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Publication Date
1962-09-01
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PHOTOSYNTHESE: ENERGETICS AND RELATED TOPICS

J. A. Bassham

September 1962
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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>TPN⁺</td>
<td>Triphosphopyridine nucleotide (oxidized)</td>
</tr>
<tr>
<td>TPNH</td>
<td>&quot; (reduced)</td>
</tr>
<tr>
<td>PGA₁⁻²</td>
<td>3-Phosphoglyceraldehyde</td>
</tr>
<tr>
<td>RuDP</td>
<td>Ribulose diphosphate</td>
</tr>
<tr>
<td>PGA</td>
<td>3-Phosphoglyceric acid</td>
</tr>
<tr>
<td>DHAP</td>
<td>Dihydroxyacetone phosphate</td>
</tr>
<tr>
<td>PEPA</td>
<td>Phosphoenolpyruvic acid</td>
</tr>
<tr>
<td>G6P</td>
<td>Glucose-6-phosphate</td>
</tr>
<tr>
<td>F6P</td>
<td>Fructose-6-phosphate</td>
</tr>
<tr>
<td>FDP</td>
<td>Fructose diphosphate</td>
</tr>
<tr>
<td>3-PGA-P⁺⁻⁴</td>
<td>3-Phosphoglyceryl phosphate</td>
</tr>
<tr>
<td>DHA</td>
<td>Dihydroxyacetone</td>
</tr>
<tr>
<td>DCMU</td>
<td>Dichloromethylurea</td>
</tr>
<tr>
<td>CMU</td>
<td>Chloromethylurea</td>
</tr>
</tbody>
</table>
plants is the transfer of electrons from the oxygen of water to the elements carbon, nitrogen, and sulfur. These electrons permit the formation of new bonds amongst these elements in place of the oxide bonds. The atoms of molecular oxygen each have an orbital filled with only one electron, and molecular/reactive substance. The separation of electrons from oxygen bonded to hydrogen in water to make molecular oxygen and the transfer of these electrons to the other elements comprising organic compounds requires a large input of energy. This energy comes from the conversion of electromagnetic energy absorbed by the pigments in the chloroplast.

Energy capture, conversion, transfer, and utilization is clearly one of the principal features of photosynthesis. Hopefully, a discussion of the energetics of photosynthesis may provide some insight to the mechanism of the process itself. It is doubtless too much to expect that energetics alone can tell us a mechanism when it is not already partially elucidated. Any calculation of the energetics of a specific chemical step requires some prior knowledge or assumptions as to the nature of the step. A consideration of energetics can tell us when a proposed mechanism is impossible. Moreover, it may sometimes help us to decide which of several possible mechanisms is more plausible. Once the mechanisms of various parts of the photosynthetic process are known, a study of the energetics of the steps may help in understanding more clearly the way in which all living systems handle the transfer of energy. In the present review, discussion of energetics will serve as a unifying theme and as an aid in the selection of material to illustrate
the most recent advances in the understanding of this complex and difficult process, photosynthesis.

A second theme will be structure. A principal thesis will be the essentiality of structure and organization to the successful and efficient manipulation of energy and matter by photosynthetic plants.

Critical selection of material is essential for a review article on photosynthesis, for the quantity and diversity of published material has increased almost exponentially in recent years, and nothing less than several volumes could cope with the many important and recent contributions in this field. Fortunately, an excellent review of the enzymic aspects of photosynthesis by Vishniac, Borecker, and Ochoa has already appeared. A number of general reviews on photosynthesis are available. The proceedings of several meetings dealing with many aspects of photosynthesis have been published. Many important articles on photosynthesis are to be found in an encyclopedia of plant physiology. I shall not attempt in this review to include all recent or important papers on photosynthesis. Instead, I will select a sufficient number of published reports to illustrate what I consider to be the important aspects of the subject. Much of the literature on bacterial photosynthesis will be neglected, since the most efficient utilization of light energy is accomplished by green plants.

It was just stated that photosynthesis in green plants uses the energy of absorbed electromagnetic radiation to bring about the transfer of electrons from the oxygen of water to carbon dioxide and other oxides, thus forming organic compounds and oxygen gas. An older theory of photosynthesis in green plants proposed that light energy split carbon dioxide
into elemental carbon and oxygen gas, with the carbon subsequently combining with water. This theory was displaced when van Niel\textsuperscript{288,289,290} on the basis of his studies of photosynthetic bacteria concluded that the mechanism of CO\textsubscript{2} fixation was similar in photosynthetic bacteria and
in photosynthetic plants, and in both cases was separate from the energy-converting process. In green plant photosynthesis, both the electrons and the energy for the reduction of CO₂ were seen to come from a light reaction which split water. However, photosynthetic bacteria were pictured as using light energy to remove electrons from some other electron donor. In some cases this other electron donor could be hydrogen sulfide or other sulfur compounds; in other cases an organic compound supplied the electrons.

The proposed hypothesis for splitting water was supported by the discovery of the Hill reaction in which electrons are transferred in the light from water to an added artificial electron acceptor\(^1\)\(^3\). The evolution of oxygen from water was further substantiated by demonstration of the formation of \(^1\)\(^6\)O₂ from H₂\(^1\)\(^8\)O in the presence of HC\(^1\)\(^8\)O\(^3\), and of \(^1\)\(^8\)O₂ from H₂\(^1\)\(^8\)O in the presence of HC\(^1\)\(^3\)O\(^3\) during photosynthesis\(^2\)\(^4\)\(^5\). The mapping of the carbon reduction cycle of photosynthesis by Calvin and coworkers\(^6\),\(^9\),\(^2\)\(^4\),\(^3\)\(^1\),\(^2\)\(^6\) showed the reduction of carbon dioxide to the level of sugar phosphates to proceed via dark enzymic reactions (see review by Vishniac, Morecky and Ochoa, ref. 296) which require energy and electron inputs apparently in the form of ATP and TPNH.

In experiments with isolated chloroplasts, the photoreduction of TPNH to TPNH with simultaneous oxygen evolution was demonstrated by Vishniac and Ochoa\(^2\)\(^7\), Tolmachev\(^2\)\(^9\), and Arnon\(^1\)\(^0\). Formation of ATP in the light, called photosynthetic phosphorylation, was discovered by Frenkel\(^1\)\(^1\)\(^3\) in particles from photosynthetic bacteria and by Arnon, et al.\(^1\)\(^4\) in isolated chloroplasts. These experiments and a great body of later work from many laboratories, some of which will be discussed later, would seem to have
established clearly enough the fact that carbon dioxide reduction is a dark reaction, only indirectly dependent upon earlier light dependent reactions. Nevertheless, there are those who still maintain that a light reaction is directly involved in the conversion of carbon dioxide and that oxygen finds its immediate origin in carbon dioxide\textsuperscript{301}. Since such an hypothesis flies in the face of the vast majority of experimentally known facts about the mechanism of photosynthesis, it will be discussed only briefly (Sec. V). Rather, the burden of proof will be left with its proponents.

The photosynthetic mechanism which will be discussed is essentially a two component and two stage system. One component is a photoelectron transport system which makes ATP, TPNH, and O\textsubscript{2}. The other component is a synthetic system which uses these cofactors to reduce CO\textsubscript{2} to organic compounds.

In the photoelectron transport system, the electrons are forced to flow in the direction of increasing potential energy. The energy for this increase is derived from the conversion of electromagnetic energy. As a result of this flow of electrons, oxygen in water is oxidized and a reactive reducing agent such as TIN\textsubscript{I} is formed. Associated with one or more stages of this electron transport is the formation of adenosine triphosphate from ATP and inorganic phosphate.

The electron transport takes place in a particulate system which is in many respects analogous to the mitochondrial electron transport system. In both cases elementary particles about 100 Å to 150 Å in diameter are involved. In oxidative electron transport it has been named the elementary particle or electron transport particle by Green\textsuperscript{127}, whereas the photosynthetic electron transport particle has been named the quantaosome\textsuperscript{22d}. 
The other component of the photosynthetic mechanism is the system for reducing carbon dioxide and synthesizing organic compounds. In this system the cofactors derived from the photosynthetic electron transport system are utilized to bring about the reduction of carbon dioxide via the basic carbon reduction cycle. Other reactions use additional amounts of cofactors from the photoelectron transport system to reduce nitrate and sulfate and to bring about the conversion of intermediates from the carbon reduction cycle to various end products of photosynthesis. The enzymes catalyzing the synthesis of carbon compounds are soluble and can be rather easily separated from the pigmented structures by the fractionation of the chloroplasts\textsuperscript{263,231}. Trebst, et al.\textsuperscript{233} showed that the chlorophyll-free soluble enzyme extract, by itself able to fix only insignificant amounts of carbon dioxide, was able to fix much larger amounts of carbon dioxide upon the addition of ATP and TPNH. Moreover, when the whole chloroplast system illuminated in the absence of added CO\textsubscript{2} was subsequently added to the enzyme extract, the mixture was able to assimilate a much larger amount of CO\textsubscript{2} in the dark. A very striking demonstration of the separation of the two stages of photosynthesis between the two components was reported by Park and Pon\textsuperscript{231}. Fractionation of whole chloroplasts gave a pigmented and non-pigmented preparation, each of which was almost totally incapable of CO\textsubscript{2} fixation in the light. When these two components were mixed and illuminated, considerable fixation of carbon dioxide took place, resulting in the formation of a great many of the usual products of photosynthetic carbon reduction.

The past five years have witnessed a number of important experimental trends and alterations of theory in the mechanism of the primary light reactions and electron transport leading to cofactor formation. The
carbon reduction pathways have remained fairly unchanged during this time. However, there are continuing reports by Kandler and Gibbs159, Fewson, et al.107, Boichenko45, and a review by Stiller267 and others suggesting that either the carbon reduction cycle developed by Calvin and coworkers is incorrect or that some modifications in that cycle are required. With the increasing evidence from other biological systems for the existence of multifunctional enzymes203, and with the development of more detailed knowledge of the reactions of the carbon reduction cycle, the near future may witness the discovery of organized enzyme systems for carbon reduction which may reconcile some of these criticisms with most of the features of the Calvin cycle.

The important (and sometimes revolutionary) recent developments in the understanding of the photoelectron transport system include the following: the application of greatly improved electron microscopy to studies of the particulate apparatus; the further developments and verification of the two light wavelengths effects; the studies of the kinetics of changes of absorption spectrum of a number of pigments in a variety of organisms and the correlation of these changes with other physical and chemical properties of the photoelectron transport system; the studies of the kinetics of appearance and disappearance of electron spin resonance phenomena and association of these phenomena with other properties of the system; the implication in electron transport and oxygen evolution of a number of substances including manganese, plastoquinone, heme proteins, and non heme iron proteins; the evidence from absorption spectrum shifts, polarized fluorescence and electric dichroism for an array of oriented
pigment molecules and the proposals for semi solid state phenomena associated with these arrays of pigment molecules. An attempt will be made here to assess the implications of each of these developments to the capture, conversion and transfer of energy in photosynthesis.

II. OVERALL ENERGETICS OF PHOTOSYNTHESIS

A. Light energy

Before proceeding into the forest of results and theories that make up the literature of photosynthesis, it is appropriate to review the basic energetics of the overall process. All visible light from 400 μm to about 700 μm is to some extent effective in producing photosynthesis in green plants10h,27h. In the case of photosynthetic bacteria, light with wavelengths as long as 950 μm brings about photosynthesis. In both types of photosynthetic organisms, the effectiveness of the light falls off rapidly near the longest wavelengths.

Studies of the in vivo fluorescence spectra and of the absorption spectra90,91,112,302 showed that fluorescence in most plants is emitted almost entirely by the pigment having the longest wavelength absorption peak. This is chlorophyll a in the case of green plants and bacterial chlorophyll in the case of photosynthetic bacteria. Duyvans' studies91 seem to indicate that this type of energy transfer from one pigment to another is very general. This emission of fluorescence by chlorophyll a and by bacterial chlorophyll taken together with the similarity between the absorption peaks of these two pigments and the action spectra for photosynthesis in green plants and photosynthetic bacteria, strongly suggested that light of whatever wavelength and however absorbed is transferred to chlorophyll a (perhaps a special form of chlorophyll a)
or to bacterial chlorophyll, as the case may be. The energy is not converted to chemical energy until it has first been degraded to the energy level of the excited pigment corresponding to the longest wavelength absorption peak (or more likely, the wavelength of fluorescence).

More recently, it appears that there may be more than one form of chlorophyll a in green plants3,52,110 while a number of complexes of bacterial chlorophyll have been known for some time91. The two light theories, to be discussed later, suggest that two of these forms or one form plus one accessory pigment, are able to work together cooperatively in two different light steps. These developments make somewhat unclear the amount of energy which is available in the form of the excited state of the pigment which actually participates in the energy conversion act. In most cases of photosynthesis in green plants, it would appear that the excited state participating in the energy conversion act corresponds to an absorption peak of from 670 to 700 μm. In bacterial photosynthesis the amount of energy available may be considered as corresponding to about 900 μm.

If we apply the equation $E = hv$ where $h$ is plank's constant and $v$ is the frequency of light, and use suitable factors to convert from ergs to calories, and if we also multiply by Avogadro's number to convert from the energy of an individual photon to that of a mole of photons (an einstein), we find that light at 670 μm, 700 and 900 μm has energies of 42.7 Kcal per einstein, 40.9 Kcal per einstein, and 31.6 Kcal per einstein, respectively.

B. Energy requirements

The ultimate result of photosynthesis is the storage of some of this
energy in the evolution of oxygen and the formation of organic compounds, as, for example, the formation of glucose:

1) \[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \left(\text{CH}_2\text{O}\right) + \text{O}_2 \quad \Delta F = +114 \text{ Kcal} \]

(1/6 glucose),

This and other free energy values given here are based on the table by Burton in the appendix to monographs by Krebs and Kornberg. Solutes are 1 M, aqueous, except H\(^+\) concentration, which is 10\(^{-7}\) M.

The reduction of nitrate and of sulfate during photosynthesis also results in the conversion of electromagnetic and chemical energy.

2) \[ \text{NO}_3^- + \text{H}_2\text{O} + 2 \text{H}^+ \rightarrow \text{NH}_4^+ + 2 \text{O}_2 \quad \Delta F = +83.3 \text{ Kcal} \]

3) \[ \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{HS}^- + 2 \text{O}_2 \quad \Delta F = +189.94 \text{ Kcal} \]

Other carbon compounds besides carbohydrates are formed as direct products of photosynthesis. For example, the formation of glycemic acid

4) \[ 3 \text{CO}_2 + 3 \text{H}_2\text{O} \rightarrow \text{H}_2\text{COH} + 2 1/2 \text{O}_2 \quad \Delta F = +287 \text{ Kcal} \]

may proceed by the loss of phosphate from phosphoglycemic acid, a primary intermediate in the carbon reduction cycle. As an example of amino acid photosynthesis, glycemic acid may be converted to cysteine.

5) \[ \text{H}_2\text{C}-\text{OH} \quad \text{H}_2\text{C}-\text{SH} \]

\[ \text{HC}-\text{OH} + \text{NH}_4^+ + \text{HS}^- \rightarrow \text{HC}-\text{NH}_2 + 2 \text{H}_2\text{O} \quad \Delta F = -18.8 \text{ Kcal} \]

From the foregoing equations, the free energy of the total reaction from inorganic oxides to cysteine and oxygen may be found:

6) \[ 3 \text{CO}_2 + 2 \text{H}_2\text{O} + 3 \text{H}^+ + \text{NO}_3^- + \text{SO}_4^{2-} \rightarrow 6 1/2 \text{O}_2 + \text{HC}-\text{NH}_2 \quad \Delta F = +541 \text{ Kcal} \]

\[ \text{HC}-\text{SH} \]
Several interesting points about the energetics of photosynthesis are illustrated by these equations. First, by far the greater part of the energy required for the synthesis of organic compounds is consumed in the removal of electrons from oxygen atoms of water and the reduction of the oxides to simple organic compounds. Second, once these simple organic compounds have been formed, subsequent reactions leading to other organic molecules may require relatively small amounts of additional energy. However, if further reduction occurs, as in formation of fats from sugar phosphates, more electrons must be removed from the oxygen in water and additional large amounts of energy must be used. Also, of course, the activation of amino acids preparatory to protein synthesis requires additional energy.

The amount of energy per mole of electrons transferred from water to carbon in the formation of carbohydrates and other compounds with about the same level of reduction, is from 25 to 30 Kcal (1.1 - 1.3 volts). For more reduced compounds, such as fats, the energy required per mole of electrons transferred is less—about 20 to 25 Kcal (0.9 - 1.1 volts).

