THE QUANTUM EFFICIENCY OF PHOTOSYNTHESIS

by

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Photosynthesis is a unique endothermic photochemical reaction in which chemical energy is gained from visible light energy by the combined action of several quanta. Nothing similar is known in the nonliving world. It was first reported a quarter of a century ago[1] that in photosynthesis the greater part of the absorbed visible light energy could be converted into chemical energy under optimum conditions. Indeed, no more than four quanta of red light seemed to be necessary to produce one molecule of oxygen gas, which is close to the thermodynamic requirement of three quanta. It is easy to understand that this result, lacking any analogy, has sometimes been doubted by theoreticians, and it is a fact that certain investigators have raised methodological objections[2]. For this reason we have reinvestigated the question of the minimum quantum requirement of photosynthesis as measured by oxygen and carbon dioxide gas exchange. The present paper is a short summary of our findings by new and simplified methods.

1. CULTIVATION OF CELLS

A strain of *Chlorella pyrenoidosa*, isolated in New England and identified by Dr. FLORENCE MEIER of the Smithsonian Institution, and for many years in laboratory use, was cultivated in tall Drechsel gas washing bottles containing 200 ml of the following salt solution: 5 g $MgSO_4 \cdot 7H_2O$, 2.5 g KNO_3, 2.5 g KH_2PO_4, 2 g NaCl, and 5 mg $FeSO_4 \cdot 7H_2O$, in 1 liter of filtered, unsterilized well water (p_H 4.5–5). The cultures were maintained at a room temperature of 25–30° C, and were aerated with 5% CO_2 in air at a rate ($\sim$ 500 ml per minute) rapid enough to prevent cell settling, and were constantly illuminated with a 100-watt incandescent lamp at a distance of about 30 cm. Cells cultivated by this method gave more uniform material and more regular manometric results than when cultivated by the older method (1, p. 427) in which slowly aerated cells settled down in Erlenmeyer-shaped flasks and became partially anaerobic until reshaken up, and in which lowered light intensities were employed for the terminal cultivation phase.

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The cultures were used for the experiments in the present work after 2–10 days growth, when they contained 200–1000 μl cells, depending upon the amount of initial inoculation. Usually 50–100 μl cells per 200 ml medium were employed as inoculum, grown as just indicated. Bacterial growth during either cell culturing or manometric experiments was found with a haemocytometer to be negligible, due to the low p_H, the lack of added organic matter in the synthetic medium, and possible antibiotics produced by the *Chlorella*.

The cells for experimental use were centrifuged in an International No. 2 Centrifuge at the lowest possible speed giving nearly complete settling in 10 minutes and were taken up, with or without further washing, in fresh nutrient medium at a concentration of 30–50 μl cells per ml.

II. MONOCHROMATOR

A Steinheil glass 3-prism spectrograph operated with a focal length of 195 mm at F 3.5 for the collimator and a focal length of 710 mm for the telescope was used as a monochromator. The slit was illuminated with a 750-watt projection lamp. The image of the coiled filament at about 20° to its plane was projected onto the slit with an auxiliary lens. A 1000-watt voltage regulator was used to supply power to the lamp which operated at constant current.

The width of the entrance slit was about 2 mm, corresponding to about 20 mμ in the red. A slit was placed in the focal plane of the telescope and was adjusted to have a width of about 30 mμ covering the region 630 to 660 mμ. A lens was placed behind this slit to throw, in a weakly convergent beam, an image of the exit prism face on the bottom of the manometer vessel.

The area of the beam at the vessel was about 3 cm^2 and the energy flux was about 0.6 micro einsteins/min. This intensity was decreased when desired by placing in the light beam, just before the exit slit, blackened wire screens calibrated by the National Bureau of Standards.

III. MEASUREMENT OF LIGHT ENERGY

The energy of the light beam was measured by the recently developed chemical actinometer[3] whereby for each quantum of visible light absorbed one molecule of O_2 is consumed. In the same or similar rectangular vessel as used for the yield determinations were placed 2 mg ethyl chlorophyllide, 200 mg thiourea, 7 ml pyridine, and O_2 gas. The actinometer vessel was shaken in the thermostat at 20° C in the same manner and in the same cross-section of the light beam as the vessels with the cell suspensions were shaken during the yield determination. The total intensity of light, absorbed by the actinometer, should not exceed 0.3 microeinsteins per minute under our working conditions. Higher intensities, as used for the yield determinations, were diminished for this purpose by the calibrated screens. Several 10 minute periods were observed for every actinometer determination. When in t minutes the pressure change in the actinometer vessel is h_{O_2} mm, the total energy flux in the light beam in t minutes is $\frac{h_{O_2} \cdot k_{O_2}}{22.4}$ or $\frac{x_{O_2}}{22.4}$ microeinsteins (micromole quanta), where the vessel constant k_{O_2} is expressed

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in mm². Then, when the oxygen developed by illuminating the green algae is n μl and the oxygen absorbed in the actinometer for the same time and beam of light is n' μl, the quantum requirement per mol of O_2 developed in photosynthesis is simply $1/\varphi = n'/n$.

IV. COMMENTS ON THE 2-VESSEL MANOMETRIC METHOD

If the yield φ and the assimilatory quotient, $\gamma = \frac{-CO_2}{+O_2}$, are to be determined simultaneously, two vessels must be employed. If H be the pressure change in vessel I and H′ that in vessel II, the x_{O_2} and x_{CO_2} values can be calculated by well known equations (see [1] and section 8).

The 2-vessel method, simple when the gas-exchanges in the dark are determined, requires special attention when applied to illuminated cells. As will be shown later, the illumination of the cells is an illumination with intermittent light. This intermittency should be equal in the two vessels, and this is attainable if the liquid volumes are equal in both vessels. Furthermore, the respiration in most cell suspensions gradually changes with time, so that the pressure changes in light will also change with time. Thus the two vessels should be darkened and illuminated simultaneously so that the conditions of the aforementioned equations are fulfilled, namely

$$x_{O_2} = x'_{O_2}$$
$$x_{CO_2} = x'_{CO_2}$$

where the primed magnitudes refer to one vessel and the non-primed to the other.

