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# THE RELATION OF QUANTUM REQUIREMENT IN PHOTOSYNTHESIS TO RESPIRATION

James A. Bassham, Kazuo Shibata, and M. Calvin

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# QUANTUM REQUIREMENT IN PHOTOSYNTHESIS

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## ABSTRACT

1. The rates of photosynthesis and subsequent respiration of Chlorella pyrenoidosa were measured using an oxygen analyzer (sensitive to paramagnetism). The energy absorbed during the photosynthesis periods was determined and the quantum requirement was calculated.

2. Dark respiration rate was found to depend on the rate of light absorption during the period of photosynthesis, and increased with increasing photosynthesis rate.

3. The quantum requirement, corrected for respiration, varied from 4.9 (at a ratio of photosynthesis to respiration of 1.4) to 6.9 (at a ratio of 12). Both uncorrected and corrected quantum requirements approach an experimental value of 7.4 at high light intensity.

4. The lower quantum requirement obtained at low light intensity is believed to be due to a relatively greater importance of contribution of energy from respiration to photosynthesis. An expression is derived for the relation between this contribution and the enhancement of dark respiration due to the level of photosynthesis to which the plants are conditioned.

5. Attempts to obtain the blue-light stimulation of photosynthesis with algae photosynthesizing in red light were unsuccessful.

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## INTRODUCTION

Many experiments on the minimal quantum requirement of photosynthesis have been reported. These experiments employed a variety of analytical techniques and physiological conditions. Despite this extensive study there exists as yet no unaminity or even a consensus regarding the actual value of this important number. Opinion is still divided between the values ranging from 2.8 to 4.5, reported by Warburg and co-workers[1, 2, 3, 4] and those around 8.0 reported by a number of other workers.[5, 6, 7, 8]

Reviews questioning the low light requirement reported by Warburg have generally pertained to the interpretation of the experimental data and to a discussion of the improbability of such an efficient energy transfer in such a complex and rapid process.[9] Some criticism,[9, 10, 11, 12] however, has been directed to the manometric technique that was employed by Warburg and co-workers in most experiments.

The criticism of the interpretation of the data, which in many instances involves a consideration of assumptions made in connection with respiration,[12, 13] loses some of its force when applied to Warburg's more recent papers,[14] in which long-term experiments at high light intensities are reported. In these experiments, the ratio of photosynthesis to respiration rate is so high that respiration corrections can be neglected. As for the objections to the very great efficiency of energy utilization for photosynthesis, model systems are proposed for a four-quantum process that do not seem too unreasonable.[15] This is made easier by what is now known about the transfer of energy to the carbon-reduction cycle,[16] this transfer being about 85% efficient. Thus the ultimate resolution of this controversy may lie in the satisfactory evaluation of the manometric techniques.

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A complete evaluation of techniques is beyond the scope of this paper. It may be of interest, however, to note that manometric techniques are critically dependent on small changes in solubility of carbon dioxide in the medium, and that this solubility is influenced by changes in hydrogen ion concentration at any physiological pH. Tolbert[17] has noticed that under suitable conditions, in particular at high light intensities and high $CO_2$ pressures, some algae secrete into the medium considerable amounts of glycolic acid. It is conceivable that in some of the quantum-requirement studies at rather high light intensities, where photosynthesis exceeds respiration by a factor of forty, acid secretion of this type could gravely endanger assumptions required in any purely manometric method. In view of this and other objections frequently raised to the manometric technique, it seemed desirable to calculate quantum requirements from measurements based on unique physical properties of oxygen and carbon dioxide independently.

Furthermore, an investigation of the possible relation between respiration and photosynthesis seemed in order. Such a study was made more attractive by the work of Brown,[18] who has measured respiratory uptake of oxygen gas enriched with $O^{18}$ and found that during alternate periods of light and dark of about fifteen or twenty minutes each, the rate of respiratory uptake in the light and dark is the same for Chlorella pyrenoidosa. This was found to be true at ratios of oxygen evolution during photosynthesis to oxygen uptake during respiration which are estimated from his data to range from 0.5 to 12. Thus, Brown's study forms a basis for the assumption that during these periods of alternating dark and light, the respiration is the same, so that in quantum-yield calculations it becomes possible to apply this dark respiration rate as a correction to the observed oxygen evolution in the light. Also important to the present discussion is Brown's observation that, in some cases at least, the rate of respiration in the dark following a period of illumination was greater than the rate of respiration following a long period of darkness. This effect was found to last for some time following illumination, and the enhanced rate continued into subsequent periods of illumination. Enhancement of respiration rate in the dark following a period of illumination was also reported earlier by Emerson and Lewis,[19] by Weigl et al.,[20] and by Brackett et al.[21] It appears, therefore, that the effect of illumination on respiration is a long-lived enhancement of the rate of respiration, which remains constant during periods of light and dark of the order of fifteen minutes or longer.

