RESPIRATION AND INTENSITY DEPENDENCE OF PHOTOSYNTHESIS IN CHLORELLA

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This group of experiments was designed to study the time course of photosynthetic rate and to determine the dependence of rate on the intensity of illumination.

A modified polarographic method of measuring oxygen concentration was used which provides a time resolution of the order of 1 or 2 seconds and a precision in rate evaluation of about 1 per cent. The illumination was designed to place all the cells of the suspension under substantially the same intensity of monochromatic radiation.

The information thus gained because of improved time resolution, precision, and uniformity of illumination will be presented in two papers. Respiration changes were observed which have an important bearing on evaluation of photosynthetic rate. The related problems of intensity dependence and respiration are the subject of this first paper.

The time course of photosynthesis will be shown to provide important criteria of mechanism. This evidence and other information on quantum yield will be presented in the second paper.

The method, together with an analysis of its limitations, will be presented first in order to provide an appreciation of the significance to be attached to the findings. Of particular importance is the fact that random errors have been reduced to the point at which the precision attained is better than the knowledge and control of systematic factors.

Polarographic Methods in Photosynthesis

Application of the conventional polarographic method[1] of oxygen determination to photosynthesis was first reported by Petering and Daniels (1938), who were followed by Petering, Duggar, and Daniels (1939) and Dutton and Manning (1941). A more recent application is that of Moore and Duggar (1949). These experiments

[1] The development of this method by Heyrovsky and his school, and its application to oxygen measurements by Vitek, are covered fully by Kolthoff, I. M., and Lingane, J. J., 1941, in Polarography, New York, Interscience Publishers, Inc.

provided quantitative measurements of oxygen uptake and evolution with calibrated dropping mercury electrodes operating in a suspension of algal cells. Other physiological applications were concurrent: Baumberger (1938), Petering and Daniels (1938), duBuy and Olson (1940), Winzler (1941), and others. All these applications provided useful and quantitative data but are subject to criticism due to the possible physiological effect of mercury. Furthermore, they involve the usual difficulties in the maintenance of dropping electrodes of uniform behavior in small closed vessels. These objections can be overcome by the use of stationary metallic electrodes.

Early investigations (Wilson and Youtz, 1923; Glasstone, 1931; and Glasstone and Reynolds, 1933) employed stationary metallic electrodes to obtain data of a polarographic nature. Such measurements are, however, accurate to only ± 5 per cent at best and are by no means comparable to the ± 0.5 to 1 per cent obtainable with the dropping electrode.

Laitenen and Kolthoff (1941) determined the reproducibility of diffusion currents obtained under well defined diffusion conditions with a platinum wire microelectrode (4 mm. long and 0.5 mm. diameter). They concluded that the electrode could be used in analytical work with an accuracy of the order of 1 per cent when proper care is taken to provide the conditions necessary to attain a steady state of uniform and reproducible convection in the solution immediately surrounding the electrode.

Davies and Brink (1942) studied the reproducibility of oxygen determinations with fixed platinum electrodes for biological application. These authors used flush or "open type" platinum wire electrodes and a "recessed" electrode with the exposed end of the platinum wire fused in the bore of a capillary tube some distance (0.6 to 1.6 mm.) from the open end. They found the open type to have a day-to-day reproducibility within 15 per cent. The recessed electrode proved to have better stability (± 3 per cent over a period of weeks) but required several minutes between measurements in order to reestablish a linear diffusion pattern within the capillary bore. Further development and biological application of this work have been reported by Carlson, Brink, and Bronk (1948), Hill (1949), and others.

Several innovations have been devised to avoid the limitations of fixed platinum electrodes without sacrificing time resolution. Among these are the rotating electrodes of Laitenen and Kolthoff (1941) and the flow electrode system of Giguère and Lauzier (1945). It appears, however, from the general principles that when the diffusion wave is swept away as in flow electrodes or rotating electrodes, there is a dependence upon the reaction of adsorbed oxygen, and a non-linearity in response. Experience shows a marked sensitivity to surface conditions as well as to constancy of flow.

The first application of oxygen measurements with fixed electrodes to photosynthesis was reported by Blinks and Skow (1938) using both mercury and platinum large stationary electrodes pressed in contact with pieces of leaf and thallus tissue. Actually this was one of the first applications of the polarographic principle to photosynthesis studies, but the nature of the modified cathode and its spacial relationship to the cellular tissue make it difficult to evaluate the measurements in quantitative terms. No attempt to calibrate electrodes under these conditions or to correct for the resulting diffusion gradients and heterogeneous illumination was reported. Haxo and Blinks (1950) used a similar technique with the tissue held in contact with a

large (5 × 20 mm.) platinum electrode by a cellophane "sling" in a vessel of circulating sea water. Similarly in this work the distribution of diffusion gradients and other effects make it difficult to evaluate oxygen exchange of the tissue directly and quantitatively. Its use was devoted mainly to the intercomparison of action spectra.

Square Wave Method

Olson, Brackett, and Crickard (1949) attained improved stability and time resolution with a fixed platinum electrode of 25 μ diameter by using an alternating square wave potential and recording photographically the momentary current at the end of the negative pulse for each 10 second cycle. This provides a point-to-point record of dissolved oxygen every 10 seconds with a time resolution which proves to be less than the 10 second interval. Internal evidence indicates discrimination within 1 or 2 seconds. Calibration showed good linearity with a precision better than ± 1 per cent and in general a day-to-day stability of less than 2 per cent.

The method was first applied to photosynthesis in a preliminary study (Brackett, Olson, and Crickard (1949)). The advantages of homogeneous illumination were explored by cross-firing from two monochromators. Based on this experience the cuvette was redesigned for this study employing optical windows on opposite sides and a somewhat larger volume for the size of the electrode.

Cuvette and Electrode Assembly

The cuvette is designed to permit homogeneous illumination of all suspended cells by imposing two beams through opposite windows. It consists of a special aminco style F absorption cell surrounded by a water jacket. This jacket is compactly designed to permit measurement of radiation transmitted through the windows over a wide angle. Details are shown in Fig. 1.

The outside surface of the cuvette, with the exception of the windows and tubulations, is covered with a thick coat of evaporated aluminum in order to prevent the loss of scattered light. Cell suspensions are placed in the cuvette with a small hypodermic syringe and are removed by aspiration. The settling of the cells (about 1 mm./hr. with *Chlorella* in 0.1 M KCl) does not become limiting until it results in a heterogeneous vertical distribution of cells. This does not become appreciable for at least 1 hour, or twice the duration of most of our experiments.

The electrodes, which were 25 μ in diameter, were calibrated with solutions saturated at 22.2°C. by known oxygen-nitrogen mixtures when used for the first time, and thereafter they were checked periodically. This precaution is necessary in view of the possibility of the formation and accumulation of films on the electrode when exposed to the air during filling and rinsing of the cuvette. Accidental mechanical distortion of the delicate electrode can also affect

its deflection characteristics. An electrode correction factor was thus obtained for each experiment by measuring deflection in 0.1 M KCl air saturated at 22.2°C.

Correction for the very small amount of oxygen used in the electrode reactions was not generally necessary in these experiments, since photosynthesis involves the sum of the contribution of oxygen evolution and oxygen uptake

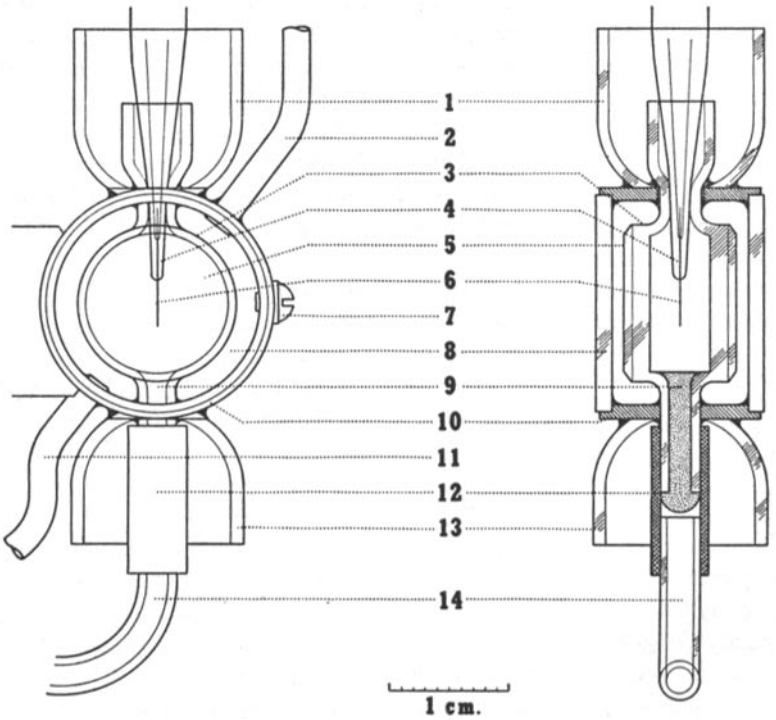


FIG. 1. Diagram of cuvette and water jacket assembly. *1*, overflow cup; *2*, cooling water outlet; *3*, cuvette body (aluminized); *4*, electrode shank; *5*, cuvette window; *6*, electrode (25 μ diameter); *7*, clean-out plug; *8*, jacket window; *9*, agar plug (cast in place); *10*, metal jacket body; *11*, cooling water inlet; *12*, gum rubber connection; *13*, glass insulating skirt (silicone-treated); *14*, KCl bridge to calomel.

in respiration. Thus any constant contribution of background slope cancels out. Only in those cases in which measurements were superimposed on a curvilinear initial decay during the first few minutes of electrode stabilization was a correction applied.

Homogeneous Illumination

In order to attain homogeneous illumination of all cells, dilute suspensions (20 to 30 per cent absorption) were used and the cuvette was illuminated by

two opposing beams arising from a single source. This provides a variation in intensity of less than 1 per cent along the optical axis between the cuvette windows. The optical system (Fig. 2) consists of two Littrow monochromator systems. The 5780 A mercury lines from a General Electric H-4 mercury arc were used in this study.[2] The aperture of the prism at A in Fig. 2 is masked by an elliptical diaphragm to provide a circular image when focused on the cuvette window by lens L_2.