C. Quantum requirement of photosynthesis

1. Possible thermodynamic limitations to efficiency.

From the foregoing sections, one might be inclined to calculate the theoretical minimum quantum requirement for the synthesis of carbon dioxide with red light as $11h/42 = 2.7$ quanta per carbon dioxide molecule reduced and oxygen evolved. However, it is to be expected that the many dark reactions of photosynthesis each proceeding at a rapid rate must each be accompanied by some expenditure of negative free energy change and that this free energy which is converted to heat must be added to the total.
energy bill, making the actual quantum requirement higher than 2.7. It will be seen subsequently that the energy efficiency of the carbon reduction cycle may be quite high, perhaps as much as 85% efficient. The energy efficiency of the photoelectron transport system cannot be calculated as yet with any certainty, owing to our lack of knowledge of the detailed mechanism of the system. Duysens\textsuperscript{57,95} has calculated that the maximum efficiency of photosynthesis can be about 70%. His thermodynamic argument is based upon radiation from the inside surface of a hollow, spherical black body through a filter which passes only a narrow band of wavelength between 660 and 680 \textmu{}m to a green plant at the center of the hollow sphere. Making an assumption of an incident light intensity of 1000 ergs per cm\textsuperscript{2}/sec., he determined that the temperature of the black body should be about 1100° K. He applied this temperature $T_1$ and room temperature of the photosynthesizing plant $T_0$, taken as 300° K in the Carnot cycle equation efficiency $= \frac{T_1 - T_0}{T_1}$, thereby arriving at his 73% efficiency. This calculation has met with some skepticism on the part of workers in this field, since many have been inclined to assume that light energy is a very "high grade" form of energy, capable of being utilized in the primary reactions of photosynthesis with nearly 100% efficiency. Moreover, Duysens seemed to be applying the principles of reversible thermodynamics to an irreversible process. Kahn\textsuperscript{153} has criticized Duysens' argument, and Duysens has answered this criticism\textsuperscript{96}. Critics and answers are: 1. Duysens ascribed a temperature to a beam of photons. Duysens disclaims this, but see formula in next paragraph. 2. Kahn: In principle a photosurface can convert light energy to work with 100% efficiency. Duysens does not accept this, and neither does the author, for
the generation of a potential by the flow of photoelectrons, necessary if work is to be done, must inevitably result in a counter flow of thermal electrons and some efficiency decrease at temperatures other than 0° K.

3. Kahn: Thermodynamics is applicable only to reversible processes.

Duysens: The efficiency for an irreversible process will be less than that for the reversible process. This is generally correct and has been shown to apply to photochemical reactions.

Mortimer and Mazo have discussed the general theory of irreversible thermodynamics of systems containing radiation and their application to photochemical reactions. They point out that Planck has said that it is "convenient to ascribe" a temperature $T_r$ to non-equilibrium radiation. This temperature is given by equation 7:

$$ T_r = \frac{hv}{k \ln \left( \frac{2hv^3}{C^2I_v} + 1 \right)} $$

where $h$ is Planck's constant, $v$ is the frequency of the light, $k$ is Boltzmann's constant, $c$ is the speed of light, and $I_v$ is the intensity of radiation per unit area per unit area per unit time per unit solid angle per unit frequency interval. Mortimer and Mazo have developed an equation (8) for the photochemical efficiency of the conversion of light energy to chemical energy, where the photochemical reaction results in a positive $\Delta F$ change. In equation 8,

$$ \eta \leq 1 - \frac{T_h}{T_r} $$

$\eta$ represents efficiency and $T_h$ represents the thermodynamic temperature and $T_r$ the radiation temperature. The limit of this efficiency is seen to be the same as the Carnot efficiency.

It appears that $T_r$ is the same temperature that Duysens found from
tables of radiation from black bodies. The mathematical derivation of the irreversible process efficiency seems to have proved only that the efficiency should be at most that of a thermodynamically reversible process, a result that seems not unexpected. Using Duysens assumptions of 1000 ergs/cm²/sec., a frequency interval corresponding to the wavelength interval 660 μm - 680 μm, and 4π geometry, the reviewer calculated an $I_v$ of $5.983 \times 10^{-12}$ ergs/cm². This gives a value $T_R$ of $1121°$ K. From this, the upper limit of the thermodynamic efficiency for conversion of the light energy to chemical energy would be 73%.

Note that the value for $I_v$ depends upon the band width of the incident light as well as upon intensity. For example, if wavelengths from only 6698 Å to 6700 Å are admitted to the plant, $I_v$ becomes about $5.983 \times 10^{-10}$ ergs/cm². However, $T_R$ depends only logarithmically on $I_v$ and becomes in this case $1440°$ K, making the calculated upper limit of thermodynamic efficiency 79%.

However, "convenient" it may be to "ascribe" a temperature to radiation in this case, there would seem to be some question as to whether it is valid to do so. Admittedly, a black body which emits enough radiation from a band one Å wide to give an intensity of 1000 ergs/cm² -sec. at a given distance has a higher temperature than a black body emitting the same total amount of energy from a band 200 Å (20 μm) wide. Does this difference in temperature make any difference in the efficiency with which this energy can be utilized in chemical reactions after the energy has been absorbed by a pigment molecule and transferred by resonance transfer to another pigment molecule where it corresponds to an excited
state that would be formed by absorption at, say, 705 m. The loss in
energy in this transfer from 670 to 705 (about 5%) would not be expected
to be necessarily different for 660 - 680 m light than for "pure 670"
light. There may well be some theoretical limitation on the efficiency
with which the energy of the excited state of the 705 pigment can be
converted to chemical free energy change of molecules in thermal equi-
librium with their surroundings, but it is hard to see how this limi-
tation could depend on the band width of the exciting light. Further
investigation of this important problem seems to be needed.

It is of importance to inquire where a limitation on the efficiency
of quantum conversion, if indeed it exists, expresses itself. Presumably
the absorption of a photon of light results in an excited electronic
state which is separated from the ground state of the pigment molecule by
the amount of energy calculated by the formula $E = hv$. This excited
pigment molecule is not in thermal equilibrium with its environment, but
rather may perhaps be considered as a "hot" molecule, even though its mode
of excitation is not as yet represented by a thermal vibration. The con-
version of the energy of this excited molecule to the stored chemical
potential of new molecules in thermal equilibrium with their surroundings
may be considered as the point at which such a limitation as just calcul-
ated should express itself.

Certainly the subsequent dark reactions occurring between molecules in
thermal equilibrium with their surroundings are not subject to this limi-
tation on the efficiency of the primary quantum conversion act. In other
words, if one is to assume the 73% limitation on the conversion of electro-
magnetic energy to chemical energy, this must be considered not as a
limitation on the overall efficiency of photosynthesis as seemingly suggested by Duysens, but rather as a limitation on the efficiency of the primary quantum conversion act. According to Calvin, this primary act is "that act or sequence of events following the absorption of the electromagnetic quantum and terminating with the appearance of a thermally-relaxed, chemically-defined individual which may then proceed by direct thermal work-performing reactions to produce the next transient and finally the ultimate products of photosynthesis."

The theoretical limit on this process of conversion of the energy of excited states of molecules to chemical energy (primary quantum conversion act) is indeed important. It may be, however, that somewhat different considerations than those used by Duysens set this limit.

If we consider that the carbon reduction cycle operates at about 85% efficiency, as will be discussed subsequently, and assume that the primary quantum conversion act is 73% efficient, then we have set a limitation on the overall efficiency of photosynthesis at 62% even before considering the inefficiencies which must inevitably accompany the other stages of electron transport, evolution of oxygen and cofactor production. This limitation already imposes a minimum quantum requirement of 4 1/2 quanta per molecules of oxygen evolved.

2. Measured quantum requirements

The quantum requirement or quantum yield of photosynthesis has been reviewed in authoritative articles by Emerson and by Kok. In each case the reviewers have gone carefully through the maze of contradictory and confusing literature on this subject and have emerged with the conclusion that the quantum requirement of photosynthesis is probably 8 quanta per molecule of oxygen evolved, although each holds out the possibility
that the quantum requirement might be as little as 7. Those familiar with this literature will recall that for over 40 years Warburg and co-workers have been reporting quantum requirements based primarily on manometric methods which range from 2.7 quanta to about 5 quanta per molecule of oxygen gas evolved. Almost without exception the many other investigators of the quantum requirement in photosynthesis have reported values of 8 or more quanta, although here and there values of 6 and 7 are reported.

The manometric methods which have been used for so many measurements are subject to a great many errors in interpretation. Among these difficulties might be listed the assumptions required for corrections for respiration, the difficulties with various buffers, and the interpretation of gas pressures resulting from photosynthesis in these buffers, the deviation of the photosynthetic quotient from -1, the transients in CO₂ exchange and oxygen exchange accompanying turning on the light, insufficiency of agitation, and so forth. To these experimental complications may be added the difficulties in interpreting Warburg’s technique of adding compensating white light and "catalytic" amounts of other lights of specific wavelengths, the many variations in culture and nutrient reported as being necessary from time to time to obtain the maximum efficiency, and so forth. All things considered, it would appear that the more reliable means for the determination of the quantum yield of photosynthesis are non-manometric methods of measuring independently oxygen evolution and carbon dioxide uptake during long periods of steady state photosynthesis at light intensities giving photosynthesis rates several times respiration. Unfortunately, there have been an insufficient number of such determinations to permit a definite conclusion as to the correct quantum yield of photosynthesis. Among
those papers which have reported some values less than 8 may be mentioned
the following: Brackett used a polarographic method for oxygen determina-
and found quantum requirements from 6.1 to 13.5. Yuan, et al. used a
paramagnetic oxygen analyzer in combination with an infrared absorption
CO₂ analyzer to follow the gas exchange in photosynthesizing Chlorella.
They obtained a few values as low as 8 and 7, but most of their values
were from 8 to 12, and they concluded that 8 was the best figure for the
quantum requirement. Bassham, Shibata and Calvin used a similar method
but worked at lower absorption and higher light intensities. Without
making any corrections for respiration they obtained quantum numbers of
7.4 quanta per oxygen over extended periods of time and at light intensities
at which photosynthesis exceeded respiration by a factor of 13. Moreover,
this value fell on a smooth curve drawn through points corresponding to
the following pairs of values in which the ratio of photosynthesis to
respiration is listed first, and the quantum requirement second: 3-9.2,
3.5-9, 4-8.3, 5.5-8.1, 7-7.9, 13-7.5. In the author's opinion, at least,
this consistent trend as represented by the smooth curve for a series of
several different experiments represents a fairly substantial indication
of a quantum requirement less than 8. However, the question of whether
the quantum requirement is 8 or 7 can certainly not be resolved on the
basis of evidence presently available.

An overall quantum requirement of 8, 7 or 8 represents an energy
efficiency of 32%, 38% or 43% respectively, if glucose and O₂ were sole
products, and 680 µm light were used.

III. THE PHOTOELECTRON TRANSPORT REACTIONS

A. Overall products of photoelectron transport

For some time it has appeared that the products of photoelectron
transport in green plants are molecular oxygen, ATP and FMNH. Over 20 years ago Ruben\(^2\) suggested that the first product of carbon reduction in photosynthesis is a carboxylic acid and that the reduction of the carboxyl group requires a phosphorylation by ATP, after which an acyl phosphate can be reduced to the aldehyde. The development of the carbon reduction cycle\(^2\) and the finding that phosphoglyceric acid was indeed a first stable product of carbon dioxide reduction and that PGA was in turn reduced as Ruben had predicted, strengthened the belief that reduced pyridine nucleotide and ATP were required as cofactors from the light reaction. In the meantime, Strehler\(^2\) had demonstrated the photoinduced formation of ATP in Chlorella in 1952. As noted earlier, the photo-reduction of TPN\(^+\) to FMNH in isolated chloroplast was reported in 1951 by Vishniac and Ochoa\(^2\), Tolmachev\(^2\), and Arnon\(^1\), while the formation of ATP by light, called photosynthetic phosphorylation, was discovered in 1954 by Frenkel\(^1\) in particles from photosynthetic bacteria and by Arnon et al.\(^1\) in isolated chloroplasts. Photophosphorylation in chloroplasts was reported to be an anaerobic process\(^3\) and to require vitamin K as a cofactor\(^1\). Subsequently the photophosphorylation was classified as cyclic and noncyclic\(^4\), and the "physiological" cofactors, vitamin K and FMN were found to stimulate the cyclic phosphorylation which was also stimulated by the nonphysiological cofactor phenazine methosulfate\(^4\). The reaction of cyclic photophosphorylation may be written as follows:

\[
\text{ADP}^{-3} + \text{HPO}_4^{2-} + \text{H}^+ \rightarrow \text{ATP}^{-4} \quad \Delta F^\circ = +6 \text{ Kcal}
\]

This value for the free energy change is based upon an assumed physiological standard free energy change of +6.) when all reactants are at unit activities except for \(\text{H}^+\) ion at \(10^{-7}\) M. This standard physiological free energy change is then corrected by assuming the activity of inorganic phosphate to be
leading to a $\Delta F$ correction for the reaction as written of $-RT \ln 10^{-3}$ which equals +4.1 Kcal, and a $\Delta F^o$ of +11 Kcal for the reaction. The reaction for the formation of oxygen and TPNH from TPN$^+$ and water is:

$$10) \quad H_2O + TPN^+ \rightarrow H^+ + TPNH + 1/2 O_2 \quad \Delta F^o = +52.6 \text{ Kcal}$$

again assuming pH 7 and activities of TPN$^+$ equal to TPNH.$^{185}$

One may expect that within the chloroplast of a photosynthesizing cell the ratios of the two forms of each of these cofactors will vary and consequently the rate of energy transfer per mole of cofactors will change considerably depending upon the balance between light intensity, carbon dioxide pressure, and other environmental factors governing the rate of photosynthesis.

Arnon.$^{11}$ has reported a stoichiometric relation between noncyclic photophosphorylation and TPN$^+$ reduction. This reaction can be expressed by the following equation (the sum of equations 9 and 10):

$$11) \quad TPN^+ + H_2O + HPO_4^{2-} + ADP^3 \rightarrow 1/2 O_2 + TPNH + ATP^4 \quad \Delta F = +63.6 \text{ Kcal}$$

Recently, Turner, Black and Gibbs reported that with spinach chloroplasts the stoichiometric ratio of 1 for ATP to TPNH was approached only at saturating light intensities and dropped sharply at lower light intensities to values as low as 0.03. This finding suggests that a proposed coupling between ATP formation and TPNH reduction.$^{19}$ might be rather easily broken at low light intensities, at least in isolated chloroplasts.

In a second important paper by this group,$^{39}$ the action spectra and quantum requirement for TPN$^+$ reduction and the formation of ATP by spinach chloroplasts were reported. The action spectrum for TPN reduction was similar to that previously reported by San Pietro et al.$^{250}$ but contained an additional peak at 500 $\mu$m in addition to the peaks at 450 $\mu$m, 675 $\mu$m,
and a shoulder at 375 μm. The action spectrum of ATP associated with TPN⁺ reduction was somewhat different, having peaks at 430 μm, and at 680 μm, with shoulders again at 375 μm and a shoulder at 500 μm. The action spectrum of ATP formation caused by FMS (cyclic photophosphorylation) had peaks at 430, 500 and 680 μm and thus resembled the noncyclic photophosphorylation except that it had a peak at 500 μm. The FMS supported phosphorylation action spectrum agreed with a report by Jagendorf but was at odds with an action spectrum for the same process published by Kok and Hoch who found a peak at 710 μm. Black et al. studied the ratio of ATP to TPNH as a function of wavelength and found that at high light intensities the ratio varied from .85 to .95 and at low light intensities from .2 to .7. In both cases, the highest ratios were found in the regions 400 to 475 μm, whereas lower values were found at 550 μm, particularly with the low light intensities.

This variation in the ratio of ATP to TPNH with wavelength, if it holds in intact photosynthesizing plants, may well explain the reported differences in the products of photosynthesis as a function of incident light wavelength, particularly variations in the ratios of protein to carbohydrate and of amino acids to other early products of photosynthesis.

From what has been said thus far, it might be assumed that TPNH with a reduction potential of -0.324 volts, is the strongest reducing agent formed by the photoelectron transport system.

\[ \text{TPN}^+ + e^- \rightarrow \text{TPNH} \quad e^+ = -0.324 \text{ volts.} \]

However, the production of molecular hydrogen by the green algae Scenedesmus was observed by Gaffron and Rubin while photoproduction of hydrogen from photosynthetic bacteria was reported by Guest and Kamen. The photo-
synthetic bacteria utilized exogenous organic compounds as electron donors. In 1961, Losada et al. reported the photoproduction of molecular hydrogen from Chromatium cells using thiosulfate as an electron donor. The discovery of the non-heme iron protein ferredoxin and the demonstration by Tagawa and Arnon of the presence of ferredoxin in chloroplasts and its ability to function as the cofactor for pyridine nucleotide reduction in the light shows that the chloroplast reaction is able to produce this stronger reducing agent which has a reduction potential of -.43 volts, at least equal to that of hydrogen (-0.42 v). Actually, ferredoxin from chloroplasts appears to be identical with PPNR, discovered by San Pietro. The presence of a reducing agent of the potential of H₂ in the chloroplasts opens up new considerations for energy transfer to carbon reduction in vivo. The equation for the formation of reduced 'ferredoxin' is:

\[
13) \quad H_2O + 2 Fd^{3+} \rightarrow 2 Fd^{2+} + 1/2 O_2 + 2 H^+ \\
\Delta F^\circ = +57.5 \text{ Kcal}
\]

if \( e' = -0.430 \text{ v} \).

That another reducing cofactor than TPNH may function in electron and energy transport in in vivo photosynthesis is suggested by reports by Miyachi et al. who found that TPNH does not decrease rapidly upon turning off the light on Chlorella cells even though the capacity for fixing CO₂ does drop sharply. However, there is a possibility that ATP, or an equivalent phosphorylating agent, may be limiting when the light is turned off.

B. Pathways of photoelectron transport and photophosphorylation

The interpretation of noncyclic photophosphorylation, reported by Arnon seems unambiguous. In the transport of electrons to TPN⁺ or Fd
from water in the case of green plant photosynthesis or from hydrogen sulfide, sulfur, thiosulfate, organic compounds, etc., in the case of bacterial photosynthesis, there must be exergonic steps, one or more of which could be coupled to the generation of ATP from ADP and inorganic phosphate.