These conditions may be satisfactorily met by the method of alternately shifting the mirror under the two vessels at periods of, *e.g.*, 10 minutes, as indicated in Fig. 1, and discussed in the next section. After two or more cycles, the pressure readings for each vessel for light and dark periods may be averaged and the light action calculated from the differences between the pressure changes in light and dark. A possible error involving noncomparability of time periods is thus eliminated. This error has been one of the main sources of difficulty in *Chlorella*-photosynthesis experiments with the 2-vessel method.

V. PROCEDURE

Simple HALDANE-BARCROFT constant-volume manometers with small capillaries (0.8 mm diameter) with rectangular vessels attached were shaken horizontally (not by arc motion) at 140–180 (usually 150) cycles per minute at an amplitude of 2.0 cm in a water bath at 20° C. The two rectangular vessels of about 2.2×3.8 μl inside width and length and 13–14 and 18–19 ml volume respectively, were filled with 200–400 μl cells in 7 ml, thus the liquid volumes were identical and the gas spaces differed. The vessels (with capillary sidearm vents) were gassed on the bath, simultaneously with aid of a manifold, and with shaking. The horizontal (not arc) shaking was so effective that physical after-effects of gas equilibration in the transition periods of dark to light and vice versa were not appreciable even when the illumination produced photosynthesis far above the compensation point and pressure changes of 5–10 mm per minute were involved. The manometers were usually read without stopping. The end of the mano-

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meter male joint was not flat rough but concave and polished, so that bubble formation in the capillary did not occur; nor did foaming.

As indicated in Fig. 1 a beam of red light (630–660 mμ) of about 3–4 cm² area, produced by means of the Steinheil monochromator, entered the side of the thermostat

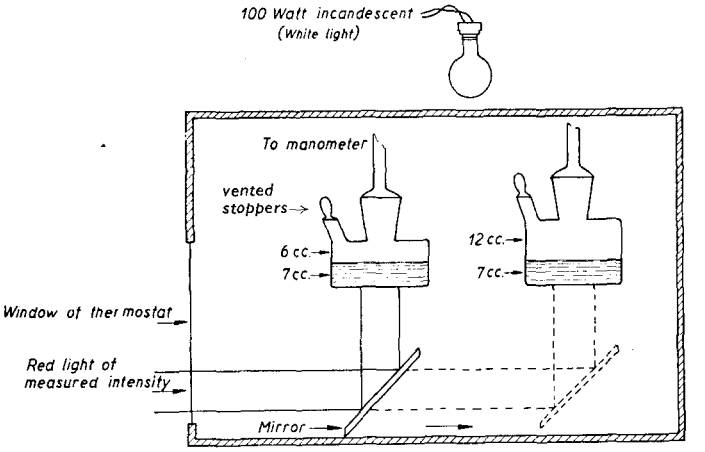

Fig. 1

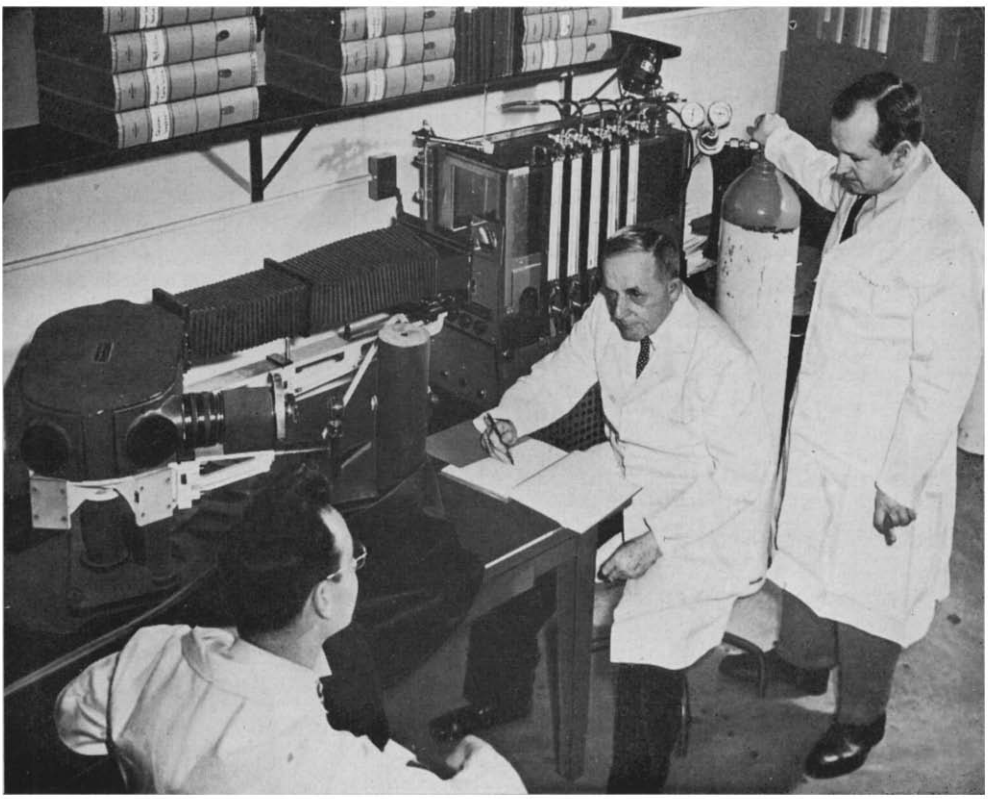

Fig. 2

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through a two walled window and was reflected by a mirror onto the bottom of a vessel, alternately in the one or the other by either shifting the mirror or the manometers, depending on the design of the experiment. The red light entering the vessel was completely absorbed. To accomplish this, the amount of cells must be sufficiently great. The amount depends upon the chlorophyll content of the cells. It was found safe, to avoid loss of light, to have 300 μl of cells in each vessel. No influence of the cell concentration on the yield was observed when light absorption was complete and shaking adequate. By this method, both O_2 and CO_2 exchanges were obtained simultaneously and independently for any and every desired period of measurement, and every yield determination was connected with an experimental determination of the relationship CO_2/O_2, so that earlier uncertainties concerning this ratio (γ) were eliminated.

