PHOTOSYNTHETIC MECHANISMS IN BACTERIA AND PLANTS: DEVELOPMENT OF A UNITARY CONCEPT[1]

R. Y. STANIER

Department of Bacteriology, University of California, Berkeley, California

I. INTRODUCTION

And here, especially, lies the importance of these "abnormal" photosynthetic processes, because a comparison of the factors and conditions which are required for their accomplishment will enable us to find those characteristics which are common to all. It will then be possible to derive the fundamental laws underlying all photosynthetic processes and to correlate these into a general view.

C. B. van Niel, 1930 (70)

The purple and green bacteria occupy an enigmatic position in the living world. In terms of their gross morphology, these two small groups can be regarded as typical unicellular representatives of the eubacteria; but whereas most eubacteria depend on chemical sources of energy, purple and green bacteria are primarily, and in many cases exclusively, dependent on light as an energy source. This abrupt intrusion of a photosynthetic mode of metabolism in a taxonomic group distinguished by the unparalleled variety of its special adaptations to the use of chemical energy sources is certainly remarkable, and has led many bacterial systematists to set off the photosynthetic bacteria in a special major sub-

[1] This essay is based on the Mueller Memorial Lecture, delivered at Harvard Medical School on October 29, 1959. Insofar as the views expressed are original, they were developed by the author during his tenure (1957–59) of a research professorship in the Miller Institute for Basic Research in Science of the University of California.

group, a treatment which cannot be justified on the basis of purely morphological considerations. Considered as members of the photosynthetic community, the purple and green bacteria are also anomalous. Both their pigment systems and their gross photosynthetic mechanisms are unique, and stand out with particular sharpness in contrast to the basically uniform pigment system and photosynthetic mechanism in algae and higher plants. It is these striking deviations from the general pattern of photosynthesis that confer on the photosynthetic bacteria their cardinal interest as biological entities.

The question, "What is photosynthesis?" is a deceptively simple one. Many different answers have in fact been proposed, and the generally accepted one has changed from time to time as understanding of the photosynthetic process has deepened. It is not improbable that a definitive answer can now at last be offered. If so, this great problem of cellular physiology will have been solved in no small measure through studies on the photosynthetic bacteria. Since the characterization of bacterial photosynthesis some 30 years ago by van Niel (70, 71), the photosynthetic bacteria have often provided the material for experimental analyses which could not have been easily undertaken, and sometimes could not have been undertaken at all, with plant material. Furthermore, the very existence of photosynthetic processes which differ from those of green plants has greatly facilitated the task, not always easy, of discriminating between the

fundamental and incidental features of this complex metabolic reaction.

This essay will review the development of ideas about photosynthesis during the 30 years which have elapsed since the recognition of the bacterial photosyntheses, and show how work with bacterial systems has contributed to the formulation of our present concepts. No attempt has been made to provide a comprehensive survey of the literature on the metabolism of photosynthetic bacteria. References are accordingly restricted to those publications which are, in my opinion, directly relevant to the main theme.

As an introduction to the subject proper, the physiological and structural features which characterize photosynthetic bacteria and distinguish them from green plants will be summarized.

II. Major Variations in Patterns of Photosynthetic Metabolism

Photosyntheses are metabolic processes which result in the conversion of radiant energy into chemical bond energy, specifically, into the phosphate bond energy of adenosine triphosphate (ATP). This is their one common denominator. Under conditions favoring growth, the ATP so generated is used for the synthesis of more cell material. Most phototrophs perform a total synthesis of cell material from inorganic nutrients; accordingly, carbon dioxide is commonly the sole carbon source. The gross conversion of carbon dioxide to cell material requires, in addition to ATP, a source of reducing power. In all green plants this reducing power is ultimately provided by water, through a special reaction, linked with the photochemical process, which results in the oxidation of water to molecular oxygen.

The green bacteria and many of the purple bacteria share with plants the ability to use CO_2 as the sole source of carbon for cellular synthesis. They cannot, however, use water as an ultimate reductant, and consequently depend on other inorganic reductants (notably reduced sulfur compounds and molecular hydrogen). Since the oxygen evolution characteristic of green plant photosynthesis is a consequence of the oxidation of water, it follows that oxygen is not evolved in bacterial photosynthesis, and that this type of photosynthesis is an anaerobic process. Indeed, the green bacteria and many

purple bacteria are strict anaerobes. Since photosynthetic bacteria cannot use fermentative mechanisms to obtain the energy needed for growth, strict anaerobiosis entails as a corollary obligate phototrophy.

The purple bacteria can also use simple organic compounds as the major carbon source for the photosynthesis of cell material. In such cases, the exogenous carbon source is similar in oxidation state to cell material, and the requirement for an exogenous inorganic reductant disappears. However, the bacterial photosyntheses of organic substrates are anaerobic processes; accordingly, a strict over-all oxidation-reduction balance must be maintained, just as in the case of a fermentation. When the organic substrate is more oxidized than cell material, strict oxidation-reduction balance is achieved by the anaerobic oxidation of part of the substrate to CO_2, which provides reducing power for the synthesis of cell material from other substrate molecules. When the organic substrate is more reduced than cell material, strict oxidation-reduction balance is achieved by partial oxidation of the substrate, coupled with reduction and assimilation of CO_2; in this case, there are two exogenous carbon sources, and assimilation of the organic substrate is mandatorily linked with CO_2 assimilation.

The major physiological distinctions between plants, green bacteria, and purple bacteria are summarized in table 1.

In terms of the chemistry of their pigment systems, the purple and green bacteria are sharply distinguished from one another, and also from all organisms that perform green plant photosynthesis. No molecular species of chlorophyll or of carotenoid is common to the photosynthetic apparatus of all three groups. In all purple bacteria, there is one molecular species of chlorophyll, bacteriochlorophyll (74). The characteristic carotenoids of purple bacteria are aliphatic (37). In the green bacteria, there occur two molecular species of chlorophyll (chlorobium chlorophylls), only one of which is present in any given strain (68). The characteristic carotenoid of green bacteria is γ-carotene (38, 80), a monocyclic compound. The pigment system of organisms that perform green plant photosynthesis cannot be so succinctly characterized; although the higher plants are strikingly uniform in this respect, marked systematic differences in the composition of the pigment system char-

TABLE 1

Major physiological distinctions between green plants, green bacteria, and purple bacteria

	Green Plants	Green Bacteria	Purple Bacteria
Source of reducing power	H_2O	H_2S, other reduced inorganic compounds	H_2S, other reduced inorganic compounds, organic compounds
Photosynthetic oxygen evolution	Yes	No	No
Principal source of carbon	CO_2	CO_2	CO_2 or organic compounds
Relations to oxygen	Aerobic	Strictly anaerobic	Strictly anaerobic or facultatively aerobic

acterize the various groups of algae (69). Nevertheless, certain pigments are common to all organisms that perform green plant photosynthesis. These pigments, which accordingly serve as chemical hallmarks of this kind of photosynthesis, are one molecular species of chlorophyll, chlorophyll *a*, and one or both of the closely related bicyclic carotenoids, *α*- and *β*-carotene. The basic features of the photosynthetic pigment system in plants, purple bacteria, and green bacteria are summarized in figure 1.