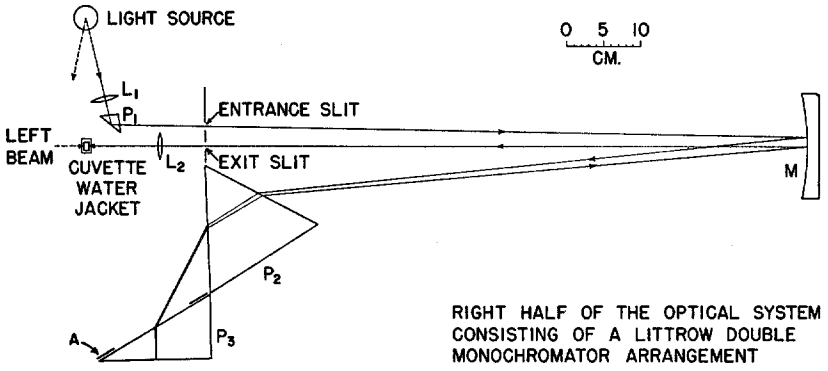


FIG. 2. Right half of illumination system. L_1, condensing lens; P_1, 90° prism; M, mirror (aspherically corrected off axis); P_2P_3, Littrow prisms; L_2, image lens; A, elliptical aperture imaged on cuvette.

Radiation Measurements

In order to determine the radiant power absorbed by the cells with a given suspension of algae in the cuvette, the power transmitted by the suspension and the power transmitted by the suspension medium alone are measured. The latter, when corrected for loss at the exit windows, gives the power incident on the cells while the ratio of the latter to the former gives the per cent transmitted. From these measurements the power absorbed by the cells may be determined.

Values of transmitted power were obtained by integrating the intensity of all the light passing through the cuvette, measured at a cylindrical surface concentric to the vertical axis of the cuvette at a radial distance of 15 mm. The intensity of radiant power per unit area at this surface on the axis of the beam was measured with a thermocouple calibrated with a 100 watt standard lamp.[3] For convenience a small photocell, calibrated by positioning at the thermocouple location, was used for intensity measurements over the cylindrical surface. The photocell, a visitron No. 51AV3, was used in conjunction with an

[2] The mercury arc was operated at 115 volts A.C. by a 1.25 KVA generator driven at constant speed by a 7.5 h.p. 1800 R.P.M. synchronous motor.

[3] National Bureau of Standards, Radiation Standard No. C-486.

electrometer type amplifier employing an RCA "acorn" tube No. 954. Readings in volts were obtained by a null method. By comparison with the calibrated thermocouple a value of 1250 microwatts/cm.^{2} was established for 1

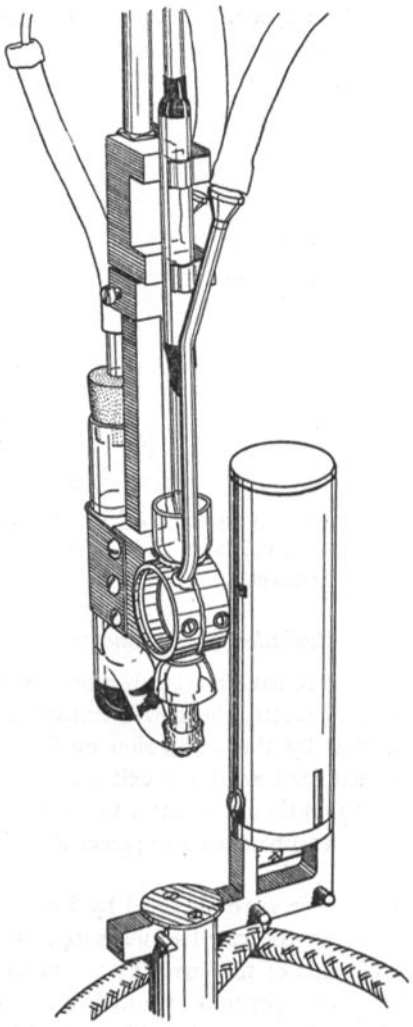


FIG. 3. Photocell traverse arrangement for measuring total power incident on enveloping surface around cuvette.

volt. The arrangement by which the photocell can be moved about the vertical axis of the cuvette at different levels with respect to the optical axis is shown in Fig. 3. Fig. 4 shows dimensions and the limits of traverse. Photocell measure-

ments were made for each beam at 5° intervals in the region of the central maximum of the beam where changes are greater and at 10° intervals in the periphery. Such traverse measurements were made at zero level (center of cuvette) and at levels of 2, 4, 6, and 8 mm. both above and below zero level. In Fig. 5 photocell readings in volts are plotted at the left for angular positions

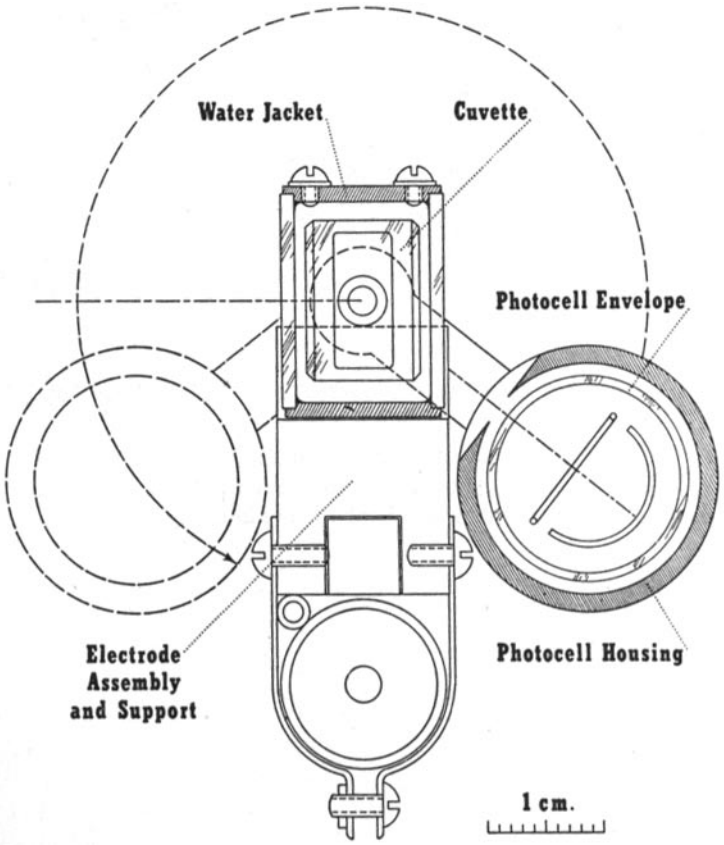


FIG. 4. Cross-section diagram of photocell traverse arrangement showing dimensions and limits of traverse.

around the cuvette or distance along the measured surface for the left beam (90° position is considered that of the optical axis of the right beam, 270° that of the left beam). To avoid confusion in the figure only the levels below the cuvette center (0 to −8 mm.) are plotted. At the right representative contours of the photocell readings are indicated, showing the distribution of intensity over the cylindrical surface measured. Some of the asymmetry which appears can be attributed to the refraction of the tapered glass electrode shank. Fig. 6

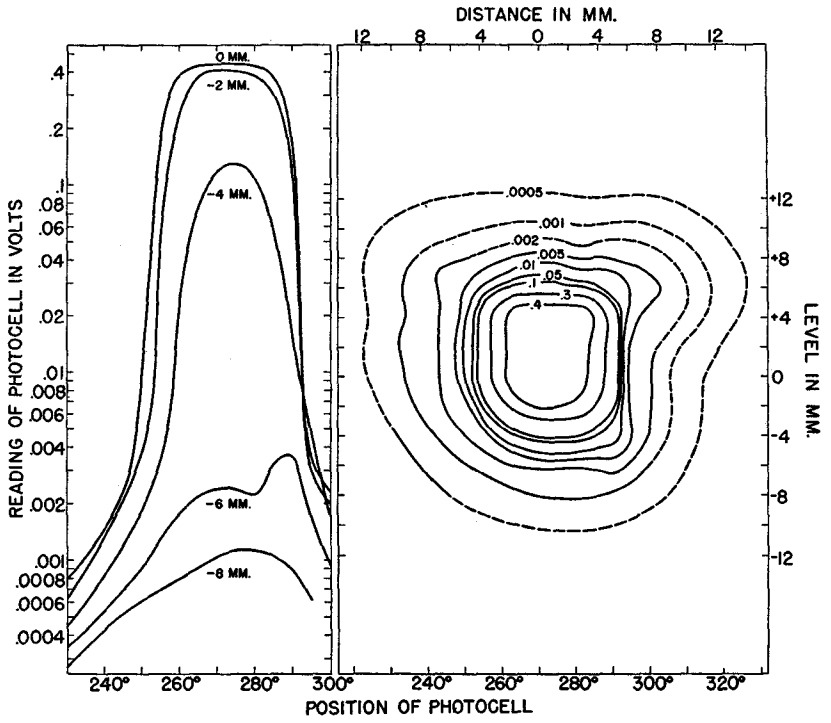


FIG. 5. Traverse plot for cuvette containing 0.1 M KCl. Left: Plot of readings for photocell angular positions at levels indicated. Right: Representative contours of photocell readings indicated showing distribution of intensity.

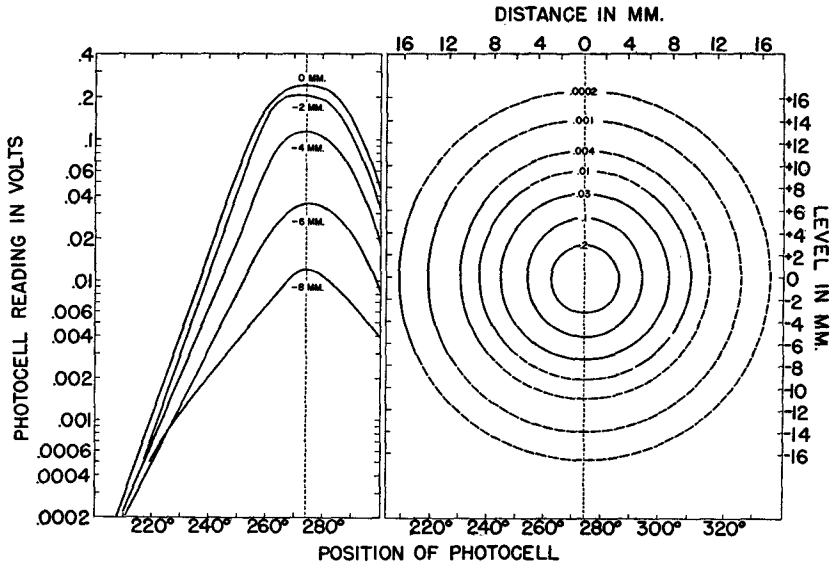


FIG. 6. Traverse plot for cuvette containing 1.26 mm.^{3} cells/cm.^{3} 0.1 M KCl. Left: Plot of readings for photocell angular positions at levels indicated. Right: Representative contours of photocell readings showing distribution of intensity.

is a similar plot for a *Chlorella* suspension containing 1.26 mm.^{3} cells/cm.^{3} in 0.1 M KCl. Here the contours approximate concentric circles showing the symmetrical distribution of intensity when cells are added to the cuvette.

