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ANOMALIES IN THE KINETICS OF PHOTOSYNTHETIC OXYGEN EMISSION IN SEQUENCES OF FLASHES REVEALED BY MATRIX ANALYSIS

EFFECTS OF CARBONYL CYANIDE *m*-CHLOROPHENYLHYDRAZONE AND VARIATION IN TIME PARAMETERS

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SUMMARY

The model of Kok et al. (Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457-475) is considered the best kinetic explanation of the damped oscillations of O_2 evolution induced in higher plants by a sequence of brief saturating flashes. Matrix analysis applied to this model shows that the parameters involved (distribution of S states at zero time, probabilities of transition between states induced by a flash) cannot be completely known from the O_2 yield sequence, Y_n. However, four quantities, with limited content of information, are readily derived from data, without additional assumptions. They are σ_1, σ_2 and σ_3, three quasi-symmetrical functions of the transition coefficients, and $\overline{Y}$, a weighed average of four consecutive Y_n values. The extent of misses and double hits and their variations can be qualitatively ascertained by inspection of the relative values of σ_1, σ_2 and σ_3. In a regular sequence (strictly obeying Kok's model), all four quantities should be constant along the time axis.

It is shown that actual sequences are seldom regular, in particular in the following conditions: (1) variable flashing frequency, (2) addition of carbonylcyanide *m*-chlorophenylhydrazone, (3) incomplete deactivation, (4) change of flashing frequency at steady state.

In order to account for these anomalies, it is proposed to modify Kok's model by introducing, in parallel to the four state storage entity (S states), a side carrier C, which can reversibly exchange a positive charge with it. In the new model, the transition coefficients are essentially time varying, thus producing a nonregular behaviour of Y_n sequences.

INTRODUCTION

The mechanism underlying the emission of oxygen in photosynthetic systems has been the subject of extensive studies since the advent of the model of Kok et al.

Abbreviation. CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

[1]. The latter was aiming at the formal explanation of the damped oscillations of O_2 yield in sequences of brief saturating flashes discovered by Joliot et al. [2]. This model stands presently as the accepted framework for current investigations in the part of the photosynthetic chain situated on the donor side of System II. It certainly owes its success to the simplicity of the basic assumptions: a four step linear mechanism of accumulation of positive charges, preceding the discharge of molecular oxygen. A great deal of experimental work has been devoted to the understanding of the nature of the storage states S_i ($i = 0, 1, 2, 3$) and significance of the parameters describing the light induced transition between states. However, a firm theoretical basis was lacking to warrant the knowledge of the above parameters from the analysis of experimental data. Recently, it has been proved that a complete solution of this problem was impossible, starting from the only knowledge of the O_2 sequence, Y_n*. One of us has proposed an analytical procedure in order to extract from data the maximum possible numerical information, without making any specific assumption concerning the parameters. For theoretical reasons, this information is much limited [3].

In this report, we apply this analytical method (henceforth called σ analysis) to experimental data obtained in our laboratory. We thus demonstrate that, in several conditions, the result are at variance with the predictions of Kok's model. We shall propose an extension of the latter which we believe may explain these anomalies.

THEORETICAL

The following summarizes a theoretical treatment already published [3]; it also includes a new relation (Eqn. 5). The essential steps of the derivation are given in the Appendix.

(1) Kok's model is a Markoff process. The effect of a flash may be described as the operation of a matrix $\tilde{K}$ on a state vector $S^{(n)}$:

$$S^{(n+1)} = \tilde{K} S^{(n)} \tag{1}$$

The components of $S^{(n)}$ are the relative concentrations of centers in states S_0, S_1, S_2 and S_3 after n flashes; the elements of $\tilde{K}$ (see Appendix, 1) are the transition probabilities (assumed to be time invariant) between states.

(2) While the problem is entirely determined when the matrix recurrence relation (Eqn. 1) and the initial state vector $S^{(0)}$ are known, giving $S^{(n)} = \tilde{K}^n S^{(0)}$, the information provided by the experiment is limited to S_3 (Y_n is assumed to be proportional to ${S_3}^{(n-1)}$). A general recurrence relation on Y_n can nevertheless be deduced:

$$Y_{n+4} - Y_n - \sigma_1 (Y_{n+3} - Y_n) + \sigma_2 (Y_{n+2} - Y_n) - \sigma_3 (Y_{n+1} - Y_n) = 0 \tag{2}$$

σ_1, σ_2 and σ_3 are functions of the transition coefficients (see Appendix, 2).

(3) The functions σ_i ($i = 1, 2, 3$) can be directly calculated from the experimental sequence Y_n: they are the solution of a linear system of three equations where seven consecutive Y_n values enter as coefficients (see Appendix, 3).

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* In what follows, we frequently use the letter Y without subscript in place of the expression "Y_n sequence" or, depending on context, to name an unspecified member of it; the same applies to the quantity $\bar{Y}$, to be defined below.

In the general problem with no double hits, the σ_i are the first three elementary symmetrical functions of the order 0 transition coefficients α_i (misses). In particular,

$$\sigma_1 = \alpha_0+\alpha_1+\alpha_2+\alpha_3 \tag{3}$$

(hence, $\sigma_1/4$ is the average miss $\bar{\alpha}$). It is shown that the α_i values, if they are all different, cannot be calculated, nor, if they were known, could they be assigned to specific states (see also ref. 4). The following unequalities must, however, hold

$$0 \leqslant \sigma_3 \leqslant \sigma_2 \leqslant \sigma_1 \leqslant 4 \tag{4}$$

In the general problem with double hits, the expressions of σ_i are more complicated. It can be predicted that, in comparison with the former problem, σ_2 will decrease, and σ_3 may increase and become larger than σ_2 (compare with Eqn. 4). Even though the σ_i system cannot be solved in terms of the transition coefficients, qualitative comparison of its components in various experimental conditions can be of much help [3].

(4) Reiterating the recurrence relation on Y_n, we find a new expression relating the steady-state O_2 yield to any four consecutive Y_n (see Appendix, 4):

$$\bar{Y}_n = \frac{(1-\sigma_1+\sigma_2-\sigma_3)\,Y_n+(1-\sigma_1+\sigma_2)\,Y_{n+1}+(1-\sigma_1)\,Y_{n+2}+Y_{n+3}}{4-3\sigma_1+2\sigma_2-\sigma_3} \tag{5}$$

(for reasons to appear later, we prefer to call this quantity "mean Y" ($\bar{Y}$) rather than "steady-state Y" (Y_{ss}); $\bar{Y}$ is in effect a weighted average).

