METABOLIC PATTERNS IN PHOTOSYNTHETIC BACTERIA

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The present review was undertaken with the aim and hope of systematizing and clarifying our present knowledge of metabolic patterns observed in photosynthetic bacteria. From a biochemical point of view, these microorganisms appear to perform a number of variations on the theme of classical photosynthesis. In spite of these apparent variations, there is little doubt that the fundamental photosynthetic mechanism in these organisms shares numerous similarities with that operating in other forms. With the current revival of interest in the mechanism of green plant photosynthesis, it seems likely that more intensive studies of bacterial photosynthesis will inevitably be stimulated and we can expect that such efforts will contribute measurably to our understanding of the general problem.

A familiarity with the remarkable metabolic versatility of the photosynthetic bacteria suggests that these organisms will also provide excellent source materials for various enzymatic systems of interest to other biochemical investigations. It is hoped, therefore, that this review will successfully acquaint microbiologists and biochemists with the significant metabolic properties of photosynthetic bacteria and thereby, with the potentialities of these organisms for such applications. In order to facilitate the accomplishment of this goal, a number of subjects normally considered in reviews on photosynthesis have been intentionally omitted. Aspects of bacterial photosynthesis which did not appear to be particularly relevant for the present purpose include, among others, discussion of kinetics and energetics of photosynthesis, properties of the photosynthetically active pigments, and techniques of isolating photosynthetic bacteria. Discussion of these (and related) topics and pertinent references can be found in several reviews by van Niel (65, 66, 67).

1. NUTRITION AND CULTIVATION OF PHOTOSYNTHETIC BACTERIA

The various genera of photosynthetic bacteria have been assorted into three subgroups, mainly on the basis of pigmentation and "nutritional" characteristics. In table 1, some of the significant descriptive features of these subgroups are summarized (cf. (65)).

Needless to say, qualitative variations are found among different genera and strains with respect to some of the characteristics listed; this classification is accordingly not used in a rigid sense, but rather for convenience in referring to the main physiological types encountered. The provisional nature of subdivision on the basis of these characteristics has been reemphasized by the recent description of new strains and types of photosynthetic bacteria (7, 28, 46, 59). It should also be noted that since the above classification is founded on physiological properties, it is to be expected that a variety of morphological forms are found in each category (66).

Most of the recent work on the metabolism of photosynthetic bacteria has been done with the nonsulfur purple organisms (*Athiorhodaceae*). In view of this fact, the present review deals mainly with this subgroup and it is therefore pertinent to consider briefly the general physiology of these organisms as well as the usual techniques employed in their cultivation. As is also true for the other two subgroups, the nonsulfur purple bacteria are pigmented and have thus far been found to require light of appropriate wave lengths in order to grow under anaerobic conditions. The usual type of light source employed is the ordinary incandescent lamp which apparently emits sufficient infra-red radiation to satisfy the illumination requirements; sources which emit little light in the infra-red region, such as fluorescent lamps, are unsatisfactory. Because of the rather large heat production from incandescent lamps, it is usually necessary to utilize some sort of cooling arrangement in order that the cultures be maintained at appropriate temperatures (30 to 37 C); the simple expedient of using fans to circulate the surrounding air is generally satisfactory for growing cultures and also for photosynthetic experiments in which the Warburg apparatus is illuminated from above.

TABLE 1

Characteristics of photosynthetic bacteria

SUBGROUP	COLOR	HYDROGEN DONORS UTILIZABLE FOR GROWTH		VITAMIN REQUIREMENTS	ABILITY TO GROW IN THE DARK
		INORGANIC	ORGANIC		
Nonsulfur purple bacteria (*Athiorhodaceae*)	Purple, red, or brown	+	+	+	+
Sulfur purple bacteria (*Thiorhodaceae*)	Purple, or red	+	+	−	−
Green bacteria (*Chlorobacteriaceae*)	Green	+	−	−	−

Stock strains of nonsulfur purple bacteria are usually maintained as stab cultures in an agar medium containing yeast extract with or without peptone. Depending on the sensitivity of the particular strain to oxygen, it may be necessary to add reducing agents to the medium or observe other precautions normally used in cultivating strict anaerobes (28). After the stab cultures have developed in the light, they can be stored in darkness for rather long periods without loss of viability (66). Preservation by lyophilization is also successful, but appears to be less frequently practiced.

Anaerobic liquid cultures are most commonly grown in completely filled glass-stoppered bottles using an inoculum from a stab or a previous liquid culture. With the latter type of inoculum and an appropriate medium, luxuriant growth in the light is ordinarily obtained in 24 to 36 hours. The use of reducing agents in liquid media and special techniques devised for preparation of "bottle cultures" have been described by a number of investigators (25, 37, 45, 52, 66).

Certain strains of nonsulfur purple bacteria can apparently use CO_2 as the primary carbon source for photosynthetic growth in the presence of inorganic hydrogen donors such as H_2 or $S_2O_3^=$ (65, 66). During growth in the presence of the so-called "organic hydrogen donors", the "nutritional pattern" is more complex. As will be shown below, organic compounds do not serve only as hydrogen donors but generally also as carbon sources, and for this reason, the designation "organic hydrogen donor" is somewhat misleading. If the organic "donor" is at an oxidation level more reduced than carbohydrate, CO_2 must be supplied in order for growth to occur; if the "donor" is more oxidized, CO_2 need not be supplied and there is in fact a *net* production of CO_2 during growth under such circumstances (52). Generally speaking, the suitability of a particular organic "donor" for growth depends on the nature of the species and strain of bacterium under consideration; compounds representative of most classes of substances biochemically important can be used in one case or another (66). Suitable nitrogen sources for growth include N_2 (44, 49), NH_4^+, and various amino acids (25, 66).

Typical nonsulfur purple bacteria differ from members of the other two subgroups in two major respects, viz. they require one or more preformed vitamins for growth and certain strains are capable of multiplying *in the dark* under the proper conditions (see Section 2. I.). The vitamins required by various genera and species have been determined by Hutner (34, 35, 37), who has devised completely synthetic media which support growth. Although there is no question that these organisms can be grown in synthetic media, Gest and Kamen (25) found that growth is usually accelerated markedly by the addition of complex materials such as yeast extract or peptone. The beneficial effect of high concentrations of yeast extract has also been noted by others (e.g., see (66)). Hutner (37) maintains that "good" growth can be obtained in certain synthetic media containing the proper relative amounts of trace metal ions, but he has not published data illustrating growth *rates* and *yields* in these media as compared with similar media containing yeast extract or peptone. The addition of yeast extract to various, but not all, synthetic media not only leads to faster growth but also markedly increases the cell yield (22). The absence of an effect of yeast extract in certain instances suggests that the supplements necessary for optimal growth may differ depending on the nature of the primary carbon and nitrogen sources provided. Although it is conceivable that the effect of yeast extract in the former cases could have been due to contaminant trace metals, it seems equally possible that yeast extract supplies organic compounds that are necessary for optimal growth. At present there is insufficient information available to permit unambiguous definition of the factors involved in optimal growth in synthetic media.

With respect to special trace metal requirements, it should be noted that the quantity of Ca^{++} present in the medium may have a striking effect on the growth of particular types or strains of nonsulfur purple bacteria. Foster (11) observed that certain *Rhodospirillum* strains showed excellent diffuse growth when Ca^{++} was present within a certain concentration range; decrease or increase of the Ca^{++} concentration beyond these limits led to poor abnormal

growth in the form of flakes or granules. Similar effects have been observed by the author with the organism *Rhodospirillum rubrum* (SI). In view of the foregoing, it appears likely that the sudden occurrence of abnormal growth (clumping, etc.) which is occasionally observed may be due to variations in Ca^{++} content of the distilled water supply used for preparation of culture media.

When the appropriate carbon and nitrogen sources, vitamins, and trace metals are provided, photosynthetic bacteria develop rapidly under anaerobic conditions in the light. The cell yields obtainable under optimal conditions in media containing organic "donors" are remarkably large in comparison with those observed in the growth of typical heterotrophic anaerobes. It appears that the photosynthetic bacteria possess an extremely efficient assimilatory system; as a consequence, end products generally do not accumulate in the medium during growth as with most anaerobic heterotrophs. Detectable formation of "excretory" end products has thus far been reported in only two instances: (a) Muller (52) found small but significant quantities of "volatile acid" in cultures of purple sulfur bacteria growing in a pyruvic acid medium, and (b) Kohlmiller and Gest (45) detected HCOOH (up to 15 mM/l) as an end product in cultures of *Rhodospirillum rubrum* growing in a malate-glutamate medium.

