 Hz). The solid dots (●), experimental values obtained by pre-illuminating the algae for 30 sec, after which they were allowed to stay in the dark for 70 sec before the weak modulating light was turned on; the triangles (△), experimental values obtained as before but with one flash given 20 msec before the weak modulating light was turned on; the open circles (○), experimental values obtained as before but with two short flashes (experimental data of Joliot *et al.*, 1969). The curves (I–III) are the theoretical curves calculated from equations (13) and (14) in this paper using the experimental oxygen yield data of Joliot *et al.* (1969). (b) Theoretical curves predicted from experimental oxygen flash yield data. Line IV is the predicted curve after three flashes of pre-illumination; line V, after four flashes; line VI, after five flashes.

evolution after the fourth, fifth and sixth flashes; these curves are very similar to the experimental curves obtained by Kok *et al.* (1970).

These above results, therefore, show that the time course of oxygen production at low light intensities, after different numbers of saturating flashes, can be calculated from the oxygen flash yield data. Hence, any model for oxygen evolution that will explain the oxygen flash yield data will also explain the kinetics of oxygen production in low light intensities. An exception is the model of Kok *et al.* (1970); they postulated that there are "double hits" which occur when the cells are illuminated by saturating light flashes. At low light intensities, "double hits" should not occur. They have taken this into account and have calculated, based on their model, the kinetics of oxygen production at low intensities (Forbush *et al.*, 1971). Their theoretical kinetic curves also agree with their experimental curves.

4. Discussion

Figure 1 shows that our models (C and D) are as much different from the models of Joliot and of Kok as they are different from each other. All four models are similar in that there are four predominant intermediates, as four is the minimum number required to explain the experimental results. One could build models with a higher number of intermediates, but that would require more parameters.

Models C and D are proposed in this paper as alternate explanations for the mechanism of oxygen evolution to the model (B) proposed by Kok *et al.* (1970) and Forbush *et al.* (1971). Our analyses (see Figs 2, 3 and 4) of the three models (B, C and D) indicate that all are valid alternatives and that none of them can yet be declared as the correct one. Thus, *no* unique model has yet been found. We feel that the fitting of the experimental data by equations derived from the first model that comes to one author's mind may create a misleading impression that a unique explanation has been found. It is accepted (see Forbush *et al.*, 1971) that the old model of Joliot *et al.* (1969) (model A) may not be considered in its present form, because it cannot explain the data of Kok and co-workers.

In Kok's model, the accumulation of four positive charges in one reaction center leads to the evolution of oxygen. Analysis of model C shows that it is possible to explain the existing experimental data on oxygen evolution by proposing that oxygen can be evolved from the accumulation of two positive charges in one reaction center. Analysis of model D shows that a four-charge hypothesis that uses two reaction centers acting together also explains the existing experimental data.

One important difference between the model by Kok and co-workers and the model C or D is the basis for oxygen evolution after the second flash.

Within the time of the flash of light (~ 10 μsec), some photosynthetic units are capable of being hit twice by photons and are able to do two photochemical reactions (Kok). It is difficult to imagine how Q^- returns to Q within this time to do the second photoreaction as Kok, Malkin, Owens & Forbush (1966) have shown that the half time of the recovery of Q is about 0·5 msec. (We have not given any further thought on this point.) Also, if the time duration of the pulse of light is kept very short, no oxygen should be evolved after the second flash. Weiss & Sauer (1970) showed that no oxygen was evolved after the second flash when 20 and 40 nsec laser flashes and 28 msec Xenon light flashes were used. The minimum dark time between flashes that they used, however, was 15 sec, so that they could not even obtain oscillations of the oxygen yield produced by a sequence of flashes. This may be the result of the fact that precursors built up by a flash of light deactivated before the next flash of light was given! Hence, part of the reason why oxygen was not observed after the second flash may be due to deactivation of the precursors built up by the first flash of light. However, if an experiment can be done with flash time of 20 nsec spaced approximately 300 msec apart, the amount of oxygen evolved after the second flash in a series of flashes following a long dark period should decide whether the two proposed models or Kok's model is the correct one. In Kok's model there should be no oxygen evolved; in models C or D oxygen evolution should be observed. This experiment should be done.†

The question of whether atomic oxygen (or a 2-electron oxidation product) can be produced before any molecular oxygen is formed is an important one. If atomic oxygen is formed first, the reaction center will need the cooperation