The interpretation of cyclic photophosphorylation both in green plants and photosynthetic bacteria, has engendered a large amount of work and theory. In general, the demonstration of cyclic phosphorylation requires the addition of cofactors themselves capable of undergoing oxidation-reduction reactions. The simplest explanation of cyclic photophosphorylation in the presence of such added electron carriers may be that the addition of such carriers short circuits the electrons produced at a high reducing potential by the light reaction to some electron transport reaction coupled with phosphorylation. As stated by Vishniac et al., the cyclic photophosphorylation can be interpreted as an electron transport phosphorylation in which electrons phototransported to a higher potential are permitted to flow back to a lower potential in a dark reaction, with the liberated energy used to drive phosphorylation. This boosting of electrons to a higher energy level by light, followed by a "downhill" flow of the electrons in the dark coupled to one or more phosphorylations, is essentially the scheme suggested by Arnon. He also proposed that cyclic photophosphorylation in some photosynthetic bacteria may be the exclusive light-requiring reaction and also the exclusive source for the production of ATP. From this he concluded that from the standpoint of evolution of photosynthesis, cyclic photophosphorylation may be the most primitive of photosynthetic activities. Stanier et al. also suggest a secondary importance for photochemically produced reductants for bacterial photosynthesis.
van Niel\textsuperscript{291} all argue against cyclic photophosphorylation as a general and exclusive light reaction of bacterial photosynthesis on the basis of known quantum requirements for bacterial photosynthesis which are 8-10 quanta per molecule, regardless of the electron donor substrate, and for other reasons. In any event, to quote van Niel\textsuperscript{291}, "However appealing the concept of cyclic photophosphorylation as a single common denominator of all photosynthesis may have seemed but a short time ago, its deficiencies have caused its originator to evoke such a process only to account for a few special types of photosynthesis.\textsuperscript{13,17} The photoproduction of molecules of hydrogen from thiosulfate by Chromatium reported by Losada\textsuperscript{196} seemed to lay to rest any doubts about the capacity for photoproduction of reductants by bacterial photosynthesis. However, there are strong arguments\textsuperscript{76} in favor of the occurrence of cyclic photophosphorylation as the only necessary photoreaction in photosynthetic bacteria which are supplied with organic electron donors with high reducing potential\textsuperscript{263}.

Nishimura\textsuperscript{219,220,221,222} has studied the kinetics of bacterial photophosphorylation during and after short light flashes (0.5 to 1.5 msec.). In the first and second of these papers he reported a separation of photophosphorylation into the light and dark phase and the effect of certain reagents and temperatures on the rates of these reactions\textsuperscript{219,222}. After developing a very sensitive and rapid method of measuring photophosphorylation, which depends upon measurement of pH change\textsuperscript{222}, and using a 0.5 msec. flash of much higher intensity\textsuperscript{221}, he found a much higher yield of ATP per single flash than previously. The amount of ATP synthesis during the flash was reported negligible compared to the total delayed photophosphorylation.
By using infrared illumination exclusively, he excludes accessory pigments as light absorbers and concludes therefore that the light absorbed per flash is limited by the number of bacterial chlorophyll molecules. His estimate of the quantum yield of delayed photophosphorylation based upon his maximum measured ratio of ATP reduced per flash to bacterial chlorophyll molecules present (0.047) seems questionable and it leads to an extremely low energy conversion efficiency (1.2%). Of more interest, after assuming a photochemical reaction of chlorophyll with cytochrome c, leading to chlorophyll reduction and cytochrome oxidation, and followed by phosphorylation accompanying a flow of electrons from chlorophyll, he calculates a ratio of ATP to photochemically oxidized cytochrome of 2. If this value is correct, it is quite interesting. If the usual type of phosphorylation accompanying the flow of two electrons occurs here, there would be four ATP molecules formed for each two electrons transported photochemically. Assuming a potential difference for each phosphorylation step of about 0.3 v (from comparisons with oxidative phosphorylation) this gives a potential difference of 1.2 v. Now suppose that one electron is transferred through this potential by one light quantum. Per mole, this represents 27.7 Kcal. If 900 μm infrared light were converted, some 31.8 Kcal per einstein are available (Sec. I. A.), which would give an energy efficiency of about 84%. If this photochemical electron transfer were from cytochrome c₂ (which has been reported in bacteria¹⁴⁵), the electron acceptor formed from BChl might have a potential of some -1.0 v or more. Biological cofactors with such extremely high reducing potentials, and systems for making ATP with electrons at such potentials are not known at present. However, Calvin⁶ has proposed the creation of such strong reducing agents in
photosynthesis for somewhat different reasons (see sec. IV. C. 3.).

Another complicating factor has been introduced by the discovery of oxidative photophosphorylation, in some cases demonstrated by means of isotopic oxygen, by Krogmann\textsuperscript{187,189} and Nakamoto et al.\textsuperscript{215} and Venables and Leland et al.\textsuperscript{292}. In this case, electrons presumably flow from the high energy reductant formed by the light all the way back to oxygen, with coupled oxidative phosphorylation analogous to that occurring in oxidative electron transport taking place. Cyclic photophosphorylation in air in the presence of either FMN or vitamin K is inhibited by orthophenanthroline and by CMU\textsuperscript{147,215,305}. Such inhibition largely disappears in an atmosphere of nitrogen. These two inhibitors are also powerful inhibitors of oxygen evolution by illuminated chloroplasts. This suggests that there may be similar steps in the processes of oxygen evolution in photosynthesis and in oxidative photophosphorylation, but does not make it mandatory that oxygen evolution occur as part of oxidative photophosphorylation, at least in air\textsuperscript{13,148}.

The demonstration of oxygen-requiring photoreactions in chloroplasts raises again the question of dark reactions of the chloroplast which may also consume oxygen, that is, respiration. Arnon et al.\textsuperscript{15} found that isolated chloroplasts were unable to form ATP in the dark by oxidizing certain hydrogen donors with molecular oxygen. Despite this finding and the fact that certain types of photosynthetic phosphorylation apparently only require catalytic amounts of oxygen\textsuperscript{14}, it is not necessary to conclude that the photosynthetic system in vivo is incapable of some type of oxidative reactions, including perhaps oxidative phosphorylation. CO\textsubscript{2} fixation in isolated chloroplasts appears to be incomplete (see discussion below), and, however efficient photosynthetic phosphorylation
may be in isolated chloroplasts, it still seems possible that cofactors, enzymes or organization required for oxidative phosphorylation may be missing from such chloroplasts and chloroplast fragments as have been so far prepared.

Blinks followed the rate of oxygen uptake in the green alga Enteromorpha in the dark and upon illumination with light of various wavelengths. He found that upon illumination with 702 μ light, there was a quick enhancement of respiration, which then fell off during the next minute or two. Illumination with shorter wavelengths showed little change in respiration at first, and then there was a striking increase in respiration. These changes he correlated with the chromatic transients described in section III. C. The point here is that the 702 μ light, as will be seen later, presumably produces reduced cofactor capable of reducing TPN+, and from this experiment it appears that electrons from this cofactor are being transported back to oxygen.

Zelisch and Barber found considerable consumption of oxygen by chloroplasts to which they had added an extraneous hydrogen acceptor. They argue that chloroplasts contain certain links of the tricarboxylic acid cycle, such as the malic and glycolic acid systems. The rapid conversion of photosynthetically formed carbon compounds to intermediates of the Krebs cycle, such as malic and citric acids, in the light and especially when the light is just turned off also support this idea.

Lundegardh showed that although cytochromes of the chloroplasts are oxidized in the light, they are not completely reduced in the dark under normal conditions, and increased reduction can be obtained upon the addition of TPNH to chloroplasts stored in the air. The oxidation
states of the chloroplast cytochromes could be changed by adding ADP or ATP or by adding TPN⁺ or TPNH. Further studies by Lundegardh on the oxidation and reduction level of cytochromes f and b₃ in chloroplasts, in the presence and absence of oxygen and with added light flashes, show considerable effects of oxygen. Since he found²²² a ratio of only about 1 to 12 between a and the total of b₃ and b as compared with a similar ratio of 1/2 in wheat root mitochondria, and a molar ratio of chlorophyll to b₃ + b of about 100, and since the oxygen consumption by his chloroplasts in a glucose media is about 1/9 that of the wheat root mitochondria, one may well conclude that even in the isolated chloroplasts there is a small but significant rate of respiration.

Lundegardh's data²⁰¹ seem to indicate short circuits between this respiratory electron flow and the photosynthetic electron transport in the opposite direction. He suggests a transfer of electrons from TPNH to b₃ to oxygen, with an indirect oxidation of cytochrome f by transfer of its electrons to b₃ or alternatively a slow flow of electrons from TPNH to Cyt b₃ to Cyt f to Cyt a and Cyt a₃ to oxygen, provided that the Cyt a and Cyt a₃ he has reported are really present in the chloroplast.

Chloroplasts are large, membrane enclosed subunits of the cell, and probably contain a great variety of biosynthetic systems²⁷,²⁵³. In the case of some unicellular algae such as Chlorella they occupy the major part of the volume of the cell. During the growth and division of cells, a chloroplast may itself grow and divide in a nearly autonomous fashion. There seems to be no reason not to suppose that the chloroplast is capable of biosynthesis in the dark, utilizing respiratory reactions to
generate the necessary ATP and reduced cofactors. Clearly there must
be some block when the light is on to prevent an excessive back reaction
of reduced cofactors with oxygen. Under a variety of special conditions
(such as low light intensity) it may be necessary for oxidative electron
transport and phosphorylation to take place during photosynthesis. The
extent of such transport may well be species dependent owing to the
nature of the biosynthetic pathways required by the particular organism,
and to its environment.

Cyclic phosphorylation and oxidative cyclic phosphorylation are
induced in isolated chloroplasts or chloroplast fragments by the addi-
tion of cofactors (either physiological or nonphysiological). Are there
similar processes taking place in photosynthesis? Trebst et al.\textsuperscript{281} have
demonstrated that the addition of a catalytic amount of cofactor such as
FMN is required to obtain a type of carbon fixation and reduction in
chloroplast fragments from sugar beet. Without such additions, only
radioactive phosphoglycerate was formed from $^{14}$CO$_2$. With added FMN,
RuA was reduced to sugar monophosphates and diphosphates.

However, this $^{14}$CO$_2$ fixation and reduction in chloroplast fragments
has a limited resemblance to carbon reduction in intact cells. The
radioautograph\textsuperscript{281}(p.3057) showing photosynthetic $^{14}$CO$_2$ products in the
presence of FMN following 30 min. illumination bears a superficial re-
semblance to the pattern obtained after 5 sec. photosynthesis of $^{14}$CO$_2$
by Chlorella\textsuperscript{22,119}(p.143). The obviously limited rate of $^{14}$CO$_2$ fixation
in the chloroplast and the limited number of products formed thereby is
also illustrated by comparison with 2 min. photosynthesis under steady
state conditions by Chlorella\textsuperscript{27}. Whatley \textit{et al.}\textsuperscript{303} reported CO\textsubscript{2} fixation rates of 2 \textmu m ole/hr/mg chlorophyll when chloroplast fragments were supplemented with chloroplast extracts. The rate in intact actively photosynthesizing Chlorella, spinach or sugar beet can be easily 200 - 300 \textmu m ole of CO\textsubscript{2} fixation/mg chlorophyll/hr. Thus the published rates of CO\textsubscript{2} fixation for chloroplasts are of the order of 1\% of those of healthy intact plants, not 20\%. Although Trebst did not report CO\textsubscript{2} fixation rates in comparable units, it appears from his radioautographs that his rates may be even less than 1\%. Later studies (Losada \textit{et al.})\textsuperscript{197,282} reported rates which on the basis just given would equal 2 or 3\% of the CO\textsubscript{2} fixation rate of healthy intact plants. Heber and Tyskiewics were able to obtain CO\textsubscript{2} fixation rates in broken chloroplasts as high as 10 \textmu m ole/hr/mg chlorophyll (5\% the in vivo rate) by careful selection of conditions and in particular by using a higher concentration of CO\textsubscript{2}-fixing enzymes. From an extrapolation of their curves of CO\textsubscript{2} fixation versus protein concentration they thought that at 100 mg protein per ml (comparable to intact cells) one might expect fixation of CO\textsubscript{2} as high as 30 \textmu g/hr/mg chlorophyll.

Thus, it would seem that studies of stimulation or inhibition of chloroplasts or chloroplast fragment \textsuperscript{14}CO\textsubscript{2} fixation while interesting in their own right, cannot necessarily limit the interpretation of \textsuperscript{14}CO\textsubscript{2} fixation in intact living cells. Indeed, it is one of the theses of the present review that when the intact chloroplast is disrupted or removed from its native environment, according to present techniques, important organizational features and enzymic activities are probably lost. Even though what remains may duplicate in some ways the products
of photosynthesis in vivo, the product production in broken systems
need not even follow the major pathways for the production of the
same product in vivo. Studies of broken systems and isolated enzymes
are important because they provide the information which helps to
interpret the results of studies with whole systems.

Considering the probable requirements of ATP and TPNH for carbon
reduction and the synthesis of organic molecules, it now appears that
a ratio of ATP to TPNH close to 1.0 may be required for complete photosyn-
thesis leading to carbohydrate, proteins, fats and all the other
products of the chloroplast's synthetic reactions. It was earlier
suggested that three molecules of ATP and two of TPNH are required
to operate the carbon reduction cycle. As will be discussed later (sec. VII),
the ATP requirement may possibly be lower in the in vivo system.
Moreover, chloroplasts of many plants synthesize considerable quantities of fats. The synthesis of fatty acids starting with intermediates
from the carbon cycle requires about two moles of TPNH for each mole
of ATP. Reductive amination to form amino acids from intermediates in
the carbon cycle requires a one-to-one ratio, while formation of the
fatty chains of the larger amino acids requires more TPNH than ATP.
The ATP requirement for carbohydrate synthesis from intermediates of
the carbon cycle is probably not more than one ATP per six carbon atoms.
From all this, it appears that the ratio of ATP to TPNH required by
complete carbon compound synthesis in the chloroplasts may be close to
one, and thus may be satisfied almost entirely by non-cyclic photophos-
phorylation, at least at high light intensities. When additional
amounts of ATP are required, and especially at lower light intensities,
cyclic photophosphorylation with natural cofactors present in the chloroplast, oxidative photophosphorylation, or ordinary oxidative phosphorylation, may be called upon to supply the additional ATP.

C. Enhancement and two light effects

Before further intricacies of electron transport and photophosphorylation can be considered, another extremely important development must be discussed. This is the apparent cooperation between two pigment systems in the electron transport reactions. Emerson and coworkers\textsuperscript{102,103} investigated the long wavelength limits of photosynthesis of Chlorella in the region of long wavelength absorption where the efficiency declines. They found that supplementary light of shorter wavelengths could bring the yield for the long wavelength red up to the level of the yield for the shorter wavelength red, that is, up to the maximum. Meyers and French\textsuperscript{208}, using an electrode to measure oxygen evolution by Chlorella, found that they could cause saturation of the enhancement of the efficiency by which 700 \( \mu \) light was used by adding a 653 \( \mu \) beam which by itself produced twice the rate that was induced by the 700 \( \mu \) beam by itself. Blinks\textsuperscript{144} found somewhat greater enhancement in red algae, possibly because the two pigments in red algae have less overlap in their absorption bands than is the case with the chlorophyll \( \alpha \) and chlorophyll \( \beta \) of Chlorella.

The existence of two quantum-conversion systems, each with its own set of pigments, might have been anticipated from the action spectra with bluegreen algae and red algae\textsuperscript{57,132,324}. Light absorbed by chlorophyll \( \alpha \) at the end of the spectrum is utilized less efficiently than light absorbed by accessory phycobilins in these organisms. Haxo\textsuperscript{133} reviewed evidence in 1980 for the cooperation of two light acts occurring in two
different pigment systems. Blinks$^{41,42,43,44}$ studied chromatic transients in the photosynthesis rate of red, green, and brown algae upon sudden change in the wavelength of incident light. In his experiments, the incident light was adjusted in intensity so that each wavelength gave the same photosynthetic rate. When the incident wavelength was suddenly changed, transient changes in the rates occurred. The transients were observed whenever the light wavelength is changed from that absorbed by an active accessory pigment to that absorbed by chlorophyll $a$, or when the wavelength is changed in the opposite direction.

Meyers and French$^{203}$ studied the Blinks effect by observing the transient increase in rate upon changing from 700 $\mu$m to another wavelength. They also studied the enhancement of photosynthesis (Emerson effects) by adding the other wavelength to 700 $\mu$m light. When the two effects in Chlorella were plotted on comparable scales as a function of the added wavelength, their action spectra are found to be identical.

French, et al.$^{111}$ studied action spectra for photosynthesis in Chlorella, using a background light of 700 $\mu$m and in other experiments a background light of 650 $\mu$m. All of these effects suggest the possibility that light absorbed by different pigments is used in cooperative reactions to produce the ultimate electron transport of photosynthesis. Such results do not, however, prove that all photosynthesis takes place via two light reactions.

French$^{110}$ concluded that the enhancement pathway involving a form of chlorophyll $a$ absorbing at 695 $\mu$m was separate from the main path of photosynthesis, which he felt involved energy transfer to shorter wavelength forms of chlorophyll $a$.

Because of light-dark shifts in absorption spectra at 700 $\mu$m, which will be discussed more fully later, Kok and Hoch$^{179}$ were led to studies
of biochemical reactions caused by light absorption in this region. They found that the action spectrum of phenazine methosulfate-stimulated photophosphorylation had a pronounced peak at 710 nm. This seems to indicate a close relation between a light reaction caused by the P-700 pigment and the transport of the electrons involved in this type of cyclic photophosphorylation. Petrack\textsuperscript{233} found that particles from bluegreen algae which had lost their phycobilin pigments and retained only chlorophyll \(a\) were still able to catalyze pms-supported cyclic photophosphorylation, but could not perform a Hill reaction, that is, oxygen evolution.