VI. INTERMITTENCY OF ILLUMINATION

The cross-section of the light beam entering the vessels was about 3 cm^2, that is, 3/8 of the bottom area of the vessel. It can be calculated, if we disregard the scattering of light, that the major part of the red light (75%) is absorbed within a distance of about 1 mm from the bottom of the vessel. This means that the light absorbing volume is only about 1/20 of the 7 ml of the cell-suspension.

Let now the intensity of the red light be so strong, that the oxygen consumption of the whole cell suspension is compensated by the oxygen evolution (compensation point for O_2). Then the oxygen development in the absorbing volume of the cell suspension may approach 20 times the point where the cells become saturated with light and the increment yield zero (with our cell conditions the saturation intensity is about 30–40 times the compensation intensity). But we obtain maximum or high yields when the vessels are shaken as described at not only compensating but even considerably higher intensities, when the latter are provided by white light. This proves that under our shaking conditions the cells alternate so frequently between darkness and illumination that the concentrations of the participants of all dark reactions virtually retain their dark values — a consideration which shows the methodological importance of the kind and rate of shaking.

VII. YIELD DETERMINATIONS ABOVE THE COMPENSATION POINT

A limiting feature of most earlier yield determinations was the low total light intensity, so low that only a fraction of the respiration was compensated for by the light action. Thus the yield determinations were in a sense determinations of inhibited or diminished respiration. We have changed this situation by illuminating the vessels from above the thermostat by a 100-watt constant-voltage incandescent lamp (as diagrammed in Fig. 1), at such a distance that the pressure changes in the vessels become zero or positive; yield determinations were then made with measured amounts of red light added in the usual manner from below the vessel. The intensity of the white light at the vessel surface was considerably smaller per unit area than that of the red light but covered a many fold greater area and hence provided much more total effective light than did the red beam. Owing to this relationship of intensities it was possible to eliminate respiration as an experimental quantity, and to start the yield experiments at positive rather than negative pressures, and yet still obtain (as experience showed)

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virtually as good yields from the red light, whether the base line were darkness or the white light.

Another limiting feature of the earlier yield experiments was the short duration of not only the periods of illumination (10 minutes) but also the total length of the experiment (commonly less than one hour). By the use of white light we have now succeeded in extending the duration of the manometric yield experiments up to at least 10 hours, if not indefinitely. The effects of this important advance are several. In general, the yields may now be determined under nearly the same conditions as obtain during the growth and cultivation of the cells, since the light intensity, temperature, medium, and gas phase during the growth and manometry are essentially the same, and furthermore we have found that the shaking does not change the cells under these conditions.

VIII. EXAMPLES OF DATA

Protocols 1, 2, and 3 provide examples of the data obtained.

PROTOCOL No. 1

Experiment of V-26-49. 20° C. 630–660 mμ. 5% CO_2 in air. 260 μl of cells per vessel. Each vessel alternating 10′ in dark and 10′ red light; thus when vessel No. 5 was dark, vessel No. 3 was illuminated, and vice versa.

Vessel No. 5	Vessel No. 3
V = 13.913 ml	V = 17.993 ml
v_f = 7.000 ,,	v_f = 7.000 ,,
k'_{O_2} = 0.665 k'_{CO_2} = 1.235	k_{O_2} = 1.046 k_{CO_2} = 1.634
80′ dark —91.5 mm	80′ dark —26.5 mm
80′ light + 1.5 ,,	80′ light + 15.0 ,,
80′ H′ + 93.0 mm	80′ H + 41.5 mm

(*Equation 1*) Action of light in 80′ $x_{O_2} = \dfrac{H \cdot k_{CO_2} - H' \cdot k'_{O_2}}{\dfrac{k_{CO_2}}{k_{O_2}} - \dfrac{k'_{CO_2}}{k'_{O_2}}} = +151\ \mu l$

(*Equation 2*) $x_{CO_2} = \dfrac{H \cdot k_{O_2} - H' \cdot k_{CO_2}}{\dfrac{k_{O_2}}{k_{CO_2}} - \dfrac{k'_{O_2}}{k'_{CO_2}}} = -168\ \mu l$

Actinometer: —8.83 μl O_2 per minute

Quantum efficiency for O_2, $\dfrac{1}{\varphi} = \dfrac{80 \cdot 8.83}{151} = \underline{4.7}$

Quantum efficiency for CO_2, $\dfrac{1}{\varphi} = \dfrac{80 \cdot 8.83}{168} = \underline{4.2}$

Assimilatory quotient, $\gamma = \dfrac{CO_2}{O_2} = \dfrac{-168}{+151} = -1.11$

If $\gamma = \dfrac{CO_2}{O_2} = -1.11$ is determined for a given cell suspension, then x_{O_2} and x_{CO_2} can be obtained by the pressure changes in light and dark in each single vessel. For example, in vessel No. 5, the following figures, taken immediately prior to the readings above, were obtained upon illumination with light of an actinometer value of —5.07 μl O_2 per minute:

Vessel No. 5

10′ dark —12.5 mm
10′ light — 2.5 ,, } 10′ H′ = + 10.2 mm
10′ dark —13.0 ,, } 10′ H′ = + 10.0 ,, } 20′ H′ = + 20.2 mm
10′ light — 1.5 ,,
10′ dark —10.0 ,,

