simpler to publicize conclusions alone, and have them accepted not because their factual origin is fully understood but because they carry the authority associated with science.

It seems to me that we dare not take this easy way out. Unless the public has sufficient information to provide a reasonable basis for independent judgment, the moral burden for the future effects of nuclear testing will rest on some smaller group. And no such group alone has the wisdom to make the correct choice or the strength to sustain it. Unless the public is made aware of the gaps and the uncertainties in our present knowledge about fallout, we cannot expect it to support the expensive research needed to minimize them. Without public understanding and support, no government policy can long endure.

Here then is our challenge. Can we, as scientists, with the help of our professional organizations, find a way to inform the public about these great issues? The raw material for such an educational campaign is available in the voluminous report of the Congressional hearings. We can distill from this material the essential facts and ideas and bring them to the people through the media of public communication: radio and television, newspaper articles, and widely distributed pamphlets.

In sum, here are the tasks which the fallout problem imposes upon us. Research into the hazards of fallout radiation needs to be more fully and widely published so that the scientific community will be constantly aware of the changes which world-wide radiation is making in the life of the planet and its inhabitants. This knowledge must be at the ready command of every scientist, so that we can all participate in the broad educational campaign that must be put into effect to bring this knowledge to the public. If we succeed in this we will have met our major duty, for a public informed on this issue is the only true source of the moral wisdom that must determine our nation's policy on the testing—and the belligerent use—of nuclear weapons.

There is a full circle of relationships which connects science and society. The advance of science has thrust grave social issues upon us. And, in turn, social morality will determine whether the enormous natural forces that we now control will be used for destruction—or reserved for the creative purposes that alone give meaning to the pursuit of knowledge.

References and Notes

1. "Social Aspects of Science," Preliminary report of the AAAS Interim Committee, *Science* 125, 143 (1957).
2. In connection with the preparation of this article, I thank my colleagues of the AAAS Committee on the Social Aspects of Science. I have relied considerably on ideas developed in the course of several committee meetings on the fallout problem.
3. "The Nature of Radioactive Fallout and Its Effects on Man," Hearings before the Special Subcommittee on Radiation of the Joint Committee on Atomic Energy, Congress of the United States (Government Printing Office, Washington, D.C., 1957), pts. 1 and 2.
4. "Joint Committee on Atomic Energy, Summary-Analysis of Hearings, May 27–29 and June 3–7, 1957 on the Nature of Radioactive Fallout and its Effects on Man" (Government Printing Office, Washington, D.C., August 1957).
5. It is not clear precisely how much of the existing fallout data is now unclassified. The statement of E. A. Martell at the Joint Committee hearings indicates that cumulative fallout data up to 1 Dec. 1955 obtained by the University of Chicago "Project Sunshine" (under contract to the Atomic Energy Commission) are reported in *Bulletin No. 11*, which is classified as "Secret." Data for the period 1 Dec. 1955 to August 1956 are reported in *Bulletin No. 12*, which is unclassified (see *3*, pt. 1, pp. 617, 618).
6. The work of the United Nations Radiation Committee, which is in session at this writing, may be expected to lead to a considerable increase in the amount of world-wide information currently available.

Assimilatory Power in Photosynthesis

Photosynthetic phosphorylation by isolated chloroplasts is coupled with TPN reduction.

Daniel I. Arnon, F. R. Whatley, M. B. Allen

The concept is firmly established in cellular physiology that adenosine triphosphate (ATP) is a universal "energy currency" acting as a link between energy-yielding and energy-consuming metabolic reactions (*1*). It was natural, therefore, that its possible role in photosynthesis, an energy transformation process par excellence, should receive early scrutiny. The participation of ATP in the over-all process of photosynthesis became clear as soon as the enzymatic mechanisms of carbohydrate metabolism were elucidated, when it was recognized that carbohydrates, the main products of photosynthesis, are formed by a series of reactions in which phosphorylation steps with ATP are essential.

The recognition that ATP was needed shed no light on its mode of formation in photosynthesis. That a portion of light energy captured during photosynthesis is transformed into ATP without being first stored in some products of CO_2 assimilation has indeed been envisaged for some time (see review, *2*). What remained obscure was the cellular site at which this special phosphorylation occurred and the mechanism by which it was accomplished. From the standpoint of cellular physiology, the important questions were whether ATP used in photosynthesis was formed by some special light-driven assimilation of inorganic phosphate catalyzed by enzymes closely bound to the chlorophyll system or, in mitochondria, by the mechanism of oxidative phosphorylation.

Direct answers to these questions became possible with the discovery of light-induced ATP synthesis (photosynthetic phosphorylation) first in isolated chloroplasts (*3*) and soon thereafter, by Frenkel, in cell-free preparations of pho-

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tosynthetic bacteria (*4*). Photosynthetic phosphorylation revealed a hitherto unrecognized major site of ATP synthesis in the chlorophyll-containing particles of photosynthetic organisms: chloroplasts in green plants and analogous cytoplasmic particles in photosynthetic bacteria. The mechanism of photosynthetic phosphorylation also appeared to differ, notably in its independence from external molecular oxygen, from ATP synthesis by mitochondria.

The purpose of this article is to assess, on the basis of current evidence, the role of photosynthetic phosphorylation in the over-all process of photosynthesis and, more particularly, to report new evidence which substantially clarifies the relation of light-induced phosphorylations to other photosynthetic events in chloroplasts.

Phosphorylation and Chlorophyll

Prior to the recognition of photosynthetic phosphorylation in chloroplasts, the only cytoplasmic particles known to synthesize ATP were mitochondria. Since green cells contain both types of cytoplasmic particles (*5, 6*), it becomes of interest to compare them as sites of phosphorylation and to appraise their relative importance as sources of ATP in photosynthesis. This will be attempted presently.

In mitochondrial phosphorylation, ATP synthesis is accomplished at the expense of energy released by the oxidation of foodstuffs. The connection between these two events is indirect. The direct effect of the oxidation of foodstuffs is the formation of reduced diphosphopyridine nucleotide ($DPNH_2$). Adenosine triphosphate is formed while $DPNH_2$ is reoxidized in a stepwise manner by molecular oxygen through the mediation of flavoproteins and cytochromes (*7*).

In early models of photosynthetic phosphorylation it was shown that if the reduction of a pyridine nucleotide was carried out by illuminated chloroplasts, the resulting reduced pyridine nucleotide could be used by added mitochondria, from either plant or animal sources, for the generation of ATP by oxidative phosphorylation (*8*). This coupled chloroplast-mitochondrial model system differed from conventional oxidative phosphorylation only in the source of the reduced pyridine nucleotide. In one case DPN was reduced by light, in the other, by a respiratory substrate. The phosphorylation reactions proper, leading to the synthesis of ATP, were in both cases dependent on enzymes localized in mitochondria.