Brief mention should be made also of the singular macromolecular structure of the photosynthetic apparatus in purple and green bacteria. Unlike most organisms that perform green plant photosynthesis, photosynthetic bacteria are devoid of chloroplasts. The pigment system is localized in much smaller structures known as chromatophores (63) which can be isolated from cellular extracts. The isolated chromatophores are 300 to 500 Å in diameter, and contain proteins, phospholipids, and respiratory pigments, in addition to the entire cellular complement of chlorophyll and carotenoids (9). The only other phototrophs which lack chloroplasts are the blue-green algae. A chlorophyll-containing cell fraction similar to the chromatophore fraction of photosynthetic bacteria can be isolated from extracts of these organisms (63, 64). The organization of the photosynthetic apparatus in the intact cell of photosynthetic bacteria and blue-green algae is not yet fully understood. This complex and somewhat controversial problem (14, 39, 56, 64, 75) will not be discussed further here.

It is customary to recognize two major subgroups within the purple bacteria: purple sulfur bacteria (family *Thiorhodaceae*); and nonsulfur purple bacteria (family *Athiorhodaceae*). The distinguishing properties are relatively minor, and difficult to apply consistently in practice. Purple sulfur bacteria are primarily photolithotrophs, which can develop with CO_2 as sole carbon source and H_2S, other reduced inorganic sulfur compounds, or H_2 as reductant. Nonsulfur purple bacteria are primarily photoorganotrophs, employing organic compounds as the principal carbon source and source of reducing power. The value of this conventional distinction is diminished by the fact that all purple sulfur bacteria so far studied in pure culture can also grow photosynthetically with organic substrates. Furthermore, although nonsulfur purple bacteria cannot use sulfide as a reductant for photolithotrophic growth, one species can use thiosulfate for this purpose, and probably all can use H_2.

A distinction between the two groups can also be made on the basis of the autotrophy of the purple sulfur bacteria, as contrasted with the heterotrophy of the nonsulfur purple bacteria, which need vitamins for growth. However, *Rhodomicrobium vannielii*, although in other physiological respects assignable to the nonsulfur purple bacteria, is an autotroph. Lastly, it should be mentioned that some nonsulfur purple bacteria can use respiratory metabolism as a means for aerobic growth in the dark, whereas all purple sulfur bacteria are strict anaerobes, and consequently obligate phototrophs.

III. RECOGNITION OF BACTERIAL PHOTOSYNTHESIS

Although purple bacteria had been observed since the earliest days of microbiology and were the subject of classical physiological investigations by Winogradsky (79) and Engelmann (19, 20) in the late 19th century, the photosyn-

CHARACTERISTIC PIGMENTS OF THE MAJOR PHOTOSYNTHETIC GROUPS			
	BIOLOGICAL GROUP		
	ALGAE AND HIGHER PLANTS	PURPLE BACTERIA	GREEN BACTERIA
CHLOROPHYLLS — MOLECULAR GROUND PLAN	SPECIAL FEATURES		
	chlorophyll a common to all. R₂ = vinyl. Ring IV reduced.	bacteriochlorophyll common to all. R₂ = acetyl. Rings II and IV reduced.	chlorobium chloro- phylls. two molecular species, only one of which occurs in any given strain. structures unknown but ring system is of the a type.
CAROTENOIDS — MOLECULAR GROUND PLAN	SPECIAL FEATURES		
	α- or β-carotene present in all. both ends of chain cyclized.	various pigments. all open chain compounds usually with methoxyl substituents	γ-carotene present in all. one end of chain cyclized.

Figure 1. Structure of the characteristic photosynthetic pigments in the three major groups of photographs.

thetic nature of their metabolism was conclusively established only in 1930, by van Niel (70). The obstacles to the recognition of bacterial photosynthesis were intellectual, rather than technical: notions, too rigidly held, about the nature of the photosynthetic process. The gross nature of green plant photosynthesis, a light-catalyzed conversion of carbon dioxide and water to cell material, with accompanying evolution of oxygen, had been recognized at the very beginning of the 19th century, although it was a good many years before the familiar stoichiometry:

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O) + O_2$$
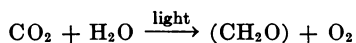
was really firmly established. By the end of the 19th century, several generations of plant physiologists had been able to convince themselves and the rest of the scientific world that all plants, from algae to flowering plants, carry out photosynthesis according to this equation. They committed the classical inductive error, illustrated in courses on logic by the statement that all swans are white, and concluded that all photosynthetic organisms mus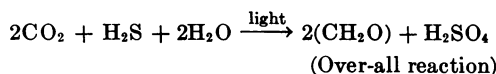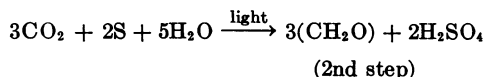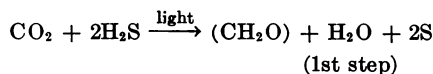t perform as prescribed by the equation for green plant photosynthesis. It then required great intellectual daring to realize that green plant photosynthesis might be merely a special case of a class of metabolic reactions; yet this realization was essential for the recognition of bacterial photosynthesis.

A second obstacle existed in the case of the purple bacteria. The invariable association of chlorophyll with the occurrence of the photosynthetic process had become clearly recognized in the 19th century. Although bacteriochlorophyll is in fact closely related chemically to the plant chlorophylls, its spectrum both in cells and in organic solvents is so radically different as to suggest a complete lack of chemical relationship. Between 1880 and 1890, Engelmann (19, 20) had defined the intracellular form of this pigment spectrally, even to its infrared band (a brilliant physical achievement for the time), and had shown that the biological response of purple bacteria to light is mediated through it. Yet the significance of these experiments, which rank among the most elegant researches in cell physiology of the 19th century, was completely lost on his contemporaries and immediate successors. The reason is obvious: the spectral properties of purple bacteria were so different from those of

plants that the functional homology of the two pigment systems appeared most improbable.

The purple sulfur bacteria develop characteristically in anaerobic environments exposed to light where hydrogen sulfide is being generated; they are abundant in sulfur springs and in grossly polluted brackish ponds where sulfide is produced biologically, as a result of the activities of sulfate-reducing bacteria. The fact that such purple bacteria are capable of oxidizing sulfide to sulfate had been established by Winogradsky in 1888 (79), but before van Niel's time they had not been grown in pure culture. Van Niel showed (71) that these purple bacteria, and also the green bacteria, will develop in a completely inorganic medium containing the necessary minerals, bicarbonate, and sulfide. They are strict anaerobes, and light is indispensable for their growth. Quantitative analyses of the chemical changes that occurred in pure cultures revealed a close stoichiometric relationship between the oxidation of sulfide and the uptake of carbon dioxide.