2. METABOLISM OF RESTING CELLS IN THE DARK

I. Utilization of O_2 as the hydrogen acceptor

Resting cells of certain strains of nonsulfur purple bacteria have been found to oxidize organic compounds in the dark with O_2 as the hydrogen acceptor.[1] The strains which display an oxidative metabolism of this kind appear to be those which can also be *grown* in the dark in the presence of oxygen.[2] The experiments of Nakamura (53) clearly showed that *Rhodobacillus palustris* could grow in the dark but only in the presence of O_2. Under anaerobic conditions, growth occurred only in the light. Similar observations with different nonsulfur purple bacteria have been subsequently made by numerous investigators. Although no one has succeeded in growing photosynthetic bacteria *anaerobically* in the dark, there is reason to believe that growth under these circumstances may be possible; this point will be amplified in the section on fermentative dark metabolism.

In general, cultures grown aerobically in the dark show only slight pigmentation as compared with cultures which have developed anaerobically in the light (66). It is of considerable interest that resting cells of such organisms (grown in the dark) display typical photosynthetic reactions after a lag period under anaerobic conditions in the light (45). Investigation of the metabolic changes occurring during this induction period may be useful in determining the nature of the enzyme systems stimulated by light.

The oxidation of various types of organic compounds in the dark by *Rhodobacillus palustris* was investigated by Nakamura (53, 57) who found that alanine,

[1] Oxygen consumption by resting suspensions of purple sulfur bacteria has been observed (e.g., see (58)), but has not been studied in any detail.

[2] It appears from the work of Hutner (37) that the same vitamins are required for growth in the dark and in the light.

lactic, pyruvic, malic, succinic, formic, acetic and several higher fatty acids stimulated utilization of oxygen. He also observed (53) that O_2 utilization, as measured by the direct method, was diminished upon illumination. By analogy with other photosynthetic systems, Nakamura interpreted this to mean that O_2 was produced during the light exposure. The suppression of O_2 utilization by light was subsequently confirmed by van Niel (65) who studied the oxidation of acetate by *Rhodospirillum rubrum*. van Niel's experiments, however, showed that the light suppression was due to an actual inhibition of O_2 uptake rather than to a balance between O_2 consumption and a postulated O_2 production initiated by illumination. Although it would appear that O_2 is not produced by photosynthetic bacteria under the experimental conditions used in the past, it seems premature to conclude that they do not produce O_2 during illumination under any circumstances.

The respiratory data obtained for oxidation of fatty acids (C_2 to C_5) by *Rhodobacillus palustris* do not agree with the theoretical values for complete oxidation (57). The data indicate the accumulation of incomplete oxidation products with or without the occurrence of oxidative assimilation. Addition of 0.0125 M KCN leads to about 50% reduction in the rate of fatty acid oxidation by this organism (57).

It has been reported (65) that *Rhodospirillum rubrum*, depleted of endogenous reserves by prolonged shaking with air in the dark, does not oxidize acetate in the absence of CO_2, i.e., with alkali present in the Warburg vessel.[3] According to later experiments of van Niel (briefly described in ref. (67)), the addition of trace amounts of succinate, fumarate, malate, or oxalacetate [but not α-ketoglutarate, citrate, or isocitrate] catalyzes the oxidation of acetate in the absence of CO_2. Tracer experiments by Barker and van Niel (quoted in ref. (67)) showed that $C^{14}O_2$ is fixed into succinic acid aerobically in the dark and that oxidation of C^{14}-labeled acetate in the presence of unlabeled succinate leads to formation of labeled succinic acid. Experiments with acetic acid labeled in the methyl or carboxyl group, or both, also showed that the two carbons of acetate were converted to CO_2 and "cell materials" to an equal extent.

These observations have been interpreted as indicating a tricarboxylic acid cycle oxidation of acetate (67). Although the results described are undeniably suggestive, they cannot be construed as proof for the occurrence of such a cycle. A conclusive proof obviously requires the critical experiments which were necessary in establishing the presence of this cycle in animal tissues. On the basis of comparative biochemical arguments, it has been suggested that the fixation of

[3] This phenomenon has been repeatedly compared with the frequently quoted experiments of Hes (30, 31) which indicated that methylene blue reduction by various microorganisms in the presence of undesignated substrates was retarded by removal of CO_2. Hes' observations have been confirmed by Gest and Stokes (27) using *E. coli* and *R. rubrum* but *only in the absence of substrates*. In the presence of oxidizable substrates, no differences in methylene blue reduction times ($\pm CO_2$) were observed. It appears that endogenous controls were not employed in the original experiments of Hes (32). In view of these observations, a direct comparison of the "Hes phenomenon" with the effect of CO_2 on acetate oxidation by *R. rubrum* is not possible.

CO_2 in photosynthesis (and chemosynthesis) also occurs primarily by reactions of the tricarboxylic acid cycle (67). From a consideration of the results of recent researches on the mechanism of green plant photosynthesis in the laboratories of Calvin (3) and Gaffron (9), it is evident that this proposal cannot be used as the only working hypothesis at the present time. Recent tracer experiments with *Rhodospirillum rubrum* have also disclosed that the metabolic conversions of acetic acid under photosynthetic conditions differ markedly from those occurring under aerobic conditions in the dark; these results are discussed in Section 3. I.

II. Fermentation of organic acids in the presence and absence of artificial hydrogen acceptors

Several investigators have noted the production of gases and acids by resting suspensions of photosynthetic bacteria under anaerobic conditions in the dark (14, 58). According to the early reports, these products were formed from endogenous reserve materials and were not produced in greater amounts upon addition of various substrates. These observations together with the unsuccessful attempts to grow photosynthetic bacteria anaerobically in the dark led to the beliefs that the fermentation reactions in these bacteria were of a special kind and that their function was simply to permit the organisms to survive during dark periods (52, 58, 66). These views are untenable as indicated by the experiments of Nakamura (54, 56, 57) and Kohlmiller and Gest (45).

Nakamura's experiments, mainly with *Rhodobacillus palustris* and *Chromatium minutissimum*,[4] showed that various added organic compounds were fermented (under an atmosphere of N_2) with the production of CO_2, H_2, or both. Compounds definitely decomposed under anaerobic conditions in the dark were formate, pyruvate, glucose, glycerol, and glycerophosphate. The immediate precursor of H_2 in the latter instances was considered to be formate, which was presumably produced as an intermediate. The detailed mechanism(s) of H_2 formation by microorganisms is still unknown. For H_2 production from formate, there is some evidence which suggests that the reaction is catalyzed by a single enzyme ("hydrogenlyase"), but the author is more inclined to support the proposal that formate decomposition is effected by an enzyme complex in which formic dehydrogenase acts in conjunction with hydrogenase (see discussion by Waring and Werkman (70)). In considering the origin of H_2 in microbial fermentations, it is also of importance to take into account those cases where an organism produces H_2 from compounds such as pyruvate, malate, amino acids, etc., but *not* from formate (4, 44a, 75). These observations suggest the existence of several mechanisms of H_2 formation. It is possible that common hydrogen carriers are involved in all of the ostensibly different "types" of H_2 formation. The comparative enzymatic studies required to explore this possibility have not yet been undertaken.