From such facts and from others which will be mentioned later, there has emerged a concept of two light-supported reactions promoted by light absorption in two different pigment systems. One of these reactions, resulting from light absorption by a pigment with a rather long wavelength peak (Kok's P-700) seems to cause the transport of electrons to a potential from which they can be used for cyclic photophosphorylation upon the addition of suitable cofactors. I shall refer to this as the P-700 reaction. The other light reaction is presumed also to transport electrons but from a potential chemically closer to the oxidation of water and the evolution of oxygen. Between these two light steps must lie some dark reactions in which electrons are transported from the product of one photo-electron transport step. Thus, two light reactions would occur along the pathway of electron transport from water to the ultimate reducing cofactor used for carbon reduction.

**D. Chemical separation of two light steps**

Krasnovsky\textsuperscript{134} and Brin\textsuperscript{50} studied the chlorophyll-sensitized transfer of electrons from ascorbic acid to pyridine nucleotide in the presence of
red light. This increase in potential from .05 v to -.32 v would, in a reverse dark reaction, release sufficient energy per two electrons to bring about the coupled formation of ATP. Vernon demonstrated the same photoreduction of pyridine nucleotide by ascorbate and chlorophyll but in aqueous medium.

Vernon and Zaug had already shown that chloroplasts which had lost their capacity for oxygen evolution were able to reduce TFM+ photochemically when electrons were supplied by the reduced dye, 2,6-dichlorophenol indophenol (DClP). Continuing this work, Ash, Zaug, and Vernon showed first that tetrazolium blue and methyl red could be photoreduced in a regular Hill reaction by spinach chloroplasts. Since no electron donor was added, it was presumed that this reaction was accompanied by the evolution of oxygen. Reduction of both dyes was strongly inhibited by the addition of dichlorophenyl methyurea (DCMU), which is thought to block photosynthetic oxidation of water leading to O₂ evolution. Reduction was restored upon the addition of ascorbate and dichlorophenol indophenol as electron donors, presumably to the other light reaction, which would not be inhibited by DCMU.

Parallel experiments with Rhodospirillum rubrum chromatophores showed these particles also to be capable of photoreducing methyl red and tetrazolium blue, but only in the presence of electron donors. In these experiments the ascorbate is presumed to act as the ultimate electron donor and the dichlorophenol indophenol as the intermediate carrier of electrons to the chlorophyll system. These results suggest that the bacterial photosynthetic process is a single-step light reaction closely analogous to the light step in green plant photosynthesis leading to the reduction of triphosphopyridine nucleotide, thought to be the step mediated
by the P-700 pigment. Since cyclic photophosphorylation is found in
bacterial photosynthesis, it might also be expected that cyclic photo-
phosphorylation is associated with the P-700 step of green plant
photosynthesis.

Losada, Whatley, and Arnon \(^{193}\) have reported the separation of two
light reactions in noncyclic photophosphorylation and TPN\(^+\) reduction of
green plants. Blocking the light reaction which oxidizes water by
omitting chloride and adding the inhibitor DCMU, and substituting tri-
chlorophenol indophenol as an electron donor, they demonstrated the
photochemical reduction of TPN\(^+\) and simultaneous noncyclic photophos-
phorylation.

Levine and Smillie \(^{192}\) have used mutants of *Chlamydomonas reinhardi* \(^{191,193}\) to show quite clearly the separation of photoelectron transport into two
phases, each with its own light reaction. Two mutant strains which are
incapable of carrying out the Hill reaction are able to photoreduce TPN\(^+\)
without oxygen evolution if supplied with DPIP as an electron donor and
ascorbate to maintain the DPIP in the reduced state. Both mutants ex-
hibited an increased cytochrome \(f\) and a decreased plastoquinone content.
Another mutant, ac-21, was able to carry out both oxygen evolution and
the photoreduction of TPN in the presence of PPNR, but only on the
addition of DPIP as an electron donor. This mutant had a normal com-
plement of plastoquinone. Levine and Smillie conclude that one light
reaction causes the transport of electrons from water to plastoquinone
and cytochrome \(b_6\), a reaction which is blocked in the two mutants which
are incapable of the Hill reaction. They propose a second light reaction
for the electron transport from cytochrome \(f\) to TPN\(^+\). The dark steps
which normally take electrons from cytochrome \(b_6\) to cytochrome \(f\) are
apparently blocked in the mutant ac-27.

It thus seems established that photoelectron transport in green plants can occur via two photochemical steps, together with some intermediate dark steps. In one photochemical step, plus one or more dark steps, electrons are removed from water and transferred to an electron acceptor. Dark reactions plus a second photochemical step, probably catalyzed by excited P-700, transfer electrons from this intermediate acceptor to another electron acceptor, which becomes a reducing agent of at least the strength of hydrogen or PPNR. Coupled to one or more stages of this transfer, ATP formation by noncyclic photophosphorylation occurs. Upon the addition of cofactors to broken systems, electrons from the strong reducing agent formed by the P-700 reaction are cycled back to the intermediate reducing agent, and in this process cyclic photophosphorylation occurs. Quite possibly the formation of ATP by cyclic photophosphorylation takes place in the identical electron transport step involved in noncyclic photophosphorylation, though this point remains to be demonstrated.

IV. PHYSICAL AND CHEMICAL PROPERTIES OF THE PHOTOELECTRON TRANSPORT SYSTEM

A. Structure of the electron transport and quantum conversion apparatus

1. Chloroplast structure

If a chloroplast is stained with osmium tetroxide or potassium permanganate, embedded, sectioned, and viewed in the electron microscope, alternate dark-staining and light layers which are called lamellae are revealed254. These lamellae extend throughout the chloroplast but are thicker and more closely packed in certain places than in others. The regions with a higher concentration of lamellae sometimes have been called
grana and correspond to the small green areas which are just visible under the light microscope. The less pigmented region of the chloroplast having fewer lamellae is called the stroma and the lamellae can be considered as embedded in a stroma matrix. The entire body is surrounded by a double membrane. Variations in this structure have been found in a wide variety of organisms with organized chloroplasts. Even in bluegreen algae, where the membrane surrounding the chloroplast is lacking, a similar lamellar system is seen. In general, the repeating interval in the closely stacked lamellae of all of these organisms is about 160 Å.

Trebst and more recently Park and Pon, have isolated chloroplasts from spinacea olereacea and have ruptured these chloroplasts, obtaining small fragments which still retain photosynthetic capacity and can slowly reduce carbon dioxide to sugar. CO₂-fixing enzymes and the photo-electron transport systems are separated by centrifugation at high speed, which causes the green portion of the chloroplast to sediment to the bottom of the centrifuge tube. The light reactions (which convert light energy to chemical energy) are found in the resuspended green sediment, whereas the reduction and synthetic reactions are to be found in the colorless supernatant protein.

Recently, Park has reviewed the properties of the green precipitate containing the photoelectron transport system. He suspended the green precipitate in water and precipitated it according to the critical point of Williams. When viewed in the electron microscope, the material was clearly lamellar in structure. When embedded and viewed in cross section, the lamellae were found to consist of two layers, each about 100 Å thick. According to Park, it is the outer portions of these two layers which
stain with osmium tetroxide or potassium permanganate. He believes the inner portion of the layer to be a mat of particles about 200 Å in diameter, as judged by the way they are revealed in the precipitated and dried lamellae. These particles were earlier noted by Frey-Wissling but were not so evident since the critical point technique was not used. These small particles are termed lamellar quantasomes. The quantasome appears as an oblate spheroid 100 Å x 200 Å, embedded in the staining membrane. Park has discussed ways in which the various lamellar systems described for different chloroplasts might be made up of these units. He notes that the top and bottom stained layer of a stack of lamellae is single, whereas the interior layers are of double thickness. He suggests that the conversion of light energy to chemical energy takes place in this lamellar system consisting of these 200 Å oblate spheres, attached to the membrane. A quantasome consists of one of these spheres with its attached membrane.

Park studied the capacity of lamellar fragments of different sizes to perform the Hill reaction and to support CO₂ fixation when the soluble enzymes are added. He found that all fractions investigated down to the smallest fragments (800 Å in diameter) are equally effective in energy conversion. The smallest fragments studied consist of single layers containing about 6 to 8 quantasomes, and these are able to utilize energy to perform electron transport resulting in oxygen evolution, TPN⁺ reduction, and coupled ATP formation. Park discussed the question of the smallest lamellar structure capable of carrying out energy conversion. He criticized the previous study of Thomas et al. on the grounds that Thomas' preparations were not chemically homogeneous, and
may well have incurred a sizable fraction of particles which were not electron transport particles. However, he concludes from recent work in his own laboratory that the dimension observed by Thomas is approximately correct, and that this smallest unit may well be the quantasome. Since the size of the photosynthetic unit first proposed by Emerson and Arnold\textsuperscript{101} has been more recently estimated by Kok\textsuperscript{177} to contain only 300 chlorophyll molecules and by Bassham and Shibata\textsuperscript{29} to contain 480 chlorophyll molecules, it is interesting to speculate whether the quantasome may be identical with the physiological photosynthetic unit.

2. Chemical composition  

(a) Quantasomes

Park has investigated\textsuperscript{229} the chemical nature of the quantasome. They can be lyophilized and, upon resuspension in water, retain their Hill activity. However, if the lyophilized quantasomes are extracted with hexane, acetone, and methanol, approximately 50\% of the lamellae is soluble and the residue has nitrogen content of 18\%, indicating that what is left is relatively pure protein. Under the electron microscope, the extracted protein globules appear about half the size of the original quantasomes. Spectrochemical analysis of the lyophilized material revealed the presence of magnesium, iron, manganese, copper, aluminum, and silicon. The ratio of magnesium to iron to copper to manganese is about 150 to 15 to 3 to 1. A calculation of the number of chlorophylls to be expected in a quantasome based on its calculated molecular weight of 1 million indicates that there should be around 150 chlorophyll molecules per quantasome.

The presence of manganese is of considerable importance since it is
known to be required for oxygen evolution in photosynthesis in the Hill reaction. The presence of copper might have been predicted from the report of Katoh et al., who localized plastocyanine in the chloroplast. Copper might be involved in the reported oxidative photophosphorylation. Plastoquinone has been found to be concentrated in chloroplasts and is implicated in photoelectron transport and probably in photophosphorylation. More recently, Kegel et al. reported the presence of two additional quinones with absorption spectra identical to the original plastoquinone but different chromatographic behavior. The significance of several forms of plastoquinone may lie in the possibility of several sites for photophosphorylation (see section VI) and the suspected connection of plastoquinones with ATP formation. Vitamin also may play such a role. A flavone-like natural cofactor isolated from chloroplasts stimulates photophosphorylation.

The similarities between the photoelectron transport particle or quantasome and the electron transport particle or elementary particle described by Green are great. Both particles have a diameter between 100 Å and 200 Å, and molecular weights between 1 million and 1 1/2 million. Both perform electron transport, albeit in opposite directions, between the oxygen of water and pyridine nucleotide. In both cases some steps in the electron transport system are coupled to phosphorylation of ADP to give ATP. Both contain a complement of heme and non-heme iron and copper compounds, and various quinones. With respect to structure, both units are roughly spheroidal particles attached or embedded in unit membranes. In each case the particle con-
tains major amounts of lipid and protein. The case for an evolutionary relation ship between the two kinds of particles becomes stronger as more is known about them.

(b) Chloroplasts (lamellar system)

Very little is known about the structure of the continuous layer to which the particles which Park has described are attached or embedded. This lamellar membrane presumably is mostly lipid in character. Benson has recently discussed the function of lipids in the photosynthetic structure. The major fraction of lipids in the chloroplast are surfactant molecules which are concentrated in the grana. Of these, galactolipids are predominant, comprising about two thirds of the chloroplast lipids. The rapid labeling of these compounds, however, suggests that aside from any structural function they may have in the lamellae they may also be involved in carbon compound metabolism. Considering the competition for space at the lipid surface between the chlorin group of chlorophyll and the galactose moiety of the galactosyl monoglycerides and diglycerides, Benson suggests that there may be two types of lipid laminae surfaces: an outer one dominated by galactolipids in the region of carbohydrate synthesis and inner one dominated by chlorophyll and the electron transport systems.

This interesting proposal leads one to wonder if the entire process of carbon reduction could be occurring in enzymes located on the surface of lamellae in which are embedded the photoelectron transport particles. Putting the carbon dioxide reduction and some of the basic synthetic steps in or next to the lamellar structure, if it does not impose too severe a space limitation, is an attractive idea. One might imagine, for example,
that electrons having a high reducing potential (generated by the photo-electron transport system) might be conducted by some mechanism directly through the lipid layer to the carbon reduction cycle system, which might itself be an organized system of enzymes (see sec. VII). This might then be thought of as a primary in vivo type mode of carbon reduction in contrast to another less active or efficient mode of carbon dioxide reduction by soluble enzymes utilizing TPNH. In the latter case, TPN in its reduced and oxidized forms would shuttle back and forth from the soluble enzyme systems such as triose phosphate dehydrogenase and the photoelectron transport system, carrying reducing power. Other functions of the non lamellar region might well be the synthesis of a wide variety of secondary products, particularly amino acids and proteins.

The movement of electrons across the lipid barrier from the photoelectron transport system to the primary carbon reduction system as just suggested, or to TPNH, requires some conduction mechanism. Perhaps this role might be played by a conjugated molecule such as the carotenoids, as suggested by Bassham and Calvin. Of course carotenoids also function to some extent in light absorption and they have been assigned a role as protectors against photooxidation, but these need not be their exclusive roles. Moreover, the fact that a carotenoidless mutant of the photosynthetic bacteria can still photosynthesize anaerobically need not be a bar to an electron conduction function for carotenoids in green plants, since the requirement for such a function is much more likely in green plants because of the lamellar structure.

A role in oxygen evolution has also been suggested for carotenoids, particularly the oxygenated carotenoids lutein and violaxanthin.
However, incorporation of oxygen into carotenoids from \( \text{O}_2 \) and from \( \text{H}_2\text{O} \) in light and dark\(^{323} \) fail to support such a hypothesis.

3. Physical orientations of molecules

In the development of lamellae in cells of plants grown in the dark and then allowed to green in the light, chlorophyll is necessary for the development of normal lamellae.\(^{142,214,247} \) Butler\(^{59} \) has reported a study of energy transfer from carotenoids to chlorophyll during the development of lamellae. In examining such energy transfer he has used the method of French and Young\(^{112} \) and Duysens\(^{91} \) who followed energy transfer by looking at the excitation spectrum for chlorophyll a fluorescence. He found that energy absorbed by carotenoids was not transferred to chlorophyll a in etiolated leaves. The capacity for such energy transfer developed during a dark period following the photosynthesis of protochlorophyll to chlorophyll. This, he believes, is the time during which the formation of lamellae takes place while incorporating both carotenoid and chlorophyll into the same structure. He suggests that the light reaction is the formation of chlorophyllide from protochlorophyllide and that during the subsequent period of an hour, the chlorophyllide is esterified with a phytol tail. This lipophilic tail permits the chlorophyll to come in close contact with the lipid layer and hence brings it into juxtaposition with the carotenoid pigments in the lipid layer, thus permitting resonance transfer of energy between the carotenoid and the chlorophyll.

This aggregation of pigment molecules is presumed to result in orientation of at least some of the chlorophyll molecules. In addition, these oriented molecules of pigment are thought to have a maximum absorption peak at longer wavelengths than ordinary chlorophyll a. Such
a long wavelength form of chlorophyll was proposed by Brody\textsuperscript{51} who discovered a 720 \textmu m fluorescence band in algae at -193° C and by Butler\textsuperscript{60} who employed fluorescence excitation spectra and absorption spectra to show that the 720 \textmu m fluorescence band at -193° C is due to a pigment which absorbs at 705 - 710 \textmu m. Moreover, the transfer of excitation energy from ordinary chlorophyll a to this 705 \textmu m absorbing pigment at -198° was reported. Olson, Butler, and Jennings\textsuperscript{224} examined fluorescence emission microscopically in the far red region of intact chloroplasts from Euglena. Using an image converted to make visible the longer wavelengths and viewing the fluorescence through a nicol prism, they found that with unpolarized exciting light there was considerable polarized fluorescence. This polarized fluorescence was of longer wavelength than the unpolarized emission. From their studies they concluded that the long wavelength polarized fluorescing molecules are highly oriented and can accept energy from other chlorophyll molecules.

These workers also reported\textsuperscript{225} further evidence for the orientation of chlorophyll molecules \textit{in vivo} based upon observation through a microscope and nicol prism of intact Euglena chloroplasts illuminated with unpolarized light. In this case photographs were taken with infrared-sensitive film. The maximum extinction of light transmitted by the nicol prism occurred when the plane of vibration of light which could be transmitted by the nicol prism was parallel to the plane of the lamellae. Minimum extinction was found in the case of the lamellar plane perpendicular to the vibration plane of light passed by the analyzer. Dichroic ratios greater than 4 were found at 695 \textmu m. The effect is reported to be visible between 690 and 710 \textmu m. Assuming the
electric vector of the oriented chlorophyll oscillator to be in the plane of the porphyrin ring, it was concluded that these findings showed the plane of the porphyrin ring in the "oriented chlorophyll" molecules to be in the plane of the lamellae.