This intensity distribution from traverse measurements is integrated from the plot of the area enclosed by each contour against its voltage. The integral in area volt values from the area under this curve gives the total power incident on the enveloping surface when the resulting volt cm.^{2} are multiplied

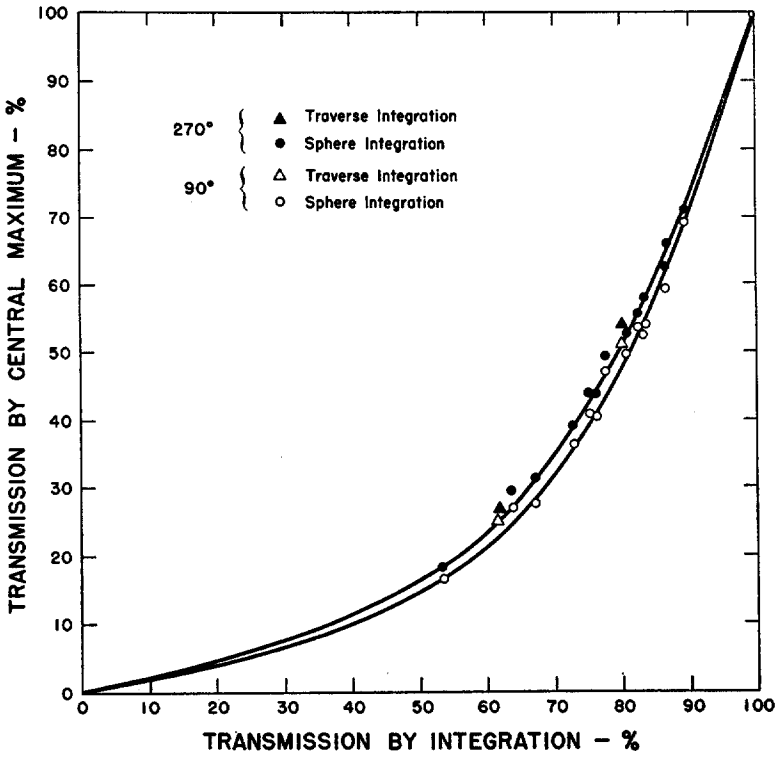


FIG. 7. Plot of central maximum transmission as a function of transmission by integration.

by the calibration constant. This value for the 0.1 M KCl in the cuvette when corrected for exit window loss gives the total power available for cells suspended. Since the total transmission of the cuvette is in effect the product of the transmission of each pair of windows this correction becomes the square root of the transmission of the cuvette when filled with KCl. This was applied for each experiment in order to correct the energy incident on the cells for changes in window transmission due to progressive filming and periodic cleaning.

Transmission measurements of suspended cells obtained from integrating

area volts from traverse data were checked by direct measurements with an integrating sphere. While the integrating sphere method is more rapid it requires components that are too bulky to be used in the limited space around the cuvette in the optical system described here. It was used, therefore, in a separate system as a check on the traverse method and to provide transmission data under a variety of conditions and suspension densities. Agreement was found to within 1 per cent.

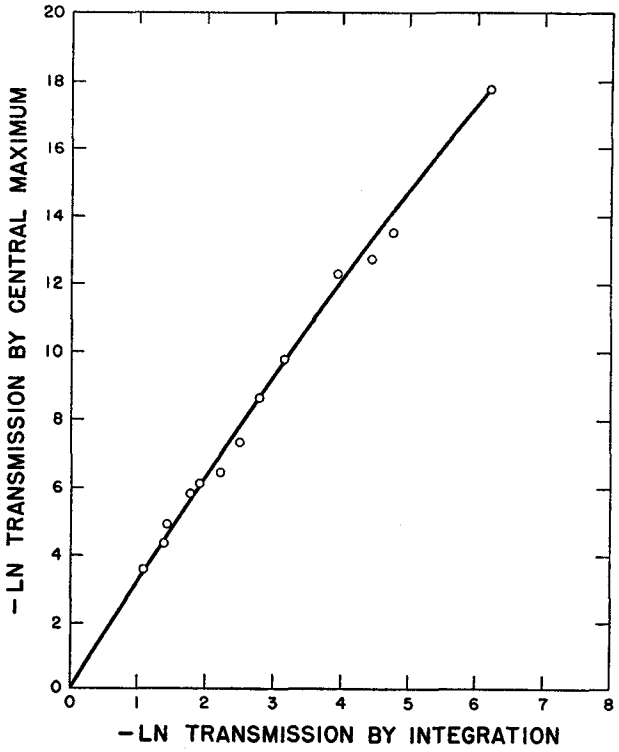


FIG. 8. Natural logarithmic plot of the data in Fig. 7.

Back scattering by suspended cells was measured by locating the cuvette outside the sphere at an opening cut out opposite the entrance of the beam, and was found to be less than 1 per cent.

A complete determination of traverse integration requires a large number of individual measurements. Because of the considerable amount of time required, it was necessary to use a shorter method for frequent checks. A single measurement of the intensity on the optical axis was found to be in substantially constant relation to the total power on the cylindrical surface, provided the illumination was undisturbed.

Without cells in the cuvette, the relation of central intensity to total power is simply linear. With cell suspensions of different densities values differing slightly for the two beams were obtained as shown in Fig. 7. Points obtained by the integrating sphere form a continuous curve which extrapolates through 0 and 100. Points by traverse integration are shown and check within 1 per cent. The data plotted logarithmically (Fig. 8) show a nearly linear relationship. Thus optical densities measured in the two ways are nearly in constant ratio. A departure from the linearity seen in the upper part of the curve is to be expected from the increase in path for light scattering of this character where the angle does not for the most part exceed 45°. The validity of using central maxima for the determination of incident power and transmission of cells depends upon the constancy of beam alignment and pattern. Photocell monitors were used to continually check the alignment as well as the intensity. The fact that little variation was experienced led us to believe that this method introduces no serious error.

Culture of Chlorella

Pure liquid cultures of *Chlorella pyrenoidosa*[4] were grown in sterile 250 mm. Drechsel gas-washing bottles, each containing 200 cc. of sterile medium of the same composition as that used by Warburg and Burk (1950) except that an equivalent amount of ferric citrate is substituted for the ferrous sulfate in order to avoid the loss of soluble iron. The orifice of the bubbling tube in each bottle was modified to provide a uniform bubble size at a rate sufficient to keep the cells in suspension when bubbled with a gas mixture of 5 per cent CO_2 in air at approximately 250 cc./minute. Each bottle was provided with cotton plugs and sterilized prior to use. A new culture was inoculated daily, taking full aseptic precautions, with about 0.5 mm.^{3} of cells from an uncontaminated culture slant on 4 gm. agar, 0.1 gm. peptone, and 0.1 gm. glucose in 100 cc. of culture medium described above. The average yield per culture at the end of 6 days was about 500 mm.^{3} of packed cells.

The cultures were maintained at 22°C. and illuminated from above by three Westinghouse 30 watt tubular white fluorescent lamps at an average distance of 18 cm. Cultures were checked for contamination after harvest by direct plating on nutrient broth agar or by inoculating nutrient broth slants with the supernate of an aliquot aseptically centrifuged.

Preparation of Cell Suspensions

Cell suspensions were centrifuged first for 5 minutes at 170 × g. Cells separated in this manner were discarded and the remainder of the suspension recentrifuged for 10 minutes at 690 × g. The supernate, which contained very

[4] Emerson strain obtained from Dr. Dean Burk.

few cells, was discarded and the centrifuged cells resuspended in 0.1 M KCl, buffer, or other suspension medium. All these operations were carried out as rapidly as possible, avoiding the prolonged state of cells in the packed or pellet condition. Immediately after the cells had been resuspended and diluted to the required absorption range the resulting suspensions were saturated with 5 per cent CO_2 in air at 22°C. for a minimum of 5 minutes up to the full time of dark or light conditioning desired for the experiment.

Experimental Procedure

Immediately before the start of an experiment photocell measurements were taken at the 90° and 270° position for the open beam (no cuvette) and for the cuvette filled with 0.1 M KCl in order to determine the incident power. This was followed by photocell measurements at 90° and 270° with the cuvette rinsed and filled with a sample of the suspension in order to determine from the per cent transmission the power absorbed by the cells. With a replicate sample of the suspension in the cuvette and after a period of 2 to 3 minutes to allow for temperature equilibrium, the recording of oxygen tension was begun.

Calculation of Quantum Efficiency

In calculating the number of quanta per molecule of oxygen evolved, it was convenient to introduce all the constants in a single factor. Thus the calculation only required multiplying this factor by a value for the incident intensity, the absorption, and dividing by the rate of oxygen production.

This factor was obtained as follows: From our thermocouple calibrations of the photocell, we obtained the sensitivity 1250 $\frac{\mu \text{ watts}}{\text{cm.}^2}$ for 1 volt of nulling potential on photocell. Thus expressing the energy in quanta we have

$$3.7 \times 10^{15} \frac{\text{Quanta}}{\text{Volt cm.}^2 \text{ sec.}}.$$

Since our experiments generally involved 3 minute intervals, it proved convenient to base our calculations on a 9 minute period. Our slope-measuring device was constructed to read directly the equivalent electrode change for 9 minutes. Since our electrode was calibrated by reference to equilibration with air, this 9 minute change was expressed as a fraction of air saturation from the ratio of deflections.

Thus we obtain our factor

$$\frac{3.7 \times 10^{15} \times 9 \times 60}{n_o} = 28.5 \frac{\text{Quanta incident}}{\text{Volt cm.}^2 \times 9 \text{ minutes} \times n_o}$$

in which n_o = the number of O_2 molecules dissolved in the cuvette when air-saturated at 22.2°C. This is calculated as follows:—

$$n_o = \frac{\alpha V N_o x}{N} \cdot \frac{760 - p}{760}$$

in which

α = Bunsen solubility coefficient for O_2 in 0.1 M KCl at 22.2°C. (0.0285 cc./cc.)
V = volume of the cuvette (0.445 cc.)
N_o = Avogadro's number (6.06×10^{23})
N = gram molecular volume at NTP (22,500 cc.)
x = mol fraction of O_2 in air (0.21)
p = vapor pressure of water at 22.2°C. (20 mm.)

