COMPUTER SOLUTIONS FOR PHOTOSYNTHESIS RATES FROM A TWO PIGMENT MODEL

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ABSTRACT A kinetic model for a two pigment mechanism of photosynthesis based on observations made on the red alga *Porphyridium cruentum* is developed. The model supposes that different products are formed by chlorophyll and phycobilin pigments and that these products react to produce oxygen. The product of the chlorophyll reaction is also rapidly utilized for O_2 consumption both in light and in the dark. Rate equations based on the model reactions were derived and put into an analog computer. The effect of varying parameters on the time course curves for oxygen exchange resulting from the model was studied. Appropriate selection of parameters yielded computer curves which resembled fairly closely the oxygen exchange curves obtained using *Porphyridium*. The model showed the Emerson enhancement effect, chromatic transients, "respiratory stimulation," and other effects observed with live algae.

INTRODUCTION

Many different bits of experimental evidence have led to the idea that there are two distinct primary photochemical reactions in the process of photosynthesis. These have been summarized in a review article (1). Particularly clear support for this concept is the finding that the action spectrum in *Porphyridium cruentum* for the respiratory stimulation following a light period matches the absorption spectrum of chlorophyll *a* while the action spectrum for the rate of O_2 evolution in light matches the absorption spectrum of phycoerythrin (2). Evidence for interaction of the products of the reactions driven by these two pigments was also obtained and an outline of a kinetic scheme was proposed (2). A model identical with this one but without the inclusion of the respiratory stimulation was independently proposed by Brody and Brody (3) during the course of this investigation.

The kinetic model developed in this paper states that a product formed by one pigment system reacts with a product made by a second pigment system to produce oxygen. One product but not the other is rapidly utilized for O_2 consumption both in light and in the dark. This model is intended to represent only the rate-limiting steps in the over-all process. Each reaction is presumed to consist of several individual steps. Because of the conflicting and rapidly changing concepts of the intermediary biochemistry of photosynthesis we have avoided assigning specific chemical names to the rate-limiting intermediate substances under discussion.

The effects of the various factors that control the rate of O_2 exchange are related to each other and are interdependent in a complex way so that the effect of varying one parameter is determined by the values of the others. Not only the amount of change produced by a certain variation of one parameter but even its sign may be dependent on the values of the other parameters. By the use of an analog computer these interrelations can be understood and the predictions of a kinetic model can be compared with actual data.

A partial study was made of the properties of the kinetic scheme by a Donner D. C. analog computer. The results showed that by appropriate choice of constants, the proposed scheme can account surprisingly well for many of the well known effects on rates of O_2 exchange caused by exposing algae to light of various wavelengths.

THE MODEL AND ITS CORRESPONDING RATE EQUATIONS

The two primary photochemical reactions are represented thus:

Reaction (1) $A \xrightarrow{F_1} x$, "Long wavelength chlorophyll (LWC) reaction,"

Reaction (2) $B \xrightarrow{F_2} y$, "Accessory pigment (AP) reaction."

Here A and B represent substances transformed by light into their corresponding products x and y. F_1 is the effective light flux absorbed by the appropriate form of chlorophyll a and F_2 is that absorbed by the accessory pigment. $F_1 = I_1\alpha_1\phi_1$ where I_1 is the light intensity expressed in quanta per second incident on a thin layer of algae. α_1 is the absorption coefficient of the active long wavelength form of chlorophyll *a in vivo* and ϕ_1 is the quantum efficiency of Reaction 1. Similarly $F_2 = I_2\alpha_2\phi_2$ for the accessory pigment system. There is no reason to assume that $\phi_1 = \phi_2$.

The next step in the scheme is the interaction of the products x and y to liberate O_2 and to regenerate their precursors.

$$\text{Reaction (3) } x + y \xrightarrow{K_3} A + B + O_2$$

In Reaction 3, x and y need not be the actual molecular species produced by Reactions 1 and 2 but may equally well be later products derived from them by intermediate reactions that do not themselves become rate-limiting. The available concentrations of the reactants in Reaction 3 are, however, presumed to be equal or proportional to the amounts of x and y produced by Reactions 1 and 2, so they are designated by the same letters for rate calculations. Reaction 3 presumably uses up water, which being constant in concentration, is omitted from the rate equation.

