PHOTOSYNTHESIS*

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THAT isolated chloroplasts are capable of forming carbohydrates from CO_2 and H_2O simultaneously evolving O_2 without any energy other than visible light has given a spectacular lead and paved the way for investigating the fundamental problem of photosynthesis, *i.e.*, the ability to convert light into chemical energy. The theory that photolysis of water is the basic reaction has now been re-examined using isolated chloroplasts and cell-free particles of the photosynthetic bacterium, *Chromatium*, and the results of these experiments are indicative of photosynthetic phosphorylation as a more likely factor in the two systems than photolysis of water as hitherto supposed (photolysis in the botanical sense means "the grouping of chloroplasts in relation to the amount of light falling on plant"; in the chemical sense it means "the decomposition or dissociation of a molecule as a result of the absorption of light"). Isolated chloroplasts have, therefore, provided an effective experimental approach to the separation of the light-dependent phase of photosynthesis in which O_2, TPNH and ATP are formed and the dark phase during which CO_2 is assimilated. The conversion of absorbed light energy into chemical energy, used for CO_2 assimilation in the dark, occurs during the photochemical reactions in which chloroplasts evolve O_2 and form assimilatory power, *i.e.*, ATP and TPNH.

With this background let us now examine the various views put forward in understanding many aspects of this phenomenon of photosynthesis. It is generally recognized that in photosynthesis oxido-reduction takes place by the utilization of hydrogen from water for reduction of CO_2. Therefore, if chlorophyll were involved, as it seems to, in the chain of reactions culminating in the transfer of hydrogen from water to CO_2, the obvious thing to expect is a reversible photochemical oxidation or reduction of chlorophyll resulting in the *storage* of light energy in its photoproducts presumably as reversibly acting unstable quanta. Indeed, this has been shown early as feasible (reversible photochemical oxidation of the pigment in the presence of ferric ions) but whether this is what happens *in vivo* is a moot point. Chloroplast reaction in relation to hydrogen transport is well summarized in Fig. 1. It shows reducing