A value in volt cm.^{2} for the intensity times area was obtained from photocell readings as follows:—

The value for the intensity at the central maximum A was converted by means of a plot of central maxima in volts against volts cm.^{2}, to a value E' in volts cm.^{2} for the incident power and corrected for window transmission and photocell change giving the radiant power E incident on the cuvette contents. Introducing the deflection for the electrode at air saturation H, and J the change in deflection or yield for 9 minutes, we have

$$q/m = 28.5 \cdot E \cdot F \cdot \frac{H}{J}$$

in which F is the per cent absorption.

In the following experiments approximately the same basic or highest illumination intensity was always used. Lower levels used in determining the influence of intensity were produced by inserting neutral filters.

This basic intensity may be expressed approximately as follows: A total of 870 microwatts/cm.^{2} incident on suspension in two nearly equal beams, and 775 microwatts, total power incident upon suspension or 1.37×10^{17} quanta/minute incident upon the suspension at λ 578 mμ, giving 2.7×10^{16} quanta/minute absorbed for 20 per cent absorption, the order of magnitude commonly employed. We can express these values

as 2.6 mm.^{3} O_2 gas volume NTP in cuvette
and 1 mm.^{3} quanta/minute absorbed.

Generally the volume of centrifuged cells was about 1.5 mm.^{3}/cc. or 0.66 mm.^{3} in the cuvette, though this was sometimes varied.

Expressed in Einsteins, E

0.23 μ E/minute incident upon the suspension (about 0.25 μ E/cm.^{2} minute)
0.045 μ E/minute absorbed

giving

0.068 μ E/minute absorbed per mm.^{3} cells.

While this may seem low compared with manometric experiments such as those of Burk and Warburg (1951) in which some 0.6 μ E have been reported, when one divides their value by the volume of cells, say 49 mm.^{3}, one finds 0.0123 μ E/minute per mm.^{3} cells so that some 2.5 to 5 minutes are required for the volume equivalent of absorbed quanta to equal that of the cell volume. Correspondingly our time is from 0.6 to 1 minute.

Thus effectively our basic intensity is relatively high, though as we will see no CO_2 or physiological limitation has been reached. Some of our observations were extended to $\frac{1}{16}$ of this basic intensity, thus covering a very wide range.

Significance of Data

In judging the significance of our data on respiration it is particularly important to distinguish between the variations in apparent rate of oxygen exchange which may be attributed to instrumental deficiencies and those changes which characterize the experimental material.

Since our limiting errors prove to be of systematic nature rather than random, statistical criteria would be valueless and even misleading. Instead we will indicate the nature of the data, its reproducibility, and the absolute errors to which it is subject.

Our original data are photographic recordings from an entirely automatic system. Fig. 9 is an unretouched reproduction of such data obtained when the algae are exposed to alternations of 3 minutes' light and 3 minutes' dark, and a blank run without cells superimposed for comparison.

Fig. 10 shows an enlarged portion of such a record as it appears in the field of the instrument for measuring slope. Each dot represents a galvanometer registration at the end of a 10 second cycle. The light illuminating the suspension is turned on or off in precise phase with the recording cycle and a fiducial line registered on the record.

The reticle line in the slope-measuring device may be set either (*a*) as the best approximation for a large number of points, or (*b*) as determined by two adjacent points, or (*c*) as a tangent to three points referring the data to the central point.

While a great deal of work was initially done with the first procedure, we found that much of importance was overlooked and systematic errors incurred which could be eliminated by more careful scrutiny of the data. The three point tangency gave closer reproducibility than the two point tangency.

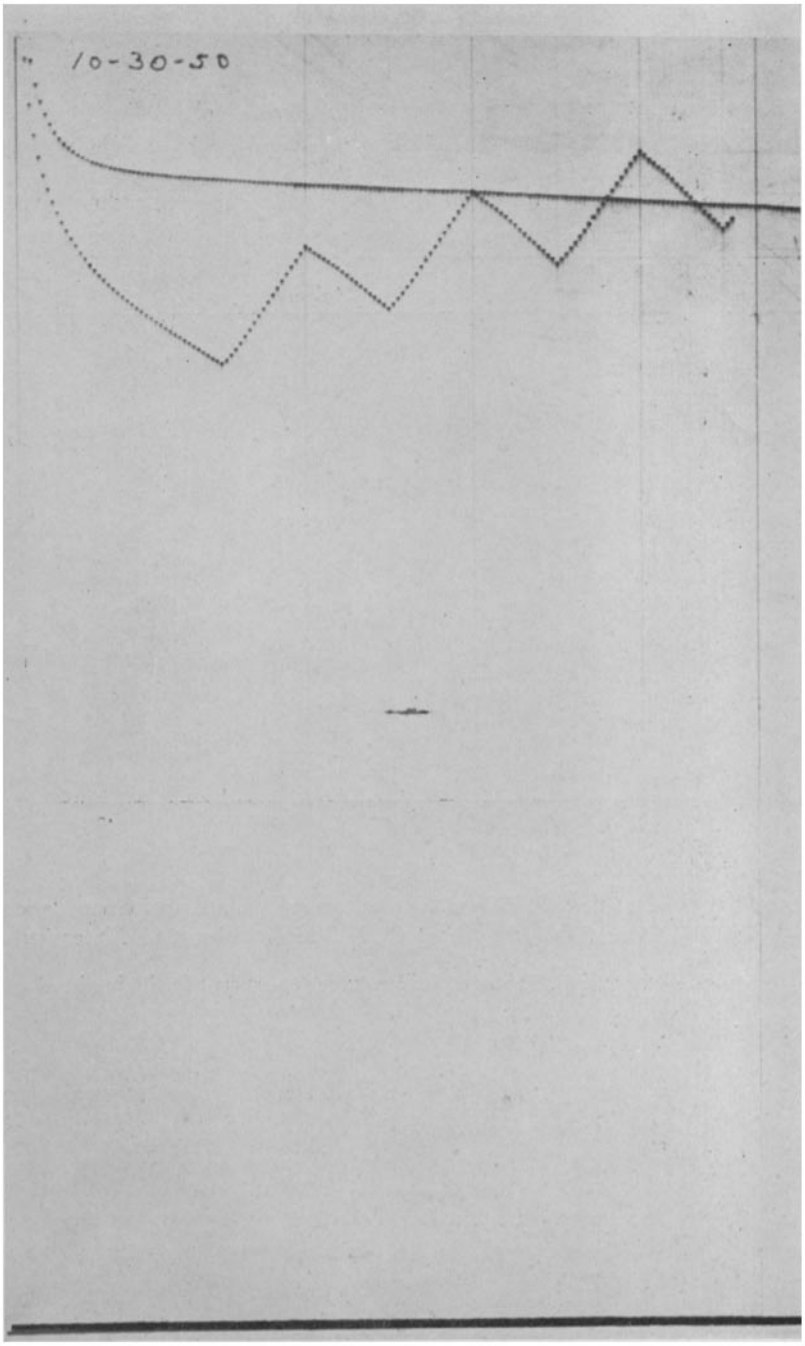


FIG. 9. Unretouched record of *Chlorella* suspension exposed to alternations of 3 minutes' light and 3 minutes' dark, with a superimposed blank run in air-saturated 0.1 M KCl for comparison.

Measured slopes are plotted in Fig. 11 as open circles; thus the rate of evolution of oxygen (millimeter deflection in 9 minutes) appears as a positive number in the light, and rate of oxygen uptake as a negative value in the dark. The data in this figure are quite typical.[5]

In order to test the reproducibility of such data the experiment was repeated in the afternoon of the same day with a new alga sample from the same preparation. Circles indicate the points from the morning run and tri-

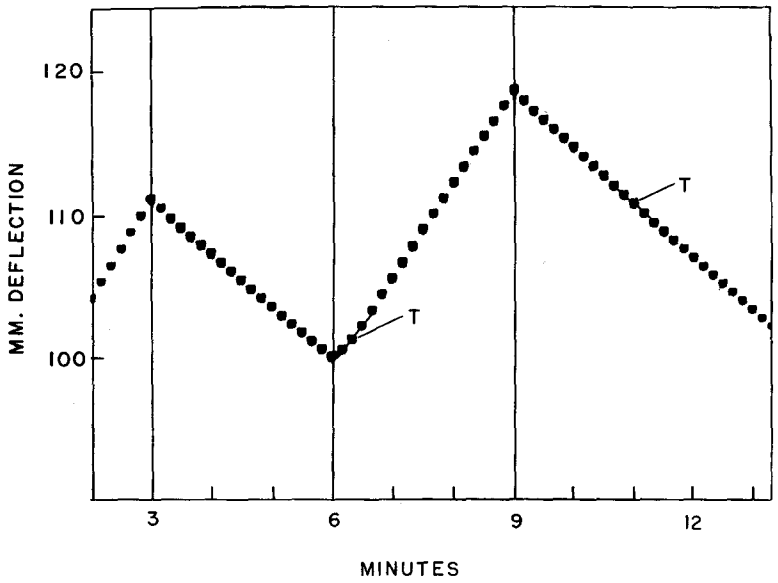


FIG. 10. Photographic enlargement of a record using 3 minutes' alternate dark and light intervals. 1.95 mm.^{3} cells/ml. 0.1 M KCl. 23.1 per cent absorption. Intensity, 835 $\frac{\mu w.}{cm.^2}$ incident on suspension. Photograph is unretouched except for inking in of scales and index lines.

angles from the afternoon run. The only change in procedure was to omit the third light period.

Minor ripples not exceeding ± 2 units appear in both runs, but displaced. These are instrumental and were traced to disturbance in temperature regulation (they were later reduced but never eliminated). A given observer could generally reset on the slope within ± 1 unit. Different observers sometimes showed a consistent difference throughout of about 1 slope unit.

In general character the two runs are remarkably alike. Nevertheless real

[5] The slope expressed in units divided by 1.6 gives an approximate value of change in per cent per minute of air saturation oxygen concentration.

differences are observable. After the first light period the triangles become about three units lower than the circles. Since this increment is the same in both light and dark, no difference in photosynthetic rate results. The difference is explained by a greater rise in respiration during the second run. One also observes a difference in the first few points during the light which may be an actual difference in the induction.

