thermodynamic forces toward the most probable form, native ribonuclease. Some of the less likely possibilities mentioned above can only be rigorously excluded upon completion of current experiments on the nature of the pairing of half-cystine residues during the lag phase.

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*PHOTOSYNTHETIC PHOSPHORYLATION AND MOLECULAR OXYGEN**

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Oxygen and photosynthesis were first linked about 200 years ago when both were discovered almost simultaneously. The earliest concept of photosynthesis was that of planetary ventilation in which illuminated plants exchanged CO_2 or "bad air" for O_2 or "vital air" (see historical review[1]). A mechanism for this gas exchange was proposed in 1796 by Ingenhousz.[2] Green plants, he suggested, absorb from "carbonic acid in the sunshine, the carbon, throwing out at that time the oxygen alone, and keeping the carbon to itself as nourishment." [2]

For over a hundred years afterward, the view that CO_2 assimilation always involved a liberation of oxygen gas was so firmly entrenched that it was even extended to the dark CO_2 assimilation by chemosynthetic bacteria.[3–5] The idea

of photosynthesis without oxygen evolution seemed a contradiction of terms until the conclusive work of van Niel[6, 7] with photosynthetic bacteria firmly established that, basically, the assimilation of CO_2 at the expense of light energy does not depend on the liberation of oxygen or on the presence of oxygen. Bacterial photosynthesis occurs under strictly anaerobic conditions and includes organisms that are obligate anaerobes (for example, *Chlorobium* and *Chromatium*[6, 7]).

Although molecular oxygen was shown to be unnecessary and often even detrimental to bacterial photosynthesis, its role in the energy transformations that occur during photosynthesis in green plants has gained more attention with time. In 1939, Hill postulated[8] that the consumption of molecular oxygen is necessary for photosynthesis of green plants in order to supply additional energy for CO_2 assimilation. He envisaged "first a reduction of a substance by chloroplasts giving oxygen and then a reoxidation of the reduced substances by part of the oxygen together with a simultaneous reduction of the necessary equivalent of carbon dioxide." [9] In 1951 Burk and Warburg[10] reported the separation of photosynthesis into a light reaction and a dark reaction on the basis of manometric measurements with intact *Chlorella* cells. In the words of Warburg, "in the light reaction, one molecule of O_2 will develop per molecule of chlorophyll . . . whereas during the dark period following the end of illumination it can be observed manometrically . . . that two-thirds of the oxygen gas developed during the light period undergoes a back reaction." [11] This dark consumption of oxygen is, in Warburg and Burk's view, a photosynthetically induced respiration that supplies two-thirds of the needed energy and is, therefore, indispensable for CO_2 assimilation in photosynthesis. As summed up recently by Warburg, "Keine (CO_2) Fixierung ohne Atmung." [12]

A biochemical model for oxygen consumption in photosynthesis in accordance with the views of Hill and Warburg was proposed by Vishniac and Ochoa.[13] They suggested that the ATP used for CO_2 assimilation in photosynthesis is formed by a collaboration between chloroplasts and mitochondria. In their model, chloroplasts reduced pyridine nucleotide in light and ATP was formed when mitochondria reoxidized the reduced pyridine nucleotide with molecular oxygen, by the dark process of oxidative phosphorylation. A similar model for ATP formation has been used in several general schemes of photosynthesis, as, for example, in the one proposed in 1951 by Holzer[14] and in 1954 by Calvin's group (ref. 15, Fig. 7). In all these schemes, oxidative phosphorylation by mitochondria, and hence oxygen consumption, was an essential feature of the mechanism of photosynthesis.

A mechanism of photosynthesis that includes a consumption of molecular oxygen cannot apply to the strictly anaerobic bacterial photosynthesis and leads, therefore, to the inference that the mechanism of energy conversion in bacterial photosynthesis is basically different from that in plant photosynthesis. However, the discovery of photosynthetic phosphorylation in isolated spinach chloroplasts[16] and, shortly thereafter, in cell-free preparations of *Rhodospirillum rubrum*,[17] has pointed to mechanisms for ATP formation at the expense of light energy that are common to green plants and photosynthetic bacteria. A unified concept of photosynthesis in plants and bacteria, based on photosynthetic phosphorylation as the common denominator is now possible and has been presented elsewhere.[18–20]

A key premise in this concept is that, in green plants as in bacteria, photosynthetic phosphorylation is fundamentally independent of molecular oxygen. Since this

has recently been questioned[12, 21] we have re-examined the relation of oxygen to photosynthetic phosphorylation. The purpose of this article is to bring together and add to the evidence which confirms and extends our earlier conclusions that photosynthetic phosphorylation, both in green plants and photosynthetic bacteria, is basically independent of molecular oxygen and hence of respiration.

Cyclic and Noncyclic Photophosphorylation.—Photosynthetic phosphorylation (photophosphorylation) is a term coined in 1954 to describe a light-induced ATP formation by isolated chloroplasts, without the aid of mitochondria and without the consumption of oxygen.[16] The over-all reaction of photosynthetic phosphorylation is represented by equation (1):

$$\mathrm{ADP} + \mathrm{P} \xrightarrow{\text{light}} \mathrm{ATP} \qquad (1)$$

Reaction 1, in which the sole product is ATP, was subsequently designated cyclic photophosphorylation, to distinguish it from a second photophosphorylation reaction by isolated chloroplasts (equation (2)) which was found a few years later[22] and named noncyclic photophosphorylation.[18]

$$\mathrm{TPN} + 2\mathrm{H}^+ + \mathrm{ADP} + \mathrm{P} + 2\mathrm{OH}^- \xrightarrow{\text{light}} \mathrm{TPNH_2} + \mathrm{ATP} + \mathrm{H_2O} + {}^1/_2\mathrm{O_2} \qquad (2)$$

The terms "cyclic" and "noncyclic" refer to the electron flow mechanisms which have been proposed[18, 19] for these two reactions that now jointly constitute the process of photosynthetic phosphorylation. In cyclic photophosphorylation all of the biochemically effective light energy is used for ATP formation. In noncyclic photophosphorylation only a portion of the biochemically effective light energy is used for the formation of ATP; the remainder is used for the formation of a reductant, $\mathrm{TPNH_2}$, and the excretion (evolution) of oxygen. A nonphysiological variant of noncyclic photophosphorylation (reaction 2) is reaction 3, in which TPN is replaced by ferricyanide[22, 23] (represented here by Fe^{3+}).

$$2\mathrm{Fe}^{3+} + \mathrm{ADP} + \mathrm{P} + 2\mathrm{OH}^- \xrightarrow{\text{light}} 2\mathrm{Fe}^{2+} + \mathrm{ATP} + \mathrm{H_2O} + {}^1/_2\mathrm{O_2} \qquad (3)$$

The significance of cyclic and noncyclic photophosphorylation to the over-all mechanism of photosynthesis in green plants is that they supply—aside from oxygen as the excreted by-product—the two products, ATP and $\mathrm{TPNH_2}$, which alone suffice for converting $\mathrm{CO_2}$ into carbohydrates in the dark.[24, 25] Both ATP and $\mathrm{TPNH_2}$ (also oxygen) are formed by noncyclic photophosphorylation (equation (2)) but cyclic photophosphorylation (equation (1)), which supplies only ATP, is also needed for $\mathrm{CO_2}$ assimilation because the ATP formed in noncyclic photophosphorylation is insufficient to convert $\mathrm{CO_2}$ to carbohydrate.[26]

Chloroplast fragments ("grana") do not respire[27, 28] and hence, according to Warburg, would not be expected to fix $\mathrm{CO_2}$ ("Grana atmen *nicht* und fixierien *nicht*" [12]). However, by fractionating isolated chloroplasts we have shown that grana fail to assimilate $\mathrm{CO_2}$ not because they are unable to respire but because they lack the requisite chloroplast enzymes. These are water-soluble and are readily leached out in the preparation of "grana."[29] The soluble chloroplast enzymes, either *in situ* or when extracted from chloroplasts, assimilate $\mathrm{CO_2}$ to the level of carbohydrates and do so solely[24] at the expense of ATP and $\mathrm{TPNH_2}$—

the two compounds formed in chloroplasts by cyclic and noncyclic photophosphorylation (equations (1) and (2)) and not by respiration.

