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QUANTUM REQUIREMENT IN PHOTOSYNTHESIS AND THE EFFECT OF LIGHT QUALITY ON PHOTOSYNTHETIC PRODUCT DISTRIBUTION.

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Publication Date
1968-09-01
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Kam-sik Ng
(Ph.D. Thesis)
September 1968

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QUANTUM REQUIREMENT IN PHOTOSYNTHESIS AND THE EFFECT OF LIGHT QUALITY ON PHOTOSYNTHETIC PRODUCT DISTRIBUTION

Kam-sik Ng

(Ph. D. Thesis)

Sept. 1968
TO MY PARENTS

and

DR. M. S. TSAO
ACKNOWLEDGMENTS

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ACKNOWLEDGMENTS

During the course of conducting the research described in this thesis, many individuals have provided me with assistance in different ways. I would like to thank all of them. Dr. J. A. Bassham's continuous suggestions in the experimental design and his valuable discussion and help in interpreting the results are greatly acknowledged. I would like to thank Professor M. Calvin for giving me the unmatched opportunity of working in the Laboratory of Chemical Biodynamics. I thank Professor K. Sauer for providing me with much helpful assistance in the measurement of the quantum requirement. Mr. A. M. El-Badry has read the entire manuscript of this thesis and has provided me with valuable criticism and enlightening discussion, and he has my gratitude. I would like to thank Mrs. E. Litton and Mrs. J. Onffroy for their help in preparing the manuscript. To Mrs. M. Kirk, Mrs. B. Mann, and Mrs. D. Roadman I owe a word of gratitude for their technical assistance.

The work described in this thesis was supported, in part, by the United States Atomic Energy Commission.
ABSTRACT

Among the different opinions on the photoelectron transport scheme, the "photolyt" hypothesis and the Hill-Bendall scheme of photoelectron transport are the major ones. These two hypotheses were discussed and compared on several aspects of photosynthesis. In order to test these two hypotheses, the quantum requirement in photosynthesis and the effect of light quality on photosynthetic product distribution were studied.

Measurement of the quantum requirement in photosynthesis consists of measuring the amount of oxygen evolved and the amount of light energy absorbed by the algae. The amount of oxygen was measured electronically with an oxygen analyzer, which determines the paramagnetism of the gas mixture. Therefore, the ambiguities stemming from the interpretation of manometric data are avoided. Light measurement requires the use of a photocell, which is calibrated against a calibrated thermopile. The calibration of the photocell against the thermopiles requires a reference light beam, which has to be (i) monochromatic, (ii) constant, (iii) uniform, and (iv) large enough to cover the entire detecting surface of the thermopile. The reference light beam which meets all these requirements was achieved by using filtered sunlight. The probable error in all aspects of the determination were discussed and uncovered a likely cause of error in the previously reported quantum requirement by a similar method. In the present study, all of the obtained values of quantum requirement are above 8, which favors the Hill-Bendall scheme of photoelectron transport.

The study of the effect of light quality on photosynthetic product distribution requires the comparison of photosynthetic products formed.
by algae illuminated with different wavelengths of light. Such comparison requires algae of the same kind and the same age to photosynthesize under identical conditions with one variant, the wavelengths of the illuminating light. Thus, the differences are solely due to the difference in the wavelengths of the illuminating light. This is achieved by having the algae carry their photosynthesis in a pair of "twin cells" which consist of two compartments containing the algae suspension. Algae photosynthesis in these two compartments of the twin cells have a common gas phase. Therefore, algae suspensions from the same batch can photosynthesize under identical conditions, with the exception of the wavelengths of the illuminating light.

Since the results of the second experiment do not support the conclusion of the first one, several explanations are proposed, and experiments on far red light stimulated cyclic photophosphorylation were done to test the validity of these explanations.
I. INTRODUCTION

"Photosynthesis", as the word implies, can be divided into two parts\(^1\)\(^-\)\(^3\)--a "photo" part in which light energy is converted into chemical energy, and a "synthesis" part in which the generated chemical energy is used for the reduction of CO\(_2\). The study of photosynthesis can, therefore, be divided into areas:

1. The study of photochemical reactions in which light energy is converted into assimilatory power.\(^4\)

2. The study of biosynthetic reactions in which the generated assimilatory power is utilized for the reduction of CO\(_2\) into various compounds.\(^5\),\(^6\)

The second process in which CO\(_2\) is reduced to various compounds has been studied in great detail. The photosynthetic carbon reduction cycle proposed by Calvin and his colleagues,\(^7\),\(^8\) together with the various known biosynthetic pathways\(^9\)-\(^12\) have provided us with a clear picture of the path of carbon in photosynthesis.

However, the first process in which light energy is converted into assimilatory power is not so clearly understood.\(^13\)-\(^15\) Among the various hypotheses concerning the photochemical reactions, there are two major ones. They are the "photolyt" hypothesis proposed by Warburg\(^16\)-\(^18\) and the "two light reactions" hypothesis based on the Hill-Bendall scheme\(^19\),\(^20\) of photoelectron transport.

The two hypotheses have different assumptions with regard to the course of primary photochemical reactions. They differ in several aspects of photosynthesis--e.g., the origin of the oxygen evolved, the
value of quantum requirement, and the generation of assimilatory power.

The rest of this chapter will be devoted to the discussion of these two hypotheses. Experiments included in Chapters II and III are designed to verify the validity of these two hypotheses. However, a comparison between the results of Chapters II and III shows inconsistency. This apparent inconsistency is investigated in Chapter IV.

A. The "Photolyt" Hypothesis

1. The quantum requirement in photosynthesis

Warburg and Negelein15,21,22 were the first to study the quantum requirement of photosynthesis. The quantum requirement obtained in their first series of determinations was 4-5 einsteins of photons per mole of CO₂ fixed.22 Based on the free energy calculation of the following equation

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2 \quad \Delta F = +112 \text{ kcal/mole} \]

the absorption of one mole of CO₂ represents the fixation of 112 kcal of energy. The efficiency in energy conversion by photosynthesis can be calculated by the following formula:

\[ \frac{\Delta F}{\text{Q.R.} \times \text{NhC}/\lambda} \]

Since the wavelength of the red light used by Warburg was 660 μm, the efficiency in energy conservation by photosynthesis is

\[ \frac{112,000 \text{ cal}}{4 \times (\text{NhC}/660 \mu \text{m})} = 65\% \]

Since many investigators14,23-25 outside the Warburg school claimed that they were unable to reproduce the low quantum requirement reported by Warburg, Warburg and coworkers redetermined the quantum requirement
using a modified technique, and arrived at a quantum requirement of 2.76,26 which represented an efficiency of 93% in energy conversion.

2. The hypothesis

Burk and Warburg16-18 considered that the fixation of CO₂ into carbohydrates is a primary photoreaction. It should require only one photon for the fixation of one molecule of CO₂. However, the free energy change upon the conversion of CO₂ into carbohydrates, together with the evolution of O₂, is 112 kcal per mole, and the energy of one einstein of red light (= 660 μν) is only 43 kcal; one photon is therefore unable to promote the following reaction:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2 \quad F = +112 \text{ kcal/mole}$$

(assuming the (CH₂O) is at the same reduction level as carbohydrates)

Burk and Warburg accounted for the energy deficit in this reaction by their "photolyt" hypothesis.16,18

A "photolyt"27,28 is an activated CO₂ complex, which is formed by activating CO₂ (or H₂CO₃) by the following reactions:

$$(\text{CH}_2\text{O}) + \text{O}_2 \xrightarrow{\text{respiration}} \text{CO}_2 + \text{H}_2\text{O} + \text{energy}$$

$$\text{---H}_2\text{CO}_3 + \text{energy} \rightarrow \text{---H}_2\text{CO}_3^*$$

where (CH₂O) represents compounds oxidized in respiration, ---H₂CO₃ is the bicarbonate ion bound inside the cell, and ---H₂CO₃* is the "photolyt" formed by utilizing the energy released in respiration. According

*The "photolyt" has never been isolated. ---H₂CO₃ is a hypothetical intermediate, namely, H₂CO₃ bound in a complex which is converted into ---H₂CO₃*, the "photolyt", upon the absorption of the energy released in respiration.
to the hypothesis, the "photolyt" is an oxygen precursor which splits into \((\text{CH}_2\text{O})\) and \(\text{O}_2\) upon the absorption of a photon. Since one photon of 60 mu light \((e=h\nu=43 \text{ kcal/mole})\) is able to promote the reaction of

\[
\text{\(\text{H}_2\text{CO}_3\)} + \text{hv} \rightarrow \text{\((\text{CH}_2\text{O})\)} + \text{O}_2 \quad F = 112 \text{ kcal}
\]

the free energy of formation of the "photolyt" must be \((112-43) = 69 \text{ kcal}\) higher than that of \(\text{H}_2\text{CO}_3\). This 69 kcal of free energy required for the activation of the \(\text{H}_2\text{CO}_3\) is provided by the oxidation of \(2/3\) mole of \((\text{CH}_2\text{O})\) in respiration.

Assuming the reduction level of \((\text{CH}_2\text{O})\) is the same as that of carbohydrate, the oxidation of \(2/3\) mole of \((\text{CH}_2\text{O})\) will release

\((112) \times 2/3 = 74 \text{ kcal}\) of energy, which is more than enough to make up the energy deficit in the activation of \(\text{H}_2\text{CO}_3\) into \(\text{H}_2\text{CO}_3^*\), provided that energy transfer and utilization is highly efficient.

The "photolyt" hypothesis can be summarized in the following equations:

\[
\text{\(\text{H}_2\text{CO}_3^*\)} + \text{hv} \rightarrow \text{\((\text{CH}_2\text{O})\)} + \text{O}_2
\]

\[
2/3 \text{\((\text{CH}_2\text{O})\)} + 2/3 \text{O}_2 \rightarrow 2/3 \text{CO}_2 + 2/3 \text{H}_2\text{O} + 70 \text{ kcal}
\]

\[
\text{\(\text{H}_2\text{CO}_3\)} + 70 \text{ kcal} \rightarrow \text{\(\text{H}_2\text{CO}_3^*\)}
\]

The overall process \(1/3 \text{\(\text{H}_2\text{CO}_3\)} + \text{hv} \rightarrow 1/3 \text{\((\text{CH}_2\text{O})\)} + 1/3 \text{O}_2\)

Since one einstein of photons \((Nh\nu)\) promotes the reduction of only \(1/3\) mole of \(\text{CO}_2\), the theoretical quantum requirement of \(\text{CO}_2\) uptake is

\[
\frac{1 \text{ einstein of photon}}{1/3 \text{ mole of } \text{CO}_2 \text{ consumed}} = 3
\]

If the ratio of \(\gamma = \frac{\text{CO}_2}{\text{O}_2} = 1\), the theoretical quantum requirement for \(\text{O}_2\) evolution will also be 3.
3. Experimental evidences for the "photolyt" hypothesis

The "photolyt" hypothesis indicates that the theoretical quantum requirement for the reduction of one mole of CO$_2$ is 3 or less. The value of quantum requirement in Chlorella photosynthesis first reported by Warburg and coworkers was between 4 and 5. After 1946, Warburg and co-workers determined the quantum requirement with improved technique and arrived at the value of 2.76 photons per mole of O$_2$ evolved or CO$_2$ uptake. This was indeed a very strong support of the "photolyt" hypothesis.

The validity of the "photolyt" hypothesis was further supported by the acid fluoride and initial oxygen evolution experiments, which gave indirect evidences for the existence of the "photolyt".

a. The acid fluoride experiment$^{29-32}$

Evidence concerning the existence of the "photolyt" comes from the acid fluoride (hydrogen fluoride) experiment of Warburg and his colleagues.$^{15}$ In their experiment,$^{31,32}$ four identical Warburg vessels were set up, each containing 2.0 ml of 5% Chlorella in culture medium. The gas phases of these vessels were:

- Vessel I: 20% CO$_2$ in air
- Vessel II: 20% CO$_2$ in air
- Vessel III: 20% CO$_2$ in argon
- Vessel IV: argon free of CO$_2$

After these vessels were shaken in the dark for half an hour, acid fluoride (HF) was tipped from the side arms of the vessels II, III, and IV. Vessel I was illuminated with bright red light for 5 min, and acid fluoride was then tipped in. (In all cases, enough acid fluoride was added so that the final concentration was high enough to stop respiration.)
Upon the addition of acid fluoride, there was an increase in pressure in all vessels, as indicated by the manometer. This increase in pressure was found due to an outburst of CO₂. The amount of CO₂ release in each vessel is shown in Figure I-1.

According to Warburg and his colleagues, the CO₂ released in vessel IV represents the amount of free glutamic acid in the *Chlorella* cells, because HF stimulates an enzymatic decarboxylation of glutamic acid. The CO₂ released in vessel III represents the amount of glutamic acid and CO₂ bound as bicarbonate when the gas phase contains 20% CO₂. The CO₂ released in vessel II represents:

(i) the amount of free glutamic acid in the cells,
(ii) the CO₂ bound as bicarbonate, and
(iii) the aerobically bound CO₂, which is identical to the activated complex of CO₂, the "photolyt".*

The CO₂ released in vessel I represents:

(i) the amount of free glutamic acid in the cells,
(ii) the CO₂ bound as bicarbonate, and
(iii) the small amount of "photolyt" remained in the cell after 5 min illumination in bright red light.*

The explanation¹⁵,32 for the above phenomenon is that during incubation in the dark there was respiration in vessels I and II, but not in vessels III and IV because vessels III and IV contained no oxygen for respiration. Hence, upon the addition of acid fluoride, the excess CO₂ evolved in vessel II over that of vessel III was defined as aerobically

*See appendix I for alternate explanation.
Figure I-1. The activated CO$_2$ of Chlorella.$^{32}$

A = Aerobically bound CO$_2$
G = glutamate
bound CO\textsubscript{2}. In the opinions of Warburg and coworkers, this aerobically bound CO\textsubscript{2} is identical to the "photolyt", the activated CO\textsubscript{2} complex H\textsubscript{2}CO\textsubscript{3}\textasciitilde, which can be formed in the dark if energy is provided from respiration. Upon the addition of acid fluoride, the "photolyt" was decomposed and the aerobically bound CO\textsubscript{2} was therefore released. Upon illumination of vessel I, there was an evolution of O\textsubscript{2}. The amount of this O\textsubscript{2} exceeded that of the CO\textsubscript{2} by an amount approximately equal to the difference in aerobically bound CO\textsubscript{2} released between vessels I and II. Therefore, the nature of the aerobically bound CO\textsubscript{2} can be represented by the following equation:

\[
\begin{align*}
\text{H}_2\text{O} + \text{CO}_2 & \xrightarrow{\text{HF}} \text{H}_2\text{CO}_3\text{\textasciitilde} \xrightarrow{\text{hv}} -(\text{CH}_2\text{O}) + \text{O}_2
\end{align*}
\]

By using different amounts of acid fluoride\textsuperscript{33,34} to control the amount of "photolyt" remaining in the cells, Warburg showed the stoichiometric relationship between the "photolyt" and the O\textsubscript{2} evolved.\textsuperscript{27,35,36} This provided him additional evidence to identify the "photolyt" as an oxygen precursor.

b. The initial oxygen evolution and enhanced respiration

Quantum requirement measurement on the initial oxygen evolution of Chlorella which had been incubated in the dark showed that it takes only one photon for each O\textsubscript{2} molecule evolved.\textsuperscript{26} Warburg took this as evidence for the one quantum light reaction of the "photolyt".

\[
\begin{align*}
-\text{H}_2\text{CO}_3\text{\textasciitilde} + \text{hv} & \longrightarrow -(\text{CH}_2\text{O}) + \text{O}_2
\end{align*}
\]

Moreover, he observed that there was an enhanced O\textsubscript{2} uptake right after illumination.\textsuperscript{37,38} He regarded this as evidence for the formation of "photolyt" in the dark with energy provided by respiration. This process is the dark reactions in his "photolyt" hypothesis.
\[
\frac{2}{3} \text{respiration} \quad \frac{1}{2}(\text{CH}_2\text{O}) + \frac{1}{2} \frac{1}{2} \text{O}_2 \rightarrow \frac{2}{3} \text{H}_2\text{CO}_3 + 70 \text{kcal}
\]

\[
\text{---H}_2\text{CO}_3 + 70 \text{kcal} \rightarrow \text{---H}_2\text{CO}_3^*
\]

c. Composition of the "photolyt"

An examination of Figure I-1 indicates that the amount of aerobically bound CO\textsubscript{2} and free glutamic acid is the same.\textsuperscript{31,32} Determination on the chlorophyll and free glutamic acid content of several algae showed that the ratio of aerobically bound CO\textsubscript{2}:glutamic acid: chlorophyll = 1:1:1.\textsuperscript{36} This important discovery suggests the possibility of a complex formed with CO\textsubscript{2}, glutamic acid, and chlorophyll. Hence, the "photolyt" can be represented by (chlorophyll-glutamic acid-CO\textsubscript{2}). However, it must be noted that the symbol gives no more information than the stoichiometric relationship among its components.

B. The Hill-Bendall Photoelectron Transport Scheme

Although Warburg had experimental evidences to support his "photolyt" hypothesis, many investigators outside the Warburg school\textsuperscript{14} were unable to reproduce many of the experiments reported by Warburg and his co-workers. Typical examples are the low quantum requirement\textsuperscript{23} in photosynthesis and the acid fluoride experiments. Furthermore, there are many experimental phenomena\textsuperscript{39,40} which are unable to be explained by the "photolyt" hypothesis. Among them, the enhancement\textsuperscript{41-43} and the chromatic transient\textsuperscript{44,45} effects are the typical ones.

1. The enhancement effect

In 1943 Emerson and Lewis measured the quantum efficiency of \textit{Chlorella} photosynthesis as a function of wavelength.\textsuperscript{23} The action spectrum (Figure I-2) shows that the quantum efficiency is constant over the range from 580 \textmu m to 680 \textmu m. The dip at 480 \textmu m can be accounted for
Efficiency of light absorbed by *Chlorella* sp. determined over the range of the visible spectrum. Emerson and Lewis

Figure I-2
by the absorption of carotenoid, which is unable to promote photosynthesis. However, not only the low quantum efficiency (high quantum requirement*) but also the severe drop at wavelengths slightly longer than 680 μm were unexplainable by the "photolyt" hypothesis, because the absorption of chlorophyll a is still high in that region. Furthermore, Emerson and coworkers found that the addition of short wavelength light enhances the quantum efficiency of Chlorella photosynthesis at long wavelength red (over 680 μm) to the limit of short wavelength red (580 μm to 680 μm). (See Figure 1-3).

2. The chromatic transient study

The chromatic transient (Figure 1-4) was discovered by Blinks, who adjusted the intensities of the two light beams (650 μm and 700 μm) so that they gave equal steady rates of photosynthesis when used separately. Upon substituting one beam for the other he observed a transient disturbance in the time course of oxygen evolution.

Myers and French studied the action spectra of enhancement and chromatic transients with background light of 700 μm; when plotted on comparable scale, the action spectra of these effects in Chlorella were identical. All these observed effects cast doubt on the "photolyt" hypothesis.

3. The Hill-Bendall photoelectron transport scheme

Based on experimental evidences, as well as thermodynamic calculations, Hill and Bendall proposed a photoelectron transport scheme

*Calculation from the action spectrum in Emerson's measurement shows the lowest quantum requirement is more than 10, while Warburg's values are below 4.
Figure I-3. Quantum yield of $O_2$ production as a function of actinic wavelength.

Solid curve: No supplemental light, "red-drop" experiment.