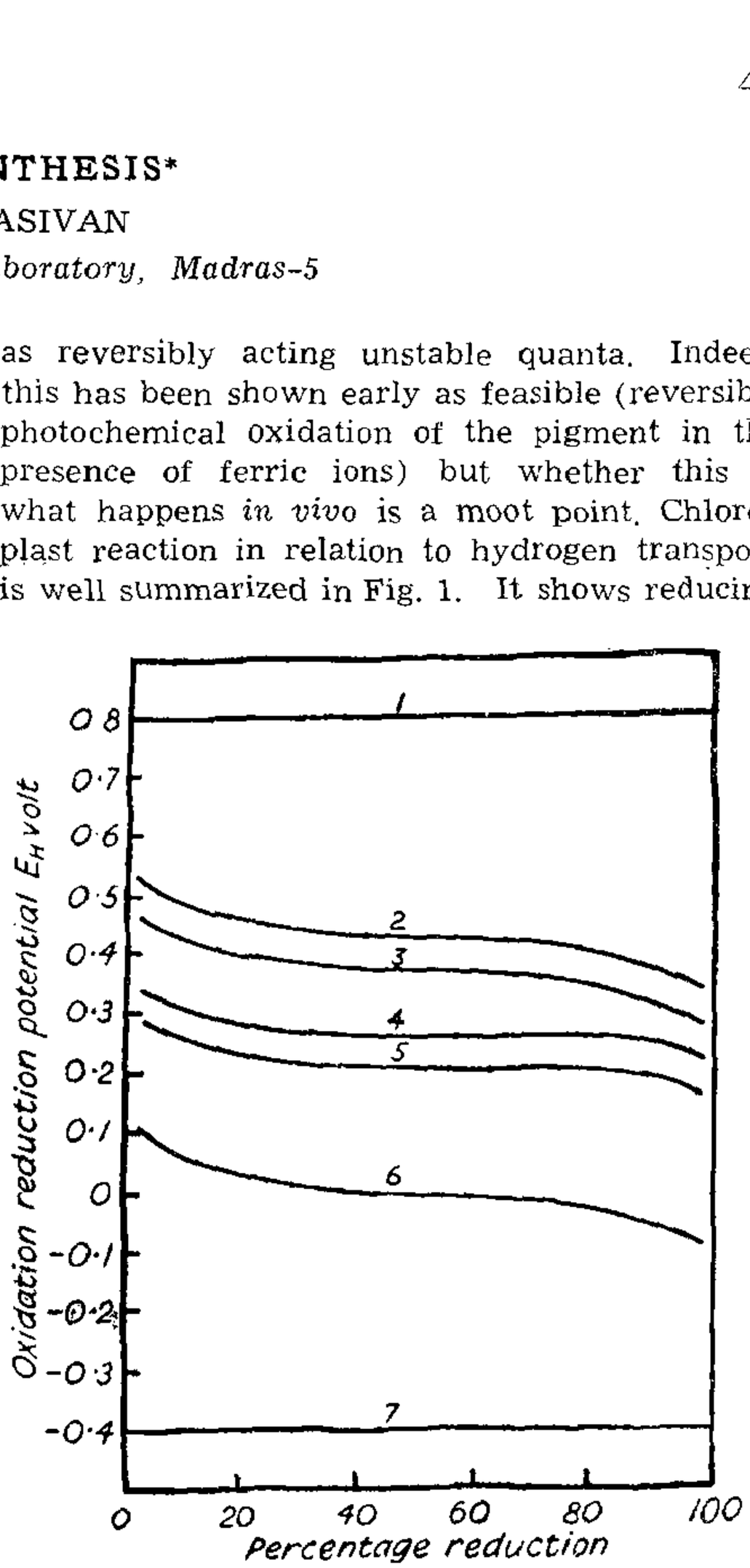

FIG. 1. Potentials and percentage reduction for substances taking part in chloroplast reaction as shown by R. Hill: 1. oxygen; 2. ferricyanide; 3. cytochrome *f*; 4. quinone; 5. 2:6 dichlorindophenol; 6. ferric oxalate; 7. hydrogen (from Hill and Whittingham, 1958).

properties of the chloroplast system, *i.e.*, relationship between the electrode potentials and the percentage reduction of the effective reagents. The reduction of ferric oxalate represents the greatest reducing potential that has yet been obtained in chloroplast reaction. Therefore, chloroplast preparations do not 'split water' to the extent required by the simple formulation of photosynthesis. Two interpretations are possible: (a) either the chloroplast reaction *in vitro* is an artefact and has no direct correlation with processes of reduction occurring in the living cell, or (b) the reduction of CO_2 requires more than the four equivalents of hydrogen chemically necessary. Illuminated

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The following abbreviations will be used in the text: ADP, ATP, adenosine di- and tri-phosphates; FMN, flavin mono-nucleotide; PGA, phosphoglyceric acid; Ru-D-P, ribulose diphosphate; R-5-P, ribose-5-phosphate; RDP, ribose diphosphate; TPN, triphosphopyridine nucleotide; TPNH, triphosphopyridine nucleotide, reduced form.

chloroplasts catalyse the production of O_2 from H_2O in the presence of certain hydrogen acceptors in a reaction which shows many of the characteristics of the O_2-producing reaction in photosynthesis. However, the reducing properties of illuminated chloroplasts are not of sufficient potential to account for a reduction of CO_2 by reaction with the minimum number of equivalents of H_2. In the present state of our knowledge this conclusion would appear fairly satisfactory although one must admit that positive proof for such oxido-reduction is yet to be found. The intermediates that store light energy subsequent to the photochemical stage are now known to be reduced forms of pyridine nucleotides and high energy phosphates. However, that does not represent the entire list as newer intermediate compounds taking part in this remarkable phenomenon of storage of light energy await discovery and characterization.