In photosynthetic phosphorylation, isolated chloroplasts were found to be capable of synthesizing ATP without the aid of mitochondria (*3, 9*). When conditions were so arranged that CO_2 assimilation was excluded, chloroplasts used light energy to esterify inorganic phosphate in accord with the over-all reaction:

$$\text{Light} + P + ADP \rightarrow ATP \qquad (1)$$

where P represents orthophosphate and ADP and ATP, adenosine diphosphate and triphosphate, respectively.

In the early experiments photosynthetic phosphorylation occurred only in intact chloroplasts; broken plastids which still retained the green water-insoluble chlorophyll pigments but had lost soluble constituents had only feeble phosphorylating activity (*3, 9*). When in subsequent experiments the phosphorylating capacity was restored to broken chloroplasts by the addition of soluble cofactors such as vitamin K, flavin mononucleotide (FMN), and ascorbate (*9*, p. 6326; *10; 11*), it became clear that the intact structure of whole chloroplasts was not essential to phosphorylation. The enzymes responsible for phosphorylation appeared bound to the water-insoluble chlorophyll pigment system. They were not removed or destroyed by breaking of the chloroplasts but were rendered inactive by the removal of water-soluble constituents (*6, 12*).

The presence of a phosphorylating system tightly bound to the chlorophyll-containing particles is also seen in experiments with cell-free preparations of photosynthetic bacteria. In these organisms there are no chloroplasts, but the photosynthetic pigments are confined to particles termed chromatophores, much smaller in size than chloroplasts (*13*). The experiments of Frenkel (*14*), Williams (*15*), Geller and Gregory (*16*), Geller (*17*), and Mattick and Lindstrom (*18*) with *Rhodospirillum rubrum* and of Anderson and Fuller (*19*) and Newton *et al.* (*20*) with *Chromatium* have shown that in these bacteria the enzymes of photosynthetic phosphorylation are also bound to the particles containing the photosynthetic pigments.

The work with photosynthetic bacteria as well as the recent confirmation of photosynthetic phosphorylation in isolated chloroplasts by Avron and Jagendorf (*21, 22*), Wessels (*23*), Krall and Purvis (*24*), and Chow and Vennesland (*25*) now provides a broad experimental base for the conclusion that in all photosynthetic organisms the cytoplasmic particles which contain the chlorophyll pigments also contain a phosphorylating system. The phosphorylating system differs from the CO_2-fixing enzymes in chloroplasts in being tightly bound to the chlorophyll pigment system; this suggests a close affinity to the early energy transformation reactions. The CO_2-fixing enzymes are water-soluble and are readily removed from the chloroplast (*6, 12, 26*).

Quantitative Importance of Photosynthetic Phosphorylation

Soon after the demonstration of photosynthetic phosphorylation in isolated chloroplasts, attempts were made to compare its rate with that of CO_2 assimilation by illuminated whole cells. Since, as with most newly discovered cell-free reactions, the rates of photosynthetic phosphorylation were rather low, there was little inclination at first to accord this process quantitative importance (*27*) as a mechanism for converting light into chemical energy.

A more reliable estimation of the potential magnitude of photosynthetic phosphorylation became possible with further improvement in experimental methods. Using these, Allen *et al.* (*28*) obtained rates of photosynthetic phosphorylation up to 170 times higher than those originally described (*3*). A comparison of these maximal rates of photosynthetic phosphorylation by spinach chloroplast fragments (500 micromoles of orthophosphate esterified per hour per milligram of chlorophyll) with the maximum rate (*29*) of carbon assimilation by intact leaves (180 micromoles of CO_2 per hour per milligram of chlorophyll) leads to the conclusion that the capacity of chloroplasts to convert light energy into ATP is significant. These high rates of phosphorylation were obtained under conditions when CO_2 fixation was excluded. However, as will be discussed later, chloroplasts are also capable of synthesizing ATP at a high rate when light energy is being used for CO_2 assimilation.

High rates of photosynthetic phosphorylation by isolated spinach chloroplasts have also been reported by Jagendorf and Avron. Under experimental conditions similar to those used by our group, they have obtained rates of 200 micromoles of orthophosphate esterified per hour per milligram of chlorophyll (*22*), but, using a nonphysiological co-

factor (phenazine methosulfate), they have observed rates up to 900 micromoles of phosphate esterified per milligram of chlorophyll per hour (*30*).

It appears therefore that nature has evolved a major mechanism for converting light into useful chemical energy, even under conditions when CO_2 assimilation is curtailed or stopped altogether. The possible physiological significance of this type of energy conversion will be discussed later.

From the standpoint of conventional photosynthesis, the main interest is in those mechanisms for ATP synthesis which can serve not as alternatives but as aids in CO_2 assimilation. The following considerations would suggest that these mechanisms also reside in chloroplasts rather than in mitochondria, which are the major site of ATP synthesis in respiration. (i) The rates of phosphorylation by chloroplasts are, on a nitrogen basis, several times greater than those reported in the literature for oxidative phosphorylation by mitochondria from either plant or animal sources (see *28*). (ii) There is a paucity of mitochondria in leaves. The mesophyll of leaves, which contains most of the chloroplasts, is a tissue remarkably specialized for photosynthesis (*31*). Within the mesophyll cells, especially in the palisade parenchyma, chloroplasts are the dominant cytoplasmic bodies; mitochondria are relatively few in number (*32*). On this basis alone, the contribution of mitochondria to the total ATP requirement in photosynthesis would not be expected to be large (*6, 33*).

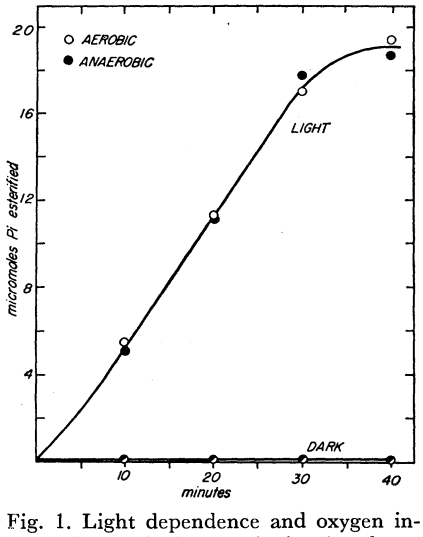

Fig. 1. Light dependence and oxygen independence of photosynthetic phosphorylation by broken chloroplasts. The reaction mixture contained, in a total volume of 3 ml, "broken" chloroplasts (chlorophyll content 0.5 mg), and, in micromoles: tris(hydroxymethyl)aminomethane, *p*H 7.4, 40; adenosine-5-phosphate, *p*H 7.4, 10; potassium phosphate (containing P^{32}), *p*H 7.2, 20; $MgCl_2$, 10; sodium ascorbate, 10; flavin mononucleotide (FMN), 0.01; and vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride), 0.3. The reaction was carried out at 15°C under nitrogen or air. Phosphorylation was measured as described previously (*9, 36*).

