The Maximum Efficiency of Photosynthesis: A Rediscovery

Dean Burk, Sterling Hendricks, Mitchell Korzenovsky, Victor Schocken, and Otto Warburg

National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland; Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland; and *Marine Biological Laboratory, Woods Hole, Massachusetts*[1]

IT WAS REPORTED in 1923 (*4*) that in photosynthesis 4 light quanta, absorbed by chlorophyll in green cells, can produce one molecule of oxygen under favorable conditions. This means that with red light about 65 percent of the absorbed radiant energy can be transformed into chemical energy. Quanta of red light are of low energy (~ 44,000 cal/mole) and several such quanta must therefore cooperate to develop one molecule of oxygen, which requires ~ 112,000 cal/mole. No analogy is known in the nonliving world, where most photochemical reactions, although simpler and effected by quanta of higher energy, are far less efficient in energy transformation, and are indeed usually exothermic. It became clear that an unknown principle, active in nature, awaited elucidation by physics and chemistry.

It is well known how the anticipated study of this new principle was interrupted about 1938, when almost every investigator in the field came to accept as the maximum efficiency of the energy transformation low values of 20 to 25 percent, that is to say, quantum values of 10 to 12 with red light. The development of this revocation has been extensively summarized in a book just published in the last few months by James Franck and Walter Loomis (*1*) and contributed to by a series of investigators.

We have reinvestigated the problem and have rediscovered the high efficiency of energy transformation in photosynthesis. The data obtained are conclusive.

In the course of this reinvestigation the methods have been simplified and improved in such a way that determinations of quantum requirements are no longer the privilege of the few. On the contrary, anyone who has a suitable light source and simple manometric equipment can now determine quantum requirements and convince himself that in green cells the greater part of the absorbed light energy can be transformed into chemical energy (see Addendum).

Improvements in Technique

Culture of cells. A strain of *Chlorella pyrenoidosa,* used in various institutes for many years and originally isolated and identified by Florence Meier, of the Smithsonian Institution, was cultivated in 300-ml gas wash bottles of the Drexel type. The culture medium was 5 g $MgSO_4 \cdot 7\,H_2O$, 2.5 g KNO_3, 2.5 g KH_2PO_4, 2 g NaCl, and 5 mg $FeSO_4 \cdot 7\,H_2O$, dissolved in 1000 cm^3 of nonsterilized well water (pH 4.5 to 5.0). Approximately 100 mm^3 of cells suspended in 200 cm^3 culture medium was added to each bottle, and aerated with 5 percent CO_2 in air so rapidly (~ 500 ml/min) that no sedimentation of the cells occurred. They were illuminated with a 100-w incandescent lamp that raised the temperature in the bottles to 25–30° C. After several days, when the cells had multiplied several fold, and the pH had risen 0.5 to 1 unit, the cultures were used for yield experiments. Such cells gave stable respiration values and high yields without exception. This culture method, which had been employed by one of us (D.B.) for many years in Washington, was found to be an important improvement over the method of 1923 (*4*) followed by most later investigators but which we have now discarded. In the earlier method, slowly aerated cells settled down in Erlenmeyer flasks in sediments that were inade-

[1] We wish to acknowledge the facilities and laboratory accommodations for research and demonstrations provided at the Marine Biological Laboratory by Professor E. S. Guzman-Barron and by Dr. Charles Packard, Director. Valuable aid in these investigations was provided by Mrs. Lois B. Macri, Mrs. Clara F. Smith, and C. R. Newhouser. The culture of *Chlorella pyrenoidosa* was obtained from Dr. F. E. Allison of the Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland. We wish to thank E. Machlett and Son, New York City, for special facilitation of provision of the manometric glassware, and the American Instrument Company, Silver Spring, Maryland, for the specially adapted thermostat and shaking mechanism employed.

quately illuminated and inadequately supplied with carbon dioxide and oxygen until reshaken up.