Simultaneous action of light of different wavelengths could favour the course of photosynthesis as for example blue-green light of very low intensity was shown to greatly increase the quantum yield of photosynthesis by *Chlorella* in the presence of red light and also due to the action of blue light the long wave limit of *Chlorella* photosynthesis was noted to shift to extreme red region. Nevertheless, joint action of quanta of red and blue light is not indispensable for photosynthesis as, indeed, plant growth in red light alone is known. A further point of interest is that in the region of 450 to 580 mμ O_2 uptake has been noted to increase in plant organs containing no chlorophyll. Light could also affect activity of photoreduced forms of chlorophyll.

Quite recently, Arnon and his collaborators discovered that isolated chloroplasts could synthesize ATP in light. They succeeded in separating the light and dark phases of reaction resulting in the formation of the reduced form of TPN and ATP on illuminating "grana" in the absence of oxygen. The interesting point is that, on illuminating isolated chloroplast, inorganic phosphate gets incorporated into ATP without liberation or uptake of O_2 and this Arnon designated as "cyclic phosphorylation". The rate at which this process proceeded was accelerated if co-factors like FMN, ascorbic acid, vitamin K or similar oxido-reductive systems that could initiate electron transfer were added. Three products of the light reactions in chloroplasts, O_2, ATP and TPNH, are now known to be formed (Fig. 2). Reactions 1 and 2 reveal an unknown capacity of chloroplasts to use light energy for the formation of energy rich pyrophosphate bonds of ATP and is now designated as photosynthetic phosphorylation as distinguished from oxidative phosphorylation by mitochondria. Reaction 1 is now known as "cyclic" and reaction 2 as "non-cyclic" photophosphorylation where only part of the light energy absorbed by chlorophyll is used for the formation of ATP; the remainder is used for formation of a reductant, TPNH, which provides the 'hydrogens' needed for the conversion of CO_2 to sugars. Reaction 3 is a non-physiologic variant of reaction 2 in which TPNH is replaced by ferricyanide.

$$ADP + H_3PO_4 \longrightarrow ATP \qquad (1)$$

$$2TPN + 2H_2O + 2ADP + 2H_3PO_4 \xrightarrow{\text{reductase}} 2TPNH_2 + O_2 + 2ATP \qquad (2)$$

$$4Fe^{3+} + 2H_2O + 2ADP + 2H_3PO_4 \longrightarrow Fe^{2} + O_2 + 2ATP + 4H^{+} \qquad (3)$$

FIG. 2. Photochemical reactions as postulated by Arnon (1959).

Arnon went further and gave a new orientation to the non-cyclic electron transport process which in photosynthesis of green plants results in a light dependent reduction of TPN, evolution of O_2 and the formation of ATP (Fig. 3). It