Calculation of $\bar{Y}$ complements σ analysis: a train of seven consecutive Y_n is used to calculate σ_i and then $\bar{Y}$. In principle, the first train (Y_1 to Y_7) should suffice; actually, it has always been found instructive to use also the successive trains (Y_2 to Y_8, Y_3 to Y_9, etc., until the differences in Y_n values become too small for a precise calculation of σ_i), a procedure called "piecewise progressive analysis" [3]. σ analysis will be understood in this sense. We define a regular sequence as one producing invariant sets of σ_i and $\bar{Y}$ through σ analysis of successive trains. Very few actual sequences are regular.

MATERIAL AND METHODS

The experiments on O_2 yield in sequences of flashes were performed with the alga *Chlorella pyrenoidosa* essentially as described in a recent report [5]. Since the reliability of σ analysis was shown to depend much upon the precision of data [3], care was taken to minimize as much as possible the experimental error by averaging several consecutive Y_n sequences recorded on a numerical analyser (Didac 800, Intertechnique). Table I illustrates the precision of σ analysis. With relative standard deviation of the raw Y_n data between 10 % (Y_2) and 1 % (Y_3), it is seen that the relative standard deviations on σ_1, σ_2 and σ_3 are at best 2.6 %, 14 % and 24 %, respectively, and that the precision decreases rapidely along the sequence. $\bar{Y}$ is a much less sensitive quantity (relative standard deviation < 1 %)*.

* It must be pointed o ut that a part of the standard deviation on $\bar{Y}_n$ and Y_n must be attributed to a systematic drift of the O_2 response of biological origin from one sequence to the following.

TABLE I

PRECISION OF σ ANALYSIS

Eight consecutive Y_n sequences with the same sample of *Chlorella*; dark adaptation time, 3 min; 15 flashes; flashing period, 0.3 s.

	Y									
	1	2	3	4	5	6	7	8	9	10
Mean	−1	36.5	580	476	257	157	332	405	333	248
Standard deviation	1.7	4.3	6.3	4.6	7.5	3.7	5.8	1.5	4.5	5.5

Train	Mean (standard deviation)			
	σ_1	σ_2	σ_3	$\bar{Y}$
Y_1 to Y_7	0.649 (0.017)	0.091 (0.013)	0.032 (0.008)	315.6 (2.2)
Y_2 to Y_8	0.655 (0.030)	0.097 (0.033)	0.035 (0.016)	315.7 (2.2)
Y_3 to Y_9	0.694 (0.042)	0.182 (0.053)	0.132 (0.039)	313.6 (2.3)
Y_4 to Y_{10}	0.568 (0.044)	0.059 (0.052)	0.037 (0.037)	311.9 (2.4)

Calculations of σ_1, σ_2, σ_3 and $\bar{Y}$ from Y or generation of synthetic Y_n sequences (with given unequal transition parameters α_i, β_i, γ_i and initial distribution $S^{(0)}$) are conveniently performed on a programmable pocket calculator (HP65, programs available upon request).

RESULTS

To test the possibilities of σ analysis, a number of published sequences have been previously surveyed [3]. Among other conclusions, it was noticed that many sequences displayed two kinds of anomalies: an initial increase of σ_i during the first two or three trains and a period 2 oscillation of σ_i as a function of the rank of trains. With the help of the $\bar{Y}$ formula (Eqn. 5), the latter anomaly is now better understood in terms of a slow drift in magnitude of Y_n. To illustrate this point, Fig. 1 shows a synthetic sequence modulated by a simple drift function (Y_n replaced by $Y_n(1+n\varepsilon)$ where ε is a constant). Notice that the oscillation of σ_i is a very sensitive property which permits the detection of a drift barely visible to the eye. As we shall see, the above example does not necessarily imply that the experimental effects result from such a trivial modulation. The following properties have been empirically verified. The amplitude and phase of the σ oscillations depend directly upon the magnitude and sign, respectively, of the drift constant ε; all three σ_i oscillate in synchrony, but their mutual differences are almost invariant; when the oscillations are small, the unperturbed σ_i may be recovered by averaging an even number of consecutive values; $\bar{Y}$ is found to be modulated by the same drift function. It must be stressed that, when dealing with strongly irregular sequences, σ_i may assume local values (for instance $\sigma_1 < 0$) having no direct mechanistic meaning. In the following, we report on several selected experimental conditions producing interesting anomalies as revealed by σ analysis.