The oxidation of sulfide characteristically proceeds in a stepwise fashion; sulfide is first converted almost quantitatively to elemental sulfur, and the accumulated sulfur is then further oxidized to sulfate:

$$CO_2 + 2H_2S \xrightarrow{\text{light}} (CH_2O) + H_2O + 2S$$
$$(\text{1st step})$$
$$3CO_2 + 2S + 5H_2O \xrightarrow{\text{light}} 3(CH_2O) + 2H_2SO_4$$
$$(\text{2nd step})$$
$$2CO_2 + H_2S + 2H_2O \xrightarrow{\text{light}} 2(CH_2O) + H_2SO_4$$
$$(\text{Over-all reaction})$$

As is particularly clearly shown by the equation for the first step, this reaction is coupled oxidation-reduction, in which carbon dioxide serves as the oxidant and H_2S as the reductant. At first sight, it bears little resemblance to the classic equation for green plant photosynthesis, which implies that oxygen is derived in part from water and in part from carbon dioxide. Van Niel perceived, however, that a strict formal homology could be achieved if the equation for green plant photosynthesis were rewritten with the addition of a molecule of water to both sides:

$$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + H_2O + O_2$$
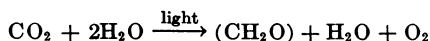
This apparently gratuitous emendation stated what was then a novel hypothesis about the mechanism of green plant photosynthesis; namely, that it is a coupled oxidation-reduction in which CO_2 is reduced to cell material while water is oxidized to molecular oxygen. As van Niel saw, both this equation and the various equations which can be written for bacterial photosynthesis at the expense of reduced sulfur compounds can be regarded as special cases of a general photosynthetic reaction:

$$CO_2 + 2H_2A \xrightarrow{\text{light}} (CH_2O) + H_2O + 2A$$
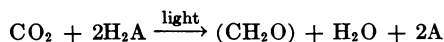
The validity of this interpretation of green plant photosynthesis was subsequently confirmed. Tracer studies with O^{18} demonstrated that the oxygen evolved originates from water, and not from carbon dioxide (12, 61, 77). Furthermore, it was discovered (40) that isolated chloroplasts can evolve O_2 upon illumination in the absence of CO_2, if provided with an artificial electron acceptor. Such a modification of green plant photosynthesis is now known as a Hill reaction.

IV. Concept of the Photolysis of Water

Van Niel's original studies on bacterial photosynthesis led to a new and more correct interpretation of the essential nature of green plant photosynthesis. They also forced a re-evaluation of the central problem of photosynthesis, namely, the nature of the light-catalyzed reaction. If one examines the equation for bacterial photosynthesis with sulfide, the reason for this is immediately evident. Of the two half-reactions, the oxidation of hydrogen sulfide to elemental sulfur (and thence to sulfate) was well known to be a dark reaction, which can be carried out by a variety of nonphotosynthetic bacteria. Furthermore, the reduction of CO_2 to organic compounds can also clearly proceed in the dark. Even in 1930, this was evident from the existence of the chemolithotrophic bacteria, and in the years that followed, many instances of CO_2 reduction by chemo-organotrophic organisms were discovered. Light is therefore not intrinsically necessary for either of the two half-reactions of bacterial photosynthesis. However, when van Niel (73) made a similar analysis of the two half-reactions of green plant photosynthesis, a different conclusion emerged. At least in biological systems, the oxidation of water with the formation of

molecular oxygen is not known to occur as a dark reaction. It was therefore tempting to postulate that light in some way intervenes in this cleaving of water. Despite repeated attempts by many investigators, the evolution of oxygen by illuminated photosynthetic bacteria has not been demonstrated; in these organisms, photosynthesis is never accompanied by oxygen release. Hence if one wished to assume a common primary light reaction in all types of photosynthesis, it could not be a cleaving of water with oxygen formation. These considerations led van Niel, some years after his original work on the purple and green bacteria, to propose a further generalization about the nature of photosynthetic processes (73). He suggested that the common primary light reaction in all photosyntheses is a cleaving of water, with the formation of an oxidized and a reduced moiety, which are conventionally designated as [H] and [OH]. It is not thermodynamically possible for these entities to be free radicals, and the square brackets were therefore symbolic representations of ignorance concerning their real physical nature. The beauty of this suggestion was that it permitted a very simple mechanistic interpretation of the photosynthetic process which applied alike to green plant and bacterial photosynthesis, as shown in figure 2.

In all types of photosynthesis, the reduced moiety from the photochemical cleavage of water is ultimately applied to the reductive step in the conversion of CO_2 to cell material. The basic difference between bacterial and green plant photosynthesis must then reside in the fate of [OH], the oxidized moiety. Van Niel assumed that green plants possess a special enzyme system capable of generating O_2 from this entity; the bacteria, lacking such an enzyme, dispose of [OH] by using it as a terminal oxidant for the external electron donor, H_2S (or, more generally, H_2A).

V. Role of Organic Substrates in Bacterial Photosynthesis

The photosynthetic metabolism of organic substrates was first systematically studied by Gaffron (28, 29), with nonsulfur purple bacteria, shortly after van Niel's work on the green and purple sulfur bacteria. Gaffron showed that many fatty acids can be rapidly metabolized

anaerobically in the light by nonsulfur purple bacteria, with a relatively small accompanying metabolic uptake or release of CO_2, the sign and magnitude of which are correlated with the oxidation level of the organic substrate. Careful measurements of the stoichiometry of photosynthesis with fatty acids as substrates led Gaffron to the conclusion that the metabolic product is a cellular reserve material somewhat more reduced than carbohydrate, with the empirical formula $C_4H_6O_2$. He was able to isolate a small amount of polymeric substance with this empirical formula from the bacterial cells (29), but did not determine its structure. Gaffron interpreted the photometabolism of organic acids by purple bacteria as a *light-catalyzed assimilation of the organic substrate*, fixation of CO_2 occurring only when surplus reducing power is made available, as a result of the assimilatory product being more oxidized than the substrate. In effect, according to Gaffron's interpretation, CO_2 reduction serves as a means for preserving the oxidation-reduction balance in this anaerobic type of metabolism.

His interpretation was vigorously challenged by van Niel (72, 73) who showed that the actual data could be explained differently by assuming that the organic substrate acts as a hydrogen donor in accord with the generalized photosynthetic equation:

$$CO_2 + 2H_2A \xrightarrow{\text{light}} (CH_2O) + H_2O + 2A$$
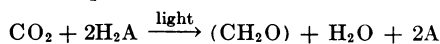
Van Niel did not exclude the possibility that part of the carbon of the organic substrate might be directly assimilated by the cell, but he insisted that it must always in part serve as an ultimate hydrogen donor for the reduction of CO_2. Insofar as an organic substrate serves the function of a hydrogen donor, it will normally be oxidized to CO_2. Carbon dioxide will therefore be both a reactant and a product of the photosynthetic reaction, and gross analysis of the over-all process cannot be expected to reveal its mechanism.