The fermentation of externally added substrates by *Rhodospirillum rubrum*

[4] The ability of other sulfur purple bacteria to ferment organic compounds in the dark is indicated by experiments of Gaffron (16).

was also studied by Kohlmiller and Gest (45). Photosynthetically-grown cells of this organism decompose pyruvate (under an atmosphere of He) with the production of CO_2, acetic, and propionic acids.[5] The utilization of pyruvate, and consequently the formation of acetate and propionate, is inhibited by removal of CO_2 from the system. This suggests that the metabolism of pyruvate by this organism may involve the intermediate formation of a dicarboxylic acid as has been proposed by Wood and Werkman (74) and others (6, 38, 39) for the fermentation of C_3 compounds by various propionic acid bacteria.[6] Cells of *Rhodospirillum rubrum* grown aerobically in the dark also ferment pyruvate with the formation of CO_2, a small amount of H_2, acetic, propionic, butyric, valeric, and caproic acids. With this type of cells, removal of CO_2 (by absorption with alkali)

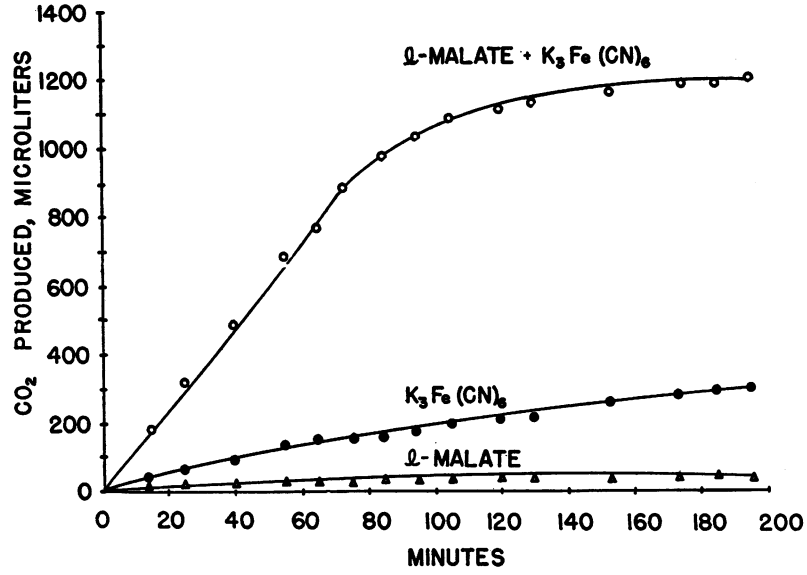

FIG. 1. Malate decomposition by *Rhodospirillum rubrum* (SI) in the dark in the presence and absence of $K_3Fe(CN)_6$. Where indicated, 20 μM of *l*-malate with or without 200 μM of $K_3Fe(CN)_6$ were supplied to 43 hour-old cells in 0.2 M phosphate buffer pH 6.8 at 30 C; the gas phase was 100% helium.

does not affect the extent of disappearance of pyruvate but does affect the relative amounts of acids produced. It is of interest that similar pronounced effects of CO_2 tension on the propionic fermentation of lactate by *Veillonella gazogenes* have been reported by Johns (38).

Although lactate, succinate, fumarate, and malate are rapidly metabolized by photosynthetically-grown cells of *Rhodospirillum rubrum* in the light, these compounds are fermented very slowly in the dark. It is difficult to ascribe the low

[5] Cell-free preparations of *Rhodospirillum rubrum* prepared by sonic disruption also rapidly ferment pyruvate in the dark (22).

[6] Larsen (46) has recently observed the formation of succinate from propionate and CO_2 by suspensions of green bacteria during illumination under anaerobic conditions.

fermentation rates in the dark to permeability effects since the addition of an artificial hydrogen acceptor under these conditions leads to rapid decomposition. An example of this phenomenon is given in figure 1 which illustrates the fermentation of malic acid in the presence and absence of $K_3Fe(CN)_6$ (22).

Other organic compounds and also H_2 are rapidly metabolized in the dark when $K_3Fe(CN)_6$ is supplied (19). The anaerobic oxidation of various fatty acids and glucose by resting suspensions of *Rhodobacillus palustris* in the presence of methylene blue has been demonstrated by Nakamura (53, 54, 57). Using decolorization times as a criterion of activity, his experiments indicated that a "coenzyme" preparation from yeast accelerates the activities on acetate, propionate, butyrate, and valerate. KCN, NaF, and iodoacetate did not influence the methylene blue reduction times significantly, but ethylurethane did show an inhibitory effect.

Similar examples of anaerobic oxidations have been reported by van Niel (67) who used undesignated substrates in the presence of acceptors such as methylene blue or quinone and by Gaffron (13) for fatty acids in the presence of KNO_3. One interpretation of these results is that photosynthetic bacteria, in contrast to heterotrophic anaerobes, have limited mechanisms for reoxidation of reduced hydrogen carriers in the dark. A corollary of this suggestion is that suitable "physiological" oxidants are normally produced as a result of photochemical activity.

From the foregoing, it seems probable that *Rhodospirillum rubrum* can ferment pyruvate in the dark (in the *absence* of artificial acceptors) by a mechanism similar to that operating in propionic acid bacteria. Since the latter organisms can obtain the energy and carbon requirements requisite for growth from such a process, it would seem that under the appropriate conditions photosynthetic bacteria—at least certain strains—should also be able to do the same. It is possible that incorporation of the proper vitamins or cofactors in media containing substrates fermentable by resting cells in the dark will facilitate the growth of these organisms in the absence of light under anaerobic conditions. The conclusive demonstration of growth under such circumstances would be of considerable interest since it might lead to information on the nature of the growth-requisite intermediates normally produced during illumination. An experimental indication that dark anaerobic growth is possible is given by the observation that cells of *Rhodospirillum rubrum* in anaerobic cultures maintained in the dark remain viable for long periods,—as shown by the fact that exposure of such cultures to light results in rapid and extensive growth (22).

III. Utilization of molecular hydrogen

Since the discovery of H_2 oxidation by the autotrophic "hydrogen bacteria", hydrogenase activity has been demonstrated in a suprisingly wide variety of microorganisms. Organisms possessing hydrogenase oxidize H_2 when they are provided with a suitable acceptor, which may be any one of a number of compounds, e.g., CO_2, O_2, NO_3^-, NO_2^-, $SO_4^=$, fumaric acid, amino acids, dyes such as

methylene blue. In the photosynthetic bacteria, H_2 utilization by intact cells is generally observed to be dependent on or stimulated by illumination when the acceptor is CO_2 or NO_3^- (15, 53, 55, 58).[7] With numerous other acceptors, however, oxidation of H_2 occurs in the absence of light. The oxidants which are reduced in the dark include: O_2 (15, 55, 77), $K_3Fe(CN)_6$ (19), S (55, 56), $SO_3^=$ (56), $SO_4^=$ (56), $S_2O_3^=$ (55, 56), NO_2^- (55, 56, 77), NO_3^- (53, 56, 77), fumarate (55, 56, 77), malate (56), aspartate (56), glyceraldehyde (56), hexosediphosphate (56), and methylene blue (19, 53).

Since the oxidation of H_2 in the presence of these compounds occurs in the dark and is unaffected by illumination, it is probable that the activity of hydrogenase, *per se*, is not light-dependent even when CO_2 is the acceptor. The ability of a substance to act as a terminal acceptor for H_2 appears to depend on the presence of specific enzyme systems which "activate" the acceptor (77). In the case of CO_2, it is reasonable to suppose that initial fixation of CO_2 occurs by dark reactions and that light is required for the further conversion of dark intermediates to the "acceptor" which is reduced by H_2 (in a dark reaction).

It seems to be generally assumed that the reduction of terminal acceptors by H_2 is mediated by some sort of coenzyme, which is reduced directly as a result of hydrogenase action. Recent experiments with a cell-free hydrogenase obtained from *Rhodospirillum rubrum* by a variety of procedures (glass grinding, sonic disruption, etc.) indicate that a readily dissociable cofactor is not involved in the reaction: H_2 + methylene blue $\rightarrow$ leucomethylene blue (20, 21). Rhodospirillum hydrogenase is gradually inactivated when stored in the presence of air, but can be preserved for long periods if maintained under an atmosphere of H_2; similar observations on the stability of a cell-free hydrogenase from *E. coli* have been made by Back *et al.* (2).

Various types of studies on hydrogenase in a variety of organisms have led to the suggestion that the air-inactivation of the enzyme is due to oxidation of a ferrous porphyrin prosthetic group to the inactive ferric form (33, 47). In this connection, it is of interest that the Rhodospirillum enzyme, as well as that from *E. coli*, is not appreciably affected by 0.001 M p-chloromercuribenzoate, which is considered to be a specific inhibitor of "sulfhydryl" enzymes (20, 21). This observation and other experiments reported by Gest (20) do not support Joklik's suggestion (40, 41) that the inactivation can be attributed to oxidation of essential sulfhydryl groups on the enzyme. The available information indicates that the hydrogenase of *Rhodospirillum rubrum* is very similar to that of *E. coli*. Spectroscopic observations have implicated participation of a porphyrin enzyme (perhaps as a *carrier*) in hydrogenase activity in the latter organism (20). At the present time it appears that further definition of the nature of the hydrogenase prosthetic group and of the role of known coenzymes in hydrogenase activity will require studies with more highly purified enzyme preparations. Such

[7] In contrast to the observations of Gaffron (15), Nakamura (53) has reported that oxidation of H_2 with nitrate as the acceptor occurs in the dark and is not stimulated by light.

experiments will no doubt contribute to our understanding of the mechanism of the numerous reactions in microorganisms which involve utilization or liberation of H_2 (these reactions have been discussed by Stephenson (64)).