Dotted curve: Short wavelength supplemental light, "enhancement" experiment.
Figure 1-4. Demonstration of chromatic transients and enhancement in Chlorella. A 700 μm and a 650 μm light beam were adjusted to yield equal steady-state rates. An initial excess rate occurred upon switching from 700 to 650 μm and an initial lag after switching back. The three last exposures demonstrate the amount of enhancement indicated Δ under steady illumination as well as a slow adjustment. Rapid alternation in experiments D and H shows the survival of an "enhancing" intermediate after the light (Myers and French).
which consists of two light reactions instead of one. The photoelectron transport scheme which is now called the Hill-Bendall scheme of photoelectron transport, can be represented by the following diagram.\textsuperscript{20} (Figure I-5).

The exact nature of $X$, $Y$, $P$, and $Q$ is not known. $X$ must be a very strong reducing agent with a potential at least $-0.74$ V, since isolated chloroplast was found to be able to reduce viologen V-740 $^{50}$ ($1,1'$-trimethyl-4,4'$'$-dimethyl-2,2'$'$-diphridylium dibromide), which has a reduction potential of $-0.739$ V. It has been suggested that $Q$ is a kind of plastoquinone with a potential of $0.0$ V. P-700 may be identical to Kok's P-700,$^{56}$ which is an aggregate of chlorophyll $a$ molecules in special binding states, and can be oxidized only by chemicals of oxidation potentials above $+0.45$ V. Very little is known about $Y$, which is probably also an aggregate of chlorophyll $a$ molecules and its oxidized form should have a potential strong enough to oxidize water to oxygen ($0.815$ V at pH 7 and 25°C).

4. **Explanation of the "Red Drop" and "Enhancement" effect**

Both the "red drop" and "enhancement" effect strongly suggest the existence of two light reactions in the overall photoelectron transport.$^{57}$ Long wavelength red light is probably inefficient in promoting one of the photoreactions which requires higher energy, so that the quantum yield of *Chlorella* photosynthesis drops severely for wavelengths longer than 680 nm. Apparently, the short wavelength red light is able to promote both photoreactions efficiently. If both the long wavelength and the short wavelength red light are present, the short wavelength red light will be used to promote the more difficult photoreaction, leaving the long wavelength red light to promote the other one. In this case,
Figure I-5. Diagrammatic representation of photoelectron transport in chloroplasts as a composite of two light reactions and dark reactions. (The light reactions are indicated by open arrows, the dark reactions by black arrows. Standard redox potentials of some of the intermediates are indicated on the adjoining redox scale.)
the long wavelength red light is used as efficiently as the short wavelength one. This explains the "red drop" and "enhancement" effect in photosynthesis. 58

5. Explanation of the "Chromatic Transient" effect 58

The work of Myers and French42,49 indicated that both the enhancement and chromatic transient expressed identical action spectra. In the overall photoelectron transport, the product of one light reaction may be the substrate for the other. Illumination by light, which preferentially promotes one of the light reactions, may result in an accumulation of a substrate for the other. Therefore, upon changing to the other light beam, which preferentially promotes the other light reaction, there is a transient in the time course of oxygen evolution. This explanation is further supported by the fact that the shape of the transient is somehow reversed, if the sequence of the light beams applied is reversed. It indicates that illumination by one light beam may lead to an "overdraw" of the substrate. Consequently, a reverse transient occurs upon switching to the other light beam.

Studies on chloroplasts lead to the identification of two light reactions.3,55,59,60 Photoreaction I can be promoted by long wavelength red, which is unable to promote photoreaction II. Inhibitors studies61 indicate that photoreaction I is coupled to the reduction of $\text{NADP}^+$ while photoreaction II is coupled to the oxidation of water. The action spectrum of chromatic transients as well as studies on chloroplasts62-64 indicate that the center for photoreaction I is rich in chlorophyll a, while that for photoreaction II is rich in chlorophyll b. The center for photoreaction I together with its pigment system is called photosystem I, and that for photoreaction II with its pigment system is...
called photosystem II.

C. **Phosphorylation**

According to the "photolyt" hypothesis, all absorbed light energy is used to split the "photolyt" into $O_2$ and $(CH_2O)$. The energy required to activate the $CO_2$ comes from respiration. Additional energy in the form of ATP required for the conversion of $(CH_2O)$ also comes from respiration.$^{65,66}$ Therefore, there is only one kind of phosphorylation--oxidative phosphorylation in respiration.

In the Hill-Bendall scheme of photoelectron transport, some of the energy released during the downhill electron transport from $Q$ to $P$ is conserved as ATP. Since the phosphorylation of ADP to ATP accompanies the photoelectron transport, it is called photophosphorylation. It is also called reductive phosphorylation to distinguish it from oxidative phosphorylation, in which the energy required for phosphorylation comes from oxidation of substrates.

Studies in chloroplasts have led to the discovery of two kinds of photophosphorylation.$^{67}$ Arnon defined non-cyclic photophosphorylation as a process by which ADP is phosphorylated to ATP when electrons are transported from water to NADP$^+$.\(^1\) Additionally, photophosphorylation may occur in a cyclic process involving no substrate reduction.$^{68,69}$ The action spectra of these two kinds of phosphorylation indicates that non-cyclic photophosphorylation requires the cooperation of both photoreactions I and II,$^{59}$ while cyclic photophosphorylation requires only photoreaction I.$^{59}$
II. QUANTUM REQUIREMENT IN PHOTOSYNTHESIS

A. Introduction

According to the "photolyt" hypothesis, the quantum requirement in photosynthesis is 4 or less; whereas the Hill-Bendall scheme of photo-electron transport indicates that the theoretical quantum requirement is 8, and the actual value will probably be more. Based on his inhibitor studies on chloroplasts, Arnon proposed a photoelectron transport scheme which consists of only one reaction. This will also lead to a quantum requirement below 8.

Quantum requirement with values below 4 had been reported by Warburg and his colleagues; whereas values above 8 had been reported by Emerson et al. Values between 4 and 8 had been reported by Bassham and his coworkers. In order to explain the result of their measurements, Bassham had suggested that photoreaction II in the Hill-Bendall photoelectron transport scheme might be a "two electrons per photon" reaction, so that the theoretical quantum requirement should be 6.

1. Theoretical quantum requirement of various proposed photo-electron transport schemes

The various proposed photoelectron transport schemes can therefore be divided into three types in terms of stoichiometry, if light is included as a reactant (Figure II-1).

The photoelectron transport scheme shown in Figure II-1A consists of two light reactions: photoreaction I (hv) and photoreaction II (hv). In the photochemical process of photosynthesis a photon is required to promote one electron from water to Q (plastoquinone?) by way of photoreaction II. The electron is then transported by some exogenous reactions
Figure II-1. Various proposed photoelectron transport schemes.
to P (P-700?). An additional photon is required to promote that electron from P to X by way of photoreaction I. Therefore, the overall process requires two photons for the transport of one electron from water to the terminal electron acceptor X.

The photoelectron transport scheme shown in Figure II-1B also consists of two light reactions. However, in photoreaction II, a photon is able to promote two electrons from water to Q. These two electrons are then transported from Q to P by some exogenous reactions. Similar to the photoelectron transport scheme shown in Figure 1A, the excitation of each of these two electrons from P to X requires one photon. Therefore it requires two photons from the transport of two electrons from P to X. The overall process requires \( 1 + 2 = 3 \) photons for the transport of two electrons from water to the terminal electron acceptor X.

In Figure II-1C-a, the photoelectron transport scheme consists of only one light reaction. It requires only one photon for the complete transport of one electron from water to the terminal electron acceptor X.

The scheme shown in Figure II-1C-b is similar to that in Figure II-1C-a except that the origin of oxygen is from \( \text{H}_2\text{O} \) instead of \( \text{CO}_2 \). This scheme is proposed by Arnon\(^70,71\) to account for his observation that some inhibitors have different effects on cyclic and noncyclic photophosphorylation. In fact, his observation only indicates that the sites for cyclic photophosphorylation are different from those for non-cyclic photophosphorylation.

Since one molecule of oxygen is produced for every four electrons removed from water,\(^*\) the quantum requirement of green plants may be

\[
\begin{align*}
4\text{H}_2\text{O} & \quad 4\text{H}^+ + 4\text{OH}^- \\
4\text{OH}^- & \quad 2\text{H}_2\text{O} + \text{O}_2 + 4\text{e}^- \\
4\text{X}^- + 4\text{H}^+ + 4\text{e}^- & \quad 4\text{XH}^+ \\
4\text{H}_2\text{O} + 4\text{X} & \quad 4\text{XH} + 2\text{H}_2\text{O} + \text{O}_2
\end{align*}
\]
defined as the number of photons required to generate one molecule of oxygen from water,

\[ O. R. = \frac{\text{number of photons absorbed (in units of \( \mu \text{ein} \)}}{\text{number of oxygen molecules evolved (in units of \( \mu \text{mole} \)}} \]

Each of these proposed schemes has a different quantum requirement for the evolution of one molecule of oxygen from water. The scheme shown in Figure II-1A gives a quantum requirement of 8, the scheme shown in Figure II-1B gives a quantum requirement of 6, and the scheme shown in Figure II-1C gives a quantum requirement of 4.

2. Values of previous measurements on quantum requirement

A quantum requirement measurement would be a way to rule out some of the proposed schemes. However, despite the many reports on the quantum requirement of photosynthesis, there is still no conclusive opinion on the actual values of this important number.\(^\text{24}\)

Values ranging from 2.8 to 4.5, which can be used to rule out the first and second photoelectron transport schemes, have been reported by Warburg and coworkers.\(^\text{22,26}\) Values around 7, which favors the second scheme, have been reported by Bassham and Shibata.\(^\text{24,70,71}\) Values above 8, which favors the first scheme, have been reported by other workers.\(^\text{24}\)

It is true that only the minimal quantum requirement bears theoretical significance, as it reveals the intrinsic properties of the photoelectron transport apparatus. However, the value of the quantum requirement reported must also be reliable. For example, the low quantum requirement reported by Warburg is subject to uncertainties in his manometric measurement of oxygen evolution. Manometric measurement critically depends on the solubilities of carbon dioxide in solution. It is found
that some algae secrete into the medium a considerable amount of glycolic acid, especially at high light intensity. This will certainly affect the reliability of the manometric measurement. Using an oxygen analyzer which measures the paramagnetism of oxygen, the rate of oxygen evolution measured by Bassham and Shibata was free from such uncertainties. However, the opal glass technique which they employed was based on the assumption that the same portion of light would reach the detecting surface regardless of the diffuseness of the light incident to the opal glass. Whether or not this assumption is correct depends on whether the diffusing power of the glass used scatters the incident light sufficiently. Since it is found that the thin piece of opal glass they used is unable to diffuse the light sufficiently, their measurements are subject to some uncertainties in the calibration of the photocell.

Many other reported values of low quantum requirement (below 8) were the result of arbitrary correction for respiration during photosynthesis. The rate of respiration during photosynthesis has been measured by Brown using $\text{^{18}CO}_2$ and $\text{^{14}CO}_2$ isotopes. However, the various assumptions he made and the scattering of his results prevent one from taking his data as quantitatively reliable.

Studies on chloroplasts by Sauer, Park, and Kelly have given evidence for the existence of two light reactions, and it requires one photon for each of the two light reactions in the photoelectron transport scheme. It therefore favors the photoelectron transport scheme shown in Figure II-1A. This will lead to a quantum requirement of 8 for the evolution of one oxygen molecule. However, studies on chloroplast or other subcellular particles are subject to the possibility of working
on a system which has lost some of its original activity. The addition of artificial electron acceptors and inhibitors in most investigations disturbs the physiological environment of the plant.

Therefore, it is the purpose of the present experiment to study the quantum requirement of photosynthesis in vivo so as to test the validity of each of the schemes proposed and to reinforce the observations found in chloroplast and other subcellular particles.

B. Discussion of the Experiment

1. Choice of plant

Chlorella pyrenoidosa was chosen for quantum requirement because:

(i) it is so small that a large number is used every time; therefore, individual variation is eliminated,
(ii) it is readily available and can be handled easily,
(iii) it has a very fast rate of oxygen evolution so that measurement of oxygen evolution can be done in a short time, and
(iv) it has the ability to grow under varying conditions of nutrient supply, level of carbon dioxide, temperature, and light intensity.

The photosynthetic rate of the algae depends on:

(i) temperature,
(ii) the supply of nutrients,
(iii) the supply of carbon dioxide, and
(iv) the supply of light energy.

In the quantum requirement measurement the only limiting factor should be the supply of light energy, because only under such conditions can the yield of energy conversion be determined by the intrinsic
properties of the photosynthetic apparatus. At the same time, the light intensity must be high enough to produce a photosynthesis rate several times greater than the dark respiratory rate, so that the uncertainty in the light respiration rate becomes less of a limitation on accurate quantum requirement measurement.

2. Effect of temperature

Between 15°C and 25°C the effect of temperature becomes appreciable only at high light intensities (I>I_5 in Figure II-2). At light intensities at which the rate of oxygen evolution is linearly proportional to the light intensity, the rate of oxygen evolution at 15°C and 25°C is practically the same. The quantum requirement measurement was done at a temperature of 20°C, which was the same temperature at which the algae culture was grown at our laboratory.

3. The supply of nutrients

The medium used to suspend the algae is a buffer with a pH of 5.1. It is similar to Myer's medium, which contains all nutrients known for optimal growth of the Chlorella. The composition of this buffer and that of Myer's medium is shown in Table II-1.

4. Carbon dioxide level during photosynthesis

Bassham and Shibata found that the rate of photosynthesis at 4% CO₂ was only 5% higher than that at 1% CO₂. Therefore, the photosynthetic rate under the light condition used for quantum requirement measurement can be considered to be saturated with respect to CO₂ at levels between 1.5% and 4%. The CO₂ level was about 3.5% at the beginning of each measurement, and about 2% at the end of each experiment.
Figure II-2. Rate of oxygen evolution as a function of light intensities.
Table II-1
Composition of Myer's medium and the medium used for suspending the algae

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration of medium used in the present experiment</th>
<th>Concentration of modified J. Myer's medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>5 x 10⁻³ M</td>
<td>12 x 10⁻³ M</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1 x 10⁻³ M</td>
<td>10 x 10⁻³ M</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1 x 10⁻³ M</td>
<td>9 x 10⁻³ M</td>
</tr>
<tr>
<td>Fe Versenol</td>
<td>1 x 10⁻³ M</td>
<td>1 x 10⁻³ M</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>0.1 x 10⁻³ M</td>
<td>1 x 10⁻³ M</td>
</tr>
<tr>
<td>*Arnon's A-4 with Cu and Mo</td>
<td>1 x 10⁻³ M</td>
<td>1 x 10⁻³ M</td>
</tr>
<tr>
<td>pH</td>
<td>5.1</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Arnon's A-4 with Cu and Mo:
- CuSO₄•5H₂O: 0.079 g/1
- H₃BO₃: 2.86 g/1
- MnCl₂•4H₂O: 1.8 g/1
- ZnSO₄•7H₂O: 0.222 g/1
- CoCl₂•6H₂O: 0.040 g/1
- MoO₃: 0.015 g/1

5. The supply of light energy

In order to insure that the limiting factor during quantum requirement measurement is the intensity of the illuminating light, we measured the rate of oxygen evolution at various light intensities. The curve shown in Figure II-2 shows that the rate of oxygen evolution is directly proportional to the light intensities until it reaches a value of 18 μmoles/min g of wet packed algae. Since the amount of algae in the cell is 30 x 0.4% = 0.12 g, the rate of oxygen evolution during quantum requirement measurement should not exceed 2.16 μmoles/min.
Monochromatic light has to be used so that the number of photons absorbed by the algae can be measured by a photocell. In the present experiment, the monochromatic light is provided by an incandescent lamp and sets of interference filters. Infrared absorption filters (Corning 1-69) were employed to cut down the large amount of infrared from the incandescent lamp. Water cooling is applied to protect the infrared absorption and interference filters.

The desired wavelength of the monochromatic light used is 625 μm. This is provided by a combination of two sets of interference filters (from Baird-Atomic Incorporated). The first set consists of four pieces (each 2" x 2") of interference filters (B-3X) while the second set consists of four pieces (each 2" x 2") of infrared blocking filters (B-2X). The transmission curves for the interference filter, infrared blocking filter, infrared absorption filter, and a combination of these three filters are shown in Figure II-3.* The transmission curve shown in Figure II-3D shows that the light provided by such a set-up has a wavelength of 627 μm with a bandwidth of 7 μm at half-peak value. The light provided by such a set-up can be considered as monochromatic for the present purpose. A piece of translucent plate is put between the sets of filters and the cell containing the algae suspension.

The reason why 627 μm light is chosen for quantum requirement study and the function of the translucent plate will be discussed in Appendix II-A.

*All spectra were obtained by using Cary 14 spectrometer.
Figure II-3. (a) Transmittance curve of the interference filter (B3-X). (b) Transmittance curve of the IR blocking filter (B2-X).
Figure II-3. (c) Transmittance curve of the IR absorption filter (Corning 1-69).

(d) Transmittance curve for the combination of interference filter (B-3X), IR blocking filter (B2-X), and IR absorption filter (Corning 1-69).
C. Experimental Set-up

The system used in this experiment is shown schematically in Figure II-4. The essential parts of the system are:

(i) a cell containing the suspension of algae,
(ii) a carbon dioxide analyzer,
(iii) an oxygen analyzer,
(iv) a bubbler for indicating the pressure inside the system as well as detecting leakage in the system,
(v) R-C elements to reduce pressure fluctuation, and
(vi) a light source provided by an incandescent lamp and sets of filters.

1. The cell

The cross-section of the cell is shown in Figure II-4B. This cell (5 mm thick) is thinner than that used by Bassham and Shibata (9 mm thick). The advantage of using a thinner cell is to reduce side scattering effect of the light, because the area of the edge of the cell is small when compared to the cross-sectional area of the cell.

With the exception of the illuminating area, the entire surface of cell wall A was masked with black tape so that only the desired area was illuminated. Scattering light was reflected forward by having an aluminum reflective surface between the surface of cell wall A and the black tape. Table II-2 shows the result of two light measurements, one done on the outer surface of cell wall B, and the other on the inner surface of cell wall A.* The results of these two measurements indicate that all

*By replacing the cell with a lucite plate with the shape and dimension of cell well A.
Table II-2

Measurements by Photocell

<table>
<thead>
<tr>
<th>A. Light Energy Measurements on Cell Wall A (in units of mV)</th>
<th>Sum of Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45 0.80 0.77 0.69 0.43 0.47 0.73 0.86 0.68 0.52</td>
<td>6.40</td>
</tr>
<tr>
<td>5.80 9.70 8.56 7.00 4.20 4.60 7.40 8.00 8.30 1.15</td>
<td>64.80</td>
</tr>
<tr>
<td>6.10 10.10 8.00 6.40 4.00 3.70 6.00 6.40 7.00 1.80</td>
<td>59.50</td>
</tr>
<tr>
<td>4.00 5.60 4.60 3.90 2.40 2.40 3.50 3.70 4.00 1.00</td>
<td>35.10</td>
</tr>
<tr>
<td>6.80 9.60 7.80 6.60 4.00 4.50 6.20 6.20 6.40 1.70</td>
<td>59.80</td>
</tr>
<tr>
<td>7.20 12.00 9.90 8.70 5.10 5.10 7.20 7.20 7.50 1.80</td>
<td>71.70</td>
</tr>
<tr>
<td>1.00 8.10 7.50 7.20 5.70 3.00 5.40 5.40 5.40 3.60</td>
<td>52.30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>349.60</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Light Energy Measurements on Cell Wall B (in units of mV)</th>
<th>Sum of Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32 0.51 0.72 1.26 1.44 1.62 0.96 1.00 1.38 1.15 0.96 0.39</td>
<td>11.39</td>
</tr>
<tr>
<td>2.52 1.02 4.20 7.40 6.70 5.60 3.90 3.70 5.00 5.70 5.00 1.05</td>
<td>49.27</td>
</tr>
<tr>
<td>2.82 1.40 5.60 8.00 6.50 5.60 4.00 7.20 9.80 5.40 4.60 1.20</td>
<td>59.30</td>
</tr>
<tr>
<td>2.94 1.15 4.80 6.40 5.00 4.40 3.10 2.80 3.65 3.90 3.35 1.00</td>
<td>39.55</td>
</tr>
<tr>
<td>2.88 1.35 6.40 9.00 7.30 6.40 4.40 4.15 5.00 5.20 4.60 1.40</td>
<td>55.20</td>
</tr>
<tr>
<td>3.00 1.50 7.40 11.40 10.00 8.70 5.80 5.10 5.70 6.50 5.90 1.65</td>
<td>69.65</td>
</tr>
<tr>
<td>3.78 2.30 6.50 7.10 6.20 5.00 3.40 3.20 3.80 4.10 1.90 0.40</td>
<td>43.90</td>
</tr>
<tr>
<td><strong>19.26</strong></td>
<td><strong>328.26</strong></td>
</tr>
<tr>
<td>x 2</td>
<td>+ 38.52</td>
</tr>
<tr>
<td><strong>38.52</strong></td>
<td><strong>Total: 328.26 + 38.52 = 366.78</strong></td>
</tr>
<tr>
<td></td>
<td><strong>366.78</strong></td>
</tr>
</tbody>
</table>

The light passing through cell wall A was measured by measurements done on cell wall B. Therefore, our measurements which were done on cell wall B represented the amount of light illuminating the algae.