Gaffron's interpretation of his own data fitted the observations then available just as well, and perhaps slightly better, than the interpretation of van Niel. Nevertheless, Gaffron's hypothesis failed to gain general acceptance, and was eventually abandoned even by its author (23). The principal reason for this was undoubtedly the impossibility of incorporating it into any general interpretation of photosynthetic pro-
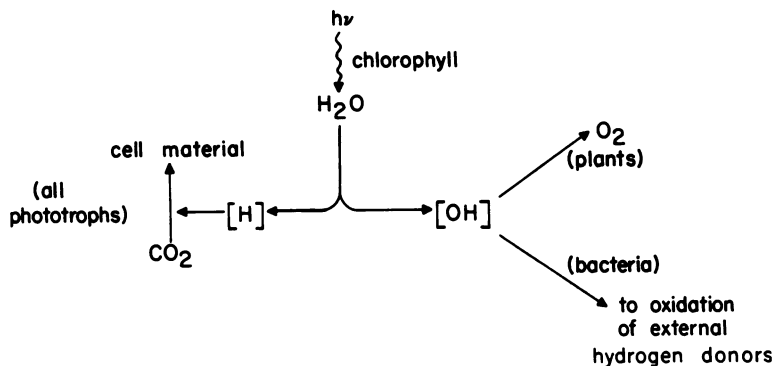
Figure 2. A diagrammatic illustration of the unitary hypothesis of van Niel concerning the mechanism of photosynthetic processes.

cesses, as these were understood at the time. The concept of a direct photoassimilation of organic compounds left no necessary place either for a fixation of carbon dioxide or for a photochemical cleavage of water. Its acceptance would thus have caused the abandonment of van Niel's superb generalizations, the intellectual beauty and logical force of which were evident to all.

There was, furthermore, one experimental observation which lent support to van Niel's interpretation of the role of organic substrates in photosynthesis. In 1940 Foster (22), then working in van Niel's laboratory, isolated a *Rhodopseudomonas* strain which performed an incomplete oxidation of isopropanol, with the stoichiometric formation of acetone. Foster was able to show that in this special case, the photometabolism of an organic compound conformed closely to the generalized equation of van Niel:

$$CO_2 + 2\,\text{isopropanol} \xrightarrow{\text{light}} (CH_2O) + H_2O + 2\,\text{acetone}$$
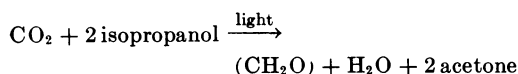
Further progress in the understanding of photosynthesis with organic substrates was slow. As time went on, however, a number of facts emerged which suggested that the isopropanol reaction of Foster was not a satisfactory general model. When $C^{14}O_2$ was used to measure the extent of CO_2 fixation during the photometabolism of organic substrates, some surprising results were obtained. For example, Glover and Kamen (36) and Ormerod (57) observed that during the photometabolism of acetate by *Rhodospirillum rubrum*, the rate of CO_2 fixation was actually *lower* than in control cells not furnished with an exogenous organic substrate.

It will be recalled that many nonsulfur purple bacteria can develop aerobically in the dark at the expense of organic substrates; under these circumstances they carry out a purely respiratory metabolism (73) with the use of the tricarboxylic acid (TCA) cycle as a mechanism of terminal substrate oxidation (16). Van Niel's interpretation of the photosynthetic process implies that when the same substrates are metabolized anaerobically in the light, they undergo a broadly similar type of degradation, except that the oxidized product of water cleavage, [OH], replaces molecular oxygen as terminal oxidant. One might therefore expect that the TCA cycle should operate in a like manner in the respiratory and photosynthetic metabolism of organic substrates. This aspect of the problem was analyzed experimentally a few years ago by Elsden and Ormerod (18), who studied the effects of fluoroacetate on the light and dark metabolism of many organic substrates by *Rhodospirillum rubrum*.

Fluoroacetate, the mode of action of which was first elucidated by the classic studies of Peters *et al.* with mammalian systems (58), inhibits terminal respiration as a consequence of its ability to replace acetate in the first step of the TCA cycle, the condensation of acetyl-CoA and oxalacetate to citrate. The product of the fluoroacetyl-CoA condensation, fluorocitrate, inhibits the enzyme aconitase, which catalyzes the ensuing step of the cycle, and thus rapidly brings terminal respiration to a halt. Elsden and Ormerod showed that the classic effects of fluoroacetate poisoning, inhibition of respiration and accumulation of citrate, were readily demon-

strable in purple bacteria during the dark oxidation of all organic substrates tested. However, an entirely different picture emerged when the effect of fluoroacetate on photosynthetic metabolism of the same organic compounds was examined. Only with acetate and pyruvate were the typical symptoms of fluoroacetate poisoning observed. The metabolism of propionate, succinate, and malate was scarcely affected. With butyrate, an inhibition of photosynthesis was found, but this was unaccompanied by the accumulation of citrate. From these data one may conclude that the TCA cycle, universally operative in the respiratory metabolism of purple bacteria, ceases to be a major pathway in the light except with acetate and closely related substrates.

Thus, there emerged a series of facts which could not be easily reconciled with van Niel's interpretation of the photometabolism of organic substrates. Dislike of this interpretation was voiced (32), but mere dislike is not an adequate weapon for the destruction of a well entrenched hypothesis. As often happens in science, it was chance discovery rather than deep thought which eventually provided the solution of this problem. In the course of other studies, our colleague Dr. Germaine Cohen-Bazire observed that nonsulfur purple bacteria growing photosynthetically with acetate accumulate massive intracellular stores of sudanophilic granules. Isolation and analysis of these granules (13) showed that they consisted largely of poly-β-hydroxybutyric acid, $(C_4H_6O_2)_n$. Gaffron's postulated assimilatory product was thus rediscovered and identified chemically. In fact, this polyester had been known as a major bacterial cell constituent for over 30 years. It was originally found in a *Bacillus* species by Lemoigne (45), and has subsequently been detected as a reserve material in many other chemotrophic bacteria (13, 21, 46, 53).

Studies of the photometabolism of C^{14}-labeled acetate and butyrate by resting cells of *Rhodospirillum rubrum* (13) showed that these fatty acids are incorporated into the intracellular depot of poly-β-hydroxybutyrate with high efficiency, and virtually without dilution of their specific activity. On the physiological level, polymer synthesis can be regarded as a mechanism for the intracellular storage of large quantities of fatty acid carbon; as a result of polymerization, the acidic substrate is neutralized

and made osmotically inert. Upon removal of the external organic carbon source, the cell can draw on this internal carbon store for general biosynthesis. The process is formally analogous to the formation of starch as a primary photosynthetic assimilate in plants.

