

ON THE PURPLE BACTERIA AND THEIR SIGNIFICANCE FOR THE STUDY OF PHOTOSYNTHESIS

BY

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INTRODUCTION.

There is hardly any group of unicellular organisms that has attracted the interest of so varied a group of biologists as the purple bacteria. Their shape, their size, their color, their peculiar reactions, chemotactic as well as phototactic, their mass-occurrence under specific conditions, all have furnished material for the taxonomist, the physiologist, the ecologist and the biochemist. Not less than five monographic papers on this group of organisms have appeared ¹⁾, besides an overwhelming number of shorter articles dealing with the various aspects of the biology of these bacteria.

On perusal of this extensive literature one becomes struck by the peculiar controversies that have existed and for the greater part still do exist concerning the ideas on this group;

¹⁾ E. Warming, *Om nogle ved Danmarks Kyster levende Bakterier*; Videnskabelige Meddelelser Kopenhagen, 1875, Nr. 20—28, p. 30—116; 1876 p. 1—36.

S. Winogradsky, *Beiträge zur Morphologie und Physiologie der Bakterien*; Heft 1. *Zur Morphol. u. Physiol. der Schwefelbakterien*; Leipzig 1888, pp. 1—120.

H. Molisch, *Die Purpurbakterien nach neuen Untersuchungen*, Jena 1907, pp. 1—95.

Joh. Buder, *Zur Biologie des Bakteriopurpurins und der Purpurbakterien*; *Jahrb. f. Wiss. Bot.* 58, 525—628, 1919.

Werner Bavendamm, *Die farblosen und roten Schwefelbakterien des Süß- und Salzwassers*; Jena 1924, pp. 1—156.

and this with respect to morphological as well as taxonomic and biochemical problems.

Especially in the last-mentioned field, the ideas that have been advanced are remarkably divergent, as will appear from section 2. Photosynthetic activity, chemosynthetic mode of life, and more or less purely heterotrophic nutrition have all been proposed and defended to explain the characteristic behavior of the purple bacteria.

Controversies in scientific concepts usually are conditioned by the overemphasis of a limited number of observations. These controversies tend to disappear after extensive observations have made possible the formulation of broader concepts which include the apparent contradictions as special cases, definitely governed by different, although equally definite conditions.

An investigation of the purple bacteria carried on by the senior author since 1923 has led to results which to some extent seem to be in agreement with the statement made in the last paragraph. The present paper is meant to be an outline of the concept to which the biochemical side of the study has led; and, also, to give an outlook on the problems suggested by it. Rather than entering into details as far as methods of investigation are concerned ¹⁾ we will content ourselves here with a consideration of some of the results obtained in their mutual connection.

SECTION I.

SUMMARY SURVEY OF THE IDEAS PUT FORWARD TO EXPLAIN THE METABOLISM OF THE PURPLE BACTERIA.

Without going into a detailed discussion of the literature, it is deemed advisable at least to mention the main theories put forward in an attempt to explain the behavior of the

¹⁾ A more extensive publication will appear shortly.

purple bacteria. Engelmann¹⁾ who first observed the very strong positive phototaxis of a purple bacterium, which even led to the name *Bact. photometricum*, concluded that light plays an important part in the metabolism of this organism: i. e. the purple bacteria would be photosynthetic. Since this would imply the evolution of oxygen from illuminated cultures, Engelmann tried to furnish experimental support for his viewpoint by the use of motile bacteria to detect small quantities of oxygen. Although the first results of these experiments were negative throughout, in 1888 Engelmann claimed to have demonstrated the oxygen production by the same method; the bacteria then used were seemingly more sensitive to small amounts of oxygen.

Beijerinck in 1901²⁾ published his ingenious method for detecting oxygen by the use of luminous bacteria, a method which may well be considered superior to Engelmann's motile bacteria method. This method was then used by Molisch³⁾ in an attempt to settle the question whether or not purple bacteria were photosynthetic. However, Molisch obtained negative results, and thus was led to refute Engelmann's idea of the photosynthetic activity of the purple bacteria.

Repeated experiments by one of us (M) have also given the same negative result, although the material used was extremely sensitive.

Nadson⁴⁾, in 1912, advanced as a different argument

¹⁾ Th. W. Engelmann, Pflüg. Arch. f. d. ges. Phys., 30, 95—124, 1883; Ibid. 42, 183—186, 1888; Bot. Ztg., 46, 661 ff., 1888; Arch. Néerland., 23, 151—198, 1888.

²⁾ M. W. Beijerinck, Proc. Kon. Akad. v. Wet., Amsterdam, 4, 45—49, 1901.

³⁾ H. Molisch, Die Purpurbakterien, Jena 1907.

⁴⁾ G. A. Nadson, Bull. d. Jard. Imp. St. Petersb., 12, 83—89, 1912.

against the photosynthetic activity of the purple bacteria, and also of the green bacteria, the fact that although they occur in black mud, yet this never shows the characteristic signs of oxidation which are found surrounding colonies of algae. Baas Becking ¹⁾ in 1925 used the same argument.

A different concept of the metabolism of purple bacteria was advanced by Winogradsky ²⁾ in 1887. This was based upon an extensive investigation of the bacteria occurring in sulphide waters, the sulphur bacteria. The experiments were for the greater part carried out with colorless forms. They led Winogradsky to his brilliant concept of chemosynthetic processes in which the energy necessary for the life of the organism is furnished by the oxidation of inorganic compounds.

Since the purple forms in Winogradsky's cultures behaved similarly to the colorless forms, the former were also considered as chemosynthetic bacteria, especially because their occurrence and development was fully dependent upon the presence of hydrogen sulphide in the medium.

The concept of purple bacteria being chemosynthetic and deriving their energy, like the colorless sulphur bacteria, from the oxidation of hydrogen sulphide to sulphate, required, however, the presence of oxygen. Winogradsky had already observed that the purple bacteria behave like true anaerobes, and therefore postulated the green bacteria, which in his cultures always accompanied the purple bacteria, as „oxygen-furnishers”, thus explaining the favorable effect of these green organisms on the development of the purple bacteria and, also, the favorable effect of light.

Beijerinck ³⁾, in his remarkable studies on „Atmungsfiguren”, also concluded that the purple bacteria were true

¹⁾ L. G. M. Baas Becking, *Ann. of Bot.*, 39, 613—650, 1925.

²⁾ S. Winogradsky, *Bot. Ztg.*, 45, 489 ff., 1887; *Zur Morph. u. Phys. der Schwefelb.*, Leipzig 1888; *Ann. Inst. Past.*, 3, 49—60, 1889.