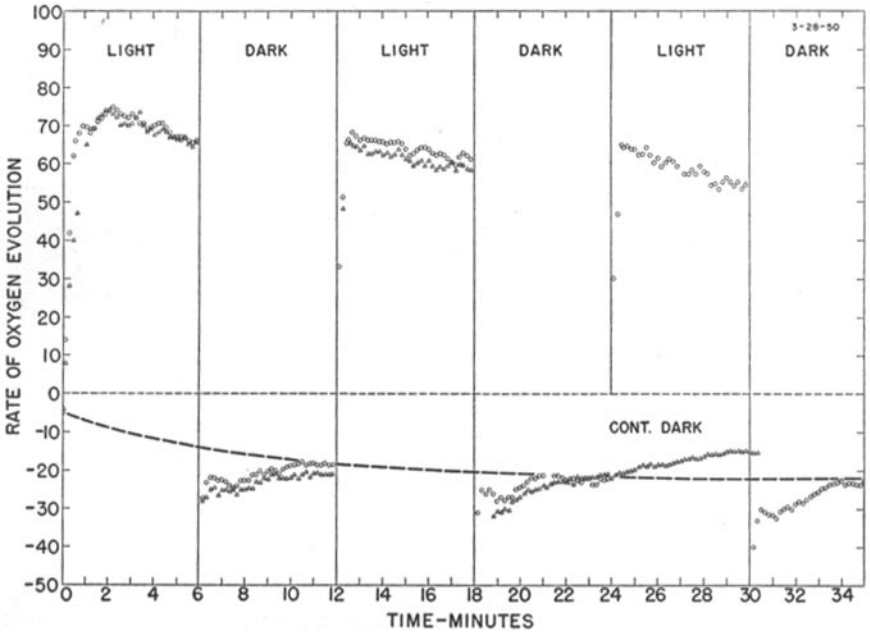


FIG. 11. Two separate runs on the same algae showing the duplication of every important feature. The second dark period for the second run, the triangles, was extended to follow the respiratory decay. The circles represent the same data as shown in Fig. 15.

This reproducibility of the steady rate reached by photosynthesis was verified in at least 20 experiments. Variations were always less than ± 5 per cent and generally within ± 2 per cent.

In contrast both respiration and induction showed wide variations in these same experiments.

Thus, based upon the reproducibility for known conditions without cells and the reproducibility of total photosynthetic rates with cells, we feel confident that our errors may be summarized as follows: (1) random errors of slope—less than ± 1 unit; (2) systematic uncertainties of slope measurement —± 1 unit; (3) periodic thermal fluctuations—less than ± 2 units; and (4)

reproducibility from sample to sample—usually better than ±3 slope units. Since a unit of slope represents about 1 per cent of the total photosynthetic rate at our full intensity these errors expressed in slope units may be considered as percentage errors.

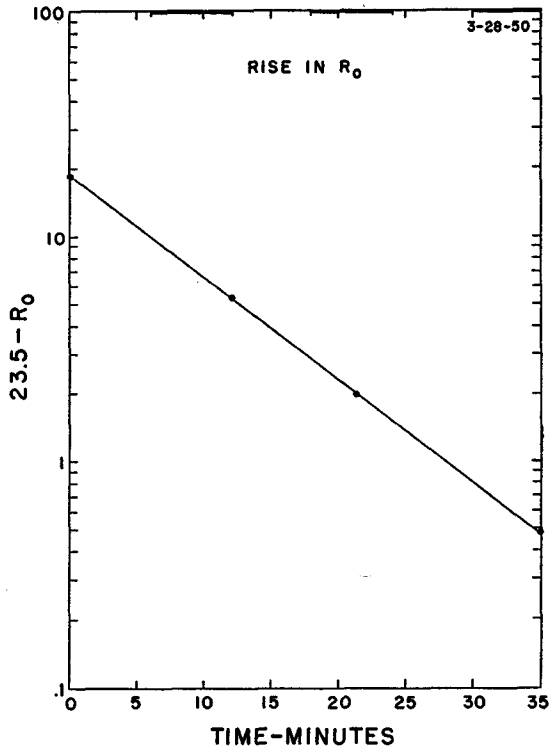


FIG. 12. Logarithmic rise of the "background" or 6 minute respiration value R_o to steady value $R = 23.5$ (arbitrary slope units) shown as a dotted curve in Fig. 11.

So long as we are concerned with relative considerations such as time variations and intensity influences, these are the only errors of importance. In our second paper we will consider the absolute errors incurred in the various radiation measurements.

Respiration

Respiratory changes are a striking feature of most of our recordings. Such changes may sometimes be as much as ten times our likely error. Furthermore, these changes follow a recognizable pattern. Not only are these characteristic changes in respiration of interest in themselves, but they present a serious difficulty in evaluating photosynthetic rate for small changes in light intensity.

Gradual changes in respiration have been observed manometrically and by other slow methods. The shorter time changes appearing with our time resolution are of particular concern.

In Fig. 11 we see that respiration decays during each dark period, but that the terminal values gradually rise toward an asymptote, thus having increased from an initial 5 to about 23 as shown by the dotted line.

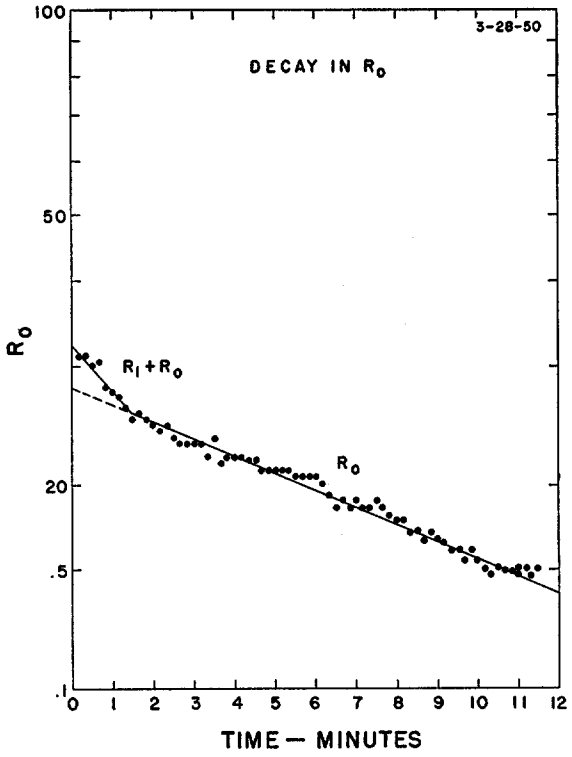


FIG. 13. Shows the initial decay of the pulse R_1 followed by the slower decay of the background respiration R_0.

Despite the roughness (± 2 units) of our slope data, a systematic form is discernible. In each dark period we observe repeated characteristics: The first two or three initial points start at a very high value and decay sharply—these we will ignore for the moment; a maximum is reached 1 or 2 minutes after the start of darkness; a subsequent decay appears which we can arbitrarily represent by a log curve.

To obtain a satisfactory representation of the respiratory behavior which is shown in our records three aspects should be considered:—

1. A background R_0 which rises during either steady or intermittent illumination and decays slowly in the dark.

2. An additional superimposed pulse R_1 which rises owing to each light period and decays rapidly in each dark period.

3. An initial, sharply decaying transient lasting less than a half minute.

From the dotted curve in Fig. 11 replotted in Fig. 12, we have a time constant for the rising background (R_o) of $\tau = 9.7$ minutes (of the type in which $R_o = R_o'(1 - e^{-(t/\tau)})$).

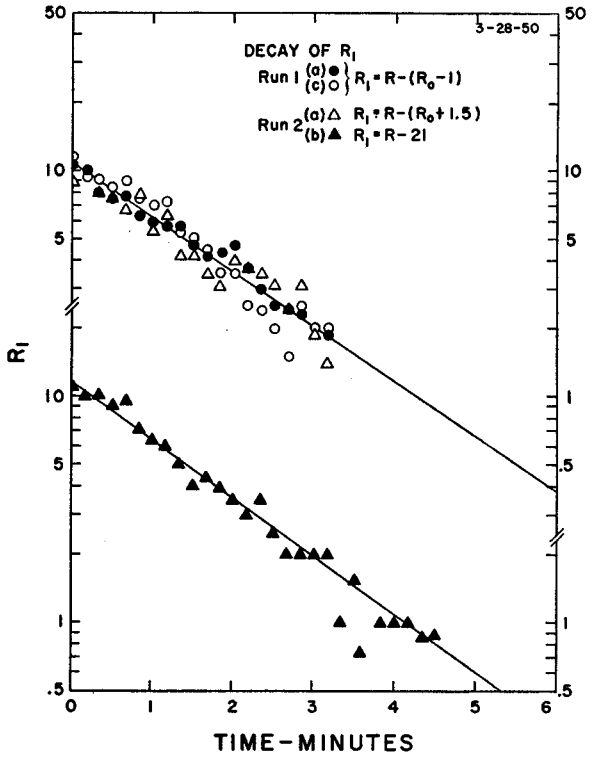


FIG. 14. Logarithmic decay of pulse toward the background rate R_o (run 2(b) displaced one log unit).

From the prolonged dark period in Fig. 11 we find a decay time constant as in Fig. 13 of $\tau_o = 16$ minutes (as in $R_o = R_o'e^{-(t/\tau_o)}$).

If now the background R_o is subtracted from the respiration observed in each dark period we may plot the residual data on semilog paper as in Fig. 14. All these data give a time constant for R_1 of about 1.8 minutes and the adjustment of asymptote is not more than 1.5 units from those found in Fig. 11.

While our data are too rough to prove that a logarithmic form is inevitable,

they are satisfied by this representation and the time constants are determined on this assumption within at least 10 per cent.

Our pulse (R_1) decay time $\tau = 1.8$ minutes is nearly ten times faster than the background (R_o) decay time $\tau = 16$ minutes, suggesting two quite different phenomena.