This assessment of the quantitative importance of ATP synthesis by chloroplasts is reached from measurements of rates of phosphorylation in cell-free systems. A different conclusion about the importance of photosynthetic phosphorylation to the over-all process of photosynthesis was recently reached by Kandler (*34*) from experiments with whole cells. However, in these, the estimation of the rate of photosynthetic phosphorylation can only be made indirectly, since it is difficult if not impossible to isolate, with certainty, from the over-all phosphorus metabolism of the intact cell, those phases which are solely linked to photosynthetic events.

Independence from Molecular Oxygen

In view of the strict dependence of mitochondrial phosphorylation on oxygen (*1, 8*), one of the most striking features of photosynthetic phosphorylation is its independence from molecular oxygen. This was perhaps to be expected for cell-free particles of the photosynthetic bacteria mentioned earlier, since in *Rhodospirillum rubrum* normal photosynthesis proceeds anaerobically without any evolution of oxygen, and *Chromatium* is an obligate anaerobic photoautotroph. It was wholly unexpected, however, in chloroplasts of such an eminently aerobic plant as spinach.

The independence from oxygen of photosynthetic phosphorylation by chloroplasts was not apparent in the early experiments (*3*) prior to the identification of the required cofactors of phosphorylation (*9*, p. 6326; *10; 11*). In later experiments, photosynthetic phosphorylation proceeded anaerobically at rates in no case lower, and in many cases substantially higher, than those observed aerobically, but only when the requisite cofactors of phosphorylation were added. The indepedence of photosynthetic phosphorylation from molecular oxygen, shown in Fig. 1, was established by manometric experiments. This conclusion has recently received strong support from the experiments of Krall *et al.* (*35*) with labeled oxygen.

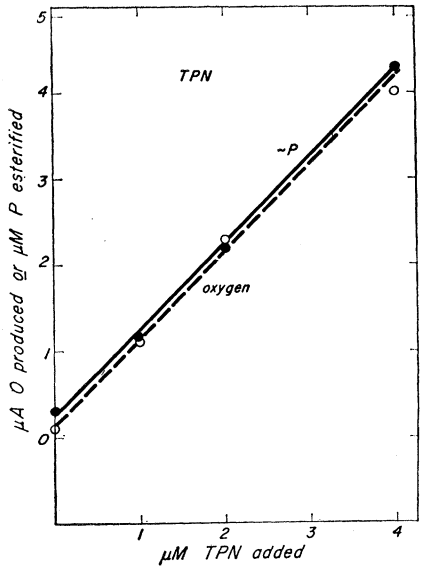

Fig. 2. Stoichiometry of oxygen evolution and adenosine triphosphate (ATP) formation resulting from the photochemical reduction of triphosphopyridine nucleotide (TPN). The relation between moles of TPN reduced, moles of orthophosphate esterified, and atoms of oxygen produced is 1:1:1. The reaction mixture contained, in a final volume of 3 ml., "broken" chloroplasts, (R_{1s}) containing 0.25 mg of chlorophyll, chloroplast extract (CE) equivalent to 2 mg of chlorophyll, and the following, in micromoles: tris, *p*H 8.3, 80; $MgCl_2$, 5; ADP, 10; $K_2HP^{32}O_4$, 10; NaCl, 35; and TPN as indicated. The reaction was run at 15°C for 33 minutes, at which time the reduction of TPN was complete. Oxygen evolution was measured manometrically under nitrogen, and the esterification of inorganic phosphate was measured as described previously (*36*).

Since chloroplasts are able to evolve molecular oxygen in light, it may be useful to define with more precision what we mean by independence from molecular oxygen. We mean that the synthesis of ATP by illuminated chloroplasts occurs (i) when absorption of molecular oxygen cannot be detected either by manometric techniques or by a mass spectrograph (*35*) or, (ii) in an atmosphere of nitrogen or argon when conditions are so arranged that traces of oxygen originally present or formed during the reaction are eliminated (*10*). The possibility that a portion of the oxygen generated in photosynthetic reactions may be immediately consumed in back reactions, before it escapes into the gas phase, is further considered unlikely since chloroplasts are unable to carry out the mitochondrial type of phosphorylation with appropriate substrates or reduced pyridine nucleotides when supplied with oxygen in the dark (*2, 6, 36*).

The independence of photosynthetic

phosphorylation from oxygen had certain general implications. First, it distinguished at once between the light-dependent phosphorylation by chloroplasts and the oxidative phosphorylation by mitochondria and suggested that, in the two kinds of cytoplasmic particles, different enzyme systems were involved in at least some of the intermediate steps in ATP synthesis. Second, it pointed to another fundamental similarity in the photosynthetic mechanisms between photosynthesis in aerobic green plants and anaerobic bacteria, in addition to the photodecomposition of water envisaged by van Niel (*37*).

From the standpoint of photosynthesis in green plants, an interesting question was whether ATP synthesis by chloroplasts is compatible with oxygen *evolution,* which normally accompanies CO_2 assimilation in isolated chloroplasts (*5, 38*), as in intact leaves. Evidence for photosynthetic phosphorylation accompanied by oxygen evolution has recently been obtained (*39, 40*). Since the new work clarifies and modifies the previous concept of photosynthesis by chloroplasts (*5, 6, 36*), it will now be examined in more detail.

Generation of Assimilatory Power

Role of pyridine nucleotides. Di- and triphosphopyridine nucleotides (DPN and TPN) were found to enhance CO_2 fixation by isolated chloroplast fragments (*26, 12*), but experiments to test their participation in photosynthetic phosphorylation had previously yielded only negative results (*5, 6, 12, 26*). Diphosphopyridine nucleotide occupies a key position in oxidative phosphorylation by mitochondria as the hydrogen or electron carrier in oxidations which lead to phosphorylation. The lack of response to added DPN was therefore regarded in earlier discussions (*2, 5, 6*) as another feature which distinguished photosynthetic from oxidative phosphorylation. The lack of response to added TPN was not considered significant, since TPN, unlike DPN, was known to be incapable of serving as a hydrogen or electron carrier in phosphorylation by mitochondria (*41*).