CO_2 assimilation proper in photosynthesis, whether in whole cells[30] or in isolated chloroplasts,[24, 25] consists of the same exclusively dark, enzymatic reactions[31] that are now known to operate in nonphotosynthetic cells.[32-34] There is, so far as we know, no valid experimental basis, for postulating in photosynthesis a peculiar photochemically produced "reducing power" that is capable of direct reduction of CO_2 to the level of carbohydrate by some special mechanism unknown elsewhere in biochemistry (see, for example, ref. 35 and 36). If we abandon the notion of an unspecified, hypothetical "reducing power" and interpret photosynthesis in terms of known biochemical mechanisms, then the unique feature of photosynthesis as a biological process is not CO_2 assimilation—a dark process driven, in both photosynthetic and nonphotosynthetic cells, by ATP and reduced pyridine nucleotide—but the formation of these two compounds by cyclic and noncyclic photophosphorylation.

The discovery of photosynthetic phosphorylation was part of a coincident, broader finding[16, 27] that isolated chloroplasts are capable of carrying out a complete extracellular photosynthesis, i.e., a conversion of CO_2 to starch and sugar, at physiological temperatures and with no energy supply except visible light. The view that chloroplasts are the sites of complete photosynthesis was once widely held without the support of critical experimental evidence and was later abandoned because of evidence to the contrary, only to be formulated anew on the basis of improved methods for isolating functional chloroplasts from leaves (see review[1]).

The discovery of cyclic and noncyclic photophosphorylation by isolated chloroplasts (also of CO_2 assimilation, see review[1]) was confirmed and extended in other laboratories, notably those of Jagendorf,[37-40] Wessels,[41, 42] Vennesland,[43, 44] and Hill.[45] Most of the work has been done with spinach chloroplasts but recently Whatley *et al.*[46] have also demonstrated cyclic and noncyclic photophosphorylation (and CO_2 assimilation) in chloroplasts isolated from several other species of plants.[47]

Of the two photophosphorylation reactions (equations (1) and (2)), the cyclic type (equation (1)) appeared the more basic from the standpoint of a general mechanism of energy transformation in photosynthesis since this reaction was found in representatives of all the different groups of photosynthetic organisms. Williams[50] found photosynthetic phosphorylation in the obligately anaerobic photosynthetic bacteria, *Chromatium* and *Chlorobium*. Cyclic photophosphorylation in algal preparations was found by Thomas and Haans[51] and Petrack and Lipmann.[52] (For a more complete review of literature see ref. 53.)

Special Features of Cyclic and Noncyclic Photophosphorylation.—The unique features of cyclic photophosphorylation are that ATP is formed with no added electron donor and no added electron acceptor. Cyclic photophosphorylation consumes neither respiratory substrate nor molecular oxygen. Both are replaced by light[53] which provides the free energy required for the synthesis of pyrophosphate bonds of ATP. This seemed at first not to be the case in photosynthetic bacteria. Frenkel's cell-free preparations of *R. rubrum* became substrate-dependent after washing; the rate of phosphorylation was doubled on adding α-ketoglutarate.[17] But in subsequent experiments Frenkel[54] ruled out the dependence on an added chemical substrate and once this fundamental point was clarified, the equivalence

of cyclic photophosphorylation in chloroplasts and bacterial particles seemed probable.[55]

The independence of photosynthetic phosphorylation from a respiratory substrate is confirmed by the independence of this process from CO_2 assimilation. Photosynthetic phosphorylation occurs when the enzymes of the reductive carbon cycle are removed by washing the chloroplasts, CO_2 is not supplied to the reaction mixture, and the reaction vessels contain KOH in the center well. This would not exclude a possible *catalytic* participation of CO_2 in photophosphorylation (cf. Warburg *et al.*[12] and Vennesland *et al.*[56]). What can be definitely excluded is that substrate amounts of carbon compound(s) are first synthesized in light from CO_2 and are then used as electron donors for the formation of ATP.

While cyclic photophosphorylation was recognized as a common denominator of all photosyntheses, noncyclic photophosphorylation as represented by equation (2)—a reaction that liberates oxygen—seemed at first destined to remain a special feature of plant photosynthesis, since oxygen is not evolved in bacterial photosynthesis. However, Losada *et al.*[57] (cf. also Duysens *et al.*[58]) have recently separated reaction 2 into two distinct photochemical reactions (*a*) a photooxidation of hydroxyl ions that yields oxygen (equation (4)) and (*b*) a photophosphorylation reaction proper in which the photoreduction of triphosphopyridine nucleotide is coupled with the formation of adenosine triphosphate (equation 5).

$$2A + 2OH^- \xrightarrow{\text{light}} 2A^- + H_2O + {}^1/_2O_2 \qquad (4)$$

$$TPN + 2H^+ + ADP + P + 2A^- \xrightarrow{\text{light}} TPNH_2 + ATP + 2A \qquad (5)$$

$$\text{Sum: } TPN + 2H^+ + ADP + P + 2OH^- \xrightarrow{\text{light}} TPNH_2 + ATP + H_2O + {}^1/_2O_2 \qquad (2)$$

Only the photooxidation of hydroxyl ions (equation (4)) is peculiar to green plants since only green plants seem capable of using OH^- as an electron donor with the resultant excretion of molecular oxygen. Reaction 5, the noncyclic photophosphorylation reaction proper, occurs also in photosynthetic bacteria.[59] Since they use inorganic or organic electron donors, such as thiosulfate or succinate instead of OH^-, oxygen evolution does not occur in the reaction.

Figure 1 depicts the over-all scheme for noncyclic photophosphorylation in green plants that we now envisage. The intermediate *A* in Figure 1 is both the electron acceptor for the first light reaction and the electron donor for the second light reaction (cf. equations (4) and (5)). We have used indophenol dyes as an experimental device to separate the two photochemical reactions. The natural intermediates with which the dyes have interacted have not been identified. They may include cytochrome components[60] or a quinone.[61, 62] The terminal physiological electron acceptor in noncyclic photophosphorylation (B in Fig. 1) is pyridine nucleotide.

Our original scheme for noncyclic photophosphorylation (ref. 22, Fig. 3) envisaged the phosphorylation step as occurring between the unknown reductant (H), formed by the photolysis of water and TPN, whereas our present scheme, based on the electron flow theory,[18, 53] links the phosphorylation step with the oxidation of cytochrome by photoactivated chlorophyll.

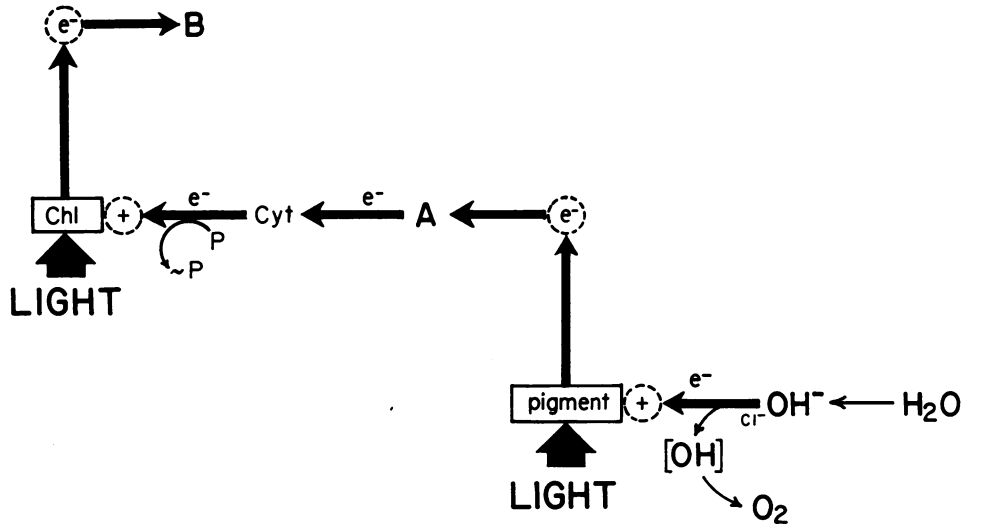

FIG. 1.—*Scheme for noncyclic photophosphorylation of the green plant type.* In the first light reaction, the pigment molecule (see text) becomes excited by the absorption of a quantum of light. The excited pigment donates its high-energy electron (e^-) to an intermediate electron acceptor (A) and accepts an electron from an hydroxyl ion. The oxidation product (OH) of the hydroxyl ion is the precursor of molecular oxygen. In the second light reaction, the chlorophyll molecule (Chl), excited by the absorption of a quantum of light, donates its high-energy electron (e^-) to the terminal electron acceptor (B) and accepts, via the cytochrome system (Cyt), an electron from the reduced intermediate (A^-) formed in the first light reaction. The phosphorylation step is linked with the transfer of the electron from cytochrome to chlorophyll.