The sides of the cell are beveled so that scattering and divergent light can also be measured.

2. The carbon dioxide analyzer

Carbon dioxide was analyzed by a Lira Model 200 carbon dioxide analyzer measuring the infrared absorption by carbon dioxide.
3. The oxygen analyzer

Oxygen was determined by a Beckman oxygen analyzer measuring the paramagnetism of oxygen. The oxygen analyzer is very sensitive to total pressure and flow rate. It works properly only at a certain pressure range and flow rate. Therefore, it was connected to the carbon dioxide analyzer in parallel, so that the gas flow rate to the oxygen analyzer could be adjusted by turning the stopcock, R. The flow rate in the present experiment was 62 ± 3 cc/min.* The pressure of the system was always adjusted to that of the atmosphere by opening the stopcock L.

4. The bubbler

Figure II-4A shows the construction of the bubbler, which serves as a pressure indicator as well as a leakage detector in the system.

If the pressure inside the system is higher than that of the atmosphere, the glycerine in the bubbler will rise. Therefore, it serves as a pressure indicator. High pressure inside the system can be released by opening stopcock L.

In the absence of leakage in the system, no air will get into the system through the bubbler if the pressure inside the closed system equals that of the atmosphere. If gas leaks out from the system, air will get into the system through the bubbler, because the bubbler is close to the inlet of the pump. This results in the formation of bubbles in the glycerine. Therefore, leakage in the system can be detected.

5. The R-C elements

The volume of the system used for quantum requirement measurement was made as small as 150 cc by using glass tubing with a diameter

*The flow rates recommended by the company are 50 to 75 ± 5 cc/min.
Figure II-4A. Schematic diagram of the apparatus for quantum requirement study.
Figure II-4B. Photosynthesis cell for quantum requirement measurements.

a. Blocking filter (B-3X); b. interference filter (B-3X);
c. translucent plastic cell walls; d. suspension of *Chlorella* or clear nutrient solution; e. translucent plastic sheet for light diffusion; f. reflecting aluminum surface.
of only 2 mm. However, the use of small glass tubing led to pressure fluctuation in the system. This was overcome by putting an "R-C" element in series with the tubing. The "R-C" element was a piece of glass tubing with smaller diameter (1 mm) which contained a 5 cc bulb at the center. The small diameter functioned as a resistive element while the bulb worked as a capacitive element. The small size of the tube reduced the flow rate and the bulb decreased the temporary pressure gradient. Together they greatly reduced the pressure fluctuation.

6. The light source

Light for the measurement was provided by an incandescent lamp and sets of filters. Various intensities were obtained by changing the input voltage. The curve in Figure II-3D, which was obtained by a Cary 14 spectrophotometer, shows that all detectable infrared is cut off when the interference filter is combined with the infrared blocking filter and the infrared absorption filter. However, the infrared from the incandescent lamp is of much larger quantity than that from the Cary 14 spectrophotometer lamp. Therefore, there might be some infrared passing through this set-up of filters.

In order to insure that there was no infrared passing the cell, the following measurement was done. The thermopile (and then the photodiode) was put inside a long black box (6" x 6" x 4' 0") which was placed in front of the light source. Infrared absorption filters were put between the light source and the thermopile. The output signals of the thermopile with different numbers of infrared absorption filters were compared. The constancy of the (n+1)/n ratio* in Table II-3 indicated that there

*See Appendix II-B for explanation.
Table II-3

Infrared from the Light Source

<table>
<thead>
<tr>
<th>No. of IR absorption filters put between the light source and the detecting surface</th>
<th>Signal from the thermopile (mV)</th>
<th>( (n+1)/n )</th>
<th>Signal from the photodiode (mV)</th>
<th>( (n+1)/n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.52</td>
<td>0.726</td>
<td>7.40</td>
<td>0.804</td>
</tr>
<tr>
<td>2</td>
<td>1.83</td>
<td>0.781</td>
<td>5.95</td>
<td>0.795</td>
</tr>
<tr>
<td>3</td>
<td>1.43</td>
<td>0.791</td>
<td>4.72</td>
<td>0.822</td>
</tr>
<tr>
<td>4</td>
<td>1.14</td>
<td>0.800</td>
<td>3.78</td>
<td>0.800</td>
</tr>
<tr>
<td>5</td>
<td>0.912</td>
<td>3.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

was practically no infrared from the light source, so that the infrared absorption filters acted as if they were attenuation filters.

The difference in the \( (n+1)/n \) ratio probably arose from the absorption difference of the IR absorption filters. This can be seen from the fact that the \( (n+1)/n \) ratio is 0.800 for both thermopile and photodiode when \( n = 4 \) (all infrared absorption filters were used).
D. Experimental Procedures

The experimental procedures can be summarized by the following diagram:

- calibrated by a standard carbon lamp
- calibrated by a thermopile
- reference light source
- large enough to cover the entire detection surface of the thermopile
- uniform
- constant
- monochromatic
- measured by silicon photocell

\[ O.R. = \frac{\text{number of photons absorbed/min (units of \text{w}en/min)}}{\text{number of oxygen molecules evolved/min (units of \text{\textmu}moles/min)}} \]

- measured by an oxygen analyzer
- calibrated by a carbon dioxide analyzer which also measures the rate of CO₂ uptake
- sensitivity of the oxygen analyzer

- oxygen volume of the system
1. Measurement of light absorption

The steps in the measurement of light absorption are: Calibration of thermopile, calibration of photocell against the calibrated thermopile, and measurement by photocell of light absorbed by the algae during photosynthesis.

a. Calibration of thermopile (Figure II-5A)

(i) Characteristics of the thermopile

The thermopile consists of a group of thermocouples which are made of platinum black. Therefore, the sensitivity of the detecting surface may not be uniform. Hence, when the intensity of a light beam is to be measured by a thermopile, the light beam must be uniform and large enough to cover the entire area of the detecting surface. That is why the thermopile is placed at a distance of 200 cm from the light source during its calibration against the standard carbon lamp.

(ii) Result of the calibration

The thermopile was calibrated against a standard carbon lamp (C-1070 and C-1071) by the method recommended by the National Bureau of Standards (in literature supplied with their standard lamp). Table II-4 shows the result of the measurement.
The thermopile is fixed in position by Styrofoam.

Figure II-5A. Cross section of thermopile.

This mark on the holder related the center of the photocell to markings on the rulers.

Figure II-5B. Photocell and holder.
Table II-4
Calibration of Thermopile by Standard Carbon Lamp

<table>
<thead>
<tr>
<th>Lamp No.</th>
<th>Power (input)</th>
<th>Power density at 200 cm (watts/cm²)</th>
<th>Power received by thermopile</th>
<th>Signal given by thermopile</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amperes</td>
<td>Volts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1070</td>
<td>0.300</td>
<td>80.9</td>
<td>45.3 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1070</td>
<td>0.350</td>
<td>91.1</td>
<td>61.9 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1070</td>
<td>0.400</td>
<td>100.7</td>
<td>80.5 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1071</td>
<td>0.300</td>
<td>79.5</td>
<td>42.8 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1071</td>
<td>0.350</td>
<td>89.4</td>
<td>58.7 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1071</td>
<td>0.400</td>
<td>98.9</td>
<td>76.7 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1070</td>
<td>0.300</td>
<td>79.6</td>
<td>44.57 x 10⁻⁶</td>
<td>111.5 x 10⁻⁶</td>
<td>13.95 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31.95 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.93 ergs/sec cm² µV</td>
</tr>
<tr>
<td>C-1070</td>
<td>0.350</td>
<td>90.6</td>
<td>61.56 x 10⁻⁶</td>
<td>153.9 x 10⁻⁶</td>
<td>20.04 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.72 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.80 ergs/sec cm² µV</td>
</tr>
<tr>
<td>C-1070</td>
<td>0.400</td>
<td>100.0</td>
<td>79.94 x 10⁻⁶</td>
<td>199.9 x 10⁻⁶</td>
<td>26.40 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.28 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75.07 ergs/sec cm² µV</td>
</tr>
<tr>
<td>C-1071</td>
<td>0.400</td>
<td>98.5</td>
<td>76.40 x 10⁻⁶</td>
<td>191.0 x 10⁻⁶</td>
<td>25.56 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29.89 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74.73 ergs/sec cm² µV</td>
</tr>
<tr>
<td>C-1071</td>
<td>0.350</td>
<td>88.8</td>
<td>58.30 x 10⁻⁶</td>
<td>145.8 x 10⁻⁶</td>
<td>19.50 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29.90 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74.75 ergs/sec cm² µV</td>
</tr>
<tr>
<td>C-1071</td>
<td>0.300</td>
<td>78.3</td>
<td>42.20 x 10⁻⁶</td>
<td>105.5 x 10⁻⁶</td>
<td>13.80 µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.55 ergs/sec cm² µV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.38 ergs/sec cm² µV</td>
</tr>
</tbody>
</table>

The mean value is 29.86 ± 0.30 ergs/sec cm² µV out of 15 measurements.
b. Calibration of photocell versus the calibrated thermopile (Figure II-5B)

(i) Wavelength dependence of the photocell

The thermopile can be considered as a black body. It can be used to measure the intensity of any light source because its output signal depends only on the light energy falling on its detecting surface and is independent of the wavelength of the light. However, the sensitivity of the photocell varies with the wavelength of the light falling on its detecting surface. Figure II-6 is a sensitivity curve of the photocell. The curve was obtained by measuring the energy output of the light from a monochromator first by the thermopile and then by the photocell.

(ii) Light source used to calibrate the photocell

In order to calibrate the photocell against the thermopile, a reference light source is required. Ideally, the light source used should be the same as that used to illuminate the algae. As the area of the photocell (15 mm x 10 mm) is different from that of the thermopile (25 mm x 10 mm), the light beam used for calibration must be uniform and large enough to cover the whole detecting surface of the thermopile. The light provided by the incandescent lamp and filter sets is too scattered to meet these requirements.

The 625 mu beam provided by a monochromator has the desired wavelength. However, only the central portion of the beam can be considered to have uniform intensity. Therefore, during the calibration, the thermopile has to be placed far enough from the monochromator so that its detecting surface sees only the central portion of the light beam.
Figure II-6. Sensitivities of the photocell at various wavelengths.
However, the intensity of the light beam becomes so low that the noise from the environment becomes a problem. Furthermore, when compared with the light for quantum requirement measurement, the light intensity provided by the monochromator is so low that it is questionable whether the sensitivity of the photocell is linear over such a long range. (The intensity of the light provided by the incandescent lamp and filter sets is about 100 times as strong as that from the monochromator.)

(iii) The sun as a light source for the calibration of the photocell

The sun can be considered as a point source of light millions of miles away from Earth. The light beam directly from the sun is therefore parallel and uniform. Hence, it is an ideal light source for the calibration of the photocell.

In order to eliminate the scattering light in the sky, a long black box was built (6" x 6" x 4' 0") (Figure II-7). There are two windows (2 ft apart) in the black box so that only collimated light can pass down the length of the box and reach the detecting surface of the thermopile or photocell placed there. An interference filter and a blocking filter were put on one window while an infrared absorption filter was put on the other. The areas of all the filters are larger than that of the windows. Both windows have an opening area of 50 mm x 25 mm, so that the light beam passing through the windows is large enough to cover the entire detecting surface of the thermopile (25 mm x 10 mm) or the photocell (15 mm x 10 mm).

The black box was mounted on a wooden stand which allows the box to be tilted so that it can face the sun as desired. An 8-ft long tube
long tube to direct the box toward the sun

thermopile

clamp

electric wiring

white paper

shutter

IR absorption filter

interference filter and IR blocking filter

clamp

wooden stand

Figure II-7. Long box built to calibrate the photocell.
with a diameter of 3/4 in. was attached to the box with its axis parallel to the latter. This tube serves as a device to direct the box towards the sun.

The box was adjusted until we saw a clear circular image of the tube on a white cardboard at the back of the tube. This indicated that the box was facing the sun. The shutter on the box was then opened, and the signal from the thermopile was recorded. The shutter was shut and opened again for three times, so that three readings were obtained from each set of measurements. As each measurement takes only a few seconds, the movement of the sun does not affect the precision of the measurement. The thermopile was then replaced by the photocell and the position of the box adjusted for another set of measurements.

The amount of infrared reaching the detecting surface was negligible. Table II-5 gives the change in output signals upon the addition of infrared filters between the light source and the detecting surface. The constance of the \((n+1)/n\) ratio* \((n\) is the number of infrared absorption filters added) indicates that there was practically no infrared reaching the detecting surface of the thermopile, so that the infrared filters acted as if they were attenuation filters. Furthermore, when a Corning 7-69 filter (which cuts away all light with wavelengths longer than 700 \text{mu}) was put in front of the detecting surface, the thermopile gave no signals.

Table II-5 shows the sensitivities of the photocell thus obtained, and that obtained by using the monochromator as the light source. They

*See Appendix II-B for explanation.
**Table II-5**

<table>
<thead>
<tr>
<th>No. of IR absorption filters (n)</th>
<th>Signal from the thermopile (mv)</th>
<th>Ratio of St/Sp (n+1)/n</th>
<th>Signal from the photodiode (mv)</th>
<th>Ratio of St/Sp (n+1)/n</th>
<th>Ratio of St/Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.298</td>
<td>0.772</td>
<td>3.35</td>
<td>0.78</td>
<td>0.089</td>
</tr>
<tr>
<td>2</td>
<td>0.230</td>
<td>0.795</td>
<td>2.61</td>
<td>0.78</td>
<td>0.088</td>
</tr>
<tr>
<td>3</td>
<td>0.183</td>
<td>0.781</td>
<td>2.04</td>
<td>0.79</td>
<td>0.090</td>
</tr>
<tr>
<td>4</td>
<td>0.143</td>
<td>0.775</td>
<td>1.61</td>
<td>0.77</td>
<td>0.089</td>
</tr>
<tr>
<td>5</td>
<td>0.111</td>
<td>1.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The first set is done in the morning (11:00), and the second set is done in the afternoon (2:00), without changing the position. The third set was done in the afternoon after the position had been changed by 10 in.

Mean ratio of the sensitivities of the thermopile to that of the photodiode is 0.0893 ± 0.0007. However, the value of 0.0895 was chosen because this eliminated the error due to the difference in the attenuation effect of the infrared filters, as all infrared filters were used.

The St/Sp ratio obtained by using the monochromator as the light source was 25.6 μV/285 μV = 0.0898.

are practically of the same value. Figure II-8 is a plot of the sensitivities of the thermopile versus that of the photocell over various intensities. It shows that their sensitivity ratios are constant from 0 to 3400 μV (3.4 mV) on the photocell scale. This indicates that both
Figure II-8. Sensitivities of thermopile and photocell at various light intensities.
the photocell and the thermopile vary linearly over such a range of light intensities.

The calibration of the thermopile was done by a standard carbon lamp, which caused the thermopile to give signals around 30 μV. However, during the measurement of light intensities by the photocell, the light source provided by the incandescent lamp and filters caused the photocell to give signals around 4 mV. The linearity of such a plot shows that the calibration of the photocell by a thermopile which had been calibrated by the standard carbon lamp is valid.

c. Measurement by the photocell

The silicone photocell (Hoffman 2A) is a circular plate with a diameter of 25 mm. It was fixed on lucite bar and then covered with a thin piece of glass (1 mm). A piece of screen (with negligible thickness) which serves as a neutral density filter to lower the light intensity so that the output signals are not too high to be read from our millimicrovolt meter (Keithley 149). During the measurement, the photocell was pressed as close as possible on the cell surface, so that all light coming from an area of 15 mm x 10 mm on the surface of the vessel was measured.

Rectangular plastic rulers, which had been marked for every 10 mm, had been made with widths of 15 mm, 30 mm, and 45 mm. Therefore, an area of 15 x 10 mm was mapped each time without overlapping on the former or latter measurements. The thickness of these plastic rulers was the same as that of the holder of the photocell. The total area of the vessel where the algae was illuminated was \((8.8 \text{ cm})^2\) or 77.44 cm², while the area mapped included 7 rows of 11 rectangles (each 10 x 15 mm).
or 115.5 cm². By mapping a larger area than that through which the light was actually passing, we were able to measure the small amount of light scattered to the sides as well as the light directly transmitted.

The edge of the vessel was beveled so that light scattered to the side could be measured (Figure II-4B). The light that passed through the edges was found less than 2% of the total amount transmitted. Therefore, the error due to scattering and divergence of the beam is small. Hence, instead of measuring all four edges, we multiplied the value of one edge by 2. This corresponded to the two sides.

The results of two sets of measurements are shown in Table II-6, A and B.