More recently, by changing experimental conditions, we have obtained evidence that TPN, but not DPN, is a catalyst of photosynthetic phosphorylation (*39,* also compare *42*). Substrate amounts of TPN were reduced, and this was accompanied by the evolution of oxygen in accordance with reaction 2. This reaction also requires a TPN-reducing factor which we have identified in an aqueous extract of chloroplasts (CE).

$$2TPN + 4H_2O \xrightarrow[\text{chloroplasts} + CE]{\text{light}} 2TPNH_2 + O_2 + 2H_2O \quad (2)$$

In the presence of adenosine diphosphate (ADP) and orthophosphate (P), reaction 2 was coupled with the formation of 2 moles of ATP. The over-all reaction is summarized by reaction 3:

$$2ADP + 2P + 2TPN + 4H_2O \rightarrow 2ATP + O_2 + 2TPNH_2 + 2H_2O \quad (3)$$

Under appropriate experimental conditions, which will be described elsewhere, the evolution of 1 mole of oxygen was accompanied by the reduction of 2 moles of TPN and the esterification of 2 moles of orthophosphate.

Reaction 3 and Fig. 2 describe a new type of photosynthetic phosphorylation. One mole of orthophosphate is esterified for each mole of TPN reduced. Only part of the light energy absorbed by chlorophyll in this reaction is trapped in the pyrophosphate bond of ATP; the remainder is used for the formation of $TPNH_2$. Thus, the light reaction in chloroplasts consists of the generation of what has previously been termed "assimilatory power" (*2*), accompanied by the evolution of oxygen. Assimilatory power has two components, both of which are needed for the assimilation of CO_2: the reductant $TPNH_2$ and ATP.

The stoichiometry of reaction 3 (Fig. 2) shows that the evolution of 1 mole of oxygen and the synthesis of 2 moles of ATP accompanies the generation of four hydrogen equivalents which are required for the reduction of 1 mole of CO_2 to the level of carbohydrate

$$\left[\overset{|}{\underset{|}{CH(OH)}}\right]$$

Reaction 3 would thus account for the well-known photosynthetic ratio CO_2/O_2 of 1, also observed with isolated chloroplasts (*5, 38*), when CO_2 is assimilated to the level of carbohydrate. A general scheme of photosynthesis in chloroplasts, based on these findings, is shown in Fig. 3.

The concept of photosynthesis by chloroplasts represented by Fig. 3 shares certain similarities with, but also differs from, the earlier one which it now replaces (*5, 6, 26, 36*). It is similar in that the chloroplast is regarded as a complete photosynthetic unit containing multienzyme systems divided into three main groups, each controlling an increasingly complex phase of photosynthesis: photolysis of water, photosynthetic phosphorylation, and CO_2 fixation. Carbon dioxide fixation remains, as before, at the apex of this hierarchy and requires the participation of all three groups of enzymes. But photolysis of water is now

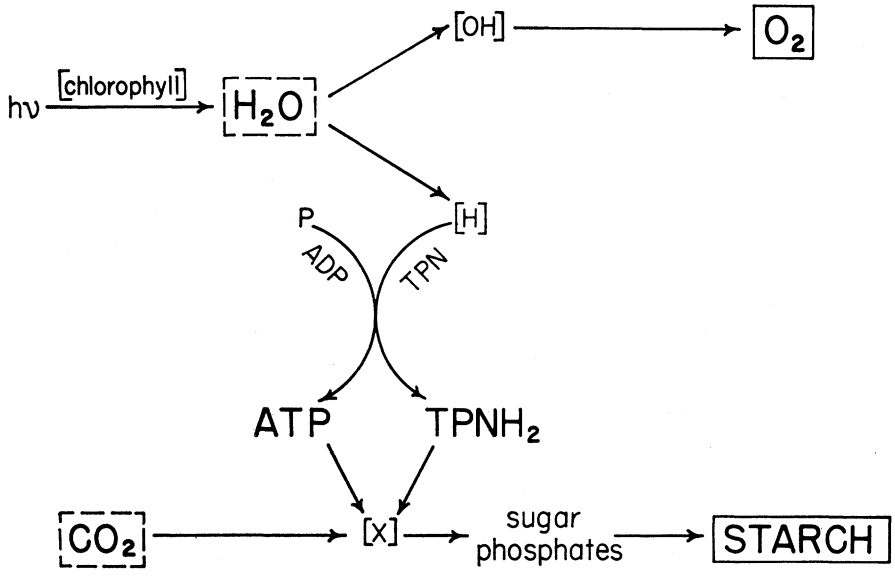

Fig. 3. Scheme for photosynthesis by isolated chloroplasts. Photolysis of water results in the evolution of oxygen and the generation of "assimilatory power" comprising two components: reduced triphosphopyridine nucleotide ($TPNH_2$) and ATP. Assimilatory power is then used for the assimilation of CO_2 in reactions independent of light. [The intermediate *X* in this scheme is not designated as phosphoglyceric acid, as would be demanded by the theory of Calvin (*43*). Experiments on carbon fixation with isolated chloroplasts have so far yielded no unequivocal evidence on this point (*26, 38*; also compare *27*, p. 294).]

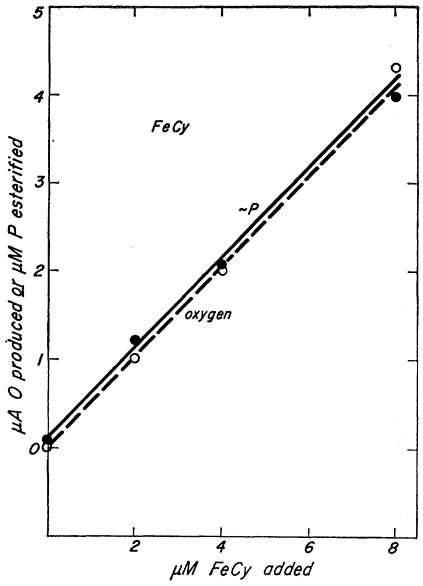

Fig. 4. Photophosphorylation and oxygen evolution with ferricyanide. The stoichiometric relation between moles of ferricyanide reduced, moles of orthophosphate esterified, and atoms of oxygen produced was 2:1:1. The reaction mixture contained, in a final volume of 3 ml, "broken" chloroplasts (P_{1s}) containing 0.1 mg of chlorophyll and the following, in micromoles: tris, *p*H 8.3, 80; $MgCl_2$, 5; ADP, 10, $K_2HP^{32}O_4$, 10; NaCl, 35; and ferricyanide as indicated. The reaction was run at 15°C for 18 minutes, at which time the reduction of ferricyanide was complete.

no longer regarded as resulting *either* in the synthesis of ATP *or* in the reduction of CO_2. Adenosine triphosphate synthesis is *coupled* with the formation of the reductant ($TPNH_2$) required for CO_2 fixation. Thus, the same light quanta which accomplish the reduction of TPN also bring about the synthesis of ATP and generate the assimilatory power needed for the conversion of CO_2 into carbohydrates or analogous end-products of photosynthesis.