Sauer and Calvin\textsuperscript{256} measured the electric birefringence and the spectrum of electric dichroism from 330 - 730 µ in spinach quantasomes. The electric birefringence was thought to be associated with protein. The dichroic ratio between 330 - 730 µ ranged from 1.03 to 1.10 except for a pronounced peak at 695 where the ratio was 1.25. This suggested that most of the pigment molecules are largely unoriented but that there is a small fraction, perhaps 5%, or 15 molecules, of chlorophyll a per quantasome which is strongly oriented. These molecules are thought to have an absorption maximum at a longer wavelength than ordinary chlorophyll a, perhaps around 695 µ. Also at this site, which they call the quantatrops, they propose the presence of electron donors such as cytochrome, and electron acceptors. Quantum conversion is pictured as migration by resonance transfer of energy from unoriented chlorophyll and carotenoid molecules to the oriented chlorophyll molecules. At that point, chlorophyll loses an electron to the electron acceptor in the primary quantum conversion act. The electron deficiency, or hole, left in the aggregate, is then presumed to migrate to the site of the electron donor, which is suggested as the cytochrome. This proposal will be discussed in more detail in section IV. C.

B. Changes in absorption spectra

1. Photo-induced changes

The action of light on the photosynthetic apparatus causes some changes in the pigments which can be detected by observing the resulting
rather small light-induced absorption changes. Two light beams are involved in such measurements. First, there must be an activating light beam to cause the change, and secondly, a measuring light beam which is partially absorbed by the pigment and partially transmitted to the monitoring device. The monitoring device, for example a photomultiplier cell, must not see the light from the activating beam. This condition can be achieved by using complementary filters which prevent the activating beam from reaching the detecting device, or by interrupting the activating light beam while the measurement is being made. In order to obtain the sensitivity necessary to measure this rather small effect, investigators often split the beam, passing one portion through the illuminated sample and another portion through a dark sample of biological materials and comparing the monitored outputs of the two samples.

The studies to 1960 have been reviewed by Hoch and Kok\textsuperscript{141} and will be mentioned here only briefly. Complementary shifts in absorption at about 480 \textmu m and 515 \textmu m were studied by Duysens\textsuperscript{91,93}, Spruit\textsuperscript{282}, Bell\textsuperscript{33}, Strehler and Lynch\textsuperscript{270,271,272}, and Chance and Strehler\textsuperscript{75}. In many of these experiments it was found that oxygen could produce the same spectral shift giving a drop in 475 absorption and an increase in 515 absorption was due to oxidation of a pigment, though in one case\textsuperscript{75} it appeared that there was an effect at high light intensity which could not be invoked by oxygen alone. A carotenoidless, pale green mutant of Chlamydomonas studied by Chance and Sager\textsuperscript{74} exhibited no 475 - 515 absorption change, leading to the conclusion that this change was a conversion of a carotenoid pigment. Since this mutant is capable of photosynthesis although damaged by prolonged illumination\textsuperscript{243}, a role somewhat removed from the
primary process of photosynthesis (such as protection against photo-oxidation) was assigned to carotenoids and to the 475 - 515 spectral shift.

Kok has developed a very elegant technique for time separation of the actinic light from the measuring light and has applied this method to studies of spectral changes throughout the visible region. In one of his experimental arrangements, the actinic flashes are given at intervals of 1/10 sec., long enough for most of the known dark reactions of the very early stages of photosynthesis to go nearly to completion. By controlling the time interval between the photo-signal and the time of measurement he can compare the absorption at any selected wavelength for any two points in the dark time following the light flash. This permits him to detect intermediates which undergo reversible color changes. With regard to the 480 - 515 μm shift, he also found a short-lived increase of absorption at 515 μm which, however, decreased upon prolonged illumination.

Of perhaps greater interest were the absorption changes which he measured in the red region using this technique. These photo-induced changes were negative at 650, 660, and 700 - 705 μm and positive at 660 μm and had a half-life time after illumination of about 5 milliseconds. For many reasons, some of which have already been discussed, the negative shift at 700 - 705 μm is of particular interest. It is considered by Hoch and Kok to represent possibly the final stage in energy transfer from one pigment to another, and it has been found in a number of photosynthetic organisms.

This 700 μm photo-induced bleaching appeared to be sensitized by light absorbed by chlorophyll a and perhaps by carotenoids. If interpreted
as bleaching of chlorophyll, the 700 shift corresponded to about one molecule of chlorophyll in 400. In Anacystis the quantum yield of the pigment bleaching was estimated to be about unity\textsuperscript{173}. The effect was rather insensitive to photosynthetic inhibitors, withstood lyophilization and rehydration, and extraction of pigments (such as phycocyanin and anacystis) or removal of carotenoids and coenzyme Q by hexane. Even partial disruption of the structure by sonic oscillation or detergent treatment with detergents and loss of up to 85\% of the chlorophyll did not remove the effect\textsuperscript{173}.

Kok and Gott\textsuperscript{173} found that the regeneration of the pigment P-700, which occurs in the dark, was stimulated by illumination with light which is absorbed mainly via accessory pigments such as phycocyanin, as well as by some chlorophyll a. This regeneration of P-700 absorption stimulated by light absorbed by accessory pigments was, unlike the photo-induced bleaching at 700 m\textmu, sensitive to aging, sonication, extraction, and heating, and was strongly inhibited by DCMU, hydroxylamine, or dinitrophenol\textsuperscript{179}. These similarities between the accessory pigment-photo-sensitized regeneration of P-700 and the oxygen evolution reaction, suggest a correlation between the two.

Following the discovery of cytochromes f, b\textsubscript{3}, and b\textsubscript{6} (or b) in green plant chloroplasts and investigation of their properties\textsuperscript{87,138,138,140}, there has been great interest in the possible role of cytochromes in photosynthesis\textsuperscript{234,157,158} which has been reviewed by Kamen\textsuperscript{154,155,156} and Gest\textsuperscript{122} as well as by Smith and Chance\textsuperscript{259} and Franken\textsuperscript{114}, the latter for bacterial systems. In the meantime, cytochrome resembling cytochrome c was isolated by Kato\textsuperscript{180,181,182} from the plastids of the red algae
Porphyra tenera. This cytochrome was characterized by absorption bands at 417, 521, and 553 m¿ in its reduced form and had a reduction potential at pH 7 of 0.335 v. Another cytochrome c type compound was isolated by Nishimura. The reduced form of this compound had absorption peaks at 417, 523, and 553 m¿, and the compound had a reduction potential estimated to be 0.36 v. Except for cytochrome b6, each of these cytochromes is a rather strong biological oxidizing agent in its oxidized form, though still some 0.4 to 0.5 v too weak to oxidize water in a dark reaction, without some additional coupled energy input.

Duyens found that illumination of photosynthetic cells caused a rapid change in their absorption spectrum such as might accompany cytochrome oxidation. Lundegardh observed that cytochromes in chloroplasts are oxidized in light, and James and Leach reported photoinduced changes in cytochrome f and b3 in green cells. Hill and Bonner observed simultaneous oxidation of cytochrome f and reduction of cytochrome b6 in plants with very low chlorophyll content. This last fact suggests the possibility that more than one cytochrome might be involved in the photoelectron transport system.

In their review, Boch and Kok published difference spectra obtained with a green and a bluegreen algae. Photosensitized bleaching occurred at 553 and 420 m¿, and there was an absorption increase below 410 m¿. However, the evidence for a direct participation of cytochrome in photoelectron transport in green plants was still tenuous, in their opinion, though there seemed little doubt that cytochromes were involved in the photosynthetic electron transport in bacteria.

Chance and Olson observing simultaneously the photooxidation
of a cytochrome c type compound and the reduction of pyridine nucleotide, measured by its fluorescence, found that the cytochrome oxidation was a much faster reaction and presumably much closer to the primary events of photosynthesis, than was the reduction of pyridine nucleotide. They also estimated a quantum requirement of two per electron transferred from this cytochrome in *Chromatium*.

Chance and Mishima studied the light-induced cytochrome oxidation in *Chromatium* and in *Rhodospirillum rubrum*. In *Chromatium*, but not in *rubrum*, they found that the photooxidation of cytochrome was just as rapid at 80° K as at 300° K under infrared illumination. The photooxidation of cytochrome was not reversible at 80° K. They concluded that infrared illumination of the chlorophyll in intact chromatium cells caused a temperature-insensitive electron or proton transfer reaction between bacterial chlorophyll and a closely associated cytochrome of type c (423.5 μ). Since a quantum requirement of two per electron transferred, which they had previously measured, is rather inefficient, they considered the possibility that the actual oxidation might be a two electron oxidation per molecule of cytochrome, and suggested that a higher oxidation state resulting from such a reaction would be unstable, so that they would only observe the one electron transfer.

Muller and Witt have observed photochemical bleaching at 425 μ which they ascribe to cytochrome oxidation at -150° with spinach chromatophores. This is but one of a number of observations made by Witt and co-workers. Since 1955, Witt has employed an ingenious method of detecting absorption changes with very fast time constants. In his method, monochromatic detecting light passes through a thin layer of sample to a
photomultiplier cell. The sample is excited by flashing illumination with wavelengths longer than 600 µm. The photomultiplier tube output was observed directly with an oscilloscope, permitting a study of the absorption spectrum during and after illumination.

The work of Witt and coworkers has been reviewed\(^{315}\), and more recent work has been summarized from time to time\(^{320,215,321,316,246,189}\). A number of types of absorption changes in green pigments and plants have been reported\(^{211}\) as follows: Type 0 is only seen when the arrangement of molecules in the photosynthetic structure is changed, i.e., in solutions of chlorophyll or pheophyrins in pyridine, or with chlorophyll precipitated on protein or plant material that has been extracted with acetone, treatment with digitonin or heated. Type 0 has a very short halftime, less than \(10^{-5}\) sec. and is unaffected by temperatures as low as \(-160^\circ\) C. It is ascribed to a \(\pi-\pi^*\) type change in the triplet state of chlorophyll \(a\) and \(b^{329,210}\). Type 0 is not considered to be an \textit{in vivo} process.

Type 1 absorption changes occur only in green particles which have not been disrupted by subjecting to treatments such as those just mentioned, and is characterized by a lifetime of \(3 \times 10^{-5}\) sec. It occurs at temperatures from \(-200^\circ\) to \(+60^\circ\) C and in all types of aerobic photosynthetic organisms, and is seen as an increase in absorption from 400 to 475 µm and a decrease in absorption at about 520 µm. This change is strongly inhibited by \(O_2\) and \(NO\). Witt ascribes this change to a photo-reaction of chlorophyll \(a\) \textit{in vivo} (Chl \(a \rightarrow\) Chl \(a\))\(^{211}\).

Type 2a consists of a negative change at about 425 µm and a small positive absorption change near 530 µm, which can be seen only in the absence of type 2b. Type 2a change occurs at \(-150^\circ\) C and is not lost
upon extraction of the chloroplasts with petroleum ether. It is not reversible at \(-150^\circ\) C. Witt ascribes type 2a absorption change as the oxidation of cytochrome \((\text{Cyt} \rightarrow \text{Cyt}^+)\). More recently they revised the reported characteristics of the postulated \(\text{Cyt} \rightarrow \text{Cyt}^+\) bleaching, which had been obscured by other changes and they now give 405 \(\text{m}_{\mu}\) as the negative peak and a temperature independent time constant of \(10^{-3} \text{ sec}\) or less. Type 2b has a negative peak around 475 \(\text{m}_{\mu}\) and a positive peak at around 515 \(\text{m}_{\mu}\) and does not occur at \(-150^\circ\) C or following extraction of the chloroplast by petroleum ether. The type 2b changes do reappear following such extraction if the extracted material is added back. They suggest that type 2b is a reduction process and may be related to a change in plastoquinone, which, however, would have to be an indirect effect since plastoquinone has an absorption at 515 and 475 \(\text{m}_{\mu}\). In their terminology, type 2b brings about a reduction of \(X_{\text{ox}}\) to \(X\). Type 2b (earlier reported as type 2, slow phase) seems to be missing in manganese-deficient Ankistrodesmus cells, suggesting that this type of absorption change is related to reactions leading to \(O_2\) evolution.

Type 3 absorption changes were found when a colored Hill reagent such as dichlorophenol indophenol was added to the green particles. Photoreduction of such added dyes is considered to be an indication of a reduction of the substance which Witt calls \(Z\), which, in his scheme, accepts electron from chlorophyll and becomes \(Z^-\) or \(ZE\). The reduction has a fast phase \((10^{-5} \text{ sec})\) and a partial back reaction \((10^{-2}-10^{-3} \text{ sec})\).

More recently, Moraw and Witt have added a type 2c absorption band change at 430 \(\text{m}_{\mu}\) and at 703 \(\text{m}_{\mu}\). They have identified this change
with Kok's P-700 bleaching on the basis of similarities in the changes of absorption at 430 and 703 nm under various physical and chemical conditions. For example, the decay time of both absorptions decreases from approximately 10⁻² sec. to 10⁻⁴ sec. when the concentration of added reduced PMS is increased from 10⁻⁶ M to 10⁻⁴ M. This type 2c absorption change is considered to be an oxidation of chlorophyll which follows the excitation of a small fraction of the chlorophyll molecules to excited states (type 1 transitions).

2. Chemically induced changes in absorption spectra

Kok and Hoch induced the P-700 shift in absorption spectrum by chemical means. Using a double beam spectrophotometer and two cuvettes, they added chemical oxidizing and reducing agents to one and observed the resulting difference in absorption spectrum. They found that P-700 could be oxidized in the dark by ferricyanide and that a half maximal peak at 700 was obtained with a ratio of Fe³⁺ to Fe²⁺ of about 3, indicating a redox potential of +0.48 v., the colored state being the reduced one. This result has been confirmed by Witt et al., who find a potential of +0.45 ± 0.01 v. at pH 6 and 8 for absorption change at 430 and 703 nm which they call type 2c and ascribe to the oxidation of chlorophyll (O → C⁺). If this redox potential is correct, the oxidized chlorophyll is a sufficiently strong oxidizing agent to oxidize any of the cytochromes so far reported from green tissues. Witt and Muller have also estimated the redox potential of the X/Z as 0.0 v. They estimate the redox potential of ZH/Z at -0.4 v., based on the observations of cofactor reduction by illuminated chloroplasts. Witt and coworkers have proposed schemes for photoelectron transport in photosynthesis. These schemes, which have been revised as more information became available, consist of a series of oxidation reduction steps...
involving the pigments and cofactors of the photoelectron transport system, and including the potentials and the time constants of the several steps involved. Among their arguments in favor of the arrangement of coupled pairs of cofactors in the schemes they have shown, are the correlations they have been able to make for these pairs in terms of time constants, temperature dependence and response to chemical and physical changes in the systems. Basically, the reaction pattern which they support is the same as the ones proposed by Hill\textsuperscript{136}. In their latest paper, Rumberg, Muller, and Witt\textsuperscript{246} have added more details to their scheme (I), including another cofactor between X and cytochrome which they call E/E\textsuperscript{+}.

They have also added an independent pathway (II) for cyclic photophosphorylation which they claim utilizes about 50% of the chlorophyll a molecules. Their argument for this latter independent pathway is based upon experiments in which a background light of 708 m\textmu is employed and is thought to keep the chlorophyll a, cytochrome, and E in the system I mostly in its oxidized form, due to absence of the shorter wavelength light reaction. Upon the addition of a flash of both 708 m\textmu and shorter wavelength light, they observed transient changes which they ascribe to rapid oxidation of the chlorophyll a which they believe to be in system II, and a slower reduction of some of the chlorophyll a in system I, due to the formation of reduced X from the shorter wavelength light reaction. In other words, the time constant of the positive charge at 703 (10\textsuperscript{-2}) correlates with the change at 515, which is considered to be the oxidation of cytochrome following its reduction by the shorter wavelength reaction. The flash-induced negative change in absorption at 703, which has a much faster time constant, is considered by them to be due to the chlorophyll a in system II.
From the short note which has been published it is not clear why it is necessary to call upon a separate set of chlorophyll a molecules in a separate system to account for the data. It is stated that a continuous background illumination of 703 μ light/μs of low intensity, so that it would seem that the 50% chlorophyll a oxidized under this circumstance might be merely fortuitous, and that the oxidation of additional chlorophyll a as well as the reduction following might be due to the additional light in the flash. Therefore, all of the chlorophyll a molecules participating in the 703 change could be the same type.

They have interpreted absorption changes at about 257 μ in spinach chloroplasts under red illumination as evidence for the reduction of plastoquinone. Bishop had previously implicated plastoquinone in electron transport during photosynthesis by showing that if plastoquinone is extracted, oxygen evolution stops, but can be restored by addition of plastoquinone.

In chromatophores from photosynthetic bacteria some studies have been made of the quantum requirements of photo-induced spectral changes. In Chromatium, a requirement of about two quanta per electron was found by Olson and Chance for light-induced cytochrome oxidation, and later Olson reported that only one quantum per electron was required.

Clayton showed that light induced a reversible alteration in a special component of bacterial chlorophyll (which he denotes as BChl) comprising 2 - 5% of the total bacterial chlorophyll. This is about the same number of molecules as Chance and Nishimura found of light-reacting cytochrome. Clayton proposes that the BChl is part of an active center in the bacterial chromatophore. Estimating the extinction coefficient of this special long
wave form of BChl$_2$, he finds that from two to four light quanta are sufficient for the reversible alteration of one molecule of BChl$_2$ even in dry preparations at 1$^\circ$ K. After further studies of the kinetics of the photo-induced change in BChl$_2$ and in cytochromes under a variety of conditions, Clayton$^{79}$ concludes that there is an intimate connection between these two photo-induced changes. He proposes a special structural configuration for BChl$_2$ which permits it to transform the exciton to a long lived localized state, and also affords trapping sites for the dissociation of excitons into separated electrons and holes (see sec. IV. C.).

