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On the Metabolism of the Thiorhodaceae.

By

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With 13 figures in the text.

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1. Introduction.

After having established, in 1929, the photosynthetic activity of *green bacteria* and *Thiorhodaceae* with the simultaneous oxidation of H_2S to S and H_2SO_4, I remarked that perhaps the difference between these organisms and the *Athiorhodaceae* might be that the latter use organic compounds instead of H_2S as the normal reducing substances in the assimilation process (1). Later experiments then showed that also the *Thiorhodaceae* in the light are capable of anaerobic development in the presence of organic substances, even when oxidizable sulfur compounds are completely lacking (2, 3), and the quantitative investigation by *Muller* (4) of the reactions occurring under such conditions seemed to establish definitely that the function of hydrogen donor for the photochemical CO_2-reduction, normally carried out by H_2S or other oxidizable sulfur compounds, can be fulfilled by simple organic substances.

Meanwhile *Gaffron* had carried on his studies on photochemical CO_2-reduction by *Athiorhodaceae* which led to the conclusion that these organisms normally photosynthesize in the presence of organic substances only (5, 6).

Thus the fundamental similarity between the two groups of *purple bacteria*, at least as far as their ability to assimilate CO_2 by a photochemical process is concerned, appeared proven beyond a doubt. One was led to formulate the characteristic metabolic function of both groups as a photochemical CO_2 reduction without the production of oxygen, but requiring the presence of various oxidizable substances as „H-donors". The main difference between *Thio-* and *Athiorhodaceae* seemed to be that the former normally use inorganic sulfur compounds as reducing substances whereas for the latter organic substances are required.

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In this connection attention should be drawn to the view expressed by *Winogradsky* (7), that the ability of *Thiorhodaceae* to develop in the absence of H_2S provided organic substances are present, must be considered as an "abnormal" mode of life for these organisms. *Muller*'s attack of this thesis (4) is not quite convincing, and *Winogradsky* is probably quite correct in believing that, under natural conditions, one does not find *Thiorhodaceae* developing except in the presence of H_2S. During the past years I have carried out innumerable enrichment cultures for *purple bacteria*, quite similar to those used for obtaining cultures of *Thiorhodaceae*, described in 1931, but substituting a large variety of simple organic substances for the Na_2S used in the media of the earlier experiments. Although *Thiorhodaceae* were frequently encountered as the main colored organisms in these cultures, it was also ascertained that the bacteria, especially in the early stages of development of these cultures, were filled with sulfur globules, and that the media contained H_2S as a result of the simultaneous development of *sulfate reducing bacteria*. The one striking example of *Thiorhodaceae* developing immediately and apparently not accompanied by *Athiorhodaceae* or *sulfate reducing bacteria*, in organic media (2, p. 27) is rather misleading because a greatly diluted water sample which contained 0.3 % H_2S and which may, therefore, have contained only *Thiorhodaceae*, was used as an inoculum for these cultures.

The fundamental similarity between *Thio-* and *Athiorhodaceae* was then, however, doubted by *Gaffron* (8), who claimed that the *Thiorhodaceae* cannot photochemically reduce CO_2 with organic substances, but only with oxidizable sulfur compounds. According to this investigator the development of *Thiorhodaceae* in media which contain organic compounds is due to the ability of these organisms to produce H_2S themselves from sulfate or sulfur and organic matter. Thus, under such conditions, one would be dealing with the following two distinctly different processes: a) production by the *Thiorhodaceae* of H_2S from sulfur-containing compounds and organic substances, a reaction which can take place in the dark, and b) a light-reaction in which the previously formed H_2S is oxidized with the simultaneous reduction of CO_2.

It is obvious that *Muller*'s results are not necessarily conflicting with this idea. For *Muller*, analyzing cultures after development had taken place, did not determine whether the chemical changes that had taken place resulted from a simple photochemical process or from a combination of the two reactions postulated by *Gaffron*.