Table II-6

<table>
<thead>
<tr>
<th>Light Energy Absorbed by Chlorella pyrenoidosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Light measurement on cell containing 25 ml of medium (118 V)</td>
</tr>
<tr>
<td>1.32 0.45 0.57 1.02 1.56 0.96 0.96 1.47 1.20 0.99</td>
</tr>
<tr>
<td>2.70 1.02 4.80 7.50 7.20 6.10 4.40 4.30 5.90</td>
</tr>
<tr>
<td>3.00 1.20 6.60 9.00 7.40 6.10 4.70 3.90 5.80</td>
</tr>
<tr>
<td>3.00 1.20 5.30 6.90 5.60 4.60 3.30 3.10 4.00</td>
</tr>
<tr>
<td>3.00 1.50 6.40 9.00 7.20 6.00 4.10 4.20 5.20</td>
</tr>
<tr>
<td>3.12 1.70 7.40 11.70 9.90 8.50 6.00 5.30 6.20</td>
</tr>
<tr>
<td>3.72 1.60 2.70 7.60 8.20 7.00 5.60 3.70 3.50</td>
</tr>
<tr>
<td>19.86</td>
</tr>
<tr>
<td>x 2</td>
</tr>
<tr>
<td>39.72 (2 side edges)</td>
</tr>
</tbody>
</table>

Total: 343.40 + 39.72 = 383.12
B. Light measurement on cell containing 25 ml of 0.6% Chlorella pyrenoidosa

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Sum of Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.08</td>
<td>0.40</td>
<td>0.70</td>
<td>1.35</td>
<td>1.56</td>
<td>1.50</td>
<td>0.90</td>
<td>0.96</td>
<td>1.50</td>
<td>1.25</td>
<td>1.05</td>
<td>0.45</td>
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<td></td>
<td>11.62</td>
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<tr>
<td></td>
<td>1.86</td>
<td>0.84</td>
<td>5.00</td>
<td>8.00</td>
<td>7.40</td>
<td>6.50</td>
<td>4.60</td>
<td>4.50</td>
<td>6.20</td>
<td>6.80</td>
<td>5.80</td>
<td>1.00</td>
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<td></td>
<td>56.64</td>
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<td></td>
<td>2.16</td>
<td>1.25</td>
<td>4.40</td>
<td>6.40</td>
<td>5.50</td>
<td>4.30</td>
<td>2.50</td>
<td>1.80</td>
<td>2.90</td>
<td>2.80</td>
<td>2.90</td>
<td>0.90</td>
<td></td>
<td></td>
<td>35.65</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>1.02</td>
<td>1.59</td>
<td>1.98</td>
<td>1.68</td>
<td>1.58</td>
<td>1.08</td>
<td>1.08</td>
<td>1.44</td>
<td>1.41</td>
<td>1.20</td>
<td>0.42</td>
<td></td>
<td></td>
<td>14.48</td>
</tr>
<tr>
<td></td>
<td>1.71</td>
<td>1.20</td>
<td>1.52</td>
<td>2.00</td>
<td>2.58</td>
<td>2.10</td>
<td>1.80</td>
<td>1.20</td>
<td>1.26</td>
<td>1.86</td>
<td>1.86</td>
<td>1.56</td>
<td>0.36</td>
<td></td>
<td>17.78</td>
</tr>
<tr>
<td></td>
<td>2.04</td>
<td>1.32</td>
<td>2.16</td>
<td>3.20</td>
<td>2.35</td>
<td>1.65</td>
<td>1.55</td>
<td>2.40</td>
<td>2.19</td>
<td>1.80</td>
<td>2.75</td>
<td>0.48</td>
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<td>21.85</td>
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<tr>
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<td>2.73</td>
<td>1.50</td>
<td>1.26</td>
<td>2.10</td>
<td>2.40</td>
<td>2.10</td>
<td>1.50</td>
<td>1.14</td>
<td>1.50</td>
<td>1.50</td>
<td>1.38</td>
<td>1.26</td>
<td></td>
<td></td>
<td>17.64</td>
</tr>
</tbody>
</table>

13.38  

x 2  

26.76  

Total: 175.66 + 26.76 = 202.42

2. Measurement of the rate of photosynthesis

The measurement of the rate of photosynthesis consists of the following steps: Calibration of the carbon dioxide analyzer, determination of the effective volume of CO₂ in the system, calibration of the oxygen analyzer, determination of the volume of oxygen of the system, and measurement of the rate of photosynthesis as the rate of oxygen evolution.

The steps are discussed in more detail in the following:

a. Calibration of the carbon dioxide analyzer

The zero point of the carbon dioxide analyzer (LIRA Model 200) was adjusted with nitrogen gas. Its sensitivity was then adjusted against a standardized mixture of air and carbon dioxide (3.80% v/v). The flow rate of the gas during the calibration was regulated to the same value as that in the measurement of photosynthesis. However, it was found later that the flow rate and total pressure had very little
b. The effective volume of CO₂ in the system

The effective volume of the system for CO₂ is defined as the volume of carbon dioxide both in the gas and in the medium (a 25 ml buffer at pH 5.1). This was determined by putting a known amount of nitrogen into the closed system which had been filled with a known amount of carbon dioxide. The change in carbon dioxide level upon the introduction of nitrogen provided the data for the calculation of the volume.

The introduction of the nitrogen into the system was done by connecting a 117.1 cc loop of nitrogen to the system. The volume of the system was calculated as follows:

\[
\begin{align*}
\text{Percentage of CO}_2 \text{ before the introduction of N}_2 & \quad \frac{90.0(4)}{100} \\
\text{Percentage of CO}_2 \text{ after the introduction of N}_2 & \quad \frac{42.0(4)}{100} \\
\text{Amount of CO}_2 \text{ in the system} & \quad 90.0(0.04) V = 42.0(0.04)(V+171) \\
\text{Effective volume of the system for CO}_2 & \quad V = 150.0 \text{ cc}
\end{align*}
\]

Calculation based on other set of data gave \( V = 149, 151, 148, 152 \). Average 150.0.

c. Calibration of the oxygen analyzer

There are three settings in the oxygen analyzer (Arnold O. Beckman, Model F3). Sensitivity from 0 to 25%, 0 to 5%, and 18 to 23% can be chosen. During photosynthesis, the oxygen level of the gas phase is close to that of the atmosphere. Hence, the setting of 18 to 23% was chosen. The sensitivity of the oxygen analyzer was to be adjusted so that a full scale change (100 divisions) on the chart corresponds to a 2% change in the oxygen level of the system. In our experiment, the oxygen level was between 20 and 22%. Therefore, the zero point in the oxygen analyzer was so adjusted that the chart gave a reading of about
50 divisions when the whole system was filled with air. (O₂ in air is 20.95%). The sensitivity of the oxygen analyzer was calibrated by changing the oxygen level of the system. The following calculation shows how this was done.

The O₂ level in air is 20.95%. In a mixture of 4% CO₂ (v/v) in air, the O₂ level is 20.95 (100-4/100) = 20.11%. Therefore, a change from air to a mixture of 4% CO₂ in air results in a drop of 20.95% - 20.11% = 0.84% in the oxygen level. Since the oxygen analyzer was to be adjusted to have a sensitivity of 100 divisions/2% change, 0.84% change should give rise to a change of 0.84% (100 divisions/2%) = 42 divisions change.

After the system was filled with 4% CO₂, it was closed and its pressure was adjusted to atmospheric pressure. After the O₂ and CO₂ levels were constant (final value of CO₂ was 3.8%), the system was opened to the atmosphere. (The air conditioning system in the room provided a very good ventilation for air, so that we can always assume that O₂ in air is 20.95%.) After the system was filled with air, as indicated by the chart, the system was closed and its pressure adjusted to the atmospheric pressure. The oxygen analyzer was so adjusted that it gave a difference of 42.0 (3.8/4.0) = 40 divisions from the original value (the oxygen level on the chart when the system was filled with 3.80% of CO₂ in air).

d. Determination of the volume of oxygen in the system

Instead of subtracting the effective volume of CO₂ in the medium from the total effective volume, the volume of oxygen was determined independently. This has the advantage of eliminating the error (if there was any) resulting from the calibration of the sensitivity of
the oxygen analyzer (see Appendix II-C for explanation). The volume was determined by calculations based on the drop in $O_2$ level when a known amount of air was replaced by pure nitrogen.

After the system was filled with air, as indicated by a constant level on the recording chart, the system was closed and its pressure adjusted to atmospheric pressure. After the oxygen level on the chart was again constant, 5.0 cc of nitrogen at atmospheric pressure was injected into the system through the medium while the stopcock $R$ was open to release the pressure increase. Since the injection was done in less than 2 sec, it is reasonable to assume that no injected nitrogen escaped from the system. The volume of the system was obtained by the following calculation:

| Oxygen level before the injection of nitrogen | 48.5 divisions | 20.95 |
| Amount of oxygen in the system before the injection | 20.95% x $V$ |
| Oxygen level after the injection of nitrogen | 2.5 divisions | 20.03 |
| Amount of oxygen in the system after the injection | 20.03% x $V$ |

As the change in the amount of oxygen resulted from the replacement of 5.0 cc of air by nitrogen, it could be calculated as follows:

$20.95\% \frac{(V-5)}{V} = 20.03$

$20.95\% (5.0) = (20.95 - 20.03\%) (V)$

$V = 10.975/0.92 = 119.3$ cc

Calculations based on other data gave values of 120 and 121. The average was therefore 120 cc.
e. Measurement of the rate of photosynthesis as the rate of oxygen evolution and the rate of carbon dioxide absorption

(i) Preparation of the algae suspension

The algae was grown in a shaking flask maintained at 20°C by a water bath. The algae was illuminated by six 90-watt fluorescent lamps. A gas mixture of 95% air and 4% carbon dioxide is bubbled through the algae culture. A modified Jack Myer's medium was used as the nutrient solution (see Table II-1). Under these conditions, the fresh weight of the algae doubled in 12 to 16 hr. The algae was harvested every day at 4:00 P.M.

The algae for the quantum requirement study was obtained from the flask. It was centrifuged and resuspended to give the desired density by the further modified medium (see Table II-1). Twenty-five ml was then put into the cell for quantum requirement study. The whole process usually required 20 min.

(ii) Health of the algae

High quantum requirement (low quantum efficiency) may be the result of measurements on unhealthy algae. In order to make sure that the algae being studied was healthy, the algae was allowed to photosynthesize in white light (by removing the sets of interference filters). The light intensity of the white light was high enough to cause light saturation. The rate of oxygen evolution was calculated and compared with known values for healthy algae. In the present series of measurements, all algae under study had a rate of oxygen evolution of 35 μmoles of oxygen/min/g of wet packed algae, or more.
Furthermore, we found that there was an induction period of about 12 min in the algae, during which its rate of oxygen evolution increased steadily towards the steady value. Illumination of the algae with high intensity white light eliminated the error of studying the quantum requirement of the algae during the induction period.

There is no literature value for the maximum oxygen evolution rate on the basis of moles per g of wet packed algae. However, experience in this laboratory showed for uncontaminated and healthy algae the rate of oxygen evolution is about 30 μmoles/min/g wet packed algae. The higher rate obtained by the present algae is probably due to a higher chlorophyll content of the algae, since the quantum requirements are still the same for algae with 35 μmoles of O₂/min/g or 40 μmoles of O₂/min/g, when saturated white light is used for illumination. Furthermore, calculation of the light-saturated rate of oxygen evolution on the basis of chlorophyll content* of the algae showed the rate of oxygen evolution was about 250 μmoles of O₂/mg Chl/hr.

Senger and Bishop80 reported a low quantum requirement for a synchronous Scenedesmus culture. The highest rate achieved by that culture was about 24.0 μmoles of O₂/min/g of wet packed algae, which was below the rate achieved by our Chlorella (35.0 μmoles of O₂/min/g of wet packed algae) at saturated white light. They gave no data to support their observation of similar results found in Chlorella. Since the quantum requirement in the present experiment is about the same as values found in their synchronous Scenedesmus culture, most of our Chlorella was at

*See Appendix II-D for extraction of chlorophylls.
its active state of photosynthesis during the quantum requirement measurement.

(iii) Rate of oxygen evolution and carbon dioxide absorption

After 12 min of photosynthesis in white light, the interference filter sets were put on. Carbon dioxide was again introduced into the system through the inlet to raise the carbon dioxide level to 3.5%. The system was then closed. The rate of oxygen evolution and carbon dioxide uptake was measured by the oxygen and carbon dioxide analyzer, and the results were automatically recorded on the chart. Meanwhile, the light energy was measured by the photocell. Figure II-9 is the recording chart which shows the evolution of oxygen and carbon dioxide absorption. The corresponding rate of energy absorption has been shown in Table II-6, A and B.

E. Calculations

1. Sensitivity of the photocell

The sensitivity of the photocell was calculated from data in Tables II-3 and II-4. Table II-3 provided the sensitivity of the thermopile which was used to calculate the intensity of the 625 μm* beam used to determine the sensitivity ratio of the thermopile and the photocell. The following calculation shows how the sensitivity of the photocell was obtained:

Sensitivity of the thermopile (from Table II-3) 29.86 ± 0.30 ergs/sec cm² μV

Power density of the 625 μm beam in No. 5 of the first series of measurements

\[(29.86 \pm 0.30 \text{ ergs/sec μV}) \times 111 \text{ μV} = 3314.60 \pm 33 \text{ ergs/sec cm}^2\]

*Actually it was 627.20 μm.
Figure II-9. Rate of oxygen evolution and carbon dioxide uptake by Chlorella pyrenoidosa.
Power of the 625 µm beam received by the photocell

\[ (3314.60 \pm 33 \text{ ergs/sec cm}^2) \times 1.5 \text{ cm}^2 = 4971.90 \pm 50 \text{ ergs/sec} \]

Sensitivity of the photocell to the 625 µm beam

\[ (4971.90 \pm 50 \text{ ergs/sec})/1.24 \text{ mV} = 4009.60 \pm 40 \text{ ergs/sec mV} \]

In order to measure the number of photons absorbed by the algae during photosynthesis, we have to express the sensitivity of the photocell in terms of µein/min mV. From Figure II-10, the weighted mean of the energies at several wavelengths can be calculated. The calculation and results are shown in the following:

Energies of one µein of photons

\[ E = 10^{-6} \text{ Ne} \]
\[ = 10^{-6} \text{ N hc/} \]
\[ = \frac{10^{-6} \times (6.03 \times 10^{23}) \times (6.63 \times 10^{-27}) \times (3.00 \times 10^{10}) \text{ ergs}}{\text{in units of cm}} \]
\[ = 119.94 \text{ ergs/λ} \times 10^{-7} \text{ (in units of µm)} = 119.94 \times 10^{7} \text{ ergs/λ (in units of µm)} \]

<table>
<thead>
<tr>
<th>Wavelengths (µm)</th>
<th>Amount of energy in one µein (x 10^5 ergs)</th>
<th>Relative amount in the beam (transmittance) (%)</th>
<th>Energy contribution (x 10^4 ergs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>615</td>
<td>19.50</td>
<td>9.0</td>
<td>17.55</td>
</tr>
<tr>
<td>620</td>
<td>19.35</td>
<td>20.0</td>
<td>38.70</td>
</tr>
<tr>
<td>625</td>
<td>19.19</td>
<td>22.0</td>
<td>42.22</td>
</tr>
<tr>
<td>635</td>
<td>18.89</td>
<td>18.3</td>
<td>34.57</td>
</tr>
<tr>
<td>640</td>
<td>18.74</td>
<td>7.4</td>
<td>13.77</td>
</tr>
</tbody>
</table>

The weighted mean of the energies of the beam

\[ E = 192.51 \times 10^4 \text{ ergs/100.65} = 191.27 \times 10^4 \]

The weighted mean of the wavelengths of the beam

\[ = 627.20 \text{ µm} \]

The transmission band of this combination of filters is so narrow that it is reasonable to assume that the average sensitivities of the
photocell from 605 μm to 645 μm is the same as the sensitivity at 627.20 μm (the sensitivity obtained from Table II-4).

Sensitivity of the photocell in units of μein/min mV

\[
(4009.50 ± 50 \text{ ergs/sec mV})/(191.27 \times 10^4 \text{ ergs}) = (209.62 ± 26) \times 10^{-5} \text{ μein/sec mV}
\]

= 0.1258 ± 0.0016 μein/sec mV

= 0.1258 ± 0.0016 μein/min mV

2. Amount of energy absorbed

The amount of energy absorbed per min was obtained by subtracting the power transmitted through the cell when it was filled with 30 ml of 0.4% algae suspension from that when it was filled with 30 ml of suspension medium.

From Table II-6A, the power transmitted through the cell filled with 25 ml of suspension medium was 383.12 mV as measured by the photocell. This corresponds to \((0.1258 \text{ μein/min mV}) \times (383.12 \text{ mV}) = 48.20 \text{ μein/min.}\) From Table II-6B, the power transmitted through the cell filled with 25 ml of 0.6% Chlorella suspension was 202.42 mV as measured by the photocell. This corresponds to \((0.1258 \text{ μein/min mV}) \times (202.42 \text{ mV}) = 25.46 \text{ μein/min.}\) Therefore, the power absorbed by the Chlorella was

\[48.20 - 25.46 = 22.74 \text{ μein/min.}\]

3. Rate of oxygen evolution

The chart in Figure II-9 provided the data for the calculation of the rate of oxygen evolution. The slope of the line corresponding to oxygen evolution is \((65-31) \text{ divisions/16 min} = 2.125 \text{ divisions/min.}\)

The volume of oxygen evolved per min is therefore:

\[(2.125 \times 91/100) \times (2.12/100) \times 120 \text{ ml} = 54.06 \times 10^{-3} \text{ ml/min}\]

= 54.06 μl/min
(There is no page 58.)
Since one umole of oxygen at 20°C and 1 atm. is 24.50 μl, the rate of oxygen evolution in terms of umoles per min is (54.06/24.5) = 2.207 umoles/min.

4. Quantum requirement for oxygen evolution

Since quantum requirement is defined as the number of photons required to evolve one molecule of oxygen from water, it is obtained by dividing the rate of energy absorption by the rate of oxygen evolution.

\[ \text{O. R.} = \frac{\text{rate of energy absorption}}{\text{rate of oxygen absorption}} \]
\[ = \frac{22.74 \text{ μein/min}}{2.207 \text{ umoles/min}} \]
\[ = 10.30 \text{ μein/umole} \]

F. Results

1. Accuracy of the measurements

The uncertainty in the light measurement due to the calibration of sensitivity of the photocell is estimated to be 0.1258 ± 0.0016 μein/min mV, or ± 1.2%. The measurement of light energy transmitted through the algae cell when it contains only nutrient medium was found to vary less than 1% in measurements made before and after the photosynthesis experiment. In a typical experiment, about half as much light would be transmitted through the algae suspension. In this case, there would be still a 1% possible error in measured light, leading to an error of ± 0.5% of the light energy coming through the cell without algae. Consequently, this gives a maximum error of ± 1.5% of the light transmitted through the cell without algae or ± 3% of the light energy calculated to be absorbed by the algae. The combined uncertainty in the measurement of photosynthetic rate all comes from the uncertainty in the determination of the oxygen volume of the system, which is at most 2%.
Therefore, the maximum error of the quantum requirement measurement is estimated at ± 6%.

2. Quantum requirements

The results of a number of quantum requirement measurements made under the conditions described are summarized in Table II-7. Although the respiration rates found in the darkness following a period of photosynthesis are shown, it is emphasized that no correction for respiration has been made and that the quantum requirements shown in the 6th and 7th columns of the table are based solely on gas exchange rate in the light and light absorption. The 2nd column shows the incident light intensity which varies over a twofold range of intensity, while the 1st column shows the algae density which also was varied over a twofold range. The 3rd column shows that the rate of energy absorption varied over a threefold range. The relative narrowness of this range is dictated by the fact that the only really valid region for studying quantum requirements is between those intensities where the rate of photosynthesis is already several times dark respiration and those intensities at which further increase in light does not cause a proportional increase in photosynthetic rate (approaching saturation). The values of the quantum requirement for oxygen evolution range from 9.0 up to 14.0, with the lower values generally being found with the lower density of algae (44 to 51% absorption). There is considerable variation in the photosynthetic quotient with the value of the ratio CO2/O2 generally decreasing at higher light intensities.