C. Solid state phenomena and the primary quantum conversion act

1. Introduction

The particulate nature of the photosynthetic apparatus, the kinetic requirements of the photosynthetic unit, the two-light enhancement effects, the existence of special forms of pigments grouped and oriented in special ways, discrete ratios of pigments and cofactors, all of which have been discussed, strongly suggest the existence of a complex set of molecules cooperating in the absorption and conversion of light energy into chemical energy in photosynthesis. Such a structure, involving as it does special relations and juxtapositions of molecules, may properly be described as a solid state system, even though it may not be truly crystalline in character. It has been suggested$^{109}$ that such a structure, bound as it is in lipid and protein, may in certain areas exclude water.

Katz$^{168}$ proposed that semiconduction in a chlorophyll crystal might serve as a mechanism in photosynthesis. Calvin and coworkers$^{47,25,61,62,63}$ have discussed and elaborated the idea of exciton migration to a special
aggregated site followed by photoconductivity trapping and charge separation\textsuperscript{64,63}.

Much of the evidence of solid state phenomena in photosynthetic tissues has been reviewed by Calvin\textsuperscript{63} and by Clayton\textsuperscript{76}. Besides the properties mentioned above, other properties of photosynthetic tissues which might be considered as evidence for solid state phenomena may be listed as follows: delayed light emission (photoluminescence)\textsuperscript{271,5,9,269,53,277,278,279}; thermal luminescence and thermal conductivity\textsuperscript{7,8}; photoconductivity\textsuperscript{7}; photo-induced electric polarizability\textsuperscript{6} and photoconductivity\textsuperscript{7}; photo-induced electron spin resonance signals\textsuperscript{31,80,250,276,277,63,2,284}, when correlated with one or more other "solid-state" properties, and especially when occurring at very low temperatures\textsuperscript{250,276,4}.

Theories based upon the semiconductor light properties of aggregated systems of pigments may be briefly stated. First, the pigments of a photosynthetic unit (a quantasome or chromatophore) are able to transfer energy from one pigment molecule to another via exciton migration. This permits the energy to be transported to the particular group of pigment molecules where the charge separation is to take place. This site contains a special array of chlorophyll molecules in close contact with electron donors (such as cytochrome) and electron acceptors.

Let us consider first a semiconductor model based on an inorganic crystal. Within a special group of chlorophyll molecules which might be thought of as a small crystal, the excited states of the interacting molecules fuse into a single conduction band which is thought to be delocalized over the entire crystal. Thus, a photoexcited electron might be in the conduction band and thus be a conduction electron. In similar
fashion the valence band would be a fusion of ground state levels which might conduct the "hole" (positive charge left by the migration of the electrons). In this system, the conduction electron and the hole could move independently since they have no energy binding them to one another. Alternatively, an exciton in which the electron is coupled to its hole could move through the crystal. An exciton with weak binding between the electron and the hole might become dissociated under the influence of an external electric field or upon thermal collision, thereby giving rise to a conducting electron and conducting hole. An electron or hole can be trapped. This trapping may occur if either species loses some of its energy or interacts with an electron acceptor or electron donor, respectively.

2. ESR measurements

Unpaired electrons such as might be formed by charge separation between electrons and holes, might exhibit paramagnetism, since their spins would be uncoupled. Sogo et al. studied the photoproduction of ESR signals in spinach chloroplasts and in Rhodospirillum rubrum from room temperature down to a -150°C. In chloroplasts, the ESR-induced signal is reversible upon turning off the light at 25°C but decays very slowly or not at all on turning off the light at -150°C. However, the photo-induced ESR signal in chromatophores of R. rubrum decayed rapidly even at -112°C.

Andrees et al. studied the action spectra for ESR production in green plant quantasomes and in R. rubrum chromatophores. In the spinach chloroplast there is a hint of an inflection point on the long wavelength side of the peak, suggesting the possibility of ESR production in special long wavelength absorbing pigments. The ESR signals seen in the whole chloroplasts had two different growth and decay times. These have been separated
into a fast growth and decay found in the quantasomes, and a much slower growth and decay signal found in the colorless protein (preilluminated in the presence of quantasomes).

Commoner has shown the existence of a dark ESR signal with a very broad half width of 19 gauss and 5 hyperfine peaks. This signal was centered at $g = 2.005$. In the light he finds another signal centered at $g = 2.002$ with a half width of 9 gauss and no hyperfine structure. He has obtained similar signals from Chlorella. Though a dark signal, the broad band ESR signal seems to be characteristic of green tissue since it is not found in colorless mutants. He reports on studies of Chlorella cultured in D$_2$O. Both signals are very much narrowed and affected by the presence of D$_2$O instead of H$_2$O, an indication that in each case the unpaired electron is influenced by hydrogen atoms. He concludes that this association precludes the possibility that unpaired electrons which give rise to the two ESR signals are conduction electrons in a semiconductor.

It may well be that the semiconductor model outlined above is not precisely applicable to the quasi-crystal organic lattice of photosynthetic tissue. The probability of such differences have been recognized.$^{76, 83}$ In Calvin's model$^{86}$ the unpaired electron and the hole would not have orbitals delocalized over several pigment molecules but instead would hop from one molecule to another and would not travel for more than 2 or 3 molecules in any event. Under such circumstances it is to be expected that the unpaired electron would be affected by the local environment of protons or deuterons in the pigment molecule with which it is associated.$^{75}$

Kok and Beinert et al.$^{32}$ have studied a particulate fraction from chloroplasts of algae which contains the 700 mµ pigment complex in a more concentrated form, but with only the first photochemical reaction remaining
intact. In the dark it acts as a reversible single electron transfer redox system with $E_0'$ at pH 7 = +0.43 v. The photochemical transfer of an electron of the P-700 pigment complex to some associated by unidentified moiety has been observed from liquid nitrogen temperatures to +50°C.

In particles obtained from red algae, they estimate the ratio of ordinary chlorophyll to P-700 as 100. These particles exhibited an ESR signal in the dark at $g = 2.0025$ with a band width of 7 gauss. The signal can be increased by ferricyanide or by light and decreased by FMN addition. Addition of $10^{-4}$ M ferricyanide oxidized all the pigment and produced a radical concentration similar to that caused by light and equal to the estimated concentration of P-700. Studies of the signal with mixtures of ferro- and ferricyanide gave $E_0'$ value at pH 7 of +0.43 v., in agreement with the value obtained spectrophotometrically.

Calvin and Andrews studied the chemically induced and photo-induced electron spin resonance signals in spinach quantaosomes and chromatophores from R. rubrum. In both bases, a dark ESR signal could be produced at oxidizing potentials of about +0.44 v. Similar ESR signals were produced by light with the difference between light and dark signals falling off in a fashion complementary to the increase in dark signals at more oxidizing potentials. At highly reducing potentials (less than -0.3 v.), the light signal again fell off, although in this region no dark signal could be produced. Presumably the chemical reduction in this case does not lead to an unpaired electron-containing species. It may also be inferred from this experiment that the ESR signal is due to a positively charged pigment (a "hole") rather than a negatively charged pigment, since it is formed by oxidation and not by reduction.
3. Quantum conversion

Considering these results as well as those already reported, Calvin
and Andrees68 were led to propose a mechanism of quantum conversion
similar to those which Calvin has proposed earlier47,25,61,62 and also
to the mechanisms recently proposed by Butler60 and by Clayton79. Fol-
lowing the absorption of light by the pigment, exciton migration is
thought to bring the energy to the site of electron transfer. In bacteria
BChl+ is produced and the electrons are transferred to an electron ac-
ceptor at a high reduction potential. The charge on the BChl+ ion would
then migrate by hole migration to a site where it may recover its electron
from a donor such as a cytochrome.

In green plants a similar process would occur, presumably involving
the oxidation of a long wavelength form of chlorophyll such as Kok's
P-700, and the transfer of an electron from this pigment to the high
potential acceptor, followed by the migration of the hole in the P-700
pigment array to the donor such as cytochrome. One difference from other
hypotheses is the suggestion by Calvin and Andrees66 that the primary photo-
chemical act results in a charge separation corresponding to nearly the
entire energy available in the quantum or at least to that available in
the final excited state prior to charge separation. For a one electron
transfer, which this appears to be, this would be 1.4 v. for 900 nm light
(bacterial photosynthesis) and 1.7 v. for P-700 (green plant photosynthesis).
The primary electron acceptor would thus have a reduction potential of ap-
proximately -0.9 v. and -1.2 v. respectively. This is more than half a volt
stronger than required to reduce such substances as hydrogen ion, ferridoxin,
lipoic acid, and pyridine nucleotide. Thus, one or more ATP molecules
could be formed in the process of dark electron transport from the primary
electron acceptor to these cofactors, provided a mechanism for such phosphorylation exists (see discussion in sec. III. B. of Nishimura's results).

Calvin and Aronson propose that in green plants a second chlorophyll system undergoes similar excitation and exciton migration to the site of electron transfer. In this case, however, what is needed is the production of an oxidizing agent sufficiently strong to oxidize water to molecular oxygen. Calvin and Aronson suggest that in this case the primary quantum conversion act involved the electron transfer from this donor to the pigment and a subsequent migration of the electron through the chlorophyll system to the site at which it is given up to the electron acceptor. In this case, presumably, the electron donor would have a redox potential greater than +1 v. and the electron acceptor a potential somewhat more negative than 0 v. Electrons would then flow by a series of dark reactions between the two chlorophyll systems.

V. THE OXIDATION OF WATER

Attractive though this and other such schemes are, it must be admitted that although there is a great body of evidence for the process just described occurring in the formation of a strong reducing agent, there is at present very little evidence for this or any other specific mechanism for the oxidation of water. All that can be said about the oxidation of water from a physical standpoint is that it can be separated by chemical and kinetic means from the other light reaction, that it seems to require slightly shorter wavelengths absorbed sometimes by a different pigment system, it does occur in a particulate system, and there is the need for separation of the products of the primary light reaction.
Chemically we know that this transfer of electrons from water requires manganese, is inhibited by such substances as DCMU and DCMU, is affected by aging and disruption of the particulate system more quickly than the other light reactions, and thus far has not been characterized by any temperature independent reactions. Of course we cannot expect the oxidation of water to take place at temperatures significantly below 0° C. However, it might be expected that the primary light reaction, even at low temperatures might manifest itself by some type of spectral change. The reductive changes due to the shorter wavelength light reaction described by Witt (type 2b) are temperature dependent.

Several reports have appeared concerning the sudden evolution of O₂ upon illumination with the light wavelengths absorbed by pigments associated with water oxidation, as well as evidence for oxygen uptake upon illumination with the light absorbed by the long wavelength reaction. These transient phenomena suggest perhaps that there are dark reactions which prepare water for its oxidation. This might have been predicted on energetic grounds considering the difficulty of oxidizing water. Quite conceivably, water is activated in some way, perhaps through reactions which use ATP, or by other steps utilizing the energy of dark reactions. For example, if more ATP is made by a long light wavelength reaction than is required for the biosynthetic reactions of carbon reduction, some ATP might be utilized in the oxidation of water.

There is far more than adequate energy in red light (680 μm) to bring about the transfer of one electron from water to a cofactor with a redox potential of 0 v. If water is activated for its oxidation, it may mean that there is a photochemical reaction in which one quantum of light energy
brings about the net transfer of two electrons from water to such a cofactor. The 40 Kcal per einstein available in red light could, in a very efficient process, bring about the transfer of two moles of electrons through a potential difference of 0.8 v. With some activation of the water by dark reactions, the required potential difference for the transfer of electrons from water to a cofactor such as cytochrome could perhaps be lowered to 0.6 v.

A two electron transfer per light quantum might be visualized either as: a) a light-induced one-electron transfer through very high potential difference followed by a thermal (dark) one-electron transfer to form stable compounds, or b) a one-step photochemical reaction causing directly the transfer of two electrons. In either case, the primary reaction may not involve water itself, but may result in the formation of an oxidant of sufficiently high oxidizing potential to oxidize water, or an activated form of water. It has also been suggested\(^\text{23}\) that \(\text{Mn}^{4+}\), perhaps a chelated analogue of cytochrome, might oxidize water and transfer two electrons photochemically to some acceptor. This idea is made attractive by the strong oxidizing potential of \(\text{Mn}^{4+}\), the ability of \(\text{Mn}^{4+}\) to undergo two-electron oxidation reaction, and the fact that it could form chelated compounds of the cytochrome type.*

Yet another possibility is a photochemical formation of a molecule of oxygen, \(\text{O}_2\), with one light quanta. In this four-electron transfer process an average of only some 0.4 v. per electron would be available from quantum conversion at best. In theory, the 42 Kcal available in an einstein of 680 mp light would be sufficient to transfer four electrons from water to a mole of cytochrome with a redox potential of

*Unpublished work from this laboratory by P. Loach and A. Yamamoto.
0.4 v. \[4 \times 23.07 \times (0.8 - 0.4) = 37 \text{ Kcal}\]. In fact, considerable activation of the water by dark reactions, perhaps utilizing ATP from the other photochemical step, would probably be needed.

At this point should be mentioned the observations of Warburg et al.\textsuperscript{301,300,299}. Burk and Warburg\textsuperscript{55} had reported that the true quantum yield of oxygen evolution in photosynthesis is one, but that \(3/4\) of the reaction product is combusted again in a dark reaction involving the uptake of oxygen. The claim of a one-quantum requirement for oxygen evolution has been convincingly refuted by Kok and Spruit\textsuperscript{100}, and Kok\textsuperscript{176} has recently reviewed evidence against this and other manometrically determined quantum requirements reported by Warburg et al. However, Warburg\textsuperscript{299} has gone on to elaborate a hypothesis in which he proposes a one-quantum photoreaction between a chlorophyll-carbon dioxide complex and carbon dioxide, which is supposed to give oxygen gas and reduced carbon, with \(2/3\) of the reduced carbon subsequently reacting with oxygen to form \(\text{CO}_2\), while liberating energy which is utilized to regenerate the chlorophyll-\(\text{CO}_2\) complex. (This last reaction takes 20 min.).

In experimental support of this theory, Warburg and Krippel\textsuperscript{301,300} report the stimulation of oxygen evolution in the Hill reaction by \(\text{CO}_2\).

This \(\text{CO}_2\) effect on the Hill reaction has been investigated and partially confirmed by Stern and Vennesland\textsuperscript{292,266,265} and by Abeles et al.\textsuperscript{1}. Though Stern and Vennesland observed a \(\text{CO}_2\) effect on oxygen evolution from chloroplasts from a variety of plants, the rates, even with \(\text{CO}_2\), ranged from 16 to 70 microliters of oxygen/hr/mg chlorophyll, compared with 600 microliters of oxygen/hr/mg chlorophyll possible for
the Hill reaction with chloroplasts. Abeles et al. did obtain CO₂ stimulation of the Hill reaction with these higher rates of oxygen evolution. The effect was measured both with manometry and by using a mass spectrometer (which obviates many of the difficulties of manometry). In their case, however, augmentation of the Hill reaction was found with kohlrabi chloroplasts but not with sugar beet chloroplasts. With kohlrabi the CO₂ effect was reduced to half by the presence of phosphate and variable in the presence of sucrose. This variability in the CO₂ effect suggests that it is not a general phenomenon like the Hill reaction and not involved in the primary steps in oxygen evolution in photosynthesis. A tracer experiment with C¹³ gave negative results for the possible participation of CO₂ in the Hill reaction.

In summary, chemical evidence does not at the present time allow us to decide whether the net number of electrons transferred by one quantum of light from water to the electron donor for the other light reaction is one, two or four. The overall quantum requirement for the transfer by two light steps of four electrons from water to TPN⁺ to make two molecules of TPNH with the simultaneous formation of at least two molecules of ATP (thus storing 127.2 Kcal as chemical free energy) would be 5, 6 and 8, respectively. If measured quantum requirements can tell us anything (sec. II. C. 2) they suggest that the quantum requirement is not less than about 7. Possibly the quantum requirement might be six for the process just described, with additional quanta used to permit some cyclic photophosphorylation to provide additional ATP for biosynthetic reactions. The possibility of transfer of one or two (but not four) electrons from water to intermediate cofactor by one light quantum is thus permitted by quantum requirements of 6 or 7 respectively.

It is conceivable that in far red light alone, transfer of electrons
from water to the intermediate cofactor (such as cytochrome) could be a completely dark reaction, utilizing only ATP from the P-700 reaction, plus perhaps ATP from oxidative phosphorylation in the chloroplast. (See sec. III. B.). Such a mechanism could account for the low quantum low yield (when uncorrected for respiration) and saturation at/ light in-
tensity/O2 production by far red light reported by French110 and by Gowinges125,126.

VI. A PHOTOELECTRON TRANSPORT SCHEME

A. Green plant photosynthesis

In Fig. 1 there is presented a scheme which attempts to correlate and summarize a great many of the ideas which have emerged from the preceding discussions.

In this scheme redox potentials are plotted with the negative potentials at the top and the positive potentials at the bottom. In the case of photoelectron transport, increasingly negative potential (compared with the fixed H2O/O2 starting point) represents increasing amount of energy stored. For the sake of simplicity, only the reduced form of each substance is shown. Thus, water but not oxygen appears at plus 0.8 v. Removal of electrons from water, of course, will produce oxygen.