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(*Equation 3*) Action of light in 20; $x_{O_2} = H' \frac{k'_{CO_2} \cdot k'_{O_2}}{k'_{CO_2} + \gamma \cdot k'_{O_2}} = 20.2 \cdot 1.62 = +32.8$

$x_{CO_2} = -1.11 \cdot x_{O_2} = +32.8 \cdot -1.11 = -36.4$

Quantum efficiency for O_2, $\frac{1}{\varphi} = \frac{20 \cdot 5.07}{32.8} = \underline{3.1}$

Quantum efficiency for CO_2, $\frac{1}{\varphi} = \frac{20 \cdot 5.07}{36.4} = \underline{2.8}$

PROTOCOL No. 2

Experiment of V-30-49. 20° C. 630–660 mμ. 5% CO_2 in air. 270 μl of cells per vessel.

Experiment I. Alternately dark and light each 10′. Actinometer for the red light (total) 5.4 μl O_2 per minute. When vessel No. 5 was dark, No. 3 was illuminated and vice versa.

No. 5		No. 3	
Constants as in Protocol 1		Constants as in Protocol 1	
10′ dark	−10.5 mm	10′ light	+0.5 mm
10′ ,,	−10.0 ,,	10′ ,,	0 ,,
10′ ,,	−9.0 ,,	10′ ,,	+1.5 ,,
10′ ,,	−8.5 ,,	10′ ,,	0 ,,
10′ ,,	−9.0 ,,	10′ ,,	+1.0 ,,
10′ ,,	−8.0 ,,	10′ ,,	+1.0 ,,
60′ dark	−55.0 mm	60′ light	+4.0 mm
60′: H′ = 4 + 55 =	+59 mm		

No. 3			
10′ dark	−2.0 mm	10′ light	+3.0 mm
10′ ,,	−3.5 ,,	10′ ,,	+2.0 ,,
10′ ,,	−2.5 ,,	10′ ,,	+3.5 ,,
10′ ,,	−2.5 ,,	10′ ,,	+3.5 ,,
10′ ,,	−0 ,,	10′ ,,	+3.0 ,,
10′ ,,	−1.0 ,,	10′ ,,	+5.0 ,,
60′ dark	−11.5 mm	60′ light	+20.0 mm
60′: H = 20 + 11.5 =	+31.5 mm		

Experiment II: Both vessels were now constantly illuminated with a 100-watt incandescent lamp of nonmeasured* light intensity and red light of measured intensity added for alternating periods of 5′. Actinometer for the red light (total) 5.4 μl O_2 per minute.

No. 5				No. 3			
5′ white	+18.5 mm	5′ white + red	+22.0 mm	5′ white	+14.0 mm	5′ white + red	+15.0 mm
5′ ,,	+18.0 ,,	5′ ,,	+22.5 ,,	5′ ,,	+14.0 ,,	5′ ,,	+16.5 ,,
5′ ,,	+16.5 ,,	5′ ,,	+22.0 ,,	5′ ,,	+12.5 ,,	5′ ,,	+16.5 ,,
5′ ,,	+17.5 ,,	5′ ,,	+20.5 ,,	5′ ,,	+14.0 ,,	5′ ,,	+14.0 ,,
5′ ,,	+17.0 ,,	5′ ,,	+23.0 ,,	5′ ,,	+11.5 ,,	5′ ,,	+15.0 ,,
				5′ ,,	+12.0 ,,	5′ ,,	+14.5 ,,
25′ white	+87.5 mm	25′ white + red	+110 mm	30′ white	+78 mm	30′ white + red	+91.5 mm
				30′: H = 91.5 − 78 =	+13.5 mm		
25′: H′ = 110 − 87.5 =	+22.5 mm			25′: H =	11.3 mm		

Calculation of quantum efficiency for experiment I (Dark ± Red)

In 60′: H + 31.5 mm H′ + 59 mm

Applying equations (1) and (2), protocol (1)

In 60′ x_{O_2} = +70.4 μl, x_{CO_2} = −56.0 μl $\Big\}$ $\gamma = \frac{CO_2}{O_2} = -0.8$

Quantum efficiency for O_2, $\frac{1}{\varphi} = \frac{60 \cdot 5.4}{70.4} = \underline{4.6}$

Quantum efficiency for CO_2, $\frac{1}{\varphi} = \frac{60 \cdot 5.4}{56} = \underline{5.8}$

Calculation of quantum efficiency for experiment II (White ± Red)

In 25′: H + 11.3 mm H′ + 22.5 mm

Applying equations (1) and (2), protocol (1)

In 25′ x_{O_2} = +30.3 μl, x_{CO_2} = −27.2 μl $\Big\}$ $\gamma = \frac{CO_2}{O_2} = -0.90$

Quantum efficiency for O_2, $\frac{1}{\varphi} = \frac{25 \cdot 5.4}{30.3} = 4.5$

Quantum efficiency for CO_2, $\frac{1}{\varphi} = \frac{25 \cdot 5.4}{27} = 5.0$

* But kept constant by a 500-watt voltage regulator.

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Experiment III, with the same cells, was performed between experiments I and II, the white light being, however, of somewhat lower intensity. Here only *one* vessel (No. 5) was used; but if we take as γ the average value of experiments I and II, that is —0.85, x_{O_2} can be calculated according to equation (3), protocol (1). The readings in vessel (5) were:

No. 5

5′ white + 5.0 mm	5′ white + red + 11.5 mm
5′ ,, + 6.5 ,,	5′ ,, + 9.5 ,,
5′ ,, + 6.5 ,,	5′ ,, + 9.5 ,,
5′ ,, + 5.5 ,,	5′ ,, + 13.0 ,,
5′ ,, + 7.0 ,,	5′ ,, + 15.0 ,,
25′ white + 30.5 mm	25′ white + red + 58.5 mm

$25'$: $H' = 58.5 - 30.5 = +28$ mm

and with $\gamma = -0.85$

$$25'\, x_{O_2} = +34\ \mu l$$

The quantum efficiency with the actinometer value of experiments I and II (5.4 μl O_2 per minute) was

$$\frac{1}{\varphi} = \frac{25 \cdot 5.4}{34} = \underline{4.0} \text{ for } O_2$$

The total duration of these experiments was 7 hours from the time of initial equilibration until the last yield determination that gave a value $\frac{1}{\varphi} = 4.5$ for oxygen, which was obtained at approximately 4 times the compensation point. The final p_H in the cell suspensions was 5.4.