In Table II-8 are shown the results of quantum requirement measurements which were made earlier and in which the photocell was calibrated.
<table>
<thead>
<tr>
<th>Density of Algae</th>
<th>Incident Light Intensity µein/min</th>
<th>Absorption µein/min</th>
<th>Rate of O$_2$ Evolution µmoles/min</th>
<th>CO$_2$ Absorption µmoles/min</th>
<th>Quantum Requirement for O$_2$ Evolution</th>
<th>Quantum Requirement for CO$_2$ Absorption</th>
<th>Respiration µmoles O$_2$/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3%</td>
<td>24.8</td>
<td>8.0</td>
<td>0.74</td>
<td>0.75</td>
<td>10.8</td>
<td>10.7</td>
<td><em>0.22</em></td>
</tr>
<tr>
<td></td>
<td>31.8</td>
<td>10.8</td>
<td>1.10</td>
<td>1.09</td>
<td>9.8</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.7</td>
<td>13.5</td>
<td>1.25</td>
<td>1.15</td>
<td>10.8</td>
<td>11.7</td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>14.4</td>
<td>1.60</td>
<td>1.23</td>
<td>9.0</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>0.4%</td>
<td>33.0</td>
<td>11.5</td>
<td>1.19</td>
<td>1.26</td>
<td>9.6</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.6</td>
<td>17.7</td>
<td>1.82</td>
<td>1.57</td>
<td>9.7</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.4</td>
<td>24.2</td>
<td>2.07</td>
<td>1.65</td>
<td>11.7</td>
<td>14.7</td>
<td><strong>0.28</strong></td>
</tr>
<tr>
<td></td>
<td>55.5</td>
<td>25.1</td>
<td>2.10</td>
<td>1.84</td>
<td>11.9</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>0.6%</td>
<td>39.1</td>
<td>20.0</td>
<td>16.10</td>
<td>13.8</td>
<td>12.4</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>22.7</td>
<td>22.25</td>
<td>17.7</td>
<td>10.3</td>
<td>13.2</td>
<td><strong>0.11</strong></td>
</tr>
</tbody>
</table>

*Respiration during first 4 min after photosynthesis

**Respiration between 8 min and 30 min
Table II-8
Summary of the Results of Quantum Requirement Measurements

<table>
<thead>
<tr>
<th>Density of Algae</th>
<th>Incident Light Intensity (μein/min)</th>
<th>Absorption (μein/min)</th>
<th>Rate of O₂ Evolution (μmoles/min)</th>
<th>Quantum Requirement for CO₂ Absorption (μmoles/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before correction</td>
<td>after correction</td>
<td>before correction</td>
<td>after correction</td>
</tr>
<tr>
<td>0.35</td>
<td>32.73</td>
<td>42.50</td>
<td>10.64</td>
<td>13.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.29</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.24</td>
<td>10.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td>13.0</td>
</tr>
<tr>
<td>0.4</td>
<td>30.92</td>
<td>40.20</td>
<td>10.81</td>
<td>14.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.60</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.77</td>
<td>8.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.07</td>
<td>9.20</td>
</tr>
<tr>
<td>0.5</td>
<td>20.22</td>
<td>26.00</td>
<td>10.67</td>
<td>13.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.49</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.14</td>
<td>9.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.36</td>
<td>12.20</td>
</tr>
<tr>
<td>0.85</td>
<td>48.85</td>
<td>63.51</td>
<td>24.60</td>
<td>32.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.43</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.05</td>
<td>9.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.53</td>
<td>12.40</td>
</tr>
</tbody>
</table>
by a method similar to that described by Bassham and Shibata. In that method the thermopile was calibrated first against the standard lamp, as is usually done, but then a piece of opal glass was placed in front of the standard lamp as a diffuser and a new sensitivity was determined. It was then assumed that as long as the opal glass was in place, causing diffusion of the incident light, the thermopile could be used for direct measurement of scattered light. The correctness of this method rests on the assumption that the opal glass will uniformly scatter light to the same degree irrespective of its incident scattered or parallel characteristics. Unfortunately, with the opal glass available to us in the present study, which we believed to be similar to that used in the earlier study, it was found that this assumption was not completely valid. Thus, when the photocell was calibrated against the opal glass-equipped thermopile, it was found that a 30% lower sensitivity was obtained as compared with calibration against the thermopile with no diffuser. Thus the values of the quantum requirement obtained using the low sensitivity had to be corrected upward as indicated in Table II-8. It seems likely that the values reported by Bassham and Shibata also should be corrected upward by about 30%, in which case the reported value of 7.5 would become 9.8.

6. Discussion

The reliability of measurements of the quantum requirement of photosynthesis depends on accurate determination of the rate of light absorption and on accurate determination of the evolution of oxygen and uptake of carbon dioxide. The use of non-manometric techniques of determining the gas exchange by means of instruments which independently measure the
changes in oxygen and CO₂ tension are well established and reliable. Provided care is taken in the calibration of the instruments and determination of the effective volume of the system, measurements with an accuracy of ± 2% can be obtained. However, the use of these instruments entails the requirement for photosynthesis by a substantial amount of plant material, and this imposes new problems of arranging a light source with a narrow wavelength band and measured intensity over a relatively large area to provide rates of photosynthesis which exceed several-fold rates of dark respiration. At the same time, many problems arise with respect to accurate determination of the absorbed light energy, since both the cell to contain the large sample of plant material and the plants themselves tend to diffuse and scatter the supplied light. Finally, the measurement of the scattered light itself presents some problem since the primary detector, the thermopile used for the measurement of light energy, cannot be used directly to measure scattered light. By using a secondary detecting device, the surface of which can be placed directly in contact with the area over which light flux must be measured, and by carefully calibrating the secondary detector against the thermopile, and by accurate mapping of all of the light flux passing through the cell containing the algae, it is possible to determine the absorbed light with an error of not greater than 4%.

Equally serious for considerations of the theoretical minimum quantum requirement of photosynthesis is the requirement that the most active and efficient possible plants be selected. This requirement can at least be approached by the selection of Chlorella pyrenoidosa cultured in such a
way that their rate of oxygen evolution per mg chlorophyll is close to the maximum that has been reported for any green plants. After all this has been done, we find the minimum measurable requirement for oxygen evolution to be around 9, similar to what many other workers have reported in the past.

In Figure II-10, all quantum requirements from Tables II-7 and II-8 have been plotted versus rate of light energy absorption per cm$^3$ of algae. There appears to be no dependence on rate of light absorption over the range used in our experiments. Thus we can rule out the possibility that partial self-shading at the lower light intensities caused higher quantum requirement. In fact, the independence of quantum requirement versus rate of light absorption also argues against the need for any respiration correction, since such a correction would be more important at the lower light absorption rate as compared with the higher light absorption rate.

Probably, light respiration in *Chlorella pyrenoidosa* is insignificant compared with the rate of photosynthesis. Tracer studies with $^{32}$p and $^{14}$C during photosynthesis and in darkness with *Chlorella pyrenoidosa* indicated that there is a switch from photosynthetic metabolism to dark metabolism. Glycolysis and oxidative pentose phosphate cycle are apparently inactive in the light but become active in the dark, using the same metabolic pools as were used in photosynthesis in the light.

It appears that ATP can diffuse rapidly in and out of chloroplasts. Thus, ATP is available to the entire plant cell from photosynthesis in the light and from respiration in the dark. The requirement for ATP for non-photosynthetic processes, as well as synthesis inside the chloroplast,
Figure II-10. Rate of oxygen evolution vs. rate of light energy absorption for several experimental conditions. The rates of oxygen evolution and of light absorption have been calculated per cm$^3$ of packed Chlorella pyrenoidosa to permit comparison of all experimentally determined values.
is probably met in part by cyclic photophosphorylation. Depending on such additional requirement for ATP and on the quantum efficiency of cyclic photophosphorylation, the total quantum requirement of photosynthesis will be increased above the theoretical 8 predicted by the two light steps, one photon per electron scheme.

Part of this chapter has been published in Biochim. Biophys. Acta 162, 254 (1968).
III. THE EFFECT OF LIGHT QUALITY ON PHOTOSYNTHETIC PRODUCT DISTRIBUTION

A. Discussion of the Experiment

1. Comparison between the two hypotheses on photoelectron transport

In Chapter I, it has been shown that among the different opinions on the primary quantum conversion process,\(^1\)−\(^6\) the "photolyt" hypothesis\(^7\)\(^,\)\(^8\) and the Hill-Bendall scheme are the two typical ones. The following table is a comparison between these two hypotheses on several aspects of photosynthesis:

<table>
<thead>
<tr>
<th>Aspects of Photosynthesis</th>
<th>&quot;Photolyt&quot; Hypothesis</th>
<th>Hill-Bendall scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary process</td>
<td>(3[H_2CO_3]^* \xrightarrow{hv} 3[CH_2O] + 3 \text{O}_2)</td>
<td>(\text{P-700 h} \rightarrow \text{P-700}^+ + \text{e})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\text{Fd}^{+++} + \text{e} \rightarrow \text{Fd}^{++})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Q \xrightarrow{hv} Q^+ + \text{e})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Q^+ + 2\text{OH}^- \rightarrow \frac{1}{2} \text{H}_2\text{O} + \frac{1}{4} \text{O}_2 + Q)</td>
</tr>
<tr>
<td>Origin of oxygen evolved</td>
<td>from \text{CO}_2</td>
<td>from \text{H}_2\text{O}</td>
</tr>
<tr>
<td>Quantum requirement</td>
<td>4 or less</td>
<td>at least 8</td>
</tr>
<tr>
<td>of photosynthesis</td>
<td>Oxidative. The energy</td>
<td>Reductive and cyclic. In</td>
</tr>
<tr>
<td></td>
<td>required for phosphy-</td>
<td>non-cyclic photophosphory-</td>
</tr>
<tr>
<td></td>
<td>rolation is provided by</td>
<td>lation ATP is generated with</td>
</tr>
<tr>
<td></td>
<td>the oxidation of carbo-</td>
<td>the reduction of NADP to</td>
</tr>
<tr>
<td></td>
<td>hydrates.</td>
<td>NADPH. Cyclic photophos-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phorylation ATP generation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without any net electron</td>
</tr>
<tr>
<td></td>
<td></td>
<td>transport.</td>
</tr>
</tbody>
</table>

Table III-1 indicates that the validity of these two hypotheses can be verified by

(i) isotopic studies on the origin of the \(\text{O}_2\) evolved
(ii) quantum requirement measurements
(iii) dependence of photophosphorylation on the quality of the illuminating light
Isotopic studies on the origin of O₂ evolved has been done. However, it is subjected to the argument that there is a rapid exchange of the oxygen atoms between H₂O and CO₂. Since inside the living plant the amount of H₂O is always more than that of CO₂, isotopic studies on the origin of the O₂ evolved will always give the isotopic species of H₂O even if the O₂ evolved is originated from CO₂.

The quantum requirement measurement is certainly one of the best ways to test the validity of these two hypotheses. However, in spite of 40 years' work on the determination of quantum requirement in photosynthesis, there is still no conclusive opinion on this important number. In Chapter II, the quantum requirement of photosynthesis has been determined and all of its values are above 8. However, there are always arguments on the correction for respiration which will change the value of this number.

The dependence of photophosphorylation on the quality of light provides a new way to test these two hypotheses, and therefore provides the impetus for the present experiments.

2. Dependence of photophosphorylation on the quality of light

According to the "photolyt" hypothesis, the energy required for phosphorylation is provided by oxidation. Therefore, the amount of ATP produced should be independent of the wavelength of the light used, so long as the light does not stimulate any special enzymatic reactions. However, the Hill-Bendall scheme of photoelectron transport consists of two light reactions, photoreaction I and photoreaction II. Some of the energy released in the dark reactions connecting the two light reactions is conserved as ATP. This process is called photophosphorylation.
Studies on chloroplasts have shown that there are two kinds of photophosphorylation. Non-cyclic photophosphorylation is defined as a process in which ATP is generated from ADP and inorganic phosphate when electrons are transported from water to the terminal electron acceptor $\text{NADP}^+$. There is also cyclic photophosphorylation mediated only by photoreaction I, which can be selectively promoted by far red light with wavelengths too long to promote photoreaction II.\(^7\) (See Figure III-1.) If this is also the case in vivo, the amount of phosphorylation in vivo can be varied by light with different wavelengths which promote different amounts of photophosphorylation I and lead to different amounts of cyclic photophosphorylation. Hence, a study of the effect of light quality on the variation of cyclic photophosphorylation can serve to distinguish between the two hypotheses.

3. Measurement of photophosphorylation

Since ATP serves as an energy currency for reactions requiring phosphorylation, it is generated, used, and regenerated during the process of photosynthesis. It is therefore impractical, if not impossible, to measure the amount of photophosphorylation by determining the amount of ATP formed. Hence, the amount of phosphorylation has to be determined indirectly.

Since ATP and NADPH are the assimilating power of photosynthesis, a variation of their ratio will lead to a variation of the photosynthetic products, because the synthesis of different products requires different amounts of them. Based on the photosynthetic carbon reduction cycle proposed by Bassham and Calvin,\(^7\)\(^8\) and the known pathways for the synthesis
Figure III-1. Scheme of photoelectron transport in photosynthesis showing sites of cyclic and non-cyclic photophosphorylation. Redox potentials of electron-carrying cofactors along this path is indicated by scale on the left.
of various products, the cofactor requirement for the incorporation of one carbon atom into sugars, polysaccharides, amino acids, proteins, and lipids has been calculated and shown in Table III-2.*

Table III-2

Cofactor Requirement for the Synthesis of Carbon Compounds

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cofactors</th>
<th>Ratio ATP/NADPH</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharides</td>
<td>ATP 3-1/6</td>
<td>NADPH 2</td>
<td>1.58 e.g., starch, sucrose</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>ATP 4</td>
<td>NADPH 3</td>
<td>1.33</td>
</tr>
<tr>
<td>Amino acid</td>
<td>ATP 2-1/3</td>
<td>NADPH 1-3/4</td>
<td>1.33 NH₃ is used as source of nitrogen</td>
</tr>
<tr>
<td>Protein</td>
<td>ATP 2-2/3</td>
<td>NADPH 1-3/4</td>
<td>1.53 Assuming 3 carbon atoms per nitrogen</td>
</tr>
<tr>
<td>Amino acid</td>
<td>ATP 2-1/3</td>
<td>NADPH 3</td>
<td>0.77 NO₃⁻ is used as source of nitrogen</td>
</tr>
<tr>
<td>Protein</td>
<td>ATP 2-2/3</td>
<td>NADPH 3</td>
<td>0.89 Assuming 3 carbon atoms per nitrogen</td>
</tr>
</tbody>
</table>

4. Indirect measurement of photophosphorylation

Table III-2 shows the cofactor requirement for the production of fatty acids, and polysaccharides starting from CO₂. The energy required for the transport of these compounds from one part of the cell to the other is assumed to be small and is neglected. Hence, Table III-2 gives only a qualitative idea about the cofactor requirement for the production of various compounds. However, it does show that a lower ratio of ATP/NADPH favors the synthesis of fatty acids and proteins while

*See Appendix III for calculation.
a high ratio favors the synthesis of polysaccharides.

Since a high ratio of ATP/NADPH favors the production of polysaccharides and a low ratio, amino acids, and lipids, a comparison of the photosynthetic products can be used as an indication of additional ATP production by cyclic photophosphorylation. Since cyclic photophosphorylation is linked to photoreaction I, a high ratio of ATP/NADPH could be achieved by illuminating the plant with long wavelength red light which is preferentially absorbed by photosystem I and used for photoreaction I. Hence a comparison of the photosynthetic product distribution of plants illuminated with light of long wavelength red and of short wavelength red would provide evidence concerning the existence of cyclic photophosphorylation in vivo, which in turn gives us additional evidence of the existence of two light reactions.

B. Materials and Methods

1. Choice of plant

The unicellular algae Chlorella pyrenoidosa is usually chosen for the studies of photosynthesis because

(i) they are small, so that a large number is used for the study and, therefore, the uncertainties due to individual variation are eliminated;

(ii) they have a very high photosynthetic rate, so that enough products are formed in a short period; and

(iii) they are easily available and can survive over a wide range of variation in temperature, pH, and nutrient supply.

In order to study the effect of light quality on product distribution, one would like to choose the kind of green plant in which the
absorption peaks of the major pigment (chlorophyll $a$) and that of the accessory pigment* are far apart, so that a larger difference in product distribution can be observed. However, the accessory pigment in *Chlorella* is chlorophyll $b$, the absorption peak of which overlaps with that of chlorophyll $a$ to such a great extent that there is only one major absorption peak which appears in the absorption spectrum of *Chlorella pyrenoidosa*. Therefore, *Chlorella pyrenoidosa* does not seem to be a suitable algae for the present purpose.

Algae with accessory pigments whose absorption peaks are far apart from that of chlorophyll $a$ are available. However, some of them are so delicate that they cannot survive a small change in their environmental conditions. For example, the absorption peaks of phycocyanine and chlorophyll $a$ are far apart in *Porphyridium cruentum.* However, *Porphyridium* has to be suspended in a solution whose salt content is so high that they are not suitable for later analysis by paper chromatography.

Among algae with the accessory pigment separated from chlorophyll $a$, *Anacystis nidulans* was found to be the most suitable one for the present purpose. The absorption spectrum of *Anacystis nidulans* (Figure III-2A) shows that the absorption peaks of chlorophyll $a$ and the accessory pigment have a wider separation than those in *Chlorella* (Figure III-2B). Furthermore, the salt content of the suspension medium for *Anacystis nidulans* is low enough for later analysis of photosynthetic products by paper chromatography.

*The accessory pigment in photosystem II. Light absorbed by this accessory pigment can be used to promote photoreaction II effectively.*
Figure III-2A. Absorption spectrum of *Anacystis nidulans*.

2B. Absorption spectrum of *Chlorella* cells.85

(Obtained using the Cary Model 14M spectrometer equipped with a Model 1462 scattered transmission attachment)
In the present experiment, both Chlorella and Anacystis nidulans were chosen because we wanted to see if a wider separation in the absorption peaks of chlorophyll $a$ and the accessory pigment leads to a greater difference in photosynthetic product distribution.

2. Light sources

Of the two light beams used to illuminate the twin cell, one should be strongly absorbed by photosystem I while the other by photosystem II. The transmission spectra of the filter sets used to provide these two light beams are shown in Figure III-3. The 680 ± 10 μm beam should be strongly absorbed by photosystem I, which contains mostly chlorophyll $a$, while the 630 ± 10 μm beam by photosystem II, which contains mostly the accessory pigment for photosynthesis.*

Light sources are provided by incandescent lamps with interference filter sets to get the desired wavelengths. Infrared absorption filters are placed between the incandescent lamp and the interference filter sets to cut down the large quantity of infrared from the incandescence lamp. Both the interference filter sets and the infrared absorption filters are cooled by running water. This set-up also provides cooling for the algae suspension in the twin cell.

3. Experimental set-up

Figure III-4 is a schematic diagram of the apparatus used for the present experiment—i.e., the steady-state apparatus—with the twin

*In Chlorella, the accessory pigment is chlorophyll $b$, while that in Anacystis nidulans is phycoerythrin.
Figure III-4. Steady-state apparatus with the "twin cell" modification.
cell modifications.

In order to ensure that the difference in photosynthetic product distribution is solely due to the difference in the quality of the light used, the photosynthetic product should be obtained from algae photosynthesizing under all other identical aspects of photosynthesis. The algae should be of the same age, photosynthesizing under the same level of CO₂ and temperature. The ideal way to achieve these requirements is to have the algae photosynthesize in a "twin cell", where algae in different compartments are illuminated by different light beams but with all other conditions identical.

The advantages of using the twin cell are:

(i) Since the algae in the two compartments can be originated from the same suspension, they are identical with respect to age and density.

(ii) Since there is only one gas phase, the algae are photosynthesizing under the same level of CO₂ and O₂.

(iii) The present CO₂ analyzer has a different sensitivity for ¹⁴CO₂ and ¹²CO₂. Therefore, there are always ambiguities in the radioactive specificity of the CO₂ inside the system. This leads to uncertainties in calculating the absolute amount of photosynthetic products formed. Since the "twin cell" has only one gas phase, such ambiguities are eliminated.

4. Experimental procedures
   a. Preparation of algae suspension

Two hundred ml of the algae suspension from the culture flask was centrifuged at 1000 g for 5 min. The algae from the centrifuge bottle were then transferred to a 12-ml centrifuge tube and
centrifuged at 1500 g for 10 min. The algae were then resuspended to
the desired density* by a suitable medium. One-thousandth \( \text{M} \) of \( \text{KH}_2\text{PO}_4 \)
washed used for *Chlorella pyrenoidosa*, and the medium for *Anacystis nidulans*
has the following composition:

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} )</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>( \text{KNO}_3 )</td>
<td>9.9 mM</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.165 g/liter</td>
</tr>
<tr>
<td>( \text{KH}_2\text{PO}_4 )</td>
<td>7.3 mM</td>
</tr>
<tr>
<td>Arnon's A-4**</td>
<td>1.0 ml/liter</td>
</tr>
<tr>
<td>Fe EDTA</td>
<td>0.01 g/liter</td>
</tr>
</tbody>
</table>

b. Cold experiments

Prior to the studies of photosynthetic product distribution
by using radioactive \( ^{14}\text{CO}_2 \) as a tracer, a "cold experiment" was run so
as to find out the light intensities at which the algae in different
compartments of the twin cell photosynthesize at similar rates. This
was done by filling one cell with the algae suspension and the other with
the suspension medium. The intensity of the beam illuminating the algae
was varied by changing the voltage applied to the lamp. The photosyn-
thetic rates of the algae at various voltages of the lamp were recorded.
After this was done, a fresh batch of algae was put into the other com-
partment, and the photosynthetic rates were recorded in a similar manner.