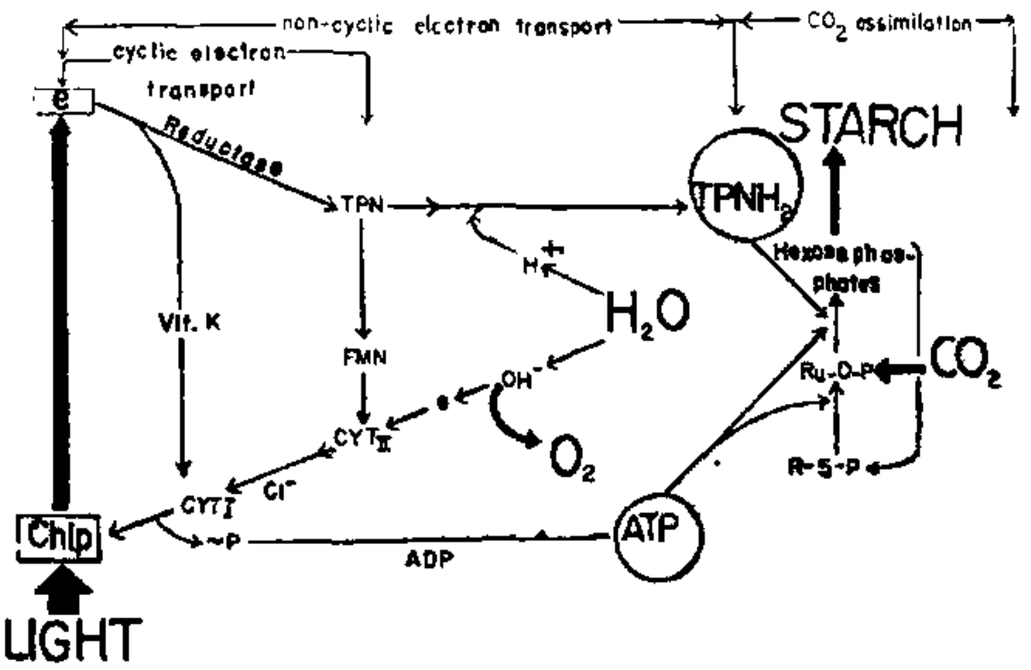

FIG. 3. Scheme for photosynthesis proposed by Arnon (1959).

differs from the cyclic pathway in that an electron expelled does not return to the chlorophyll molecule but is removed from the cyclic pathway by TPN and used either for the reduction of CO_2 and the formation of carbohydrates or under special conditions for the production and accumulation of TPNH. This scheme of Arnon brings out that, chlorophyll, on absorbing a light quantum, becomes 'excited' and expels high-energy level electrons. In both

green plants and photosynthetic bacteria this energy is converted into the pyrophosphate bond-energy of ATP during a 'downhill' return of electrons to chlorophyll through a cyclic electron transport system of the vitamin K type. In the green plants, there is a second cyclic electron transport system of the FMN type for forming ATP and also an open non-cyclic mechanism of electron transport to TPN and this results in evolution of O_2. Cytochrome components are also visualized in electron transport of both cyclic and non-cyclic mechanisms. Photosynthetic bacteria seem to use light energy only for formation of ATP by the vitamin K type of cyclic photophosphorylation. Green plants, however, require both cyclic and non-cyclic electron transport systems for formation of assimilatory power, ATP and TPNH (photosynthetic triphosphopyridine nucleotide reductase, the reducing factor for chloroplasts has now been crystallized ; this protein is considered to occupy a prominent place in the reducing properties of light-activated chloroplasts). ATP and TPNH are then used for assimilation of CO_2 to form sugar and starch as dark reactions with regeneration of R-5-P and Ru-D-P. Thus, the formation in light, of reduced phridine nucleotides and high-energy phosphates and the subsequent dark reactions of CO_2 assimilation using these compounds is the new concept but how these reaction-conjugation mechanisms link up with the primary photochemical process occurring in chlorophyll remain largely speculative.

During the last decade Calvin and his collaborators using $C^{14}O_2$ showed that CO_2 reduction by plants resulted essentially in the formation of Ru-D-P subsequently converted into PGA (Figs. 4, 5). For one turn of the

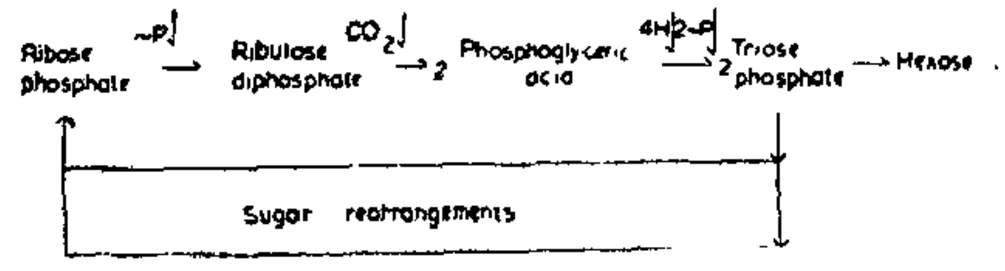

FIG. 4. Photosynthetic carbon cycle of Calvin (from Hill and Whittingham, 1958).