This scheme contains two light steps. One, mediated by the shorter wavelength light (sometimes described as "accessory pigment"-absorbed light) is a transport of electrons from water to an intermediate electron carrier such as cytochrome. The second light step (P-700) is the transport of an electron from cytochrome to a very high negative potential. The principal path of photoelectron transport is shown by the heavy
Fig. 1. Scheme for photoelectron transport in green plants.
The vertical scale represents increasingly negative redox potentials. Heavy line is principal path of electron flow.
lines and except for the two light steps all other reactions representing electron flow are indicated by downward pointing arrows.

As pointed out by Calvin, the photoreaction (700 μ) leading to the production of the unpaired electron and its subsequent trapping, is apparently reversible all the way to 1° K. Consequently, the energy gap between the chlorophyll hole and the trapped electron must be very nearly equal to the energy available in the absorbed light quantum, which at 700 μ is 40.9 Kcal per einstein (see sec. II. A.) and represents 1.77 v. for a single electron shift. Subsequent to trapping, the electron must find its way to some reasonably stable species which can undergo biochemical coupling reactions. While not fully agreeing with the thermodynamic limitations that have been proposed (see sec. II. C. 1) the reviewer is inclined to feel that there may be some energy gap (ΔE₁) between the trapped electron and this first stable species. This gap might perhaps be thought of as resulting from a kind of thermodynamic limitation on the efficiency with which the energy of a species not yet in thermal equilibrium with its environment can be utilized by chemical reactions at room temperature. Rather arbitrarily, this limitation has been chosen as 75%, ΔH₂, the first stable species, is thus placed at about .4 v. below the energy of the excited electron. Still, there is ample energy in the flow of two electrons from ΔH₂ to PPNR (Fd) for the generation of a molecule of ATP. Subsequently, the PPNR can reduce TPNF. The Chl⁺ hole left behind by this photooxidation of chlorophyll in turn is reduced by an electron from cytochrome f.

The other light reaction utilizing 680 μ light and resulting in the oxidation of water might also be a one-electron photo step as suggested by Calvin and Androes and indicated here by a dashed line. For reasons
already given, particularly quantum requirement measurement, I have shown it as a two-electron step accomplished by a single light quantum, and therefore resulting in the movement of electrons through a potential difference of 0.91 v. As discussed in sec. V, it may be that this photo-step begins with water which has already been activated in some way, or other forms of chemical energy. Presumably by utilizing ATP/ Again it is assumed that there is some limitation on the efficiency with which the energy of the excited electrons may be utilized, and the return of these electrons to a stable product, here indicated as cytochrome b8, is accompanied by an uncoupled energy drop denoted by ΔE0. The subsequent flow of electrons from cytochrome b8 to cytochrome f (probably via a plastoquinone) is accompanied by a coupled formation of ATP.

The result of this flow of electrons via two light reactions is that for two quanta of light at 700 μ and one quantum of light at 680 μ, two electrons are transported from water to PPNR and two molecules of ATP are produced while one is used up, thus resulting in a net formation of one molecule of ATP per two electrons transported. This is therefore a process requiring six quanta per O2 evolved.

Cyclic photophosphorylation is viewed as being for the most part unnecessary in in vivo photosynthesis, although at times a requirement of a ratio of ATP to TPNR greater than one might be encountered for specific biosynthetic pathways and so some cyclic photophosphorylation might then occur in vivo in order to supply the additional ATP.

When only long wavelength light (700 μ) is administered to green plants there may be a pathway, marked "evolutionary link", by which electrons can be made to flow from the water to the donor to the P-700
light reaction by dark reactions only. This pathway would use up more ATP (two molecules) than it would produce (one molecule). Therefore, much of the reducing agent such as PFR would have to be consumed again via oxidative phosphorylation to produce additional ATP, both for carbon dioxide reduction and to keep the electron flow going. In theory, at least, eight quanta of 700 m light could produce a net of two TPNH molecules and two ATP molecules via this route. Of the eight electrons transported by eight quanta, four would be required to cycle all the way back to oxygen, making water and producing eight molecules of ATP in the process. Two more molecules of ATP would be produced during the noncyclic transport of electrons to two molecules of TPN and eight molecules of ATP would be used to bring eight electrons from water to cytochrome f. Though an eight-quantum mechanism in theory, in actuality this pathway would probably be considerably less efficient, since it would not utilize all the mechanism of the photoelectron transport system. Thus, the low light intensity saturation and low quantum yield for oxygen evolution by far red light could easily be explained.110,125,126.

If at low light intensities with red light most of the photoelectron transport followed this path, and if the ATP molecules could be made by respiratory reactions in the chloroplast, then conceivably a "quantum requirement" of four could be calculated, if correction for respiration was made. The "corrected" quantum requirement of Bassham et al.30 extrapolated towards four at zero light intensity.

This one light step pathway may be thought of as an evolutionary link with bacterial photosynthesis, for it might represent the first type of green plant photosynthesis utilizing water to appear in the course of evolution.
B. Bacterial photosynthesis

Fig. 2 shows a similar scheme for photoelectron transport during bacterial photosynthesis. This is a one light reaction pathway similar to the P-700 reaction of green plant system. In this case, however, only 1.38 v. is available, which means that if we once again assume a thermodynamic energy drop (ΔE₃), there will not be sufficient energy to permit the formation of a molecule of ATP with the flow of two electrons from the primary light reaction to PPFR. In this case, however, this ATP formation is not needed, because a molecule of ATP is formed during the flow of electrons from the electron donor to bacterial chlorophyll. Since the electrons do not come from water, there is no ATP requirement for its activation. The difference in maximum wavelength for green plant and bacterial photosynthesis thus becomes understandable. The high measured quantum requirement of bacterial photosynthesis, however, provides an objection to this scheme.

VII. THE CARBON REDUCTION REACTIONS

The carbon reduction pathways and the enzymes catalyzing these pathways have been reviewed in this series²³⁶. The development and description of the carbon reduction cycle in photosynthesis has been described by Bassham and Calvin²⁶ and other biosynthetic reactions leading from the carbon cycle have been recently discussed¹⁵⁹. The cycle, as it has been usually presented, is shown in Fig. 3. It is important to note at this point that this cycle was developed primarily on the basis of kinetic tracer studies of in vivo systems. From such studies it was possible to put together a pattern of cyclic carbon fixation which consists of the following stages:
Fig. 2. Scheme for photoelectron transport in photosynthetic bacteria.
Fig. 3. The carbon reduction cycle (Calvin Cycle) of photosynthesis.
1) Ribulose-5-phosphate is phosphorylated by ATP to give ribulose-1,5-diphosphate.

2) Ribulose-1,5-diphosphate is carboxylated to make a six carbon unstable intermediate which is hydrolytically split to make two molecules of PGA (3-phosphoglyceric acid).

3) 3-Phosphoglyceric acid is first phosphorylated with ATP and then reduced with TPNH to make triose phosphate. At this point the input of energy from the cofactors made by the light is at an end.

4) Subsequent steps accomplish the conversion of five molecules of triose phosphate to three molecules of ribulose-5-phosphate. None of the steps in this conversion require any input of energetic cofactors formed by the light.

Comparing the reactions of the proposed cycle with known isolated enzyme reactions, it is possible to make a tentative assignment of specific enzymes to each of the steps in the carbon reduction cycle. With such an assignment before them, enzymologists began to investigate whether there were sufficient activities in these enzymes and sufficiently wide distribution of the enzymes throughout the plant kingdom for them to be enzymes of the primary carbon reduction cycle of photosynthesis. While adequate amounts and distribution of some enzymes postulated have been found, certain deficiencies have been reported for other enzymes proposed for the cycle. Peterkovsky and Racker reported carboxydismutase (ribulose diphosphate carboxylase) activity in algae extracts which would support somewhat less than the normal rate of CO₂ assimilation in intact cells under natural conditions. They also reported low activities for transaldolase (which is not in the postulated carbon reduction cycle) and
sedoheptulose diphosphate-1-phosphatase as well as fructose diphosphate-1-phosphatase. Richter and later Fewson et al. reported a lack of fructose diphosphate aldolase, the enzyme postulated for the formation of fructose diphosphate from triose phosphate.

It is noteworthy that many of these observations were made on cell free extracts. If one believes the basic formulation of the reductive cycle to be correct, one is led to the suspicion that there may be some organization of the various enzymes and that this organization may be associated with the particulate structure of the chloroplast. One might also be led in this direction by an examination of the observations of Kandler and Gibba, who found an asymmetry in the labeling of hexoses isolated from green plants which had photosynthesized in the presence of \( \text{CO}_2 \). It appears that if hexoses are formed by condensation of two trioses there must be some way in which the two triose phosphates are kept apart so as not to become equally labeled before they condense. This might be accomplished by an organized group of enzymes in which one or the other triose moieties is bound chemically until it is used. However, other purely kinetic explanations are possible.

Another indication of differences between the isolated systems and the in vivo system is found in the kinetics of the formation of PGA from ribulose diphosphate. Bassham and Kirk studying the kinetics of appearance of \( \text{C}^{14} \) in intermediates of the carbon cycle in Chlorella photosynthesizing under steady state conditions, concluded that only one molecule of free phosphoglyceric acid could arise from each carboxylation of ribulose diphosphate. In the isolated enzyme system, as well as in broken chloroplasts, carboxylation of ribulose diphosphate gives two molecules of PGA per molecule of \( \text{CO}_2 \) or of ribulose diphosphate. In intact
algae, when the light is turned off, the transients in PGA concentration indicate that two molecules of PGA are being formed per carboxylation. The formation of less than two free PGA molecule per carboxylation reaction, if true, requires both the in vivo system and light. The requirement for light suggests that the carboxylation is reductive and dependent upon a high energy reducing agent formed by the photoelectron transport system. This makes attractive the suggestion (see sec. IV. A. 2. b) that the carbon reduction cycle system might be located on the opposite side of a lipid membrane from the photoelectron transport system, thus permitting direct high-potential electron flow to the reductive reactions. Some demanding structural requirement also would be indicated if the reductive carboxylation occurs only in vivo. It may be that in the isolation of even whole chloroplasts there is a tendency for the lamellar structures to separate and for the soluble proteins or the carbon reduction cycle to separate from the lipid surface, with which they might be in contact in vivo.

Another explanation for the apparent requirement of the in vivo system might be that only in such a system is a sufficiently high reducing potential maintained in a localized space by means of a very high ratio of reduced to oxidized cofactors. If this is so, and if reductive carboxylation is a real reaction in photosynthesis, it might be possible to demonstrate it in broken systems by using strong reducing agents such as hydrogen and suitable transhydrogenases, provided the enzymic system is still intact.

The postulation of a reductive carboxylation does not explain all
the difficulties previously outlined regarding the inadequacies of enzymic turnover rates for the specific steps in the postulated cycle. Considering the job that has to be done by the photosynthetic organism, namely, the extremely rapid and efficient reduction of carbon dioxide by a cyclic series of biochemical reactions, only one known type of biochemical system seems adequate. This would be a multifunctional enzyme with which the various specific enzymic functions are grouped together in a single organized protein molecule. After the magnificent discoveries by Lynen\textsuperscript{203} of such a system for the biosynthesis of fatty acids, it seems almost required that such an organized system is also involved in the primary carbon reduction reactions of photosynthesis.

The finding that over half the total soluble protein from the chloroplast is represented by a single homogeneous species, called fraction I\textsuperscript{193,204}, is itself an indication of the possibility of a multifunctional enzyme for the carbon reduction cycle. Park and Pon\textsuperscript{231} have studied the colorless supernatant after centrifugation of the green particles from ruptured spinach chloroplasts. An electronmicrograph of some of this supernatant frozen-dried showed mostly protein particles about 100 - 200 Å in diameter. Fraction I protein prepared according to the method of Littleton and Ta'o\textsuperscript{204}, and then studied with the electron microscope, showed identical particles. The colorless supernatant obtained by Park and Pon by centrifugation contains carboxydismutase and is able to convert fructose-6-phosphate to many of the other intermediates of the carbon reduction cycle. Since this preparation is largely Fraction I protein, they suggest that Fraction I protein may contain a number of enzymic sites of the photosynthetic cycle. However, they have
not as yet rigorously purified this fraction and then tested it for the various enzymic activities.

A multifunctional enzyme system, if it is to transfer substrate moieties from one function to another, must have some kind of biochemical handle with which to attach the substrate moiety. In the case of the fatty acid-synthesizing system described by Lynen\textsuperscript{203}, the handles are the sulfhydryl groups. Considering the nature of the reactions involved in the carbon cycle in photosynthesis, and the importance of sulfhydryl groups to enzymic catalysis of reactions involving carbonyl and acyl functional groups, one would expect that one or more of the handles required by the multifunctional enzyme system postulated for photosynthesis must be sulfhydryl.

Another type of handle so far encountered only with the coenzymes thiamine pyrophosphate is the acidic carbon of the thiazole ring. (See Breslow\textsuperscript{48,49} and Krampitz\textsuperscript{182}) Whether in the case of a multifunctional enzyme proposed for CO\textsubscript{2} reduction the handle would be thiamine pyrophosphate itself bound to the enzyme, or a similar grouping of NCS atoms as an integral part of the enzyme, cannot be decided. In the transketolase reactions of the carbon reduction cycle, the carbons numbers 1 and 2 of the ketose monophosphates add to the acidic carbon of thiamine pyrophosphate, forming a thiamine pyrophosphate glycoaldehyde compound. Datta and Racker\textsuperscript{85,86} isolated a "glycoaldehyde-enzyme-intermediate" which must be an enzyme bound thiamine pyrophosphate glycoaldehyde addition compound. Holtzer \textit{et al.}\textsuperscript{144} have isolated thiamine pyrophosphate glycoaldehyde upon incubation of thiamine pyrophosphate with fructose-6-phosphate in the presence of transketolase.
Calvin and Bassham \(^67\) proposed that glycolic acid formed during the carbon reduction in photosynthesis is actually a product of oxidation of the thiamine pyrophosphate glycolaldehyde intermediate of the transketolase reaction. Further, it was suggested that the formation of glycolic acid is an indication of a biosynthetic pathway in which some of the glycolaldehyde thiamine pyrophosphate compound is oxidized by lipoic acid (analogous to the oxidation of active acetaldehyde from the carboxylation of pyruvic acid) and gives, upon subsequent transfer to coenzyme A dihydro-lipoic acid and glycolyl CoA. The glycolyl CoA is then considered to be a useful starting point for further biosynthetic pathways. Also it was supposed that acetyl phosphate (the starting point for fatty acid synthesis) was formed in the chloroplast by the phosphoketolase reaction from glycolaldehyde thiamine pyrophosphate compound.

Now consider the mechanism of the decarboxylation and oxidation of pyruvic acid\(^{132,130,143,238}\). Pyruvic acid reacts with thiamine pyrophosphate by addition of the carbonyl carbon to the carbon atom of the thiazole ring between the nitrogen and sulfur atoms, after which decarboxylation gives an acetaldehyde thiamine pyrophosphate compound. This compound reacts with oxidized lipoic acid to give acetyl hydrolipoic acid and thiamine pyrophosphate.

Suppose that ribulose-1,5-diphosphate could add to thiamine pyrophosphate bound to the enzyme. We must suppose that the thiamine would be such a bound form for addition of thiamine pyrophosphate to isolated carboxydismutase does not stimulate its activity -- it may in fact inhibit. There would result a phosphoglycolaldehyde thiamine pyrophosphate addition compound and a 3-phosphoglyceraldehyde moiety. In a transketolase type
reaction, this aldehyde would be released, but in the case of ribulose there is a different configuration of hydrogen and hydroxyl at the number 3 carbon atom (compared with fructose and sedoheptulose). Besides, the phosphoglyceraldehyde must be kept bound for the subsequent in vitro reaction which is about to be described.

Therefore, let us suppose that the thiazole ring opens and that the phosphoglyceraldehyde moiety transfers electrons to the thiazole carbon by the rupture of the C-S bond of the thiazole ring and simultaneous formation of a phosphoglycerol-S bond. Now the glycolaldehyde carbon bonded to the thiazole carbon can carry a partial negative charge, and carboxylation can take place, analogous to a reversal of the decarboxylation of pyruvic acid. Thus carboxyphosphoglycolaldehyde thiamine pyrophosphate would be formed.

In vitro, or in the dark, with no strong reducing enzyme functional group present, hydrolysis of the phosphoglycerol-sulfur bond (OH⁻ attack) and of the thiamine carboxyglycolaldehyde bond (H⁺) with ring closure to reform the thiazole ring would result in the effective transfer of electrons to the carboxyglycolaldehyde moiety and the formation of two molecules of 3-phosphoglyceric acid.

In vivo in the light, if there were present a strong reducing group such as enzyme disulfhydryl, with a potential of hydrogen (-0.42 v.) it could accept the phosphoglycerol moiety from the thiamine pyrophosphate sulfur (forming sulfhydryl) and hold it as 3-phosphoglycerol-5-enzyme-SH (which could in time react with free phosphodihydroxyacetone to give enzyme-disulfide and fructose-1,6-diphosphate).

The thiamine pyrophosphate sulfhydryl, carboxyphosphoglycolaldehyde
would, if hydrolyzed, give thiamine pyrophosphate and free 3-phosphoglyceric acid. However, kinetic studies indicate that some phosphoglyceric acid must be bound and reduced without equilibrating with the free pool. If the thiamine pyrophosphate were converted to thiamine triphosphate, which has been reported present in plants, this high energy phosphate anhydride could very easily be in position to phosphorylate the carboxy group, forming the acyl phosphate. The conversion of thiamine pyrophosphate to TFP could utilize ATP or even by some high energy intermediate from the quantaosome (photoelectron transport system) be transported directly across the lamellar membrane.