Further work (67) showed that *Rhodospirillum rubrum* accumulates large stores of poly-β-hydroxybutyrate only when furnished with even-carbon fatty acids (acetate, β-hydroxybutyrate, butyrate). Photometabolism of such substrates as succinate, malate, and propionate by either growing or resting cells leads to the accumulation principally of a second photosynthetic assimilate, a glycogen-like polysaccharide. This polysaccharide is also the major primary product of photosynthetic CO_2 assimilation in the presence of H_2. The paths of carbon assimilation by purple bacteria are consequently complex, the nature of the primary assimilatory product being determined by the chemical nature of the substrate.

The photosynthetic formation of poly-β-hydroxybutyrate from acetate and butyrate is biochemically a relatively simple process which illustrates in the clearest possible fashion the essential nature of the photometabolism of organic substrates. The conversion of acetate to polymer is a reductive synthesis:

$$2n\ C_2H_4O_2 + 2nH \rightarrow (C_4H_6O_2)_n + 2n\ H_2O$$
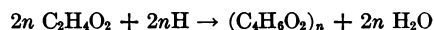
It therefore cannot proceed without an input of electrons. These are normally provided by the oxidation of some acetate via the TCA cycle, the balanced equation for the gross reaction being:

$$9n\ C_2H_4O_2 \xrightarrow{\text{light}} 4(C_4H_6O_2)_n + 2n\ CO_2 + 6n\ H_2O$$
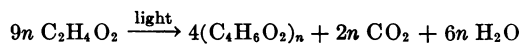
This obligatory coupling of synthesis with oxidation explains the observation of Elsden and Ormerod (18) that the photometabolism of acetate, unlike that of most other organic substrates, is inhibited by fluoroacetate with an accompanying accumulation of citrate. Far from being coupled with a reduction of CO_2, the photosynthesis of poly-β-hydroxybutyrate from acetate actually competes with CO_2 reduction for the limited reducing power available. This explains the observations (36, 57) that the rate of CO_2 fixation during the photometabolism of acetate may be less than the endogenous rate.

The anaerobic oxidation of some acetate becomes unnecessary if an external reductant for

polymer synthesis can be provided. Indeed, as Gaffron first showed (29), the photometabolism of acetate can be coupled with an uptake of molecular hydrogen. We have restudied the kinetics of this interesting photosynthesis and have found that assimilation of acetate is much more rapid in hydrogen than in helium, no doubt because the rate of its photosynthetic assimilation is normally limited by the rate at which acetate can be oxidized through the cycle (67). The acetate-hydrogen reaction can be represented by the equation:

$$2n \text{ acetate} + n\,H_2 \xrightarrow{\text{light}} (C_4H_6O_2)_n + 2n\,H_2O$$
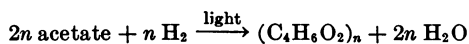
β-Hydroxybutyrate is rapidly photometabolized by *R. rubrum* without any uptake or formation of CO_2 (*unpublished observations*). Its photosynthetic conversion to polymer can be represented by the equation:

$$n\,C_4H_8O_3 \xrightarrow{\text{light}} (C_4H_6O_2)_n + n\,H_2O$$
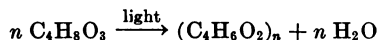
This is the purest example of the direct photoassimilation of an organic substrate. There is no photoreduction, for the simple reason that nothing is oxidized or reduced. Hence the reaction leaves no obvious place for the photolysis of water with the generation of an oxidation-reduction system, which is the central common event of photosynthesis according to van Niel's hypothesis. Yet it is undoubtedly a photosynthetic process, since light is indispensable for its occurrence. Ten years ago it would not have been possible to propose a unitary hypothesis capable of furnishing a common link between this reaction and the more familiar photosynthetic reactions. Fortunately, the situation today is not so bleak. Since 1950, advances in other directions have revealed a new common denominator of photosynthesis, which was almost unsuspected and completely unsupported by experimental evidence when van Niel proposed the photolysis of water.

VI. Photophosphorylation

Between 1945 and 1954, the path of photosynthetic carbon dioxide assimilation in green algae was mapped by tracer methods, largely by Calvin and his group (8). During the same period, the previously unknown steps of this sequence were independently elucidated at the enzymatic level (60, 78). The details of this

pathway need not concern us here, since the central importance of this body of work for the understanding of photosynthesis lies not in the biochemical step reactions so revealed, but rather in the definitive establishment of the *net chemical requirements* for the reduction of a mole of carbon dioxide to the carbohydrate level. These requirements are 2 moles of reduced pyridine nucleotide and 3 moles of ATP. The formation of reduced pyridine nucleotide did not pose an intrinsically new problem; in theory, the photolysis of water postulated by van Niel could furnish an unlimited quantity of reductant at this potential level. The formation of the required ATP was another matter, however. For the first time, the bioenergetics of photosynthesis was removed from the dense and rather smoggy atmosphere of quantum efficiency measurements and thermodynamic argumentation in which it was then shrouded and faced squarely as a *biochemical* problem. We need not retrace here in detail the rather tortuous path which was followed in the search for the mode of origin of ATP in green plant photosynthesis (4). The correct answer was eventually provided by Arnon *et al.* (7), with the discovery that plant chloroplasts perform a hitherto unknown light-induced synthesis of ATP from ADP and inorganic phosphate. During the past few years, this group has succeeded in demonstrating two distinct ATP-generating reactions catalyzed by chloroplast material (5). The first of these is cyclic photophosphorylation, a reaction which can be formulated as:

$$ADP + P_i \xrightarrow{\text{light}} ATP$$
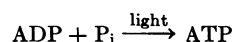
and which can occur under strictly anaerobic conditions, in the absence of photosynthetic oxygen evolution. The second type of ATP-generating reaction in chloroplasts can be expressed by the over-all equation:

$$TPN + 2H_2O + ADP + P_i \xrightarrow{\text{light}}$$
$$TPNH_2 + \tfrac{1}{2}\,O_2 + ATP + H_2O$$
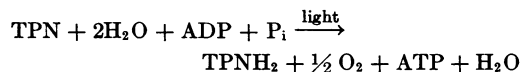
As suggested by the above equation, the esterification of inorganic phosphate is in this case linked with photosynthetic oxygen evolution and with the generation of reduced pyridine nucleotide. In effect, it represents a coupled Hill reaction.

Almost simultaneously with the discovery of

photophosphorylation in chloroplast prepara-
tions, Frenkel (24) discovered the same phenom-
enon in preparations of bacterial chromatophores.
Despite considerable subsequent work on photo-
phosphorylation with cell-free bacterial prepara-
tions (2, 25, 26, 31, 55), the only light-induced
reaction for the synthesis of ATP which has been
found is cyclic photophosphorylation. Since the
second, noncyclic reaction characteristic of chlor-
oplast preparations is intimately linked with
their capacity for oxygen evolution, its absence
in bacteria is, of course, readily understandable.
Cyclic photophosphorylation thus emerges as
the one common biochemical denominator of
all types of photosynthesis.