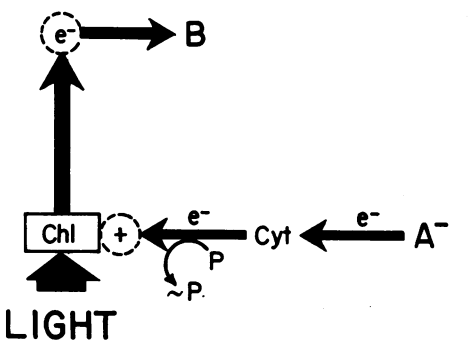

FIG. 2.—*Scheme for noncyclic photophosphorylation of the bacterial type.* The chlorophyll molecule (Chl) becomes excited by the absorption of a quantum of light. The excited chlorophyll donates its high energy electron (e^-) to the electron acceptor (B) and accepts, via the cytochrome system (Cyt), an electron from an external electron donor (A^-). The phosphorylation step is linked with the transfer of the electron from cytochrome to chlorophyll.

TPN reduction in noncyclic photophosphorylation (reaction 2), although normally coupled with, may also be uncoupled from, ATP formation. Trebst *et al.* (ref. 63, Table 1) have shown that ammonia[64] suppresses almost completely the formation of ATP in reaction 2 in light without inhibiting the formation of $TPNH_2$. These results speak against the suggestion of Chance and Olson[65] that the photoreduction of TPN may be driven by ATP, formed perhaps by cyclic photophosphorylation (cf. also Marrè and Forti[66]). The contrary is, in fact, the case. Elec-

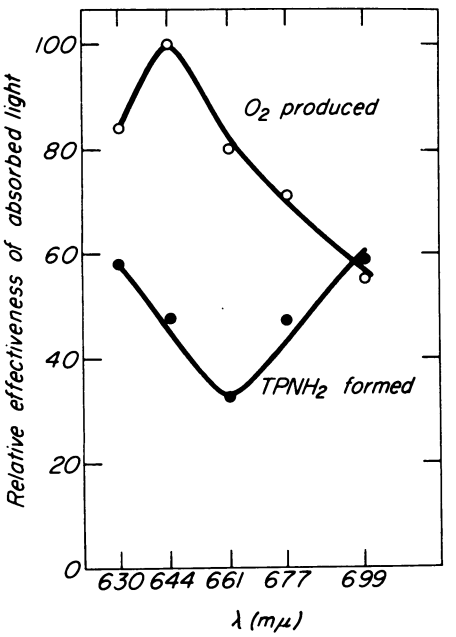

FIG. 3.—*Effectiveness of monochromatic light, in the red region of the spectrum, on oxygen evolution and TPN reduction, occurring as separate chloroplast reactions.*[57] Oxygen was measured manometrically when the photochemical reaction was limited to the photooxidation of OH^- (equation (4)) in a reaction mixture which included, in a final volume of 3 ml, chloroplast fragments (C_{18}) containing 1 mg. of chlorophyll and the following in μmoles: tris/acetate buffer, pH 8, 40; potassium ferricyanide, 15; and 2, 6 - dichlorophenol indophenol (DCPIP), 0.2. TPN reduction (measured spectrophotometrically at 340 mμ) was carried out in a parallel experiment in which the photooxidation of water was blocked by the omission of chloride and the addition of 2×10^{-5} *M* CMU. The electron donor system for TPN reduction consisted of 20 μmoles ascorbate and 0.05 μmoles of 2,3',6-trichlorophenol indophenol (equation (5)). The reactions were run for 15 min at 15°C. Gas phase, nitrogen.

100 on the ordinate scale is equivalent to 0.15 μatoms oxygen evolved per μmole quanta of light absorbed. 58 on the ordinate scale is equivalent to 0.09 μmoles $TPNH_2$ formed per μmole quanta of light absorbed.

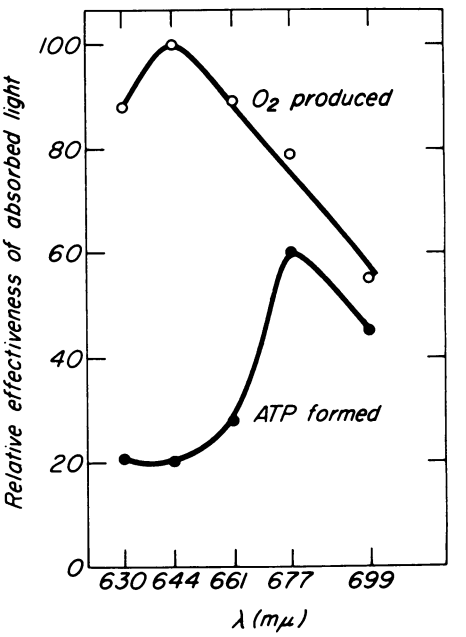

FIG. 4.—*Effectiveness of monochromatic light, in the red region of the spectrum, on oxygen evolution and cyclic photophosphorylation, occurring as separate chloroplast reactions.*[57] Oxygen evolution was measured as described in Figure 3. ATP formation by cyclic photophosphorylation (equation (1)) was measured in a parallel experiment in a reaction mixture which included, in a final volume of 3 ml, chloroplast fragments (C_{18}) containing 1 mg chlorophyll and the following in μmoles: tris/HCl buffer, pH 8.3, 80; $MgSO_4$, 5; ADP, 10; $K_2HP^{32}O_4$, 10; and vitamin K_3 (menadione), 0.3.

100 on the ordinate scale is equivalent to 0.16 μatoms oxygen evolved per μmole quanta of light absorbed. 60 on the ordinate scale is equivalent to 0.10 μmole ATP formed per μmole quanta of light absorbed.

tron transport, resulting in TPN reduction, may proceed without ATP formation, but no photophosphorylation can occur unless it is coupled with electron transport.

Experimentally, it is possible to convert noncyclic photophosphorylation in chloroplasts (equation (2) and Fig. 1) to a "bacterial" type (equation (5) and Fig.

2) by replacing OH^- with an exogenous electron donor at the level of A^-. Reduction of pyridine nucleotide is then coupled with the oxidation of the exogenous electron donor and a simultaneous ATP formation but *without* oxygen evolution.[57]

We have not definitely identified the "pigment" shown in Figure 1 that is involved in the photooxidation of hydroxyl ions. Recent experiments on the effectiveness of monochromatic light on the photochemical reactions of chloroplasts suggest that this may be a special photosynthetic pigment such as chlorophyll *b* or another accessory pigment that is found only in oxygen-evolving organisms, i.e. higher plants and algae, rather than chlorophyll *a* (or the closely related bacteriochlorophyll) found in all photosynthetic cells. (See review of related literature in ref. 57.) In the red region of the spectrum the photooxidation of OH^- (equation (4)) was most effective around 644 mμ, which corresponds to the maximum absorption peak[67] in the red for chlorophyll *b* (Figs. 3 and 4). The effectiveness of monochromatic light in the red region on photoreduction of TPN (equation (5)) and cyclic photophosphorylation (equation (1)) was distinctly different from that on oxygen evolution (equation (4) and Figs. 3 and 4), thus supporting the view that these are different light reactions. The experiments with monochromatic light favor the conclusion that the photochemical reaction which produces oxygen is different from the other photochemical reactions which produce ATP and reduced pyridine nucleotide. The relation of oxygen produced to ATP formation in noncyclic photophosphorylation will now be examined in more detail.

