TIME COURSE AND QUANTUM EFFICIENCY OF PHOTOSYNTHESIS IN CHLORELLA

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This is the second of two papers on respiration and photosynthesis in *Chlorella pyrenoidosa* with improved time resolution and precision of rate determination. The modified polarographic method of oxygen determination used, and evidence of its accuracy were presented in the first paper. In that paper it was shown that respiratory changes occur which present a serious problem in evaluating photosynthetic rate at low light intensities and for small changes in intensity.

While the Kok effect was confirmed on the basis of average respiration rates, it was shown that plausible values of respiration in the light which can be interpolated on simple assumptions of continuity make quantum yield independent of light intensity over a wide range (from about 0.25 compensation to 4 times compensation for $\lambda = 5780$ A).

In this paper we will present data, from the same experiments, on the magnitudes of quantum yield and the time course of yield. These experiments include the study of some 50 cultures from which over 150 photographically recorded runs were made. Of these some 99 have been completely analyzed for rate and quantum yield.

As a result of these experiments the most striking observation is the reproducibility of the efficiency for a given culture. Not only is the same rate repeated within 1 or 2 per cent in period after period for the same sample, but a new sample from the same culture gives about as good an agreement (usually within 2 to 5 per cent). Even after an overnight delay there is seldom a pronounced change (more than 5 per cent). This is to be contrasted with the great differences exhibited by different cultures, which vary from 6.1 to 13.5 quanta per molecule of oxygen evolved.

This constancy of efficiency is also in sharpest contrast to the widely variable respiration and induction. As an example, when the total rate of photosynthesis may be, say, 100 units, respiration may vary from 5 units to 35 units without changing the total rate by more than the 1 or 2 units to which our data are uncertain. Neither our somewhat larger systematic error nor our

assumptions concerning interpolation for respiration in the light can materially affect this observation.

It is clear that these widely different values of quantum yield constitute specific attributes of particular cultures. When plotted on cumulative prob-

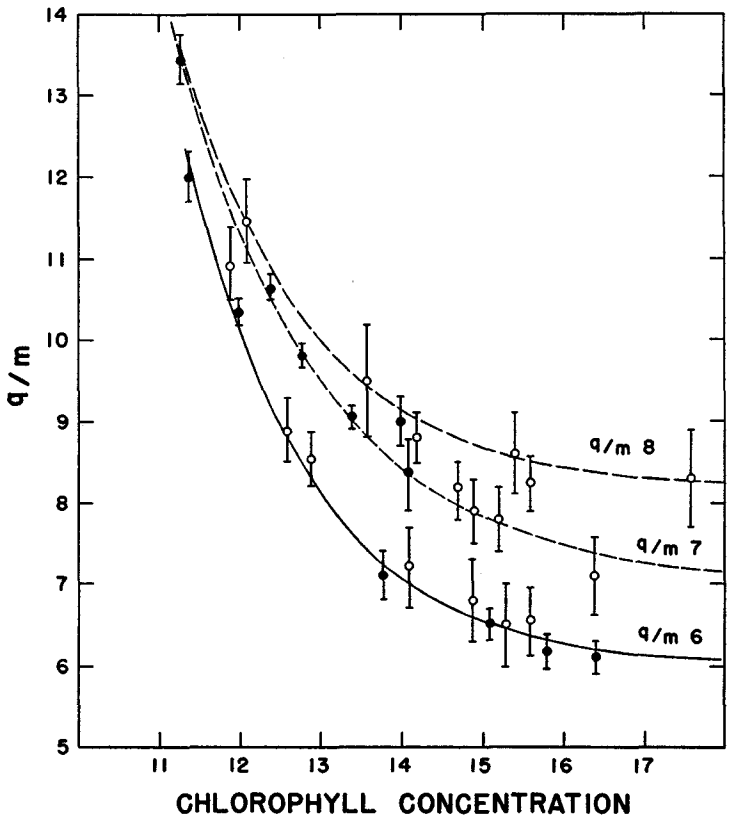

FIG. 1. Relation between q/m of O_2 and chlorophyll concentration for all well established q/m values determined in the course of this investigation. Chlorophyll concentration c is proportional to molar concentration and is equal to $\frac{-\ln T}{\delta}$ when T equals the transmission of the suspension.

ability paper they form a simple linear pattern with such departures as are to be expected for such a limited number of points. A median value of 8.5 q/m is found.

Many possible factors have been examined for correlation with these apparently random variations in yield, such as age of culture, growth rate, condition of culture, chlorophyll content, etc.

Chlorophyll concentration proved to be the only factor which showed a significant correlation. All our well established values of q/m for different cultures are plotted in Fig. 1 against a number c which is proportional to the chlorophyll concentration within the cell. This number c is obtained from our routine data: $c = \frac{-\ln T}{\delta}$ in which T is the transmission of the cell suspension and δ is the volume of cells in cubic millimeters per milliliter of suspension from centrifugation. We will show that our empirical value of c yields an approximate value of molar concentration: $C = \frac{c}{8.5}$ moles/liter.

