Enhancement of the Photosynthesis of Chlorella pyrenoidosa as a Function of Far-Red and Short-Wave Illuminations 1, 2

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Introduction

Photosynthesis shows an enhancement when the value of the function

\[ E = \frac{p_{12} - p_2}{p_1} \]

I

exceeds 1.0. Here \( p_1 \) is the rate of \( O_2 \) evolution in monochromatic far-red light, \( p_2 \) the rate in monochromatic light of shorter waves, and \( p_{12} \) the rate in both beams given simultaneously. Stated differently, simultaneous illumination can yield an extra rate

\[ D = (p_{12} - p_1 - p_2) \]

II

in excess of the sum of the rates in the separate beams.

The nature of the dependence of enhancement on far-red and short-wave illuminations, i.e., the nature of the functions \( E (I_1, I_2) \) and \( D (I_1, I_2) \), is of interest. On the one hand, for choosing optimum illuminations for subsequent studies and, on the other hand, for clarifying the essential nature and mechanism of the phenomenon. In previous studies, the properties of these 2 functions have not been entirely resolved.

The qualitative character of \( E (I_1, I_2) \) is only partly known. The dependence of \( E \) on short-wave illumination, with fixed far-red illumination, is relatively well established (1, 2, 3, 4) : \( E \) increases with \( I_2 \) until \( p_2 \) is several-fold greater than \( p_1 \). As \( I_2 \) increases further, \( E \) remains constant or declines gradually. In contrast, the dependence of \( E \) on far-red illumination is barely known. The only observations on this dependence seem to be those of Hoch (5) who measured enhancement in Anacystis in 3, smaller than compensating, far-red illuminations. His results seem too limited to help much in establishing the general shape of the surface \( E (I_1, I_2) \).

The function \( D \) has not received any explicit attention, and, cannot be evaluated for most studies because rates \( (p_1, p_2, p_{12}) \) have not been reported along with enhancements \( (E) \). At present it is unclear whether \( D \) increases as illuminations rise to high levels, or whether it reaches a maximum at very low illuminations, and then remains constant, or even declines, with further increases in illumination. It is worth emphasizing that, whatever its nature, the function \( D \) is not in general paralleled by the function \( E \). For example, a maximum value of \( E \) at very low illuminations, and its decline at higher illuminations, could be consistent with a continuously increasing \( D \), albeit the rate of increase of \( D \) with illumination, at high illuminations, would have to be smaller than that of \( p_1 \).

For lack of information about compensating and light-saturating photosynthetic rates, relating enhancement to the illumination curve of photosynthesis is uncertain. Moreover, being unable to compare \( D \) with respiratory rates, a contribution of light-induced respiratory changes to enhancement seems possible. Only the limited data of Hoch (5) and Myers and Graham (1) indicate that excess rates may be several-fold larger than dark respiratory rates, and hence could not be due to respiratory depressions in light.

The present work, concerning enhancement in Chlorella pyrenoidosa strain 3, is devoted to clarifying the dependence of \( E \) and \( D \) on far-red (696 mp) and blue (482 mp) illuminations. Our results have permitted us to construct graphic representations of the 2 surfaces \( E (I_1, I_2) \) and \( D (I_1, I_2) \) over a range of illuminations, and to relate these functions to compensating and saturated photosynthesis.

Materials and Methods

Oxygen evolution rates were measured with a horizontal Pt electrode, similar to that described by Myers and Graham (6). The Pt surface \((2.0 \times 17.0 \text{ mm}^2)\) was recessed about 0.5 mm below a dialysis membrane, which separated the electrode compartment from a large external solution \((\sim 300 \text{ ml})\) containing 0.1 M KCl, 0.05 M potassium phosphate buffer pH 7.0, and 0.01 M KNO\textsubscript{3} and 0.005 M MgSO\textsubscript{4}. The solution was maintained at 22 to 25\(^\circ\), and was continuously stirred and equilibrated with gases containing \( N_2, 5\% \text{ CO}_2, \) and 0, 1, or 20 \% \( O_2 \).