Oxygen as an Excreted By-Product of Noncyclic Photophosphorylation.—The essence of noncyclic photophosphorylation, both in plants and in photosynthetic bacteria, appears to be the formation of ATP which is coupled with an electron transport from an external electron donor to pyridine nucleotide acting as the electron acceptor. As already mentioned, in plants, as in photosynthetic bacteria, oxygen is *not consumed* in these essential aspects of noncyclic photophosphorylation. Oxygen may be *liberated* in this process but only in the specific instance, that is characteristic of plant but not of bacterial photosynthesis, when OH^- is the external electron donor.

This concept is supported by the experimental findings that ATP formation greatly *increases* the rate of oxygen evolution and the concomitant reduction of the electron acceptor, whether it is ferricyanide or TPN[22, 23, 39, 68, 69] (equations (2) and (3)). These results run counter to Hill's original hypothesis that in chloroplasts "the reduced product formed in the light might be reoxidized by molecular oxygen to give a phosphorylating system similar to that in chemosynthetic bacteria."[45] As Hill and Bonner have recently pointed out, "In such a hypothesis one would expect that addition of inorganic phosphate and ADP to the system would result in *decreased* rates of (H) acceptor reduction and of oxygen production"[60] (italics ours).

Hill has accepted the new experimental findings and concluded that "the above... hypothesis has to be abandoned."[60] Warburg,[12] however, has retained the hypothesis of oxygen consumption in energy transformations in photosynthesis and questioned the new experimental facts. Specifically, Warburg *et al.*[12] found that with naphthoquinone sulfonate as the hydrogen acceptor, the stoichiometry of oxygen evolution by chloroplasts is not affected by phosphorylation. Warburg *et al.*[12] suggested that the increase in photoproduction of oxygen as a consequence of

photophosphorylation—first observed by other investigators[22, 68] with ferricyanide (equation (3))—was the result of an uncontrolled change in acidity.

Since the increased production of oxygen that accompanied photophosphorylation in the ferricyanide system was found in solutions that were strongly buffered with tris (hydroxymethyl) aminomethane buffer,[23] it seemed unlikely that the observed effect was the result of uncontrolled pH changes as suggested by Warburg *et al.*[12] Moreover, Davenport[69] has recently found that the rate of photoreduction of TPN, the physiological hydrogen acceptor in noncyclic photophosphorylation (equation (2)), was also greatly increased at two different pH's when a phosphate-accepting system was present.

We have now reinvestigated the effect of photophosphorylation on oxygen evolution by isolated chloroplasts at different hydrogen ion concentrations, using either TPN or ferricyanide as electron acceptors. The pH value of each reaction mixture was checked at the end of the experiment. The results are shown in Figures 5 and 6. With either TPN or ferricyanide as the electron acceptor, we observed a signifi-

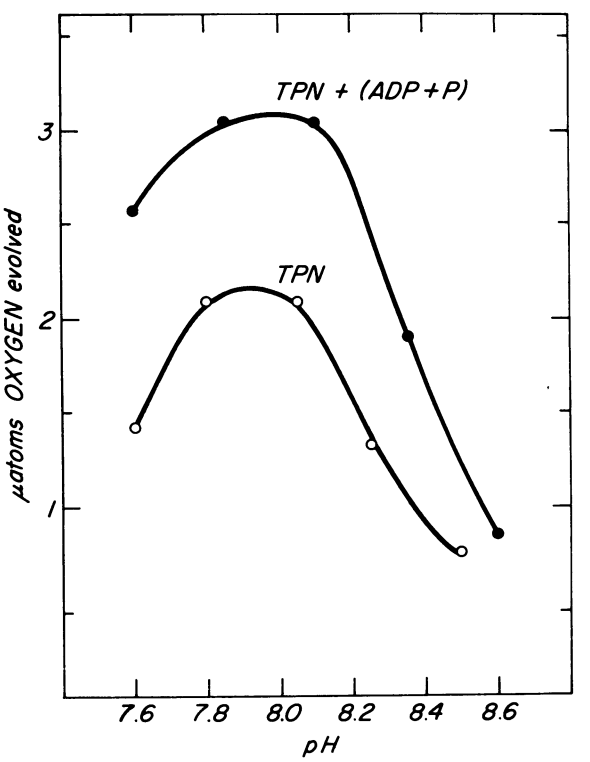

FIG. 5.—*Effect of photophosphorylation at various pH values on oxygen evolution accompanying TPN reduction.* The reaction was carried out in Warburg manometer vessels at 15°C. Illumination, 25,000 Lux for 6 min.; gas phase, nitrogen. In all vessels the reaction mixture contained, in a final volume of 3 ml, 200 μmoles tris-HCl buffer, 4 μmoles TPN, purified TPN-reductase from spinach, and broken chloroplasts (P_{18}) containing 0.2 mg chlorophyll. To the phosphorylating series [TPN plus (ADP plus P)] were added 10 μmoles $MgSO_4$, 10 μmoles potassium phosphate and 10 μmoles ADP. Tris buffer, potassium phosphate, and ADP were each adjusted to the selected pH; the TPN was adjusted to pH 7.5. The pH shift, as determined at the end of the reaction, was found not to exceed 0.1 unit.

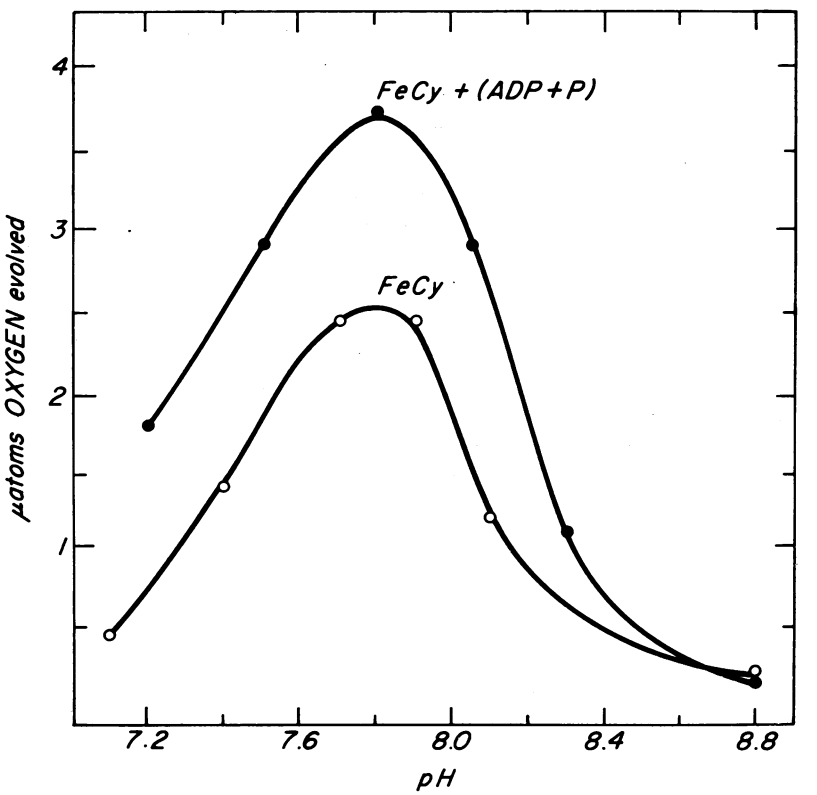

FIG. 6.—*Effect of photophosphorylation at various pH values on oxygen evolution accompanying ferricyanide (FeCy) reduction.* Experimental conditions as described for Figure 5 except that TPN and TPN reductase were omitted and 15 μmoles $K_3Fe(CN)_6$ were added to each vessel.

cantly higher photoproduction of oxygen by isolated chloroplasts when the reaction was coupled with ATP formation. The increase in oxygen evolution occurred over a wide range of pH (cf. Avron *et al.*[68]).