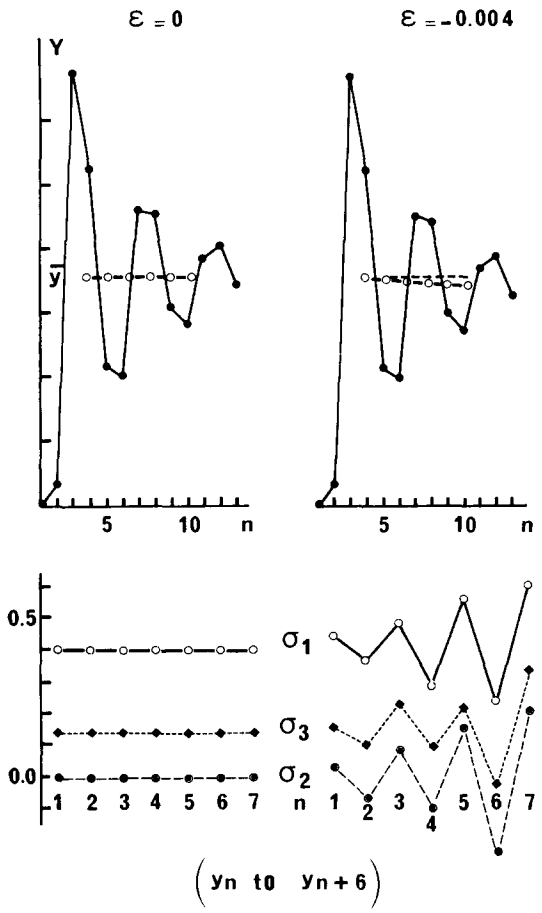

Fig. 1. Effect of drift on Y_n sequence (synthetic) and σ analysis. Top, synthetic Y_n sequence (●, concentration of state S_3 as a function of the rank of flashes n), $\bar{Y}$ (○) calculated from Y_n and σ_i (Eqn. 6). Bottom, σ_1 (○), σ_2 (○), σ_3 (◆) obtained through piecewise progressive σ analysis (see text), n is the starting point of the train, i.e. $n = 1$ corresponds to the train Y_1 to Y_7. Left, unperturbed sequence with parameters: $S_0^{(0)} = 0.3$, $S_1^{(0)} = 0.7$, $S_2^{(0)} = S_3^{(0)} = 0$, $\alpha_0 = \alpha_1 = \alpha_2 = 0$, $\alpha_3 = 0.4$, $\gamma_i = 0.05$; notice that $\bar{Y}$ is exactly equal to Y_{ss} (steady state). Right, the preceding Y_n sequence has been modulated by a negative drift, i.e. each Y_n is replaced by $Y_n(1+n\varepsilon)$, $\varepsilon = -0.004$.

(1) *Effect of CCCP at different flashing periods*

Recently, Lemasson and Etienne [5] have explained the effect of CCCP ($4 \cdot 10^{-6}$ M) by a reversible "photoinactivation" of centers in their S_2 and S_3 states. This view is corroborated by σ analysis of the same set of data (Fig. 2). At short flashing periods (T_*), the σ_i sequences are strongly oscillating and $\bar{Y}$ is rapidly decreasing from a high initial level, a situation very similar to the theoretical one of Fig. 1. At longer flashing periods, the rate of decrease of $\bar{Y}$ tends to zero and the initial value is much reduced; at $T_* = 1$ s, looking at the $\bar{Y}_n$ sequence it could be concluded that the Y_n sequence is regular; however, this is not strictly true, since the σ_i still display some long period oscillations (Fig. 2, insert). The decay of $\bar{Y}$ and

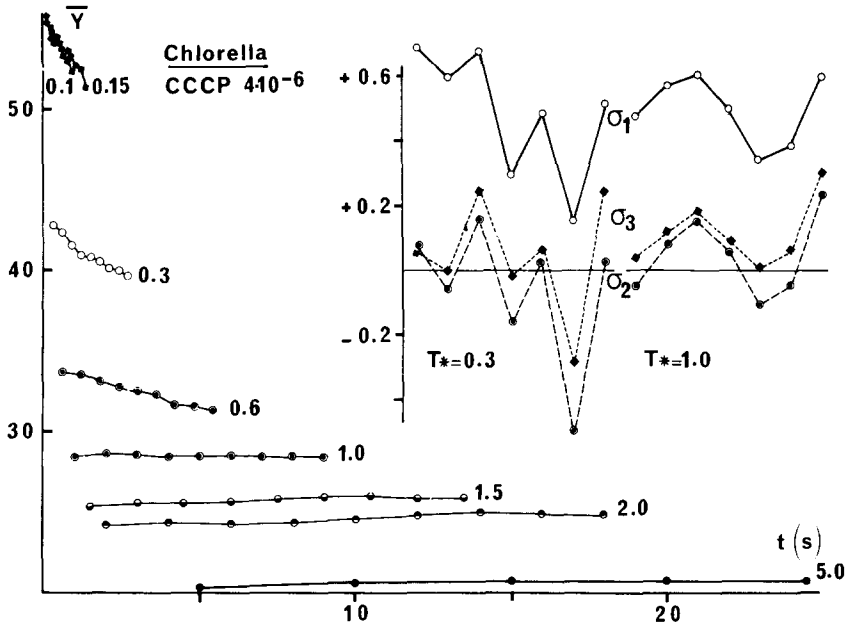

Fig. 2. σ analysis of Y_n sequences at different flashing periods in *Chlorella* in the presence of CCCP $4 \cdot 10^{-6}$ M. $\bar{Y}$ (arbitrary unit) of successive trains as a function of time, (compare with Fig. 1, top). The flashing period T_* (s) is given at the right of each curve. The time axis represents $n\ T_*$, n being the index of the train used to calculate $\bar{Y}_n$. Insert: σ_i of successive trains for two values of T_*. Original Y_n sequences taken from ref. 5.

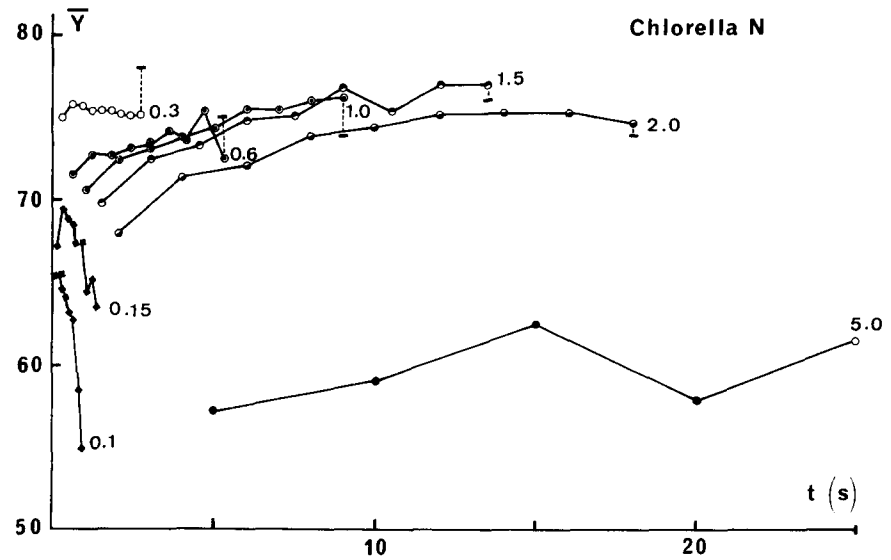

Fig. 3. σ analysis of Y_n sequences at different flashing periods in *Chlorella* (control). See legend of Fig. 2; the horizontal bar at the end of each curve for $T_* = 0.3$–2.0 is the steady-state value, Y_{ss}. Original Y_n sequences taken from ref. 5.

its dependence on T_* are readily explained by the above-postulated mechanism. If T_* is shorter than the time constant of photoinactivation, the centers must complete several cycles before they can reach the photoinactivation equilibrium. The latter is governed by two factors: the sensitive fraction of the cycle (i.e. 1/2 assuming that S_2 and S_3 are equally inactivated by CCCP) and the time dependence of the kinetics of photoinactivation. Obviously, with long enough T_*, the photoinactivation equilibrium* is already reached during the first and second flashing period. From the $\bar{Y}$ decay at $T_* = 0.1$ s and assuming that the equilibrium situation obtains at $T_* = 1.0$ s, a pseudo first order rate constant of 0.3 s^{-1} may be estimated for the photoinhibition reaction.