The anode was a large area Ag/AgCl electrode, mounted in a hollow plastic cylinder, which made contact with the solution across an agar plug of about 5 mm thickness and 20 mm diameter. The agar plug was intended to trap Ag\textsuperscript{+} and thus prevent poisoning such as described by Myers and Graham (6). No poisoning was evident, and photosynthetic activity as indicated by saturating rates gradually increased when cells were left for periods of 2 to 3 days. Most ex-

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experiments, however, were conducted in the first 24 hours after mounting fresh cells.

The sum of electrical resistances in the electrode circuit was always less than 1% of the resistance of the Pt electrode itself. The polarizing voltage was 0.55 volt. Currents were recorded with a Keithley 150 Micro-Micro-Ammeter and Varian recorder.

Illumination was provided by a Köhler projector having two 1000 W tungsten lamps, 2 condensers, a beam combining plate, and a projection lens. Identical superimposed images of the diaphragms at the condensers were directed by a prism and plexiglass light pipe to the Pt electrode, or by turning the prism, through an identical light pipe to a calibrated thermopile. Light-saturated currents were measured with white illumination of \(1.0 \times 10^5\) ergs cm\(^{-2}\)sec\(^{-1}\) (about twice saturating). Baird interference filters with peak transmissions of about 80% at 482 mp and 696 mp, and with bandwidths of about 15 mp at 40% transmission and about 30 mp at 1% transmission were used to obtain the monochromatic beams. Water, glass, and additional interference filters were employed to remove infra-red from the white, blue, and far-red beams.

After mounting cells, a period of 2 to 3 hours was allowed for gas equilibration. For experiments in which the gas used was 5% CO\(_2\) in N\(_2\), the cells were exposed to illuminations of 696 mp, sufficient to support photosynthesis, during the equilibration period. In enhancement measurements, a sequence of far-red, dark, blue, far-red and blue, dark, far-red, and dark was employed. The length of the periods was 4 to 8 minutes with 1% and 20% O\(_2\) (insuring > 95% of steady state rate), and 8 to 15 minutes without O\(_2\) (giving > 90% of steady state rate). The timing and sequence of the periods was controlled automatically by a programming device operating electrical shutters.

Like Myers and Graham (6), we found that photocurrents in saturating light corresponded to rates of photosynthesis typical of those obtained by other methods. From the volume (~14 µl) of algal suspension confined in the electrode compartment, and from the packed cell density (usually ~0.005 µl µl\(^{-1}\)) of the suspension, both the total volume of cells (~0.090 µl) and the thickness (~4 µ) of the cell layer sedimented on the Pt were calculated. Since the mean distance from Pt to cells was about 1% of the distance to the external solution, approximately 99% of the O\(_2\) evolved by the cells was expected to diffuse to, and be reduced at, the Pt surface. Essentially quantitative recovery of evolved O\(_2\) indeed seems to have occurred. With saturating white light, the photocurrents varied from 8 to 23 µamps. Assuming 4 electrons per O\(_2\), the corresponding rates of evolution were 20 to 60 µl O\(_2\) per µl cells per hour, in agreement with reported maximum rates for Chlorella.

In order to reduce the dark current (~8 µamps with 20% O\(_2\)) and thereby improve somewhat the precision of the photocurrent measurements, most of the enhancement experiments were carried out with 1% O\(_2\) in the external solution. In addition, some experiments were performed with 20% O\(_2\) and without O\(_2\). While the induction period following darkness was longer with the reduced O\(_2\) concentrations, (only slightly longer with 1% O\(_2\)), there was no evidence of modifications due to anaerobiosis. Undoubtedly, the presence of illumination during the equilibration period of the experiments carried out in the absence of O\(_2\), and the avoidance of unnecessary dark periods during measurements helped to prevent anaerobic adaptation.

The absence of O\(_2\) and the employment of 1% O\(_2\) led, however, to a distortion of illumination curves, which provided an independent means of estimating dark respiratory rates. Numerous illumination curves of photosynthesis in far-red and blue lights were measured in the course of enhancement experiments. At other times, illumination curves in white light were measured. Always with 20% O\(_2\), and in many experiments with 1% O\(_2\), the illumination curves, for the 3 lights, were linear up to about 1/3 of saturated rate, and the linear portion passed through the origin. In many cases, the degree of linearity was high, the average deviation of points from a straight line being ≤ 0.2% of the saturated rate.