These results confirm and extend those reported earlier[22, 23] and are in harmony with the view that oxygen is an excreted by-product of electron transfer in noncyclic photophosphorylation incidental to the use of OH^- as the electron donor. The electron transfer is more rapid when it is coupled with ATP formation and since OH^- is the electron donor, (compare the anode reaction in the electrolysis of water), more oxygen will be produced per unit of time when ATP is formed simultaneously.

To recapitulate, oxygen is not essential to the two main events of noncyclic photophosphorylation in plants and bacteria: the photoreduction of pyridine nucleotide and the coupled formation of ATP. In fact, oxygen gas is an excreted by-product of photosynthesis in green plants, although not in photosynthetic bacteria, because only green plants can use hydroxyl ions as the electron donor in noncyclic photophosphorylation. When other electron donors are substituted, noncyclic photophosphorylation in green plants resembles that in photosynthetic bacteria: it proceeds without oxygen evolution.[57]

Oxygen as an Electron Acceptor in Noncyclic Photophosphorylation.—When photosynthetic phosphorylation was first discovered it proceeded at a sustained rate only in the presence of oxygen (ref. 16, Fig. 2b). However, since there was no net evolution (as measured by manometric pressure change) of molecular oxygen, we concluded then[16] that oxygen acted as a catalyst in photosynthetic phosphorylation and not as a substrate as in oxidative phosphorylation. A decisive difference, then and now, between photosynthetic and oxidative phosphorylation was the inability of chloroplasts to form ATP in the dark by oxidizing substrates of oxidative phosphorylation with molecular oxygen.[70, 71]

The catalytic action of oxygen may now be explained by the mechanism for noncyclic photophosphorylation shown in Figure 1. As already stated, pyridine nucleotide, the physiological electron acceptor in noncyclic photophosphorylation (B in Fig. 1), may be replaced by non-physiological electron acceptors, such as ferricyanide [compare equations (2) and (3)]. Mehler[72–74] has shown earlier that in a Hill reaction molecular oxygen may act in the same manner as ferricyanide. When oxygen acts as an electron acceptor in a Hill reaction it is usually reduced to hydrogen peroxide. It now appears[44, 75] that the photoreduction of oxygen may also be coupled with the formation of ATP.

The reduction of oxygen to hydrogen peroxide by illuminated chloroplasts is ordinarily a sluggish reaction but it can be greatly accelerated by the addition of one of several catalysts. Among these are FMN[76] and vitamin K_3. On the basis of the mechanism for noncyclic photophosphorylation (Fig. 1), it would be expected that, in the presence of oxygen and catalytic amounts of FMN or vitamin K_3, ATP formation would be accompanied by the reduction of oxygen. Oxygen would then act as the terminal electron acceptor, like TPN or ferricyanide in equations (2) or (3) (see also B in Fig. 1). With oxygen acting as the terminal electron acceptor and OH^- as the electron donor there would be no net evolution of oxygen because the oxygen released is now offset by the oxygen consumed. The $H_2\overset{*}{O}_2$ formed by the reduction of $\overset{*}{O}_2$ (equation (6)) is then decomposed by the endogenous catalase of chloroplasts[12, 75] (equation (7)). ATP is therefore formed without any net consumption of oxygen (equation (8)).

$$\overset{*}{O}_2 + 2H^+ + ADP + P + 2OH^- \xrightarrow[\text{catalyst}]{\text{light}} H_2\overset{*}{O}_2 + ATP + H_2O + {}^1/_2O_2 \qquad (6)$$

$$H_2\overset{*}{O}_2 \xrightarrow[\text{endogenous catalase}]{\text{dark}} H_2\overset{*}{O} + {}^1/_2\overset{*}{O}_2 \qquad (7)$$

$$\text{Sum: } \overset{*}{O}_2 + 2H^+ + ADP + P + 2OH^- \xrightarrow[\text{catalyst}]{\text{light}} H_2\overset{*}{O} + {}^1/_2\overset{*}{O}_2 + ATP + H_2O + {}^1/_2O_2 \qquad (8)$$

The over-all reaction 8 becomes an oxygen-dependent photophosphorylation without a manometrically measurable oxygen consumption. The oxygen consumed ($\overset{*}{O}_2$) is exchanged with the oxygen produced (O_2). We have previously called this an "oxygen-catalyzed cyclic photophosphorylation."[53] We prefer now to abandon this term and to identify the mechanism of this pseudocyclic process

more precisely as a special case of an oxygen-dependent noncyclic photophosphorylation, one in which molecular oxygen acts like ferricyanide in replacing TPN as the terminal electron acceptor. As a result, oxygen becomes reduced to hydrogen peroxide. Superficially, reaction 8 is of the cyclic type since its over-all balance is the same as reaction 1. What characterizes the pseudocyclic reaction 8 and indicates that it has a mechanism different from the truly cyclic reaction 1 is a dependence on oxygen.

The hydrogen peroxide formed in reaction 6 can be trapped with the use of an ethanol-catalase trapping system.[72] In this case reaction 9 would replace reaction 7 and ATP formation would be accompanied by oxygen consumption, as shown by equation (10), which is a sum of reactions 6 and 9.

$$\overset{*}{O}_2 + 2H^+ + ADP + P + 2OH^- \xrightarrow[\text{catalyst}]{\text{light}} H_2\overset{*}{O}_2 + ATP + H_2O + {}^1/_2O_2 \quad (6)$$

$$H_2\overset{*}{O}_2 + C_2H_5OH \xrightarrow[\text{added catalase}]{\text{dark}} 2H_2\overset{*}{O} + CH_3{\cdot}CHO \quad (9)$$

$$\text{Sum: } \overset{*}{O}_2 + 2H^+ + C_2H_5OH + ADP + P + 2OH^- \xrightarrow[\text{catalyst, added catalase}]{\text{light}} 2H_2\overset{*}{O} + CH_3{\cdot}CHO + ATP + H_2O + {}^1/_2O_2 \quad (10)$$

Figure 7 shows that the stoichiometry of these reactions has been experimentally verified. In an atmosphere of oxygen, ATP formation in noncyclic photophosphorylation (*a*) was accompanied by oxygen evolution when TPN or ferricyanide were added in substrate amounts as electron acceptors, (*b*) gave no significant oxygen evolution or uptake when FMN was added in catalytic amounts (equation (8)), and (*c*) was accompanied by oxygen consumption (equation (10)) when catalytic amounts of FMN were added jointly with substrate amounts of ethanol and excess catalase.

These results demonstrate the fundamental independence of noncyclic photophosphorylation of chloroplasts from molecular oxygen. Depending on the composition of the reaction mixture, the same ATP formation has occurred whether oxygen is produced (equations (2) or (3)), consumed (equation (10)) or merely exchanged (equation (8)).

Krogmann[77] has recently investigated oxygen-dependent photophosphorylations using indophenol dyes as cofactors. He concluded that his results could not be satisfactorily explained by the reduction of molecular oxygen to hydrogen peroxide. However, the recent work of Trebst and Eck[75] and our own evidence using ethanol-catalase as a hydrogen peroxide trapping system[72] (Fig. 7) shows that oxygen is indeed reduced to hydrogen peroxide.