PROTOCOL No. 3

Comparison of the yield in carbonate-bicarbonate mixtures and in culture medium

Experiment of VI-1-49. 20° C. 630–660 mμ. Three vessels, in each 7 ml cell suspension, containing 200 μl of cells. Cultures centrifuged, then washed once in, and taken up in, carbonate-bicarbonate mixture. Intensity 5.4 μl O_2 per minute.

I. Vessel No. 7.

$V = 13.824$ ml
$v_f = 7.00$ ml
$k_{O_2} = 0.657$

Gas space air. Solution 85 parts M/10 $NaHCO_3$ + 15 parts M/10 K_2CO_3; p_H 9.2. *At compensation point with white light.*

15′ white light	0
15′ ,, ,, + red light	+ 11.5 mm
15′ ,, ,,	0
15′ ,, ,, + ,, ,,	+ 11.5 ,,
15′ ,, ,,	— 0.5 ,,
Light action 30′	+ 23 + 0.5 = + 23.5 mm = 15.4 μl

$$\frac{1}{\varphi} = \frac{30 \cdot 5.4}{15.4} = \frac{162}{15.4} = \underline{10.5}$$

II. Vessels Nos. 3 and 5, containing 7 ml culture medium, p_H 4.9, with 200 μl of cells each. Cultures centrifuged, then washed once in, and taken up in, fresh culture medium. Gas space 5% CO_2 in air. Mirror shifted every 10′ from one vessel to the other; actinometer 5.4 μl O_2 per minute for red light.

No. 3

$V = 17993$ ml
$v_f = 7000$,,
$k_{O_2} = 1.046$
$k_{CO_2} = 1.634$

15′ white light	+ 11.0 mm
15′ ,, ,, + red light	+ 17.0 ,,
15′ ,, ,,	+ 10.5 ,,
15′ ,, ,, + ,, ,,	+ 16.0 ,,
30′	H = + 11.5 mm

No. 5

$V = 13913$ ml
$v_f = 7000$,,
$k'_{O_2} = 0.665$
$k'_{CO_2} = 1.253$

15′ white light + red light	+ 29.5 mm
15′ ,, ,,	+ 15.5 ,,
15′ ,, ,, + ,, ,,	+ 29.0 ,,
15′ ,, ,,	+ 17.5 ,,
30′	H′ = + 25.5 mm

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$$\left.\begin{array}{l} x_{O_2} = +41.3 \text{ mm} \\ x_{CO_2} = -43.0 \text{ ,,} \end{array}\right\} \gamma = -1.04$$

$$\frac{1}{\varphi} = \frac{30 \cdot 5.4}{41.3} = \frac{162}{41.3} = 3.9$$

III. Vessel No. 7, with same cells as before but without white light (below compensation-point). pH 9.2

10′ dark	−33.5 mm
10′ red light	−23.5 ,,
10′ dark	−30.5 ,,
10′ red light	−22.5 ,,
10′ dark	−30.0 ,,
20′ dark	−60.5 ,,
20′ dark	−62.7 mm, 20′ red light −46.0 mm

Light action 20′ $62.7 - 46 = +16.7 \text{ mm} = 11\ \mu l$

$$\frac{1}{\varphi} = \frac{20 \cdot 5.4}{11} = \underline{9.8}$$

IV. Again Nos. 3 and 5, but no white light (under conpensation point) pH 4.9

No. 3		No. 5	
10′ dark	−4.0 mm	10′ red light	− 5.0 mm
10′ red light	−1.5 ,,	10′ dark	−12 ,,
10′ dark	−5.0 ,,	10′ red light	− 4.5 ,,
10′ red light	−1.5 ,,	10′ dark	−13 ,,
10′ dark	−5.0 ,,	10′ red light	− 4.0 ,,
30′ dark	−14.0 mm	30′ dark	−37.6 mm
30′ red light	− 4.5 ,,	30′ red light	−13.5 ,,

Light action $H = +9.5$ mm $\qquad H' = +24.1$ mm

30′

$$\left.\begin{array}{l} x_{O_2} = +45.6 \\ x_{CO_2} = -53.0 \end{array}\right\} \gamma = -1.18$$

$$\frac{1}{\varphi} = \frac{30 \cdot 5.4}{45.6} = \underline{3.6}$$

V. Again No. 7, but with half light intensity (Actinometer, 2.75 μl O_2 per minute), pH 9.2.

10′ red light	−24 ,,
10′ dark	−28 ,,
10′ red light	−24.5 ,,
10′ dark	−27.0 ,,
10′ red light	−23 ,,
Light action 10′	$27.5 - 23.8 = +3.7$ mm $= 2.42\ \mu l$

$$\frac{1}{\varphi} = \frac{10 \cdot 2.75}{2.42} = \underline{11.3}$$

The total duration of these experiments was 8 hours.

IX. SUMMARY AND CONCLUSION

Since development of the new methods and procedures described, in a sequence of thirty experimental days, almost without exception quantum efficiencies of 3 to 5 quanta per molecule of O_2 produced by the action of red light have been obtained. The simultaneously observed quotients of $\frac{CO_2}{O_2}$ for light action lay between −0.8 and −1.3, which means that the quantum efficiencies for CO_2 consumption in red light were essentially the same as those for O_2 production.