In some experiments with 1% O\(_2\), and always in the absence of O\(_2\), the curves were only linear above a limit corresponding to 0.5 to 3% of saturated rate, and the linear portion extrapolated to a negative photocurrent at zero illumination. This effect is due, we think, to evolved O\(_2\), produced at compensating and smaller rates, being consumed within the cells by respiration. In this case, the magnitude of the negative photocurrents, found by extrapolation of the linear part of the curves for no O\(_2\), is a measure of the respiratory rate supported by photosynthetically evolved O\(_2\). The magnitudes of the negative photocurrents in the absence of O\(_2\) were roughly 1/40 the photocurrent in saturating light, corresponding to a respiratory rate of approximately 1.0 µl O\(_2\) µl\(^{-1}\) cells hr\(^{-1}\). This value is typical of respiratory rates found by other methods.

The preceding explanation of the illumination curves is supported by an analysis of the oxygen concentrations in the electrode maintained by diffusion, provided one makes the plausible assumption that Chlorella respiration is mediated by cytochrome oxidase, the activity of which is saturated by about 10\(^{-2}\) M O\(_2\) (7). We assume that in the electrode compartment, one-dimensional diffusion occurs across 3 layers, a 2.5 µ layer free of cells next to the Pt, a 5 µ layer of cells, and a 492.5 µ layer between the cells and the external solution. The O\(_2\) concentration at the Pt surface is zero, that at the interface with the external solution is maintained at a constant value. The diffusion equation is then applied to the 3 layers, appropriate boundary conditions being employed. Results of the analysis show that the steady state concentration of O\(_2\) in the cell layer is equal to the sum of 2 terms. One is the concentration maintained
by diffusion from the external solution in the absence of respiratory or photosynthetic activity, that is \( \sim 2 \times 10^{-6} \), \( \sim 1 \times 10^{-7} \), and \( < 1 \times 10^{-7} \text{ M} \) in 20%, 1% and 0% \( \text{O}_2 \), respectively. The other term depends on the respiratory or photosynthetic rates. For an \( \text{O}_2 \) evolution at the rate of 30 \( \mu \text{l} \text{O}_2 \text{ ml}^{-1} \text{ cells hr}^{-1} \), the term is approximately \( 1 \times 10^{-6} \text{ M} \). For respiration at 1 \( \mu \text{l} \text{O}_2 \text{ ml}^{-1} \text{ cells hr}^{-1} \), the term is negative and equal to about \( 3 \times 10^{-8} \text{ M} \). Then, the total concentration of \( \text{O}_2 \) at the cells, in darkness and with 1% \( \text{O}_2 \), would be roughly \( 7 \times 10^{-8} \text{ M} \). Hence, a respiration mediated by cytochrome oxidase would be slightly inhibited or just barely saturated. In the absence of \( \text{O}_2 \), respiration would be completely inhibited in darkness, but would become saturated when photosynthesis reaches 3 to 4 times compensating. Both predictions are in complete accord with our observations.

In all that follows, \( \text{p}_1 \), \( \text{p}_2 \), and \( \text{p}_{12} \) designate rates of photosynthesis relative to the saturated rate in white light. For experiments in which the illumination curves extrapolated to zero, the enhancement \( (E) \) and excess rate \( (D) \) were calculated from equations I and II. For experiments in which illumination curves extrapolated to an intercept a distance \( d \) below the origin, the apparent photosynthetic rates \( (\text{p}_1, \text{p}_2, \text{p}_{12}) \) are too small by an amount corresponding to the increase in respiratory rate in light. Provided the illuminations, and hence the photosynthetic rates, are always high enough to saturate the respiratory capacity and increase the respiratory rate by the full amount \( d \), the true photosynthetic rates will be \( \text{p}_1 + d, \text{p}_2 + d, \text{p}_{12} + d \). Then enhancement and excess rate are given by

\[
E = (\text{p}_{12} + d) - (\text{p}_2 + d) = \text{p}_{12} - \text{p}_2, \quad \text{III}
\]

\[
D = (\text{p}_{12} + d) - (\text{p}_1 + d) - (\text{p}_2 + d) = \text{p}_{12} - \text{p}_1 - \text{p}_2 - d. \quad \text{IV}
\]

These equations were used to calculate \( E \) and \( D \) for all experiments in which \( d \neq 0 \). With 20% \( \text{O}_2 \) and in many experiments with 1% \( \text{O}_2 \), \( d = 0 \).