The method here reported involves a rather direct measurement of oxygen evolution and light absorption during photosynthesis, and of oxygen uptake during respiration, without recourse to complex corrections. Special precautions and a new technique were employed in the measurement of absorbed light. The observations of the quantum requirement were made with a variety of light intensities, the lowest intensity being near the compensation point. This made possible an investigation of the contribution of energy from respiration to photosynthesis and of the validity of corrections for respiration in quantum requirement calculations.

An attempt was also made to observe the effect on the quantum requirement of adding a small increment of blue light to the red light which was used for all of the quantum requirement measurements. This study was suggested by the recent paper of Warburg,[22] who found that such an addition decreased the quantum requirement very markedly. Here again, however, it is possible that the blue light effect may involve a photoactivation of some acid-secreting enzyme.

## METHODS AND MATERIALS

The system used in this experiment is shown schematically in Fig. 1. The essential parts of the system are a cell C containing the suspension of algae, a carbon dioxide analyzer ($CO_2$ in Fig. 1) and in oxygen analyzer ($O_2$ in Fig. 1), which are connected with each other by glass tubing in a gas-circulating system. By a stopcock A, we can either open the system for the introduction of a gas or close it for the observation of the rate of photosynthesis or respiration. The main flow of gas, which is indicated by large solid arrows, is produced by the pump $P_1$. A smaller gas flow is necessary for the oxygen analyzer and this is provided by a branched system with a capillary K and another pump, $P_2$. The oxygen analyzer is very sensitive to any change of the total pressure of gas. To maintain the system at atmospheric pressure and to reduce small pulsations of pressure, a rubber membrane H between the system and the atmosphere and a bulb D were used. J is the inlet for introduction of the suspension of algae and $M_1$ and $M_2$ are flow meters.

Oxygen was determined by a Beckman oxygen analyzer measuring the paramagnetism of oxygen, and carbon dioxide was determined by a Liston-Becker carbon dioxide analyzer measuring the infrared absorption by carbon dioxide. The two signals from the analyzers were automatically recorded by a multipoint recorder. The sensitivity of the recording system and the amplification of input signals were so adjusted that full scale on the recorder paper represented a 2% range for oxygen and a choice of either 2% or 5% for $CO_2$. There is a close linear relationship between the reading obtained from the oxygen analyzer and the true partial pressure of oxygen, but the response from the carbon dioxide analyzer deviates considerably from linearity. Therefore, the amount of oxygen generated in photosynthesis or absorbed in respiration was used for the calculation of quantum requirement, while the change of carbon dioxide was used only for reference.

In order to calculate the total oxygen evolved or absorbed, it is necessary to know not only the sensitivity of the system but also the total volume for oxygen. Both calibrations are accomplished by using two tubes G of known volume, from which pure nitrogen gas can be added to the system in two increments. Observation of the change in scale reading with each addition of nitrogen provides the two equations from which the total volume and the sensitivity can be calculated. The volume of the system for oxygen was

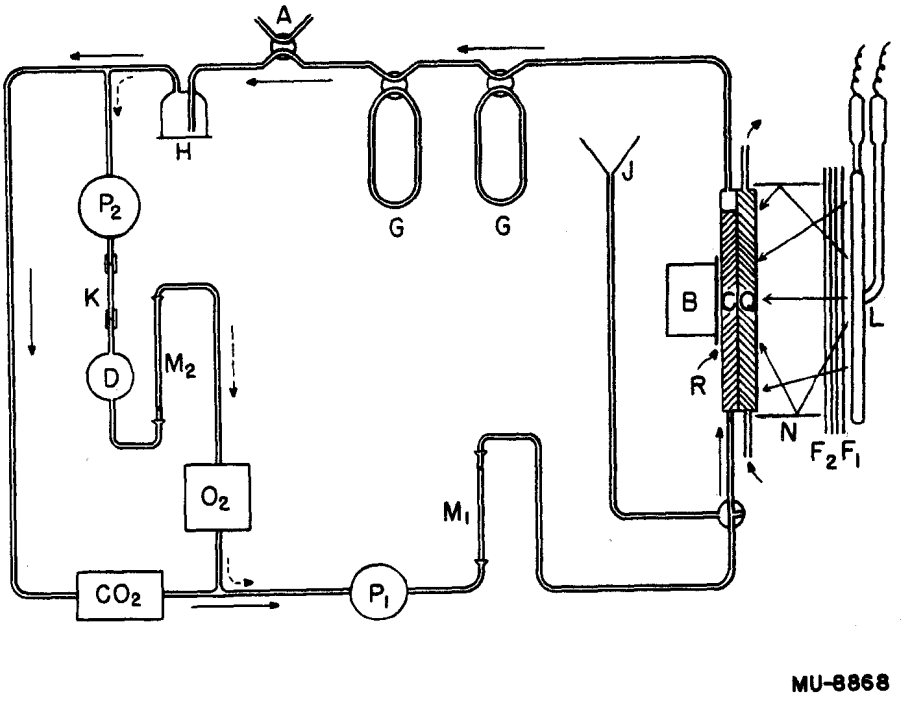


Fig. 1. Schematic Diagram of Apparatus for Measuring Quantum Requirement of Photosynthesis.