A further reaction, the consumption of O_2 by the product of Reaction 1 and the regeneration of A, was found to be of appreciable significance in *Porphyridium* and in a *Chlamydomonas* mutant but may be less important in other species.

$$\text{Reaction (4)}\ x + O_2 \xrightarrow{K_4} A$$

Fig. 1 shows the model in the form of a block diagram.

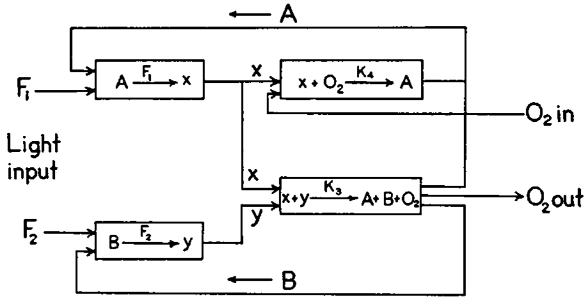

FIGURE 1 A block diagram to illustrate the reaction sequences of the model.

The following rate equations follow from the above reactions:

$$\text{Eq. (1)}\ dx/dt = F_1A - K_3xy - K_4x$$
$$\text{Eq. (2)}\ dy/dt = F_2B - K_3xy$$
$$\text{Eq. (3)}\ dA/dt = K_3xy + K_4x - F_1A$$
$$\text{Eq. (4)}\ dB/dt = K_3xy - F_2B$$
$$\text{Eq. (5)}\ dO_2/dt = K_3xy - K_4x$$

For a given sample of photosynthetic plant material, K_3 and K_4 are temperature-sensitive rate constants independent of light intensity. dO_2/dt is measurable as a function of time, temperature, and light intensities. A and B, as well as x and y, are variables when the material is first illuminated; however, they may reach steady-state concentration levels in continuous light, and are then called A_∞, etc. In the dark a different steady-state level of each, designated, A_0, etc. is reached. For calculating the time course of $+dO_2/dt$ during illumination A_0, B_0, x_0, y_0, are used for the initial conditions while A_∞, B_∞, x_∞, y_∞, are the initial conditions for calculating the $-dO_2/dt$ time course in the dark following a light exposure.

Computer Solution. These equations were put directly into a Donner D. C. analog computer. The computer output, generally dO_2/dt, was plotted as a function of time on a Nesco recorder. In addition to dO_2/dt plots were also made of A, x, and y as functions of time for various choices of the parameters F_1, F_2, K_3, K_4, A_0, and B_0, while x_0 and y_0 were set to 0.

A simplified set of equations was found adequate for most purposes except as will be discussed later. This simplification is based on the assumption that B is large enough so that small variations in its concentration do not influence the rate. Substituting F'_2 for F_2B reduces the computer requirements from four integrators to three by omitting Equation 4. Most of the work was done with this arrangement.

A further simplification required only two integrators and a servo multiplier in our curve analyzer (4).

For this purpose we may write:

Eq. (6) $A = (A_0 - x)$

hence avoiding the necessity for evaluating A by integration.

Then:

Eq. (7) $dx/dt = F_1 (A_0 - x) - K_3xy - K_4x$ transformed Eq. 1

Eq. (8) $dy/dt = F'_2 - K_3xy$ $(F'_2 = F_2B)$ transformed Eq. 2

Eq. (9) $dO_2/dt = K_3xy - K_4x$ same as Eq. 5

Some results were obtained in this way before the Donner computer, which was far more convenient, became available.