The coupling of ATP synthesis with TPN reduction simplifies the concept of CO_2 assimilation by chloroplasts. If the ATP generated during the TPN reduction step (reaction 3) is sufficient for CO_2 assimilation, it is no longer necessary to visualize a competition for light energy between photosynthetic phosphorylation and CO_2 fixation and to search for appropriate regulatory mechanisms for keeping the two in balance (*6*). The generation of the two components of assimilatory power, $TPNH_2$ and ATP, goes up and down simultaneously, in accordance with the rate of CO_2 fixation.

Specificity of TPN. The specificity of TPN in photosynthetic phosphorylation (*39*), together with its previously demonstrated effect on CO_2 assimilation by isolated chloroplasts (*12, 26*), argues in favor of TPN, rather than DPN, as the pyridine nucleotide *directly* associated with photosynthesis [indirectly, of course, DPN could also be effective in the presence of transhydrogenase (*43*)].

The preference for TPN rather than DPN in photosynthetic events was hitherto not well established. It was tied to the phosphoglyceric acid theory of carbon assimilation (*44*) and rested on evidence for the cyclic emergence of light-induced TPN-dependent enzymes (*45*) concerned in the metabolism of that acid: glyceraldehyde-3-phosphate dehydrogenase (GPD) (*46–48*) and glyceraldehyde-3-phosphate-TPN reductase (GTR) (*49*).

The GTR enzyme is known only to oxidize glyceraldehyde-3-phosphate irreversibly to phosphoglycerate (*49*); the emergence of this enzyme in photosynthetic tissue would thus support not the phosphoglyceric acid theory of Calvin (*44*) but rather Warburg's view (*50*) that phosphoglyceric acid is a product of carbohydrate degradation during photosynthesis. The TPN-dependent GPD enzyme which can reduce phosphoglyceric acid offers no compelling evidence for the role of TPN in photosynthesis, since the same function could be performed by the DPN-specific GPD also present in green cells (*46–48*). However, its specificity in photosynthetic phosphorylation, apart from its other conceivable functions, gives TPN a direct role in photosynthesis, regardless of the validity of the phosphoglyceric acid theory of carbon assimilation.

Improbability of lipoic acid as a TPN reductant. Calvin and his associates (*51*) have assigned to lipoic (thioctic) acid a key role in photosynthesis as a compound concerned in the primary conversion into chemical energy of the light quanta absorbed by chlorophyll. They have proposed that the reduced lipoic acid (dithiol form) could, in turn, reduce DPN or TPN (*52*). Lipoic acid could thus fulfill the role of hydrogen carrier in the water-splitting reaction leading to the formation of $TPNH_2$ (Fig. 3).

Reactions in which lipoic acid is a cofactor are very sensitive to arsenite inhibition; the inhibition can be reversed by the addition of dithiols but not of monothiols (*53*). Peters *et al.* (*53*) and Gunsalus (*54*) observed inhibition of the pyruvic oxidation system by concentrations of arsenite of the order of $3 \times 10^{-5}M$; the inhibition was reversed by the dithiol 2,3-dimercaptopropanol (BAL) but not by the monothiol glutathione. In the chloroplast system, $10^{-3}M$ arsenite failed to inhibit the TPN reduction and its coupled phosphorylation. It seems unlikely, therefore, that lipoic acid is a cofactor in this reaction. The TPN reduction and its coupled phosphorylation are sensitive, however, to other sulfhydryl inhibitors, as is evidenced by their inhibition by *p*-chloromercuribenzoate, an inhibition which was reversed by glutathione.

The improbability that lipoic acid is a participant in photosynthetic phosphorylation is also indicated by the results of Geller (*17*): $10^{-2}M$ arsenite failed to inhibit phosphorylation by illuminated particles of *Rhodospirillum rubrum.*

Hill reaction as a fragment of a phosphorylating system. Isolated chloroplasts are known (*55*) to evolve oxygen when they are illuminated in the presence of an artificial electron acceptor (Hill reaction) in accordance with reaction 4, in which A represents a nonphysiological substance such as ferricyanide or benzoquinone (*56*).

$$\text{Light} + A + H_2O \rightarrow H_2A + \tfrac{1}{2}O_2 \quad (4)$$

The finding of a phosphorylation which is coupled with TPN reduction and oxygen evolution permits us to view the Hill reaction as a measure of photochemical electron transport which is proceeding without its normally associated phosphorylation reaction. The Hill reaction would thus be analogous to those electron transport reactions studied in particulate systems of animal origin in

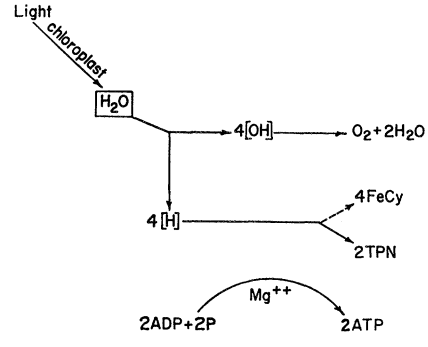

Fig. 5. Diagram representing the generation of "assimilatory power" and the evolution of oxygen by isolated chloroplasts. The components of assimilatory power are reduced triphosphopyridine nucleotide ($TPNH_2$) and adenosine triphosphate (ATP). In the absence of carbon dioxide, $TPNH_2$ and ATP can be generated in substrate amounts in the light by the green fraction of chloroplasts and used subsequently for sugar synthesis in the dark by a colorless chloroplast extract supplied with CO_2 (*72*). (Compare also Figs. 2, 3, and 4.)

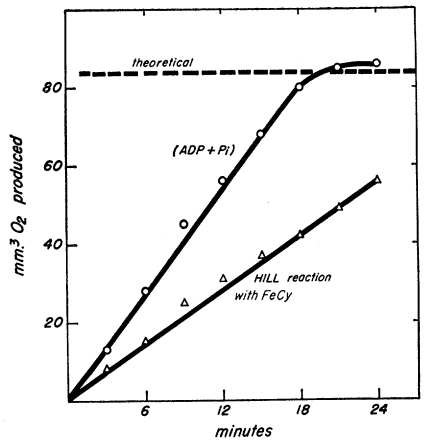

Fig. 6. Effect of phosphate acceptor system on the Hill reaction with ferricyanide. The reaction mixture contained, in a final volume of 3 ml, "broken" chloroplasts (P_{1s}), containing 0.1 mg of chlorophyll; and the following in micromoles: tris(hydroxymethyl)aminomethane, 80; sodium chloride, 35; potassium ferricyanide, 15. The vessel with the phosphate acceptor system received, in addition (in micromoles): ADP, 10; K_2HPO_4, 10; and $MgCl_2$, 5. The oxygen evolution was measured manometrically at 15°C. Gas phase, nitrogen; KOH in the center well of the vessel. Illumination as described previously (*38*).

which oxidation has been separated from its normally associated phosphorylation —for example, in the Keilin-Hartree preparations (*57*) or in the electron transport particles of Green (*58*).