In other experiments with 1% \( \text{O}_2 \), \( d \) varied between 0.001 and 0.025 relative to saturated rate. In the best experiments with 1% \( \text{O}_2 \), from which the functions \( E(I_1, I_2) \) and \( D(I_1, I_2) \) were characterized, \( d \leq 0.010 \) and the respiratory capacity was saturated with the lowest illuminations employed. In the absence of \( \text{O}_2 \), \( d \) was roughly 0.030. In all cases where \( d \) was not zero, spuriously high enhancements, as high as \( E = 8.0 \) without \( \text{O}_2 \), were calculated by equation (1), whereas by equation III typical values, \( E = 1.0 \) to 2.0, were obtained.

Results

Since only 1 to 2 determinations of enhancement could be obtained per hour, individual experiments (8-12 determinations) were usually confined to one plane intersecting the enhancement surface. Three kinds of planes corresponding to A) variable blue and constant far-red, B) variable far-red and constant blue, and C) constant ratio of blue to far-red illuminations, were investigated. In a few longer experiments, the trace of the enhancement surface in 2 or 3 planes was obtained.

Altogether 16 experiments were carried out with 1% \( \text{O}_2 \). Not all gave the same results. In 4, enhancement was absent or negative (i.e., \( E \leq 1.0 \)). In a few other experiments, relatively small enhancements were found \( (E \leq 1.5) \). In these, the maximum enhancement occurred with comparatively low blue illuminations and \( E \) fell quickly at higher blue illuminations.

In 6 experiments, involving 8 planes, relatively large enhancement \( (E \geq 2.0) \) occurred with optimum illuminations, and the data for all 8 planes were self-consistent. Some additional experiments gave essentially similar results but with some inconsistencies.

The principal characteristics of \( E(I_1, I_2) \), as indicated by the experiments giving high maximum enhancement, were as follows. First, in agreement with Myers and Graham (1), \( E \) increased with blue illumination (far-red being constant), and only reached a maximum when \( p_2 \) was about five-fold larger than \( p_1 \) (fig 1). At higher blue illuminations, \( E \) declined. Secondly, enhancement increased

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**Fig. 1 (upper).** \( E(p_2) \) with fixed far-red illumination.

**Fig. 2 (lower).** \( E(p_1) \) with fixed short-wave illumination.
markedly as far-red illumination decreased, blue illumination remaining constant (figs 1, 2). In this case, $E$ did not pass through a maximum but increased continuously as $p_1$ approached zero. Finally, figure 1 shows that, as far-red illumination increased, the height of the enhancement maximum decreased, while the position of the maximum shifted in such a manner that the ratio $p_2/p_1$ (approximately 5) remained approximately constant.

Both the qualitative and approximately quantitative characteristics of $E$, as found in the 6 best experiments, are summarized in figure 3, which represents the surface $E(I_1, I_2)$. In the figure, contour lines tie together points of equal enhancement. The figure emphasizes that maximum enhancement was restricted to a small area for which far-red light was very weak ($p_1 = 0.005-0.01$) and blue illumination was such that $p_2$ was approximately 0.05 to 0.15. From this region of maximum values, $E$ fell gradually as $p_2$ was increased, somewhat more rapidly as $p_1$ was increased, and very rapidly as $p_2$ was decreased.

Using the same data, the contours of the excess rate ($D$) relative to saturated rate were also constructed (fig 4). The dependences of $D$ on blue and on far-red illuminations are qualitatively similar. As one illumination increases, the other being constant, $D$ rises sharply, then more gradually, then becomes constant. At very high illumination, $D$ apparently decreases. When both illuminations increased in a fixed ratio, $D$ continued to increase, no maximum being clearly reached with the illuminations employed. The highest values of $D$ obtained were approximately 0.04 and occurred in the neighborhood of $p_2 = 0.2$ to 0.3, $p_1 = 0.1$. With still higher illuminations, corresponding to the curved portion of the illumination curve, $D$ must decline and eventually become negative.