These results were obtained not only with low light intensities below the compen-

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sation point and for short periods of time (minutes), but also with light intensities well above the compensation point (several fold), and in experiments lasting many hours. It is important to emphasize that with the same cell suspension the same quantum yields may be obtained both below and far above the compensation point.

The new results resolve several uncertainties left open by the experiments of 1923. At that time the light intensities were so low that only a fraction of the respiration was compensated by the light. Thus the objection could never have been refuted that light inhibited respiration anticatalytically, that is, without expenditure of energy. But now, in the experiments above the compensation point, this question is eliminated, and chemical energy, corresponding to positive O_2 production and CO_2 consumption, is in fact clearly gained.

It was a further shortcoming of the experiments of 1923, that the yields had been determined only for short periods of time (*e.g.*, 10 minutes). But now, in the experiments above the compensation point, the cells are so nearly under their natural culture conditions, that there is no evident time limit to yield determinations. Thermodynamically this is a noteworthy advance since the longer the experiments the surer becomes the necessary condition of all calculations of yield: that the absorbed light energy is the sole source of energy for the photosynthetic processes.

Finally, we may point out that the methodology has been so simplified that efficiency determinations can be carried out wherever simple manometric equipment and a suitable light source are available, without the need of a bolometer, thermopile, cathetometer or special differential manometer. In fact, demonstration of the high quantum efficiencies reported in this paper may readily be made in the laboratory classroom.

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RÉSUMÉ ET CONCLUSIONS

Depuis le développement des nouvelles méthodes et des nouveaux procédés décrits, nous avons trouvé, à peu près sans exception, une efficience de 3 à 5 quanta par molécule d'oxygène produite par l'action de la lumière rouge. Les coefficients $\frac{CO_2}{O_2}$ observés simultanément pour l'action de la lumière se trouvaient entre —0.8 et —1.3, ce qui signifie que l'efficience en quanta pour la lumière rouge est à peu près la même pour la consommation de CO_2 que pour la production de O_2.

Ces résultats ont été obtenus non seulement pour de faibles intensités et de courtes périodes, mais aussi pour des intensités bien au-dessus du point de compensation (plusieurs fois) et pour des expériences durant plusieurs heures. Il est intéressant de noter que l'on peut obtenir les mêmes rendements en quanta pour une même suspension cellulaire au-dessous et au-dessus du point de compensation.

Les nouveaux résultats résolvent plusieurs incertitudes qui avaient subsistées après les expériences de 1923. A cette époque, les intensités de lumière étaient si faibles que seule une fraction de

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la respiration était compensée par la lumière. C'est pourquoi, l'objection n'a jamais pu être réfutée selon laquelle la lumière empêcherait la respiration anticatalytiquement, c.à.d. sans dépense d'énérgie. Actuellement cette question se trouve éliminée par les expériences au-dessus du points de compensation et on a vraiment un gain en energie chimique correspondant à une production positive de O_2 et une consommation de CO_2.

Une autre insuffisance des expériences de 1923 est due au fait que les rendements avaient été déterminés seulement pour des périodes brèves (p. ex. 10 minutes). Actuellement, où l'on travaille au dessus du points de compensation, les cellules se trouvent si près de leurs conditions de culture naturelles qu'il n'y a pas de temps limité évident pour les déterminations de rendement. C'est un sérieux avantage du point de vue thermodynamique, car plus les expériences sont longues, et plus sûrement la condition nécessaire pour toute détermination de rendement sera remplie, c.à.d. que la lumière absorbeé soit la seule source d'énérgie pour le processus photosynthétique.

Finalement, nous avons, tellement simplifié la méthodologie que des déterminations d'efficience simplifiées peuvent être effectuées facilement partout où l'on dispose d'un simple manomètre et d'une source de lumière adéquate. On n'a pas besoin de bolomètre, de thermopile, de cathétomètre, ni de manomètre différentiel spécial. En effet, l'on peut démontrer l'efficience quantique élevée, rapportée dans ce mémoire, dans un laboratoire de classe.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

Seit die hier beschriebenen neuen Methoden und Verfahren entwickelt worden sind, haben wir in einer Reihe von 30 Arbeitstagen fast ohne Ausnahme Quantumleistungen von 3 bis 5 Quanta pro Molekül O_2 (gebildet unter der Einwirkung von rotem Licht) gefunden. Gleichzeitig wurden Quotienten $\frac{CO_2}{O_2}$ für die Lichtwirkung gefunden, die zwischen —0.8 und —1.3 lagen; dies bedeutet dass die Quantumleistung in rotem Licht für CO_2-Aufnahme und O_2-Abgabe ungefähr gleich war.

Diese Ergebnisse wurden nicht nur für niedrige, unter dem Kompensationspunkt gelegene Lichtintensitäten und für kurze Zeitspannen (Minuten) gefunden, sondern auch für hohe, weit über dem Kompensationspunkt gelegene Lichtintensitäten und für Versuche von mehreren Stunden. Mit der gleichen Zellsuspension kann man unter- und oberhalb des Kompensationspunktes dieselbe Quantumausbeute erhalten.

Die neuen Ergebnisse beheben einige Unsicherheiten der Versuche von 1923. Damals waren die Lichtintensitäten so gering, dass nur ein Teil der Atmung durch das Licht kompensiert wurde. Der Einwand, dass das Licht die Atmung antikatalytisch, also ohne Energieverbrauch hemme, konnte daher nie widerlegt werden. Nun aber, in den Versuchen oberhalb des Kompensationspunktes, ist diese Frage erledigt; es wird wirklich Energie entsprechend der Abgabe von O_2 und Aufnahme von CO_2 gewonnen.