found to be 303 cc. After the volume was measured, the sensitivity of the system was adjusted to 0.2% per large division, or 2% full scale, and again checked.

The volume of algal suspension used was 93.5 cc in a disc-shaped cell 12.55 cm in diameter and 0.84 cm thick (inside dimensions), giving a total volume of 103.5 cc. The vessel is made of clear colorless plastic (lucite) and is equipped with a cooling-water jacket Q.

The light source consisted of two spiral neon tubes made of special red glass to prevent the transmission of the light other than nearly monochromatic red light (6300 Å). An examination of the spectral distribution of energy coming from this source indicated that more than 99% of the emitted energy lay in the wave-length region between 6150 and 6550, with most of the energy between 6260 and 6390. A weighted mean of the energies at the several wave lengths gave 6376 Å, and this was used in the calculation. Energy at wave lengths shorter than 6000 Å is less the 0.02% of the total. Two sheets of infrared-absorbing glass $F_1$ were set in front of the light source to absorb thermal radiation. At the same position, two sheets of plastic polaroid filters $F_2$ were inserted, so that the incident light intensity could be controlled by rotating these filters relative to each other. The incident light intensity was varied from $7.47 \times 10^{-5}$ to $1.478 \times 10^{-3}$ watt/cm$^2$ sec. Between these filters and a cell, an aluminum reflecting tube N was used to provide a more uniform field of diffuse light. The energy of light absorbed was observed with a Kurlbaum large-area bolometer B. A sheet of opal glass R was attached to the face of the bolometer.[22] This is necessary because the detecting surface is far from the quartz window of the bolometer, so that not all the diffuse light entering the window reaches the detecting surface. The proportion of light entering the window which reaches the detecting surface is kept constant regardless of the diffuseness of the light incident to the opal glass, because the opal glass scatters all incident light uniformly.

The calibration of the bolometer was carried out with three different standard lamps without an opal glass. The ratio of the sensitivities with and without opal glass was obtained by an experiment in which the same neon light with infrared-absorbing filters was placed far from the bolometer in both cases to provide a parallel light. This ratio was then used, together with the directly measured sensitivity of the bolometer without opal glass, to give the sensitivity with opal glass.

The bolometer with attached opal glass was placed close by the cell

wall, which was itself only 0.3 cm thick. The incident light was measured by placing water in the cell, whereas the transmitted light was measured with algae in the cell. The light absorption by the cell itself was measured and found to be negligible. Because the cell was thin in comparison with its area, scattering of light to the sides was negligible. The order of back-scattering (reflection) of the light was roughly checked and found to be small enough to be disregarded. The small nonuniformity of both the incident and transmitted light fields was carefully mapped in each case with a small photocell, and a correction was applied to the reading obtained with the bolometer at the center area of the field, which was quite uniform.

The 93.5-cc suspension of algae occupied 110.6 $cm^2$ of the cell cross-sectional area, while there was an unoccupied area of 12.7 $cm^2$ above the suspension when no gas was passing through. When the gas pump was turned on, causing a gas flow rate of about 1,100 cc/min, the bubbling of gas through the suspension resulted in a redistribution of the unoccupied area among many bubbles. The gas pump was turned off momentarily during readings of the transmitted light. The light energy absorbed was then calculated as the difference between incident and transmitted light in watts per $cm^2$, multiplied by the above "rest area," 110.6 $cm^2$. This calculation involves the assumption that the rest area is the same as the "bubbling area." In other words, it is assumed that each bubble occupies the entire thickness of the cell, an assumption that is in agreement with the appearance of the bubbles during the experiment. The greatest error that could result from this assumption can be calculated by considering the extreme case in which the bubbles would occupy the entire area, but only 12.70/123.3 of the thickness of the cell. In this case, the absorbed light energy would be about 4% greater. Since from the appearance the former case is much nearer to reality, the actual error cannot be more than about 2%.

The energy calculated above, together with the wave length of the light, 6376 Å, gives directly the number of quanta of light per second absorbed by the algae. The slope of the oxygen evolution curve, taken over 20 to 30 minutes, together with the sensitivity of the instrument and the volume of the system gives the number of molecules of oxygen evolved per second of photosynthesis. A photograph of a typical chart printing is shown in Fig. 2.