The validity of this hypothesis was tested with a Hill reagent which is compatible with the phosphorylating activity of chloroplasts. Quinone was found not to fulfill this requirement well, but ferricyanide did. Fig. 4 shows the esterification of inorganic phosphate coupled with oxygen evolution and the reduction of ferricyanide by illuminated chloroplasts. The stoichiometry of this reaction is the same as in the case of TPN: 1 mole of oxygen is evolved and 2 moles of orthophosphate are esterified in a transfer of four electrons to ferricyanide (compare with reaction 3 and Fig. 2).

The phosphorylation coupled with the photochemical reduction of ferricyanide can thus be regarded as a nonphysiological model for the generation of assimilatory power (Fig. 5). (The TPN-reducing factor in chloroplast extract is not required for the reduction of ferricyanide.)

Further support for the proposal that the Hill reaction is part of an uncoupled phosphorylation system is seen in Fig. 6. With ferricyanide as a reductant, the rate of oxygen evolution in the Hill reaction was increased by coupling it with phosphorylation. It seems reasonable to interpret this effect as indicating that the electron transport system of chloroplasts is able to function more effectively when it is coupled, as it would be under physiological conditions, to the synthesis of ATP.

Cyclic Photophosphorylation

In measuring the generation of assimilatory power, the phosphorylation coupled with the reduction of TPN was accomplished without the addition of either flavin mononucleotide (FMN) or vitamin K, both of which have previously been identified as cofactors of photosynthetic phosphorylation (*10, 11*). The addition of catalytic amounts of either FMN or vitamin K altered the system profoundly. The results are shown in Figs. 7 and 8.

Phosphorylation was sharply increased, whereas oxygen evolution and the accumulation of reduced TPN were abolished. The most direct explanation of these results is that the addition of catalytic amounts of either FMN or vitamin K brought about additional phosphorylation accomplished at the expense of energy liberated by the reoxidation of $TPNH_2$ by [OH], the oxidized product of photodecomposition of water.

Expressed in another way, the addition of either FMN or vitamin K has brought about a recombination of the photodecomposition products of water, [H] and [OH], and a conversion into ATP of all of the light energy originally trapped in the "water-splitting" reaction. Under these conditions, when CO_2 assimilation did not occur, the hydrogen atoms which would have been used in the reduction of CO_2 became a part of a reconstituted water molecule instead of a newly formed sugar molecule. This type of light-induced phosphorylation is the same as we reported previously under the general name of photosynthetic phosphorylation (*2, 5, 6, 10, 26*). It will now be designated by the more specific name of *cyclic photophosphorylation* (Fig. 9) to distinguish it from the "one-step" phosphorylation associated with the generation of assimilatory power when only part of the captured light energy is converted into ATP (Fig. 5).

Another explanation of the observed effects of FMN and vitamin K on the "one-step" phosphorylation is possible, and is being tested experimentally, but it appears at this time to be unlikely. It would limit phosphorylation only to the electron transfer step coupled with TPN reduction and would explain the increased phosphorylation which follows the addition of FMN or vitamin K (Figs. 7 and 8) as resulting from a more rapid turnover of the TPN rather than

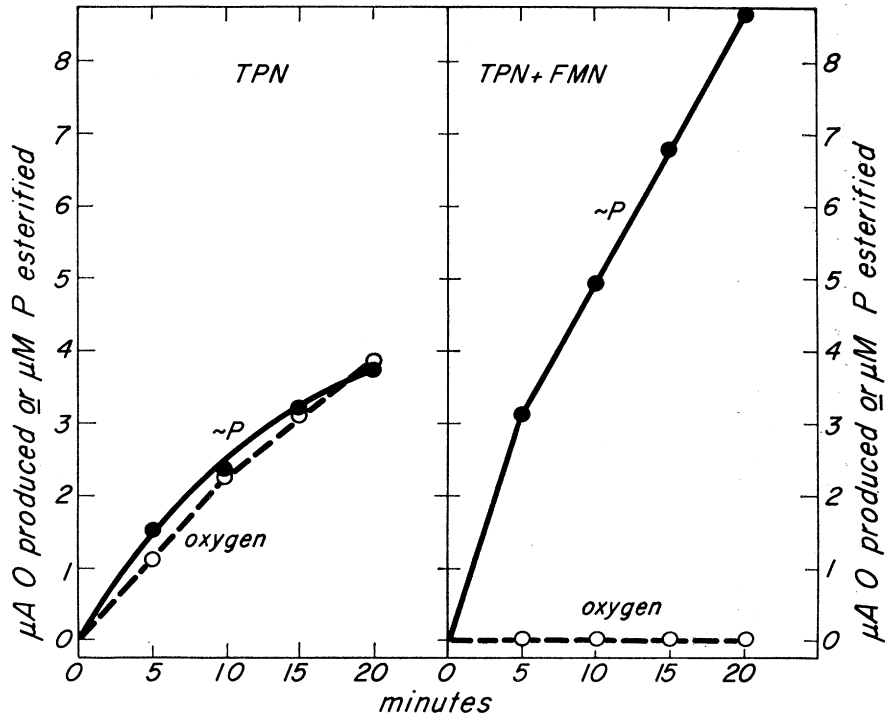

Fig. 7. Photophosphorylation and oxygen evolution with TPN in the presence and absence of FMN. The reaction mixture was the same as that described in Fig. 2, except that 15 μmole of K_2HPO_4, 15 μmole of ADP, and 4 μmole of TPN were used in each vessel. In the "TPN + FMN" series, 0.1 μmole of FMN was added.

from the activation of additional phosphorylation sites.

Physiological significance. In previous discussions (*5, 6, 26, 36*), cyclic photophosphorylation was regarded as the sole photosynthetic phosphorylation and its physiological significance was sought in its contribution of ATP to CO_2 assimilation. The identification of a second type of photosynthetic phosphorylation, as a component of assimilatory power, renders the earlier interpretation of cyclic phosphorylation too narrow.

It is tentatively proposed that another physiological role of cyclic photophosphorylation might be the conversion of light energy into ATP under conditions when CO_2 assimilation is, for one reason or another, reduced or even stopped altogether. This might arise during the well-known midday closure of stomata in leaves of higher plants (see *59*) when the supply of CO_2 becomes restricted. The midday closure of stomata often occurs as a result of a water deficit in the plant. Cyclic photophosphorylation, unlike CO_2 assimilation, provides a mechanism for the utilization of light energy without the consumption of water.