*In the present study, the suspension density of *Chlorella* is 0.6% and
that of *Anacystis nidulans* is 0.4%.

**Composition of Arnon's A-4:**

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} )</td>
<td>0.079 g/l</td>
</tr>
</tbody>
</table>
From these two sets of data, the voltages of the lamps at which the algae in the two different compartments photosynthesize at similar rates were chosen for later studies.

c. Hot experiments (experiments with radioactive material as tracer)

Thirty-five ml of algae suspension were added to each compartment of the twin cell. The algae were allowed to photosynthesize in their corresponding light beams for a period of 15 min so as to see whether they are healthy or not. The lamps were then turned off for 3 min, during which $^{14}$CO$_2$ was introduced into the system and mixed with the $^{12}$CO$_2$ already present. Light was turned on again and samples were taken every 5 min for a period of 50 min.

Sampling was done by using a syringe-like filler which was able to transfer a preset volume of algae suspension to a preweighed test tube containing 4 ml of methanol to kill the algae. The test tubes were weighed again to obtain the exact weight of the algae suspension.

5. Analysis

a. Total $^{14}$C fixed

The algae suspension in methanol was shaken vigorously and 500 µl of the suspension was transferred to a test tube, where 30 ml of 1 N sulfuric acid was added. After vigorously shaking, 500 µl of this suspension was transferred to a scintillation vial, where 18 ml of scintillation solution B* was added. After vigorously shaking, the vial was put into the scintillation counter to be counted for 1 min. It

*Composition of scintillation solution B: 13.5 g PPO 750 ml ethyl alcohol
0.3 g POPOP in 1050 ml toluene
150.0 g naphtha- 1200 ml p-dioxane

PPO is trade name for 2,5-diphenyl oxazole.
POPOP is trade name for 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene.
was found that the suspension did not settle out too much in a period of 1 hr, because the counting rate was practically the same after a period of 1 hr. The rate of $^{14}$C fixation was obtained by plotting the moles of $^{14}$C fixed per gram of algae versus time.

b. Sugars and free amino acids

Sugars, amino acids, and organic acids were isolated by two-dimensional paper chromatography as described by Bassham et al. The various compounds on the paper were located and identified by autoradiography. Portions of the paper containing the various compounds were cut out and counted by Geiger-Müller counter to determine the total amount of $^{14}$C in that compound.

c. Proteins (as insoluble amino acids)

The proteins were analyzed as insoluble amino acids by methods described by Bassham et al.

d. Lipids

The lipids were extracted by a solution of chloroform and methanol (chloroform:methanol = 2:1) in the following way:

To 500 µl of the sample (as algae suspension in methanol) a drop of 3 N sulfuric acid was added. This changed all free amino acids into the positively charged ammonium ion while the fatty acids were in the uncharged form at this pH. Eight-tenths ml of chloroform was added so that the ratio of chloroform to methanol in solution was 2:1. Enough chloroform:methanol solution was added until a one-phase solution resulted. The solution was again let stand for 2 hr with occasional shaking. The organic layer (chloroform) was transferred to a preweighed
culture tube. The inorganic layer was later extracted three times with chloroform. The extract was also transferred to the culture tube and combined with the previous extract in the organic layer. The culture tube was weighed to obtain the total amount of chloroform extract transferred.

Five-tenths gram of anhydrous sodium sulfate was added to each tube and shaken to remove any water that was in the extract. The tubes were then centrifuged at 2500 g for 15 min. Five hundred µl of the extract was transferred to a scintillation vial and let stand overnight to evaporate all the chloroform, a very strong quenching agent. Eighteen ml of scintillation solution A* was added to each vial for scintillation counting.

C. Results

1. Total 14C fixation
   a. By Anacystis nidulans

   Figure III-5A shows the rates of 14C fixation by Anacystis nidulans illuminated by the 630 µw and 680 µw light. The points representing total 14C fixation by the algae under these two kinds of illumination practically fall on one line. This indicates that the rate of 14C fixation is the same under either source of light.

   b. By Chlorella

   Figure III-5B shows that the rate of total 14C fixation by Chlorella is faster under the illumination of 680 µw light than that

*Composition of scintillation solution A: 4.5 g of PPO and 0.1 g of POPOP in 1.0 liter of toluene.
Figure III-5A. Total $^{14}$C incorporation by *Anacystis nidulans*.

$x = 680$ mu

$o = 630$ mu
Figure III-5B. Total 14C incorporation by Chlorella pyrenoidosa.

X = 680 mu

o = 630 mu
under the 630 μm one. This must be put into consideration for later comparison of the photosynthetic product distribution.

2. The incorporation of $^{14}$C into lipids
   a. By Anacystis nidulans

   Figure III-6A shows the rates of $^{14}$C incorporation into the algae lipids by Anacystis nidulans illuminated by 630 μm and 680 μm light. The two curves are parallel with the 630 μm curve slightly above the 680 μm one. The fact that these two curves are parallel indicates that the modes of lipid synthesis are identical in the two cases. Since the two curves are parallel, the rates of $^{14}$C incorporation under the illumination of two kinds of light are the same. However, the 630 μm curve is above the 680 μm one; the induction period of lipid synthesis is probably shorter under 630 μm light illumination than that under the 680 μm one.

3. The incorporation of $^{14}$C into proteins (as insoluble amino acids)
   a. By Anacystis nidulans

   The curves shown in Figure III-7 (A to C) give the rates of $^{14}$C incorporation into some of the insoluble amino acids. It can be seen that the rates of $^{14}$C incorporation into these insoluble amino acids are practically the same for algae under the illumination of 630 μm and 680 μm light.

   b. By Chlorella

   The rates of $^{14}$C incorporation into some of the insoluble amino acids are shown in Figure III-8 (A and B). In Figure III-8A the points of both the 630 μm and 680 μm light fall on one line, while in
Figure III-6A. Incorporation of $^{14}$C into lipids by *Anacystis nidulans*.

$\circ = 630$ $\mu$u

$\times = 680$ $\mu$u
Figure III-68. Incorporation of $^{14}$C into lipids by *Chlorella pyrenoidosa*.

$X = 680 \text{ m\mu}$

$O = 630 \text{ m\mu}$
Figure III-7A. Incorporation of $^{14}C$ into insoluble amino acids by \textit{Anacystis nidulans}.

- Alanine: $\square = 630 \text{ mu;}$ $\blacksquare = 680 \text{ mu.}$
- Proline: $\vartriangle = 630 \text{ mu;}$ $\vartriangle = 680 \text{ mu.}$
- Tyrosine: $\circ = 630 \text{ mu;}$ $\bullet = 680 \text{ mu.}$
Figure III-7B. Incorporation of $^{14}$C into insoluble amino acids by *Anacystis nidulans*.

Aspartic acid: ○ = 630 mu; ● = 680 mu.
Glycine and Serine: □ = 630 mu; ▼ = 680 mu.
Lysine: □ = 630 mu; ■ = 680 mu.
Threonine: △ = 630 mu; ▲ = 680 mu.
Figure III-7C. Incorporation of $^{14}$C into insoluble amino acids by *Anacystis nidulans*.

Glutamic acid: $X = 680 \text{ mw}$

$O = 630 \text{ mw}$
Figure III-8A. Incorporation of $^{14}$C into insoluble amino acids by Chlorella.

Alanine: $\bullet = 680 \text{ mu}$
Glutamic acid: $\triangle = 630 \text{ mu}$

$\circ = 630 \text{ mu}$
Figure III-8B. Incorporation of 14C into insoluble amino acids by Chlorella.

Aspartic acid: $\alpha = 630$ $\mu$g
$X = 680$ $\mu$g
Figure III-8B the two curves are parallel with the 630 μm one above the 680 μm one. Judging from the fact that the total $^{14}$C fixation was faster for the 680 μm light illuminated algae, the rate of $^{14}$C incorporation into these insoluble amino acids are slightly faster in photosynthesis by Chlorella under the illumination of 630 μm light. Nevertheless, within expected experimental error, one cannot take such difference as a very significant one.

4. The incorporation of $^{14}$C into polysaccharides
   a. By Anacystis nidulans
   Sucrose and UDPG are taken as representative compounds of polysaccharides. Figure III-9 (A and B) shows the rates of $^{14}$C incorporation into sucrose and UDPG during Anacystis nidulans photosynthesis. According to the predictions in Chapter IV, more sucrose and UDPG are expected to be formed in the 680 μm light. However, the rates of $^{14}$C incorporation into these compounds are the same for 630 μm and 680 μm light illuminated algae.
   b. By Chlorella
   Figure III-10 (A and B) shows that more $^{14}$C was incorporated into sucrose and UDPG in the photosynthesis carried out by Chlorella under the illumination of 680 μm light. However, compared with the rate of total $^{14}$C fixation, the differences in the relative rates of $^{14}$C incorporated into sucrose and UDPG during the photosynthesis by Chlorella are very small.
Figure III-9A. Incorporation of $^{14}$C into sucrose by *Anacystis nidulans*.

- $\bigcirc$ = 630 m\(_\mu\)
- $\bigtimes$ = 680 m\(_\mu\)
Figure III-9B. Incorporation of $^{14}C$ into UDPG by *Anacystis nidulans*.

$\circ = 630$ $\mu u$

$X = 680$ $\mu u$
Figure III-10A. Incorporation of $^{14}$C into sucrose by *Chlorella*.

- $\circ = 630$ mu
- $\times = 680$ mu
Figure III-10B. Incorporation of $^{14}$C into UDPG by Chlorella.

o = 630 mw

X = 680 mw
5. The incorporation of $^{14}$C into sugar phosphates and organic acids

a. By *Anacystis nidulans*

The patterns of $^{14}$C incorporation into various photosynthetic intermediates during photosynthesis, carried out by *Anacystis nidulans*, are shown in Figure III-11. Figure III-11 (A, B, and C) shows that the initial rates of incorporation, as well as the final steady levels of $^{14}$C in PGA, sugar monophosphates, and sugar diphosphates, are practically the same for algae under the illumination of either the 630 mu or the 680 mu light. Figure III-11 (D and E) shows that the same results are found in malic and citric acids photosynthesized by algae under the illumination of 630 mu or 680 mu light.

b. By *Chlorella*

The $^{14}$C labeling patterns and the final steady-state levels of PGA, sugar monophosphates, and sugar diphosphates under the illumination of 630 mu and 680 mu light are shown in Figure III-12. The higher steady-state levels of these compounds under the illumination of 680 mu light are probably due to the higher $^{14}$C fixation rate under the illumination of 680 mu light.

Together with the results of these compounds formed in *Anacystis nidulans* photosynthesis, it indicates that the pool size of the various intermediates in the photosynthetic reduction cycle are closely related to the overall photosynthetic rate. This result is consistent with the opinion of Bassham *et al.* that most of the CO$_2$ goes into the algae via the photosynthetic carbon reduction cycle.
Figure III-11A. Incorporation of $^{14}$C into PGA by *Anacystis nidulans*.

$O = 630 \text{ mu}$

$X = 680 \text{ mu}$
Figure III-11B. Incorporation of 14C into sugar monophosphates by Anacystis nidulans.

- $o = 630 \text{ mu}$
- $X = 680 \text{ mu}$
Figure III-11C. Incorporation of $^{14}$C into sugar diphosphates by *Anacystis nidulans*.

\[ o = 630 \text{ mw} \]
\[ X = 680 \text{ mw} \]
Figure III-11D. Incorporation of $^{14}$C into malic acid by *Anacystis nidulans*.

$\Delta = 630$ $\mu$u

$X = 680$ $\mu$u
Figure III-11E. Incorporation of $^{14}C$ into citric acid by *Anacystis nidulans*.

$\Delta = 630 \text{ mu}$

$X = 680 \text{ mu}$
Figure III-12A. Incorporation of $^{14}$C into PGA by Chlorella.

$\Delta = 630 \text{ mu}$

$X = 680 \text{ mu}$
Figure III-12B. Incorporation of $^{14}$C into sugar monophosphates by Chlorella.

$\Delta = 630 \text{ mu}$

$X = 680 \text{ mu}$
Figure III-12C. Incorporation of $^{14}$C into sugar diphosphates by Chlorella.

$O = 630 \text{ mW}$

$X = 680 \text{ mW}$
6. The incorporation of $^{14}$C into amino acids

a. By *Anacystis nidulans*

The $^{14}$C incorporation curves of alanine, glutamic acid, glycine, and serine are shown in Figure III-13. These curves indicate that there is hardly any difference in the rate of synthesis of these amino acids under the influence of these two different kinds of light. This implies that the algae provide the same ratio of ATP/NADPH for amino acid synthesis under the illumination of these two kinds of light.

b. By *Chlorella*

In Figure III-14, the $^{14}$C incorporation curves of glutamine, glutamic acid, and aspartic acid are shown. The "rise and fall" pattern of these curves indicates that the protein synthesis did not start immediately after the light was turned on. The levels of the free amino acids therefore rose to a maximum and then declined to a steady-state level. The fact that the peaks of the 630 mu curves are higher than those of the 680 mu ones suggested one of the following two possibilities.

(i) The protein synthesis of 680 mu light illuminated *Chlorella* reached its maximum rate faster than those of the 630 mu light illuminated one. Therefore, the levels of the free amino acids of the 680 mu light illuminated *Chlorella* began to drop earlier than those of the 630 mu illuminated one. Consequently, the peaks of the 680 mu curves are lower than those of the 630 mu ones. If this is the case, the rate of $^{14}$C incorporation into insoluble amino acids (proteins) should be higher when *Chlorella* is illuminated by 680 mu light. However, the
Figure III-13A. Incorporation of $^{14}$C into alanine by *Anacystis nidulans*.

$X = 630$ mu

$\Delta = 680$ mu
Figure III-13B. Incorporation of $^{14}$C into glycine and serine by *Anacystis nidulans*.

$X = 630 \text{ mu}$

$\sigma = 680 \text{ mu}$
Figure III-13C. Incorporation of 14C into glutamic acid by *Anacystis nidulans*.

\[ X = 630 \text{ mu} \]

\[ \sigma = 680 \text{ mu} \]
Figure III-14A. Incorporation of $^{14}$C into glutamin by Chlorella.

- $\square = 630$ mu
- $\blacksquare = 680$ mu
Figure III-14B. Incorporation of $^{14}$C into glutamic acid by Chlorella.

- $o = 630$ mu
- $• = 680$ mu
Figure III-14C. Incorporation of $^{14}$C into aspartic acid by Chlorella.

$\Delta = 630$ mw

$\Delta = 680$ mw
present data of $^{14}$C incorporation of insoluble amino acids do not support this explanation.

(ii) The rate of free amino acid synthesis reached its maximum earlier in 630 mu light, so that the initial levels of free amino acids synthesized by Chlorella were higher in the 630 mu light. Since the rate of amino acid incorporation into proteins is proportional to the amount of free amino acids available, then the levels of amino acids synthesized by Chlorella under illumination of 630 mu light decreased more rapidly than those under the illumination of 680 mu light. If this is the case, the rate of $^{14}$C incorporation into insoluble amino acids (proteins) should be higher when Chlorella is illuminated by 630 mu light. An examination of the $^{14}$C incorporation curves of insoluble glutamic acids and aspartic acids shows that this may be the case. The points on the glutamic acid curve (Figure III-8B) are too scattered to support such an explanation. However, in Figure III-8B, the curves show that the incorporation of $^{14}$C into aspartic acid is faster when the Chlorella was under the illumination of 630 mu light.

D. Discussion

Tables III-3A and III-3B are summaries of the photosynthetic product distribution of Anacystis nidulans and Chlorella pyrenoidosa. Table III-3A shows that there is no observable difference in the photosynthetic product distribution of Anacystis nidulans under the illumination of 630 mu and 680 mu light. Table III-3B shows that there are some differences among the photosynthetic product distribution between Chlorella under the illumination of 630 mu and that under 680 mu light.
Although the differences are in line with the prediction on Table III-2, they are too small to be given serious consideration.

Although the separation between the absorption peak of chlorophyll a and that of the accessory pigment is bigger in *Anacystis nidulans* than in *Chlorella*, a bigger difference in photosynthetic product distribution is not found in *Anacystis nidulans*.

Together with the fact that the difference in photosynthetic product distribution of *Chlorella* is too small to be of any significance, it may be concluded that the 630 μm and 680 μm light caused no observable difference in the photosynthetic product distribution.

**Table III-3A**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rate of formation under the illumination of 630 μm light</th>
<th>Final levels under the illumination of 630 μm light</th>
<th>Rate of formation under the illumination of 680 μm light</th>
<th>Final levels under the illumination of 680 μm light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total 14C fixed</td>
<td>6.09</td>
<td>6.09</td>
<td>6.09</td>
<td>6.09</td>
</tr>
<tr>
<td>2. Lipids</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>3. Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) alanine</td>
<td>0.058</td>
<td>0.058</td>
<td>2.25</td>
<td>2.25</td>
</tr>
<tr>
<td>b) proline</td>
<td>0.028</td>
<td>0.028</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>c) tyrosine</td>
<td>0.025</td>
<td>0.025</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>d) aspartic acid</td>
<td>0.062</td>
<td>0.062</td>
<td>2.54</td>
<td>2.54</td>
</tr>
<tr>
<td>e) glycine and serine</td>
<td>0.057</td>
<td>0.057</td>
<td>2.18</td>
<td>2.18</td>
</tr>
<tr>
<td>f) lysine</td>
<td>0.031</td>
<td>0.031</td>
<td>1.11</td>
<td>1.11</td>
</tr>
<tr>
<td>g) threonine</td>
<td>0.031</td>
<td>0.031</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>h) glutamic acid</td>
<td>0.031</td>
<td>0.031</td>
<td>1.07</td>
<td>1.07</td>
</tr>
<tr>
<td>4. Polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) sucrose</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
</tr>
<tr>
<td>b) UDPG</td>
<td>5.08</td>
<td>5.08</td>
<td>5.08</td>
<td>5.08</td>
</tr>
</tbody>
</table>
### Table III-3B

Photosynthetic Product Distribution by Chlorella

<table>
<thead>
<tr>
<th>Compounds synthesized</th>
<th>Rate of $^{14}$C incorporation under the illumination of 630 μm light (μmoles $^{14}$C/min g)</th>
<th>Steady-state levels under the illumination of 630 μm light (μmoles $^{14}$C/g)</th>
<th>ratio $^+$</th>
<th>Steady-state levels under the illumination of 680 μm light (μmoles $^{14}$C/g)</th>
<th>ratio $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total $^{14}$C fixed</td>
<td>9.11</td>
<td>9.66</td>
<td>0.943</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>2. Lipids</td>
<td>1.13</td>
<td>1.19</td>
<td>0.950</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Proteins (as insoluble amino acids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) alanine</td>
<td>0.0625</td>
<td>0.0625</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) glutamic acid</td>
<td>0.055</td>
<td>0.055</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) aspartic acid</td>
<td>0.016</td>
<td>0.015</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) sucrose</td>
<td>**</td>
<td>29.0</td>
<td>0.906</td>
<td>32.0</td>
<td>0.906</td>
</tr>
<tr>
<td>b) UDPG</td>
<td></td>
<td>2.20</td>
<td>0.887</td>
<td>2.48</td>
<td>0.887</td>
</tr>
</tbody>
</table>

$^+$The ratio between compounds formed under 630 μm light illumination and those under 680 μm light illumination.