cycle one molecule of CO_2 is fixed and this requires the transfer of four hydrogen atoms and formation of three active phosphate groups. This double requirement has been shown with particulate preparations of spinach leaves. All the energy requirements for the operation of the cycle were present together with added ATP. No reaction occurred until the addition of one of the sugar phosphates as primer after which there was continued fixation of CO_2 and formation of hexose phosphate. A rapid decrease in CO_2 concentration was accompanied by a rapid fall in PGA and rise in RDP. This is a notable contribution as it is the first analytical study of changes in intermediates *in vivo* within times approaching those of individual reaction velocity constants.

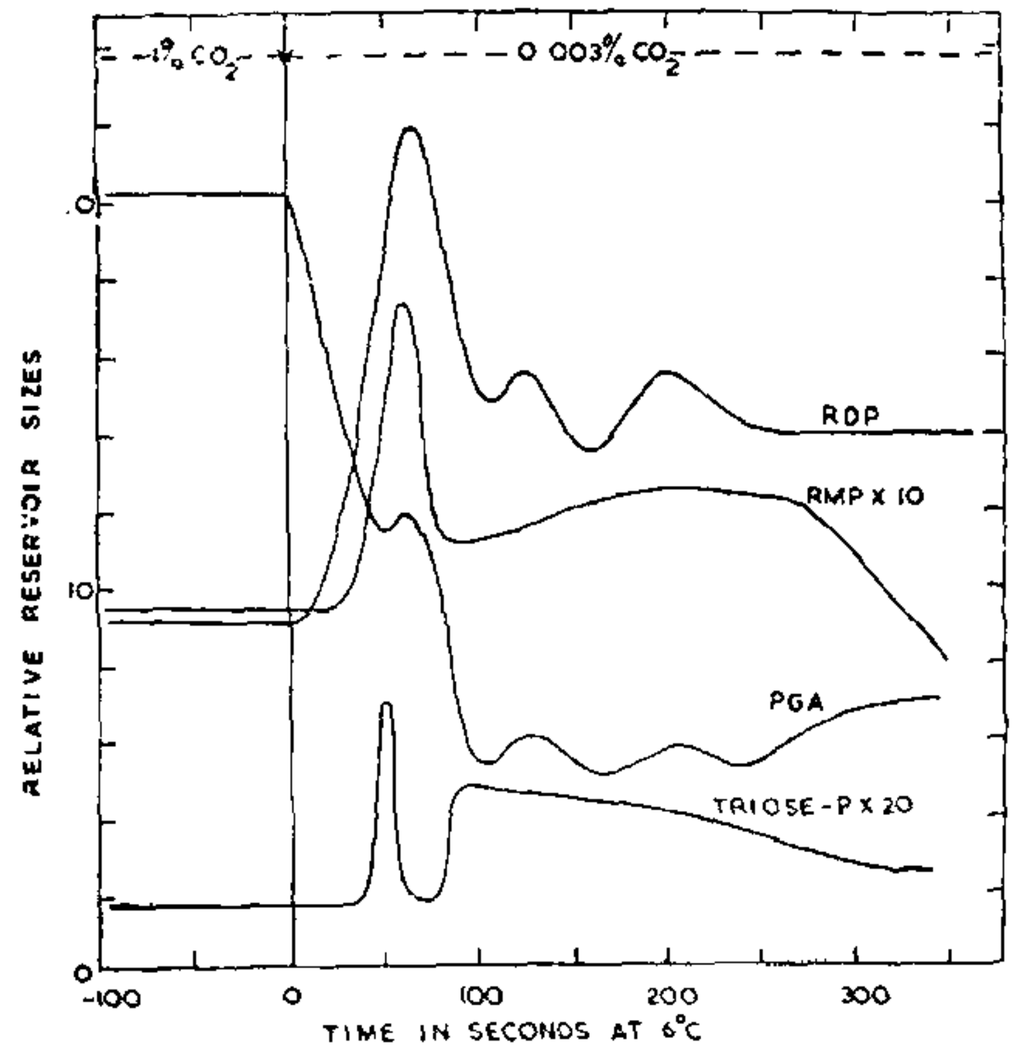

FIG. 5. Changes in the concentrations of phosphoglyceric acid, ribose, di- and mono-phosphate and triose phosphate consequent to a reduction in CO_2 concentration as shown by A. T. Wilson and M. Calvin (from Hill and Wittingham, 1958).

The nature of the enzyme systems taking part in the formation of the intermediates of the cycle of photosynthesis are being better understood now. In the green plant, Coenzyme II is postulated as the hydrogen acceptor for chloroplast functioning as a photochemical system. Coenzyme II reduction involves an intermediary which has been shown to be a soluble protein separable from the green insoluble system containing chlorophyll and cytochrome. The insoluble portion seems to possess the ability to initiate phosphorylation since H_2-acceptors could replace Coenzyme II and soluble protein (*see* Hill and Bendall). The chloroplast system seems to act by reversing a stepwise H_2 transfer and phosphorylation is a light reaction of a reductive nature against a thermochemical gradient. The emphasis laid by Hill and his collaborators is on a two step light-driven reaction involving cytochrome b_6 and *f*, somewhat analogous to hydrogen transfer characteristic of mitochondria. Admittedly, such a phosphorylation step between cytochrome b_6 and *f* has not found experimental evidence

in green plants. Indeed, one of the drawbacks of the Arnon scheme is that the properties ascribed to cytochromes do not seem to agree with their *in vitro* known properties but that is no argument to underestimate the major discovery that the rate of production of O_2 doubled in the presence of ADP, magnesium and inorganic phosphate. Therefore, it becomes obvious that, if it is asssumed that hydrogen (or electron) transfer is the basic mechanism by which cytochrome functions in chloroplasts, there ought to be more than one light-driven reaction acting against the thermochemical gradient.