The smooth curves in Fig. 1 are arbitrary logarithmic curves found from semilog plots and shown for comparison. It will be noted that the points form a band about 2 quanta per molecule wide. The lower limit of this band is quite well defined, yielding a curve whose constants can be determined within about ± 5 per cent. However, a number of analytical forms can express the data for this limiting curve within the experimental error.

Two such forms may be of interest. The first:

$$q/m = 5.7(1 + e^{-(c/a)+b}) \tag{1}$$

in which

$$a = 0.0175$$

and

$$b = 6.6$$

A second is found when one inquires as to the contribution of added increments of chlorophyll density thus,

$$\frac{m}{q} = \lim \frac{\sum \frac{\Delta c}{x}}{\sum \Delta c} = \frac{\int \frac{dc}{x}}{\int dc} \text{ or } \frac{mc}{q} = \int \frac{dc}{x}$$

in which x is the value of q/m for the increment. Plotting $\frac{mc}{q}$ against c as in Fig. 2, these limiting points fall upon a straight line:

$$\frac{m}{q} = \frac{1}{2.75}\left(\frac{c - 0.088}{c}\right) \tag{2}$$

$c = 0.088$ is about half of the greatest value observed, hence the fraction $\frac{c - 0.088}{c}$ has a maximum value of $\frac{1}{2}$.

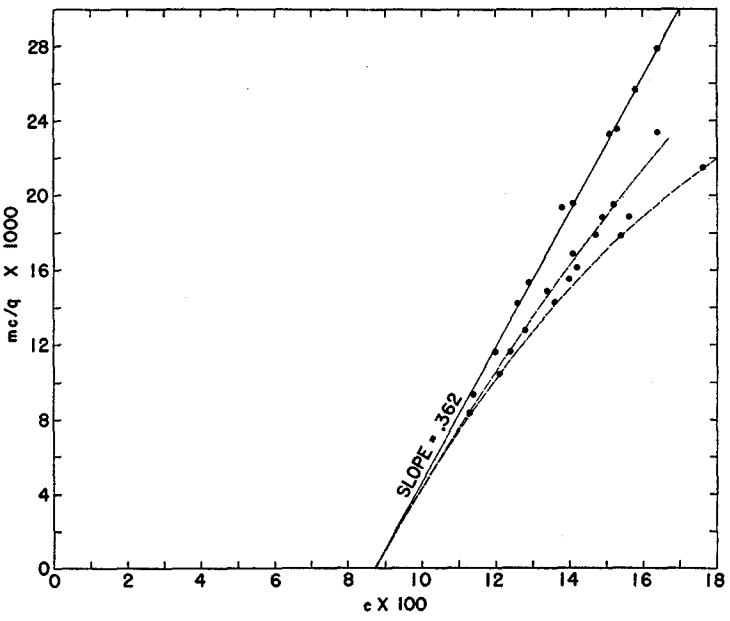

FIG. 2. Data of Fig. 1 plotted to show a relation between $\frac{mc}{q}$ and c. The fact that the limiting curve is a straight line suggests an alternative mathematical formulation which describes the data equally as well as do the curves of Fig. 1.

Both these expressions suggest partitioning of chlorophyll into contributing and non-contributing categories, thus from (1)

$$\frac{q}{5.7m} - 1 = e^{-(c/a)+b} \text{ or } \frac{\frac{5.7m}{q}}{1 - \frac{5.7m}{q}} = \frac{e^{-b}}{e^{-c/a}} = \frac{\left(\frac{m}{m'}\right)}{\left(1 - \frac{m}{m'}\right)}$$

$m' = \frac{q}{5.7}$ may be thought of as the number of molecules produced at maximum efficiency. Then $\frac{m}{m'} = \frac{5.7m}{q}$ is a contributing or efficiency fraction. Thus our expression is the ratio of the contributing to the non-contributing fraction and the non-contributing chlorophyll diminishes with increasing c. A partition into equal contributing and non-contributing portions occurs when $\frac{c}{a} = b$.

The second form indicates by extrapolation that in our experiments only one-half, at most, contribute and that no photosynthesis would occur for values less than $c = 0.088$.

In either case we can write $\frac{m}{q} = \frac{1}{\gamma_o}\frac{P}{2}$ in which $\gamma_o = 2.75 \pm 0.3$ and P approaches 1 for our highest concentration.

Thus our values of q/m approach $2\gamma_o$ for the most efficient cases observed and a probability or partition function P appears which is dependent upon chlorophyll concentration.

One is tempted to speculate as to the possible meaning of this probability function P. However, it is possible that the explanation is a matter of biological condition rather than one of physical mechanism.

The fact that Willstätter and Stoll (1918) have reported no influence of chlorophyll concentration when the chlorophyll concentration was varied in higher plants perhaps gives weight to the view that this is indeed a problem of the condition of the alga cells.

The exponential form suggests that we have inadequately accounted for the contribution of scattered light. Two lines of evidence argue against so trivial an explanation. First, low efficiency occurs for low concentration, which is just the opposite of what would arise from such an error. Second, one finds no correlation of efficiency with over-all cell density.