Molecular oxygen can also serve as the terminal electron acceptor B (Fig. 1) in an experimentally contrived system in which the use of water as the electron donor for chloroplasts is blocked by inhibitors and an external electron donor is supplied instead. Krogmann and Vennesland[21] have described a system of this kind in which ATP formation was accompanied by the simultaneous consumption of O_2 and of an external electron donor. An apparent "oxidative photosynthetic

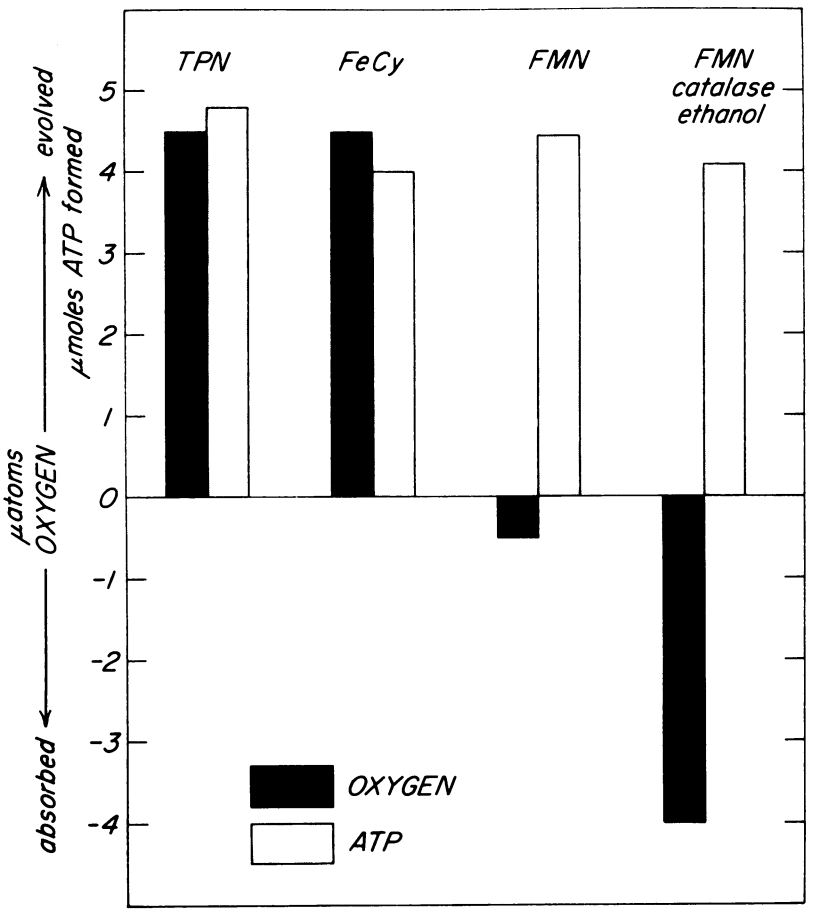

FIG. 7.—*ATP formation accompanied by oxygen evolution, oxygen exchange, or oxygen consumption, in noncyclic photophosphorylation by chloroplasts.* Oxygen was evolved when TPN or ferricyanide was the electron acceptor. In the presence of FMN, oxygen evolution was balanced by oxygen absorption (equation (8)). (We consider the slight oxygen absorption found in this treatment to be within the limits of experimental error.) In the presence of FMN, ethanol and catalase, ATP formation was accompanied by oxygen uptake (equation (10)).

The reaction mixtures contained, in a total volume of 3 ml, the following in μmoles: tris buffer, pH 7.8, 80; $MgCl_2$, 10; ADP, 10; $K_2H^{32}PO_4$, 10; and broken chloroplasts (P_{1s}) containing 0.4 mg chlorophyll. The following were added (in μmoles) where indicated: TPN, 6; $K_3Fe(CN)_6$, 15; FMN, 0.1; ethanol, 600; and crystalline catalase, 1 mg. TPN was added together with purified TPN-reductase from spinach. The experiment was carried out in Warburg manometer vessels, at 15°C. Gas phase, air; illumination, 25,000 Lux for 6 min.

phosphorylation" resulted that resembled oxidative phosphorylation by mitochondria, especially when $DPNH_2$ was used as the electron donor. However, the analogy between such "oxidative photosynthetic phosphorylation" and oxidative phosphorylation by mitochondria appears to be fortuitous. The role of $DPNH_2$ in this chloroplast system was not that of a physiological electron donor but that of a nonspecific reducing agent for the dye, one of several possible reducing agents.

That the consumption of oxygen was artificially induced and was not a physiological component of photophosphorylation is made clear by the observations of Krogmann and Vennesland[21] that "there was ample energy released by the dark oxidation of the DPNH to form the high energy phosphate bonds. Nevertheless, the reaction gave no phosphorylation unless the system was illuminated, even though the light caused no increase in the rate of oxygen consumption." We interpret this example of "oxidative photosynthetic phosphorylation" in chloroplasts as an instance of noncyclic photophosphorylation of the "bacterial type" in which oxygen acts as a nonspecific terminal electron acceptor (B in Fig. 2).

Nonparticipation of Oxygen in Cyclic Photophosphorylation.—In the preceding discussion, noncyclic photophosphorylation in chloroplasts was shown to be fundamentally independent of oxygen in that oxygen was either merely an excreted by-product of photooxidation of hydroxyl ions or a nonspecific electron acceptor. An even more direct demonstration of the independence of photosynthetic phosphorylation from oxygen is afforded by cyclic photophosphorylation. Here oxygen does not take part in the process at all, since, as already mentioned, neither an external electron acceptor nor an external electron donor is involved; oxygen thus cannot participate in the process as a substitute for one or as the by-product of the other. In cyclic photophosphorylation both the electron donor and the electron acceptor are endogenously generated by the light reaction.

Investigations of the mechanism of cyclic photophosphorylation in chloroplasts and bacterial particles have led to the conclusion that the formation of ATP is coupled with a cyclic, "closed circuit" transport of electrons from an endogenous electron donor to an endogenous electron acceptor, through a chain of carriers associated with the photosynthetic apparatus (cf. review[53]). Experimentally, a vigorous cyclic photophosphorylation by chloroplasts *in vitro* requires the addition, in *catalytic* amounts, of an electron carrier which can be either a known component of chloroplasts (vitamin K or FMN) or a nonphysiological agent such as phenazine methosulfate. (Fresh bacterial particles require no addition of external cofactors for vigorous cyclic photophosphorylation, but on aging respond to the addition of vitamin K and PMS[53].) The role assigned to the added electron carriers in cyclic photophosphorylation is illustrated diagrammatically for phenazine methosulfate in Figure 8.

The full identity of the photosynthetic electron carrier system is not yet established but the main components appear to include cytochromes,[18, 53] quinones (a naphthoquinone[78] or a benzoquinone[61, 62]) and flavin compounds.[78, 79] There is thus a parallelism between cyclic photophosphorylation of chloroplasts and oxidative phosphorylation of mitochondria with respect to several components of the electron transport chain although, it will be recalled, not with respect to the electron donor and electron acceptor. Nevertheless, suggestions have come recently, mainly from the laboratory of Vennesland,[21] that photophosphorylation by chloroplasts usually resembles oxidative phosphorylation in mitochondria in being dependent on molecular oxygen. Vennesland *et al.* have recently expressed their position in these words: "It is our opinion that chloroplasts which have not suffered damage to their oxygen-evolving mechanism prefer a cyclic process in which oxygen is evolved when FMN or menadione are cofactors. A truly anaerobic cycling involving neither production nor reconsumption of molecular oxygen *can apparently*

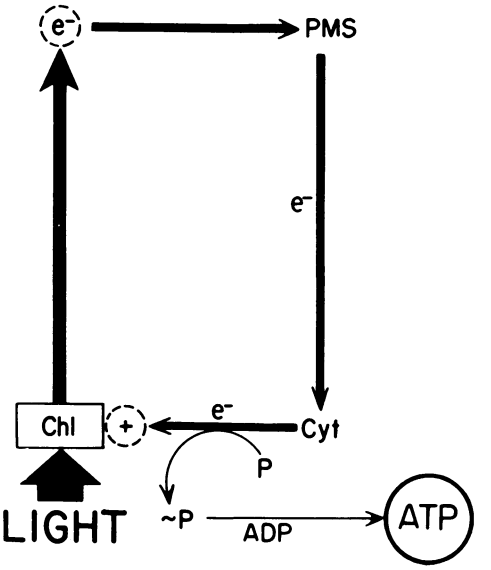

FIG. 8.—*Scheme for cyclic photophosphorylation.* The chlorophyll molecule (Chl) becomes excited by the absorption of a quantum of light. The excited chlorophyll donates its high-energy electron to oxidized phenazine methosulfate (PMS) and accepts an electron from reduced cytochrome (Cyt). The cycle is completed when the reduced PMS donates an electron to the oxidized cytochrome. The phosphorylation step is envisaged as being linked with the transfer of the electron from cytochrome to chlorophyll.

occur also, and is most pronounced with pyocyanine and phenazine methosulfate, less so with vitamin K_3, and hardly at all with FMN" [56] (our italics).