*After 50 min, the steady-state levels of lipids and proteins had not been established. (In fact, they might not exist.)

**The steady-state levels of sucrose and UDPG were established in a short period. It is therefore impractical to compare their rates of synthesis.
The absence of the effect of 630 m\textmu{} and 680 m\textmu{} light on the photosynthetic product distribution can be accounted for by the following possibilities:

(i) The photoelectron transport scheme consists of only one light reaction, as described in the "photolyt" hypothesis.

(ii) The photoelectron transport scheme still consists of two light reactions. However, the "spillover" mechanism regulates the distribution of absorbed photons, so that there is no excessive photoreaction to generate additional ATP by cyclic photophosphorylation in the algae illuminated by the 680 m\textmu{} light.

(iii) Far red light stimulates no additional cyclic photophosphorylation in vivo.

The strongest support for the "photolyt" hypothesis is the low value of quantum requirement. Arguments against Warburg's low number of quantum requirement are always concerned with his manometric method in measuring oxygen evolution in the presence of carbon dioxide. Measuring the oxygen pressure with an oxygen analyzer which measures the paramagnetism of the oxygen gas, Bassham et al. also found that the quantum requirement was below 8. However, later quantum requirement determinations in this laboratory show that the value of quantum requirement is above 8 and has revealed a possible error in the measurement by Bassham et al. Although the present experimental results seem to be consistent with the "photolyt" hypothesis, quantum requirement measurement and other evidences such as the enhancement effect and chromatic transients proved this hypothesis no longer valid. Therefore, the "photolyt" hypothesis cannot be used to account for the present experimental results.
The experiment was based on the fact that 680 μm light would be preferentially absorbed by photosystem I and used for photoreaction I. Since cyclic photophosphorylation is linked to photoreaction I, additional ATP might be generated by cyclic photophosphorylation. This changes the ratio of ATP/MADPH, which in turn affects the photosynthetic product distribution. This assumption works only if the "separate package" mechanism regulates the distribution of absorbed photons. If the "spillover" mechanism regulates the distribution of photons, there will be no excess of photoreaction I even if 680 μm light is preferentially absorbed by photosystem I. Since there is no observable effect found in the present experiment, it seems that the "spillover" regulates the distribution of absorbed photons. However, this is true only if it can be shown that far red light can promote additional photoreaction I for the generation of ATP by cyclic photophosphorylation. Hence, far red light-stimulated cyclic photophosphorylation is studied in the following chapter.
IV. RED LIGHT-STIMULATED CYCLIC PHOTOPHOSPHORYLATION IN VIVO

Although cyclic photophosphorylation in chloroplasts has been shown to occur in vitro, very little is known about this important process in vivo. Inhibitor studies could provide only some indirect evidence. Furthermore, the use of inhibitors can alter more than one reaction of the living cell, adding ambiguity to the results. Far-red light might provide another way to stimulate cyclic photophosphorylation and since far-red light might affect fewer sites in the living cell, it seemed useful for the study of cyclic photophosphorylation in vivo.

Measurement of light scattering provides an indirect method to measure photophosphorylation. By measuring scattering changes, U. Heber has shown that far-red light hardly stimulates any cyclic photophosphorylation in vivo. However, light scattering changes are attributed to shrinkage or swelling of chloroplasts. This in turn is believed to be due to the hydrogen ion exchange between the chloroplasts and the environment. Since phosphorylation may be related to a change in pH, measurement of scattering change might provide an indirect method for the measurement of phosphorylation. However, the shrinkage or swelling of the chloroplasts, or even the exchange of hydrogen ion with the environment, may be due to some other physiological phenomena, and may not be related to phosphorylation. Thus, the absence of a scattering change may not be an indication of the absence of phosphorylation. Therefore, it is important to investigate the possibility of far-red light-stimulated cyclic photophosphorylation in vivo by direct measurement.
The action spectrum, published by Arnon et al.,\textsuperscript{71} (Figure IV-1) indicated that the quantum yield of cyclic photophosphorylation in chloroplasts increases sharply from 680 mu to 720 mu. Since the action spectrum of \textit{Chlorella} photosynthesis\textsuperscript{3} (Figure IV-2) shows that light of wavelengths longer than 690 mu is unable to promote photosynthesis, it may be possible to induce cyclic photophosphorylation \textit{in vivo} by shining light of wavelengths longer than 690 mu onto the algae which is photosynthesizing under the illumination of light with wavelengths where the quantum yield of cyclic photophosphorylation is low--e.g., 630 mu. Hence, a measurement of the levels of ATP and ADP before and after the introduction of this long wavelength red light could provide the evidence concerning the occurrence of cyclic photophosphorylation \textit{in vivo}.

Although ATP is not stored as the end product of photosynthesis, any additional amount of ATP provided by cyclic photophosphorylation could be measured by the change in photosynthetic product distribution, as well as in the steady-state levels of ATP and ADP.

\textbf{B. Experimental}

The experimental procedures are similar to those of Bassham's inhibitor studies\textsuperscript{81} on the photosynthetic carbon reduction cycle, except that the algae suspension was illuminated only on one side, and instead of added inhibitors, light with wavelengths longer than 690 m was used to illuminate the other side.

Two experiments were done. In the first one, the algae suspension was illuminated on one side by blue light and the other by long wavelength red light. Light was provided by incandescent lamps filtered by
Figure IV-1. Effect of red and far-red monochromatic light on ferredoxin-catalyzed photophosphorylation.
Efficiency of light absorbed by *Chlorella* sp. determined over the range of the visible spectrum. Emerson and Lewis.\(^{23}\)

**Figure IV-2.**
Corning color filters, the transmission spectra of which are shown in Figure IV-3.

The algae was allowed to photosynthesize in the blue light (color filter 4-96) for a period of half an hour after the introduction of $^{32}\text{P}$, so that the pools of ATP, ADP, and PGA are all saturated with $^{32}\text{P}$. $^{14}\text{C}$ was then introduced as $\text{CO}_2$ into the system. After the introduction of $^{14}\text{CO}_2$, the algae were allowed to photosynthesize for 20 min before the addition of long wavelength red light (combination of color filters 4-77 and 2-58) by turning the other lamp on. Samples were taken before and after the red light was turned on.

The second experiment was similar to the first one, with some modifications on the light sources. Instead of using blue light, 630 nm light provided by interference filter sets was employed. Long wavelength red light was provided by the combination of Corning color filters 2-58 and 7-59. The transmission spectra in Figures IV-3C and IV-4B show that such a combination can abolish all light with wavelengths shorter than 690 nm. Since the intensity of the 630 nm light provided by interference filter sets was very low, the algae were allowed to photosynthesize for one and a half hours in $^{32}\text{P}$ before the introduction of $^{14}\text{C}$. After the introduction of $^{14}\text{C}$, the algae were allowed to photosynthesize for 20 min before the long wavelength red light was turned on.

C. Results and Discussion

The $^{32}\text{P}$ incorporation curves of ATP, ADP, UDPG, and PGA in the first experiment are shown in Figure IV-5. It can be seen that upon
Figure IV-3A. Transmission spectrum of the Corning 4-96 color filter.

3B. Transmission spectrum of the Corning 4-77 color filter.

3C. Transmission spectrum of the Corning 2-58 color filter.
Figure IV-4A. Transmission spectrum of the interference filter set (Baird Atomic BX-3).

4B. Transmission spectrum of the Corning 7-59 color filter (When combined with the Corning 2-58 color filter, the transmission band between 280 m and 520 m is cut off, and allows only the long wavelength to pass through.)
Figure IV-5A. $^{32}$P incorporation curves of ATP, UDPG, and ADP.
Figure IV-5B. $^{32}\text{P}$ incorporation curve of PGA.
the addition of red light, the level of ATP and that of ADP dropped slightly and then reached the slightly higher steady state. Compared with the amount of red light added, the rise in ATP and ADP levels is too small to be attributed to the occurrence of cyclic photophosphorylation. However, the fact that all the levels of ATP, ADP, UDPG, and PGA increased to a higher steady-state value prevents us from drawing the conclusion that red light stimulates no cyclic photophosphorylation in vivo during aerobic photosynthesis.

The uncertainties in this experiment probably come from the light sources:

Blue light used: The broad transmission band of the Corning color filter might have provided too much light for photosystem I, so that photoreaction I is saturated. Since cyclic photophosphorylation is linked to photoreaction I, it will not occur if photoreaction I is saturated by overall photoelectron transport.

Red light used: An examination of the transmission spectra in Figure IV-3 shows that a small amount of light with wavelengths from 680 mu to 690 mu can pass through the combination of filters 4-77 and 2-58. This may account for the small increase in the ATP and ADP levels after the red light was turned on, because the levels of ATP and ADP are proportional to the overall rate of photosynthesis.

In the second experiment, instead of using blue light, 630 mu interference filter sets are employed so that the light provided is preferentially absorbed by chlorophyll b, which is the predominant pigment in photosystem II. Furthermore, the light intensity was so low that the
rate of CO₂ uptake was only 4 μmole/min per algae,* which was only one-fourth of that in the first experiment. Hence, the light intensity would not be so high as to saturate system I, and therefore not be too high for cyclic photophosphorylation to be performed by photoreaction I.

The transmission spectra in Figure IV-4 show that no light with wavelengths shorter than 690 mJλ can pass through the combination of the color filters 7-69 and 2-58. Furthermore, experiments had shown that the addition of this red light had no observable effect on the rate of photosynthesis or dark respiration. Therefore, the possibilities of enhancing the levels of ADP and ATP by non-cyclic photophosphorylation could be avoided. Hence, in theory this far-red light is suitable for the stimulation of cyclic photophosphorylation.

The ³²p incorporation curves of ATP, ADP, UDPG, and PGA are shown in Figure IV-6. It can be seen that neither the addition nor the removal of the long wavelength red light had any effect on the levels of these compounds. In fact, the levels of ATP and ADP dropped slightly when the red light was turned on, and rose slightly when the red light was turned off. (This might be due to the fact that the voltage of the lamps serving as the light source for the 630 mJλ light dropped slightly (about 1%) when the lamp for the red light was turned on. However, the differences among those points on the curves were too small to be taken into serious consideration.)

Another possibility for the absence of observable cyclic photophosphorylation stimulated by the long wavelength red light is that the amount

*The saturation rate of Chlorella photosynthesis is about 40 μmole CO₂/min per g algae (see Figure II-2).
Figure IV-6A. $^{32}$p incorporation curves of ATP, UDPG, and ADP.

- $\circ$ = ATP
- $\times$ = UDPG
- $\Delta$ = ADP
Figure IV-6B. $^{32}$P incorporation curve of PGA.
of red light absorbed by the algae is too small, because the absorption spectrum of Chlorella drops sharply for wavelengths longer than 680 μm. Comparison of the measured light intensities of the 630 μm and the long wavelength red light is impractical, because the Corning color filters let through a large amount of infrared light which is unable to promote cyclic photophosphorylation. Therefore, the amount of "useful" long wavelength red light* absorbed by the Chlorella has to be calculated from the transmission spectra of Figure IV-4 and the absorption spectrum of Chlorella in Figure IV-5. In order to make a comparison, the amount of 630 μm light absorbed by the algae has also to be calculated. Together with the relative intensities of the two incandescent lamps, the amount of 630 μm and long wavelength red light absorbed by the Chlorella were thus calculated and tabulated as follows:

**Calculation.** The relative amount of light absorbed by the algae depends on (i) the relative amount of light available to the algae, which in turn depends on (a) the intensities of the incandescent lamp, and (b) the percentage of transmission of the filters; and (ii) the relative amount of absorption by the algae.

(i) **The relative amount of light available to the algae.** During the experiment, the incandescent lamp for the Corning color filters was operated at 115 volts while that for the interference filter set was at 109 volts. Both lamps were at the same distance from the corresponding

*Useful long wavelength red light is defined as the red light with wavelengths from 690 μm to 740 μm, because studies in chloroplasts have shown that 740 μm red light is still able to promote photoreaction I.*

98-100
surfaces of the cell. With the light meter at a fixed distance and location, the light intensities of the incandescent lamp at 115 and 109 volts were found to be 817 FC and 7000 FC, respectively. Hence, the relative intensity of the light for the Corning color filters was taken as 8175 FC and that for the interference filter set, 7000 FC. Actually, a larger value should be assigned to the color filters, because the peak of the emission spectrum of the incandescent lamp is about 800 μm, which is closer to the transmission band of the color filters than that of the interference filters.

The relative amount of transmission of the filters is expressed in area units of μm X percentage of transmission.

Relative amount of transmission by the combination of Corning color filters 7-59 and 2-58 (in area units of % X μm)

<table>
<thead>
<tr>
<th>Wavelength (μm)</th>
<th>Color Filter 7-59</th>
<th>Area units</th>
</tr>
</thead>
<tbody>
<tr>
<td>740</td>
<td>80</td>
<td>10 μm X (80 + 75)%/2 = 775</td>
</tr>
<tr>
<td>730</td>
<td>75</td>
<td>10 μm X (75 + 65)%/2 = 700</td>
</tr>
<tr>
<td>720</td>
<td>65</td>
<td>10 μm X (65 + 50)%/2 = 575</td>
</tr>
<tr>
<td>710</td>
<td>50</td>
<td>10 μm X (50 + 30)%/2 = 400</td>
</tr>
<tr>
<td>700</td>
<td>30</td>
<td>10 μm X (30 + 0)%/2 = 150</td>
</tr>
<tr>
<td>690</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Total 2600 area units

Since the percentage of transmission of the color filter 2-58 is 90% for wavelengths from 680 μm to 800 μm, the relative amount of transmission by the combination of Corning color filters 7-59 and 2-58 is 2600 X 90% = 2340 area units.
Relative amount of transmission by the Baird-Atomic interference filter set* (BX-2 and BX-3 combined)

<table>
<thead>
<tr>
<th>Wavelength (mu)</th>
<th>% of transmission</th>
<th>Area units</th>
</tr>
</thead>
<tbody>
<tr>
<td>620</td>
<td>22.5</td>
<td>$10\text{ mu} \times (22.5 + 22.5)/2 = 225$</td>
</tr>
<tr>
<td>630</td>
<td>22.5</td>
<td>$10\text{ mu} \times (22.5 + 22.5)/2 = 225$</td>
</tr>
<tr>
<td>640</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 450 area units</td>
</tr>
</tbody>
</table>

(ii) The relative amount of absorption by Chlorella. The relative amount of absorption by Chlorella was calculated from the absorption spectrum shown in Figure IV-7. However, according to Figure IV-8, the optical density of spinach chloroplasts is zero for wavelengths longer than 750 mu. The optical density for wavelengths longer than 750 mu in the absorption spectrum of Figure IV-7 is probably due to light scattering rather than absorption. Therefore, a value of 0.27, which is the optical density of Chlorella at 750 mu, was subtracted from all measured optical densities.

*By replacing the two small triangular area units from 610 mu to 620 mu and from 640 mu to 650 mu with similar ones from 620 mu to 630 mu and from 630 mu to 640 mu, the transmission band of the interference filter set is assumed to have a rectangular shape. This facilitates the calculation.
Figure IV-7. Absorption spectrum of Chlorella.\textsuperscript{98}
(There are no pages 136, 137, and 138.)
Relative amount of light absorbed.

The relative amount of light absorbed by the Chlorella is calculated by the following formula:

\[
\text{Relative amount of light absorbed} = (\text{Intensity of the incandescent lamp}) \times (\text{Area units of transmission}) \times (\text{Area units of absorption}).
\]

Since the percentage of transmission of the Corning color filter 2-58 is 90% from 680 µm to 800 µm, the intensity of the incandescent lamp for the color filter is taken as 8175 FC X 90% = 7358 FC.
Table 1.

<table>
<thead>
<tr>
<th>Wavelength (mu)</th>
<th>Relative amount of light absorbed by the algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>620</td>
<td>$7000 \text{ FC } \times 225 \times 4.75 = 7.48125 \times 10^6$</td>
</tr>
<tr>
<td>630</td>
<td>$7000 \text{ FC } \times 225 \times 4.75 = 7.48125 \times 10^6$</td>
</tr>
<tr>
<td>640</td>
<td>$7000 \text{ FC } \times 225 \times 4.75 = 7.48125 \times 10^6$</td>
</tr>
</tbody>
</table>

$14.96250 \times 10^6$

(630 mu light)

<table>
<thead>
<tr>
<th>Wavelength (mu)</th>
<th>Relative amount of light absorbed by the algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>690</td>
<td>$7358 \text{ FC } \times 150 \times 5.47 = 5.92687 \times 10^6$</td>
</tr>
<tr>
<td>700</td>
<td>$7358 \text{ FC } \times 400 \times 0.26 = 7.65232 \times 10^6$</td>
</tr>
<tr>
<td>710</td>
<td>$7358 \text{ FC } \times 575 \times 0.13 = 5.50011 \times 10^6$</td>
</tr>
<tr>
<td>720</td>
<td>$7358 \text{ FC } \times 700 \times 0.078 = 4.01746 \times 10^6$</td>
</tr>
<tr>
<td>730</td>
<td>$7358 \text{ FC } \times 775 \times 0.045 = 2.5661 \times 10^6$</td>
</tr>
</tbody>
</table>

$25.6629 \times 10^6$

(long wavelength red light)

Although the results of the above calculation may not be very accurate, it shows that the amount of long wavelength red light absorbed by the algae is greater than that of the 630 mu light. It therefore removes the doubt that the amount of long wavelength red light absorbed by the Chlorella is too small to promote an observable amount of photoreaction I for cyclic photophosphorylation.

Hence, the result of the present experiment is consistent with U. Heber's observation that long wavelength red light stimulates no observable cyclic photophosphorylation in vivo during aerobic
photosynthesis. This also supports the opinions of Izawa and Good that during photosynthesis the ATP requirement can be fulfilled by non-cyclic photophosphorylation alone. Since the ratio of ATP/NADPH is greater than one for the overall process of photosynthesis, the stoichiometric relationship in non-cyclic photophosphorylation has to be more than one ATP for each NADPH produced.
V. SUMMARY

The "photolyt" hypothesis and the Hill-Bendall scheme are the two controversial hypotheses on photoelectron transport. These two hypotheses were discussed, and two sets of experiments were designed to test their validity. They are (a) the quantum requirement of photosynthesis and (b) the effect of light quality on the photosynthetic product distribution.

The measurement of quantum requirement consists of (a) measurement of oxygen evolution and (b) measurement of energy absorption. Most criticisms on Harburg's low value of quantum requirement are his interpretation of manometric data. However, measuring oxygen evolution with an oxygen analyzer, Bassham et al. also obtained values of quantum requirement below 8. After correction for respiration, the value is about 4, which also favors Harburg's "photolyt" hypothesis on photoelectron transport, which consists of only one light reaction. Without correction for respiration, the values of some of the measurements are still below 8. This led Bassham to propose that in the Hill-Bendall scheme, one of the two light reactions promotes two electrons upon the absorption of one photon.

Using the oxygen analyzer and a new method to calibrate the photocell, the quantum requirement was determined with reliable accuracy. Furthermore, a possible error in Bassham's experiment was revealed. The result of the measurement favors the Hill-Bendall scheme of photoelectron transport.

The basic assumptions in the study of the effect of light quality on the photosynthetic product distribution are (a) the photoelectron
transport scheme consists of two photoreactions as described by the Hill-Bendall scheme, (b) far-red light can stimulate cyclic photophosphorylation in vivo, and (c) the 'separate package' mechanism regulates the distribution of absorbed photons. The result of the present study indicates that light with different wavelengths has no significant effect on the photosynthetic product distribution.