Attributing this variation in efficiency to biological condition, however, deserves more serious consideration. It is true that the variations in chlorophyll density arose in an accidental fashion, the cause being often unknown. Dr. Franck has suggested[1] that the value of respiration might furnish evidence of condition.

A striking fact, however, which is frequently observed in our experiments is that for a given culture the respiration can be modified over a range of fourfold by light and dark adaptation without changing the quantum efficiency. Hence, if we are to find a valid index of condition the most likely choice would be to use the fully light-adapted rate of respiration, and divide it by the cell volume.

Unfortunately we do not have values of respiratory rate which have been obtained with due attention to securing complete light adaptation to a chosen high intensity. However, our maximum values approach this condition and offer some information.

From these none too satisfactory data, we observe that all the points which form the upper limit of q/m or poorest efficiency for a given chlorophyll density do show, in fact, unusually low maximum respiration values. This suggests that both high respiratory capacity and high chlorophyll density are required for maximum efficiency, but that the two requirements may not be interdependent.

Unfortunately for a mechanistic explanation, one also observes that the

[1] Personal communication.

lower values of chlorophyll density do show some tendency toward lower respiratory value with diminishing chlorophyll density.

Since both factors may have been operative we cannot assume the validity of the analytical forms of our expression for the dependence upon chlorophyll density. Despite the intriguing possibility of a quantum statistical explanation we must regard the apparently significant values as possibly coincidental.

Furthermore, since our experiments were designed primarily for the study of the influence of intensity and the time course of photosynthesis, they were not so well designed to establish absolute magnitudes of quantum efficiency. The choice of bilateral illumination prevented the direct use of an integrating sphere. The tedious method of traverse and contour integration precluded the frequent recheck required for the best evaluation of absolute magnitudes. It was necessary, therefore, to rely upon the constancy of the relation between the integrated value and the central intensity which was measured as routine.

However, it is our opinion that the reported values are not likely to be in error by more than 10 per cent.[2]

The lowest values approached in these experiments are, therefore, significantly higher than the values of 3.5 to 4.0 reported by Warburg and Burk (1950).

While the median value found, 8.5 q/m, agrees well with findings of other observers, our evidence suggests the importance of a lower limit of about 6 q/m for steady state, which is less than the value of 8 found by many observers, and gives weight to values of about 7 occasionally reported by Kok (1948, 1949) and others.

However, we prefer to defer the matter of absolute magnitude to the completion of further experiments now in progress designed for absolute measurement.

Since our data indicate a dependence, direct or indirect, of quantum yield on chlorophyll density, it may prove profitable to reopen this question despite earlier observations to the contrary by Willstätter and Stoll (1918) and later by Emerson and Arnold (1932), and Emerson, Green, and Webb (1940). A systematic study of the factors affecting yield seems desirable. Chlorophyll density, which varied in our experiments in a random fashion despite the maintenance of presumably good culture conditions, must be varied in a controlled and systematic fashion with some reliable measure of physiological condition. Indices of such condition, such as light-adapted respiratory capacity, therefore seem essential to such an undertaking.

Aerobic Induction

The initial behavior of photosynthesis at the beginning of illumination is of great theoretic importance. Our automatically recorded 10 second points

[2] Note added after submission of manuscript: This opinion is now supported by preliminary findings from new experiments designed for systematic accuracy of magnitude.

(with a time resolution which appears to be small compared to the interval) disclose initial changes in oxygen exchange rate of considerable interest.

Drastic measures have been taken in these experiments to secure equal illumination of all cells in order that the observations of time course be significant of the events within the cells. Experiments which place the cells in a variety of different intensities not only suffer from the averaging of a variety of conditions but may yield distorted curves due to differences in diffusion distance to regions of different behavior. Many of the idiosyncrasies of aerobic induction reported in the literature were found in preliminary experiments under inhomogeneous illumination. These are found to disappear when uniform conditions are secured by the measures described. Observations in gas phase, of course, further complicate the picture by imposing time delays which may be of the order of minutes and of uncertain and variable character.

The phenomena of aerobic induction have been the subject of extensive study (Osterhout and Haas, 1918; Warburg, 1928; van der Paauw, 1932; Smith, 1937; McAlister, 1937; Blinks and Skow, 1938; van der Veen, 1949). Nevertheless, advances in time resolution and the possible elimination of diffusion anomalies through homogeneous illumination make a reexamination of these phenomena worth while.

In almost all our experiments the initial rate is less than the steady rate attained later in the same period of illumination. The initial rate is lowest, probably zero, for most complete dark adaptation with which one also observes very low respiration. The first induction after dark adaptation under aerobic conditions may occupy 3 minutes or more and appear somewhat logarithmic in form.

In succeeding periods of illumination, the induction shows a progression of forms—starting at successively higher initial rates, rising more rapidly, and making progressively sharper transitions to the steady rate. This is well illustrated in Fig. 3, in which the initial 3 minutes of successive light periods are plotted on a larger scale from an intermittent run of 6 minutes' light and 6 minutes' dark. Thus the initial capacity to perform photosynthesis may be carried over many minutes (at least 6 in our observations) but is gradually lost in the dark.