The σ analysis of the control case (from the same report [5]) is also very instructive (Fig. 3). Three domains of T_* may be distinguished. At $T_* < 0.3$ s, a fast decay of $\bar{Y}$ is observed, reminiscent of the CCCP case. The likely explanation, however, must be different (possibly a transient rate limitation similar in origin to the O_2 burst in continuous light). Notice the initial increase of $\bar{Y}$ (at all T_* values from 0.15 to 2.0 s) which is the corresponding aspect of the initial σ_i increase already mentioned (first anomaly). At $T_* = 0.3$ s, the sequence is almost regular (except for the initial rise). From $T_* = 0.3$–2.0 s, the Y_n sequence shows an increasing initial deficit but reaches essentially the same steady-state level (we shall refer to this time range as the "Emerson plateau"). The effect of CCCP clearly indicated that $\bar{Y}$ can be chosen as an objective measure of the number of active centers; however, variations of $\bar{Y}$ may also occur for a different reason, namely because of variations of the transition parameters (see Discussion). Whatever the reason, the behaviour of $\bar{Y}$ within the Emerson plateau shows that a deficit either in active centers or in available stored positive charges is initially induced by this particular timing protocol, but that it is only momentary and is readily compensated during sustained flash illumination. At $T_* > 2.0$ s, $\bar{Y}$ is depressed due to deactivation [1, 2, 6].

σ_i values have been compared in the absence or presence of CCCP as a function of T_*. σ_1, a direct measure of the average miss coefficient, is a decreasing function of T_*; especially at low T_* values, σ_1 is distinctly smaller in the presence of CCCP than in its absence.

(2) *Anomaly of the Emerson plateau*

The T_* dependency and induction effects of $\bar{Y}$ shown above in the control case convey the impression that a small pool distinct from, but communicating with, the System II centers modulates the O_2 emission and that the dark time between flashes somehow controls its effect on the centers. Another protocol producing a similar effect consists in a sudden increase in T_* at the steady-state flash illumination, T_* staying within the Emerson plateau time range (Fig. 4). This also results in a transient deficit in Y. It should be noted that the effect cannot be explained by a trivial rate limitation due to overloading the plastoquinone pool during the preillumination flash sequence, since the relaxation of this limination would produce an increase of Y above the steady-state level. We believe that the anomaly is somehow associated with the mechanism of deactivation (see Discussion).

* It is assumed [5] that S_2 and S_3, once inactivated by CCCP, decay slowly into active S_0 and S_1.

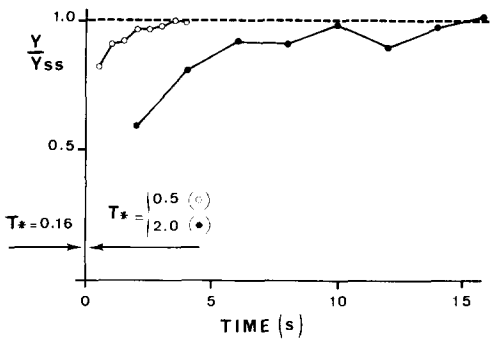

Fig. 4. Anomaly of the Emerson plateau in *Chlorella*. The sample (dark-adapted 3 min) was given 16 flashes at a flashing period $T_* = 0.16$ s, then (origin of time scale) T_* was suddenly shifted to 0.5 s (○) or 2.0 s (●). Ordinate, Y normalized to the steady-state Y_{ss}.

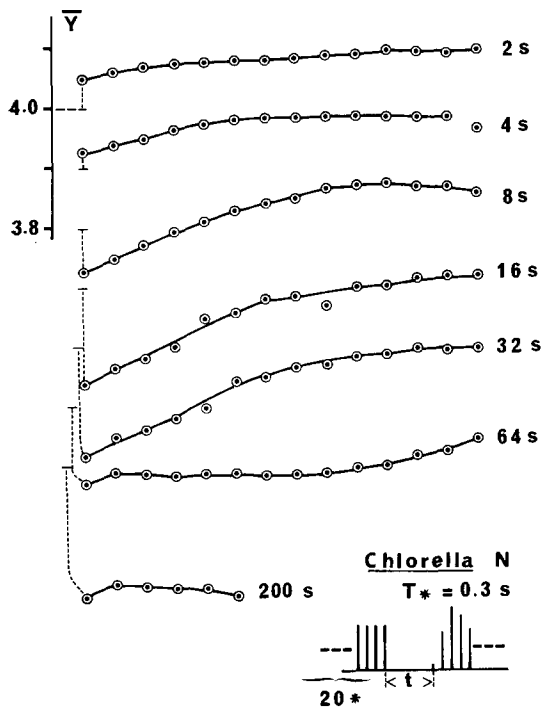

Fig. 5. Induction of $\bar{Y}$ during deactivation in *Chlorella*. Consecutive sequences of 20 flashes spaced 0.3 s apart were given with interval t between sequences (see scheme of protocol at bottom). Average of 10 sequences ($t = 2, 4, 8, 16$), 5 sequences ($t = 16, 32$), 2 sequences ($t = 200$). For clarity the vertical scale has been regularly shifted for each curve (a dashed line connects the beginning of each curve with the same value of 4.0 arbitrary units).