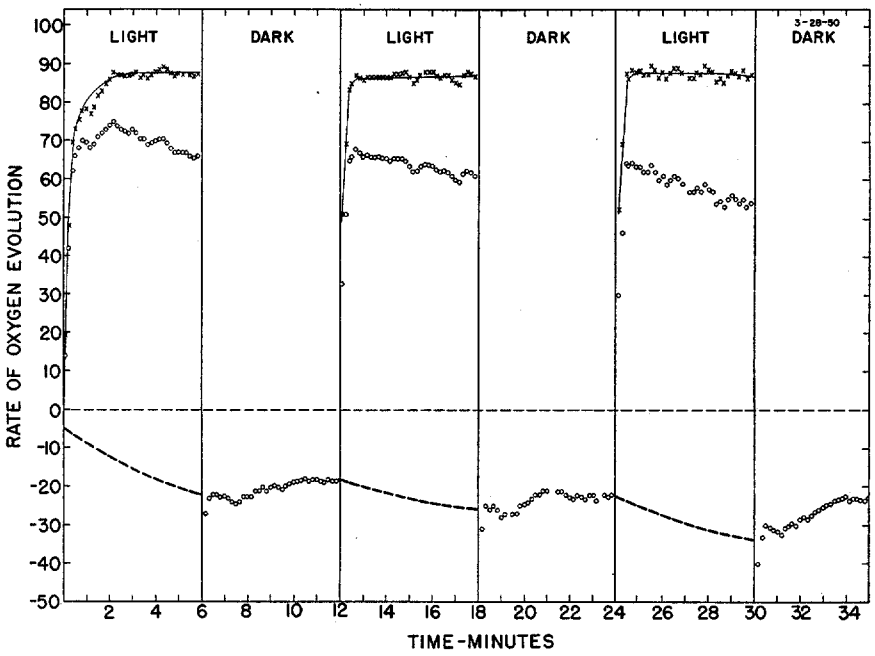


FIG. 15. Point-by-point tangent slope plot (open circles) showing the time course of oxygen evolution rate for a typical experiment. Broken lines represent logarithmic interpolation of respiration during light. Upper curves, indicated by crosses, represent the implied rate of photosynthesis obtained from interpolated light respiration.

Evaluation of Photosynthesis

If we are to compute total rate of photosynthesis it is necessary to know the respiration taking place during the light. During the first light period in Fig. 15, if we assume continuity, respiration must rise from about six units as observed beforehand to about 24 units as observed afterward, ignoring the first point or two. Let us assume that the rise is simply logarithmic as indicated by the dotted curve in Fig. 15. Adding these values to the observed points (open circles) we obtain points (crosses) above, and similarly for the other light periods.

Ignoring the small ripples of less than 2 units which appear quite uniformly

throughout, we find total photosynthetic rate exhibiting a quite simple behavior: (1) a brief induction period which becomes less pronounced, sharper, and of shorter duration in succeeding periods; followed by (2) a steady constant rate after induction is over. This is repeated in each light period.

This type of interpolation between the known dark values of respiration seems very satisfactory and is similar to expedients used by Emerson and Lewis (1943) to meet a similar though slower time change of respiration observed in their experiments.

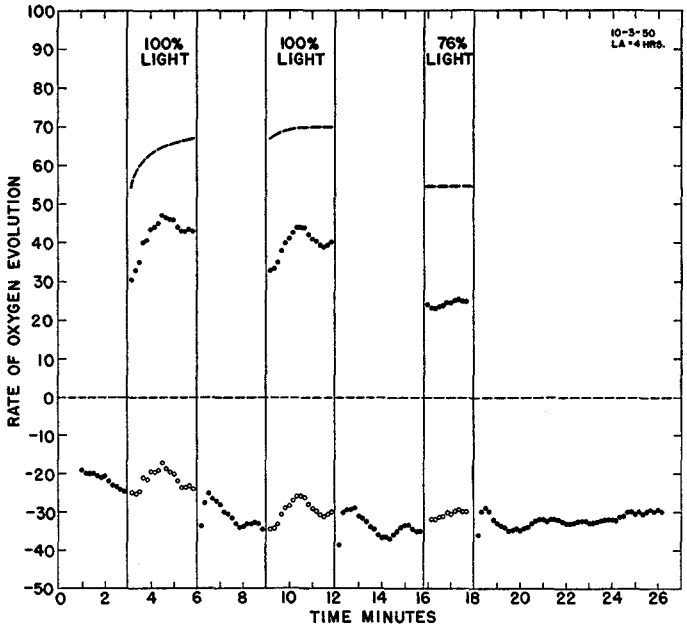


FIG. 16. Slope plot of 76 per cent intensity experiment using the standard procedure for interpolation described in text.

Unfortunately, respiratory behavior is not always so simple, especially when shorter periods of illumination are used.

Figs. 16 to 18 are a series obtained in order to determine the influence of intensity. If average respiration values are used or, as we actually did at first, the best tangent to all the points is measured for slope, one obtains the points given in Fig. 19.

The separate values for evolution and the corresponding values for respiration are shown separately with the points coded to correspond. Even this raw data uncorrected for electrode consumption and difference of one run to the next show both evolution and respiration varying in a roughly linear manner with light intensity. However, the two lines do not extrapolate to the same point for zero light.

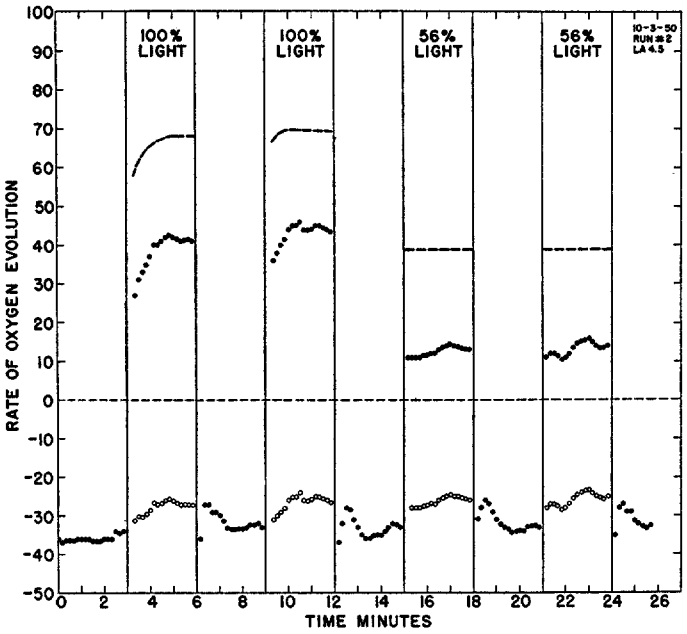


FIG. 17. Slope plot of 56 per cent intensity experiment using the standard procedure for interpolation described in text.

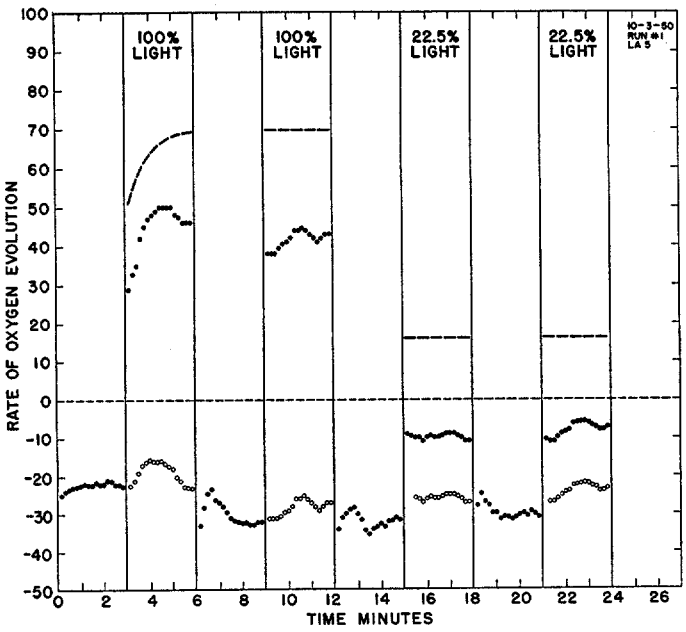


FIG. 18. Slope plot of 22.5 per cent intensity experiment using the standard procedure for interpolation described in text.

When corresponding respiration and evolution points are added, a remarkably linear dependence is found for total photosynthesis. However, the failure to extrapolate to zero is still more marked.

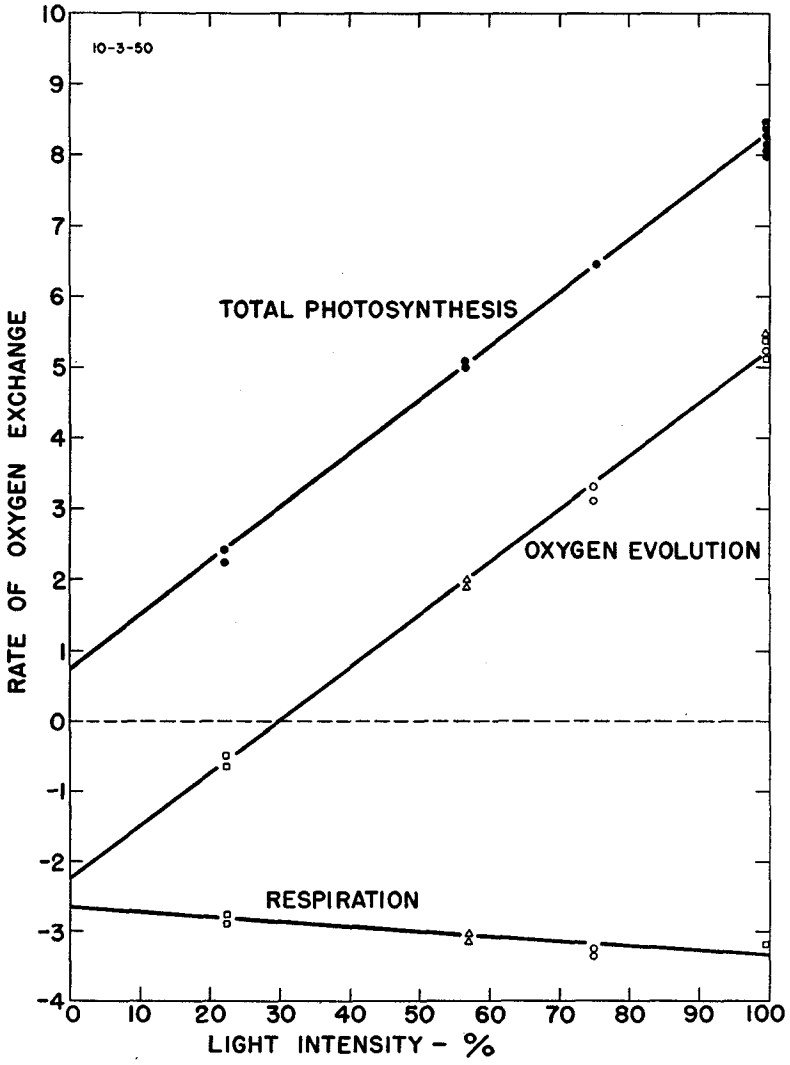


FIG. 19. Relation between oxygen evolution and light intensity, with rates of evolution based on slope of best straight line through points of record.