Ein anderer Mangel der Versuche von 1923 bestand darin, dass die Ausbeuten nur über eine kurze Zeitspanne (z.B. 10 Minuten) bestimmt wurden. Nun aber, in den Versuchen oberhalb des Kompensationspunktes, befinden sich die Zellen so nahe den Bedingungen einer normalen Kultur, dass eine offensichtliche Zeitgrenze für Bestimmungen der Ausbeute nicht besteht. Thermodynamisch gesehen ist das ein wichtiger Fortschritt, denn je länger die Versuchszeit, desto sicherer wird die für alle Berechnungen der Ausbeute notwendige Bedingung erfüllt sein: dass nämlich die absorbierte Lichtenergie die einzige Energiequelle für den photosynthetischen Vorgang sei.

Endlich können wir darauf hinweisen, dass wir die Methodologie so vereinfacht haben, dass Leistungsbestimmungen mit einem einfachen Manometer und einer passenden Lichtquelle, ohne Bolometer, Thermoelement, Cathetometer und Spezial-Differentialmanometer ausgeführt werden können. So können die hier mitgeteilten hohen Quantumleistungen im Schullaboratorium nachgewiesen werden.

APPENDIX

I. EMERSON has objected[2,5] to the yield determinations of 1923[1] and 1948[4] on the ground that the assimilatory $\gamma = CO_2/O_2$ was not determined simultaneously with the yield φ; *i.e.*, that the value of γ employed, —0.91, which had been determined gas analytically, may not be the γ during the different φ-determinations carried out for different periods of time, light intensities, and cell cultures.

As has been mentioned, we have observed experimental fluctuations of γ from —0.8 to —1.3 If we had used these γ-values in 1923 for the computation of φ, let us see what the values of φ would have been.

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The volume of our vessel was 37.0 ml and the volume of the liquid phase 16.53 ml. For 10° C

$$k_{CO_2} = 5.67 \qquad k_{O_2} = 1.70$$

$$K_{O_2} = \frac{k_{CO_2} \cdot k_{O_2}}{k_{CO_2} + \gamma\, k_{O_2}} = \frac{5.67 \cdot 1.70}{5.67 + \gamma\, 1.70}$$

Therefore

γ	K_{O_2}	Quantum requirement $\frac{1}{\varphi}$
—0.8	2.24	4.20
—0.91	2.34	4.00
—1.3	2.78	3.40

where the underlined values are the values used and obtained in 1923. This calculation shows that EMERSON's objection was not very significant and could not explain the divergent quantum requirements of 4 against 10 to 12.

II. In an effort to avoid difficulties caused by fluctuations of γ. EMERSON AND LEWIS made quantum-efficiency measurements in carbonate-bicarbonate solutions at p_H 9.1, which kept the CO_2-pressure constant instead of using culture medium at p_H 4.9. They claimed[5] that in such alkaline solutions the quantum-efficiency was the same as in the acid culture medium: "then we find the yields measured in acid phosphate culture medium are in good agreement with those measured in carbonate mixture".

But the experimental data were not presented to substantiate this important statement. We can confirm EMERSON's finding that in the carbonate-bicarbonate mixtures the quantum-requirement is 10 to 12, but we cannot confirm that the same quantum efficiency is obtained in the acid culture medium. Data presented in protocol 3 show that very different quantum-efficiencies are obtained if we determine the quantum efficiency of aliquot portions of a cell suspension in carbonate mixture at p_H 9.1 and in culture medium at p_H 5. The quantum values observed in the following time sequence were

	$\frac{1}{\varphi}$
In carbonate mixture at p_H 9	10.5*
In culture medium at p_H 5	3.9*
In carbonate mixture at p_H 9	9.8
In culture medium at p_H 5	3.6
In carbonate mixture at p_H 9	11.3

where the asterisked values were obtained above the compensation point and the others below the compensation point.

Maximum yields should therefore not be determined in the carbonate mixture, as has been done frequently during the last 10 years.

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3 O. WARBURG AND V. SCHOCKEN, *Arch. Biochem.*, 21 (1949) 363.
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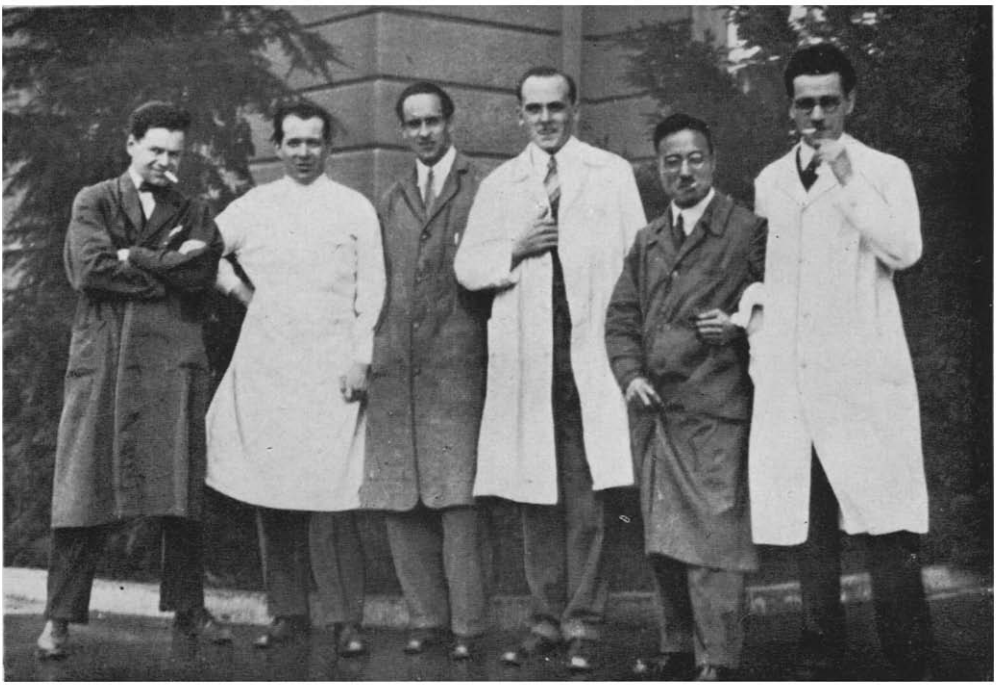

Fig. 1. Left to right: F. Lipmann, D. Nachmansohn, S. Ochoa, F. O. Schmitt, K. Iwasaki, P. Rothschild. Kaiser Wilhelm Institut für Biologie, Berlin Dahlem, 1928.