³⁾ M. W. Beijerinck, *C. f. Bakt.* 14, 827—845, 1893.

anaerobes ¹⁾. He does not enter into a discussion of the metabolism of these organisms but only states:

„Die zahlreichen auf ihre Lebensbedingungen bezüglichen Fragen sind angeregt, jedoch durchaus noch nicht als gelöst zu betrachten.“

Molisch, in 1907, discussed and refuted also Winogradsky's concept. His experiments had shown that certain forms of purple bacteria developed in the absence of hydrogen sulphide but in the presence of organic matter, and with an astonishing disregard of Winogradsky's evidence claims that hydrogen sulphide is not necessary for the growth of the purple bacteria, organic matter, however, indispensable. Although light in many cases has a favorable effect on the development of the purple bacteria, the organisms can grow in the dark. Molisch' ideas on the meaning of these results are not at all clear, and for the present it seems advisable to leave them out of further consideration.

In a remarkably clear and complete discussion of facts and viewpoints Buder in 1919 ²⁾ summarizes the results as follows:

„Die Schwierigkeit, die gegenwärtig der Stoffwechsel der Thiorhodaceen einer Erklärung bietet, scheint mir hauptsächlich darin begründet zu sein, dass man geneigt ist, dem Pigment im Anschlusse an Engelmann eine assimilatorische Rolle zuzuweisen, andererseits aber auch im Anschluss an Winogradsky in der Oxydation des Schwefelwasserstoffes ein wesentliches Glied des Stoffwechsels zu sehen, zumal

¹⁾ Beijerinck's conclusion: „Die beiden von mir untersuchten Arten haben die merkwürdige Eigenschaft, dass ihre Individuen auf verschiedene Sauerstoffspannungen gestimmt sind, je nachdem sie mit mehr oder wenig konzentrierten H₂S-Lösungen in Kontakt gewesen sind, oder wenn sie im Tropfen verschiedene Konzentrationen dieses Stoffes vorfinden“ may not be fully justified since this behavior can also be explained on the basis of H₂S tolerance. A more detailed discussion of this point will appear elsewhere.

²⁾ Joh. Buder, Jahrb. Wiss. Bot., 58, 525—628, 1919, esp. p. 587.

auch die Versuche Skenes und die Analogie mit den farblosen Schwefelbakterien dafür sprechen. Wir hätten dann also zwei Energiequellen für die Gewinnung des Kohlenstoffes aus der Kohlensäure zur Verfügung: das Licht und die bei der Oxydation des H_2S zu S und SO_2 freiwerdende chemische Energie. Bisher scheint man sich nun gescheut zu haben, das Bestehen einer Photosynthese und Chemosynthese nebeneinander anzunehmen. Von energetischen Gesichtspunkten aus kann ich dafür keine Schwierigkeit sehen. Denn es ist sehr wohl denkbar, dass der gleiche chemische Prozess sich unter Zufuhr sowohl von Licht als auch von Wärme oder anderen Energieformen abwickelt."

Exactly the same ideas are to be found in Bavendamm's monograph ¹⁾. Bavendamm concludes:

„Damit sind wir zu der Engelmannschen Hypothese, die jetzt — abgesehen von der noch nicht ganz geklärten Frage der Sauerstoffausscheidung — keine Hypothese mehr ist, zurückgekommen."

The facts do not justify this conclusion. For the fate of the oxygen, as well as the necessity of the hydrogen sulphide for the purely autotrophic development of the purple sulphur bacteria cannot entirely be explained on the basis of Engelmann's hypothesis. Moreover, not only the fate of the oxygen has to be explained, but also and primarily the necessity of *both* H_2S and light. The assumption of photosynthetic organisms which also can develop chemosynthetically; or of chemosynthetic organisms which under certain circumstances also can live photosynthetically, requires the presence of light *only* or of H_2S and oxygen *only*. However, Bavendamm states quite definitely that:

„Für beide Gruppen (colorless and purple sulphur bacteria) ist der Schwefelwasserstoff unbedingt notwendig.

¹⁾ Werner Bavendamm, Die Schwefelbakterien, Jena 1924, p. 88.

Ohne ihn können keine Lebens- und Bewegungserscheinungen stattfinden. Die Schwefelbakterien sind also als Anorgoxydanten anzusehen.

Während das Licht für die farblosen Schwefelbakterien ohne Belang ist, entwickeln sich die roten nicht ohne Licht. Der rote Farbstoff übt dieselben oder ähnliche Funktionen aus wie das Chlorophyll der höheren Pflanzen." ¹⁾

In their highly important contribution to the study of bacterial metabolism, which is partly based upon Wieland's theory of the mechanism of oxidation-reduction processes, Kluver and Donker ²⁾ refer to the metabolism of the purple sulphur bacteria as follows:

„Wenn man diese Betrachtungsweise einmal akzeptiert, fällt aber die Notwendigkeit, dass der Akzeptor bei der Dehydrierung des Schwefelwasserstoffes oder des Schwefels gerade Sauerstoff sein soll, weg; denn es kann ebensogut irgendein anderer Akzeptor im Spiele sein. Nur muss das Prinzip beibehalten bleiben, dass die gebildete Akzeptor-Wasserstoffverbindung mit Hilfe der Lichtenergie imstande ist, den Wasserstoff auf die Kohlensäure zu übertragen."

This viewpoint is later also taken up by Baas Becking and Parks ³⁾ and by Buchanan and Fulmer ⁴⁾.

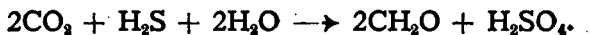
If, for the moment, we leave out the intermediate acceptor-hydrogen compound which will be discussed in section 4, we come to the following formulation of the metabolism of the purple sulphur bacteria under the influence of the light:

¹⁾ W. Bavendamm, Die Schwefelbakterien, Jena 1924, p. 89.

²⁾ A. J. Kluver and H. J. L. Donker, Die Einheit in der Biochemie, Chem. d. Zelle u. Gew., 13, 134—190, 1926, esp. p. 175—176.

³⁾ L. G. M. Baas Becking and G. S. Parks, Physiol. Rev. 7, 85—106, 1927.

⁴⁾ R. E. Buchanan and E. I. Fulmer, Physiology and Biochemistry of Bacteria, Baltimore, 1928—1930, esp. Vol. I, p. 413, and Vol. III, p. 203.