The recent writings of Nichiporovich *et al.* (*see* Krasnovsky) indicate that relative rapidity of entry and subsequent distribution of labelled carbon in carbohyrates, amino-acids, organic acids and proteins vary with plant species, age, nutrition and intensity and spectral composition of the incident light. They claim that red light promoted increased carbohydrate formation while blue light promoted amino-acid and protein formation. These workers presume that this is attributable to the activation of cytochrome, flavin systems and respiration in blue light and its consequent effect on alteration of the ratio of oxidative to reductive reactions of photosynthesis. Be that as it may, it is quite obvious that, functioning carbon cycles must have an uninterrupted supply of products such as reduced pyridine-nucleotides and ATP, which, indeed, are resultant products of photochemical reactions. The active H_2 is lost by the reduced pyridine nucleotide in the carbon cycle and DPN (diphosphopyridine nucleotide) and TPN return to the system of photochemical reactions. Similarly, ADP returns to the photosynthetic phosphorylation cycle.

Deuterium (D_2O) and tritium (T_2O), the heavy hydrogen isotope and radioactive hydrogen, respectively, have been used alongside $C^{14}O_2$ in experiments on photosynthesis to understand the role of H_2 in the process. Accumulation of tritium was shown in glycolic, phosphoglyceric and glutamic acid in light experiments and in the dark, accumulation was greater in amino-acids. Much of the experimental work in this field remains inconclusive and generally point to the remote possibility of primary reversible dehydrogenation being the venue of participation of chlorophyll in photosynthesis. The use of heavy oxygen (O^{18}) in photosynthesis showed its accumulation in sugar phosphates and even here much work seems imminent if we were to understand the many mechanisms involved. However, the use of isotopes of H_2 or O_2 have so far yielded no tangible results and the mechanism of liberation of molecular oxygen which is the bed-rock of the phenomenon is still hypothetical. It has been suggested that intermediates of the oxidation of water resulting in the formation of molecular oxygen are evanescent and, since the process is a high velocity reaction, have defied detection. Despite all these results that have accrued by using isotope techniques, no decisive stand could be taken other than the path of carbon in photosynthesis being a multi-step cyclic process where CO_2 is bound by a compound (this according to Calvin is Ru-D-P subsequently converted into PGA) which is continually regenerated.