The data for 20% and 0% $O_2$ were too few to show conclusively that the $E$ and $D$ functions with these gases are the same as with 1% $O_2$. Nevertheless, a few remarks can be made. In the absence of $O_2$, values of $E$ ($<2.0$) and $D (<0.02)$ were similar to those with 1% $O_2$, under comparable conditions of illumination. The same was true with 20% $O_2$. Moreover, a comparison of several experiments with 20% $O_2$, in which blue illumination was varied while far-red was held constant at several different values, indicated a fall-off of $E$ with increasing $p_2$ similar to that found with 1% $O_2$. The highest values of $D$ (approximately 0.03) were again similar to those with 1% $O_2$.

**Discussion**

If one accepts that the rate of respiration in darkness is roughly 1/40 of that of saturated photosynthesis, it follows that the excess rates never exceeded about twice the respiratory rate. Moreover, for illuminations giving maximum $E$, the excess rates were roughly 1/3 the respiratory rate. From these considerations, an inhibition of respiration by light, if it occurred, could be an important source of an apparent photosynthetic enhancement. We do not believe this is the case. The linearity of the illumination curves is not consistent with light-induced respiratory changes, and such light-induced inhibitions

![Fig. 3 (left). $E(p_1, p_2)$. Contours of $E$ estimated from experimentally determined profiles (dashed lines).](image1)

![Fig. 4 (right). $D(p_1, p_2)$. Contours of $D$ estimated from experimentally determined profiles (dashed lines).](image2)
of respiration as have been observed are saturated by about compensating illuminations; with higher illuminations only respiratory stimulations have been discerned (5, 8, 9). These respiratory effects could not lead to the gradual increase of D up to illuminations many-fold greater than compensating, and could not give maximum values of D in excess of the respiratory rate in darkness. These considerations confirm the view of other authors, that enhancement is primarily a photosynthetic, not respiratory phenomenon.

Remarks on Interpretation. We now consider whether the experimentally determined functions \( E(I_1, I_2) \) and \( D(I_1, I_2) \) are in accord with previously proposed explanations of enhancement. Two hypotheses, the spill-over model and the separately-packaged pigment-systems model, have been offered. Both Myers and Graham (1) and Duyvans and Amsz (10) have discussed both models. Neither model has been given an analytical formulation. In the following, simple analytical formulations are presented. Both models lead to equivalent functions for \( E \) and \( D \); thus our results support one model as well as the other. Subsequently, we will note discrepancies between the experimental and predicted functions, and offer explanations for these.

Common Features of the Models. According to both the spill-over and separate-package hypotheses, the rate of steady state photosynthesis is limited to the rate of the more slowly sensitized of 2 required photoreactions. In both hypotheses, absorbed far-red quanta are presumed to be distributed in a fixed ratio between far-red and short-wave photoreactions, distribution to the far-red reaction predominating. Accordingly, the rate (\( p_{12} \)) of photosynthesis in far-red light will be limited to the rate of the short-wave reaction. Then,

\[
p_{12} = a i_1 + b i_2
\]

V

where \( i_1 \) is the rate of absorption of far-red quanta and \( a \) is the fixed fraction \((< 1/2)\) of absorbed quanta acting in the short-wave reaction. Of the rate \((1 - a) i_1\) of absorption of far-red quanta by the far-red system, \((1 - 2a) i_1\) in excess of the rate of sensitization of the short-wave reaction, and is, therefore, wasted unless balanced by short-wave illumination.

Spill-over Model. According to this hypothesis, short-wave quanta are absorbed predominantly by pigments directly associated with the short-wave photoreaction, but the excitation energy can be transferred to the long-wave photoreaction when this reaction tends to be the slower. Thus one supposes that short-wave quanta are distributed in a flexible manner which tends to equalize the rates of both reactions. In short-wave light alone, it is supposed that the distribution is perfectly balanced, half the absorbed quanta going to each reaction; then,

\[
p_2 = (1/2) i_2
\]

VI

where \( p_2 \) is the rate of photosynthesis and \( i_2 \) is the rate of absorption of short-wave quanta. In short-wave and far-red lights given simultaneously, the absorbed short-wave quanta go predominantly to the short-wave reaction thus tending to balance the predominant sensitization of the far-red reaction by far-red quanta. In the presence of far-red and limiting short-wave illuminations, the overall rate \( (p_{12}) \) will still be limited by the rate of the short-wave reaction:

\[
p_{12} = a i_1 + b i_2
\]

VII

Here, \( b \) \((> 1/2)\) is the maximum fraction of absorbed short-wave quanta which can be utilized in the short-wave system.