The work that has been done in the past few
years on the mechanism of cyclic photophos-
phorylation with both plant and bacterial sys-
tems (reviewed in references 6 and 27) suggests
that formation of ATP is coupled with the trans-
port of electrons through a chain of carriers
associated with the photosynthetic apparatus.
Cyclic photophosphorylation thus has a certain
analogy with oxidative phosphorylation, the
essential difference being that in cyclic photo-
phosphorylation both oxidant and reductant
are endogenously generated by the primary light
reaction. The detailed structure of the carrier
system is not yet clearly established. However,
cytochromes comprise an important component,
just as in oxidative electron transport. The first
evidence for the association of cytochromes with
the photosynthetic apparatus was provided in
1951 by Hill and Scarisbrick (41), who described
a new plant cytochrome of the c type, cyto-
chrome f, which is specifically localized in chloro-
plasts. Shortly afterwards, Vernon (76) reported
large amounts of a c type cytochrome in extracts
of *Rhodospirillum rubrum*. Similar pigments have
subsequently been found in other photosynthetic
bacteria (17, 35, 43, 54), including the obliga-
tory anaerobic purple sulfur and green bacteria,
in which they obviously cannot play a role in
respiratory electron transport.

Direct evidence for the participation of these
pigments in the events of photosynthesis has
been obtained by examination of the spectral
changes that occur when suspensions of algae
or photosynthetic bacteria are illuminated (10,
11, 15, 66; summary in 65). The spectral changes
caused by illumination are rather complex, since
light also induces marked shifts in the absorp-
tion bands of the other respiratory carriers and
of the carotenoid pigments of the photosynthetic
apparatus. However, it is clear that in several
kinds of photosynthetic cells, illumination evokes
an immediate oxidation of cytochrome, which
is rapidly reversed when the light is turned off.

VII. ARNON'S MODEL FOR PHOTO-PHOSPHORYLATION

On purely formal grounds, it is possible to
reconcile recent observations concerning photo-
synthetic ATP generation and cytochrome func-
tion with van Niel's hypothesis that the primary
photochemical event in photosynthesis is the
photolysis of water. The recombination of the
reduced and oxidized moieties through an inter-
nal transport system could provide the physical
basis for cyclic photophosphorylation, and the
photosynthetic generation of [OH] could account
for light-induced cytochrome oxidation. How-
ever, Arnon (6) has suggested a more attractive
hypothesis. He proposes that the absorption of a
light quantum by chlorophyll results in the
expulsion of an electron at a high energy poten-
tial, created at the expense of the energy con-
tained in the absorbed light quantum. The chloro-
phyll molecule thereby becomes electropositive
and immediately accepts an electron from cyto-
chrome, which is accordingly oxidized. In cyclic
photophosphorylation, the electron which has
been expelled from chlorophyll passes through
the photosynthetic electron transport chain and
is finally captured by the oxidized cytochrome,
part of its energy being drained off in passage
as ATP (figure 3). This, then, would be the es-
sential sequence of events common to both bac-
terial and green plant photosynthesis. In green
plants a second possible fate awaits the expelled
electron. It may be accepted by TPN, which
simultaneously picks up a hydrogen ion from
water to form reduced TPN. At the same time,
the oxidized cytochrome is reduced by the trans-
fer of an electron from a hydroxyl ion, a reaction
which is envisaged as resulting in ATP formation
and concomitant oxygen evolution (figure 4).

VIII. PHOTOSYNTHETIC HYDROGEN EVOLUTION AND NITROGEN FIXATION

As discovered by Gest and Kamen (33, 34),
purple bacteria can under certain conditions
evolve molecular hydrogen upon illumination.
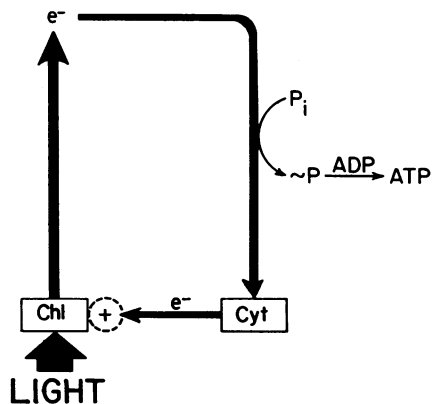Such hydrogen production requires the presence

Figure 3. A simplified version of the model of Arnon (6) to illustrate the mechanism of cyclic photophosphorylation.
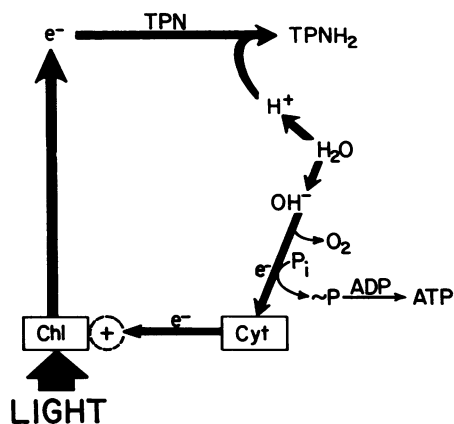

Figure 4. A simplified version of the model of Arnon (6) to illustrate the mechanism of noncyclic photophosphorylation in green plants.

Figure 5. The mechanism postulated by Losada, Nozaki, and Arnon (49) to account for photohydrogen evolution from thiosulfate by purple bacteria.

of an oxidizable substrate, and is rigorously dependent on light. Most of the work on photohydrogen production by purple bacteria has been done with organic substrates. Recently, Losada, Nozaki, and Arnon (49) have shown that in *Chromatium* a light-dependent evolution of hydrogen can be obtained at the expense of a simple inorganic oxidizable substrate, thiosulfate. They propose that the mechanism involves a reduction of hydrogen ions by electrons expelled by light from chlorophyll, oxidation-reduction balance being preserved by electron transfer to chlorophyll from thiosulfate via the transport system of the photosynthetic apparatus (figure 5).
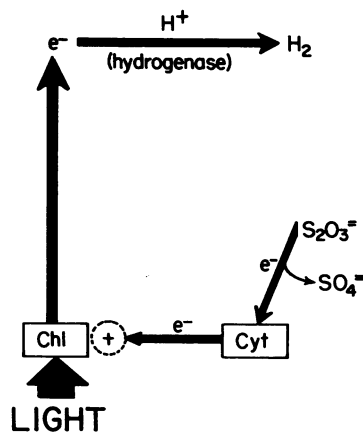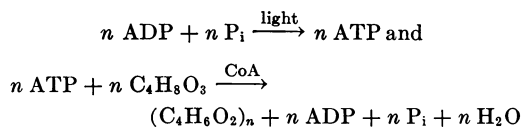
Early in the work on photohydrogen production, it was observed (42) that molecular nitrogen acts as a repressor. This observation led to the discovery that both purple and green bacteria are capable of nitrogen fixation when growing anaerobically in the light (47). The capacity for nitrogen fixation by facultatively aerobic purple bacteria under aerobic conditions in the dark is, in contrast, of negligible magnitude (48, 59). This phenomenon can also be readily interpreted in terms of Arnon's model. The fixation of molecular nitrogen represents, in effect, another possible way of utilizing the electrons expelled from chlorophyll by light (Arnon, *et al.*, 7a).