The suspension of Chlorella pyrenoidosa was cultured with 4% carbon dioxide in air with shaking under the illumination of white fluorescent lamps. At the same time each morning, 900 cc of the 1,100 cc volume of culture was

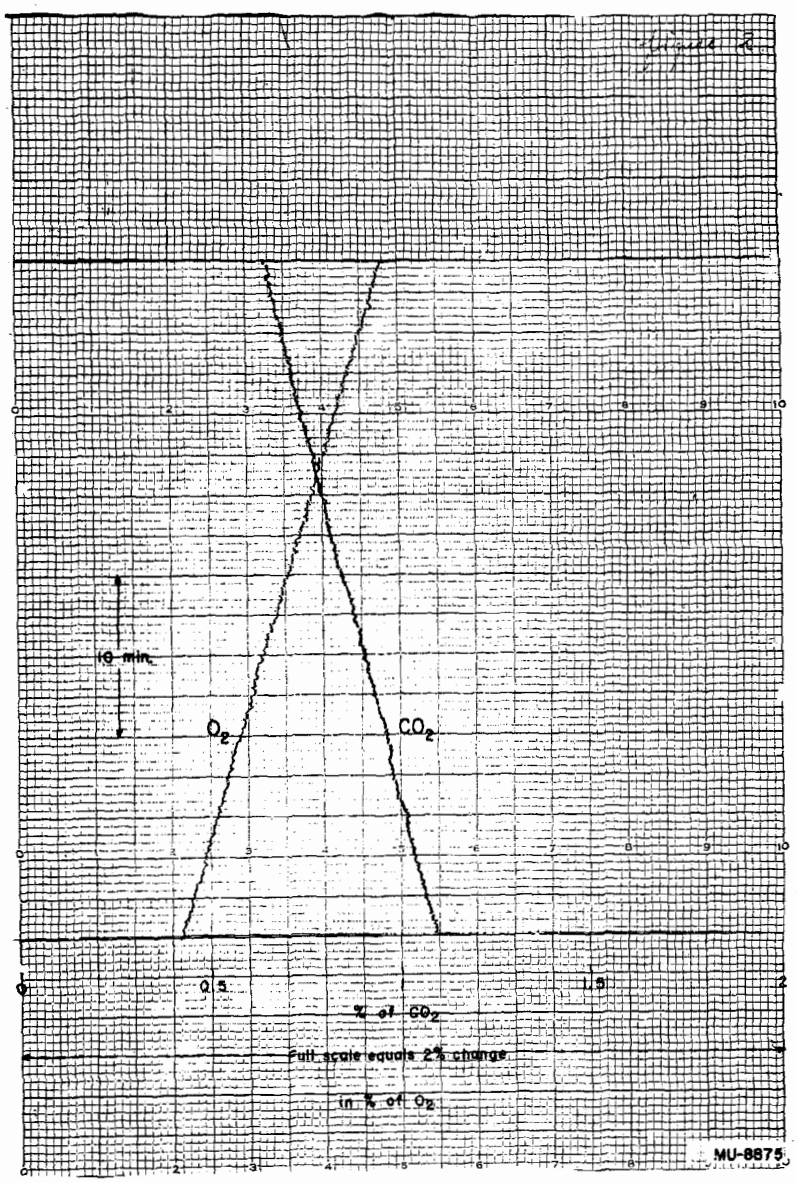


Fig. 2. Chart recording of oxygen and carbon dioxide concentration changes during photosynthesis.

drawn out, and the same amount of culture medium was added to the remaining 200 cc, so that a reproducible state of culture was obtained at each harvesting, several days after the initial inoculation. The effect of the period between harvesting on the quantum requirement was checked, and every 24 hours' harvest was found to be reproducible for the measurement of quantum requirement and to give a higher efficiency than algae grown for either shorter or longer periods of time under our growth conditions. The harvested suspension was used immediately without further manipulation for the observation of the quantum requirement. The absorbence (optical density) of the suspension of algae used was between 0.502 and 0.615, and the pH was 7.0 to 7.1.

The effect of the concentration of carbon dioxide in air on photosynthesis rate was tested at 1% and 4%. The rate at 4% $CO_2$ was only 5% higher than the rate at 1% $CO_2$, so that the photosynthetic rate under the light condition used can be considered to be saturated with respect to carbon dioxide at 4% $CO_2$. The data described below are all obtained with 4% $CO_2$ in air.