RESULTS AND DISCUSSION

Some Time Course Curves Derived from the Model with Various parameters. A set of values for the constants was found that gave dO_2/dt time course curves similar to some found for live algae, and also led to equal steady-state concentrations of x_∞ and y_∞. Sets of time course curves for O_2 exchange with light on and off were then plotted for systematic variations of A_0, F_1, F'_2, K_3, and K_4, one at a time, with the others at the standard values. The results in Fig. 2 show how each of these parameters influences the curve shapes, using the scheme without Equation 4. A much expanded time scale was used for the computer records. Only the relative not the absolute values of the constants are of significance.

To clarify the relation of the model to actuality Fig. 2 will be discussed in terms of the effect of red and of green light on O_2 exchange of *Porphyridium* (2). Red light activates mainly Reaction 1 while green activates primarily Reaction 2. There is some absorption by both pigment systems at all wavelengths so we do not have a situation where F_1 or $F_2 = 0$. However, in red light the ratio F_1/F'_2 is larger than it is in green light.

Increasing F'_2 and keeping other parameters constant is thus the equivalent of increasing the effective light flux absorption by the accessory pigment system while holding the flux absorption by the long wavelength chlorophyll system constant. The increase of F'_2 from a low to a high value as shown in Fig. 2 changes the time course curve from the shape of that for red light to that typical for green light. The steady-state level of dO_2/dt is seen to approach a limiting value as F'_2 is increased. To raise dO_2/dt above this limiting value would require an increase in F_1.

Thus the model appears to provide the possibility of correlating the saturating intensity action spectrum of McLeod (5) with the low intensity phenomena of enhancement by two simultaneous beams (6-9).

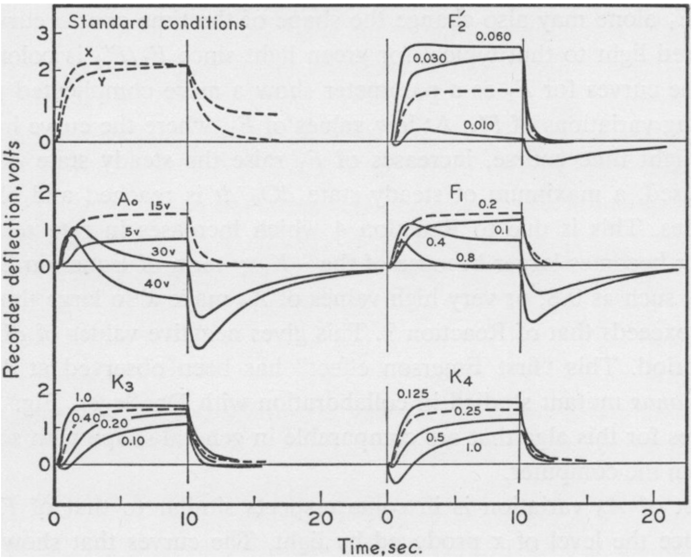

Figure 2 The time courses for O_2 exchange calculated from the model with each constant varied separately. The curve for the standard conditions is shown dotted in each set. For these standard conditions the time courses of x and of y are also shown. The first part of each record is for the "light" period, the second part for "dark." Standard conditions: $A_0 = 15$ v, $F_1 = 0.20$, $F'_2 = 0.0196$, $K_3 = 0.40$, $K_4 = 0.25$. Equations 1, 2, 3, and 5 of the text were used. $F'_2 = F_2B$. The part of the records for dark is with $F_1 = F'_2 = 0$.

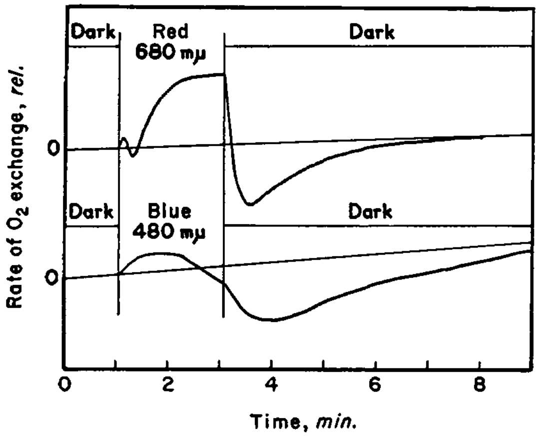

Figure 3 Time course curves for O_2 exchange in a *Chlamydomonas* mutant, Sager No. 95, measured in collaboration with Dr. Ruth Sager. A platinum electrode was used at 19.1°C in Knops solution with 5 per cent CO_2 in air. Red light, 680 mμ, blue light, 480 mμ. At 480 mμ the O_2 exchange becomes negative during the light period as in Fig. 2 with $A_0 = 40$ v.