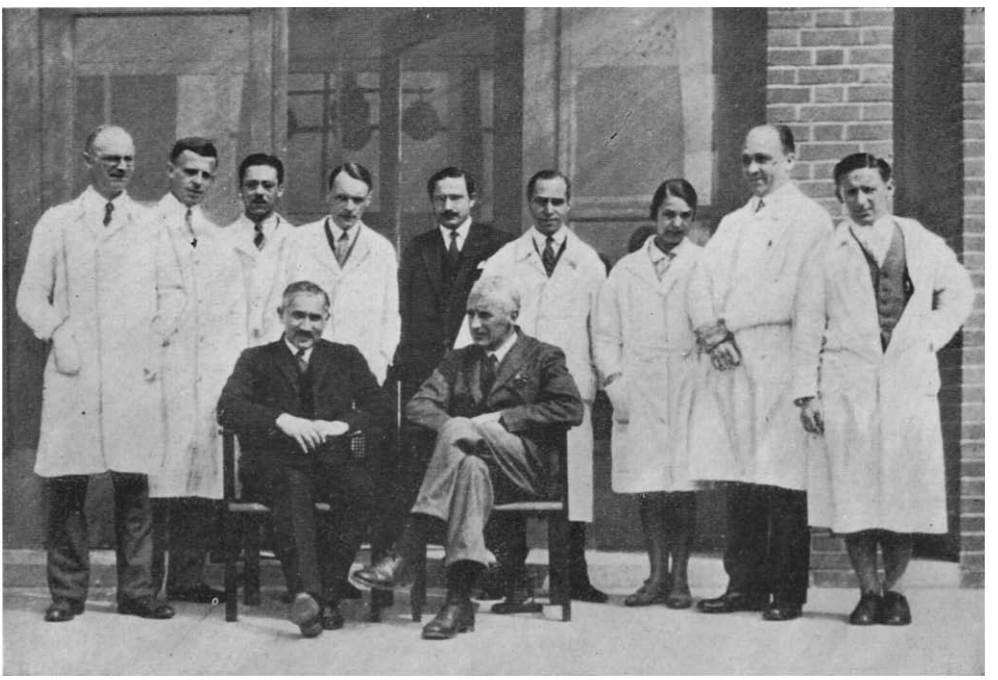

Fig. 2. Left to right: Sitting: O. Meyerhof and A. V. Hill. Standing: K. Lohmann, A. v. Muralt, G. Benetato, H. Blaschko, A. Grollman, H. Laser, Miss Wagner, W. Schulz, E. Boyland. Kaiser Wilhelm Institut für Medizinische Forschung, Heidelberg, 1931.

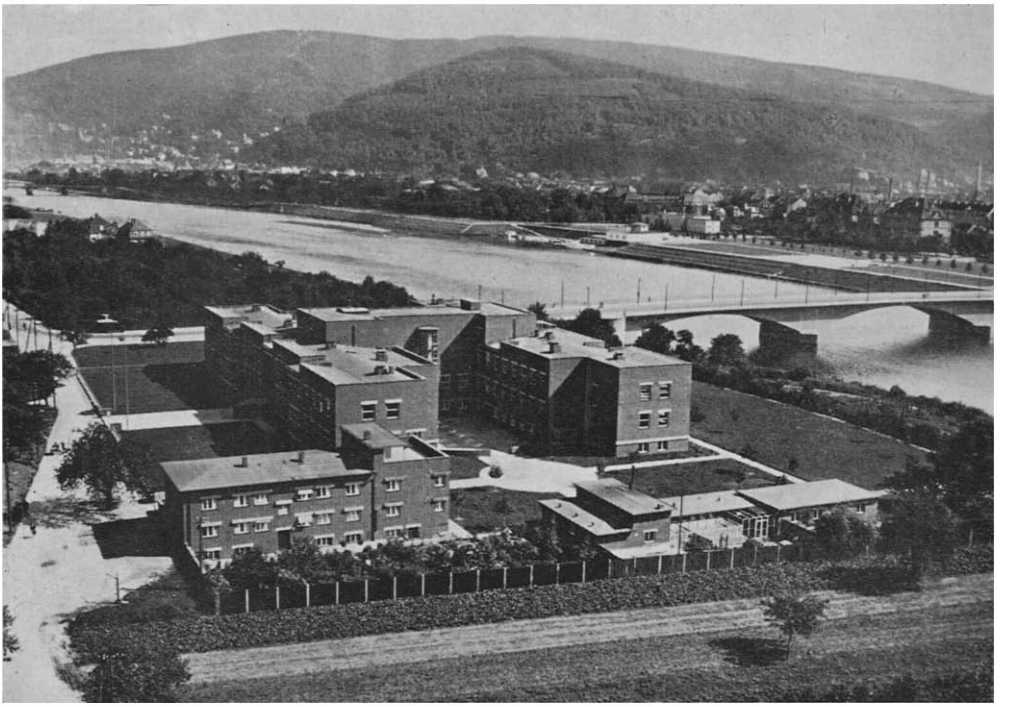

Fig. 3. Kaiser Wilhelm Institut für Medizinische Forschung, Heidelberg.

Fig. 4. Left to right: S. Korey, D. Nachmansohn, D. Burk, A. v. Szent-Györgyi, O. Warburg,