The first step in this reaction:

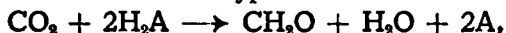


shows the greatest similarity with the well-known equation of photosynthesis:



The only difference is that in the former case sulphur appears as the excretion product or final metabolite, whereas in the latter case this is replaced by O_2 .

In analogy with the well-established situation in the oxidative metabolism of microorganisms where different organisms require different compounds as hydrogen donors this has led to the formulation of a general group of photosynthetic reactions of the type:



thus opening the possibility for the existence of a number of different and special photosynthetic processes, each of which requires one or more special hydrogen donors for the reduction of the CO_2 ¹⁾. In the case of photosynthesis by the green plants this means that there the usual hydrogen donor is the water, that the oxygen evolved during this process is the dehydrogenation product of this reaction. This viewpoint has already been postulated in 1923 by Thunberg²⁾.

The experimental evidence given in a previous paper¹⁾ for the purple sulphur and green bacteria has strongly supported the viewpoint that in the process of photochemical carbon dioxide reduction by these organisms H_2S replaces the H_2O as a hydrogen donor.

It could also be shown that the green and purple sulphur bacteria in this respect possess a remarkable difference. The green bacteria, namely, can dehydrogenate the hydrogen

¹⁾ C. B. van Niel, *Contrib. to Marine Biol.*, Stanford Univ. Press, 1930, p. 161—169.

²⁾ T. Thunberg, *Z. f. physik. Ch.*, 106, 305—313, 1923.

sulphide with a simultaneous reduction of the carbon dioxide; this dehydrogenation of the H_2S leads to the formation of elementary sulphur, which for these organisms is the final oxidation product. The purple sulphur bacteria, however, can still further dehydrogenate this first oxidation product of the H_2S to sulphuric acid and also this dehydrogenation is intimately linked up with the simultaneous reduction of the CO_2 . This has been found to be the case both when the intermediary produced sulphur is stored inside the cells or when it is excreted.

These facts, together with some preliminary results obtained with *Thiorhodaceae* and *Athiorhodaceae* under various conditions, are well worth a closer consideration in relation to the general problem of photosynthesis.

SECTION II.

THE SPECIFICITY OF THE GREEN AND PURPLE SULPHUR BACTERIA.

In the foregoing section the statement was made that the green bacteria can only utilize H_2S as a H-donor in the photochemical CO_2 -reduction, whereas sulphur formed as an intermediate product can serve the same purpose in case of the purple sulphur bacteria. This statement was substantiated by the fact that excellent cultures of purple sulphur bacteria could be obtained in media where H_2S was completely lacking, but where elementary sulphur, sulphites or thiosulphates were present. Also could it be shown by quantitative determinations that here, too, the carbon dioxide functions as the only final H-acceptor. The green bacteria did not show any development under those conditions. It then became necessary to determine whether these purple bacteria required the presence of inorganic S-compounds that might be conceived as intermediate products in the dehydrogenation of H_2S or whether also other hydro-

gen compounds would function for the performance of their activities.

In these experiments it was found that leuco-methylene blue and reduced indigocarmin were dehydrogenated by the purple bacteria ¹⁾ under completely anaerobic conditions. With respect to Molisch' statement, that for the *Thiorhodaceae* the H₂S was not at all indispensable, other organic compounds were then tried with the result that a good development was obtained in yeast extract media.

However, in all these media sulphur is still present, in the latter case even in the form of sulphhydryl-groups ²⁾. Hence the fact that an abundant development of pure cultures of *Thiorhodaceae* was secured in media which — besides inorganic constituents and with sulphates as the only source of sulphur — contained sulphur-free organic compounds, becomes of special importance. The quantitative investigation of the changes which these organic compounds (lactates, pyruvates, etc.) undergo is being carried out by one of us (M). The fact is mentioned here because it brings about a connection between the *Thiorhodaceae* and the *Athiorhodaceae*.

It is obvious, then, that under completely anaerobic conditions various substances can serve the purple sulphur bacteria for building up their organic cell constituents in the light. In the case of inorganic compounds these organic cell constituents are built up from carbon dioxide; in the case of organic substrata this assimilation, of course, is not necessarily connected with a reduction of the CO₂ of the medium. That the metabolism of the organic substances

¹⁾ In the following mention will only be made of experiments with the purple bacteria. The pure culture of the green bacterium, *Chlorobium limicola* Nadson perished soon after the experiments with the sulphur compounds had been concluded and a new isolation, although under way, has not yet been completed.

²⁾ Cf. e.g. J. B. van der Lek, *Onderzoekingen over de butyl-alkoholgisting*, Diss., Delft, 1930, p. 128.

may not be considered as a „fermentation“ ¹⁾ of these compounds is clearly shown by the fact that in the same media but in the absence of radiant energy which can be absorbed by the bacteria ²⁾ no development takes place.

This serves to re-establish the connection between *Thiorhodaceae* and *Athiorhodaceae* ³⁾. Molisch had considered the sulphur-bearing and the sulphur-free purple bacteria as fundamentally the same organisms because he refuted the idea that the *Thiorhodaceae* did not need organic matter for their development. As has been shown before, the typical *Thiorhodaceae* belong to the autotrophic organisms; they can build up their cell constituents from CO₂ only. However, organic matter also will enable them to grow. Under anaerobic conditions, however, this growth can take place only with the simultaneous supply of radiant energy. And since none of the *Thiorhodaceae* isolated thus far is capable of development in the presence of even small amounts of oxygen, no development has been observed as yet in the dark.

The above-mentioned connection between *Thio*- and

¹⁾ The term fermentation is used here in the strictly Pasteurian sense to designate such metabolic processes which occur in the absence of atmospheric oxygen.

Cf. Kluyver and Donker, loc. cit.

²⁾ It is preferable to use the designation „radiant energy“ instead of „light“, since Engelmann's, Buder's and Dangeard's beautiful experiments have shown that the purple bacteria absorb radiant energy in the infra-red region and develop there. Cf. the interesting article by P. A. Dangeard; *Le Botaniste*, 19, 1—422, 1927, esp. pp. 200—204 and 290—303.