Photohydrogen production and photosynthetic nitrogen fixation appear to be of universal occurrence in photosynthetic bacteria, but are not strictly confined to these phototrophs. As was shown many years ago by Gaffron and Rubin (30), "hydrogen-adapted" green algae (*i.e.*, algae in which formation of the enzyme hydrogenase has been induced by exposure to molecular hydrogen) are capable of hydrogen evolution in the light, presumably at the expense of endogenous electron donors. It may be supposed that any photosynthetic organism is potentially able to perform this reaction, if it contains hydrogenase as a constitutive enzyme or can form it by induction. Photosynthetic nitrogen fixation is characteristic of many blue-green algae (1). A similar argument can be applied in this case.

Any phototroph, if capable of synthesizing the enzymatic machinery for nitrogen fixation, will be able to use the reducing power available from the light activation of chlorophyll to reduce molecular nitrogen. The ubiquity of photohydrogen evolution and photosynthetic nitrogen fixation in photosynthetic bacteria and the rarity of these processes in green plants therefore probably reflect a general difference between these two groups with respect to the "dark" enzymatic constitution of the cell, and not a difference with respect to the photosynthetic machinery itself.

IX. REINTERPRETATION OF GROSS REACTIONS OF BACTERIAL PHOTOSYNTHESIS

The advances in our knowledge of the central events of photosynthesis which have been outlined in the preceding sections permit a new interpretation of the various gross reactions of bacterial photosynthesis. Let us reconsider the photosynthetic conversion of β-hydroxybutyrate to poly-β-hydroxybutyrate. Merrick and Doudoroff (52) have recently shown with cell-free preparations of *Rhodospirillum rubrum* that the immediate substrate for polymer synthesis is β-hydroxy butyryl-CoA. Polymer formation with β-hydroxybutyric acid as an exogenous substrate thus requires a minimal input of 1 mole of ATP per mole of acid assimilated. The reaction is rigorously light-dependent, for the simple reason that under anaerobic conditions cyclic photophosphorylation is the only mechanism available to the cell for making ATP. This photosynthesis can therefore be more correctly represented by the coupled reactions:

$$n \text{ ADP} + n \text{ P}_i \xrightarrow{\text{light}} n \text{ ATP and}$$

$$n \text{ ATP} + n \text{ C}_4\text{H}_8\text{O}_3 \xrightarrow{\text{CoA}}$$
$$(\text{C}_4\text{H}_6\text{O}_2)_n + n \text{ ADP} + n \text{ P}_i + n \text{ H}_2\text{O}$$

An additional problem arises in connection with those bacterial photosyntheses in which the gross synthetic reaction is reductive, whether this be the photometabolism of acetate or of CO_2 in the presence of an oxidizable inorganic substrate such as H_2S or H_2. In green plants, reducing power in the form of reduced pyridine nucleotide is provided ultimately by water, through intervention of the photochemical system. In bacteria, however, a chemical reductant other than water must be furnished. *Provided*
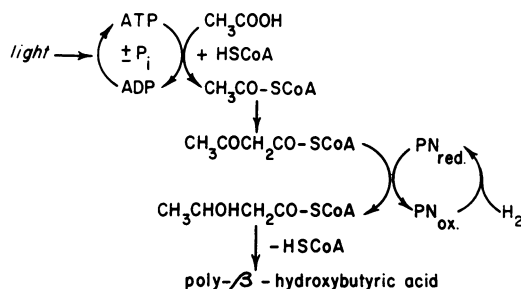

Figure 6. The mechanism postulated by Stanier et al. (67) to account for the acetate-hydrogen reaction in purple bacteria discovered by Gaffron (29).
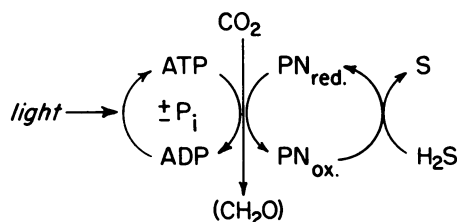

Figure 7. A modern interpretation of the gross mechanism of photosynthetic CO_2 assimilation with H_2S as reductant by purple and green bacteria. Compare with the classical interpretation shown in figure 2.

that electrons can be removed from this reductant at the pyridine nucleotide level by purely enzymatic means, there is no logical need to invoke the interposition of the photochemical system for the provision of reducing power. This is illustrated (figure 6) by our concept of the acetate-hydrogen reaction (67). Here, just as in the photoassimilation of β-hydroxybutyric acid, the *only* role which must be attributed to light is ATP synthesis. The photoassimilation of CO_2 with energy-rich inorganic electron donors such as H_2S and H_2 can be similarly interpreted, as shown diagrammatically in figure 7.

The situation is not so simple in the case of a donor such as succinate, which transfers electrons at a potential level below that of the pyridine nucleotides. Electrons from succinate cannot serve for the direct reduction of DPN or TPN. Frenkel (26) has observed that bacterial chromatophores are capable of performing a light-dependent reduction of DPN, coupled with the oxidation of either succinate or reduced flavin
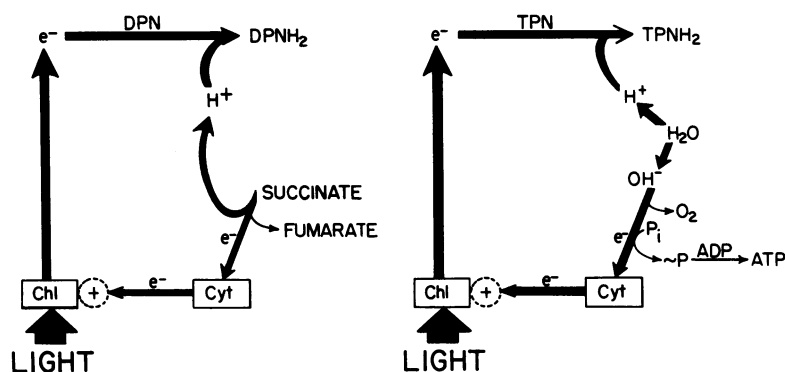
Figure 8. Mechanisms for the photochemical generation of reducing power in purple bacteria (left) and in green plants (right).

mononucleotide. This reaction proceeds independently of cyclic photophosphorylation. Evidently, in this situation radiant energy can intervene to boost the potential of the electrons from donors that are incapable of direct coupling with pyridine nucleotide. The Frenkel reaction, interpreted in terms of the Arnon model (figure 8), has a certain formal analogy with the water-linked generation of reduced TPN in green plant photosynthesis, and might indeed be regarded as an evolutionary precursor of it.