Varying F_1 alone may also change the shape of the time course curve from that typical for red light to that typical for green light since F_1/F'_2 is color-dependent. However, the curves for F_1 as a parameter show a more complicated pattern than those showing variations of F'_2. At low values of F_1, where the curve has the shape of a green light time course, increases of F_1 raise the steady state dO_2/dt value. As F_1 is raised, a maximum of steady state dO_2/dt is reached and above this its value declines. This is due to Reaction 4 which increases in rate relative to Reaction 3 as x becomes larger because of the $-K_8xy$ term of Equation 2. Very large values of F_1 such as 0.8, or very high values of A_0 make x so large that the rate of Reaction 4 exceeds that of Reaction 3. This gives negative values of dO_2/dt during the light period. This "first Emerson effect" has been observed at 480 mμ in a *Chlamydomonas* mutant studied in collaboration with Dr. Sager. Fig. 3 gives time course curves for this alga that are comparable in general shape with some of those in Fig. 2 from the computer.

The effect of A_0 variation is in some respects similar to that of F_1 since they both influence the level of x produced by light. The curves that show a high rate after the initial rise and then slowly drop to a lower steady-state level are produced by a large A_0 that later drops to a lower value of A_∞ in the steady-state condition. This effect may be seen by recording the time course of A or of x since $A = A_0 - x$. For large values of A_0 a smaller value of F_1 will lead to the same amount of respiratory stimulation ($-dO_2/dt$) following the light exposure.

The value of K_3 primarily influences the initial rate of rise of dO_2/dt. The influence of K_3 on the steady-state rate at low intensities (where F_1 and F_2 primarily determine the rate of Reaction 3) appears only when K_3 is so low that Reaction 4 becomes significantly large in relation to Reaction 3. The rapid rate of decline of dO_2/dt immediately after the light is off is proportional to K_3, x_∞, and y_∞.

The influence of K_4 is best seen in the dark period following red illumination. This does not show in the K_4 series of Fig. 2 since the standard conditions about which the parameters were varied were those representing green light exposures. However, in the series of K_4 variations of Fig. 2 the decrease of steady-state dO_2/dt caused by increased rate of Reaction 4 is evident. The approximately exponential rate of return to the normal base line after the minimum dO_2/dt following red light as seen in Fig. 4 is used for determining the value of K_4 in fitting the equations to real data.

The Negative Spike. When K_4 is large in relation to K_3, dO_2/dt goes negative briefly at the start of an illumination period. This effect, called the negative spike (2), is due to the rate of Reaction 4 being initially greater than that of Reaction 3. The negative spike is prominent in red light which produces an excess of x in relation to y. Since the influence of K_4 on the negative spike can be seen better in red light than in green light a set of standard conditions different from those of Fig. 1 was chosen to illustrate in Fig. 4 the controlling effect of K_4 on the nega-