Thus there seems to be general agreement that a "truly anaerobic" cyclic photophosphorylation by isolated chloroplasts occurs in the presence of phenazine methosulfate. The question seems to turn on the role of oxygen in cyclic photophosphorylations catalyzed by vitamin K and FMN. Vennesland *et al.*[56] suggest that these are mainly of the oxygen-dependent type. We consider the participation of oxygen to be a special case of noncyclic photophosphorylation, where oxygen acts as a nonspecific substitute for TPN (Fig. 7). From our point of view, the main interest in vitamin K and FMN as catalysts of photophosphorylation lies not in their ability to catalyze an electron flow in which oxygen participates as a nonspecific electron acceptor (equations (6) and (8)) but rather in their ability to catalyze a truly anaerobic cyclic electron flow in which oxygen does not participate (equation (1)). We consider the anaerobic cyclic photophosphorylation as a common denominator of plant and bacterial photosynthesis and we regard the oxygen-dependent pseudocyclic type as merely a special case of noncyclic photophosphorylation in chloroplasts.

The experimental conditions required for demonstrating an anaerobic, cyclic photophosphorylation catalyzed by vitamin K and FMN differ from those required for demonstrating the oxygen-dependent, pseudocyclic photophosphorylation. Aside from the absence of oxygen, the effective operation of the anaerobic system requires higher concentrations (catalytic versus "microcatalytic") of vitamin K and FMN, and, particularly for the anaerobic FMN system, high concentrations of the chloroplast material in relation to illumination. The need

for a relatively high concentration of chloroplast material suggests that the anaerobic FMN and vitamin K systems require more chloroplast factor(s) than the pseudocyclic oxygen-dependent system. No similar requirements were observed for the anaerobic phenazine methosulfate system.

Despite the similarity of the over-all reactions (equations (1) and (8)), the anaerobic, cyclic photophosphorylations catalyzed by vitamin K and FMN can be distinguished experimentally from their oxygen-dependent, pseudocyclic analogues. The latter depend on the photoproduction of oxygen (equations (6), (7), and 8)) which requires chloride[53, 80] and are sensitive to two well-known inhibitors of oxygen evolution in photosynthesis, CMU[81] (*p*-chlorophenyldimethyl urea) and *o*-phenanthroline.[80]

Table 1 shows that, under experimental conditions favorable for each system,

TABLE 1

EFFECT OF CHLORIDE ON PHOTOPHOSPHORYLATION IN NITROGEN OR AIR

Treatment	μmoles ATP Formed	
	Minus chloride	Plus chloride
Nitrogen, FMN	5.1	5.7
Nitrogen, vitamin K_3	9.7	9.9
Air, FMN	0.5	6.1
Air, vitamin K_3	0.4	5.5

In the nitrogen series the illumination was 2,000 Lux for 30 min, and the reaction mixture included, in a final volume of 3 ml, chloroplast fragments (C_{18}) containing 2.5 mg chlorophyll and 0.3 μmoles of FMN or vitamin K_3. In the air series, the illumination was 50,000 Lux for 30 min and the reaction mixture included, in a final volume of 3 ml, chloroplast fragments (C_{18}) containing 0.2 mg chlorophyll and 0.003 μmoles of FMN or vitamin K_3. Other components of the reaction mixture were, in μmoles: tris buffer, pH 8.3, 80; $K_2H^{32}PO_4$, 15; ADP, 15; and $MgSO_4$, 5. Chloroplasts were prepared in 0.5 *M* sucrose and chloride-free reagents were used. In the plus chloride series 10 μmoles NaCl were added. Nitrogen gas was purified by passing through a zinc-methylene blue solution (A. T. Jagendorf, personal communication).

the omission of chloride had scarcely an effect on the anaerobic cyclic photophosphorylation with FMN or vitamin K, but severely inhibited their oxygen-dependent, pseudocyclic counterparts. Likewise, CMU (also *o*-phenanthroline[53]) inhibited the oxygen-dependent, pseudocyclic photophosphorylation but had relatively little effect on their anaerobic, cyclic counterparts (Table 2).

TABLE 2

EFFECT OF CMU ON PHOTOPHOSPHORYLATION IN NITROGEN AND AIR WITH HIGH CHLOROPHYLL AT LOW LIGHT INTENSITY

Treatment	μmoles ATP Formed			
	Nitrogen		Air	
	Control	CMU	Control	CMU
FMN	7.0	6.6	4.2	1.7
Vitamin K_3	8.1	7.6	3.3	1.3
PMS	2.7	2.4	2.9	2.8

The reaction mixture included, in a final volume of 3.0 ml, chloroplast fragments (C_{18}) containing 2.5 mg chlorophyll and, in μmoles: tris buffer, pH 8.3, 80; $MgSO_4$, 10; $K_2H^{32}PO_4$, 15; ADP, 15; and, where indicated, FMN, 0.3; vitamin K_3, 0.3, or, phenazine methosulfate (PMS), 0.1. The final concentration of CMU was 2×10^{-6} *M*. The reaction was run for 30 min at an illumination of 2,000 Lux. The nitrogen gas was purified as described in Table 1.

When some experimental conditions are unfavorable for anaerobic, cyclic photophosphorylation, the high affinity of reduced FMN or vitamin K_3 for molecular oxygen may still give rise to a pseudocyclic, oxygen-dependent photophosphorylation. Thus, we have observed in the presence of 10^{-4} *M* FMN or vitamin K_3 at a high light intensity, and with a relatively low concentration of chloroplast material (0.25 mg chlorophyll/3 ml) that photophosphorylation became oxygen-dependent, even when the reaction was started in purified nitrogen gas that was

TABLE 3

EFFECT OF CMU ON PHOTOPHOSPHORYLATION IN NITROGEN AND AIR WITH LOW CHLOROPHYLL AT HIGH LIGHT INTENSITY

Treatment	μmoles ATP Formed			
	Nitrogen		Air	
	Control	CMU	Control	CMU
FMN	5.6	1.5	5.4	1.1
Vitamin K_3	8.6	4.7	5.3	1.0
PMS	9.3	9.9	8.9	8.6

Experimental conditions as in Table 2 except that less chloroplast material (C_{18}) was used (0.25 mg chlorophyll) and the illumination was increased to 20,000 Lux. Reaction time, 15 min.

treated to remove oxygen impurities (Table 3). Phosphorylation became oxygen-dependent when the catalytic amounts of FMN or vitamin K_3 were photochemically reduced by chloroplasts. An equivalent amount of oxygen was then liberated which was sufficient to sustain an oxygen-dependent photophosphorylation (cf. Trebst and Eck[75]). The oxygen dependence of the system was determined by blocking the oxygen evolution reaction with CMU. ATP formation then became strongly inhibited. (It seems likely that a similar dependence on photochemically generated oxygen accounts for the results reported by Vennesland *et al.* in ref. 56, Table 2.) Photophosphorylations catalyzed by phenazine methosulfate were resistant to inhibition by CMU and *o*-phenanthroline,[53] regardless whether the reaction was carried out under conditions favoring the anaerobic cyclic, or the oxygen-dependent pseudocyclic pathway (Tables 2 and 3).