Not only does this type of aerobic induction change from period to period as light adaptation progresses, but differences in behavior are observed for different cultures. The persistence of induction in particular appears to be different in different cultures. In some cases induction almost disappears after several periods of light (for 3 minutes' light and 3 minutes' dark). In other cases induction may still be quite pronounced.

These phenomena of aerobic induction occupy too short a time to be faithfully observed by manometric methods. They are not to be confused with the

phenomena of anaerobic induction observed by Gaffron (1940), which occupy much more time.

The observations of Emerson and Lewis (1940, 1941) are interesting. Making allowances for manometric delay, the CO_2 bursts which they observe may coincide in time with our induction. Similarly their deduced curves for oxygen

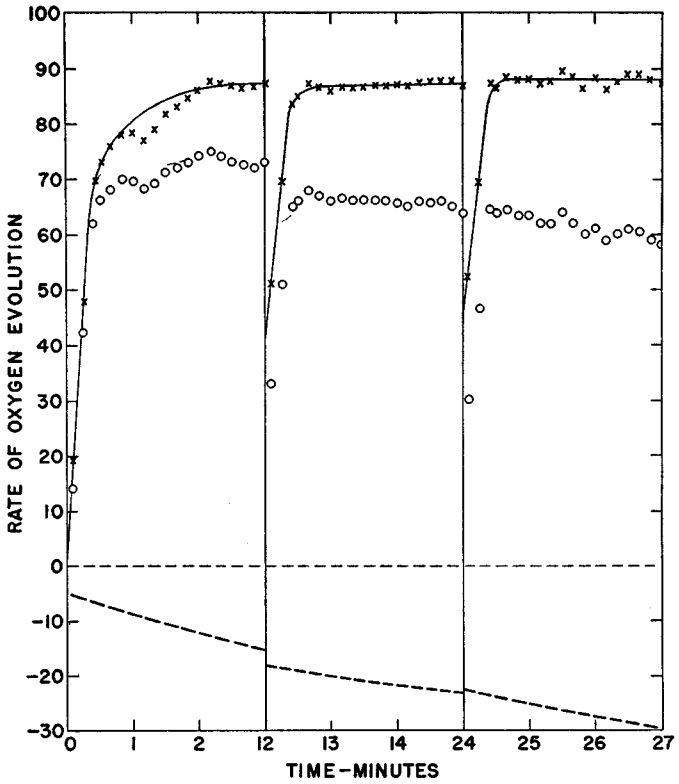

FIG. 3. Aerobic induction. Plot of rates for the first 3 minutes of three successive periods of illumination during an intermittent exposure of 6 minutes' light and 6 minutes' dark.

appear compatible. If one computes the amount of gas which might be produced at unit quantum efficiency by the energy which fails to make its contribution during induction as we observe it, one can indeed satisfy their suggested requirements for CO_2 burst. Most striking is the fact that just as our induction progressively changes in character, so does their burst if thought of as complementary.

Our experiments certainly exclude the possibility that photosynthesis starts at a high initial rate as postulated by Burk and Warburg (1951). There is

only one circumstance under which we observe an initial rate which might be slightly higher than the subsequent steady rate. This occurs when the culture is intermittently illuminated first with a high intensity and then with a lower intensity. In the first period after the change, there appears to be a possible inverse induction, but it lies on the border of our error (1 or 2 units).