3. METABOLISM IN THE LIGHT

I. Photochemical metabolism of organic acids

In contrast to the photosynthetic requirements of green plants, photosynthetic bacteria demand in addition to CO_2 and water an "accessory" inorganic or organic hydrogen donor. This "accessory donor" may be any one of a wide variety of compounds, depending on the type and strain of bacterium under consideration (e.g., H_2, H_2S, $H_2S_2O_3$, fatty acids, alcohols, etc. (65)). In the presence of such oxidizable compounds, the organisms are capable of rapidly utilizing CO_2 under anaerobic conditions when illuminated properly (13, 15). The exact role of the "accessory donor" in bacterial photosynthesis has not yet been clearly defined; the function of this "reactant" is discussed below in the light of recent experimental findings.

Most of the recent investigations on the mechanism of photochemical CO_2 utilization by bacteria have been conducted using nonsulfur purple organisms and organic acids or alcohols as "hydrogen donors." It was noted above that anaerobic photosynthetic growth of these bacteria in a proper medium containing organic "donors" more reduced than carbohydrate (e.g., fatty acids or alcohols) is successful only if CO_2 is supplied. Media containing more oxidized carbon sources (e.g., malate or pyruvate) support excellent growth in the absence of a CO_2 supplement. In fact, a considerable *evolution* of CO_2 occurs during growth on the "oxidized" substrates. Entirely analogous phenomena have been observed in metabolic studies with resting cell suspensions, i.e., upon illumination in the presence of fatty acids (propionic, etc.), CO_2 is consumed, whereas in the presence of more oxidized organic acids, excess CO_2 is produced.

Since the earlier metabolic experiments were concerned primarily with manometric measurement of gas exchanges, it is not difficult to appreciate the probable reasons for the apparently preferred use of fatty acids as "hydrogen donors". The direct observation of light-stimulated CO_2 *consumption* in these instances no doubt appeared to be a more satisfactory criterion of photosynthetic activity (from a comparative biochemical viewpoint) than light-catalyzed *evolution* of CO_2 from more oxidized substrates. As typically expressed, the over-all result of such an experiment for the case where butyric acid is the "hydrogen donor", is given below (65).

$$C_4H_7O_2Na + 2H_2O + 2CO_2 \xrightarrow{\text{light}} \underset{\text{"cell material"}}{5(CH_2O)} + NaHCO_3$$

Aside from indicating that a certain amount of CO_2 is utilized in the presence of butyrate, an equation of this type obviously has limited significance from a metabolic standpoint. The term (CH_2O) has been generally used to symbolize the reduction product(s) of the CO_2 consumed in photochemical reactions. As is

well known to all students of microbial metabolism, the same designation has been bestowed on the cellular product(s) of oxidative and fermentative assimilation. Occasionally it is noted in the literature that the symbol (CH_2O) is obviously a simplification which is employed only to facilitate expression of overall experimental results in equation form. It is unfortunate that many investigators tend to overlook the arbitrary nature of this term in striving for balanced equations to express assimilatory activity. From the numerous isotopic tracer studies being currently reported, it is becoming increasingly evident that assimilated carbon is usually rapidly incorporated into many of the diverse components of microbial cells. Because of this fact, it seems desirable in discussions of intermediary metabolism to place less emphasis on simple balanced equations of the kind shown above.

It was indicated above that the photochemical *consumption* of CO_2 in the presence of fatty acids has been studied in considerable detail using manometric techniques (for references see (57) and (65)). These experiments were mainly concerned with quantitative determination of the amount of CO_2 consumed as a function of the fatty acid used and with the kinetics of this process under various conditions. Interpretation of the results from the point of view of the *mechanism* of the photochemical reaction is difficult and has not been particularly rewarding. Although the amount of CO_2 consumed from the gas phase can be readily determined by manometric measurements, the quantity of CO_2 actually *reduced* by the cells cannot be directly estimated by using such procedures. The greatest uncertainty in this respect has been that the so-called organic "hydrogen donor" (i.e., fatty acid) is undoubtedly not only a hydrogen donor. The "donor" may be in part oxidized to CO_2 and the latter subsequently reduced. Such events could not be detected by measurements which disclose only *net* changes in gas production or consumption. In addition to this serious uncertainty, it is also probable that a portion of the "donor" may become directly assimilated, the extent of this process depending on the chemical constitution of the "donor" molecule.

In spite of the aforementioned difficulties, the results of such experiments have been interpreted (65, 67) primarily in terms of a generalized equation for photosynthesis, viz.,

$$CO_2 + 2H_2A \xrightarrow{\text{light}} (CH_2O) + H_2O + 2A$$

where H_2A is a substance such as H_2O, H_2S, organic compound, etc. which undergoes a one-step oxidation. This equation was devised in an attempt to reconcile the *apparently* different forms of photosynthetic processes observed in green plants and the various types of photosynthetic bacteria. There is no question that this representation has been of value as a rough first approximation of the overall process, which is unquestionably a complicated mechanism composed of numerous reactions. It is evident, however, that this type of formulation cannot be expected to contribute more to the understanding of the essential features of the photosynthetic process than Lavoisier's original equation for sugar fermentation did for the elucidation of the glycolytic mechanism.

The generalized equation indicated above cannot be applied with any satisfaction when the "donor" substance is an organic acid. This is clearly illustrated by the results observed when acetate is the "hydrogen donor". When cells of *Rhodospirillum rubrum* are suspended in a bicarbonate buffer solution containing acetate and exposed to light under an atmosphere of 5% CO_2 in N_2, a small net production of CO_2 is observed (approximately 0.2 mole per mole of acetate added (65)). The overall process under these conditions has been represented as follows:

$$C_2H_3O_2Na + H_2O + CO_2 \xrightarrow{\text{light}} 2(CH_2O) + NaHCO_3$$

As indicated previously, the amount of CO_2 actually reduced, if any, cannot be determined from manometric experiments alone. In addition to this uncertainty, it should be noted that the disappearance of acetic acid (or any other fatty acid "donor") has apparently never been directly measured in this type of experiment. Using $C^{14}O_2$, Barker and van Niel (quoted in (67)) found that *growing* cells of nonsulfur purple bacteria reduce CO_2 to unknown cell materials when developing in a medium containing unlabeled acetate. The amount of C^{14} incorporated, however, was apparently considerably smaller than would be expected on the assumption that acetic acid acts only as a "hydrogen donor" for reduction of $C^{14}O_2$.

Direct assimilation of carbon from acetate by photosynthesizing resting cells of *Rhodospirillum rubrum* has been recently demonstrated by Kamen *et al.* (43). Using $C^{14}H_3COOH$, it was found that the methyl group of acetate was incorporated to a large extent into "insoluble" cell material. Similar experiments with $CH_3C^{14}OOH$ disclosed that the major portion of the carboxyl group is converted to CO_2. These results are markedly different from those observed during the dark aerobic oxidation of labeled acetate (refer Section 2. I.) and indicate that the transformations of the acetic acid carbons are certainly not identical in light and dark. The two important conclusions that can be drawn from these experiments are:

(*a*) The accessory (organic) "hydrogen donor" required in bacterial photosynthesis ordinarily does not undergo a simple one-step oxidation; it generally supplies carbon intermediates, *other than* CO_2, which are directly used by the cell for synthetic purposes. This is apparently true for practically all classes of organic "hydrogen donors." (*b*) The metabolic fate of carbon from the accessory "hydrogen donor" is not necessarily the same in photosynthetic metabolism and dark oxidative metabolism.