As short-wave illumination increases, a point is reached where the rate \((a i_1 + b i_2)\) of introduction of quanta into the short-wave system equals the rate \([ (1 - a) i_1 + (1 - b) i_2]\) of introduction into the far-red system, and both rates equal \(1/2 (i_1 + i_2)\). Then,

\[
p_{12} = 1/2 (i_1 + i_2) = a i_1 + b i_2 = (1 - a) i_1 + (1 - b) i_2
\]

VIII

At this point no far-red quanta are wasted, and any additional increment of short-wave illumination will be divided equally between both reactions. Hence, at this balance point, and for all stronger short-wave illuminations, \( E \) and \( D \) have fixed maximum values.

From equations V, VI, VII, expressions for \( p_{12} \), \( E \) and \( D \) are readily derived in terms of either \( i_1 \) and \( i_2 \), or \( p_1 \) and \( p_2 \). For \( i_2 \) less than balancing, one obtains

\[
p_{12} = p_1 + 2b p_2, \quad \text{IX}
\]

\[
E = (p_{12} - p_2)/p_1 = 1 + [(b - 1/2)/a] (i_2/i_1)
\]

X

\[
D = p_{12} - p_1 - p_2 = (b - 1/2) i_2 = (2b - 1) p_2, \quad \text{XI}
\]

From equation VIII, the ratios \( i_2/i_1 \) and \( p_2/p_1 \) for short-wave illumination just strong enough to balance the reaction rates are found:

\[
i_2/i_1 = (2a - 1)/(1 - 2b)
\]

\[
p_2/p_1 = (2a - 1)/(1 - 2b)
\]

XII

Substituting XII in X and XI leads to expressions for \( E \) and \( D \) for just-balancing, as well as for higher than balancing, short-wave illuminations:

\[
E = 1/2a, \quad \text{XIII}
\]

\[
D = [(1/2a) - 1] p_1, \quad \text{XIV}
\]

Separate-Package Hypothesis. In this case, one supposes no energy transfer is possible between the pigment system of the short-wave reaction and that of the far-red reaction. As a result, absorbed short-wave quanta, like far-red quanta, are presumably divided in a fixed ratio between short-wave and far-red reactions, the preponderance of short-wave quanta acting in the short-wave reaction. Then the rate
of photosynthesis in short-wave light is limited to the rate of the far-red reaction, giving in place of equation VI,

$$p_2 = (1 - b)i_2.$$  

VIa

A quantity \((2b - 1)i_2\) of short-wave quanta are wasted.

Evidently, simultaneous short-wave and far-red illuminations again permit a balancing of rates. With short-wave illumination limiting, the rate \((p_2)\) is equal to the rate of the short-wave reaction, and is again given by equation VII. Likewise, at the balance point, \(p_{12}\) is again given by equation VIII. Short-wave quanta in excess of the quantity needed to balance the far-red reaction will be wasted in the same measure as with short-wave illumination alone.

From equations V, VIa, VII, and VIII, expressions for \(p_{12}, E,\) and \(D\) are obtained as before:

$$p_{12} = p_i + bp_2/(1 - b),$$  

 IXa

$$E = (p_{12} - p_2)/p_1 = 1 + [(2b - 1)/a](i_2/i_1) = 1 + [(2b - 1)/(1 - b)](p_2/p_1),$$  

 Xa

$$D = p_{12} - p_1 - p_2 = (2b - 1)/i_4 = [b/(1 - b) - 1]p_2,$$  

 XIa

$$i_2/i_1 = (2a - 1)/(1 - 2b), p_2/p_1 = [(1 - b)/(2b - 1)][(1 - 2a)/a],$$  

 XIIa

$$E = (1/a) - 1,$$  

 XIIIa

$$D = [(1/a) - 2]p_1,$$  

 XIVa

Comparison of Models. Our results with far-red of 696 m\(\mu\) indicate a maximum enhancement of about 2.5; from this value \(a\) can be calculated from equations XIII or XIIIa. Also the ratio \(p_2/p_1\) needed to obtain maximum enhancement is approximately 5; then from equations XII or XIIIa, \(b\) can be evaluated. For the spill-over hypothesis, \(a\) is approximately 0.20 and \(b\) is 0.65, the latter value indicating that the flexibility of utilization of short-wave quanta is limited. Apparently of the order of 35% of short-wave quanta are constrained to act in the far-red system; since another 35% must then always be used in the short-wave reaction, only 30% remain to be flexibly distributed to whichever reaction tends to be the slower. For the separate package hypothesis, \(a\) is 0.29 and \(b\) is 0.57.