Summing up it could be stated that TPN-coupled phosphorylation, *i.e.*, synthesis of ATP, without evolution of O_2, is the new concept of Arnon that warrants attention and it has much experimental proof. Therefore, the conversion of light into chemical energy is fundamentally linked with phosphorus than with carbon. The view that efficient transfer of light energy into high-energy phosphate by a process recombination of products of photolysis cannot yet be thrown overboard. But in what way and how much of this high-energy phosphate is utilized in reduction of CO_2 may well have to be explained. Comparing chloroplast and chromatophore reactions there is undoubtedly similarity in the photophosphorylation process of both systems ; in essence, it is an anaerobic process depending on electron transport between photochemical reductant and oxidant (Fig. 6).

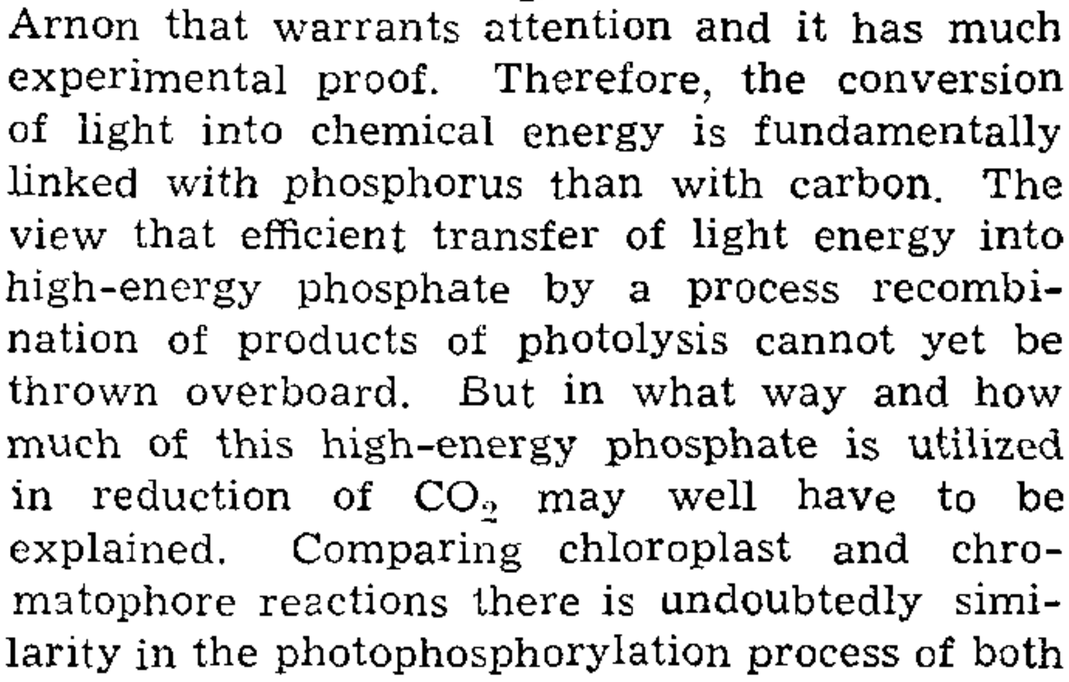

FIG. 6. Comparison of photosynthetic systems in green plants and photosynthetic bacteria (after Arnon, 1959).