The effect of blue light on the quantum requirement of photosynthesis was studied. A pure cadmium lamp, which was used as a blue light source, was set on the opposite side of the algae vessel from the red neon light. To remove light of red and yellow wave lengths as well as ultraviolet, a light-blue filter was inserted between the blue light source and the cell. The energy of the blue light was varied from 3% to 10% of the energy of the main red light, which was $7.49 \times 10^{-4}$ watts/$cm^2$ sec. The concentration of the suspension of algae was also varied to permit from 25% transmission to 80% transmission of red light. The rate of generation of oxygen was increased with added blue light, but the increment of the rate was found to be just proportional to the additional quanta of blue light, so that, in our experimental condition, no catalytic blue-light effect was observed.

## RESULTS AND DISCUSSION

The results of one series of experiments are shown in Table I. The number of quanta absorbed per second by the algae suspension q, together with the net number of oxygen molecules evolved per second, G, and the number of oxygen molecules evolved per second corrected for respiration, P, give the uncorrected $(1/\phi)$ and corrected quantum requirements $(1/\phi)_c$ respectively. The different experimental values were obtained at different incident light intensities, $I_o$. Since the optical density D ranged only from 0.502 to 0.615, the number of quanta absorbed depends principally on the incident light intensity.

Table I

Light absorbed and gas evolved in photosynthesis

| D | $I_o$ (Watts/cm$^2$ sec) | q | G | R | P=G-R | $(1/\phi)$ | $(1/\phi)_c$ |
|---|---|---|---|---|---|---|---|
| 0.502 | $0.747 \times 10^{-4}$ | $1.80 \times 10^{16}$ | $+0.107 \times 10^{16}$ | $-0.26 \times 10^{16}$ | $+0.367 \times 10^{16}$ | 16.8 | 4.91 |
| 0.598 | 1.82 | 4.78 | 0.516 | 0.33 | 0.846 | 9.26 | 5.65 |
| 0.615 | 2.57 | 6.83 | 0.756 | 0.43 | 1.19 | 9.04 | 5.74 |
| 0.608 | 3.25 | 8.59 | 1.03 | 0.39 | 1.42 | 8.34 | 6.05 |
| 0.584 | 5.29 | 13.7 | 1.70 | 0.41 | 2.11 | 8.05 | 6.49 |
| 0.605 | 7.49 | 19.8 | 2.55 | 0.42 | 2.97 | 7.77 | 6.67 |
| 0.524 | 14.8 | 36.3 | 4.77 | 0.45 | 5.22 | 7.61 | 6.95 |

These results are shown graphically in Fig. 3. The points for the evolution of oxygen uncorrected for respiration lie on a straight line that does not pass through the origin. This line can be represented by

$$G = \phi_{\infty} - R_o,$$

where $\phi_{\infty}$ is the slope of the uncorrected line and corresponds to the reciprocal of the quantum requirement at very high light intensity if no light saturation could occur, $R_o$ is the number of molecules of oxygen absorbed during dark respiration following a long period of darkness, and is obtained by extrapolation of the uncorrected line to zero light absorption.

The difference between the G and P value for any experiment is, of course, R, the respiration rate following photosynthesis. Values of R are plotted against quanta absorbed during the previous period of photosynthesis in Fig. 4. It can be seen that the respiration increases with the light absorbed during the previous period of photosynthesis. The value $R_o$ is not an experimental point but is derived from Fig. 3 as previously mentioned. Because the experiments reported here were all at photosynthesis rates above the compensation point, little can be said about the Kok effect[22] in this case. An important point in this experiment is that following higher light intensities the respiration rate approaches a constant maximal value which results in the corrected curve (P in Fig. 3) having the same final slope as the uncorrected line. This curve can be expressed as

$$P = \phi_{\infty} - R_o + R.$$

Since the uncorrected oxygen-evolution curve contains both the oxygen evolution by photosynthesis and the oxygen absorption by respiration in the light, there are only two possible circumstances under which it should be a straight line over the entire range studied. The first is that the quantum yield of photosynthesis be completely independent of respiration and the respiration be constant or linearly dependent on light absorption. In this case the corrected curve would also be a straight line, which it is not. The second possibility is that respiration varies with light intensity and the quantum requirement of photosynthesis depends on the amount of respiration. Furthermore, the interrelation would have to be such that the effect of oxygen absorbed by respiration would be approximately compensated by the contribution of energy from respiration to photosynthesis.