In further support of conclusion (*a*), the direct utilization of acetate carbon for synthesis of cell materials during photosynthesis has also been demonstrated in growing cultures of *Rhodospirillum rubrum* by Cutinelli *et al.* (5). In separate experiments, cells were grown in a medium containing inorganic salts, 0.05% yeast extract, and $C^{13}H_3C^{14}OONa + NaHCO_3$ or CH_3COONa and $NaHC^{14}O_3$. The incorporation of isotopic carbon into the pigments and trichloroacetic acid-precipitable proteins of the cells obtained was quantitatively determined, as well as the isotopic content of the carbonate still present at the end of the experi-

ment. Calculations from the results obtained indicate that for both the pigments and proteins approximately 45% of the total carbon is derived from the methyl groups of acetate, about 25% from the carboxyl groups, and roughly 10% from the bicarbonate. The remainder of the carbon is considered to be derived from unknown compounds present in the yeast extract added to the medium. As was also found by Kamen *et al.* (43), it was observed that the carboxyl groups of acetate are converted to CO_2 to a greater extent than are the methyl groups. All of these results emphasize again the significance of the "accessory donor" as a carbon source for synthesis of cell materials. In the instance discussed above, more cell material is derived from the "accessory donor" than from CO_2! The exact distribution of acetate-derived labeled carbon in the various constituents of *Rhodospirillum rubrum* is now under investigation in both Kamen's and Ehrensvard's laboratories; these experiments will no doubt have important implications in the field of bacterial photosynthesis as well as for the general problem of acetate metabolism.

The second conclusion is of importance in that it indicates the necessity for reconsidering previous notions concerning the pattern of metabolic conversions of the accessory organic "donor". van Niel (65) has reported that *certain* strains of nonsulfur purple bacteria may oxidize a given compound aerobically in the dark and also utilize the same compound as a "donor" anaerobically in the light *at the same rate*. His interpretation of these observations implied that the metabolic fate of the carbon from the "donor" is essentially the same in both dark and light. In view of the more recent tracer experiments just discussed, it appears that the metabolism of the accessory "donor" may be considerably different in photosynthesis and dark oxidation. It seems possible that the dissimilation of the organic "donor" in light and dark may have features in common, but that the "reducing power" generated by the action of light may significantly alter the final disposal of carbon intermediates derived from the accessory donor. In connection with the foregoing question, it is also of importance to emphasize that only a relatively small number of photosynthetic bacteria are capable of oxidizing "donors" aerobically in the dark. The majority are obligate anaerobes which can only be grown under photosynthetic conditions in the absence of oxygen. Furthermore, the only experimental data reported by van Niel are for the case where acetic acid is the "donor". It would obviously be desirable to determine the generality of the correlation reported using other compounds. Still another complication is found in the fact that the manometric photosynthetic experiments were conducted using an atmosphere of 5% CO_2 in N_2, it being assumed that N_2 is an inert gas with regard to the metabolism of photosynthetic bacteria. It has been recently found that N_2 is *not* an inert gas for these organisms and that its presence significantly alters the quantitative and qualitative aspects of photosynthetic conversions. (see Section 3. III.)

II. Photochemical metabolism of alcohols and ketones

Various strains of nonsulfur purple bacteria can develop photosynthetically in media containing mineral salts, CO_2, vitamins (yeast extract), and primary

or secondary alcohols (66). Such strains can be readily isolated from natural sources by appropriate enrichment culture procedures. The photochemical decomposition of primary and secondary alcohols, as well as the corresponding ketones, by these organisms has been studied in some detail (10, 11, 61, 62).

The *Rhodopseudomonas* strains which Foster studied (10, 11) decomposed lower aliphatic primary alcohols (methyl, ethyl, and *n*-propyl) during growth. Since these alcohols were presumed to play the role of "hydrogen donors" only, cultures were analyzed for the presence of the corresponding fatty acids which would necessarily result from simple one-step oxidation. Fatty acids were not found in any instance in appreciable quantity.

Similar studies with secondary alcohols, however, indicated that certain strains attacked these substances with the production of the corresponding ketones. With isopropanol, analyses of cultures for CO_2 and isopropanol disappearance and for acetone formation, provided data which supported the following equation (10):

$$2CH_3CHOHCH_3 + CO_2 \xrightarrow{\text{light}} 2CH_3COCH_3 + (CH_2O) + H_2O$$

Attempts to demonstrate that the amount of carbon in the cells obtained was quantitatively equivalent to the amount of CO_2 which disappeared were not entirely successful due to complications introduced by the presence of yeast extract in the medium. Disregarding this point, it would appear that in this instance, isopropanol acts exclusively as a hydrogen donor for reduction of CO_2 to "cell material". In addition to isopropanol, several other secondary alcohols (e.g., methylethyl carbinol, methyl-*n*-propyl carbinol, etc.) were found to be converted to the corresponding ketones under similar conditions. Quantitative balance experiments have as yet not been reported for alcohols other than isopropanol.

Photochemical attack of alcohols in the presence of CO_2 (and N_2) by cell suspensions in bicarbonate buffer was first studied by Foster (10, 11). With the primary alcohols the manometric data showed that acids were not produced, in conformity with the results noted in growing cultures. The decomposition of primary alcohols appeared to be catalyzed by adaptive enzymes as indicated by the fact that cells derived from a malate medium utilized CO_2 in the presence of the alcohols with the kinetics characteristically observed in instances of enzymatic adaptation. Photooxidation of isopropanol by cell suspensions was also investigated and the results obtained (10) were in general agreement with the equation given above for decomposition of this compound in growing cultures.

The results of Foster's isopropanol experiments have been frequently construed as "unequivocal" proof for the validity of the generalized photosynthesis equation as applied to the nonsulfur purple bacteria. As a result of the continuous emphasis on the significance of the isopropanol reaction, Siegel and Kamen (62) have recently studied this reaction in more detail. The strains originally isolated by Foster were found to have lost the ability to decompose isopropanol and an active strain of *Rhodopseudomonas gelatinosa* was therefore isolated by

enrichment culture techniques. In contrast to Foster's original strains, the new isolate metabolized acetone as well as isopropanol. Nevertheless, small amounts of acetone accumulate during anaerobic photosynthetic growth of the organism in an isopropanol medium. The source of the acetone produced (during growth) was studied by growing cells in a medium containing isopropanol, yeast extract, and $NaHC^{14}O_3$. The acetone distilled from the grown culture was found to be unlabeled. On the other hand, the harvested *cells* were labeled to a considerable degree. The radioactive organisms were then exposed, in the "resting" state, to unlabeled isopropanol anaerobically in the light; the acetone produced by these cells was also found to be unlabeled. Although these results strongly suggest that isopropanol is directly dehydrogenated to acetone, a rigorous proof requires the demonstration that C^{14}-labeled isopropanol is converted by the organism to labeled acetone, both compounds having identical specific radioactivities (counts/minute/millimole).

Assuming that isopropanol is directly converted to acetone in this photosynthetic conversion, it is of importance to assess the significance of this reaction for the mechanism of bacterial photosynthesis. Although it would provide an instance where an organic compound acts solely as a hydrogen donor, it is apparent from the foregoing discussion that this case represents an exception rather than the rule. In the opinion of the reviewer, the data at hand certainly do not justify extrapolation from this instance to the general thesis that organic compounds serve primarily as hydrogen donors for the reduction of CO_2 to cell materials. Since all organic compounds used by these organisms are "oxidized" during photosynthetic metabolism, these substances are *by definition* hydrogen donors. In practically all instances they unquestionably serve as sources of carbon also and must, therefore, be considered from this point of view.

Siegel's recent study (61) of acetone utilization in the light by *Rhodopseudomonas gelatinosa* is of interest from a general biochemical standpoint as well as with respect to bacterial photosynthesis. The strain used grows well in a medium containing acetone, CO_2, a small amount of yeast extract, and mineral salts. During photosynthetic growth in such a medium, the cells quantitatively convert acetone ($+CO_2$) into cell materials as shown by carbon balance experiments. The mechanism of this photochemical conversion was studied using cells suspended in bicarbonate buffer under an atmosphere of 5% CO_2 in N_2. Cells derived from an acetate medium attacked acetate rapidly but isopropanol and acetone at significant rates only after a prolonged lag period (10 to 15 hours). Cells derived from acetone or isopropanol media, on the other hand, utilize acetate, acetone, and isopropanol immediately and rapidly. Siegel assumes that these observations indicate typical enzymatic adaptations and has applied the technique of "simultaneous adaptation" to a study of the mechanism. The results are considered to support the following scheme:

$$\text{Isopropanol} \rightarrow \text{acetone } (+CO_2) \rightarrow \text{acetoacetate} \rightarrow \text{acetate} \rightarrow \text{cell material}$$

Although this may well be the actual sequence, this scheme must be accepted with reservations for the present. First, because of inherent limitations in the

technique of "simultaneous adaptation", independent evidence of a more direct nature is desirable. In addition it must be pointed out that the observed kinetics of "adaptation" to isopropanol and acetone by acetate-grown cells do not appear to be entirely typical for a "true" adaptive phenomenon; isopropanol appears to be utilized by such cells *immediately*, at a very low rate to be sure, and the rate increases only after rather long incubation. In view of the foregoing, and considering the fact that N_2, a utilizable nitrogen source for growth, was present in the gas phase, the classification of these particular reactions as "adaptive" becomes somewhat questionable. There is no doubt that growth on a particular substrate provides cells which utilize that substrate (and metabolically related compounds) immediately and at a rapid rate, as has been observed in numerous other instances, but the significance of such phenomena in terms of formation of adaptive enzymes is still not entirely clear and warrants further study.