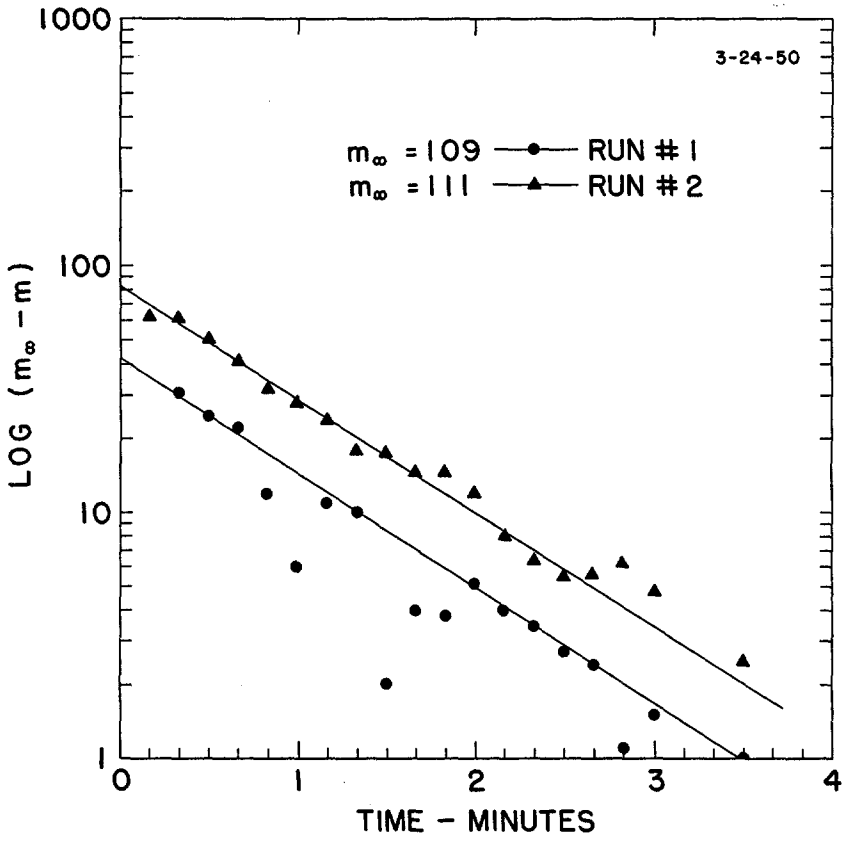

FIG. 4. Induction in the first light period for two successive runs on the same algae. Each run used a small part of a large sample, the only difference being in time of adaptation.

The precise shape of the rate curve during induction is of theoretical importance. Even though a 10 second interval between points leaves much to be desired in studying induction which sometimes lasts only 30 seconds, the first induction after protracted darkness may yield up to 20 points. This is sufficient to show marked departures from logarithmic form and give rough values of constants. Using the slope value (m_∞) determined by steady state as an

asymptote, one may subtract the momentary slope values (m) and plot the difference on semilog paper as in Figs. 4 to 8. These give a good idea of the variety of form exhibited in induction by different cultures.

Fig. 4 shows the striking reproducibility of time constant for two runs from the same culture. A difference in dark adaptation is shown by the difference

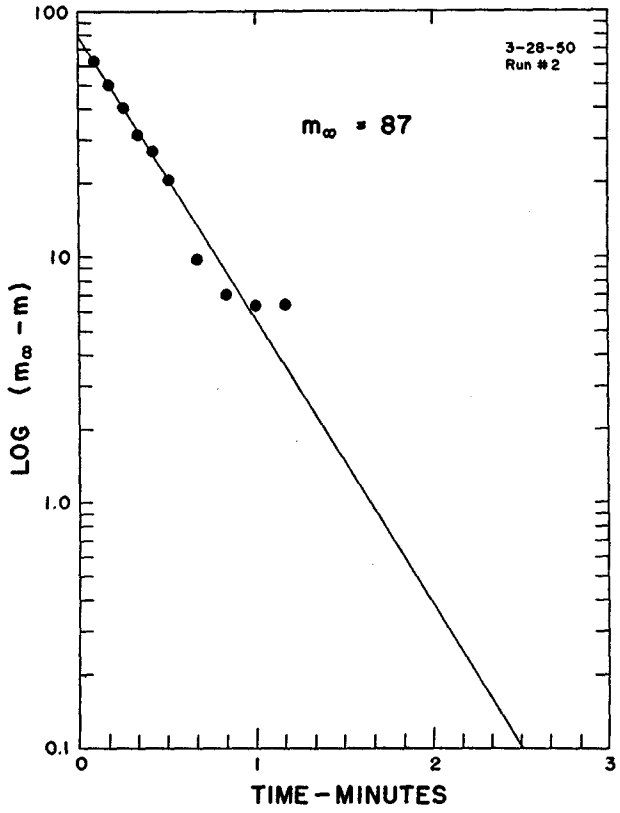

FIG. 5. Induction (final rate minus momentary rate) plotted logarithmically against time. Data obtained from the first light interval shown in Fig. 3.

in initial rate. Thus the more dark-adapted the culture, the more nearly the intercept or first value of $m_\infty - m$ approaches m_∞ as illustrated by Fig. 5 and also Fig. 8.

That induction is not simple, is shown by the departures from logarithmic approach to steady state: One notes breaks, changes in slope, (Fig. 7), and changes in asymptote. Fig. 6 shows how sensitive this type of plot is to the choice of asymptote which in this case required a slightly different value for linearity from the final steady state.

Even this limited information makes crucial demands upon any theory.

A preparatory photochemical process involving chlorophyll seems inescapable. This may or may not be a part of the steady state mechanism.

A possible mechanism which explains many of the quantitative and qualitative observations is one in which chlorophyll performs two acts in the course