Manometry. Approximately 300 mm³ of cells, resuspended in 7 cm³ of a fresh culture medium of pH 4.9, were put into rectangular vessels of 14 to 20 cm³ volume and saturated with 5 percent CO_2 in air. Two vessels were used to determine, as described in 1924 (*3*), the oxygen as well as the carbon dioxide exchange. The gas volumes in the two vessels were different, but the volumes of the solutions and therefore the concentrations of the cells were equal. The vessels were shaken at 20° C by horizontal motion, with an amplitude of 2 cm and a frequency of 150 per min. In spite of this rapid motion, which moved the vessels 600 cm per minute, no splashing or foaming occurred, even in experiments of more than 20 hr duration. It was a further improvement owing to this motion that physical transition effects were not observed upon change from dark to light and vice versa, that is, the gas equilibration was virtually perfect for our purposes.

The well known requirement of the two-vessel method, that the metabolism in the two vessels must be identical ($x_{O_2} = x'_{O_2}$ and $x_{CO_2} = x'_{CO_2}$) was complied with by eliminating a dangerous differential time factor as nearly as possible. The light beam of measured intensity (630 to 660 mμ) was shifted by a mirror alternately from one vessel to the other at intervals of 10–60 min or these vessels were shifted alternately into and out of the fixed light beam. Thus in every case the one vessel was illuminated when the other vessel was dark and vice versa, and when many light and dark periods followed each other and the pressure changes of all dark periods and of all light periods were summed, the metabolism in the two vessels was virtually identical for the same periods of elapsed time. The total pressure changes effected by light were usually of the order of magnitude of + 20 to + 50 mm. These figures were differences between two rates of oxygen consumption (negative pressure changes) in the experiments with noncompensated respiration. But when the respiration was compensated by white light (see following section) the figures + 20 to + 50 mm were the directly observed positive pressure changes, produced by the added red light. To measure such great pressure changes simple Haldane-Barcroft blood-gas manometers could be used instead of the special differential manometer heretofore employed.

Measurement of the quantum intensity. The bolometer used in 1923 was replaced by the chemical actinometer described a few months ago (*6*). This resulted not only in a simplification but in an improvement in accuracy. When the oxygen produced by the red light had been measured, the vessel containing the cells was replaced by a similar vessel containing 2 mg of ethyl chlorophyllide, 200 mg of thiourea and 7 cm³ of pyridine, with tank oxygen as the gas phase. The light absorption being complete in the cell suspension as well as in the ethylchlorophyllide solution, the quantum requirement per molecule oxygen was obtained by the very simple equation:

$$\frac{1}{\phi} = \frac{h \cdot \nu}{O_2} = \frac{O_2 \text{ consumed by the actinometer}}{O_2 \text{ produced by the cells}},$$

where the consumption and the production of the oxygen must be calculated for equal time periods.

Complete absorption is the only method so far devised by which the *absorption* of the light and the *action* of the light can be measured manometrically under the same conditions. Thus far all manometric determinations of quantum requirements using incomplete absorption are contradictory and uncertain. On the other hand, the main objection against the method of complete absorption was the existence of too great a respiration relative to the measured light action; this objection is no longer valid because today respiration can be eliminated by compensating with white light or components thereof (see the following section).

A New Principle

Let us consider a *Chlorella* suspension, shaken in a beam of red light that is absorbed completely, to be a machine that transforms light energy into chemical energy. The efficiency of this machine will be known if we know the light energy entering the vessel and the amount of oxygen developed in it, one mole of oxygen being equivalent to the production of about 112,000 cal. No theory of the mechanism of this energy transformation can alter the observed result of such an efficiency determination.

Yet there are possible objections to be answered. When at low light intensities respiration still exceeds photosynthesis, then our machine does not produce a net gain of chemical energy, and it might be considered that the light merely inhibits the loss of chemical energy. Since respiration is a catalytic process, conceivably its inhibition by light could be merely anticatalytic, requiring no expenditure of energy. Hence the calculation of the efficiency might be safe thermodynamically only when the light intensities are so high that oxygen is in fact given off from the cell suspensions into the gas space.

To comply with this requirement we illuminated the cell suspensions with white light (of nonmeasured intensity) over the greater part of the vessel surface, the light intensity per unit area being relatively small, but the influx of light energy through the total area

of the vessel being sufficient to compensate or overcompensate the respiration of the cell suspensions. As the compensating light intensity we define here that light intensity which effects the result that no oxygen enters or leaves the cell suspension. It is very likely that in this state the oxygen exchange of all cells in the suspension is zero, owing to the rapid motion of the cells. It is obvious that our compensating light intensity varies with the amount of cells in the vessel and is not to be confused with the compensating light intensity for very thin cell suspensions.