III. Photoproduction of H_2 and N_2-fixation

Photochemical production of molecular hydrogen by *Rhodospirillum rubrum* was first observed in nutritional experiments which were designed to determine the minimal phosphate level required for maximal growth of these organisms. To avoid complications introduced by the presence of phosphate in the yeast extract routinely added to growth media, various synthetic media suggested by Hutner (36) were employed. The important components of these media were (*a*) a dicarboxylic acid (malate, fumarate, or succinate)[8], (*b*) glutamic or aspartic acid as the nitrogen source, and (*c*) biotin, which is a required growth factor. Anaerobic photosynthetic development of *Rhodospirillum rubrum* in such cultures was accompanied by vigorous evolution of both CO_2 and H_2 (24, 25). The *net* formation of *both* CO_2 and H_2 was considered unusual and was certainly not expected in view of the fact that these organisms can utilize H_2 together with CO_2 by the reaction: $2H_2 + CO_2 \xrightarrow{\text{light}}$ "cell material" (15, 65, 66).

Since H_2 had never been observed as a photosynthetic product during growth or in resting cell experiments with these organisms, the possibility was entertained that the gas production was due to a heterotrophic contaminant. Suitable bacteriological control tests, however, demonstrated that the cultures were not contaminated and, accordingly, that purple bacteria produce H_2 during photosynthetic growth under the proper circumstances. Addition of NH_4Cl or high concentrations of yeast extract (or peptone) to the medium described above provides media which support excellent growth, but H_2 is not observed as a metabolic product when these supplements are present (25). These latter observations adequately explain the failure of previous workers to note photochemical H_2 production, since ammonium salts were universally used as nitrogen sources and yeast extract (or peptone) was generally also added as a source of unknown growth factors.

The second phase of the study of photochemical H_2 evolution by these organisms began with attempts to demonstrate the phenomenon manometrically using resting cell suspensions. Numerous exploratory experiments using 100% N_2 as

[8] Pyruvate can be used in place of a dicarboxylic acid (22).

the gas phase gave negative results. During these discouraging attempts, the H_2 metabolism of *Rhodospirillum rubrum* was frequently compared with that of *Diplococcus glycinophilus* (4). The latter organism appeared to behave exactly opposite to *Rhodospirillum rubrum* in that it (*a*) did not produce H_2 during fermentations in stationary culture in a closed system with small gas space and (*b*) produced H_2 in Warburg vessels when N_2 was the gas phase. Cardon and Barker (4) also noted that an atmosphere of 100% H_2 completely inhibited H_2 formation by *Diplococcus glycinophilus*, as might be expected. The apparent qualitative differences between the two organisms with respect to (*a*) and (*b*) ultimately, and somewhat reluctantly, led to an attempt to observe photoproduction of H_2 from malate by *Rhodospirillum rubrum* under an atmosphere of 100% H_2. Contrary to expectations, a clear-cut production of H_2 *induced by illumination* was observed under these conditions (and also when noble gases were used to establish anaerobiosis). This observation led to the inescapable conclusion that N_2 was not an inert gas with respect to photoevolution of H_2 (24).

Subsequent control experiments and tests with N_2^{15} indicated clearly that *Rhodospirillum rubrum* contains an active N_2-fixing system which permits the organism to grow, using N_2 as the primary nitrogen source (26, 44). These observations have been confirmed and extended by Wilson and Burris and their colleagues at the University of Wisconsin (48, 49, 50, 51, 69). The efforts of this group have led to several pertinent findings of general interest. They have found that: (*a*) N_2 fixation is widespread among *all* types of photosynthetic bacteria (49, 50), (*b*) N_2 fixation by these organisms is most pronounced under anaerobic conditions in the light as compared with anaerobic exposure in the dark or with aerobic exposure with or without light (48, 51), and (*c*) the products of N_2^{15} fixation appear to be very similar to those observed in *Azotobacter vinelandii* (69).

The inhibition of photochemical H_2 evolution by N_2 in photosynthetic bacteria superficially appears to be the counterpart of the competitive inhibiting effect of H_2 on N_2 fixation observed in Azotobacter and in symbiotic N_2 fixation (72, 76). Although there is no direct supporting evidence available, it appears that the former phenomenon can be most reasonably explained on the basis of a competition, during transfer of metabolic hydrogen, between hydrogenase (which would yield H_2) and the N_2-reducing system. The intermediate in N_2 reduction which presumably competes successfully for the metabolic hydrogen is not known; since NH_4^+ also abolishes photochemical H_2 evolution in the presence of various substrates (in resting suspensions and in growing cultures), it is possible that reductive amination of keto acids represents one "locus" for the competition (26). At the present time, it appears that the obscure relationship between H_2 and N_2 metabolism observed in various N_2-fixing systems (73) will probably be most profitably attacked by closer examination of the phenomena occurring in photosynthetic bacteria. The discovery of N_2 inhibition of photoproduction of H_2 in the latter organisms has had the effect of refocusing attention on this relationship. Recently Lindstrom *et al.* (49) have reexamined the tentative earlier suggestion that all organisms containing hydrogenase might be

potential N_2 fixers. It now appears that in N_2-fixing organisms, there is a definite relationship between hydrogen transfer reactions involving hydrogenase and N_2 reduction. On the other hand, hydrogenase is also present and fulfills necessary biochemical functions in organisms devoid of the N_2-fixation system.

The identity of the H_2 precursor in photochemical H_2 evolution was investigated by Gest *et al.* (26) on the basis of substrate specificity. In these studies, it was found that resting cells evolved H_2 in the presence of certain dicarboxylic acids (malate, fumarate, oxalacetate) or pyruvate, but not in the presence of a variety of other compounds. Succinate alone did not induce H_2 formation, a result which was unexpected in view of observations made on growing cultures. The relatively small production of H_2 from pyruvate and the lack of H_2 formation from formate (in the light) appeared to rule out reactions of the types (*a*) $CH_3COCOOH + H_2O \rightarrow CH_3COOH + CO_2 + H_2$ and (*b*) $HCOOH \rightarrow CO_2 + H_2$. These reactions must be reconsidered as possibilities as a result of recent work from several laboratories. Kohlmiller and Gest (45) found that young active suspensions of *Rhodospirillum rubrum* decompose pyruvate photochemically with the production of relatively large quantities of H_2 (approximately 0.8 mole/mole of pyruvate) in contrast to the results noted previously with older cell suspensions. With respect to HCOOH as a possible intermediate, it was also observed that this acid accumulates as an end product in "bottle" cultures of *Rhodospirillum rubrum* actively producing H_2 (in a malate + glutamate medium), but does not in cultures growing without H_2 formation (in the presence of NH_4^+). Experiments on HCOOH decomposition by resting cells, however, have given results which at the moment appear to contradict the possibility that HCOOH is an intermediate. Although HCOOH is decomposed in the dark by resting cells with the production of CO_2 and H_2, relatively little gas formation is observed during illumination. This suppression of gas production by light, which was first described by Nakamura (54), has been previously interpreted as being due to photosynthetic *recombination* of CO_2 and H_2 (liberated from HCOOH by "dark" hydrogenlyase activity) to "cell material". The experiment illustrated in figure 2 indicates that this explanation may not be valid (22).