Many repetitions failed to eliminate the extrapolated zero-light discrepancy. We judged the difficulty to be many times our likely error. However, we

found that the magnitude was different depending upon whether the observations at reduced intensity preceded or followed those at high intensity.

Extending the observations to lower light intensities and inverting the order of procedure, the values shown in Fig. 20 were obtained. Here the limits

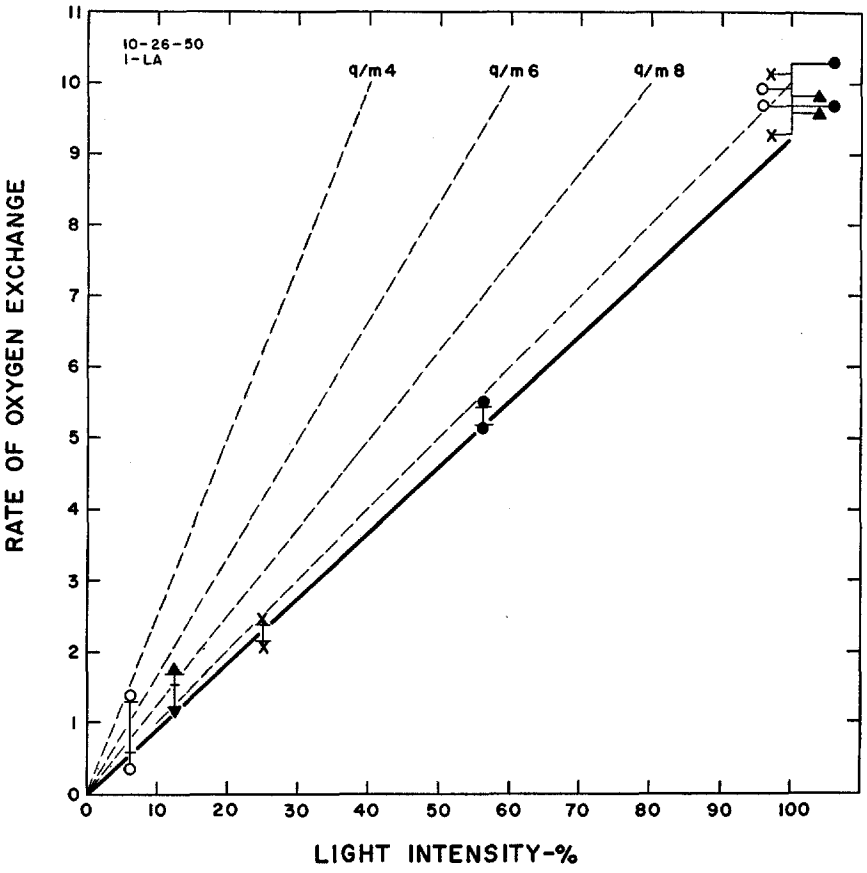


FIG. 20. Plot of experimental data similar to that in Fig. 19 but extended to lower intensities and with sequence of intensities reversed (low light first). The broken lines express the rate of oxygen exchange for the quantum efficiencies indicated and serve to show the implied efficiencies.

arising from using higher or lower possible respiration values are shown. The lower extrapolate quite satisfactorily through zero but the upper imply progressively higher efficiencies for lower intensities as shown in Fig. 21. The bend in the curve occurs at about compensation.

These higher efficiencies implied by the higher respiration limits are very

similar to those found by Kok (1948) manometrically. We have verified the Kok effect repeatedly and find quantitative agreement with his findings when our rates are based upon average respiration.

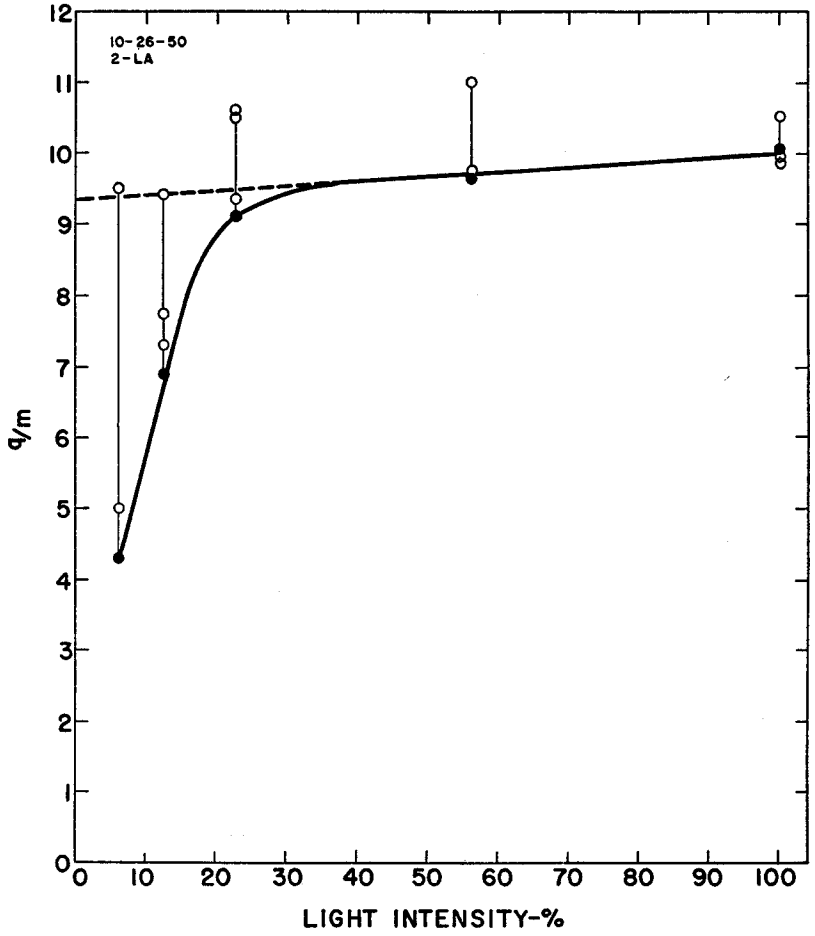


FIG. 21. Implied high quantum efficiencies at low light intensity by using the upper limits in Fig. 20.

However, we find that the nature of the experiment affects the magnitude and the magnitude lies within the range which can be accounted for by respiratory variation.

Let us now attempt to find some form of interpolation which takes account of the observed changes in respiration.

Formalizing our observations as to respiration and photosynthesis suggests

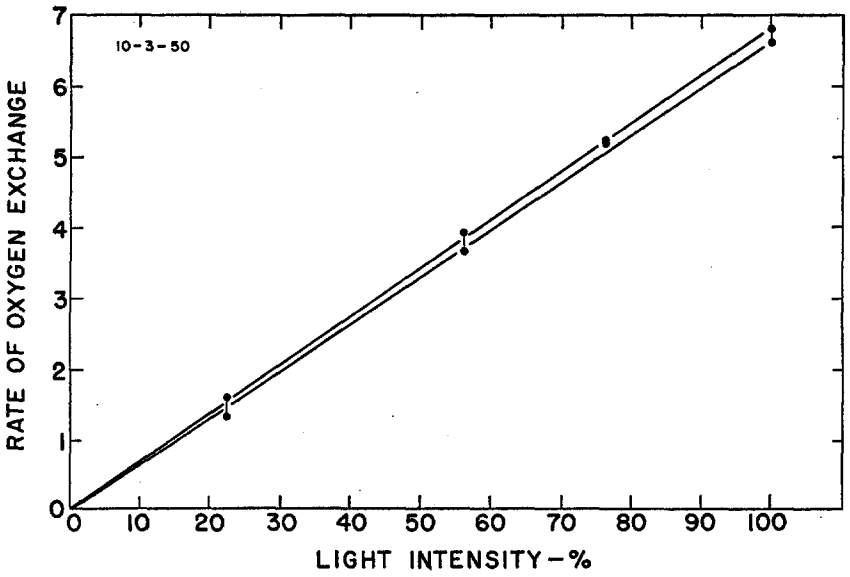


FIG. 22. Relation between oxygen evolution and light intensity, with rate of evolution based on the point-by-point slope plots of Figs. 16, 17, and 18.