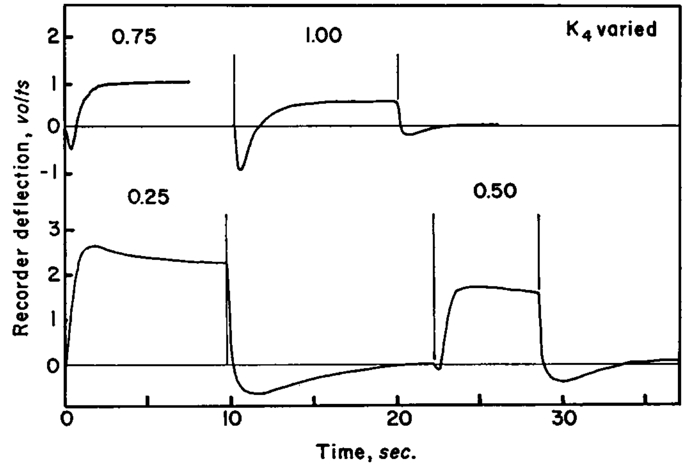

Figure 4 Time course curves calculated from the model with constants selected to show the "respiratory stimulation" following a light period and the "negative spikes." Conditions: $A_0 = 15$ v, $F_1 = 0.6$, $F'_2 = 0.036$, $K_3 = 0.4$, K_4 varied.

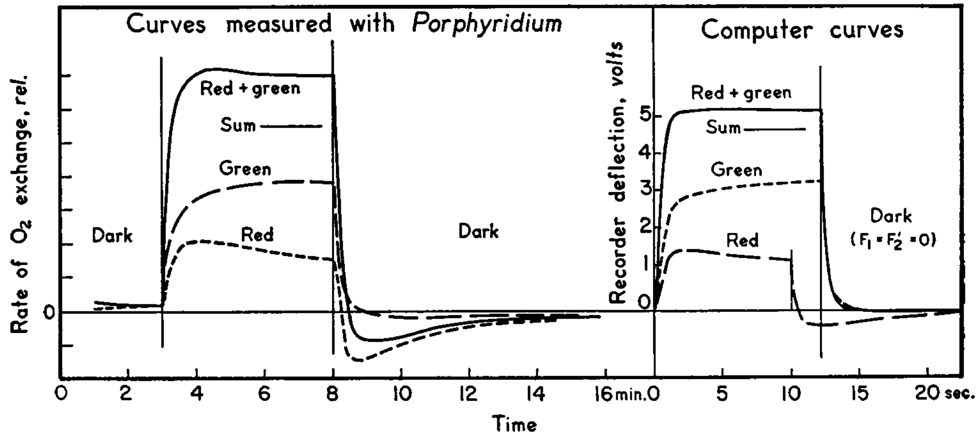

Figure 5 Time course of O_2 exchange in *Porphyridium cruentum* compared with computer curves. The measured curves were made with red light, 680 mμ, and with green light, 567 mμ, as well as with both beams together. The constants used for the computer curves were selected to give an approximate match to the red and the green measured curves keeping A_0, K_3, and K_4 the same for all curves. F_1 and F'_2 values were selected to approximate the red and the green light experimental data while for red + green the sum of F_1 and F'_2 for green and for red alone was used.

The Emerson enhancement effect is the ratio of the steady-state rate with both beams together to the sum of the two separately. Its value is larger for the algal records than for the calculated curves. The constants used were: $A_0 = 20$ v, $K_3 = 0.40$, $K_4 = 0.25$, $F_1 = 0.25$ for both red and green, $F_1 = 0.50$ for red + green. $F'_2 = 0.024$ for red, $F'_2 = 0.040$ for green, $F'_2 = 0.064$ for red + green.

tive spike. It also shows the decrease in steady-state rate as Reaction 4 increasingly competes with Reaction 3.

In Fig. 4 the more rapid recovery from the respiratory stimulation is also evident as K_4 increases. While the rate of Reaction 4 goes up in proportion to K_4, its higher rate uses up the residual supply of x more rapidly so that the respiratory stimulation goes through a maximum, then declines as K_4 is increased.

In the experiments showing the negative spikes (2) a momentary deflection of the recorder in the direction of O_2 evolution was seen at the time the light was turned off. The model provides no explanation for this effect.

Enhancement. One of the earlier reasons for believing two separate photochemical reactions to be involved in photosynthesis was the Emerson enhancement effect. This effect is defined as the ratio of photosynthetic rates produced by simultaneous illumination by two appropriate wavelengths to that of their sum when given separately. By means of enhancement measurements it has been found that the long wavelength chlorophyll forms and the accessory pigments supplement each other (6-9). The model described here leads to enhancement effects as shown in Fig. 5.