Since respiration in the dark does vary with the light intensity to which the plants are accustomed, not only in these experiments but also in those of

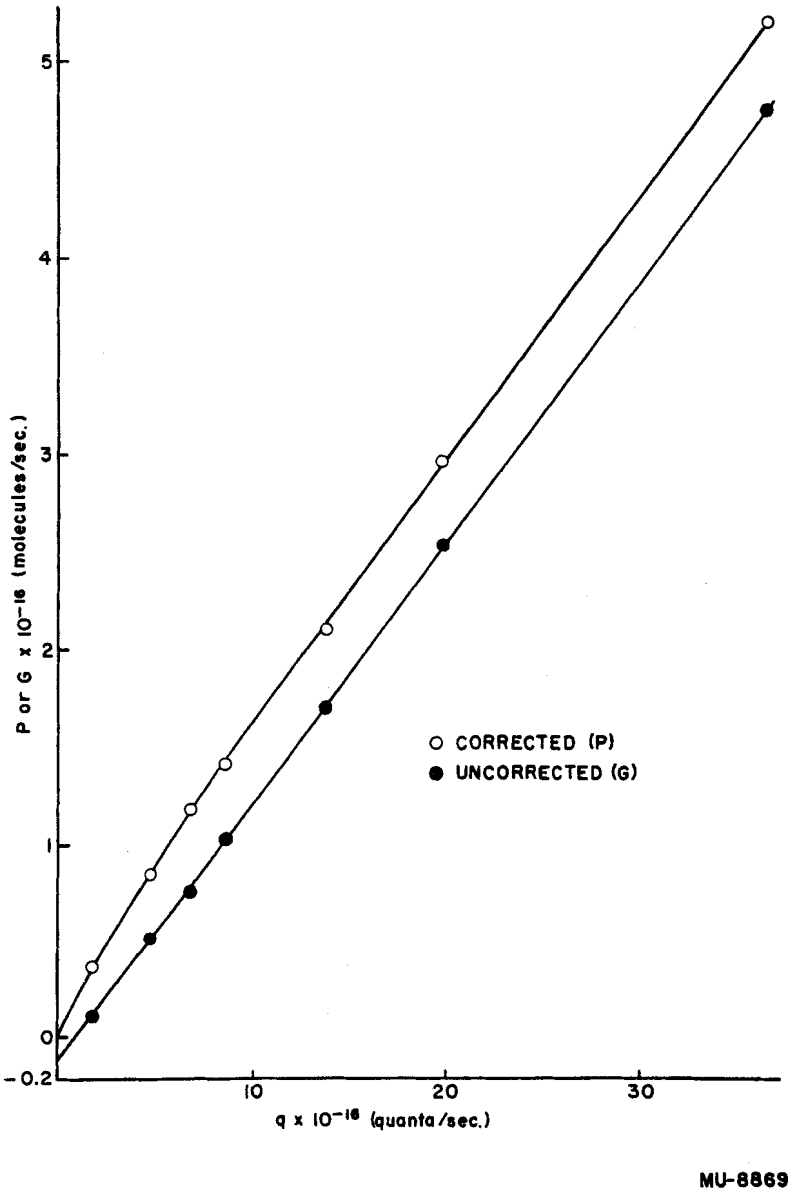


Fig. 3. Corrected and uncorrected rates of oxygen evolution during photosynthesis as a function of rate of light absorption.

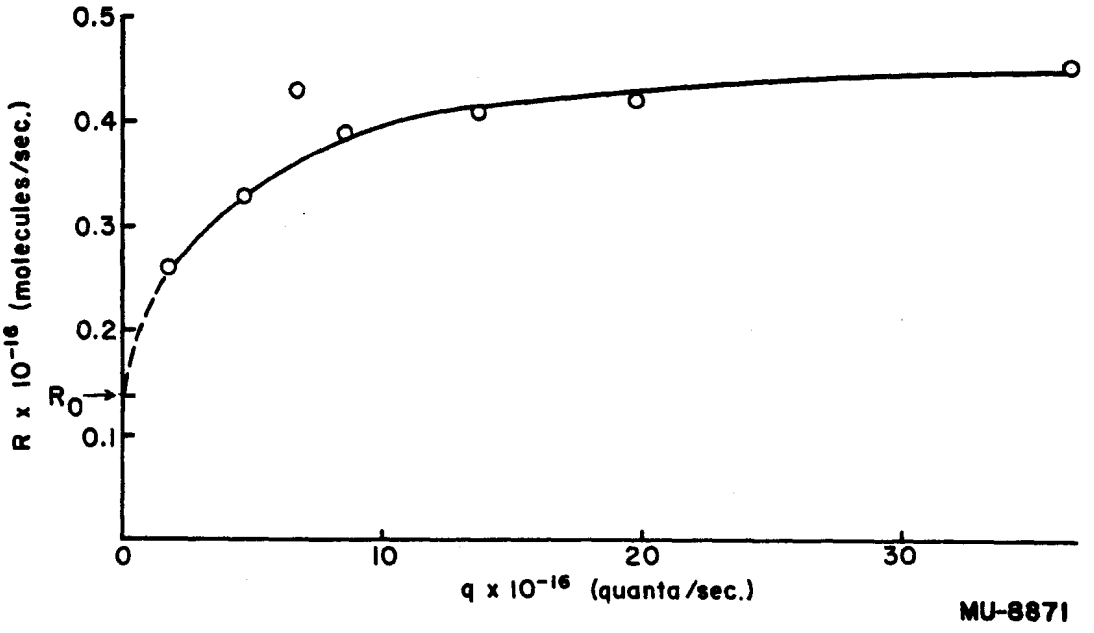


Fig. 4. Rate of oxygen absorption during respiration as a function of previous light-absorption rate.