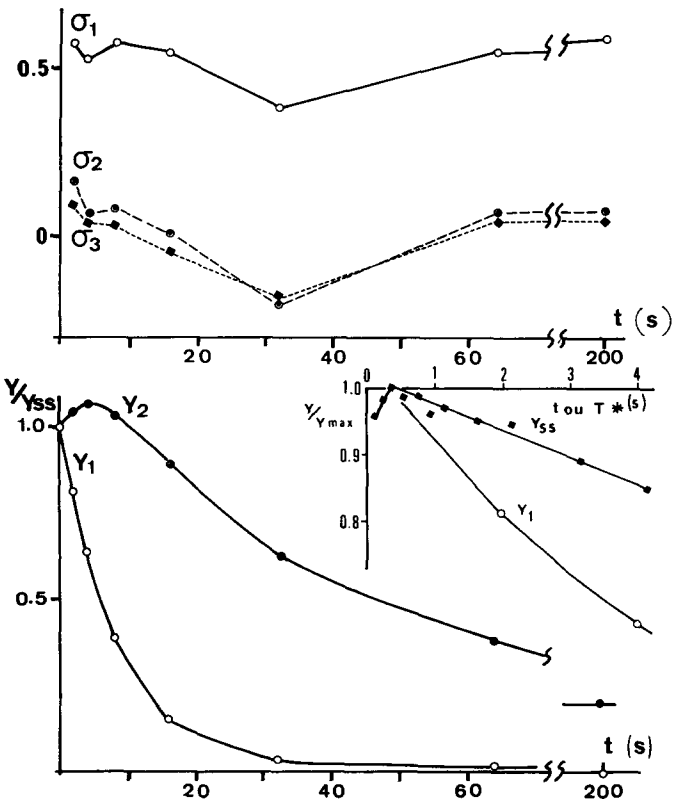

Fig. 6. Deactivation in *Chlorella* (continuation of Fig. 5). Top, σ_1 (○), σ_2 (⊙), σ_3 (◆) as a function of deactivation time t (σ_t averaged over 4 trains spanning Y_1 to Y_{10}). Bottom, decay of Y_1 (○), Y_2 (●) as a function of deactivation time t. Bottom insert, comparison of deactivation of Y_1 (same data as bottom left) and Y_{ss} (◆) as a function of flashing period (T_*); Y_{ss} averaged over 20 samples.

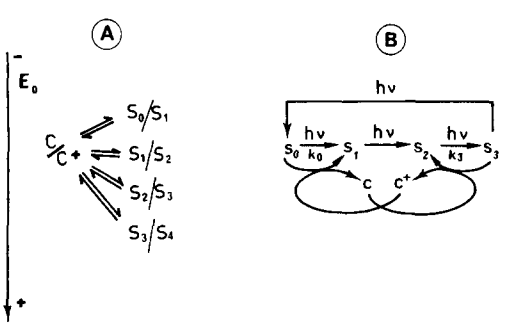

Fig. 7. A model with lateral carrier C (see text). A, schematic positions of C/C^+ and the four levels of the S system on the redox scale. B, simplified version of the model with the free diffusing carrier C.

(3) *Deactivation after steady-state flash illumination*

During deactivation, the states S_2 and S_3 slowly disappear [2]. Various assumptions concerning the detailed mechanism (one step or two steps) have been made and seem to be partly supported by the experiments [7, 8]. We used deactivation as a simple trick to modify the state distribution in order to test the theoretical independency of σ_i and $\overline{Y}$ on the initial state vector $S^{(0)}$. Actually, the analysis again reveals an anomaly (Fig. 5). As deactivation proceeds, an increasing deficit in $\overline{Y}$ is found. We must point out that this deficit is by no means a consequence of deactivation: the latter is generally understood as a conservative mechanism of redistribution of states, i.e. $\Sigma S_i =$ constant during deactivation, which also implies that $\overline{Y} =$ constant. Two phases may be noted in the development of the $\overline{Y}$ deficit: a first part is completed at 32 s and a second, larger part requires up to 200 s. These two phases might be correlated with the deactivation of Y_1 and Y_2 (Fig. 6), i.e. with the decay of S_3 and S_2, respectively. σ_i values are also dependent upon the deactivation time; however, no correlation is apparent with the $\overline{Y}$ deficit; in particular the σ_i are practically the same at short and long times before and after the $\overline{Y}$ deficit has developed (Fig. 6).

DISCUSSION

The kinetics of O_2 evolution in sequence of flashes offer an interesting example of the role played by hidden variables in scientific investigation. The outstanding features of the standard Y_n sequence (periodicity of order 4, damping, position and height of first maximum) have been constraints stringent enough to discourage the development of many concurrent models. Among the few so far proposed [2, 1, 9, 10], only Kok's model [1] has managed to survive, in large part because of its minimum character and its ready correspondence with the chemistry of water oxidation into oxygen. Its necessary weakness, however, (which is shared by other models in equivalent ways) is to introduce the hidden state variables S_0, S_1 and S_2. The theory of σ analysis shows that the information on the model that may be drawn from experiment is much limited, precisely because of the weight of hidden variables. It is clear that the problem is bound to remain unsolved as long as no experimental access to the hidden states will become feasible.

Another important result of σ analysis is that it provides several sensitive tests for the qualitative validity of Kok's theory. We have seen that in many circumstances the actual Y_n sequences are not regular, as they should be according to the latter theory. It is thus proved that Kok's model, besides not being subject to strict verification, has only an approximate validity if taken stricto sensu. Our opinion is that the basic scheme is right, but that it must be complemented with additional assumptions.

The most striking manifestation of nonregularity was found in the behaviour of $\overline{Y}$, which was often of the induction type (i.e. slow increase during the sequence to a steady-state value). We do not believe that this induction effect is at all connected to the so-called adaptation phenomena (for instance the state 1 – state 2 transition [11]) because of its small amplitude and occurrence at the very beginning of flash illumination. A priori, this type of nonregularity cannot be interpreted unambiguously: bearing in mind that the meaning of $\overline{Y}$ is close to that of Y_{ss}, we know (see Appendix, 5) that

$$Y_{ss} = \Sigma S_i / \Sigma (1-\alpha_i)^{-1} \tag{6}$$

(assuming negligible double hits) and, therefore, the induction of $\bar{Y}$ may reflect a transient change either in ΣS_i, i.e. the totality of active centers, or in some function of the transition parameters, analogous to $\Sigma(1-\alpha_i)^{-1}$. The choice may not be clarified by the simultaneous consideration of the time course of σ_i, because we have seen that the strong oscillations of the latter associated to the $\bar{Y}$ drift forbids a precise evaluation of the unperturbed σ_i. In fact, we shall see below that the point is perhaps irrelevant, in as much as the model may be modified to produce variations which may be interpreted at will as pertaining to the states or to the parameters.