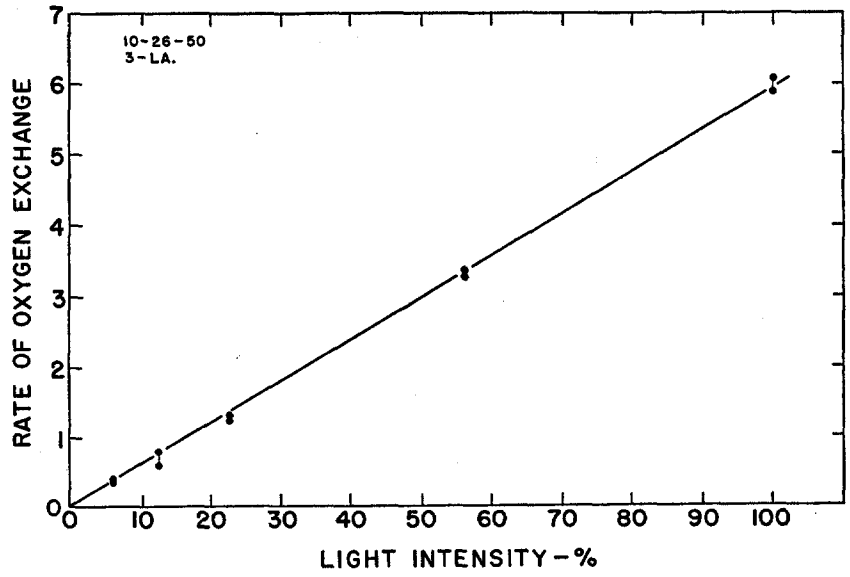
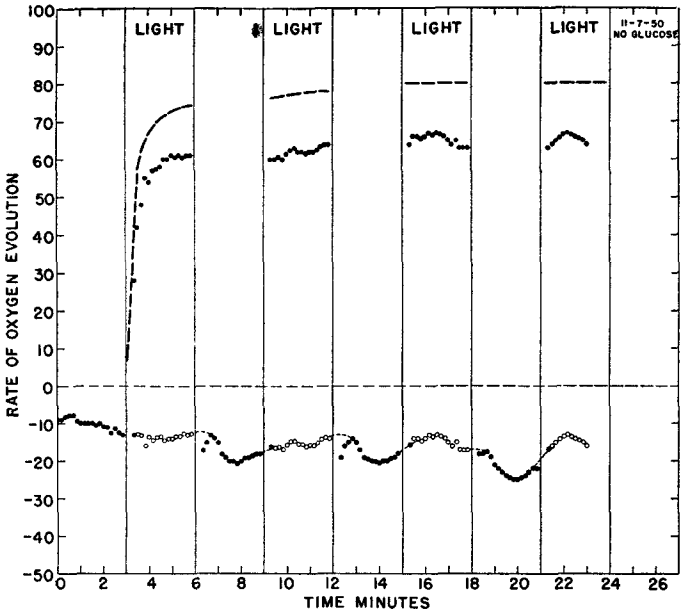
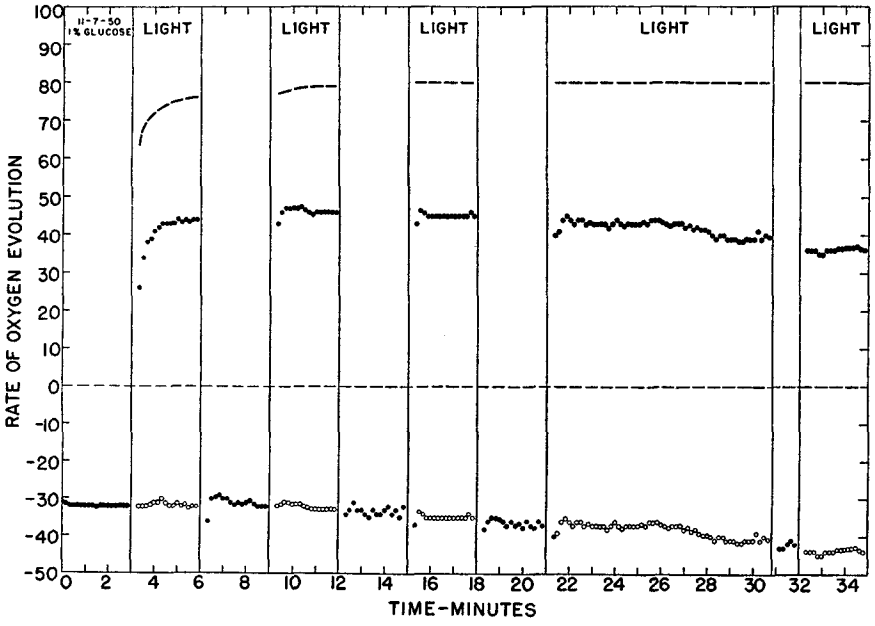


FIG. 23. Replot of data in Figs. 20 and 21 using the interpolation procedure, thus eliminating the failure to extrapolate to zero and the implication of high efficiency at low light intensity.



(*a*)



(*b*)

FIG. 24. Comparison of behavior without and with glucose. (*a*) 0.1 M KCl—no glucose. (*b*) 0.1 M KCl + 1 per cent glucose. Respiration has been doubled by glucose. The pulses have disappeared. The photosynthetic rate is unchanged. The induction time is reduced. Solid circles, observed points; open circles, interpolated points, broken line, implied photosynthetic rate.

the following procedure: (*a*) ignore the first two or three points in the dark; (*b*) fill in respiration points in the light by working backward from the subsequent dark period on the assumption that the photosynthetic rate is essentially constant after induction is over; (*c*) connect to the previous dark values as simply as possible; (*d*) adjust to secure reasonable continuity but for expected error of ± 2 per cent.

The result in every case is a quite regular fluctuation of respiration many times that which one observes in control blanks.

A larger part of the amplitude of this short period wave or fluctuation in respiration usually occurs in the dark where there can be no doubt of its reality. The amount varies with the duration of light and dark—with dark adaptation and other attributes of the culture. In all cases, however, it is repetitive and shows a definite pattern.

With this type of interpolation we can reevaluate our determinations. The data in Fig. 19 become those in Fig. 22. Similar experiments extended to lower intensities are given in Fig. 23. All evidence of non-linearity has disappeared. Instead we have assumed that an appreciable fluctuation of respiration occurs in the light, as well as in the dark where it is observed.

If one still doubts the reality of these respiratory fluctuations let us consider an experiment with and without glucose.

When average slopes were used the quantum efficiency was found to be appreciably higher without glucose than it was with glucose. Point-by-point slope plots are shown in Figs. 24 *a* and *b*. The well defined fluctuations found without glucose disappear with glucose where only our typical instrumental variations remain except for the first transient point or two, which is still to be found. Computed rate is now the same for both.

DISCUSSION

In darkness and in the absence of substrate respiration falls to a hardly measurable rate. Glucose or a prolonged exposure to light raises the rate to about the same high level.

Intermittent exposure (1 to 6 minute periods) results in a periodically varying respiration rate, a maximum respiration occurring after 1 to 2 minutes of darkness.

Decay of respiration in the dark exhibits two components—a fast decay of the order of 2 minutes time constant, and a slow one some ten times greater. Thus several hours are required for respiration to reach a dark-adapted low value.

The behavior of respiration suggests that the rate is controlled by substrate. One might postulate that photosynthesis supplies at least two materials, one which is used up rapidly and the other slowly. The supply of the first reaches a maximum after an appreciable delay of 1 to 4 minutes while the maximum of the second is approached more slowly.

This picture of respiratory behavior seems quite plausible. The implications of such a picture are important in evaluating quantum efficiencies whenever the respiratory contribution becomes a substantial part of the calculation.

Because of the delay or phase lag in the periodic variation of respiration for intermittent illumination, the average respiration in the dark may be greater than the average in the light. This results in assuming a higher photosynthetic rate than actually occurs. This error becomes important only when low illumination levels or small differences in level are studied.

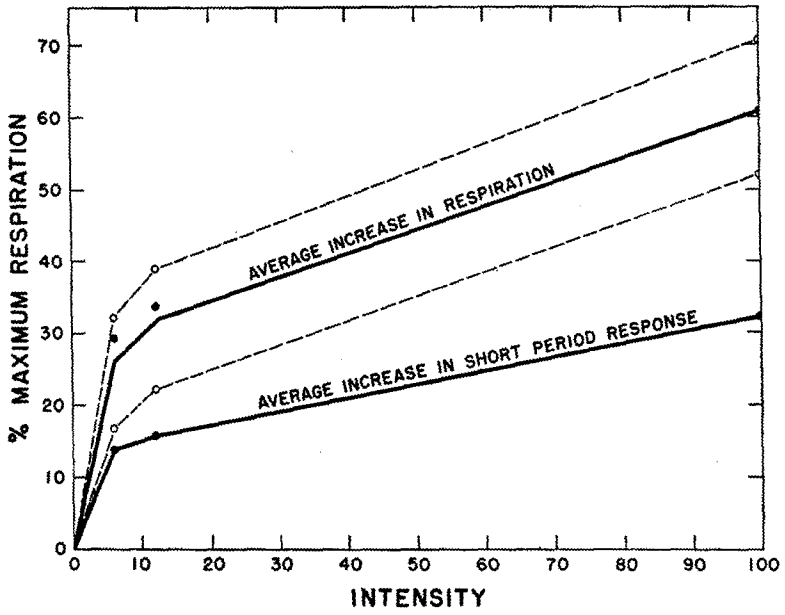


FIG. 25. Intensity dependence of respiration for dark-adapted algae. Upper solid curve, increase in respiration, R_o—average of several experiments. Points, values for three separate experiments, broken lines, limits, lower solid curve, average increase in short period or pulse response, R_1.

The dependence of respiration on intensity shows another reason why this error becomes important below compensation. Despite the capriciousness of respiratory behavior, one observes certain consistent trends. In Fig. 25 we have plotted the increases in respiration due to intermittent illumination against intensity. In one curve we show the increase in background (R_o) which occurs after several periods of illumination; in the other the amplitude of fluctuation (R_1), which also results. The solid curves are the average of many experiments. The individual experiments all show similar trends but differ greatly in magnitude. The limits of this variation are shown by the dotted curves, open circles being actual points for particular experiments.

The similarity of the curves is striking. Both undergo most of the change below light levels of compensation. It suggests that respiratory demands exert a prior claim on the products of photosynthesis.

The fact that respiration rises with increased light intensity, so rapidly below compensation and so slowly above, explains the bend in apparent efficiency curve (Fig. 21).

In our discussion so far we have ignored the first few points obtained in the dark. Respiration appears to start at a very high value and then sharply decays to a slower pattern in a matter of 10 to 30 seconds. The fact that these initial transients are unaffected by glucose indicates that they are a different mechanism than either type of respiration (R_o or R_1). By neglecting these transients we have found q/m to be independent of intensity. If they imply a higher instead of a lower respiration during the light we are in trouble as to any evaluation of photosynthesis. Our first point is subject to larger uncertainty than others and makes extrapolation difficult. However, it should be particularly noticed that this phenomenon is in the opposite direction to an instrumental lag, and so is not likely to be an artifact.

Nevertheless, these sharp transients present a pattern occurring markedly under some conditions and to a less extent under others. The fact that they are unaffected by glucose sets them apart from the general respiratory pattern and encourages us to postulate that this is not respiration. Possibly it is a "back phenomenon" occurring only when photosynthesis stops. In this case there would be no implication of occurrence in the light and we have incurred no error as a consequence in our evaluation of photosynthesis. Since the time integrals of oxygen exchange due to these transients would be small they would not make a large contribution to average respiration excepting for very small time intervals. It is of particular interest to note that these transients correspond very well with Strehler and Arnold's (1951) observed phosphorescence, thus giving further support to the idea of a "back reaction."

CONCLUSIONS

1. Respiration changes as a result of illumination.
2. In the absence of glucose or other supply of substrate, respiration decays in the dark showing at least two types—a fast decay in a few minutes and a slow decay lasting hours.
3. Respiratory response to illumination is delayed.
4. Intermittent illumination (in the absence of glucose, etc.) produces a periodic variation in respiration with a delay or phase lag.
5. Periodic variation of respiration may produce a higher average value in the dark than in the light due to the lag and depending upon the period of intermittent illumination.
6. Based upon average respiration values our data confirm the Kok effect.

7. Interpolated values of respiration, however, result in photosynthetic rates which are linearly dependent upon intensity of illumination.

8. Thus the quantum efficiency is found to be independent of intensity, over the wide range of intensities investigated.

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