Inspection of the flow sheet diagram of the model (Fig. 1) shows three possible mechanisms for enhancement:

(*a*) Reduction of losses through Reaction 4: With the system driven by red light, which makes $x > y$, the proportion of x lost through Reaction 4 would be smaller when green light was added, thus increasing the level of y, hence the rate of Reaction 3.

(*b*) Raising the steady level of A: With the system driven by red light A_0 becomes depleted to a lower steady value A_∞, thus lowering the rate of x production. If now the proportion of y to x is increased by adding green light, the rate of Reaction 3 goes up, thus running x through Reaction 3 faster than before. This raises the steady-state level of A so x production is increased for a given F_1 value.

(*c*) Raising the steady-state level of B: The exact inverse of case *b*. This could only happen when the simplifying assumption of B being large and not rate-limiting was invalid.

The computation of enhancement at the steady state can be done more easily from simplified equations applying only to that condition rather than by using the time course equations.

Chromatic Transients. The transient change of photosynthetic rate produced by shifting the light from that absorbed by one pigment to that absorbed by another (10) was also one of the effects leading to the two pigment concept. The present model also shows transient rate changes, but of a simpler character than those commonly seen in live algae.

Fig. 6 shows the transients produced from the model by switching between an F_1/F'_2 ratio representing green light and a higher ratio representing red light. The

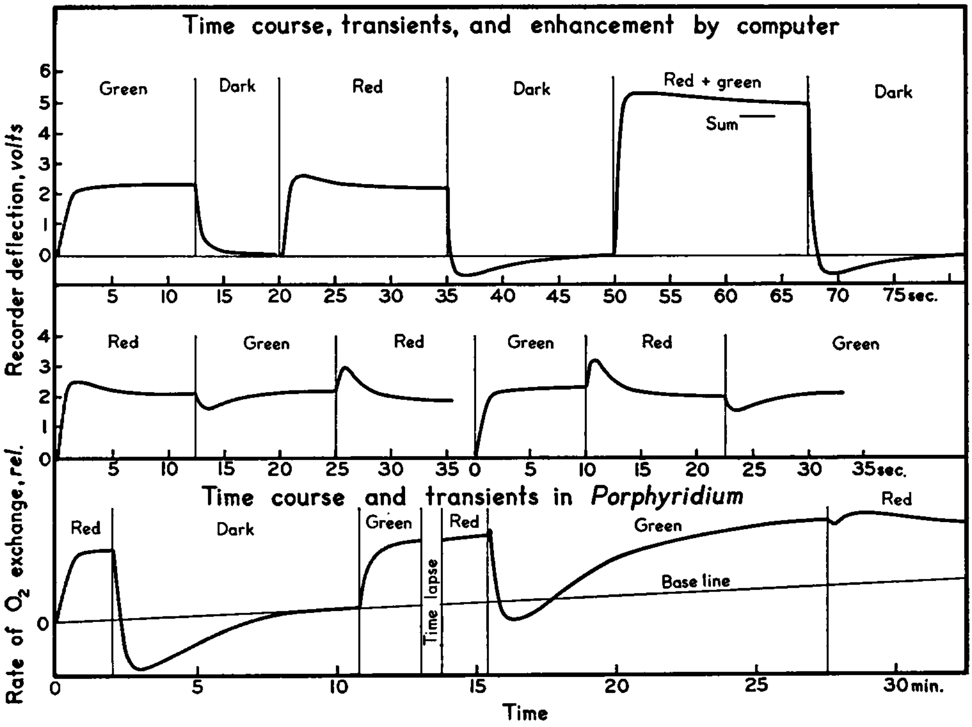

Figure 6 The time course curves and chromatic transients observed in *Porphyridium cruentum* as compared with computer curves showing the time course, chromatic transients, and enhancement. The algae were exposed to red, 680 mμ or to green, 567 mμ as indicated; the two beams were adjusted in intensity to give equal rates of O_2 exchange in the steady state. The constants used for the computer curves were: $A_0 = 15$ v, $K_3 = 0.40$, $K_4 = 0.25$, for red light $F_1 = 0.60$, $F'_2 = 0.036$, for green light $F_1 = 0.30$, $F'_2 = 0.0294$. The rise in level of the green → red transient in the algae is preceded by an initial dip not given by the model. The small initial rise of the red → green transient of the algae also remains unexplained by the model.