Once the acyl phosphate were formed, the thiazole sulffhydryl could bond to the acyl carbon, releasing inorganic phosphate and forming a seven-membered ring. This compound would then react with another enzyme disulfhydryl, giving back the thiamine pyrophosphate and phosphoglyceryl-8-enzyme-SH, identical with the one already formed. Hydrolysis of this compound would give only phosphoglyceric acid. In vivo, some molecules of this compound would be converted to phosphodihydroxyacetone (DHAP), and others would react with DHAP to give fructose diphosphate as mentioned above. At very short exposures of plants to $^{14}\text{CO}_2$, radioactive carbon in the DHAP pool would be diluted, and the resulting fructose would be more labeled in carbon atom number 4 than in carbon atom number 3.

A reaction of thiamine pyrophosphate with fructose-6-phosphate would give glyceraldehyde thiamine pyrophosphate and 4-phosphoerythryl-8-enzyme-SH. In this case it is the oxidized form of a disulfide function of the enzyme which would accept the aldehyde moiety, and this might be a different functional grouping from the phosphoglyceryl carrier. In any
event, if this mechanism is correct we could now understand the long puzzling fact that phosphoglyceraldehyde and erythrose phosphate, alone among the postulated intermediates of the carbon reduction cycle, are seldom seen in tracer experiments, and have been detected in only minute amounts. Of course, these compounds can be produced from the intermediates discussed here. For example, 4-phosphoerythryl-3-enzyme-SH, if allowed to accumulate, would no doubt give enzyme disulfide and erythrose-4-phosphate, though this reaction would compete with the hydrolysis to give phospho-erythronic acid and enzyme disulfhydryl.

In any event, the foregoing speculative mechanism indicates how an organized multifunctional enzyme system might bring about most or all of the reactions of the carbon reduction cycle, with a very high turnover, and using electrons and perhaps high energy phosphate precursor direct from the photoelectron transport system. Attempts to isolate enzymic activities from such a system would no doubt result in variable and sometimes negative results, depending on the assay, the species, and the isolation technique. It is to be expected that some purified enzymic activities could be isolated and might be associated with smaller protein molecules than the parent complex. It would not be surprising if they would also require cofactors and exhibit sensitivities to inhibitors uncharacteristic of the parent complex, for they would be but fragments of the original, possibly with unprotected functional groups and partial catalytic deficiencies.

A different carbon reduction pathway has been proposed recently by Stiller267. In her postulated pathway, a two carbon sugar (diose) is synthesized de novo from carbon dioxide. It then condenses with
glyceraldehyde phosphate, giving ribulose phosphate which in turn is phosphorylated and carboxylated, making PGA. The PGA is then reduced as in the Calvin cycle to glyceraldehyde phosphate.

The arguments in favor of this proposal rest mainly on three points: 1) The reported lack of sufficient enzymic activity for some steps of the Calvin cycle (discussed above); 2) the Gibbs effect; 3) a belief that the kinetic data with $^{14}\text{C}O_2$ have been incorrectly interpreted.

A thorough discussion of these points is beyond the scope of this review, but a brief comment may be made. 1) Stiller's proposal suggests pathways from $\text{CO}_2$ to two carbon compounds for which there is at present no reported enzymic activity whatever; 2) there are many explanations possible for the Gibbs effect including that given in the previous discussion; also the Gibbs effect can be explained from purely kinetic reasons based upon the Calvin cycle$^{26}$; 3) the kinetic data$^{31,27}$ do not by any means support the idea of a de novo synthesis of diose from $\text{CO}_2$ in the manner proposed. Bassham and Kirk$^{27}$ found only 1.0 to 1.5 $\mu$m of $^{14}\text{C}$ in all unstable products, including cellular bicarbonate, preceding stable compounds in the carbon fixation pathway. This small amount cannot account for the difference in the rate of labeling of PGA carboxyl carbon as compared with the alpha and beta carbon atoms$^{326,23A}$. Many efforts have been made in this laboratory to trap volatile and unstable compounds which might precede the stable products observed by chromatography. Every effort in this direction has produced a negative result. The main unstable or volatile compound appears to be $\text{HCO}_3^-$ or some compound so closely resembling $\text{HCO}_3^-$ as to be indistinguishable by most means$^{152A}$. 
One other subject should be mentioned very briefly—the carboxylation leading to four carbon dicarboxylic acids, especially malic acid and aspartic acid\textsuperscript{253,11\textasciitilde}. This pathway, which can become relatively more important in certain species\textsuperscript{11\textasciitilde}, utilizes PEPA derived from PGA from the cycle. Hence it incorporates three carbon atoms via the cycle for each carbon atom taken in by the second carboxylation reaction. This reaction uses only TPNH from the light and is strongly exothermic. From the data given in Table I, the energy of this reaction can be calculated:

\[ \text{H}_2\text{O} + \text{CO}_2 + \text{PEPA}^-- + \text{TPNH} \rightarrow \text{Malate}^- + \text{TPN}^+ + \text{HPO}_4^{2-} \]

\[ \Delta F' = -12.8 \text{ Kcal} \]

It is interesting to note that this reaction as well as reductive reactions, leading from the cycle to alanine is prominent in preillumination experiments where the carbon \textsuperscript{14} is admitted to the plant just as the light is turned off\textsuperscript{28}. This observation seems to indicate that in these experiments when the light was turned off ATP was exhausted before TPNH.

B. Energetics of the carbon reduction cycle

A list of free energy changes useful in calculating the energetics of the carbon cycle, is given in Table I. Using these equations and values and the free energy of formation of glucose from the elements, one can easily calculate a free energy of formation of 3-PGA\textsuperscript{3} = \( \Theta \) -155.7 Kcal, where \( \Theta \) = the difference in free energy of formation between inorganic phosphate (HPO\textsubscript{4}\textsuperscript{2-}) and that of water. A similar calculation starting with pyruvate\textsuperscript{7} gives a value for the free energy of formation of 3-PGA\textsuperscript{3} of \( \Theta \) -157.5 Kcal, and the average of the two values is \( \Theta \) -156.8 Kcal.

Using similar calculations starting with glucose, one can calculate that the free energy of formation of glyceraldehyde phosphate and of dihydroxyacetone phosphate are \( \Theta \) -102.7 Kcal and \( \Theta \) -104.5 Kcal.
Free energy changes useful for calculations of energetics of carbon reduction cycle

<table>
<thead>
<tr>
<th>Reaction</th>
<th>( \Delta F' ) (Kcal)</th>
<th>Literature Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose + HPO(_4^2^-) (\rightarrow) GSP(^-2^-) + HOH</td>
<td>+3.3</td>
<td>21</td>
</tr>
<tr>
<td>GSP(^-2^-) (\rightarrow) FSP(^-2^-)</td>
<td>+0.5</td>
<td>257</td>
</tr>
<tr>
<td>FSP(^-2^-) + ATP(^-4^-) (\rightarrow) FDP(^-4^-) + ADP(^-3^-) + H(^+)</td>
<td>-4.2</td>
<td>56</td>
</tr>
<tr>
<td>FDP(^-4^-) (\rightarrow) DHAP(^-2^-) + PGA1(^-2^-)</td>
<td>+5.5</td>
<td>206</td>
</tr>
<tr>
<td>DHAP(^-2^-) (\rightarrow) FIA1(^-2^-)</td>
<td>+1.8</td>
<td>205</td>
</tr>
<tr>
<td>PGA1(^-2^-) + HPO(_4^2^-) + TPN(^+) (\rightarrow) 3 PGA-P(^-4^-) + TPNH + H(^+)</td>
<td>+1.7</td>
<td>58 (corrected for DPNH)</td>
</tr>
<tr>
<td>3-PGA-P(^-4^-) + ADP(^-3^-) (\rightarrow) 3-PGA(^-3^-) + ATP(^-4^-)</td>
<td>-4.6</td>
<td>54</td>
</tr>
<tr>
<td>3-PGA(^-3^-) (\rightarrow) 2 PGA(^-3^-)</td>
<td>+1.1</td>
<td>207</td>
</tr>
<tr>
<td>2 PGA(^-3^-) (\rightarrow) PEPA(^-3^-) + HOH</td>
<td>-0.6</td>
<td>207</td>
</tr>
<tr>
<td>ADP(^-3^-) + PEPA(^-3^-) + H(^+) (\rightarrow) Pyruvate(^-1^-) + ATP(^-4^-)</td>
<td>-6.1</td>
<td>56</td>
</tr>
<tr>
<td>CO(_2) + TPNH + Pyruvate(^-1^-) (\rightarrow) Malate(^-2^-) + TPN(^+)</td>
<td>+0.3</td>
<td>185</td>
</tr>
<tr>
<td>ADP(^-3^-) + HPO(_4^2^-) + H(^+) (\rightarrow) ATP(^-4^-)</td>
<td>+7.0</td>
<td>21 (selected average)</td>
</tr>
</tbody>
</table>

FORMATION FROM ELEMENTS:

- Glucose: -219.22, 185
- Fructose: -218.78, 185
- Pyruvate ion: -113.44, 57
- Water: -56.69, 241A
- Glycerol: -118.76, 185
- Sorbitol: -225.31, 185
- CO\(_2\): -92.31, 241A
respectively. Assuming the hydrolysis of glyceraldehyde phosphate and dihydroxyacetone phosphate are accompanied by about -3 Kcal in each case (this seems to be a good average value for this type of hydrolysis; see ref. 21), one calculates the free energies of formation for the free trioses shown in Table II. The ring energy of glucose was estimated from the work of Cantor and Peniston70, who reported 0.024% straight chain form. The ring energy of fructose was estimated from an unpublished measurement by the reviewer of the absorption at 280 m\(\mu\) (carbonyl bond) of very carefully purified fructose which gave 0.28% as the percentage fructose in the straight chain form and a ring energy of -3.3 Kcal.

From the free energies of formation of the straight chain forms of these sugars an empirical assignment of free energies of formation was made as shown in Table II, which also shows the resultant errors between calculated and measured values. Free energies of formation of other sugars of interest have been calculated as shown in the lower part of Table II. Values for the free energy of formation of ribulose-5-phosphate and ribulose-1,5-diphosphate were calculated, assuming free energy changes analogous to those involved in the formation of fructose-6-phosphate and diphosphate (Table I). The free energy of formation of ribulose diphosphate fructose/was in this manner determined to be 2\(\Delta\)G° = 171.0 Kcal. This value plus those in Tables I and II and the free energy of formation calculated for PGA permits calculation of the free energy changes in the following equations.

The energetics of the original carbon reduction cycle and of the variant discussed in VII. A. began with a common reaction, the phosphorylation with ATP of ribulose-5-phosphate:

\[
15) \text{Ru5P}^2 + \text{ATP}^4 \rightarrow \text{RuDP}^4 + \text{ADP}^3 + \text{H}^+ \]
TABLE II
Calculation of $\Delta F$ of formation of some sugars

<table>
<thead>
<tr>
<th>Sugar</th>
<th>$F_1$Maq.</th>
<th>Ring Energy est.</th>
<th>$F_1$Maq.</th>
<th>Calc.*</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-219.22</td>
<td>-4.9</td>
<td>-214.3</td>
<td>-214.2</td>
<td>+0.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>-218.78</td>
<td>-3.5</td>
<td>-215.3</td>
<td>-216.3</td>
<td>-0.1</td>
</tr>
<tr>
<td>DHA</td>
<td>-107.5</td>
<td></td>
<td>-107.5</td>
<td>-107.4</td>
<td>+0.1</td>
</tr>
<tr>
<td>Glycerald.</td>
<td>-105.7</td>
<td></td>
<td>-105.7</td>
<td>-105.3</td>
<td>+0.4</td>
</tr>
<tr>
<td>Glycerol.</td>
<td>-116.78</td>
<td></td>
<td>-118.78</td>
<td>-116.3</td>
<td>+0.5</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-225.31</td>
<td></td>
<td>-225.31</td>
<td>-225.2</td>
<td>+0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Calc.*</th>
<th>St. Chain Forms</th>
<th>Ring</th>
<th>$F_1$Maq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose</td>
<td>214.3 + 36.3 = -178.0</td>
<td>-1.5(est.)</td>
<td>-179.5</td>
<td></td>
</tr>
<tr>
<td>Ribulose</td>
<td>-215.3 + 36.3 = -179.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedoheptulose</td>
<td>-214.3 - 36.3 = -250.8</td>
<td>-3.5(est.)</td>
<td>-254.1</td>
<td></td>
</tr>
<tr>
<td>Erythrose</td>
<td>-105.7 - 36.3 = -142.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From empirical assignments: -CHO -29.0 Kcal; =C=O -27.4 Kcal;
-CH$_2$OH -40.0 Kcal.
In the cycle which has usually been written (Fig. 3) this is followed by the reaction:

16) \( \text{RuDP}^{-4} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2(3\text{-PGA}^{-3}) + 2\text{H}^+ \) \( \Delta F^\circ \) = -12.4 Kcal
and the resulting two molecules of PGA are reduced by the equation:

17) \( \text{H}^+ + 3\text{-PGA}^{-3} + \text{TPNH} + \text{ATP}^{-4} \rightarrow 3\text{PGA}^{-2} + \text{ADP}^{-3} + \text{HPO}_4^{-2} + \text{TPNH}^+ \)
\( \Delta F^\circ = +3.1 \text{ Kcal} \)

The total free energy expenditure from ribulose diphosphate to glyceraldehyde phosphate is the sum of the free energy of reaction 16 plus twice the free energy of reaction 17 or -6.2 Kcal in all. Two TPNH and three ATP molecules were used per CO\(_2\) reduced.

Consider now the energetics of the variant on the cycle discussed above. Let the reducing agent for the carboxylation reactions have the potential of hydrogen. Let the free energy of hydrolysis of TPP equal that of ATP. Assume that 3-phosphoglyceryl-S-enzyme-SH can go with zero free energy change to oxidized enzyme disulfide and 3-phosphoglyceraldehyde.

18) \( \text{RuDP}^{-4} + \text{CO}_2 + 2\text{HS-enz-SH} + \text{TPP}^{-4} \rightarrow \text{TPP}^{-2} + 2\text{PGA}^{-2} + 2 \text{S-enz-S} \)
\( + \text{HPO}_4^{-2} + \text{H}^+ \)
\( \Delta F^\circ = -5.8 \text{ Kcal} \)

Thus the reduction of the molecule of CO\(_2\) via the carbon reduction cycle with this pathway requires two molecules of ATP (reactions 15 and 16) and two molecules of reducing agent.

This free energy change is given for all reactants assumed to be 1 Maq. However, if HPO\(_4^{-2}\) concentration is 10\(^{-3}\) M, then a correction of -4.1 Kcal brings the steady state free energy change for reaction 18 to \( \Delta F^S = -9.9 \text{ Kcal} \). Correction for actual concentrations of intermediates alters the values further and shows that each step has a negative free energy change, as it of course must have if the steps postulated are correct\(^{26,23}\).
The overall efficiency of the cycle as originally proposed is remarkably high. The reduction of carbon dioxide with water and the consequent evolution of oxygen (+113.5 Kcal, reaction 1) was accomplished with the expenditure of three molecules of ATP (33 Kcal) and two molecules of TPNH (105 Kcal), or 138 Kcal free energy in all, for an efficiency of 82%. By the variant pathway just described, using two moles of hydrogen, $H_2$, (2 x 58.7 Kcal) plus two moles of ATP (22 Kcal) or a total of 135.4 Kcal, the energy efficiency is about 84%.

The high efficiency of the carbon reduction cycle is not surprising. The reactions which comprise this cycle are for the most part highly reversible. Thus, the negative free energy change in each step which provides the driving force to cause the reactions to proceed rapidly in the forward direction need not be great. Each step involves the handling of fairly small packets of energy, unlike the primary conversion steps in the photoelectron transport system. We may expect that subsequent biosynthetic reactions leading from the carbon cycle to the synthesis of end products of photosynthesis will likewise be quite efficient for the most part.

VIII. CONCLUSION

I have attempted to review the principal lines of evidence leading to our present concepts of energy capture, conversion, and utilization in photosynthesis. Throughout the whole process of photosynthesis, with all its many parts, the importance of structure and organization to the efficient utilization of energy is evident.

In trying to summarize the vast amount of accumulating information about all aspects of photosynthesis, I have put forth some schemes which in some respects are quite speculative. I am confident that all those
familiar with the literature of photosynthesis will greet these schemes with great skepticism. The initiate to the field would be well advised to do likewise, for nothing is more certain than the fact that theories of photosynthesis are often out of date by the time they are printed. In any event, few workers in the field can agree at any time on any hypothesis. Hopefully, the schemes presented here will provide some stimulation and thought.

Aside from the schemes, a fairly clear picture of the general features of photosynthesis seems to be emerging. Two systems accomplish two stages of photosynthesis. The first system is the photoelectron transport system, and it is highly organized and dependent upon its structure. Absorbing light through its pigments, it accomplishes, perhaps in two steps, the transport of electrons from water to suitable cofactors and the simultaneous formation of ATP. The second phase of photosynthesis involves a system which I believe to be also highly organized. This system utilizes the cofactors from the first stage to bring about the reduction of carbon dioxide via a cyclic series of reactions. I have suggested that this system loses some of its enzymic capacity due to some disruption in its structure when it is isolated according to techniques so far used. What we see in such isolated systems are residual traces of the original activity which may or may not duplicate in all details the in vivo process. It may be that the carbon reducing system of photosynthesis is not so far separated from the photoelectron transport system as we had generally supposed. One of the exciting prospects for the future is the elucidation of the possible structural relationships in the living cell between the various components of the photosynthetic mechanism.
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