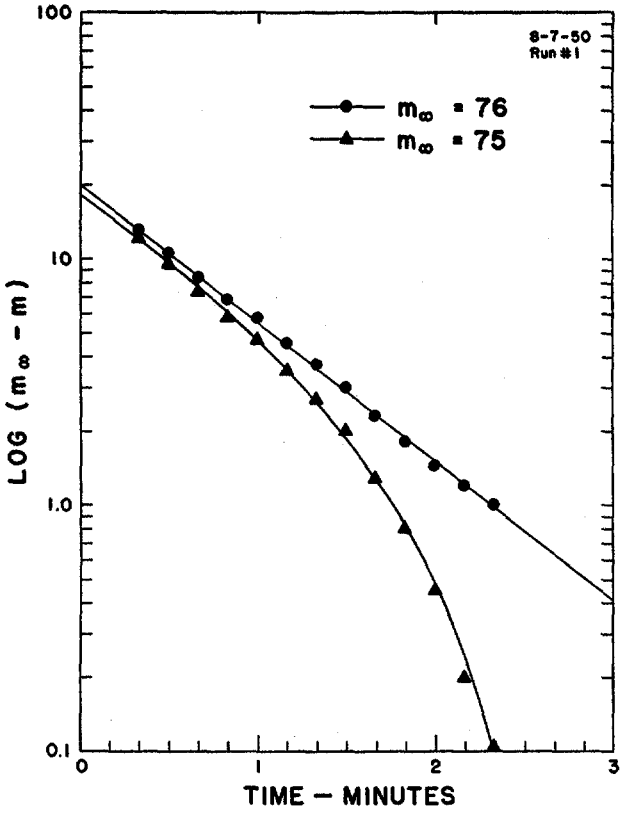

FIG. 6. Induction in the first light period in which the apparent semilog asymptote differs slightly from the observed steady rate. ▲, actual steady rate, ●, steady rate which appears to be the asymptote.

of photosynthesis after which its role is repeated as a two-step cycle. If both steps are photochemical and require a fixed number of quanta per chlorophyll molecule, it can be shown mathematically that the demand for efficiency to be independent of intensity can be satisfied. The simplest such mechanism satisfying our data would require as an optimum about 3 quanta per oxygen molecule for each step.

It is of interest, therefore, to inquire as to the number of quanta per mole-

cule of chlorophyll absorbed during induction. From the following calculations we see that the order of magnitude is reasonable.

Taking a probable value of the specific extinction exponent from Zscheile and Comar (1941) of 8.0 (when C is in grams per liter and d in centimeters)

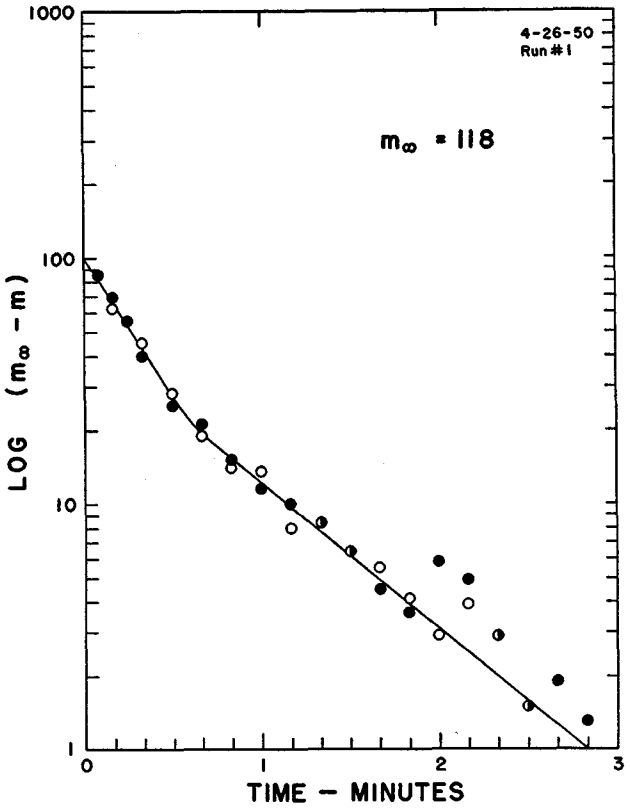

FIG. 7. Induction in the first light period showing a marked change in slope between 30 and 40 seconds. The open and closed circles are for two different observers making the original rate measurements.

and multiplying by the average molecular weight of a and b, 900, and converting to the value for base e, we obtain the molar absorption coefficient, $\alpha = 16{,}570$. Then $-\ln T = \alpha C d$, in which

d = centimeters of packed cells in suspension, and
C = moles of chlorophyll per liter of packed cells.

The volume of our cuvette is 0.445 cc. From centrifugation we have the volume of cells per cubic centimeter of suspension, $\frac{\text{mm.}^3}{\text{cc.}}$ so $\frac{\text{mm.}^3}{\text{cc.}} \times 0.445 =$

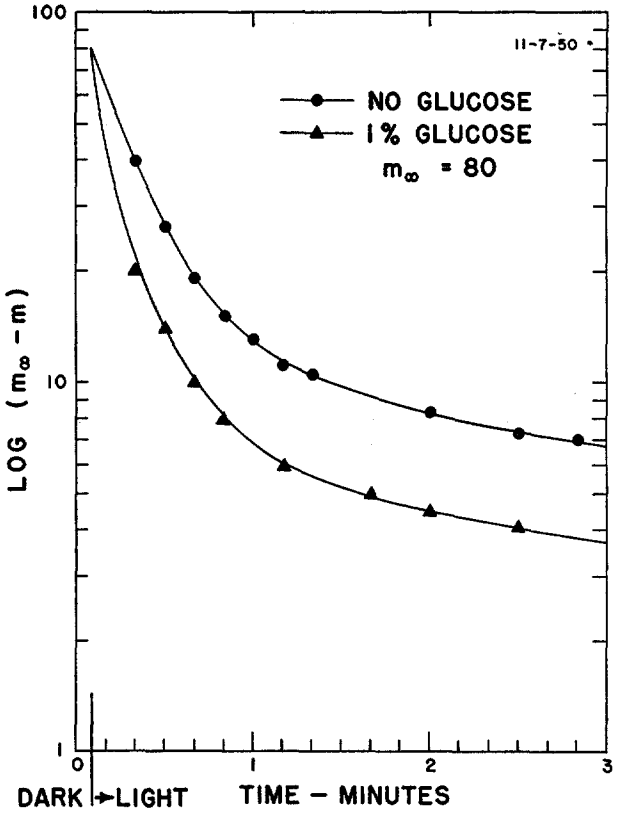

FIG. 8. Induction in the first light period for two successive runs on the same algae. Induction time was markedly reduced when 1 per cent glucose was added.