light-on, light-off, time course curves and the enhancement effect for the same condition are given in the upper part of the figure. The transients thus calculated from the model lack the sharp positive spike of those seen in actual measurements on algae. It appears that the more complex algal transients may be produced by a combination of the operation of this model with the positive light-off spike mentioned above, which is not described by the model. The transients were calculated from the simplified model not including variations in B. Presumably further complications of a more realistic nature could be introduced into the transients by the complete model with a small value of B_0.

Intensity curves. The time course of O_2 evolution for values of F_1/F'_2 representing green and red light was recorded for the range of intensities within the capacity of the computer as set for $A_0 = 15$ v. The steady-state rate was plotted against "intensity," *i.e.*, $F_1 + F'_2$ for constant ratios of the two as given in Fig. 7. The data for green light gave a linear plot that extrapolated back nearly but not exactly to zero. The region of light saturation was out of the computer range for the chosen conditions.

The data for red light also had a linear region but deviated from this at the lowest point and the linear position extrapolated back to a point far from zero. Such non-linear intensity curves have been observed in algae at low intensities, but were treated as an experimental obstacle rather than a significant effect (6). The model attributes the course of this non-linearity to Reaction 4 which competes with Reaction 3 in greater proportion at low intensity.

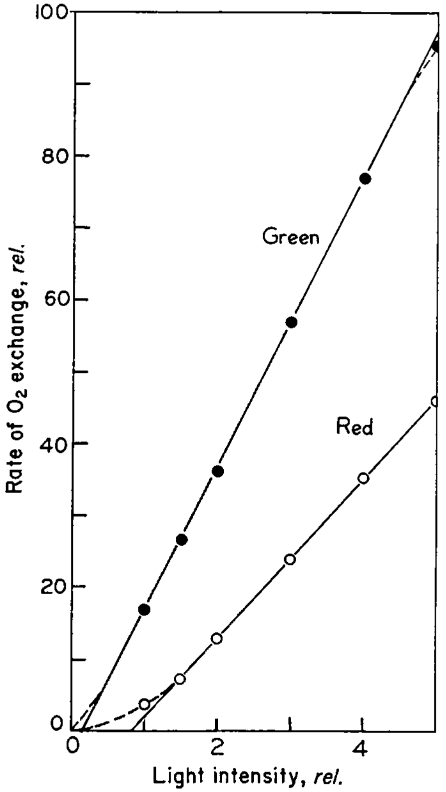

FIGURE 7 Photosynthetic rate *versus* light intensity for steady-state photosynthesis as computed from the model for "green" and for "red" light. Conditions: $A_0 = 15$ v, $K_3 = 0.40$, $K_4 = 0.250$, for red light: $F_1 = 0.20$ to 1.0, $F'_2 = 0.0120$ to 0.060, $(F_1/F'_2 = 16.67)$, or green light $F_1 = 0.2$ to 1.0, $F'_2 = 0.0196$ to 0.098, $(F_1/F'_2 = 10.2)$.

An instructive comparison was made of the time course of x and y values for the lowest and highest green light intensities. The time course curves are given in Fig. 8. At the low intensity a constant steady-state value of dO_2/dt, x, and y is rapidly reached and maintained thereafter. At the higher "intensity," however, dO_2/dt goes through a maximum, then slowly declines. The value of x increases while y decreases. This behavior of the model suggests that the long continued drift in photosynthesis rate observed under some conditions, may be an inherent property of the mechanism of photosynthesis rather than a secondary effect.

Fitting a real time course curve by adjustment of the constants A_0, B_0, K_3, K_4, F_1, and F_2 of the model is a trial and error process of considerable difficulty because each adjustment of one constant requires resetting several others. In practice K_4 is the easiest to approximate for red light curves because it alone determines the curvature of the later part of the record for the light-off period.