Another objection to Kok's model could have been formulated a priori. The choice of such words as "misses" or "double hits" for the transition parameters of order 0 or 2 restricts the understanding of the above parameters to probabilities of a center nonreacting or reacting twice. It would seem preferable to understand a transition in a broader sense as the reaction $S_i \rightarrow S_j$ ($j = i$ or $j \neq i$) occurring between successive flashes without limitation in the nature and complexity of intermediary steps (photochemical or thermal processes, number of intermediary states, etc.). For instance, a "miss" can be interpreted as the photochemical transformation $S_i \rightarrow S_{i+1}$ followed by its spontaneous partial reversal $S_{i+1} \rightarrow S_i$ [7, 12] or as consequence of a fast equilibrium between active and inactive forms of centers [4, 13]. More important, the word "miss" implies a loss in photochemical quantum yield, an assertion which actually lacks experimental proof. We also recall that any deactivation process occurring in the dark between flashes should be incorporated in the proper elements of the matrix $\tilde{K}$ [5]. Actually, the only support for the "photochemical" interpretation of the above transition parameters is found in the observation that the reduction of flash duration considerably modifies the pattern of the Y_n sequence, particularly as regards Y_2 [7], thus indicating that the extent of double hits has diminished.

To account for nonregularity, it is obviously necessary to introduce an additional degree of freedom, since Kok's model per se implies a strict regularity. For instance, we may assume that an additional side carrier C/C^+ is part of the oxygen evolving complex and is able to reversibly exchange a positive charge with the main charge storing device S_i. There result two sets of active states with the following general kinetic relationship:

$$\begin{array}{ccccccc} \xrightarrow{h\nu} & S_iC & \xrightarrow{h\nu} & S_{i+1}C & \xrightarrow{h\nu} & \\ & \downarrow\uparrow K_i & & \downarrow\uparrow K_{i+1} & & \\ \xrightarrow{h\nu} & S_{i-1}C^+ & \xrightarrow{h\nu} & S_iC^+ & \xrightarrow{h\nu} & \end{array} \tag{7}$$

(K_i, K_{i+1} are equilibrium constants). It is important to see that the equilibria-link states (e.g. S_iC and $S_{i-1}C^+$) carrying exactly the same number of positive charges. C/C^+ does not take part directly in O_2 emission; in other works, S_3C and S_3C^+ are both precursors of O_2. The abbreviated symbols S_i and S_i^+ for S_iC and S_iC^+, respectively, will be used below.

It must obviously be assumed that the K_i values are not all identical, otherwise there would result a model isomorphous to the original. In fact, if we artificially take apart the two components S_i and C, consideration of the position of redox levels

(Fig. 7A) renders this assumption quite natural*. Also the equilibria K_i must not be too slow, otherwise the two systems of substates would not interact significantly during a sequence. Thus, under the above conditions, we note at once interesting properties of this model: the reversible movement of positive charges between the two components S_i and C introduces time varying parameters into matrix $\tilde{K}$, which are clearly of the "miss" type ($S_i \xrightarrow{h\nu} S_{i+1} \rightarrow S_i^+$) or of the "double hit" type ($S_i^+ \xrightarrow{h\nu} S_{i+1}^+ \rightarrow S_{i+2}$); no photochemical loss is incurred by the above special type of "miss" and, similarly, the above special type of "double hit" does not depend upon flash duration; since the initial redox level of C may be different from its steady state, we expect the occurrence of induction features in the sequence, notably on $\bar{Y}$**.

As the mathematical treatment of the new model (Eqn. 7, completed with the usual transition parameters, possibly distinct for each subsystem) is quite difficult and hopelessly complicated in its full generality, we resorted to a simplified approximate version (Fig. 7B), where nevertheless the same general idea is at work. Accordingly, it is understood that a free diffusing*** system C may either reduce S_3 to S_2 or oxidize S_0 to S_1, thus artificially producing misses on S_2 or double hits on S_3 (see the resulting matrix in Appendix, 6). This simplified model is easy to handle and produces synthetic sequences which are then subjected to σ analysis. One such run is reproduced in Table II; it shows, as expected, that some induction behaviour (oscillations of σ_i, drift of $\bar{Y}$) is indeed a property of the model.

Barring a quantitative verification of the new model, we contented ourselves in

TABLE II

SYNTHETIC SEQUENCE AND σ ANALYSIS IN THE SIMPLIFIED VERSION OF MODEL WITH LATERAL CARRIER C

Parameters: $S_0^{(0)} = 0.3$, $S_1^{(0)} = 0.7$, $S_2^{(0)} = S_3^{(0)} = 0$, $\alpha_0 = \alpha_1 = 0$, $\alpha_2 = \alpha_3 = 0.3$, $\gamma_i = 0$, $k_3 T_* = 0.05$, $k_0 T_* = 0.01$, $x^{(0)} = 0$ (see text and Appendix, 7).

	Y									
	2	3	4	5	6	7	8	9	10	11
C*	0	0.49	0.514	0.276	0.127	0.283	0.404	0.354	0.244	0.252
Kok*	0	0.49	0.504	0.258	0.110	0.283	0.407	0.345	0.224	0.242

Train (C*)	σ_1	σ_2	σ_3	$\bar{Y}$
Y_2 to Y_8	0.6191	0.0831	−0.0024	0.3026
Y_3 to Y_9	0.6256	0.0931	0.0075	0.3025
Y_4 to Y_{10}	0.6081	0.0740	−0.0066	0.3023
Y_5 to Y_{11}	0.6269	0.0979	0.0084	0.3022

* C, model with lateral carrier C; Kok, model without lateral carrier C.

* The scheme of Fig. 7A bears only a superficial analogy with a recent modification of Kok's model [14] where S_0 and S_1 are able to donate electrons to O_2.

** In the state S_3C^+, four positive charges are present, hence the model predicts a "delayed" emission of $O_2 = S_3^+ \rightarrow S_0 + O_2$, this step presumably being irreversible.