³⁾ Cf. section 3 of this paper. Of interest is also the statement made by Schneider, *Beitr. z. Biol. d. Pflanzen*, 18, 81—115, 1930:

„In Gegensatz zu B. avendamm glaube ich, dass man bei einer Beurteilung der Verwandtschaft dieser Bakteriengruppen nach phylogenetischen Gesichtspunkten die *Thiorhodaceen* mit den *Athiorhodaceen* zusammenfassen und sie nicht wegen ihrer Fähigkeit zur Speicherung von Schwefel den farblosen Schwefelbakterien zuordnen sollte.“

Athiorhodaceae, therefore, may not yet be considered in such a sense that the organisms belonging to these groups behave exactly similarly. For up till the present no growth has been obtained with the *Athiorhodaceae* in the absence of organic matter. Apparently the H_2S can not be used by these organisms for a photosynthetic carbon dioxide reduction. This difference, however, may not be as fundamental as at first sight it seems.

For the work of Beijerinck made us familiar with the conversion of autotrophic into heterotrophic forms of life ¹⁾. In his pioneer investigations on the cultivation of algae on solid media Beijerinck could not only prove that algae isolated with the aid of purely mineral media could be gradually accustomed to the presence of organic matter, but it was also established that under these conditions the algae could grow in the dark. Moreover it was found that algae which had been grown on organic media for some time could not immediately be cultured in purely mineral media. That under certain circumstances these heterotrophic forms are constant is most clearly shown by the conversion of the green *Chlorella variegata* into the colorless *Prototheca* ²⁾.

But these conversions of autotrophic into mixotrophic or completely heterotrophic forms are not limited to the photosynthetic organisms. Again it was Beijerinck ³⁾ who could show that similar conversions can take place in bacteria. His investigations of the nitrifying bacteria led him to the conclusion that the autotrophic, chemosynthetic *Nitrobacter* could develop in the presence of organic substances, and

¹⁾ M. W. Beijerinck, Prov. Utr. Genootsch. Utrecht, 1889, 35—52; Bot. Ztg., 48, 741 ff., 1890; Centr. f. Bakt., 13, 368—373, 1893; Ibid., II. Abt., 4, 785—787, 1898.

²⁾ M. W. Beijerinck, Rec. Trav. Bot. Néerl., 1, 14—27, 1904; Folia Microbiol., 1, 1—97, 1912.

³⁾ M. W. Beijerinck, Folia Microbiol., 3, 91—113, 1914; Versl. Kon. Akad. v. Wetensch., 22, 1163—1170, 1914.

that this development gives rise to a culture which has lost the ability to lead a purely chemosynthetic mode of life.

Hence it is quite conceivable that autotrophic organisms like the purple sulphur bacteria could gradually be so modified as to require organic compounds for their growth. Since it has been shown that this group of purple bacteria can develop in organic media in the absence of inorganic oxidizable sulphur-compounds this possibility can readily be tested by experimentation.

On the other hand by repeated transfers one might succeed in culturing *Athiorhodaceae* with H_2S in the absence of organic matter. The objection might be raised here that not only physiologically but also morphologically the differences between *Thio*- and *Athiorhodaceae* are rather great. This, however, is not the case. Among the *Thiorhodaceae* ¹⁾ we have met with forms which morphologically show the greatest possible similarity with e. g. *Rhodobacillus palustris* Molisch or *Spirillum rubrum* Esmarch. These small forms never show the typical sulphur droplets inside the cells; when grown in purely inorganic H_2S -containing media the sulphur produced as an intermediate compound is excreted, and in young cultures can be found as small globules in the liquid. In appearance these droplets are identical with the sulphur droplets inside the cells of larger *Thiorhodaceae*. Also in cultures of the green bacteria the sulphur is excreted. It first appears in the form of similar droplets, which gradually become crystalline.

At the present time we are not yet in a position to decide more definitely whether *Thiorhodaceae* and *Athiorhodaceae*

¹⁾ As *Thiorhodaceae* we designate those purple bacteria which can grow in a purely mineral medium, utilising H_2S as a H-donor for the reduction of CO_2 , regardless of the ability of the organisms to store intermediary produced sulphur as droplets *inside* the cells. As *Athiorhodaceae* those purple bacteria are designated which up till now require organic compounds in the medium.

have to be considered as fundamentally different physiological groups or whether external conditions during their development have caused reversible or irreversible changes. In this connection we also want to draw the attention to the experiments of Baars¹⁾. This investigator was able to throw new light on the problem of thermophilic bacteria. For his experiments showed beyond doubt that the two strains of *Vibrio desulfuricans*, hitherto considered as different species (*Vibrio desulfuricans* Beij. and *Vibrio thermodesulfuricans* Elion) in fact were nothing but one and the same organism, more or less fixed with regard to their ability to withstand different physical conditions, *in casu* different temperatures. By repeated transfers from young cultures and exposing these transfers to gradually changing conditions Baars succeeded namely in modifying *V. desulfuricans* so that it would grow only at temperatures above 30° C, and in obtaining a transfer from a *V. thermodesulfuricans* culture which did not develop at temperatures above 30° C. Similar experiments were carried out with respect to the salt-concentration of the medium, thus establishing the fundamental identity of *V. desulfuricans* Beij. and *V. aestuarii* van Delden.

It is true that these experiments which have demonstrated the occurrence of modifications due to *physical* conditions of the medium may not be considered as a support for the viewpoint that also *physiological* changes can be induced experimentally. But we want to emphasize here that Baars' experiments have shown conclusively that the degree of adaptability is largest in young cultures. This fact, in connection with the interesting observations on „Physiological Youth in Bacteria” by Sherman and Albus²⁾ shows the

¹⁾ J. K. Baars, Over sulfaatreductie door bacterien; Diss. Delft, 1930.

²⁾ James, M. Sherman and W. R. Albus, Jour. Bact., 8, 127—139, 1923; cf. also Henrici, Rate of Growth and morphologic Variation, Springfield, 1928.

way by which experimental modifications may most successfully be studied.

The established similarities between *Thiorhodaceae* and *Athiorhodaceae* will shortly be discussed in the next section.

SECTION III.

SOME CHARACTERISTICS OF THE ATHIORHODACEAE.

Also for the *Athiorhodaceae* the favorable influence of light has been established ¹⁾. Moreover it is well known that even if these organisms are not completely anaerobic they can occur under conditions where oxygen can hardly be present. From Molisch' investigations with pure cultures it appeared, however, that light is not absolutely necessary for their growth, and also that e.g. *Rhodobacillus palustris* develops aerobically on ordinary agar slants.

These facts led the senior author in 1924—1925 to some experiments which were planned with a view to determine the relationship of oxygen and light. The experiments were carried out with pure cultures of *Rhodob. palustris* and of *Spir. rubrum* ²⁾.

It appeared that both organisms could develop under aerobic conditions, in the light as well as in the dark. But under anaerobic conditions only those cultures that were kept in the light showed growth.