An opposite effect—the slow rise in rate of O_2 evolution with time prolonged far beyond the "normal" induction period is also found in algae. Such a phenomenon appears in the computer curves of Fig. 9 showing that a continued increase in rate as well as the slow decline of Fig. 8 may be accounted for by the model.

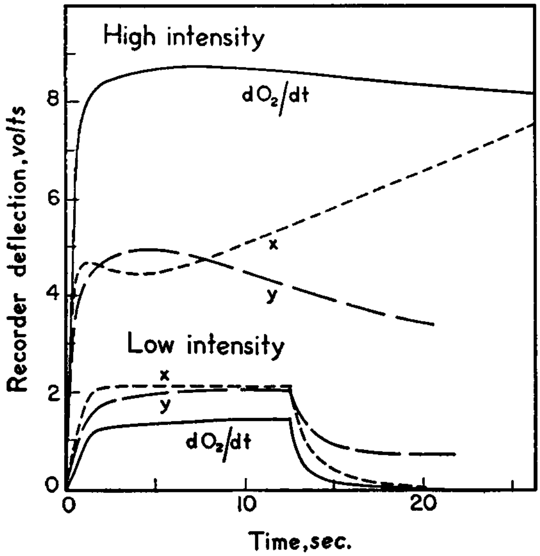

FIGURE 8 Computer time course curves for rate of O_2 exchange and for concentrations of the intermediates, x and y. The two sets are for the same conditions used for the highest and the lowest points on the green light curve of Fig. 7.

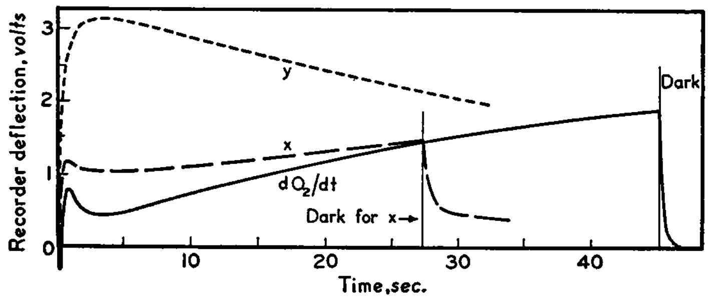

FIGURE 9 Computed time course curves for the complete set of Equations 1 to 5 with a small value of B_0. A prolonged slow increase in rate of O_2 exchange can be explained by the model. By contrast Fig. 8 shows a prolonged decrease in rate after a broad maximum. Both effects are often observed with algae. Conditions: A_0 = 22.5 v, B_0 = 5.0 v, K_3 = 1.0, K_4 = 0.50, F_1 = 0.40, F_2 = 1.00.

Fig. 9 was produced by using the complete set of Equations 1 to 5 thus treating B as a variable. This was one of a series plotted in a partially successful attempt to reproduce the small momentary positive spike superimposed on the early part of the time course curve of algae absorbing light in the accessory pigment system (*i.e.* Figs. 1 and 4 of reference 11) by the model with B_0 made small.

GENERAL DISCUSSION

The nature of the model is so elementary that it is no surprise to find that it cannot cover all the phenomena observed in the time course curves of O_2 exchange seen with live algae. The surprising thing is that so many phenomena actually are described at least in rough approximation by this kinetic scheme. The conclusion at present is that this two pigment scheme, or something reasonably close to it, is actually correct in outline form. Deviations in the behavior of real cells from that predicted by the model could well be expected from side reactions of the intermediates with other cell constituents. Furthermore, each reaction in the model is presumed to consist of a number of actual steps. In the model no account is taken of the likely possibility that under certain conditions some of these intermediate steps could be slow enough to complicate the kinetics.

It is a pleasure to thank Ault Associates of Menlo Park, California for the loan of the Model 3500 Donner D. C. computer and Donner function multiplier that made this work possible.

Received for publication, July 14, 1961.

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