Brown[18] and of Weigl,[20] and since the report by Brown shows that the respiration rate is constant in the light and in the dark for alternating light and dark periods of about twenty minutes, it can be assumed that the respiration rate does vary with the light intensity, and is equal at any given light intensity to the rate observed in a subsequent dark period. Consequently, the second of the above hypotheses seems correct. This leads to the concept of "extra respiration" given by the difference $R - R_o$, which represents the enhancement of respiration rate due to photosynthesis at any light intensity.

The effect of respiration on quantum requirement is shown in Fig. 5, where corrected and uncorrected requirements are plotted as a function of the ratio of photosynthesis to respiration. The contribution of energy from respiration to photosynthesis at any light intensity can be related to the difference between the quantum requirement at "infinite light," $1/\phi_\infty$, and the actual quantum requirement $1/\phi$. If this difference is multiplied by the number of molecules of oxygen evolved per second during photosynthesis, P, the number, $\Delta q$, of quanta "saved" by extra respiration is obtained:

$$\begin{aligned}\Delta q &= P(1/\phi_\infty - 1/\phi) \\ &= P(1/\phi_\infty - q/P) \\ &= P/\phi_\infty - q .\end{aligned}$$

If $\Delta q$ is divided by the respiration enhancement, $R - R_o$, there is obtained the number of "quanta saved" for each extra molecule of oxygen absorbed by extra respiration:

$$\frac{\Delta q}{R - R_o} = \frac{(P/\phi_\infty) - q}{R - R_o} .$$

But, it was found experimentally that

$$P = \phi_\infty q + R - R_o .$$

Therefore,

$$\frac{\Delta q}{R - R_o} = \frac{1}{R - R_o}\left(\frac{\phi_\infty q + R - R_o}{\phi_\infty} - q\right),$$

which reduces to

$$\frac{\Delta q}{R - R_o} = \frac{1}{\phi_\infty} .$$

This result, which is the consequence of the linearity of the G function

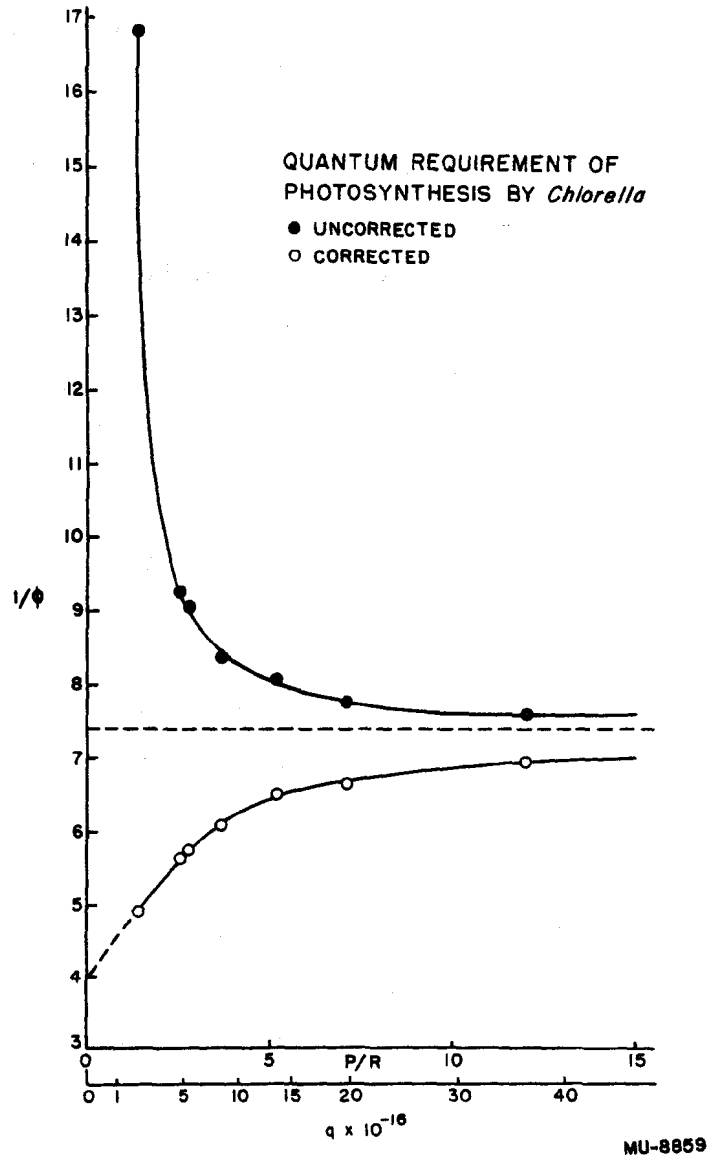


Fig. 5. Relation between quantum requirement in photosynthesis and the ratio of photosynthesis to respiration rate.