A comparison of the equations for the 2 models shows that they are of similar form. For example, maximum enhancement (equations XIII and XIIIa) is a function of \(a\) only, and, for short-wave illumination limiting, \(E\) depends only on \(b,\) not upon \(a\) (equations X and Xa). Thus, when both models are fitted to the same experimental results, both models lead to numerically identical equations for \(E\) and \(D\) as functions of \(p_2\) and \(p_1.\) These equations are, for short-wave illumination limiting,

$$E = 1 + 0.30 p_2/p_1,$$  

 XV

$$D = 0.30 p_2,$$  

 XVI

and for far-red illumination limiting

$$E = 2.5,$$  

 XVII

$$D = 1.5 p_1.$$  

 XVIII

For our experiments, the choice of far-red (696 m\(\mu\)) and short-wave (482 m\(\mu\)) wavelengths was based on previous results (2, 11, 12) suggesting that maximum enhancements can be obtained with these wave-lengths. However, Myers and Graham (1) showed recently that twofold greater enhancements are obtained with 710 m\(\mu\) than with 695 m\(\mu.\) In addition, they found a larger ratio \(p_2/p_1\) is required to obtain maximum enhancement with 710 m\(\mu\) than is the case with 695 m\(\mu.\) Both of these findings are predicted (equations XII and XIII, or XIIa and XIIIa), provided the fraction \(a\) of far-red entering the short-wave reaction declines as far-red wavelength increases. Myers and Graham found, moreover, that the slopes of curves representing \(E\) \((p_2)\) for \(p_1\) constant, in the region of small \(p_2,\) were independent of far-red wavelength. This is in agreement with equations X and Xa, which show the slope depends only on \(b.\)

Discontinuity of the Derived Functions. In figure 5, the contours of \(E\) and \(D,\) given by equations XV through XVIII, are plotted. On comparing with the experimental functions (figs 3, 4), one sees that, for small \(p_1\) and \(p_2,\) the predicted and experimentally determined surfaces are in good agreement, both with respect to the general shape of the contours as well as to the positions of contours of a given value. Moreover, even with small \(p_1\) and \(p_2,\) the predicted functions are discontinuous along the contour line \(E = 2.5.\)

In the spill-over model, this discontinuity reflects the assumption that, with \(p_2\) smaller than balancing, short-wave quanta are divided in the ratio \(b/(1 - b)\) between the 2 photoreactions, while with \(p_2\) greater than balancing, the short-wave quanta in excess of those needed to reach the balance are divided equally between the reactions. To remove the discontinuity, the ratio of division of short-wave quanta could be assumed to change in a continuous fashion as \(p_2/p_1\) increases. Offhand, it would seem possible that the energy transfer mechanism assumed in the spill-over hypothesis could lead to an increase in the value of \(p_2/p_1\%

For the separate package hypothesis, the discontinuity is more difficult to explain, since in this case \(b\) and \(a\) represent simply the fixed ratios of absorption of the 2 separate pigment systems. Possibly, a plausible explanation could be found in terms of the kinetics of dark reactions associated with the photoreactions.

Saturation Effect. Comparison of the experimental and derived functions \(E\) and \(D\) (figs 3, 4, 5) shows also an important discrepancy in the region of high \(p_1\) and \(p_2.\) Specifically, whereas the contours of \(E\) of the derived function are everywhere straight lines radiating from the origin, the contours of the experimental function are straight only for low \(p_1\)
and $p_2$, and, for higher $p_1$ and $p_2$, curve away in a counterclockwise sense. We suggest that this curvature of the contours is associated with the curvature of the illumination curve of photosynthesis.