*** The approximate character of the modified version must be stressed: C is assumed here to be free diffusing only for the convenience of mathematical study.

analysing its properties qualitatively and comparing them with our experimental results. One notices first that we seem to have deliberately chosen to interpret the induction effect as resulting from variation in the transition parameters, as opposed to variation in sum of states. It is true that, in the simplified version, ΣS_i = constant; however, due to the lateral movement of changes to and from C, it is apparent that momentary loss or gain of the average charge of the system ($\Sigma i S_i$) will have effects similar to a change in the concentration of active centers*. In particular, a rationale for a qualitative interpretation is that a shift of C to the oxidized form should produce a deficit in $\overline{Y}$.

At the steady state, $\overline{Y} \equiv Y_{ss}$ and the anomaly of the Emerson plateau induced by a sudden increase of T_* (Fig. 4) may be understood as a shift of C/C^+ to the oxidized side, which would result from a particular time dependence of the reaction of exchange of charges between C and S_i; once the ratio C/C^+ has been established to a new steady-state value, the same Y_{ss} value as before the T_* shift must obtain. The induction of $\overline{Y}$ during deactivation from the steady state (Fig. 5), although a complex phenomenon, is probably related to the foregoing. Noting that Y_1 in the deactivation experiment from the steady state (Fig. 6) is identical to the first measured Y in the T_* shift experiment (Fig. 4), one must conclude, in keeping with our interpretation, that deactivation of S_3 consists of two steps: (1) $S_3 \rightarrow S_2^+$ and (2) transfer of a positive charge of S_2^+ to a reductant. The first step is reversible and does not imply a loss of positive charge; it merely contributes to a redistribution of charges with no concurrent variation in the average charge, whereas the second step decreases it. This mechanism probably explains why the deactivation curves of Y_1 and Y_{ss} are different (insert of Fig. 6): assuming that C^+ mediates the irreversible loss of a positive charge to an endogeneous reductant R, it is seen that at steady-state flash illumination, even with long flashing period (Emerson's plateau), a favorable competition of the step $S_0 \rightarrow S_1$ for the positive charge in C^+ must exist against the step $R \rightarrow R^+$. A fast phase in the deactivation of S_3 in chloroplasts [15] could also be explained by the step $S_3 \rightarrow S_2^+$.

According to the foregoing, the dark distribution, e.g. 0.3/0.7/0/0 (for $S_0/S_1/S_2/S_3$) would result from the equilibrium $S_i \rightleftharpoons C \rightleftharpoons R$, the second equilibrium being much slower than the first. Consequently, C is rather reduced in darkness, more than in the steady-state flash illumination. Again, the change of C/C^+ from dark to light could explain the induction of $\overline{Y}$ in the sequences with varying T_* (Fig. 3); the fact that the initial $\overline{Y}$ deficit is maximum for rather large values of T_* might indicate that the equilibrium $S_i \rightleftharpoons C$ is not fast. A puzzling point is raised concerning the dependency of σ_1 on T_*, since according to Eqn. 15 (Appendix, 6) the miss coefficient on S_2 is an increasing function of T_*, whereas the actual trend is opposite (except perhaps for small T_* values in the presence of CCCP). It is possible that the decrease of σ_1 with increasing T_* mostly reflects the role of the redox level of the plastoquinone pool as discussed by several authors [15, 16].

Would it be possible to adopt the new model to explain also the corresponding experiment in the presence of CCCP (Fig. 2)? Much the same effect as in the hypothesis of Lemasson and Etienne [5] would occur in this model, with the additional

* The irreversible loss (gain) of states is achieved by the nonmarkovian condition that the sum of transition parameters relative to a state be $<1(1>)$, instead of being equal to 1 as in all markovian matrixes considered here.

assumption that, in the presence of CCCP, centers in states S_i^+ are inactive; if, furthermore, CCCP has the effect of lowering the redox potential of C, the formation of S_i^+ states will be favored. Since CCCP is known to transform cytochrome b_{559} into a low-potential form [17], the latter remark encourages us to speculate on a possible identification of C with cytochrome b_{559}. Other properties are common to the hypothetical C and cytochrome b_{559}: for instance, the side position with respect to the main electron transport path, the non-necessity of the component for O_2 evolution, the sluggishness of reaction (for b_{559}, see refs. 17–20).

APPENDIX

(1) *Matrix recurrence relation (Eqn. 1)*

The order 4 matrix $\tilde{K}$ is defined as:

$$\tilde{K} = \begin{bmatrix} \alpha_0 & 0 & \gamma_2 & \beta_3 \\ \beta_0 & \alpha_1 & 0 & \gamma_3 \\ \gamma_0 & \beta_1 & \alpha_2 & 0 \\ 0 & \gamma_1 & \beta_2 & \alpha_3 \end{bmatrix} \tag{8}$$

Where α_i, β_i, γ_i ($\alpha_i+\beta_i+\gamma_i = 1.0$) are the order 0 ($S_i \rightarrow S_i$), order 1 ($S_i \rightarrow S_{i+1}$), order 2 ($S_i \rightarrow S_{i+2}$) ($i$ modulo 4) transition coefficients, respectively. Note that the symbols are different from those in the nomenclature of Kok et al. [1] where β_i stand for the order 2 transition coefficient ("double hits"), while the order 0 transition coefficients ("misses") have the same symbol and the order 1 transition coefficients have no symbol.

(2) *Recurrence relation on Y_n (Eqn. 2)*

The derivation involves the following steps: (a) the characteristic equation of $\tilde{K}$, i.e. the determinantal equation in λ

$$f(\lambda) = |\tilde{K}-\lambda\tilde{I}| = 0 \tag{9}$$

where $\tilde{I}$ is the unit matrix; (b) the property (Cayley-Hamilton) that $f(\lambda) = 0$ implies $f(\tilde{K}) = 0$; (c) multiplication (right) by $\tilde{K}^n S^{(0)}$ yielding a vectorial identity between successive state vectors, $S^{(n)}$ to $S^{(n+4)}$, hence the relation in successive Y_n ($= \beta_3 S_3^{(n-1)}$), i.e. Eqn. 2.