However, the similarity exists only for the vitamin K pathway of cyclic photophosphorylation, the FMN pathway as well as the non-cyclic pathway being peculiar to chloroplasts. Therefore, with the recognition of cyclic photophosphorylation (vitamin K pathway) as

a common process to photosynthesis in green plants and photosynthetic bacteria, it is possible to omit photolysis of water as a common photochemical reaction. Thus, the evolution of O_2 by green plants is a consequence of some special way in which they form TPNH in light for which water is the hydrogen donor. To keep chlorophyll functioning, electrons removed by non-cyclic transport have to be continuously replenished possibly by electrons donated by the hydroxyl ions *via* a cytochrome chain and yielding molecular oxygen in the process (Fig. 3).

I have endeavoured to present much of what is known about photosynthesis in the hope that it would enthuse workers to study this fascinating subject from the biochemical, biophysical and physiological angles. It is a pleasure to acknowledge with thanks help of my colleagues : Drs. L. Saraswathi-Devi, D. Subramanian and S. Suryanarayanan for critical review of this manuscript.

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4. — and —, *Ibid.*, 1960, **187**, 417.
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6. Krasnovsky, A. A., *Ibid.*, 1960, **11**, 363.

ADDENDUM

Since writing this up for the press an excellently written and fully illustrated article on the subject by D. I. Arnon has come to my notice (*Scientific American*, 1960, 203, 104). The reader would do well to peruse this. Much of the ground I have covered is what Arnon's latest article contains except that the non-cyclic photophosporylation referred to earlier as peculiar to green plants (*see* Fig. 6) has now turned out to be common for the photosynthetic bacteria also. However, the hydrogen donor in the latter case is not water but reducing substances like succinate.

Arnon has also added a philosophic note towards the end on "Photosynthesis and Evolution" which may be summarized as : When life appeared there was little oxygen in the atmosphere but had hydrogen as a free gas. In the same way as the bacterium *Chromatium* has been shown to be capable of making ATP in visible sunlight, the chlorophyll molecule, just evolved, is presumed to do in cells under anaerobic conditions. Thus, this vestige of a functional mechanism of cyclic photophosphorylation seems still to be retained by the aerobic plants we now see despite their ability in addition to make ATP through respiration. Then perhaps came a further evolutionary step, the non-cyclic process simulating the bacterial function of reducing pyridine nucleotide without using molecular hydrogen. The acme of perfection in photosynthesis eventually came from the ability of having water to donate the needed hydrogen and liberating oxygen into the atmosphere.

SYMPOSIUM ON COLLAGEN

A SYMPOSIUM on Collagen, sponsored by the Central Leather Research Institute, Madras, was held on the 29th and 30th November, 1960. Prof. M. S. Thacker, Director-General, Scientific and Industrial Research, India, inaugurated the symposium at a function presided over by Dr. A. Lakshmanaswamy Mudaliar, Vice-Chancellor of the Madras University. The inauguration was followed by the Conference lecture on "Structure of Fibrous Proteins and Polypeptides" by Prof. G. N. Ramachandran of the University of Madras. In this lecture, Prof. Ramachandran summarized the present state of knowledge on proteins and polypeptides, and dealt in detail with the structural problems associated with collagen and feather keratin.

Thirty-five papers were contributed to the symposium, and it may be noted that more than half the number came from scientists and technologists representing nine different countries of the world outside India.

In the first session on "Structural Studies" presided over by Dr. S. Ramaseshan of the Indian Institute of Science, Bangalore, ten papers were presented and discussed. Many proteins such as insulin, tobacco mosaic virus, myoglobin, etc., form single crystals ; they have a specific sequence of amino-acid residues and are arranged in a perfect crystalline lattice array ; their X-ray patterns show hundreds of sharp spots known in conventional X-ray crystallography. On the other hand, proteins like collagen, keratin, etc., form fibres and they differ from crystalline proteins not so much in their intrinsic chemical nature as in their physical state of aggregation. In fact it is this aggregation which endows collagen, for example, with those characteristic properties which make it an important biological building block. X-ray patterns of fibrous proteins show only a few diffraction streaks, and the elucidation of the structure is beset with difficulties. The