As is evident from the figure, the rate of H_2 formation from HCOOH in the light is considerably lower than it is in the dark. When the HCOOH was almost exhausted in the dark, as indicated by the H_2 yield, the vessels in the light were transferred to the dark and the manometric measurements continued. It can be seen that H_2 was then evolved at an accelerated rate with the final production of approximately the *same amount* of H_2 as was formed by organisms kept dark throughout the experiment. This experiment suggests that light *inhibits* the net decomposition of externally added HCOOH. This remarkable effect may have some bearing on the general problem of photochemical H_2 production and is being further studied in this connection.

In the experiments of Kohlmiller and Gest (45) with active young cells of *Rhodospirillum rubrum*, lactate and succinate were found to support immediate and rapid photochemical H_2 evolution, in contrast to the results originally re-

ported. The explanation for the failure to obtain these results in the earlier experiments (26) is evident from the recent studies of Siegel and Kamen (63) with *Rhodopseudomonas gelatinosa* and *Rhodospirillum rubrum*. These investigators found that with certain substrates H_2 was not formed by resting cells if CO_2 was continually removed from the system (by absorption with alkali); in the presence of CO_2, however, the same compounds did give rise to H_2 production. In addition, it was observed that the total quantity of H_2 formed in other instances (e.g., by *Rhodopseudomonas gelatinosa* from acetate or malate) was significantly diminished when CO_2 was absorbed throughout the course of the experiment. These pronounced effects of CO_2 tension on the quantitative and qualitative aspects of the reaction are reminiscent of the similar effects already

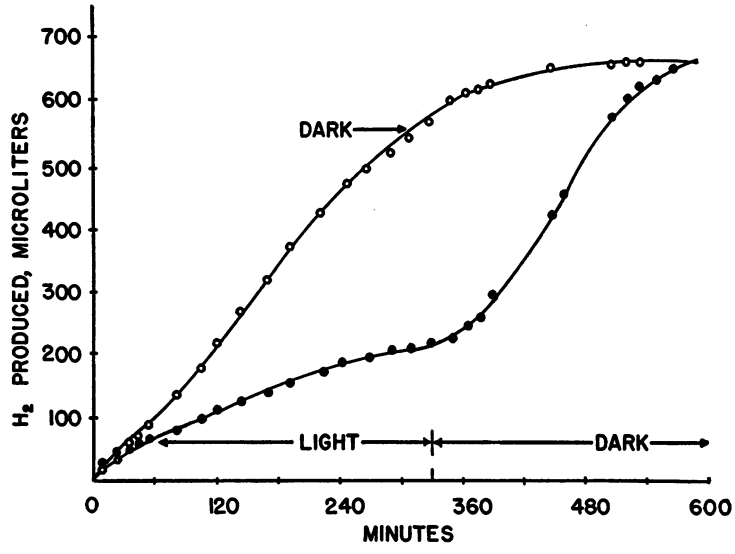

FIG. 2. H_2 production from HCOOH by *Rhodospirillum rubrum* (SI) in light and dark. Thirty micromoles of HCOONa were supplied to 26 hour-old cells in 0.05 M phosphate buffer pH 6.8 at 30 C; the gas phase was 100% helium. KOH was present in the center well.

described concerning the dark fermentation of pyruvate by *Rhodospirillum rubrum*. Evidently these effects can reasonably account for the differences noted between young and older, less active, suspensions of the same organism and between organisms of different species, i.e., the level of endogenous CO_2 formation may determine whether or not a given substance will evoke H_2 formation.

Quantitative studies of the CO_2 and H_2 yields from various substrates were reported by Siegel and Kamen (63). For the present purposes, these are of interest with respect to the total amount of H_2 that can be produced in this type of photochemical decomposition. The yield of H_2 in certain cases is remarkably large, e.g., 3 moles/mole of malate (*Rhodospirillum rubrum*). The author has also noted similar large yields in the photodecomposition of lactate and has

found it difficult to reconcile these high yields in terms of "normal" fermentation patterns.

It is of interest that *Rhodopseudomonas gelatinosa* shows a marked endogenous photoevolution of H_2 and CO_2 in contrast to *Rhodospirillum rubrum* (63). A further point of difference is found in the fact that evolution of these gases in the presence of added substrates by the former organism appears to be closely related in some unknown way to the level of endogenous activity. The explanation of this interdependence is not apparent at the present time. In all other respects, photochemical H_2 production appears to be identical in the two organisms.

It was noted above that a *net* production of CO_2 occurs during photodecomposition of "oxidized" organic compounds. Siegel and Kamen (63) have shown that the production of CO_2 from various compounds usually is inhibited by N_2 as compared with the reaction occurring under inert noble gases. As with photochemical H_2 production, CO_2 evolution under an inert gas is generally also abolished or significantly diminished by addition of NH_4^+. These observations indicate, as might be expected, that carbon transformations as well as hydrogen transfer reactions are markedly affected by the N_2-fixing system. The carbon intermediates formed during photodecomposition of oxidized organic compounds, either under N_2 or an inert gas, are as yet unknown. With the dicarboxylic acids, the manometric evidence is not inconsistent with the possibility that pyruvate is an important intermediate as was indicated previously. It is also known that fatty acids do not accumulate during photodecomposition of pyruvate (under an inert gas) in contrast to the results observed in the dark fermentation of this compound (45). It would appear that the problem of identifying the carbon intermediates produced in photodecomposition will be best approached through isotopic techniques. Preliminary experiments of this kind have been recently reported by Glover and Kamen (29) for the photodecomposition of C^{14}-labeled acetate in the presence of CO_2 by *Rhodospirillum rubrum* (see also Section 3. I.). The labeled intermediates formed in short exposure periods have not yet been identified, but do not appear to be phosphate esters, amino acids or Krebs cycle components. Their results also suggest that the transformations of acetate carbon during photodecomposition differ quantitatively, and perhaps qualitatively, from those of $C^{14}O_2$-carbon (in the presence of unlabeled acetate or H_2). The identity of the primary carbon fragments formed during photodecomposition and the relationship of these fragments to CO_2 fixation reactions remain to be established.

Siegel and Kamen (63) have suggested that *all* substrates which can be readily metabolized by purple bacteria can probably evoke H_2 formation under appropriate conditions. This viewpoint implies that H_2 is not derived from a unique conversion but rather that hydrogen from a large variety of substances, including water, can be funneled to a common intermediate carrier, which then couples with hydrogenase (yielding H_2) or with various types of acceptors, e.g., certain intermediates involved in reduction of N_2 to amino groups. The composition of the intermediate carrier is not known. It is conceivable that the same hypothetical, and perhaps hitherto unknown, carrier is involved in the formation of

H_2 from various substrates in the well-known heterotrophic fermentations. The essential unsolved problem in both types of H_2 evolution (i.e., photochemical and heterotrophic) appears to be the identification of the specific carrier system which has an oxidation-reduction potential in the region required for H_2 formation (i.e., approximately -0.4v).

Various considerations suggest that the *final* phase of "photochemical" H_2 evolution, i.e., H_2 release from the carrier, is probably not light-dependent; accordingly, light would be responsible for the *reduction* of the primary hydrogen carrier. Previous speculation (26, 42) on the mechanism of the phenomenon has been based essentially on the schemes for bacterial photosynthesis devised by Shibata (60) and van Niel (65). A hypothetical mechanism, derived from these schemes, by which H_2 could arise from water is illustrated by the following equations (26) for the case where malic acid is the "accessory donor."

1. $8H_2O + 8X + 8Y \xrightarrow{\text{light}} 8HX + 8YOH$
2. $C_4H_6O_5 + 4HX \longrightarrow (C_4H_8O_4) + H_2O + 4X$
3. $\frac{1}{2}(C_4H_8O_4) + 8YOH \longrightarrow 2CO_2 + 6H_2O + 8Y$
4. $\frac{1}{2}(C_4H_8O_4) \longrightarrow (CH_2O)_2$
5. $4HX \longrightarrow 2H_2 + 4X$

Overall: $C_4H_6O_5 + H_2O \xrightarrow{\text{light}} (CH_2O)_2 + 2CO_2 + 2H_2$

An essential feature of this scheme is a proposed *imbalance* between utilization of HX and YOH; if by some means, the number of "units" of YOH disposed of (reaction 3) is greater than the number of HX "units" disappearing (reaction 2), then an excess of HX is considered to be available as a precursor of H_2 (reaction 5). Obviously, this postulated series of events must be considered as completely speculative since there is practically no evidence available to support the reactions indicated. In particular, the concept (65) that the main function of the accessory donor is to "detoxify" YOH (reaction 3) appears to be doubtful in view of the foregoing discussions. On the whole then, the only overall scheme proposed thus far must be regarded as a very crude representation of one conceivable mechanism that might explain the phenomenon.