mm.^{3} volume of cells in the cuvette. Dividing this by the area 87 mm.^{2} gives the equivalent thickness of cells in millimeters. Thus $d = \frac{\text{mm.}^3}{\text{cc.}} \times \frac{0.445}{870}$ in equivalent centimeters, and $C = \frac{-\ln T}{\alpha d}$

$$C = \frac{-\ln T}{16{,}570 \times \frac{\text{mm.}^3}{\text{cc.}} \times \frac{0.445}{870}} = \frac{c}{8.5}$$

moles of chlorophyll per liter of packed cells in which $c = \frac{-\ln T}{\frac{\text{mm.}^3}{\text{cc.}}}$.

Since $\frac{\text{mm.}^3 \times 10^{-6}}{\text{cc.}} \times 0.445$ is the number of liters of cells in the cuvette and

6×10^{23} is the number of molecules per mole, the number of molecules of chlorophyll within the cuvette is

$$n = \frac{-\ln T}{16{,}570/870} \times 6 \times 10^{17} = 0.32 \times 10^{17} \times (-\ln T):$$

Thus for a given experiment we find

$$-\ln T = 0.221 \text{ and } 1.46 \frac{\text{mm.}^3}{\text{cc.}} \text{ cells in suspension from the hematocrit,}$$
$$c = 0.151 \text{ or } C = 0.018 \text{ molar concentration in the cells,}$$
$$n = 7 \times 10^{15} \text{ chlorophyll molecules in the cuvette.}$$

By hemocytometer count there were 44,400 cells per mm.^{3}, and since the volume of the cuvette is 445 mm.^{3} there were about 2×10^7 cells in the cuvette ($44{,}400 \times 445$), and thus 3.5×10^8 molecules/cell, implying 4.4 μ average diameter of cell.

Since this is a typical case of quite high efficiency, it is of interest to note that 2.5×10^{16} quanta/minute were absorbed Thus, 3.6 quanta/minute were absorbed by each chlorophyll molecule, and 17 seconds were required per quantum.

At the full rate 4×10^{15} O_2 molecules/minute were produced and $\frac{7 \times 10^{15}}{4 \times 10^{15}} \times 3.6 = 6.3$ q/m of O_2, when each chlorophyll molecule requires 1.75 minutes to absorb the 6.3 quanta per molecule of O_2 produced.

If we have a total of n chlorophyll molecules and of these n_a are in an intermediate state, we may think of a photochemical reaction producing n_a and another returning it to its original state. We may regard n_a as chlorophyll molecules associated with an intermediate and the return of n_a as related to the observed rate of photosynthesis, m. Thus, when q is the rate at which quanta are absorbed and γ_o the quanta required per molecule returned, $m = \frac{qn_a}{\gamma_o n}$, energy being available in proportion to the participating fraction $\frac{n_a}{n}$. Without entering into the mathematical details given below,[3] one can derive the expression for the time constant in minutes $\tau = \frac{ny}{q}$ when $y = \frac{\gamma\gamma_o}{\gamma+\gamma_o}$.

[3] From

$$\frac{dn_a}{dt} = \frac{q}{\gamma}\left(1 - \frac{n_a}{n}\right) - \frac{q}{\gamma_o}\frac{n_a}{n}$$

or

(*Footnote continued on following page*)

The values γ and γ_o are the quanta required in each of the two photochemical steps. If both are the same $y = \frac{\gamma_o}{2}$. Earlier we found $\frac{m}{q} = \frac{P}{2\gamma_o}$ when P approached 1 under optimal conditions and $\gamma_o = 2.75$. So $y = \frac{\gamma_o}{2} = 1.38$ would be predicted for the simplest case, thus being one-fourth of the optimal q/m. When the data permitted, the values of y have been determined and plotted in Fig. 9 against the corresponding value of q/m.

From this it is seen that while induction usually starts at the predicted optimal slope regardless of q/m, it generally exhibits a slower stage before steady state is reached. This is often about $y = \gamma_o = 2.75$.

It is beyond the scope of this paper to pursue the elaborations of this concept of mechanism and the evidence which encourages this line of speculation.