The σ_i are functions of the elements of $\tilde{K}$:

$$\begin{aligned} \sigma_1 &= \alpha_0+\alpha_1+\alpha_2+\alpha_3 \\ \sigma_2 &= \alpha_0\alpha_1+\alpha_0\alpha_2+\alpha_0\alpha_3+\alpha_1\alpha_2+\alpha_1\alpha_3+\alpha_2\alpha_3-\gamma_0\gamma_2-\gamma_1\gamma_3 \\ \sigma_3 &= \alpha_0\alpha_1\alpha_2+\alpha_0\alpha_1\alpha_3+\alpha_0\alpha_2\alpha_3+\alpha_1\alpha_2\alpha_3-\gamma_0\gamma_2(\alpha_1+\alpha_3)-\gamma_1\gamma_3(\alpha_0+\alpha_2) \\ &\quad +\beta_0\beta_1\gamma_2+\beta_1\beta_2\gamma_3+\beta_2\beta_3\gamma_0+\beta_3\beta_0\gamma_1 \end{aligned} \tag{10}$$

Note the quasi symmetrical character of these expressions (completely symmetrical if $\gamma_i = 0$), which implies that many permutations of the transition parameters yield identical recurrence relation in Y_n (no possibility of assigning numerical values to the parameters of a given state). Note also that, even in the simplest case ($\gamma_i = 0$), the

system Eqn. 10 cannot be solved (it can only be solved in terms of inequalities $a_i \leqslant \alpha_i \leqslant b_i$ where a_i and b_i may be calculated) because the fourth function $\sigma_4 = \alpha_0\alpha_1\alpha_2\alpha_3$ is not known [3].

(3) *σ analysis (first step)*

σ_1, σ_2 and σ_3 are solution of the system:

$$\begin{aligned}
&Y^5 - Y_1 - \sigma_1(Y_4 - Y_1) + \sigma_2(Y_3 - Y_1) - \sigma_3(Y_2 - Y_1) = 0\\
&Y_6 - Y_2 - \sigma_1(Y_5 - Y_2) + \sigma_2(Y_4 - Y_2) - \sigma_3(Y_3 - Y_2) = 0\\
&Y_7 - Y_3 - \sigma_1(Y_6 - Y_3) + \sigma_2(Y_5 - Y_3) - \sigma_3(Y_4 - Y_3) = 0
\end{aligned} \tag{11}$$

or of any equivalent system using Y_n to Y_{n+6}.

(4) *Relation of $\bar{Y}$ (Eqn. 5)*

The following infinite system is constructed by reiteration of the recurrence relation Eqn. 2:

$$\begin{aligned}
&Y_{n+4} - \sigma_1 Y_{n+3} + \sigma_2 Y_{n+2} - \sigma_3 Y_{n+1} - (1 - \sigma_1 + \sigma_2 - \sigma_3) Y_n = 0\\
&Y_{n+5} - \sigma_1 Y_{n+4} + \sigma_2 Y_{n+3} - \sigma_3 Y_{n+2} - (1 - \sigma_1 + \sigma_2 - \sigma_3) Y_{n+1} = 0\\
&\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\\
&Y_{ss} - \sigma_1 Y_{ss} + \sigma_2 Y_{ss} - \sigma_3 Y_{ss} - (1 - \sigma_1 + \sigma_2 - \sigma_3) Y_{ss} = 0
\end{aligned} \tag{12}$$

Adding all left members and simplifying yields Eqn. 5 (where Y_{ss} stands for $\bar{Y}$). This is the second step of σ analysis.

(5) *Steady-state distribution (Eqn. 6)*

It is the solution of the matrix equation

$$(\tilde{K} - \tilde{I}) S^{(\infty)} = 0$$

which is given by the minors of order 3 of the determinant $|\tilde{K} - \tilde{I}|$.
For instance (with $\gamma_i = 0$)

$$\begin{aligned}
S_0^{(\infty)} &= k\beta_1\beta_2\beta_3\\
S_1^{(\infty)} &= k\beta_0\beta_2\beta_3\\
S_2^{(\infty)} &= k\beta_0\beta_1\beta_3\\
S_3^{(\infty)} &= k\beta_0\beta_1\beta_2
\end{aligned} \tag{13}$$

(k is found by the condition $\Sigma S_i = 1.0$). Eqn. 6 follows, since $Y_{ss} = \beta_3 S_3^{(\infty)}$ (see also ref. 4).

(6) *Transition matrix with the lateral C system*

In order to emphasize the role of C in mimicking misses and double hits, we consider its effect on a simple case of Kok's model where $\beta_i = 1$ ($\alpha_i = \gamma_i = 0$), which would alone produce an undamped sequence. The resulting matrix is

$$\tilde{K}' = \begin{bmatrix} 0 & 0 & 0 & 1 - k_0 x^{(n)} T_* \\ 1 & 0 & 0 & k_0 x^{(n)} T_* \\ 0 & 1 & k_3(1 - x^{(n)}) T_* & 0 \\ 0 & 0 & 1 - k_3(1 - x^{(n)}) T_* & 0 \end{bmatrix} \tag{14}$$

where k_3 and k_0 are the rate constants of the reactions $S_3+C \rightarrow S_2+C^+$ and $S_0+C^+ \rightarrow S_1+C$, respectively, $x^{(n)}$ is the concentration of C^+ after n flashes ($[C]+[C^+]=1.0$). We further assume that the progress of both reactions is approximately given as shown, T_* being small as compared to the time constant of the reactions.

$\tilde{K}'$ should be compared with the standard matrix with misses on S_2 and double hits on S_3

$$\tilde{K} = \begin{bmatrix} 0 & 0 & 0 & \beta_3 \\ 1 & 0 & 0 & \gamma_3 \\ 0 & 1 & \alpha_2 & 0 \\ 0 & 0 & \beta_2 & 0 \end{bmatrix} \tag{15}$$

note also that in Eqn. 14 the parameters are time varying because $x^{(n)}$ evolves from one flash to the next:

$$x^{(n+1)} = x^{(n)}+k_3(1-x^{(n)})S'_3{}^{(n)}T_*-k_0x^{(n)}S'_0{}^{(n)}T_* \tag{16}$$

where the primed S symbols refer to the concentration of states immediately following the flash.

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