In concluding this discussion of light-stimulated H_2 formation, two more points are worthy of brief consideration. First, the analogous phenomenon occurring in adapted algae, described originally by Gaffron and Rubin (18), appears to differ from the reaction in photosynthetic bacteria in several respects. Although there are insufficient data available to justify a detailed comparison, the reviewer is inclined to believe that the differences may be superficial and, accordingly, that the same basic reactions are involved in both instances. The second point of importance is that practically all of the earlier manometric studies with "resting cells" were performed using N_2 in the gas phase, this practice being based on the assumption that N_2 was an inert gas for these organisms. The discovery that most, if not all, photosynthetic bacteria fix N_2 and the fact that the overall metabolism of these organisms appears to be markedly altered by

the presence of this gas obviously make it necessary to reconsider the older experiments from a different point of view. As was indicated previously, these important factors must be taken into account in assessing the significance of many of the experimental data obtained in earlier studies.

IV. Phosphorylation in photosynthesis

A number of investigators have suggested that light energy may be converted to chemical energy in the form of high-energy phosphate compounds (e.g., see (8)). Such a process together with the subsequent utilization of the energy-rich compounds for driving various synthetic reactions represents an intelligible mechanism for energy transfer in photosynthesis. Thus far, efforts to obtain evidence for the transformation of radiant energy to phosphate bond energy have been limited to experiments with intact cells. These studies have generally suffered from the disadvantage that significant net changes in the quantitative levels of phosphate compounds within intact cells are usually difficult to demonstrate because of the organization and economy of processes in such systems. Using the green alga, *Chlorella pyrenoidosa*, Emerson *et al.* (8) attempted to demonstrate and correlate changes in the distribution of intracellular phosphate compounds with the utilization of light energy. The experimental rationale was in many respects similar to that used in previous studies on the role of phosphorylation in chemosynthetic metabolism. For example, it was expected that in the absence of CO_2, a net phosphate uptake and esterification should occur upon illumination; in the presence of CO_2 ("phosphate-acceptor"), on the other hand, little change or even shifts in the other direction would be anticipated. The changes observed with respect to phosphate uptake and distribution of phosphate esters were in general not significant and a definitive conclusion could therefore not be reached.

Wassink *et al.* (71) have recently investigated phosphate exchanges between the medium and cells of the sulfur purple bacterium *Chromatium* (strain D) with exactly the same approach. The results of this study were interpreted to support the phosphorylation hypothesis in that the changes observed were analogous to those reported by Vogler and Umbreit (68) in experiments which presumably demonstrated the coupling of phosphate esterification with sulfur oxidation in *Thiobacillus thiooxidans*. It must be emphasized that the phosphate exchanges reported in both of these investigations were actually very small. Further, and more important, is the fact that the disappearance of phosphate from the medium in both instances has not been directly correlated with the formation of organic phosphate esters. It is quite possible that a variety of other phenomena, not well understood then and now, could have played a major role in the small changes which were reported (e.g., see (23)). In the opinion of the present reviewer, the *evidence* that high-energy phosphate formation is directly coupled with oxidation of the inorganic hydrogen donor in chemosynthesis is still highly circumstantial. This point is strongly emphasized here because of the increasingly frequent statements or implications in the literature to the effect that energy transfer in chemosynthesis has been shown to occur directly via adenosinetriphosphate.

Another attempt to secure experimental data concerning the "phosphorylation hypothesis" in photosynthesis was made by Aronoff and Calvin (1). These investigators used $P^{32}O_4^{=}$ as a tracer to study the effect of light on phosphate uptake and turnover in *Chlorella vulgaris*, leaves, and grana. From the data obtained they concluded that light has no significant effect on phosphorus metabolism in these systems. Careful consideration of the techniques employed, however, indicates that the inherent advantages of the sensitive tracer method were nullified by the other inadequate analytical procedures used in this research.

A more satisfactory application of the tracer technique to this problem was made by Gest and Kamen (23) in experiments with *Rhodospirillum rubrum* and two species of green algae. The results showed clearly that the uptake and *turnover* of phosphate in these organisms is markedly greater in the light than in the dark. From these studies it appears that energy-rich phosphate esters are probably formed as a consequence of light absorption but it must be emphasized again that there is no evidence for a *direct* coupling of the two processes. Further support for the concept of direct, or an indirect but closely related, coupling of phosphorylation and light absorption is given by the recent identification of phosphoglyceric acid as an early intermediate in green plant photosynthesis (3, 9).

The theoretical objections (17) to the "phosphorylation hypothesis" are in general directed against the assumption (8) that one high-energy phosphate bond (~ph) is formed for each light quantum absorbed. If it is assumed that more than one ~ph can be formed from each quantum, then the energetic requirements of the overall photosynthetic reaction (i.e., CO_2 reduction to carbohydrate) can apparently be accounted for reasonably. The arguments presented against the possibility that more than one ~ph can be formed per quantum are based on the absence of evidence for photochemical reactions in which the energy of a single quantum can be parcelled into a number of smaller "quanta" of energy with high quantum efficiency (12). If this line of reasoning is followed, the problem rapidly comes to an impasse, since the conversion and storage of light energy then could not possibly be explained by *any* type of reaction now known in biochemistry. This conclusion is difficult to accept from a comparative biochemical point of view at the present time and until further information is available, it is perhaps more appropriate to reconsider the validity of various assumptions involved in these theoretical arguments.

CONCLUSIONS

From the foregoing discussions, it is apparent that many aspects of the nutrition and metabolism of photosynthetic bacteria will require further study before we can correlate the similarities and idiosyncrasies of these organisms with the more thoroughly understood heterotrophic bacteria. The major approach employed thus far in exploring the mechanisms of photosynthetic and other types of metabolism in these bacteria has been the study of gas exchanges by means of manometric techniques. It is evident from recent developments in intermediary metabolism that the data obtainable using such procedures alone are

necessarily limited in scope. Further insight into the metabolic mechanisms operating in these organisms will no doubt be obtained by the intelligent application of other modern biochemical techniques, in particular the use of isotopic tracers, and also by more systematic studies through classical procedures.

Such approaches are necessary for further definition of the pathway of CO_2 and of carbon from the "accessory organic hydrogen donor" in bacterial photosynthesis. The available data indicate that, in general, the "accessory donor" does not undergo a simple one-step oxidation during photochemical decomposition but rather that it also provides carbon fragments for synthesis of various cellular compounds. The relationship of "accessory donor" metabolism to light-dependent fixation of CO_2 is still unknown. In certain cases, the "accessory donor" also serves as a substrate for aerobic oxidation in the dark. Although the mechanisms by which "donors" are oxidized are unknown, it appears that under aerobic conditions in the dark the fate of the carbon from the "accessory donor" is not necessarily the same as in anaerobic photosynthetic metabolism. As yet, the nature of the relationship, if any, between respiratory and photosynthetic metabolism is ill defined.

Contrary to earlier beliefs, it is now established that photosynthetic bacteria can ferment various substances in the dark; in one instance the fermentative pattern is in certain respects similar to that observed in propionic acid bacteria. Further studies are required before the spectrum of fermentative systems existing in these organisms can be clearly delineated.

The recently discovered phenomena of photochemical H_2 production and inhibition of this process by N_2 in these organisms have uncovered new aspects of their metabolic potentialities. This obvious interaction between hydrogen transport reactions and the N_2-fixation mechanism is of great interest to the general field of N_2 reduction in biological systems. It appears that photosynthetic bacteria will provide excellent experimental systems for further study of the obscure relationships between metabolism of N_2 and H_2.

The mechanism of photochemical H_2 formation is still unknown. At the present time, it seems likely that elucidation of the detailed schemes by which H_2 is produced in heterotrophic fermentations will contribute to our understanding of light-stimulated H_2 formation. In this connection, renewed studies on H_2 *utilization* with various acceptors have been made, but the relationship of this process to H_2 *evolution* is still uncertain. It seems probable that the continued study of H_2 metabolism in photosynthetic bacteria will supply valuable information concerning the hydrogen transport reactions involved in photosynthesis.

Acknowledgment. The author wishes to express his appreciation to Prof. L. O. Krampitz and Prof. H. G. Wood for critically reading the manuscript and for helpful discussions during its preparation.

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