in Fig. 3, means that in this experiment each extra molecule of oxygen absorbed by enhanced respiration results in the conservation of the same number of quanta as would be required for the evolution of a molecule of oxygen at a light intensity so high that the contribution of respiration to photosynthesis is negligible. This equivalence, although fortuitous, is not unreasonable if we consider existing biochemical evidence.

The requirements for the operation of the carbon reduction cycle leading to the reduction of a carbon dioxide molecule are now fairly well known, and include two molecules of two-electron reducing agent (reduced triphosphopyridine nucleotide, TPNH) and three molecules of adenosine triphosphate (ATP).[16] Of the latter, two molecules of ATP are required for the reduction of the two molecules of phosphoglyceric acid (PGA) formed in each carboxylation to two molecules of triose phosphate, while one ATP molecule is required for the formation of one molecule of ribulose diphosphate (RuDP) from ribulose monophosphate.

$$\text{ATP} + \text{RuMP} \longrightarrow \text{RuDP} + \text{ADP},$$

$$\text{H}_2\text{O} + \text{RuDP} + \text{CO}_2 \longrightarrow 2\ \text{PGA},$$

$$2\text{H}^+ + 2\text{PGA} + 2\text{ATP} + 2\text{TPNH} \longrightarrow 2\ \text{Triose phosphate} + 2\ \text{ADP} + 2\ \text{TPN}^+.$$

The triose phosphate molecules are rearranged to sucrose and ribulose monophosphate without further energy requirement.

While there are many possible ways in which the reducing agent might be formed with light energy, it seems reasonable to suppose that one quantum may be required for the transfer of each electron from water to the electron acceptor, which then becomes the reductant. Since two molecules of two-electron reductant are required for the reduction of one $CO_2$ molecule, only four quanta would be required, if all the ATP could be supplied from respiration. This is in agreement with the experimental result shown in Fig. 5, in which the corrected quantum requirement at the lowest light intensity is 4.9 and the corrected curve is tending toward 4 at P/R = 0.

At high light intensities, when respiration is inadequate to supply the requirement of ATP, some reductant must be oxidized with oxygen or some intermediate in oxygen evolution to provide energy for the formation of ATP. The number of ATP molecules formed from each molecule of reductant consumed is not known in photosynthesis, but

studies of oxidative phosphorylation during respiration[24] indicate that in that case the number may be two or three. In photosynthesis, since two quanta are required for the formation of one molecule of reductant, either one or 2/3 quantum respectively is required for the formation of each molecule of ATP. Therefore, the total quantum requirement to form 2 TPNH and 3 ATP molecules is either 7 or 6.

As for the value of $\Delta q\,(R - R_o)$, which is the contribution of respiratory energy to photosynthesis in terms of quanta saved compared to extra oxygen consumed, it has been estimated that about six or seven molecules of ATP are formed for each molecule of oxygen consumed by respiration. If it is true that one quantum is required for the formation of one ATP when photosynthetic reductant is oxidized, as stated above, then each molecule of oxygen taken up as a result of enhanced respiration will "save" about seven quanta. Thus the relation

$$1/\phi_\infty = 7.4 = \Delta q/(R-R_o)$$

is not unreasonable.

The increase in both light and dark respiration rate that results from conditioning of the plants to a given level of photosynthesis--as compared with the respiration rate of the algae after a long period of darkness--probably is due to the increase in availability of free sugars newly formed by photosynthesis. Thus, after a long period of darkness, the level of respiration is maintained by breakdown of starch and other storage products and is just enough to maintain the plant's "basic metabolic rate." This basic respiration would supply no energy to photosynthesis when the plants are again placed in the light. Such energy contributions would be possible only after the plant had laid down some sugars by photosynthesis and the respiration rate had increased. Thus the low quantum requirements observed when the plants are photosynthesizing at low light intensities following a long period of photosynthesis are attained at the expense of chemical energy stored during previous photosynthesis.

Failure to obtain the blue-light effect reported by Warburg may have been due to the culture conditions used for the algae. According to Warburg, the blue-light effect was found with algae grown in winter by a north window with an incandescent light for additional illumination, but not with algae grown in the summer. The winter light and incandescent lamp may be somewhat dificient in blue light. In any event, we never had algae with a very high quantum requirement in pure red light, a condition that is probably necessary if one is to observe the blue-light effect.

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