In 1929—1930 these experiments were repeated with the same organisms, and, in addition, also with a strain of

¹⁾ Cf. e. g. H. Molisch, *Die Purpurbakterien*, Jena 1907; E. Schneider, *Beitr. z. Biol. d. Pfl.*, 18, 81—115, 1930.

²⁾ These experiments were carried out in the Microbiological Laboratory of the Techn. Univ., Delft, Holland. The strain of *Rh. palustris* was newly isolated, *Spir. rubrum* was obtained through the courtesy of the National Collection of Type Cultures, Lister Institute, London, England.

Streptococcus varians Ewart ¹⁾, isolated by the same author in the Jacques Loeb Laboratory of Hopkins Marine Station. The results agreed with those formerly obtained. However, the observation was then made that in one respect at least *Str. varians* behaved differently from the other purple bacteria. The aerobic cultures had the same red color as those of *Rh. palustris* or of *Sp. rubrum*, which in the illuminated flasks was much more fully developed than in the flasks kept in the dark. But the anaerobic cultures of *Str. varians* were, in contradistinction to the red cultures of the other organisms, yellowish-brown with a green shade. If such a culture was then brought in contact with the air a rapid change in the color could be observed, and in 24 hours a deep-red sediment was found instead of the yellowish-brown, in the light as well as in the dark.

The rapidity with which this color-change takes place, together with the fact that hardly any further growth of the organism was noted, make it seem highly probable that the red pigment has originated by a simple chemical process out of a chemically closely related compound which is already present in the brownish anaerobic cultures. The assumption lies at hand that this chemical process is of an oxidative nature; under anaerobic conditions the red pigment would partly or wholly be present in a reduced state, which, as soon as oxygen becomes available, is oxidized again to the red form.

The experiments also showed that the bacteria were not killed in the absence of radiant energy under anaerobic conditions. Culture media, after inoculation with the various strains

¹⁾ A. J. Ewart, J. Linnean. Soc. Bot., 33, 123—155, 1897. The name *Streptococcus varians* given by Ewart is misleading. Although in certain cultures the organism actually resembles a *Streptococcus*, the microscopic observations on a large number of cultures has led to the conviction that it is much more closely related to the family *Pseudomonadaceae*, both with respect to the shape of the cell as to the presence of polar flagella.

and kept in the incubator in the dark for some weeks under anaerobic conditions, did not show any signs of development. But if these same cultures were exposed to the rays of ordinary electric filament lamps growth took place within a few days. Similarly, well-developed cultures could still be obtained in the dark if these media were then exposed to the air.

These observations give rise to the following considerations. In the absence of radiant energy the organisms apparently can utilize the energy obtained from oxidative metabolic processes, and, since the presence of oxygen is indispensable, only from these. Under the influence of radiant energy, however, they develop in the absence of oxygen just as well, so that they are capable of securing the energy entirely from the absorbed radiant energy.

Just as in the case of the *Thiorhodaceae* developing in organic media, the metabolism of the *Athiorhodaceae* can not be considered as a fermentation process in the Pasteurian sense. From the fact that the synthetic processes involved in the building up of new cell material are carried out in the dark under aerobic conditions only, we must conclude that the dehydrogenations of the organic substratum can only take place with oxygen as the hydrogen-acceptor in that case. It is tempting to suggest a mechanism of the same kind in the illuminated anaerobic cultures, although it is, of course, possible that the way in which the energy transfer is brought about is fundamentally different in the case where this energy is obtained from chemical reactions and in the case where it is absorbed in the form of quanta of radiant energy. In the latter case the energy taken up may, namely, result in such rearrangements in the molecules that the end-product is fit to serve as the raw material for synthetic reactions which occur spontaneously¹⁾, so that

¹⁾ Cf. for a more complete discussion of the energy exchange in katabolic and anabolic reactions: A. J. Kluyver, Arch. f. Mikrobiol., 1, 181—197, 1930.

purely chemical oxido-reductions would not be necessary. The investigation of the anaerobic metabolism of organic compounds by the purple bacteria will undoubtedly contribute much towards a more definite conclusion.

In case the metabolism of purple bacteria in organic media under anaerobic conditions and with the supply of radiant energy, is chemically similar to the metabolism under aerobic conditions in the dark, we are dealing with processes in which the dehydrogenations involved in the synthetic processes are carried out with oxygen as the final acceptor in the dark, whereas, in the light, the oxygen is replaced by some other compound which requires activation by radiant energy.

SECTION IV.

CONSIDERATIONS ON THE MECHANISM OF PHOTOCHEMICAL CARBON DIOXIDE REDUCTION IN CONNECTION WITH THE METABOLISM OF THE GREEN AND PURPLE BACTERIA.

At the present time two distinctly different viewpoints for the explanation of the photochemical carbon dioxide reduction are being held.

One theory explains the formation of the carbohydrate from carbon dioxide by postulating an intramolecular rearrangement in the carbonic acid molecule after this has combined with the chlorophyll and has been activated by the uptake of radiant energy.

This rearrangement leads to the formation of a formaldehyde-peroxide-chlorophyll complex, where the peroxide part may be more intimately connected with the formaldehyde configuration (Willstätter—Stoll¹⁾ or with the

¹⁾ R. Willstätter und A. Stoll, Untersuchungen über die Assimilation der Kohlensäure, Berlin 1918.

chlorophyll molecule (Stern, Noack, Holluta)¹⁾. By the subsequent splitting-up of this peroxide configuration, which is supposed to be enzymatic in character and is brought in in connection with the existence of a „dark reaction”, the so-called Blackman-reaction of Warburg c.s., the carbonic acid thus becomes converted into formaldehyde and oxygen.

The second theory defends the idea that the oxygen evolved is part of the H_2O molecule which in the process of photosynthesis is dehydrogenated. This type of mechanism includes the theories put forward by Kögel²⁾, Thunberg³⁾ and Weigert⁴⁾. The proposed mechanisms for this carbon dioxide reduction differ and the experimental support advanced for these various schemes has been refuted⁵⁾.

Yet the study of the green and purple sulphur bacteria has shown that, at least in the metabolism of these organisms, the only way in which one can conceive of the mechanism of the carbon dioxide reduction is by means of an oxido-reduction in which the H_2S (or S) functions as the H-donor, the CO_2 as the acceptor.