To illustrate how saturation could affect $E$ and $D$, we can assume that the spill-over and separate package models (rate equations V–IX) refer to the photochemical production of an intermediate which is subsequently converted into final products by a dark reaction which is limiting in high illuminations. In this case, the observed rates of photosynthesis, ($P_1$, $P_2$, and $P_{12}$) will be functions of the rates ($p_1$, $p_2$, and $p_{12}$) of intermediate formation. Since illumination curves of photosynthesis are, to a fair approximation, represented by rectangular hyperbolae (13), we consider the specific case where

$$P_1 = \frac{p_1}{(1 + p_1)}, \quad P_2 = \frac{p_2}{(1 + p_2)}, \quad P_{12} = \frac{p_{12}}{(1 + p_{12})}.$$  

With these 3 equations, as well as the previous equations, VIII, VIIIa and IX, IXa relating $p_{12}$ to $p_1$ and $p_2$, values of $P_1$, $P_2$, and $P_{12}$ can be calculated for...
given values of $p_1$ and $p_2$. Then $E[= (P_{12} - P_2)/P_1]$ and $D[= (P_{12} - P_1 - P_2)]$ can be computed in turn, and the surfaces $E(P_1, P_2)$ and $D(P_1, P_2)$ constructed. For both the spill-over and separate package models fitted to the experimental relations $E_{\text{max}} = 2.5$ and $P_2/p_1$ (for $E_{\text{max}} = 5$, one obtains the surfaces shown in figures 6 and 7.

Although the discontinuity previously discussed remains, the contours of $E$ and $D$ show curvatures qualitatively similar to those found experimentally. There is, however, an important qualitative discrepancy: enhancements and excess currents of a given value occur at much higher values of $P_1$ and $P_2$ in the experimental surfaces than in those of the model. We think this discrepancy reflects the often-noted (13) observation, corroborated by us, that illumination curves, in the range of low illuminations, are more linear than is a rectangular hyperbola. Evidently the assumed rectangular hyperbolic function over-corrects the simple models without saturation.

The suggested effect of saturation on enhancement would require that the illumination curve of photosynthesis be, in reality, a curve, not a straight line, even at low illuminations. We must ask then whether the experimental errors associated with our illumination curves, previously taken to be linear, could be consistent with an actual curvature sufficient to explain the observed character of enhancement. In answering this question, we recall first that the illumination curve data, for rates up to 1/3 of saturating, could be fit by a straight line, the average deviation of points from the line being 0.2% of saturated rate. Secondly, we note that, up to 1/3 of saturating rate, the rectangular hyperbola, equation (XIX), can be fitted by a straight line, the average deviation of points of the hyperbola from the straight line being essentially 1% of saturated rate. Then our data could be consistent with an illumination curve, the average curve of which (over the range 0 to 1/3 of saturating) is about 1/5 that of the rectangular hyperbola. But comparison suggests that the experimental functions $E$ and $D$ are closer to those predicted by the models without saturation than to those of the model with the rectangular hyperbolic light curve. Thus, the slight curvature, which could exist within the limits set by experimental error could essentially account for the differences between the observed enhancement and the enhancement predicted by the models without saturation.

Summary

Enhancement of photosynthesis of *Chlorella pyrenoidosa* in far-red (696 mp) and short-wave (482 mp) illuminations was studied. Both $E$ (the ratio of the rate of photosynthesis supported by far-red in the presence of short-waves to the rate supported by far-red in the absence of short-waves) and $D$ (the excess photosynthesis rate generated in enhancement) were characterized as functions of far-red and short-wave illuminations. By reference to respiratory and saturated photosynthetic rates, the functions $E$ and $D$ were related to the illumination curve of photosynthesis. The magnitude of $D$, which reached values as high as 0.04 of saturated rate, and the fact that $D$ increased with illumination up to illuminations giving approximately 30% of saturated rate, demonstrated clearly that enhancement is photosynthetic and not due to light-induced respiratory inhibitions. The essential characteristics of the observed dependences of $E$ and $D$ on illumination are in accord with both the spill-over and separate-package hypotheses of enhancement, with the modifications that A) the distribution of short-wave quanta between the 2 photo-reactions is a continuous function of illumination, and B) light saturation of photosynthesis is taken into account.

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Literature Cited