---

† *Note added in proof by Govindjee.* The present paper was written in 1970 and revised in 1971 as new data became available. In early 1972, we became aware of another paper by JOLIOT, P., JOLIOT, A., BOUGES, B. & BARBIERI, G. (1971), *Photochem. Photobiol.*, **14**, 287, in which these authors reported that the oxygen evolution in the second flash was negligible when short flashes (2 μsec at $\frac{1}{3}$ of the peak, or 15 μsec including the tail of the flash) spaced 320 msec were used. (In these experiments, chloroplasts were preilluminated with continuous light, then kept in darkness for 6 min prior to flash excitation.) This result would favor Kok's model. However, we wish to emphasize that none of the presented models should be discarded because of just one reason, as they can be modified to explain the new finding. Furthermore, we note that P. Joliot and co-workers have proposed a still newer model in which two fluorescence quenchers (Q_1 and Q_2) and two Z's are involved —this model also explains the existing data (paper presented at the 2nd International Congress on Photosynthesis Research, Stressa, Italy; paper in press, 1972). Thus, the aim of our paper in suggesting that no existing models should be accepted as unique is further emphasized. More work should be done to analyze all the available data, and no model (including Joliot's 1972 model), as yet, should be accepted as unique!

of two photons and one molecule of water. However, if a molecule of oxygen is directly formed without the intervention of a step in which atomic oxygen is formed first, the reaction center will require the cooperation of four photons and two molecules of water. The nature of the process involving the cooperation of two photons should be different from the process involving the cooperation of four photons. Hence, it is important to test any experimental data on oxygen evolution whether it can be explained only by a cooperation of four or two charges. Kok's model which assumes the cooperation of four charges explains all experimental data, while Joliot's older model which assumes the cooperation of two charges does not explain all experimental data. One of our models assuming the cooperation of two charges, however, can explain all experimental data. Hence, the present experimental data have *not* resolved this important question of whether oxygen evolution requires the cooperation of two or four charges. We, however, recognize that within the principle of finding the *simplest* model consistent with the facts, model D does not merit as high a rating as B or C because D is much more complex.

Renger (1970) has proposed a mechanistic picture of how O_2 is evolved; this picture was based on Joliot *et al.*'s older model. We feel that a mechanistic speculation based on the models described here may be too premature at this time.

REFERENCES

ALLEN, F. L. & FRANCK, J. (1955). *Archs Biochem. Biophys.* **58,** 124.
BARBIERI, G., DELOSME, R. & JOLIOT, P. (1970). *Photochem. Photobiol.* **12,** 197.
CHENIAE, G. (1970). *A. Rev. Pl. Physiol.* **21,** 467.
DELERIEU, M. (1968). Thesis, University of Paris, Orsay.
EMERSON, R. (1958). *A. Rev. Pl. Physiol.* **9,** 1.
FORBUSH, B., KOK, B. & MCGLOIN, M. (1971). *Photochem. Photobiol.* **14,** 307.
GOVINDJEE, R., RABINOWITCH, E. & GOVINDJEE. (1968). *Biochim. biophys. Acta* **162,** 539.
JOLIOT, P. (1965). *Biochem. biophys. Acta* **102,** 116.
JOLIOT, P., BARBIERI, G. & CHABAUD, R. (1969). *Photochem. Photobiol.* **10,** 309.
KOK, B., MALKIN, S., OWENS, O. & FORBUSH, B. (1966). *Brookhaven Symp. Biol.* **19,** 446.
KOK, B., FORBUSH, B. & MCGLOIN, M. (1970). *Photochem. Photobiol.* **11,** 457.
RABINOWITCH, E. & GOVINDJEE (1969). *Photosynthesis.* New York: John Wiley & Son.
RENGER, G. (1970). *Z. Naturf.* **25b,** 966.
ROSENBERG, J. (1969). *Biophys. Soc. Abstr.* **9,** A29.
STIEL, H. H. & WITT, H. T. (1969). *Z. Naturforschg.* **24b,** 1588.
WEISS, G. & SAUER, K. (1970). *Photochem. Photobiol.* **11,** 495.
WHITTINGHAM, C. P. & BROWN, A. H. (1958). *J. exp. Bot.* **9,** 311.
YOUNG, R. A., BLACK, G. & SLOUGER, T. C. (1968). *J. chem. Phys.* **49,** 4769.
ZANKEL, K. & KOK, B. (1970). Paper presented at the International Conference on the Photosynthetic Unit, Gatlinburg.