Of course it is possible that the mechanism of the CO_2 reduction in the photosynthetic process carried out by the green plants is fundamentally different from that carried out by the green and purple sulphur bacteria, because the green pigments active in these different groups are not identical. But in view of the fact that also the green pig-

¹⁾ K. Stern, Z. f. Bot., 13, 193—230, 1921; K. Noack, Ibid., 17, 481—549, 1925; J. Holluta, Samml. chem. u. chem.-techn. Vorträge, 28, 409—476, 1926.

²⁾ P. R. Kögel, Biochem. Z., 95, 313—318, 1919.

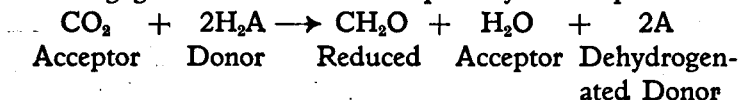
³⁾ T. Thunberg, Z. physik. Ch., 106, 305—313, 1923.

⁴⁾ F. Weigert, Ibid, 106, 313—323, 1923.

⁵⁾ Cf. for detailed discussions of the proposed mechanisms H. A. Spoehr, Photosynthesis, New York, 1926; Josef Holluta, Samml. chem. u. chem.-techn. Vorträge, 28, 409—476, 1926.

ments of the green and of the purple bacteria differ mutually, and especially the pigment of the green bacteria shows spectroscopically a much closer relationship to the chlorophyll than to the bacteriochlorin, we cannot see any reason for not generalizing this oxido-reduction principle so as to include also the photochemical CO_2 reduction by the green plants.

In section 2 it was pointed out that this leads to the following general formulation of photosynthetic processes:



It is evident that this formulation requires the activation of the acceptor as well as that of the donor¹⁾. In other metabolic processes which occur in the dark the activation of both acceptor and donor is carried out by the living protoplasm or by special parts of it. In the case of photosynthetic processes, however, it becomes necessary to ascribe the function of activator to the photochemically active pigment. Now the question arises whether this pigment activates both the donor and the acceptor or whether its action is confined to only one of them.

In the literature on photosynthesis that has appeared up to the present time the green pigment of the plants has been used to explain either the activation of the carbonic acid molecule (rearrangement to formaldehyde-peroxide), or the production of active hydrogen and hydrogen peroxide (Thunberg, Weigert). In the latter case the hydrogen is assumed to be active enough to reduce the chemically unchanged carbon dioxide.

A consideration of the metabolism of the green and purple sulphur bacteria leads to the conclusion that the green pig-

¹⁾ Cf. A. J. Kluyver und H. J. L. Donker, *Chem. d. Zelle u. Gew.*, 13, 134—190, 1926.

ment of the photosynthetic cell brings about the activation of the carbon dioxide molecule, as follows from the following consideration. The common feature of the photochemical carbon dioxide reduction by green plants, green bacteria and purple sulphur bacteria is the utilization of CO_2 as the final H-acceptor. They differ markedly in the suitability of various hydrogen compounds as a H-donor. It is of special importance to emphasize that the only compound which can serve as such in the case of the green bacteria is the hydrogen sulphide in which the hydrogen is loosely bound and, therefore, already present in an active form, as is shown e. g. by the spontaneous oxidation of H_2S in contact with the air. It is, therefore, possible that these bacteria do not need to activate their H-donor any more.

But even if the carbon dioxide reduction would require hydrogen in a more active state than as it is present in the hydrogen sulphide molecule it will be evident that for this activation the radiant energy would hardly be indispensable, the more so because the activation of the extremely stable carbon dioxide is the most difficult part of the process.

Besides the common feature of the necessity of activation of the CO_2 we also find green pigments produced in all three groups of organisms. The role of the green pigments as photochemical activators has been established beyond doubt by the numerous observations which show that photosynthesis only takes place under the influence of radiant energy which can be absorbed by these pigments ¹⁾. Hence the conclusion lies at hand that we have to look upon the green pigments as the agents which cause the activation of the H-acceptor, the carbon dioxide.

Now it may be possible that the activation of the hydrogen of the donor is also carried out by the same agency. In

¹⁾ Cf. e.g. Joh. Buder, *Jahrb. f. Wiss. Bot.*, 58, 525—628, 1919; P. A. Dangeard, *Le Botaniste*, 19, 1—422, 1927.

view of the necessity of hydrogen sulphide for the green bacteria which is only dehydrogenated to sulphur by these organisms this seems, however, hardly plausible. Yet the hydrogen of the sulphur (hydrate) ¹⁾, as well as that of the water, undoubtedly requires activation before it can be transferred to the carbon dioxide. It is well known that the colorless sulphur bacteria carry out this activation in the dark, utilizing oxygen as the main acceptor. These organisms must, therefore, bring about this activation without the interference of radiant energy. It is quite well possible that also the purple sulphur bacteria activate the hydrogen of their donors without the aid of radiant energy. But there exists another possibility which we want to discuss here.

The main difference between the green and purple sulphur bacteria lies in the fact that the former contain only a green pigment whereas in the latter this is accompanied by a large amount of red pigments ²⁾. Could this not be taken as an indication that the red pigments in the case of the purple sulphur bacteria might have some function in the activation of the hydrogen in such donors where this is more firmly bound?

We are fully aware of the speculative nature of the following considerations. That they are included here may be justified by the importance of the problem and the possibility of arriving at a more complete picture of the mechanism of photosynthesis.

The absence of yellow and red pigments in the green

¹⁾ The assumption of the dehydrogenation of sulphur in the form of its hydrate is a consequence of Wieland's theory of oxidation-reduction processes, which may be considered as well enough established to justify the omission of further support here.

²⁾ A copious pure culture of green bacteria, containing about 0.5 grams of dried organisms furnished a green solution when extracted with alcohol. Neither from this alcoholic solution, nor from the remaining bacteria could any yellow or red pigments be extracted by the use of various methods.

bacteria makes it seem improbable that these pigments have to be looked upon as decomposition products of the green pigment as postulated by Lubimenko ¹⁾. It also is not in agreement with the theory that they play a part in chromatic adaptation, since the green and purple sulphur bacteria are found in nature under the same conditions.

It is tempting to suggest a connection between the necessity of a hydrogen activation and the occurrence of red and yellow (carotinoid) pigments in the green plants and the purple sulphur bacteria. In this connection we want to mention a statement recently made by Schneider ²⁾:

„Ja es ist sogar zu erwarten, dass sich Anhaltspunkte finden lassen für die Bedeutung der roten Farbstoffkomponenten (Karotinoide) nicht nur im Sinne einer chromatischen Adaptation, sondern auch für die Assimilation. Gerade dieser Fragenkomplex, der die Rolle der Karotinoide bei der Assimilation betrifft, reicht über den engeren Rahmen der Stoffwechselphysiologie der Purpurbakterien hinaus und ist von einer gewissen allgemeinen Bedeutung.“

A cooperation of the yellow and red pigments in photosynthetic reactions might take place in two different ways; the red and yellow pigments might perform their function with or without the uptake of radiant energy.