When the steady state in the white light was reached, a beam of red light of measured intensity was sent through the bottom of the vessel into the cell suspension, the cross section of the beam being 3 cm^2 and the total energy flux about 0.25 microeinsteins/min. It was completely absorbed in the cell suspension. The increase of positive pressure effected by the red light and the intensity of the red light were the two magnitudes from which the efficiency of the energy transformation of the added red light was calculated. The efficiency so found with red light proved to be as high, at and several fold above the compensation point with white light, as the efficiency at zero or low white light intensities below the compensation point (3 to 5 quanta per O_2 molecule developed; see Examples 1–5).

This result raised the whole level of certainty in this field of investigation, and is probably owing in large part to the rapid motion of a great amount of cells. The time of illumination with red light of relatively high intensity must be so short for every cell that no Blackman or other dark reaction limits the action of the light; in other words the product of light intensity and time ($i \times t$) must be too small to alter the concentrations of dark reactions. The observed efficiency is, of course, a fact that is independent of all theories about what happens in the cell suspensions, chemically or physically.

Another factor that might limit the certainty of efficiency determinations is time of illumination. The shorter the time periods, the greater is the danger that the energy of some dark reaction contributes to the oxygen production. The longer the time periods, the more certain are we that we have reached the thermodynamically desirable state in which the concentrations of all cell constituents are kept constant.

From 10 min in 1923 (*4*) and 15 min in 1948 (*5*) we have now extended the time periods to more than 20 hr of continuous illumination with white light, producing continuous positive pressure, and have varied the periods of the efficiency determinations with added red light from 5 to 60 min. This great improvement was possible because the horizontal motion of the rectangular vessels did not damage the photosynthetic capacity of the cells and because the white light, when it overcompensated respiration, stabilized the chemical conditions in the cells. In fact, the efficiency of the energy transformation has now been measured under the conditions of growth, so that very likely the experiments can be extended to any length of time.

Nonaction of Light on Respiration

If the manometric efficiency of the red light is the same when the respiration of the cell suspensions is noncompensated, compensated, or several fold overcompensated by added unmeasured white light, then obviously every theory should be rejected that assumes that light acts on the process of respiration anticatalytically or stoichiometrically. This conclusion has now been confirmed independently by the following type of experimentation:

A rectangular vessel was set up to contain NaOH and glass beads in two side arms, 300 mm^3 of cells in 7 ml of culture medium (pH 4.5–5) in the main compartment, and air in the gas phase. The vessel was shaken at 20° C slowly or rapidly, and in the dark or in the light. By changing the rate of shaking the steady-state pressures of CO_2 in liquid and gas phases were changed, but in the dark this had no effect on the observed rate of respiration. In the light, the rate of oxygen consumption decreased in the slowly shaken vessel; but in the rapidly shaken vessel, where the steady-state pressure of CO_2 was lower, *no action of light on the rate of oxygen consumption was observed* when the intensity of the light entering the vessel was about 0.25 microeinsteins/min, an intensity that with adequately high CO_2 pressures gave high photosynthetic efficiencies (3 to 5 quanta per molecule of O_2 developed). The shaking effect was reversible, the action of the light alternately appearing or disappearing with decreasing or increasing shaking rate.

The experiments may be extended to higher light intensities, but the higher the light intensity the more effective must be the removal of the carbon dioxide, for which the light and the alkali compete. The CO_2 pressure required to yield maximum respiration is clearly below that needed for effective photosynthesis, where CO_2 functions as substrate and not merely catalytically.

Such experiments prove that the light did not interfere with the process of respiration, either anticatalytically or stoichiometrically by reduction of intermediates. Light, when it compensated respiration, did so by producing oxygen, and because the gas exchange of photosynthesis happens to be the reverse of the gas exchange of respiration. Thus a question, old as the science of photosynthesis, has been answered by the most simple of experiments.

EXAMPLES

1. Manometric pressure changes effected by red light when respiration was not compensated by white light. Two-vessel method, culture medium pH 5, 5% CO_2 in air. 20° C.