X. NATURE OF THE PRIMARY LIGHT REACTION

Arnon's model of the central events of photosynthesis represents a major departure from the model of van Niel, which has dominated most thinking in the field for the past 20 years. In the new model, water cleavage is no longer a primary event. In fact, the participation of water in photosynthesis becomes again, as it was assumed to be before 1940, a special feature of green plant photosynthesis, with the further novel proviso that water is not directly involved in the photochemical event. In place of the photolysis of water, what is now proposed is a much simpler primary electronic shift in chlorophyll, closely coupled with a transfer of electrons from the associated cytochrome.

The electronic nature of the primary event in photosynthesis was first suggested in 1949 by Katz (44), but this concept had not been integrated into a general formulation of the photosynthetic process until the model of Arnon was proposed. We must now consider what experimental evidence there is for a primary event of this sort. The expulsion of an electron from chlor-

ophyll should cause marked changes in the chlorophyll absorption bands of the photosynthetic apparatus. However, as we have already seen, the rather complex spectral changes that follow illumination of intact photosynthetic cells appear to reflect changes in the carotenoids and the respiratory pigments.

The first clear evidence that illumination provokes spectral changes attributable to chlorophyll itself has been obtained recently by Arnold and Clayton (3), with the use of dried films of undenatured chromatophores prepared from *Rhodopseudomonas spheroides*. The effects are particularly clear with chromatophores prepared from a carotenoidless mutant, and involve shifts in the positions of all the major peaks of bacteriochlorophyll. With chromatophores prepared from cells of the wild type, there are accompanying changes in the carotenoid spectrum; such changes can, however, be abolished without affecting the chlorophyll response by brief exposure of the dried chromatophores to ethanol vapor. This light-induced spectral change of chlorophyll is freely reversible and can be obtained repeatedly with a single preparation exposed to successive periods of illumination.

The response is independent of temperature; its magnitude remains unchanged between 1 and 300°K. Since no ordinary chemical reaction can take place at 1°K, the light-induced spectral changes must reflect a purely electronic event. The participation of water in this reaction is excluded by the anhydrous state of the biological material.

The question then arises, "Why cannot a similar phenomenon be observed with intact

cells?" Arnold and Clayton have also furnished a plausible answer to this question by showing that metabolic inhibitors (*e.g.*, azide and hydroxylamine) abolish the light-induced changes of the cytochrome spectrum in whole cells of *Rhodopseudomonas spheroides* and simultaneously reveal light-induced changes of the chlorophyll spectrum such as those observed with dried films of chromatophores. Probably electron transport is sufficiently rapid in the normally functioning photosynthetic apparatus to prevent the accumulation of a pool of excited electrons; the spectral changes associated with chlorophyll excitation are therefore undetectable.

XI. CONCLUSION

We may now summarize briefly the new picture of photosynthesis that is beginning to emerge. The only common *physical* event in all types of photosynthesis is the excitation of chlorophyll by light with the expulsion of electrons. This has one universal *chemical* consequence: transfer of the electrons through a closed carrier system coupled to chlorophyll, with a concomitant generation of ATP. Several alternative chemical fates for the electrons are possible (*e.g.*, reduction of TPN in green plants, reduction of DPN in purple bacteria, hydrogen evolution or nitrogen fixation in photosynthetic bacteria and certain algae), but none of these is of universal occurrence.

The use that a photosynthetic cell makes of the ATP generated through photophosphorylation is determined in part by its inherited enzymatic constitution and in part by the environmental conditions. In green plants, green bacteria, and purple sulfur bacteria, the lion's share of the ATP will normally be used to drive the synthesis of organic cell constituents from CO_2. In nonsulfur purple bacteria (and also in purple sulfur bacteria growing in the presence of organic substrates) it will be used to drive the synthesis of organic cell constituents from the externally provided organic substrate. It is probable that many green plants could partly or entirely replace CO_2 with organic substrates as a source of carbon for the photosynthesis of cell material, just as do the purple bacteria, but critical experiments to test this point do not seem to have been undertaken. However, it has been shown that the green tissues of higher plants (leaf disks) can perform a light-dependent synthesis of starch

from exogenous glucose (50). In this case, the plant cell uses photosynthetically generated ATP for a specific and limited assimilatory metabolism of an organic compound.

We have recently examined the possible utilization of organic carbon sources for general cellular synthesis by *Chlorobium limicola*, a green bacterium hitherto presumed to be an obligate lithotroph. It was found (62) that this organism can use acetate as a major source of cellular carbon in the light; acetate uptake is strictly dependent on the simultaneous provision of CO_2 and of an exogenous reductant (H_2S). The different requirements for acetate utilization by *C. limicola* and by purple bacteria can probably be explained by the absence in *C. limicola* of an enzymatic mechanism for the anaerobic oxidation of acetate. If acetate cannot be oxidized, it can provide neither the reducing power nor the CO_2 required for the over-all processes of biosynthesis, and these requirements must be furnished from other sources.

Recent findings accordingly suggest that the use of CO_2 as the sole source of carbon for the synthesis of cell material is not really a fundamental feature of *any* photosynthetic process. The fact that most phototrophs normally do use CO_2 as their sole carbon source is more satisfactorily interpreted as an evolutionary adaptation to the scarcity of organic material which has long existed on our planet. Photosynthesis falls into a special metabolic category only in terms of the *mode* of ATP formation; it cannot be placed in a special metabolic category in terms of the *use* that the cell makes of the ATP so generated.

ATP is the common energetic currency of the cell; it can be used to perform chemical, osmotic, or mechanical work. In the first study ever made of the reactions of purple bacteria to light (19), Engelmann described a biological response which can now be interpreted as a utilization of photosynthetically generated ATP to perform mechanical rather than chemical work. He observed that purple bacteria suspended in water in a sealed cover slip preparation will soon cease all movement if kept in the dark. When this preparation is illuminated, the cells become motile, and remain in active movement as long as illumination is continued. Since no exogenous substrate is available, the cells cannot perform a gross photosynthetic reaction, but light triggers the

closed cycle of events which results in photosynthetic phosphorylation. Under these severely restrictive conditions, one of the few effective uses which the cell can make of the resulting ATP is the production of flagellar contractions.

In 1845, Mayer (51) defined photosynthesis as follows: "Die Pflanzen nehmen eine Kraft, das Licht, auf, und bringen eine Kraft hervor; die chemische Differenz."

In retrospect, we can see that work on photosynthesis during the intervening century has been largely devoted to clearing away the metabolic irrelevancies which prevented the restatement of this profound insight in more precise physicochemical terms.

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