The consequences of these two possibilities will be shortly discussed.

A. The fact that a large number of microorganisms produce yellow and red pigments also in the dark has led to a number of investigations which were meant to elucidate the function of these pigments. We mention only the work of Ewart ³⁾ and of Shibata ⁴⁾ from which it appears that

¹⁾ V. Lubimenko, *Rev. gén. Bot.*, 39, 547 ff, 1927, *Ibid.*, 40, 23 ff, 1928.

²⁾ Erich Schneider, *Beitr. z. Biol. d. Pflanzen*, 18, 81—115, 1930, p. 84.

³⁾ A. J. Ewart, *J. Linnean Soc. Bot.*, 33, 123—155, 1897.

⁴⁾ K. Shibata, *Jahrb. Wiss. Bot.*, 51, 179, 1912.

these pigments, under certain conditions, can serve as a source of oxygen. The chemical nature of these pigments is not accurately known; from the general reactions, however, it is concluded that they are closely related, if not belonging to the group of pigments known as carotinoids¹⁾.

Now these pigments as occurring in various microbes can have, according to the work of Ewart and of Shibata, a definite function in the metabolism of the cell. The fact that in an atmosphere of hydrogen these cells give off oxygen suggests that this oxygen is present in a loosely bound state. Whether this is comparable with the state of oxygen in oxyhaemoglobin or whether the oxygen is combined in a different way it is impossible to determine unless the chemistry of the pigments is better known.

It is equally difficult to apply these observations to the yellow and red pigments of the photosynthetic cell. In case the cooperation of these pigments during the photochemical CO_2 -reduction were to be sought in the activation of hydrogen we have to reckon with two possibilities. The first would be a reduction of the pigment by the hydrogen donor and a subsequent dehydrogenation of the reduced pigment with CO_2 as the acceptor. Although a reduction of the CO_2 by means of this donor, which would then correspond to Kluyver and Donker's „intermediary produced acceptor-hydrogen compound" is more easily conceivable than a reduction by H_2O or S, the difficulty of accounting for the hydrogen transfer from H_2O or sulphur hydrate to the normal non-reduced pigment arises. Although the behavior

¹⁾ From the investigations of Lubimenko, *Rev. Gén. Bot.*, 39, 547 ff, 1927; 40, 23 ff, 1928, it would appear that this group contains a large number of related compounds which, in the living cell, are combined with proteins. The number of red and yellow pigments that have been studied chemically is still limited but their general configuration certainly shows the possibility of the existence of a large variety.

of *Streptococcus varians* under aerobic and anaerobic conditions in the light might seem to furnish some evidence in favor of the reduceability of the red bacterioerythrin, we hardly believe this to be the normal way in which this pigment would take a part in the hydrogen transference, especially during the CO_2 -reduction.

The second possibility is that the pigment takes up water and the hydrate so formed serves as the H-donor whereby this pigment-hydrate is dehydrogenated to a compound the structure of which might be considered peroxidic. The decomposition of this „peroxide” with the liberation of molecular oxygen would explain the „dark” or „Blackman-reaction” by a catalase. The similarity between Blackman-reaction and catalase-function has been well established, especially by Warburg’s investigations¹⁾. In this connection it is worth noting that also for the oxygen-liberation by yellow and red micrococci in a hydrogen atmosphere a heavy-metal catalysis seems to be involved, since Shibata’s experiments had already shown that this process was inhibited by carbon monoxide and hydrocyanic acid.

Although for the activation of H_2O -hydrogen this scheme might seem plausible, the configuration of the red pigments in the purple bacteria would then have to differ to such an extent from that of the green plant carotinoids as to explain the activation of sulphur hydrate hydrogen *instead of* H_2O -hydrogen.

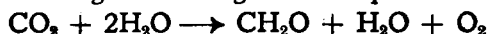
The behavior of *Streptococcus varians* would not necessarily be conflicting with this idea, since this organism is decidedly aerobic and thus in the organic medium could easily activate the hydrogen to such an extent that also the red pigment becomes available as an acceptor. Under aerobic conditions this reduced form could loose its hydrogen by transference to the atmospheric oxygen.

¹⁾ Cf. O. Warburg, Ueber die katalytischen Wirkungen der lebendigen Substanz, Berlin, 1928, pp. 287—497.

B. Let us now consider the possibility of a photochemical cooperation of the yellow and red pigments in the carbon dioxide reduction.

This leads us to a short discussion of the theoretical side of the photochemical process ¹⁾.

It has to be kept in mind that the reduction of CO₂ to the carbohydrate stage according to the equation



requires a quantity of energy equivalent to about 120.000 calories. This energy is supplied in the form of light quanta, and it has frequently been pointed out ²⁾ that at least in light of larger wavelength this transmission of energy does not seem compatible with Einstein's law of photochemical equivalents. For the quantum energy $Nh\nu$ for light of 7000 Å is only 40.000 calories, so that a minimum amount of three quanta per CO₂ molecule is necessary. If, further, it is kept in mind that

„In den meisten photochemischen Prozessen der Energiebedarf der auf den Primärvorgang folgenden chemischen Prozesse geringer ist als der Energieinhalt des absorbierten Quants“ ³⁾

it is evident that under the influence of this light four quanta would be required. The experimental determination of the energy efficiency in photosynthesis by O. Warburg and E. Negelein ⁴⁾ checks perfectly with this theoretical calculation.

The difficulty with respect to Einstein's law becomes less when we bear in mind that the carbon from CO₂

¹⁾ We are greatly indebted to Drs. H. Mestre and Ph. A. Leighton of Stanford University for their valuable aid in this field.

²⁾ Cf. e. g. O. Warburg, *Katalytische Wirkungen etc.*; Josef Holluta, *Samml. chem. u. chem. techn. Vortr.*, 28, 409—476, 1926; H. A. Spoehr, *Photosynthesis*, New York, 1926; F. Weigert, *Z. physik. Ch.* 106, 313—323, 1923.

³⁾ F. Weigert, *Z. physik. Ch.*, 106, 321, 1923.

⁴⁾ O. Warburg u. E. Negelein, *Ibid.*, 106, 191—219, 1923.

to CH_2O is reduced four valences so that one quantum per valence is required.