Vessel I $v = 13.913$, $v_f = 7.00$
Vessel II $v' = 17.993$, $v'_f = 7.00$
Actinometer 5.4 mm³ O_2/min

Vessel I	Vessel II
10 min dark −26 mm	10 min dark −12 mm
" " " −23 "	" " " −10 "
" " " −23 "	" " " −11 "
" " " −23 "	" " " −11 "
40 min dark −95 mm	40 min dark −44 mm
10 min light −17 mm	10 min light −7 mm
" " " −15 "	" " " −7 "
" " " −14 "	" " " −7 "
" " " −15 "	" " " −7 "
40 min light −61 mm	40 min light −28 mm
Light action +34 mm	Light action +16 mm

$$\left.\begin{array}{l} x_{O_2} \text{ in 40 min} + 51.6 \text{ mm}^3 \\ x_{CO_2} \text{ in 40 min} - 53.7 \text{ mm}^3 \end{array}\right\} \gamma = \frac{CO_2}{O_2} = -1.04$$

$$\frac{1}{\phi} = \frac{h\nu}{O_2} = \frac{40 \cdot 5.4}{51.6} = 4.2$$

2. Manometric pressure changes effected by red light at 20° C, when respiration was overcompensated by white light. pH 5.0. 5% CO_2 in air. Actinometer for red light 5.4 mm³ O_2/min.

Vessel I $v = 13.913$, $v_f = 7.00$
Vessel II $v' = 17.993$, $v'_f = 7.00$

Vessel I				Vessel II			
5 min white	+18.5	mm		5 min white + red	+22	mm	
" " "	+18.0	"		" " " "	+22.5	"	
" " "	+16.5	"		" " " "	+22	"	
" " "	+17.5	"		" " " "	+20.5	"	
" " "	+17.0	"		" " " "	+23.0	"	
25 min white	+87.5	mm.		25 min white + red	+110	mm.	

Action of red light +22.5 mm in 25 min.

Vessel II							
5 min white	+14	mm		5 min white + red	+15	mm	
" " "	+14	"		" " " "	+16.5	"	
" " "	+12.5	"		" " " "	+16.5	"	
" " "	+14.0	"		" " " "	+14.0	"	
" " "	+11.5	"		" " " "	+15.0	"	
" " "	+12.0	"		" " " "	+14.5	"	
30 min white	+78	mm.		30 min white + red	+91.5	mm.	

Action of red light +13.5 mm in 30 min.

$$\left.\begin{array}{l} x_{O_2} \text{ in 25 min} + 30.3 \text{ mm}^3 \\ x_{CO_2} \text{ in 25 min} - 27.2 \text{ mm}^3 \end{array}\right\} \gamma = \frac{CO_2}{O_2} = -0.9$$

$$\frac{1}{\phi} = \frac{h\nu}{O_2} = \frac{25 \cdot 5.4}{30.3} = 4.5$$

For the same culture, when the respiration was not compensated by white light, we obtained

$$\frac{1}{\phi} = \frac{h\nu}{O_2} = \frac{60 \cdot 5.4}{70.4} = 4.6$$

$$\text{and } \gamma = \frac{CO_2}{O_2} = -0.8$$

3. Manometric vessels continuously illuminated for 27 hr by white light of approximately compensating intensity. $\frac{1}{\phi} = \frac{h\nu}{O_2}$, for the added red light, at the beginning and end of the experiment.

In hours 1 to 4, respiration compensated, $\frac{1}{\phi} = 4.5$ $(\gamma = -1.0)$

In hours 20 to 22, respiration compensated, $\frac{1}{\phi} = 4.5$ $(\gamma = -1.0)$

In hours 27 to 28, respiration not compensated, $\frac{1}{\phi} = 3.5$ $(\gamma = -1.27)$

4. Comparison of same culture in M/10 carbonate buffer (pH 9) and in culture medium (pH 5).*

Red light, 630–660 mμ. $I \sim 0.25$ microeinsteins/min 20° C.
Quantum requirement $\frac{1}{\phi} = \frac{h\nu}{O_2}$ for the added red light.

In carbonate buffer, respiration compensated by white light, $\frac{1}{\phi} = 10.5$

In culture medium, respiration compensated by white light, $\frac{1}{\phi} = 3.9$ $(\gamma = -1.04)$

In carbonate buffer, respiration not compensated $\frac{1}{\phi} = 9.8$

In culture medium, respiration not compensated $\frac{1}{\phi} = 3.6$ $(\gamma = -1.18)$

In carbonate buffer, respiration not compensated $\frac{1}{\phi} = 11.3$

* It may be gathered from this example that the efficiency in the unnatural carbonate buffer is only a fraction of the efficiency in culture medium. Many investigators believed that the efficiencies in both solutions were equal, and this was one of the reasons why the quantum requirement of 4 was denied by them and the figures 10 to 12 accepted as maximal.