Concluding Remarks.—From the standpoint of cellular physiology, the role of oxygen in ATP formation in photosynthesis provides an interesting contrast to the role of oxygen in ATP formation in respiration. The high thermodynamic efficiency of respiration, and its superiority over fermentation in using the free energy of the degradation of foodstuffs for synthesis of the pyrophosphate bonds of ATP, is made possible by the key role that molecular oxygen plays as the terminal electron acceptor in oxidative phosphorylation by mitochondria. Only with oxygen can the oxidation of foodstuffs be complete (to CO_2 and water) and their free energy released in full for metabolic purposes. In the last seven years biochemical investigations of photosynthesis in cell-free systems revealed no such role for oxygen in ATP formation by chloroplasts and bacterial particles. Here, ATP formation occurs in cyclic photophosphorylation under strictly anaerobic conditions where the participation of oxygen is excluded. In noncyclic photophosphorylation, oxygen may replace TPN as a terminal electron acceptor, but, as discussed later, this results in a loss instead of a gain in the yield of physiologically useful energy.

The strictly anaerobic character of cyclic photophosphorylation in bacterial preparations needs no elaboration since photosynthesis in bacteria is firmly established as an anaerobic process.[7] In isolated chloroplasts, anaerobic cyclic photophosphorylation has been demonstrated with phenazine methosulfate as a catalyst, and also with vitamin K_3 and FMN. With the latter, a far more rigid control of experimental conditions was required than with phenazine methosulfate.

Special experimental safeguards are needed for demonstrating in chloroplasts an anaerobic cyclic photophosphorylation catalyzed by vitamin K_3 and FMN, because these substances, when reduced photochemically, readily react with small concentrations of molecular oxygen. Phenazine methosulfate also reacts readily

with oxygen, but its affinity for oxidized cytochromes[82] seems to be greater than for oxygen; it is thus able to catalyze a cyclic electron flow (Fig. 8) even in the presence of molecular oxygen.

Noncyclic photophosphorylation consists of ATP formation that is coupled with the reduction of pyridine nucleotide and the oxidation of an exogenous electron donor. The process, which was originally found in isolated chloroplasts,[22] has now also been demonstrated in bacterial particles.[59] The difference between noncyclic photophosphorylation in the two cases centers on the electron donor. For isolated chloroplasts the electron donors are hydroxyl ions which on oxidation form oxygen; this accounts for oxygen evolution in plant photosynthesis. Photosynthetic bacteria cannot use hydroxyl ions but only such electron donors as organic acids or reduced sulfur compounds[6, 7]; this accounts for the absence of oxygen evolution in bacterial photosynthesis. Instead of oxygen, oxidized bacterial electron donors are formed, which may be further metabolized or may accumulate and yield, for example, elemental sulfur.[6]

The role of oxygen in photosynthesis is, therefore, only incidental and not vital to the energy transformation reactions, as it is in respiration. Oxygen is evolved because it is an excreted product of the oxidation of hydroxyl ions—the electron donor in noncyclic photophosphorylation by chloroplasts. Experimentally, when another electron donor is substituted for hydroxyl ions, noncyclic photophosphorylation in chloroplasts becomes converted to the bacterial type: ATP formation is coupled with pyridine nucleotide reduction but no oxygen is evolved.[57]

In a cell-free system, oxygen evolution by chloroplasts will be observed manometrically only under certain conditions. First, the acceptor for the electrons "expelled" from chlorophyll in the primary photochemical reaction must not react preferentially with the components of the photosynthetic electron transport chain (cf. Trebst and Eck[83]). If it does react, as in the case of phenazine methosulfate, then cyclic photophosphorylation, in which oxygen is not evolved, will result. Second, the electron acceptor must not be easily oxidized by molecular oxygen. This is often, but not always, related to redox potential. Thus, TPN with a redox potential, at pH 7, of $E'_0 = -324$ mV shares this property with ferricyanide ($E'_0 = 360$ mV) and p-benzoquinone ($E'_0 = 293$ mV). In the intact plant, oxygen is normally evolved during photosynthesis because the $TPNH_2$ formed in noncyclic photophosphorylation is not reoxidized by the oxygen formed, but is used up in carbon assimilation.

A special case of noncyclic photophosphorylation by isolated chloroplasts occurs when oxygen acts as the terminal electron acceptor and becomes reduced to hydrogen peroxide. The photoreduction of oxygen by isolated chloroplasts, discovered by Mehler,[72] has been investigated by Brown and Good[84] and Good and Hill,[76] who suggested that it is "an artifact" [84] and that "the direct reduction of oxygen does not ordinarily occur to a significant extent *in vivo*." [76]

In a cell-free system containing microcatalytic amounts of FMN or vitamin K_3, oxygen may replace TPN as the electron acceptor in noncyclic photophosphorylation by chloroplasts and give rise to an oxygen-dependent, pseudocyclic photophosphorylation. The substitution of oxygen for TPN is analogous to the substitution of ferricyanide for TPN. ATP is still formed in each case, but the reductant produced is no longer $TPNH_2$ but either ferrocyanide, or, when oxygen

is the electron acceptor, H_2O_2 or water. Thus, the intervention of oxygen in noncyclic photophosphorylation results in a loss of biochemically useful energy which would have normally been used for forming a strong reductant, i.e. $TPNH_2$. When oxygen is the electron acceptor and water is the final product of oxygen reduction (equations (6) and (7)) the energy loss is equivalent to a redox potential of 1139 mV (difference between $E_0' = 815$ mV for the water/oxygen system and $E_0' = -324$ mV for the $TPN/TPNH_2$ system). If hydrogen peroxide is allowed to accumulate as the final product of the oxygen reduction, the computed energy loss would be equivalent to a redox potential of 1006 mV.

* The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; P, orthophosphate; TPN, $TPNH_2$, oxidized and reduced forms of triphosphopyridine nucleotide; FMN, riboflavin phosphate (flavin mononucleotide); PMS, phenazine methosulfate.

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NONCYCLIC PHOTOPHOSPHORYLATION IN PHOTOSYNTHETIC BACTERIA

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Noncyclic photophosphorylation is the formation of adenosine triphosphate in a light-induced electron flow from an external electron donor to an electron acceptor such as pyridine nucleotide. In photosynthesis of green plants the electron donors are the hydroxyl ions of water and hence the over-all reaction also produces, aside from ATP and PNH_2,[1] molecular oxygen, which is liberated when OH^- is photooxidized (see Fig. 1 in preceding article[2]).

Photosynthetic bacteria cannot use OH^- as an electron donor for photosynthesis but use a variety of reductants stronger than OH^-, among them hydrogen gas, succinate, or thiosulfate. With hydrogen gas no input of light energy is required for the reduction of pyridine nucleotide, since hydrogen gas, with the aid of bacterial hydrogenase, can reduce pyridine nucleotide in the dark.[3] The contribution of light to bacterial photosynthesis is then limited to the formation of ATP by cyclic photophosphorylation.[4, 5] With other electron donors such as, for example, succinate, the additional input of energy that is necessary to reduce pyridine nucleotide is provided by light. Thus, in bacterial photosynthesis with succinate as the electron donor, light energy serves a dual purpose: it supplies ATP by cyclic photophosphorylation and, by photooxidizing the electron donor, it also provides electrons for reducing pyridine nucleotides by a noncyclic electron flow mechanism.

Photoreduction of pyridine nucleotide (DPN) by cell-free preparations of *Rhodospirillum rubrum*[6, 7] and *Chromatium*[3] has been observed but attempts to find a simultaneous ATP formation, analogous to the noncyclic photophosphorylation in chloroplasts, led to negative results.[7, 8] As pointed out by Vernon and Ash,[8] a demonstration of ATP formation by a noncyclic electron flow mechanism is more difficult in chromatophores than in chloroplasts because in chromatophores it cannot be experimentally distinguished from a simultaneous ATP formation by cyclic photophosphorylation. In chromatophores, a vigorous cyclic photophosphorylation occurs under anaerobic conditions and without added cofactors,[3] whereas in chloroplasts cyclic photophosphorylation is always under experimental