Now in connection with the short average life-time of a molecule activated by the uptake of an energy quantum it is clear that there is a tendency among physicists and photochemists to avoid reaction-schemes which require the uptake of more than one quantum per activated molecule. It is true that the large „phyllochlorin” complex ¹⁾ cannot quite be compared with the small, mostly inorganic molecules for which this difficulty undoubtedly exists. Yet, many attempts have been made to explain photosynthesis on the basis of a series of reactions each of which requires only one quantum.

These schemes all suffer from the concept that photosynthesis consists chiefly of a rearrangement in the carbonic acid-chlorophyll complex, leading to the chlorophyll-peroxyde-formaldehyde ²⁾.

It has been shown that this concept of photosynthesis is incompatible with the results of the study of the photosynthetic process in the green and purple sulphur bacteria, where we have to consider the photochemical reduction as taking place with hydrogen from a „foreign” donor.

Is it possible, then, to conceive of this reduction in such a manner that the necessary amount of four quanta is distributed over four reacting, radiant energy absorbing molecules?

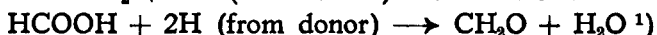
Regardless of the lack of definite proof for the existence of intermediate products in photosynthesis ³⁾ theoretical

¹⁾ H. Mestre, Contrib. to Marine Biol., Stanf. Univ. Press, 1930, p. 170—187.

²⁾ Cf. especially Josef Holluta, loc. cit.

³⁾ Although G. Klein and O. Werner, Biochem. Z. 168, 361, 1926 gave experimental evidence for the production of formaldehyde during photosynthesis, this first definite evidence does not necessarily imply that it is a *normal* intermediate product, since it was obtained by the use of Neuberg's „Abfangverfahren”, which may lead to an abnormal course of the reactions.

considerations lead to the conviction that this reduction by means of a hydrogen transference does take place in steps. Also on the basis of theoretical considerations, only one intermediate step between the CO_2 and carbohydrate (CH_2O) is conceivable, so that the reduction may be represented by:



From calculations of the free energy changes, based upon Lewis and Randall's and Parks' ²⁾ figures, it is found that the first step requires about 63000 cal., the second about 55000 cal., so that in this case each of the reactions would require 2 quanta of light of longer wavelength.

As has already been pointed out it is quite well possible that the energy-transference of two quanta in each step can be accomplished by the phyllochlorin only. But there remains the possibility that only one quantum is thus used for the activation of the carbon dioxide (acceptor) by the phyllochlorin, whereas the second quantum serves for the activation of the H_2O (donor) by the yellow pigment.

If, further, we consider that the reaction



requires an energy supply of only about 13000 cal. it is evident that the activation of the hydrogen of the H_2O molecule must require a considerable amount of energy. Then it is not so improbable that this energy may also be supplied in the form of light quanta. Whether this is done by means of the phyllochlorin or by the yellow and red pigments is not yet to be decided. As pointed out above, however, the activation of the hydrogen of the donor by some

¹⁾ With these equations we do not mean to express that formic acid and formaldehyde are produced as such, but only that the subsequent stages of the reduction lead to products of the same empirical composition.

²⁾ G. N. Lewis and M. Randall, *Thermodynamics*, New York, 1923; L. G. M. Baas Becking and G. S. Parks, *Physiol. Rev.*, 7, 90, 1927.

other agency than the green pigment has to be considered the more probable.

In case the yellow pigments are considered as active in a photochemical activation of the donor-hydrogen the difficulty of explaining the apparently normal rate of photosynthesis in light of wavelengths that are hardly, if at all, absorbed by these pigments arises. That pigments other than phyllochlorin can be photochemically active in the performance of the photosynthetic function of „green” plants was made probable by Beijerinck's observation that the highest degree of photosynthetic activity of *Porphyra vulgaris* was found in light the wavelength of which did not coincide with the absorption maximum of the phyllochlorin ¹⁾ Beijerinck concluded:

„It is obvious that the maximum of carbonic-acid decomposition is in this case determined by the co-operation of the colored rays which both pigments by preference absorb.”

This conclusion was substantially supported by the extensive and skilful investigations of Richard Harder, who concludes: ²⁾

„Die Phykochromoproteide sind also an die Assimilation beteiligt.”

It is true that in these cases the additional pigments do absorb the light in wavelengths at which photosynthesis is most active. Also it is true that here we deal with chromoproteids which can not yet be considered as having a similar function as the carotinoid pigments, although there are indications that the latter, too, are present in the living plant cell combined with proteins ³⁾. Therefore we may not consider these experiments as supporting the viewpoint that the carotinoid pigments play a photochemical part in

¹⁾ M. W. Beijerinck, Proc. Kon. Akad. v. Wet., Amst., 4, 45—49, 1901.

²⁾ R. Harder, Z. f. Botanik, 15, 305—351, 1923.

³⁾ Cf. esp. V. Lubimenko, loc. cit.

the carbon dioxide reduction of the green plants and the purple sulphur bacteria. The possibility exists that in the case of the bluegreen, brown and red algae the chromoproteids have a function similar to that of sensitizers in other photochemical reactions.

However this may be; we cannot formulate any more definite ideas before the chemical investigation of the red and yellow pigments has elucidated their chemical nature. It is to be hoped that the brilliant work on carotin and related compounds, recently carried out especially by Karrer and coworkers, Kuhn, Zechmeister and Smith ¹⁾ will soon be followed by similar studies on the red pigments of the purple bacteria.

Although we are fully aware of the highly hypothetical nature of the ideas developed in the last section, we wish, in concluding, to draw the attention to the following well established facts, which support our view regarding the activation of the donor-hydrogen in the different forms of the process of photosynthesis:

1. The absence of yellow and red pigments in those photosynthetic organisms which can only utilize hydrogen donors containing hydrogen in an already active state;
2. The presence of yellow and red pigments in those that must activate the donor-hydrogen;
3. The presence of catalase only in the latter organisms ²⁾.

The junior author is much indebted to the "Nederlandsch-Amerikaansche Fundatie" for granting him a fellowship which enabled him to work on these problems at the Hopkins Marine Station.

Hopkins Marine Station of Stanford University, Pacific Grove, California.

¹⁾ Cf. James H. C. Smith, *Contrib. to Marine Biol.*, Stanford Univ. Press, 1930, p. 145—160.

²⁾ The anaerobic purple sulphur bacteria show a very strong catalase reaction; the green bacteria, however, are catalase-negative.