5. Results by 2-vessel method for a series of experiments that were carried out from May 26 to June 16 of this year, at 20° C, in culture medium at pH 5, with an intensity of red light not exceeding 0.25 microeinsteins/min. No experiment is omitted.

	$\frac{1}{\phi}$	γ
	$\left[\frac{h\nu}{O_2}\right]$	$\left[\frac{CO_2}{O_2}\right]$
	3.2	−1.08
	4.6	−0.80
	4.5	−0.90*
	3.9	−1.04
	3.6	−1.18
	4.2	−0.97
	2.8	−1.16
	2.8	−1.23
	2.5	−1.25
	4.5	−1.00†
	4.7	−0.93†
	3.5	−1.27
	4.2	−1.00
	3.0	−1.33
Average	3.7	−1.08

* Respiration overcompensated by white light from above.
† Respiration compensated by white light from above.

It may be gathered from Example 5 that the quantum requirement for carbon dioxide consumed can be equal to or somewhat greater or smaller than the requirement for oxygen produced. For we have

$$\frac{1}{\phi} \cdot \frac{1}{\gamma} = \frac{h\nu}{O_2} \cdot \frac{O_2}{CO_2} = \frac{h\nu}{CO_2},$$

a magnitude which here for the first time, to our knowledge, has been determined in nutrient medium at low pH (about 5), and has been found to approximate $\frac{h\nu}{O_2} = 3$ to 5.

Yet the quantum requirement for oxygen is the more fundamental energy measure, because there is only one reaction in photosynthesis that produces oxygen—the photochemical reaction (decomposition of water); but there are many different dark reactions that consume carbon dioxide to form a great variety of products. Thus if, as an extreme case, oxalic acid were formed from water and carbon dioxide γ for this compound would be -4 and the quantum requirement for carbon dioxide would be only one-fourth of the requirement for oxygen.

Conclusion

It follows from the data obtained[1] that in the spectral region 630 to 660 mμ no more than 4 quanta are required to produce one molecule of oxygen gas. A requirement of 3 quanta is open to serious consideration, although thus far the average value in our experiments has been nearer 4 than 3.

A quantum requirement of 4 means that the efficiency of the energy transformation in our experiments was approximately $\frac{112{,}000 \times 100}{4 \times 44{,}000} = 65$ percent, whereas the quantum number 3 would mean an efficiency of about 85 percent.

In any event, under favorable conditions the greater part of absorbed light energy can be transformed in green cells into chemical energy, and this is, to quote the man who laid the foundation of quantitative photochemistry, "a marvellous achievement of nature" (*2*).

[1] Reported in greater experimental detail in *Biochem. et Biophys. Acta*, October 1949 (Meyerhof-Festschrift volume); *Arch. Biochem.*, September 1949; and at meeting of Society of General Physiology, Woods Hole, Massachusetts, June 1949.

Addendum

Quantum requirement determinations were demonstrated to, and carried out with the aid of, the students of the physiology class at the Woods Hole Marine Biological Laboratory on July 19 and 20. Requirements of 3 to 5 quanta per molecule of oxygen gas produced in photosynthesis were observed on these two days. The respiration was overcompensated several fold by light of unmeasured intensity from an incandescent lamp used with or without red filter (cut-off at ≦ 560 mμ). In two closely comparable, consecutive experiments, quantum requirements of 3.0 and 3.5 were obtained from measured red light (630–660 mμ) when the overcompensating light entered the manometric vessel as, respectively, white light from above and filtered red light from below. In the latter instance the cells mainly responsible for overcompensation by unmeasured light were identical at any given moment with those receiving the added measured red light. The absorbed measured red light was but a fraction of the total energy flux absorbed, most of which was absorbed in the first millimeter of depth.

The aid of students Jack Durell, Richard Klein, Burlyn Michel, and Martin Schwartz in carrying out various of the class experiments is acknowledged gratefully.

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