It should be pointed out that while we have been led to these concepts by

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$$= \frac{q}{\gamma} - \frac{n_a q}{n}\left(\frac{1}{\gamma} + \frac{1}{\gamma_o}\right) = F$$

$$dF = -dn_a \frac{q}{n}\left(\frac{1}{\gamma} + \frac{1}{\gamma_o}\right) = -\frac{dn_a}{\tau}$$

$$\frac{dF}{F} = -\frac{dt}{\tau}$$

$$F = F_o e^{-(t/\tau)} \quad \text{when} \quad \tau = \frac{n}{q}\left(\frac{\gamma\gamma_o}{\gamma + \gamma_o}\right) \quad \text{and} \quad F_o = \frac{q}{\gamma} - \frac{n_{ao}}{\tau}$$

$$\frac{n_a}{n} = \frac{(1 - e^{-(t/\tau)})}{1 + (\gamma/\gamma_o)} + \frac{n_{ao}}{n} e^{-(t/\tau)}$$

so

$$m = \frac{q(1 - e^{-(t/\tau)})}{\gamma + \gamma_o} + \frac{q}{\gamma_o}\frac{n_{ao}}{n} e^{-(t/\tau)}$$

writing

$$\tau = \frac{ny}{q} \text{ when } y = \frac{\gamma\gamma_o}{\gamma + \gamma_o} \text{ then for } \gamma_o = \gamma = 2.75 \; y = 1.38$$

As pointed out, the total number of chlorophyll molecules, n, can be computed from the chlorophyll density; q, the number of quanta absorbed per minute, is a known condition of the experiment; and τ can be found from the semilog plot. It is therefore possible to test the prediction of y wherever there is a linear segment in the semilog plot.

purely formal reasoning from our data, Dr. Franck[4] developed a much more complete theory from other and broader evidence which has many elements of similarity.

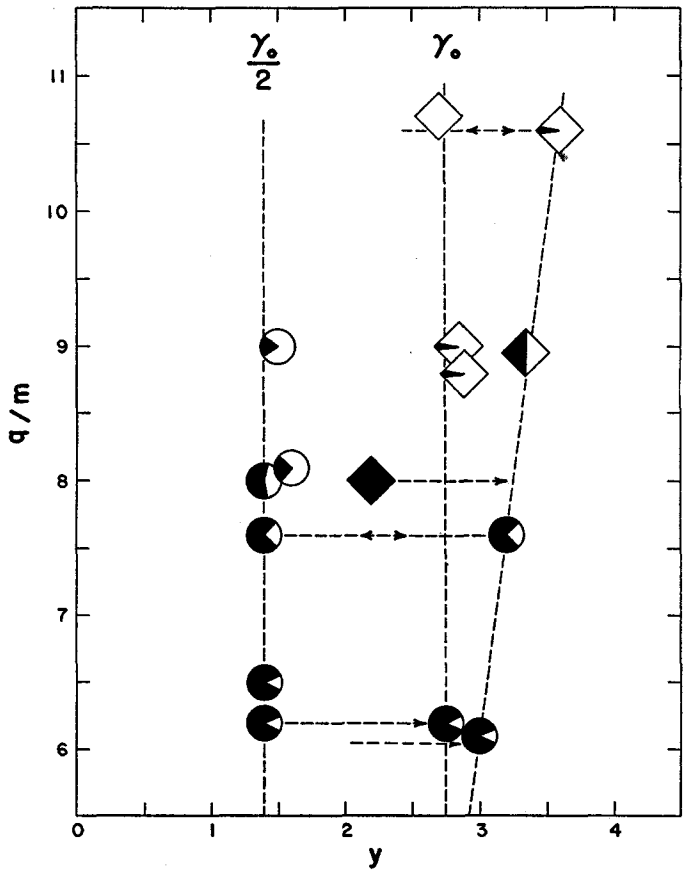

FIG. 9. Effective component quantum value, y, in general shows little influence of q/m, the number of quanta required per O_2 molecule produced. Arrows show range of values in particular experiments. Degree of black or white shows the degree of dark or light adaptation.

CONCLUSIONS

1. Though the quantum yield remains constant for different samples of the same culture despite great changes in respiration due to dark adaptation, the quantum requirement for different cultures varies from 6.1 to 13.5 quanta per molecule of oxygen evolved (q/m).

[4] Presented at the Conference on Photosynthesis, Gatlinburg, Tennessee, October 25–31, 1952.

2. This variation from one culture to another appears to depend upon chlorophyll concentration, though other paralleling factors cannot be ruled out.

3. Both chlorophyll concentration and quantum requirement show a random distribution. A statistical median for 50 cultures and 99 determinations gives $q/m = 8.5$ with a systematic uncertainty of perhaps 10 per cent. Since the variations are real, the median is regarded as less important than the lower limit approached (about $q/m = 6$).

4. Dark adaptation under aerobic conditions produces an initial photosynthetic rate of nearly zero. The immediate rise to steady state is somewhat logarithmic in character and may require over 3 minutes.

5. In intermittent light (of periods from 1 to 6 minutes) the induction observed in subsequent light periods starts from a finite initial rate and occupies a shorter time, often as little as 30 seconds.

6. The theoretical importance of aerobic induction is discussed. A chlorophyll cycle of two photochemical steps is found to satisfy most of the observed characteristics and to be compatible with an efficiency independent of intensity.

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