It is conceivable that devices other than closure of stomata are also available, both to higher and to lower plants without stomata, for curtailing CO_2 assimilation when the normal photosynthetic products accumulate in the cell. Under such conditions it would greatly benefit the cell to have a supply of ATP generated at the expense of light energy. This ATP of photochemical origin could then be used to drive the many ATP-dependent reactions, notably protein and fat synthesis (*1*). Another example is the synthesis of glutamine, which provides an important mechanism for the incorporation and transfer of nitrogen in plants, and which was also shown by Elliott (*60*) to depend on ATP in accordance with reaction 5:

$$\text{Glutamate} + NH_3 + ATP \rightarrow \text{glutamine} + ADP + P \quad (5)$$

One is tempted to suggest that cyclic phosphorylation itself may, under certain conditions, be a device for diverting light energy into channels other than CO_2 assimilation. Cyclic phosphorylation would thus represent a pattern evolved by photosynthetic cells to use light energy for accomplishing cellular work independently of CO_2 assimilation.

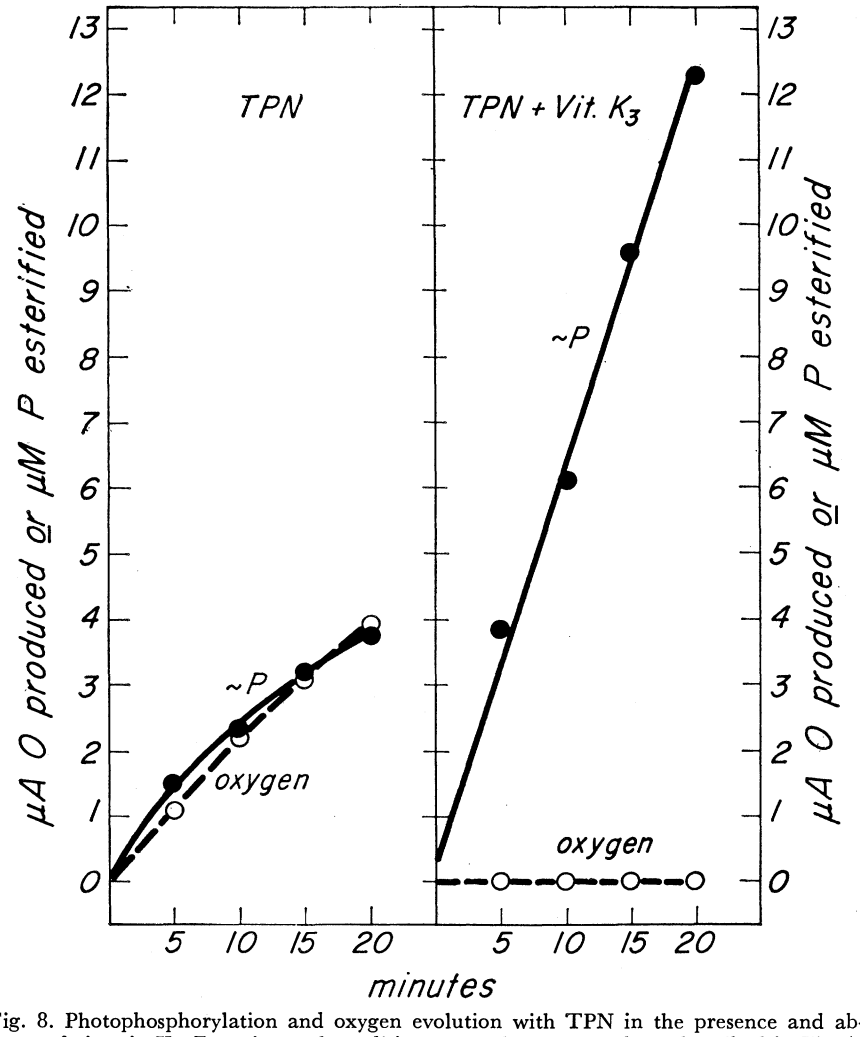

Fig. 8. Photophosphorylation and oxygen evolution with TPN in the presence and absence of vitamin K_3. Experimental conditions were the same as those described in Fig. 7. In the "TPN + vit. K_3" series, 0.2 µmole of vitamin K_3 (menadione) was added.

Cyclic Photophosphorylation and Quantum Efficiency Measurements

If cyclic photophosphorylation is concurrent with CO_2 assimilation, then the portion of light energy which it consumes will not be accompanied either by oxygen evolution or by CO_2 uptake. Quantum efficiency values for the over-all process of photosynthesis, dependent as they usually are on the measurements of these two gases, would therefore be low in proportion to the share of light energy which is diverted to cyclic photophosphorylation. One possible explanation, attractive because of its simplicity, of the high quantum efficiency yield obtained by Warburg *et al.* (*61*) with cells grown in fluctuating light is that they divert little if any of the absorbed light energy from CO_2 assimilation to cyclic photophosphorylation. On the other hand, the seemingly less efficient cells grown under constant illumination may already contain an accumulation of carbohydrates or of other photosynthetic products and would then use a larger share of the absorbed light energy for cyclic photophosphorylation to generate ATP, to be used for protein synthesis and other endergonic processes.

Catalysts of photosynthetic phosphorylation. In previous formulations it was tentatively proposed (*5, 6, 26, 36*) that FMN and vitamin K are members of the same electron transport chain of what is now termed cyclic photophosphorylation. Whatley *et al.* (*62*) have shown, however, that under modified experimental conditions maximal rates of cyclic photophosphorylation are obtained with either FMN or vitamin K as catalysts (Table 1). On the basis of present evidence, vitamin K and FMN are therefore tentatively regarded as participating in alternative pathways for cyclic photophosphorylation. The postulated "FMN pathway" is shown in Fig. 9. The "vitamin K pathway" differs from the "FMN pathway"; for example, it is insensitive to $10^{-3}M$ dinitrophenol. Other differences and similarities between these two postulated pathways are now under investigation. This subject is discussed more fully elsewhere (*63, 64*).

The marked stimulating effect of phenazine methosulfate on photosynthetic phosphorylation is sometimes interpreted as casting doubt on the specificity of vitamin K and flavins as the natural cofactors of photosynthetic phosphorylation (*30*). Analogous effects of phenazine methosulfate as an electron carrier in the succinic and other dehydrogenase systems (*65*) have led to no questions about the specificity of the natural cytochrome system in these reactions. We know of no valid reason at this time for questioning the experimentally demonstrated role of vitamin K (*11*, Table 1) and FMN (*10*) in photosynthetic phosphorylation. Both of these substances are normal constituents of chloroplasts; in fact, Dam has shown that in green leaves vitamin K is concentrated in chloroplasts (see review, *63*). We are therefore inclined to favor Geller's interpretation (*17*) that, in photosynthetic phosphorylation, phenazine methosulfate merely "serves as a fast 'by-pass' or 'short circuit' for electron transport around the site which is rate limiting in the system."