The absence of any significant effect of light quality on the photosynthetic product distribution raised questions on the validity of the above assumptions. The present quantum requirement measurement and other studies on photosynthesis have shown that the photoelectron transport scheme consists of two light reactions. Far-red light-stimulated cyclic photophosphorylation has been studied and reported in chloroplasts by Arnon. The validity of the 'separate package' mechanism in regulating the distribution of absorbed photons can be tested, if it can be shown that far-red light-stimulated cyclic photophosphorylation also exists in vivo.

Experiments on far-red light-stimulated cyclic photophosphorylation were carried out. The wavelengths of the far-red light used were too long to promote photoreaction II. Thus it can only be used to promote photoreaction I for cyclic photophosphorylation. In contrast to the result of such studies on chloroplasts, we found no far-red light-stimulated cyclic photophosphorylation in vivo.

Although the absence of far-red light-stimulated cyclic photophosphorylation prevents us from drawing any conclusion on the mechanism of quantum distribution, it accounts for the absence of any significant effect of light with different wavelengths on the distribution of
photosynthetic products. Furthermore, it shows that results from the
in vitro study may be different from those in vivo.
REFERENCES


APPENDIX I

An Alternate Explanation for the Acid Fluoride Experiment

Instead of postulating the existence of "photolyt", the acid fluoride experiment can be explained by the following reactions:

\[
\begin{align*}
\text{O} & \text{C} - \text{CH}_2 - \text{CH}_2 - \text{C} = \text{C} \text{H} \text{OH} \xrightarrow{\text{F}^-, \text{enzyme}} \text{O} & \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_3 + \text{CO}_2 \\
\text{O} & \text{C} - \text{CH}_2 - \text{C} = \text{O} \text{H} \xrightarrow{\text{F}^-, \text{enzyme}} \text{O} & \text{C} - \text{CH}_2 - \text{C} - \text{NH}_3 + \text{CO}_2
\end{align*}
\]

\[
\text{transamination}
\]

\[
\text{O} & \text{C} - \text{CH}_2 - \text{CH}_2 - \text{C} = \text{C} \text{H} \text{OH} \xrightarrow{\text{Light} [\text{H}]} \text{O} & \text{C} - \text{CH}_2 - \text{C} - \text{C} = \text{C} \text{H} \text{OH}
\]

Assuming the fluoride activated enzyme catalyzes not only the decarboxylation of glutamic acid but also aspartic acid, the results of the acid fluoride experiment can be explained as follows:

The CO₂ evolved from vessel II consists of CO₂ released from the following sources:

(i) CO₂ bound as bicarbonate
(ii) glutamic acid
(iii) aspartic acid

(Aspartic acid is in equilibrium with oxaloacetic acid, which can be formed by the oxidation of malic acid during respiration.)

The CO₂ evolved from vessel I is less than that evolved from vessel II by an amount equal to the CO₂ released from aspartic acid. The
aspartic acid is in equilibrium with the oxaloacetic acid, which is reduced to malic acid in photosynthesis. The fluoride activated enzyme is unable to catalyze the decarboxylation of malic acid. Therefore, less CO$_2$ will be evolved from the algae if it is exposed to light before the addition of sodium fluoride.

The CO$_2$ evolved in vessel III is released from glutamic acid and the CO$_2$ bound as bicarbonate, while that evolved from vessel IV is released only from glutamic acid.
APPENDIX II

Function of Translucent Plate and Reason to Employ 630 μm Light

A. The Function of the Translucent Plate

The function of the translucent plate is to diffuse the light so that the algae suspension is illuminated by a more uniform light beam. Local saturation is therefore greatly reduced.

The transmission band of the interference filter should have a certain band width, so as to provide enough energy to the algae to give an observable rate of oxygen evolution. In the present case, the transmission band of the interference is 627 μm ± 8 μm. Since the quantum yield of Chlorella photosynthesis is at its maximum value for illuminating light with wavelength from 570 μm to 635 μm,23 the light provided by the present set-up will be optimal for quantum requirement study. If the interference filter is made with transmission band of 680 μm, some of the light passing through will have wavelengths longer than 680 μm. Since the quantum yield of Chlorella photosynthesis drops severely for wavelengths longer than 680 μm, the use of interference filter with a transmission band at 680 μm will give a high quantum requirement.

Furthermore, Chlorella pyrenoidosa has an absorption peak at 680 μm; therefore, the intensity gradient will be more pronounced if 680 μm light is used.

B. Significance of the \((n+1)/n\) Ratio

N is the number of IR absorption filters. The \((n+1)/n\) ratio is the signal ratio of the thermopile (or the photocell) with the corresponding number of IR absorption filters in front of its detecting surface. Since the IR absorption filters are not neutral density filters but absorb more infrared than visible light (transmission spectrum
shown in Figure II-3C), a constancy of the (n+1)/n ratio indicates that the light passing through has a narrow transmission band in the visible region and contains practically no infrared, so that the IR absorption filters act as if they are neutral density filters.

C. The Oxygen Volume of the System

The advantage of determining the oxygen volume of the system by using the oxygen analyzer is that it eliminates any error that may exist in calibrating the sensitivity of the oxygen analyzer. The reason is that the amount of oxygen evolved during photosynthesis is obtained by multiplying the volume of the system by the sensitivity of the oxygen analyzer. This can be seen from the following example:

Apparent sensitivity of the oxygen analyzer 2%/100 div. = 0.02%/div.

Real sensitivity of the oxygen analyzer 1.5%/100 div. = 0.015%/div.

Upon the determination of the oxygen volume of the system, the replacement of 5.0 cc of air by 5.0 cc of nitrogen results in a change of 50 divisions.

Apparent volume of the system \( \frac{5.0 \times 20.95}{0.02 \times 50} = 20.95/0.2 \)

True volume of the system \( \frac{5.0 \times 20.95}{0.015 \times 50} = 20.95/0.15 \)

During photosynthesis, the rate of oxygen evolution is found to be 2 div./min.

Apparent rate of oxygen evolution \( 2 \times \frac{1/100}{100} \times \frac{2/100}{100} \times \frac{20.95}{0.20} \)

Real rate of oxygen evolution \( 2 \times \frac{1/100}{100} \times \frac{1.5/100}{100} \times \frac{20.95}{0.15} \)

Apparent rate of oxygen evolution = Real rate of oxygen evolution = 41.90 \times 10^{-3} \text{ cc.}
D. Extraction of Chlorophylls from Chlorella

After the Chlorella was centrifuged down to a known volume in the centrifuge test tube, the supernatant was removed from the algae by a dropper. It was then transferred to the mortar by cracking the test tube, so that the least amount of water got into the mortar. (Water in the mortar makes it difficult to work.) The algae (with the glass) was ground for several minutes. Liquid nitrogen was put into the mortar to freeze the algae. After all nitrogen was evaporated, the algae was ground again for several minutes. This procedure was repeated three times to insure complete breakage. Eighty percent acetone was added to the mortar to extract the chlorophyll. The extract was centrifuged and the supernatant should contain most of the chlorophyll. The precipitate was washed once with 80% acetone and the wash was combined with the extract. The amount of chlorophylls (chlorophyll a and b) in solution can be calculated from Mackinney's formula.

\[
\text{mg chlorophyll/liter} = OD_{665 \, \text{m}u} \times 6.45 + OD_{649 \, \text{m}u} \times 17.72,
\]

where \(OD_{665}\) and \(OD_{649}\) are the optical densities at 665 \(\text{m}u\) and 649 \(\text{m}u\), respectively.

Experience shows that for 1 \(\sigma\) of wet packed algae, the extract should be diluted to a volume of 250.0 ml to get a convenient measurement by the spectrophotometer.
The Cofactor Requirement for the Synthesis of Carbohydrates, Fatty Acids, Amino Acids, and Proteins\textsuperscript{102,103}

A. The Cofactor Requirement for the Synthesis of Carbohydrates

Starting from PGAL (phosphoglyceraldehyde), the cofactor requirement for the synthesis of carbohydrates can be shown by the following pathways:

Pathway leading to the synthesis of carbohydrates

\[ \text{HOH}_2\text{C-C-CH}_2\text{PO}_3^= \xrightleftharpoons{\text{H}_2\text{O}} \text{HOCH}_2\text{C-C-CH}_2\text{OH} \]

\[ \xrightarrow{\text{fructoaldolase}} \text{HOCH}_2\text{C-C-CH}_2\text{OH} \]

\[ \xrightarrow{\text{fructose-1,6-diphosphatase}} \text{HOCH}_2\text{C-C-CH}_2\text{OH} \]

\[ \xrightarrow{\text{phosphoglucoisomerase}} \text{HO}_{\text{C}}\text{C-C-CH}_2\text{OH} \]
This pathway shows that only one ATP is required for the synthesis of one UDPG from PGAL and UDP. Hence, the cofactor requirement for the synthesis of one UDPG is $2 \times (9 \text{ATP} + 6 \text{NADPH}) + \text{ATP} = 19 \text{ATP} + 12 \text{NADPH}$.

Starting from UDPG, the synthesis of starch and sucrose involves no net consumption of any cofactors. The cofactor requirement for incorporation of one CO$_2$ into carbohydrates can therefore be regarded as equal to that into UDPG. Thus, the ratio of ATP/NADPH for the incorporation of one CO$_2$ into sucrose and UDPG is $19/12 = 1.58$.

**B. The Cofactor Requirement for the Synthesis of Fatty Acids**

Starting from malonyl-CoA the biosynthesis of a given fatty acid can be shown in the following equations: 101, 102

\[
\begin{align*}
\text{CH}_3\text{-C-SCoA} + \text{HS-ACP} & \rightleftharpoons \text{CH}_3\text{-C-S-ACP} + \text{CoASH} & \text{(1)} \\
\text{HO}_2\text{C} & \text{HO}_2\text{C} \\
\text{CH}_2\text{-C-SCoA} + \text{HS-ACP} & \rightleftharpoons \text{CH}_2\text{-C-S-ACP} + \text{CoASH} & \text{(2)} \\
\text{CH}_3\text{-C-S-ACP} + \text{CH}_2\text{-C-S-ACP} & \rightleftharpoons \text{CH}_3\text{-C-CH}_2\text{-C-S-ACP} + \text{HS-ACP} + \text{CO}_2 & \text{(3)} \\
\text{CH}_3\text{-C-CH}_2\text{-C-S-ACP} + \text{NADPH} + \text{H}^+ & \rightleftharpoons \text{CH}_3\text{-C-CH}_2\text{-C-S-ACP} + \text{NADP}^+ \text{ (D-isomer)} & \text{(4)} \\
\text{CH}_3\text{-CH=CH-C-S-ACP} & \rightleftharpoons \text{CH}_3\text{-CH=CH-C-S-ACP} + \text{H}_2\text{O} & \text{(5)} \\
\text{CH}_3\text{-CH=CH-C-S-ACP} + \text{NADPH} + \text{H}^+ & \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{-C-S-ACP} + \text{NADP}^+ & \text{(6)} \\
\end{align*}
\]
where ACP represents acyl carrier protein, and CoASH refers to coenzyme A.

Starting from PGAL, the synthesis of malonyl-CoA can be represented by the following equations:

\[
CH_3CH_2CH_2-S-ACP + HS-CoA \rightleftharpoons CH_3CH_2CH_2-S-CoA + HS-ACP \quad (7)
\]

\[
H_2O \quad CH_2-C-S-CoA + CH_3-C-S-CoA + 2NADPH + 2H^+ \rightarrow CH_3CH_2CH_2-C-S-CoA + CO_2 + CoASH + 2NADP^+ + H_2O \quad (8)
\]
La$_{SH}^S$ is regenerated by FAD as shown by the following reactions:

$$\text{La}_{SH}^S + \text{FAD} \rightarrow \text{FADH}_2 + \text{La}_{SH}^S$$

$$\text{FADH}_2 + \text{NAD}^+ \rightarrow \text{FAD} + \text{NADH} + \text{H}^+$$

and CO$_2$-biotin is generated from the biotin enzyme complex as follows:

$$\text{CO}_2 + \text{ATP} + \text{biotin-enz} \rightarrow \text{CO}_2\text{-biotin-enz} + \text{ADP} + \text{HOPO}_3^\text{=}$$

where $\text{La}_{SH}^S$ and $\text{La}_{SH}^I$ are the oxidized and reduced form of lipoic acid, E and enz are the enzymes that catalyze the corresponding reactions, and Th-PP is thiamine pyrophosphate.

Starting from PGAL, the overall cofactor production in the synthesis of malonyl-CoA can be calculated from the following equation, which is the sum of all above equations:

$$\text{O}_3\text{C}-\text{C}-\text{CH}_2\text{-OPO}_3^= + \text{ADP} + 2\text{NAD}^+ + \text{H}^+ \rightarrow \text{O}_3\text{C}\text{-CH}_2\text{-C}-\text{S-CoA} + \text{ATP} + 2\text{NADH} + 2\text{H}^+$$

Since the synthesis of one molecule of PGAL requires 9 molecules of ATP and 6 molecules of NADPH, the cofactor requirement for the synthesis of one molecule of malonyl-CoA via PGAL will be $(9-1) = 8$ ATP and $(6-2) = 4$ NADPH.

The incorporation of one -(CH$_2$-CH$_2$)- unit into the chain of a given fatty acid via malonyl-CoA requires $2(\text{NADPH} + \text{H}^+)$.

Starting from CO$_2$, the cofactor requirement for the incorporation of one -CH$_2$- unit into the chain of fatty acid can be calculated from the following table:
Cofactor requirement for the synthesis of PGAL and malonyl-CoA:

<table>
<thead>
<tr>
<th>Cofactor Requirement</th>
<th>ATP</th>
<th>NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGAL</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>malonyl-CoA</td>
<td>9-1=8</td>
<td>6-2=4</td>
</tr>
<tr>
<td>-CH₂-CH₂- in the chain of fatty acid</td>
<td>8</td>
<td>6+2=8</td>
</tr>
</tbody>
</table>

Hence, the cofactor requirement for the incorporation of molecule of CO₂ as -CH₂- into the chain of a given fatty acid is 8/2=4 ATP and 6/2=3 (NADPH + H⁺). And the ratio of ATP/NADPH for the incorporation of one CO₂ into the fatty acid chain is 4/3=1.33.

C. The Cofactor Requirement for the Synthesis of Amino Acids and Proteins

Since the algae were grown in a medium containing KNO₃, NO₃⁻ is the nitrogen source for amino acid synthesis. The reduction of nitrogen from the level of NO₃⁻ to that of NH₃ can be represented by the following diagram:

```
NADH or NADPH
  /  \
 /    \       /      \       /  \       /  \       /  \
\ NO₃⁻ ——> NO₂⁻ ——> N₂O₂⁻ ——> NH₂OH ——> NH₃
```

Assuming no ATP is required in any of the reduction steps, the cofactor requirement for the reduction of NO₃⁻ to NH₃ will be 4 NADPH. As shown in the following equation, the incorporation of an amino group into an α-keto acid to form an amino acid requires one NADH (or NADPH) as exemplified by the following equation:
Hence, with \( \text{NO}_3^- \) as the nitrogen source, the incorporation of one amino group into an \( \alpha \)-keto acid requires 5 molecules of NADPH. The cofactor requirement for the synthesis of amino acids can therefore be obtained by adding 5 NADPH to that required for the synthesis of the corresponding \( \alpha \)-keto acids. Based on biosynthetic pathways such as the photosynthetic carbon reduction cycle and the citric acid cycle, the cofactor requirement for the synthesis of several \( \alpha \)-keto acids has been calculated and shown in the following table:

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Cycle</th>
<th>ATP</th>
<th>NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGAL</td>
<td>photosynthetic carbon reduction cycle</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>pyruvate</td>
<td></td>
<td>( 9 \times 2 = 7 )</td>
<td>( 6 \times 1 = 5 )</td>
</tr>
<tr>
<td>oxaloacetate</td>
<td></td>
<td>( 7^+1 = 8 )</td>
<td>5</td>
</tr>
<tr>
<td>acetyl-CoA</td>
<td></td>
<td>7</td>
<td>5(^*1 = 4 )</td>
</tr>
<tr>
<td>oxaloacetate</td>
<td></td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>citrate</td>
<td>citric acid cycle</td>
<td>( 7^+8 = 15 )</td>
<td>( 4^+5 = 9 )</td>
</tr>
<tr>
<td>( \alpha )-keto glutamate</td>
<td></td>
<td>15</td>
<td>9(^*1 = 8 )</td>
</tr>
</tbody>
</table>

*the minus sign indicates the production of cofactors in the course of biosynthesis.

\(^+\)the positive sign indicates the production of cofactors in the course of biosynthesis.
Together with the 5 molecules of NADPH required to convert an α-keto acid into the corresponding amino acid, the cofactor requirement for the synthesis of alanine, aspartic, and glutamic acids are shown in the following table:

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>ATP</th>
<th>NADPH</th>
<th>Ratio of ATP/NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>7</td>
<td>5+5=10</td>
<td>0.7</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>8</td>
<td>5+5=10</td>
<td>0.8</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>15</td>
<td>8+5=13</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Proteins are polypeptides in which various amino acids are joined together by peptide bonds. One of the steps in the synthesis of proteins is the activation of the amino acids. This step requires one ATP for each amino acid activated. Hence, the cofactor requirement for the incorporation of one molecule of CO₂ into a given protein molecule can be calculated. The following table lists the cofactor requirement for the synthesis of some amino acids in protein molecules:

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>ATP</th>
<th>NADPH</th>
<th>Ratio of ATP/NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>7+1=8</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>8+1=9</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>15+1=16</td>
<td>13</td>
<td>1.23</td>
</tr>
</tbody>
</table>

From the above calculations, it is apparent that, starting from CO₂, the ratio of ATP/NADPH in the synthesis of protein molecules is about 1, when nitrate is the source of nitrogen for biosynthesis.
APPENDIX IV

The "Separate Package" and the "Spillover" Hypothesis on the Mechanism of Quantum Distribution

There are two hypotheses concerning the distribution of absorbed photons between photosystem I and photosystem II. These are the "separate package" and the "spillover" hypotheses. According to the "separate package" hypothesis, the photons absorbed by pigment system I are transferred to the reaction center for photoreaction I, and those absorbed by pigment system II are transferred to the reaction center for photoreaction II. There is no transfer of absorbed photons between the two photosystems. The photons available for photoreaction I and II will depend on the quality of light as well as on the amount of pigments in each system of the plant. Light preferentially absorbed by pigment system I will promote more photoreaction I than photoreaction II. If this "excess" photoreaction I is used for cyclic photophosphorylation, light with different wavelengths will promote different amounts of cyclic photophosphorylation.

According to the "spillover" hypothesis, the photons absorbed by one pigment system can be transferred to the other if the reaction center of that system is saturated. Hence, the absorbed photons are optimally distributed between photosystems I and II to give maximum efficiency of photosynthesis. Light preferentially absorbed by a certain pigment system will not be used to promote the corresponding photoreaction in excess; instead, a portion of it may be transferred to the other photosystem to balance the two photoreactions, if the wavelength of the light is short enough to promote both photoreactions.
The validity of either mechanism can be examined by the action spectrum of photosynthesis. If the "separate package" mechanism regulated the distribution of absorbed photons, the quantum efficiency of photosynthesis will have a maximum at a certain wavelength, where appropriate amount of light is absorbed by pigment systems I and II and used for these two photoreactions. On the other hand, if the "spillover" mechanism regulates the distribution of absorbed photons, the quantum efficiency should be independent of the wavelengths of the light, as absorbed photons can be transferred from one photosystem to the other.

The action spectrum of photosynthesis as determined by Emerson et al., shows that neither of these two hypotheses are perfect. The actual mechanism of quantum distribution is probably similar to the "spillover" mechanism, with the exception that the transfer of photons from one photosystem to the other is not so efficient as within itself.
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