Present evidence suggests that the role of ascorbate may be to protect some essential components of the chloroplasts against inactivation (*39*). We are now inclined to assign to ascorbate a protective action and to consider it to be perhaps a poising agent, rather than a catalyst in the electron transport chain (Fig. 9). Contrary to the report of Wessels (*23*), ascorbate, under our experimental conditions (*64*), markedly increased phosphorylation with either FMN or vitamin K, under anaerobic conditions (Table 1).

The requirements for Mg^{++} and TPN

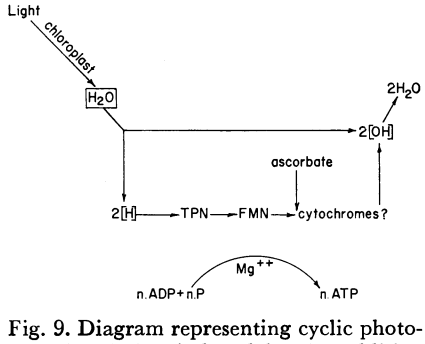

Fig. 9. Diagram representing cyclic photophosphorylation induced by the addition of catalytic amounts of riboflavin phosphate (FMN) to a chloroplast system generating assimilatory power (Figs. 3 and 7). The suppression of oxygen evolution and the increase in phosphorylation (Fig. 7, right) are visualized as resulting from additional phosphorylation steps coupled with the stepwise recombination of the elements of water.

and the possibility that there is a cytochrome requirement in photosynthetic phosphorylation are discussed elsewhere (*63, 64*).

Several soluble enzymes and other factors contained in chloroplasts have now been identified as likely components of photosynthetic phosphorylation: (i) adenylic acid kinase (*24, 28, 39, 66*); (ii) a TPN-reducing factor which has the properties of a protein (*39*) [this factor appears to be the same as that described by San Pietro and Lang (*67*)]; (iii) a diaphorase, specific for $TPNH_2$ as an elecron donor and capable of reducing FMN or vitamin K_3 (*68*).

Phosphorylation and Biochemical Evolution

The presence in green plants of anaerobic mechanisms for generating ATP at the expense of light energy provides another link in the chain of evidence for the basic unity of photosynthetic mechanisms in aerobic green plants and anaerobic photosynthetic bacteria, as proposed by van Niel (*37*). Photosynthetic phosphorylation is also compatible with the premise that the emergence of photosynthetic organisms in the evolutionary scale was accomplished by their acquisition of a pigment system capable of carrying out the photodecomposition of water (*37*).

Our present knowledge of photosynthetic phosphorylation (*69*) suggests that the capacity for harnessing light energy, when first acquired, was probably more closely associated with ATP synthesis than with CO_2 assimilation. This suggestion is based on the close structural association, in both chloroplasts and bacterial chromatophores, of the phosphorylating activity with the chlorophyll pigment system. By contrast, the enzymes responsible for CO_2 assimilation are easily dissociable from the chlorophyll pigment system in the case of chloroplasts (*6, 12, 26*) and apparently not even structurally joined together in the case of bacterial chromatophores (*70*).

The direct use of light energy for the synthesis of ATP by a recombination of the products of the photodecomposition of water would be a significant step forward in supplementing the ATP derived from anaerobic fermentations, which were most probably the oldest energy-releasing processes (see *1*). If it is agreed that, at an early period in the evolution of life forms, organic compounds were abundant and the earth's atmosphere was a reducing one (*71*; also see *1*, review), then new mechanisms for ATP formation were probably more important than CO_2 fixation to the newly emerging organisms. It is assumed that, at first, the mechanism for the reconstitution of water leading to ATP formation was simpler than that now proposed for cyclic photophosphorylation (Fig. 9).

The next step in the evolution of photosynthesis might have been the formation of assimilatory power—that is, the conversion of only a portion of the captured light energy into ATP, the remainder being used to generate a reductant for CO_2 assimilation. Here a recombination of the products of photodecomposition of water had to be prevented, since the "hydrogen" derived from the decomposition of water would be required for the reduction of CO_2. The diversion of the "hydrogen" for CO_2 assimilation would have to be accompanied by a mechanism for disposing of the [OH], the oxidized product of the photodecomposition of water. This mechanism was perhaps, at first, generally dependent on an external hydrogen donor, as it still is today in photosynthetic bacteria. In a later stage of evolution, culminating in the emergence of green plants, this mechanism became an enzyme system for the liberation of molecular oxygen (*37*).

Photosynthetic phosphorylation by chlorophyll-containing particles, being independent of molecular oxygen, could occur before oxidative phosphorylation by mitochondria, which requires molecular oxygen. The only known source for molecular oxygen on earth is photosynthesis by green plants (*37, 71*). This evolutionary sequence provides another argument against the dependence of photosynthesis on ATP generated by a mitochondrial mechanism. It is an in-

Table 1. Effect of riboflavin phosphate (FMN), vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride), and ascorbate on photosynthetic phosphorylation under anaerobic conditions.

Treatment	P esterified (μmole)	
	FMN system	Vitamin K_5 system
Complete	7.0	5.5
FMN or vitamin K_5 omitted	0.9	0.9
Chloroplasts omitted	0.2	0.2
Light omitted	0.2	0.2
Complete	6.2	6.8
Ascorbate omitted	0.9	3.8

teresting aspect of biochemical evolution that green plants, after evolving a mechanism independent of molecular oxygen for generating ATP in light, have also shared with nongreen organisms the emergence of an oxygen-dependent generation of ATP by oxidative phosphorylation.

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Arlow B. Stout, Geneticist and Plant Breeder

Arlow Burdette Stout was born at Jackson Centre, Ohio, on 10 March 1876, and died at the age of 81 at his home in Pleasantville, New York, on 12 October 1957.

His youth was spent in a rural community at Albion, Wisconsin, where most of the surrounding land was still uncultivated and offered intimate contact with nature to an eager boy whenever duties on the farm permitted. His education came the hard way, as it did for many of his contemporaries. Beginning in a one-room country school he continued his education at a local academy (Albion Academy), at Melton College, and at the State Normal School at Whitewater, Wisconsin, with frequent interruptions for longer or shorter periods, as required by the need to earn money, usually by teaching. In the autumn of 1908 he entered the University of Wisconsin, from which he received the Bachelor of Arts degree in 1909. He remained at the university as instructor of botany until his appointment as director of laboratories at the New York Botanical Garden in 1911. He was granted the Doctor of Philosophy degree by Columbia University in 1913. He retired from the New York Botanical Garden in 1947.

The major direction of Dr. Stout's research interests was determined by his first formal instruction in botany. In a course at Albion Academy in 1895–96 he used Asa Gray's *How Plants Grow* as a text, and he was especially intrigued with the description of the process of seed formation. However, he was most surprised and perplexed to note, the following summer, that two plants in his family's garden produced perfect flowers but did not fruit. These plants were a cluster of the familiar day lily (*Hem-*