Hence *Gaffron*'s explanation could not *a priori* be rejected on the basis of available information. It is true that the ability of *sulfur bacteria* to cause sulfate reduction has, already long ago, formed a most interesting controversial subject, finally solved by *Winogradsky* in a negative sense; one might also take recourse to analogies, such as the inability of *nitrifying organisms* to cause the reduction of nitrate or nitrite. On the other hand, the existing similarity between photosynthesis of green plants and of *purple bacteria* expressed in the equations

$$CO_2 + 2\,H_2O \rightarrow (CH_2O) + H_2O + 2\,O$$

and

$$CO_2 + 2\,H_2S \rightarrow (CH_2O) + H_2O + 2\,S,$$

might be used with almost equal justification in support of *Gaffron*'s idea by admitting that, in the dark, the *Thiorhodaceae* make this reaction proceed in the opposite direction, just as is found to be the case in the metabolism of the green plants.

It should be made clear that *Gaffron*'s ideas were based upon the outcome of some experiments which revealed a striking difference between the behavior of *Thio-* and *Athiorhodaceae* under similar conditions.

If one illuminates a culture of *Athiorhodaceae* in the presence of a fatty acid salt one observes an immediate and rapid uptake of CO_2, a result which is in agreement with the assumption of a photochemical CO_2-reduction for which the presence of organic reducing substances (i. e. the fatty acid) is a prerequisite. If one carries out this experiment with *Thiorhodaceae*, however, no such uptake is observed. But by adding also sulfate to a suspension of the latter organisms, and especially after leaving the culture in the dark for some time, one can observe a rapid CO_2-uptake immediately upon illumination.

Soon afterwards, *Roelofsen* published some experiments (9, 10) in which the results obtained by *Gaffron* had not been corroborated. These induced *Roelofsen* to ascribe *Gaffron*'s observations to the use of *Thiorhodaceae* cultures contaminated with *sulfate reducing bacteria*. As further support in favor of the opinion that organic substances can be used directly, according to the ideas of *van Niel* and *Muller*, and that the *Thiorhodaceae* are unable to reduce inorganic sulfur compounds to H_2S in the presence of organic materials, *Roelofsen* mentions some culture experiments which show that, in the dark, the *Thiorhodaceae* are unable to develop anaerobically with organic matter, even in the presence of sulfate. This, however, can hardly be considered a strong argument, because the same author did not succeed in culturing the organisms in the dark under any circumstances, although he believes to have shown that they carry out a "normal" fermentation process with the production of acid and CO_2.

It is, therefore, not surprising that *Gaffron* has returned to this subject (11). The results obtained in 1934 proved to be reproduceable, and *Roelofsen*'s explanation was rejected on the basis of quantitative considerations. After greatly extending the experimental material *Gaffron* concludes that his formerly expressed theory of the metabolism of the *Thiorhodaceae* is correct; organic substances cannot be used directly in a photochemical process, but must be converted primarily by a dark reaction to H_2S. The same is claimed for the function of molecular hydrogen, which *Roelofsen* (9) first had shown to be a possible hydrogen donor for carbon dioxide reduction by the *Thiorhodaceae*, an observation later extended by *Gaffron* to the *Athiorhodaceae* (6). A strong argument in favor of the theory put forward by *Gaffron* is undoubtedly the fact that he demonstrated the production of H_2S by *Thiorhodaceae* when these are incubated in the dark, particularly in the presence of molecular hydrogen. Thus the photosynthetic activities of the two groups of *purple bacteria* seemed to be quite dissimilar; the *Thiorhodaceae* would be just as specific as the *Athiorhodaceae*, and be dependent upon H_2S or some other inorganic oxidizable sulfur compound for their assimilation process, whereas these substances are unsuitable for the *Athiorhodaceae* whose specificity requires organic compounds or molecular hydrogen. *Gaffron* concludes: "daß bei aller äußerer Ähnlichkeit in der Lebensweise bisher kaum eine wichtige Reaktion aufgefunden worden ist, die *Thiocystis* und *Rhodovibrio* gemeinsam wäre" (11, p. 3). A careful inspection of the experimental results led, however, to the conviction that further researches would be necessary before the data collected by *Gaffron* could be definitely taken to support his conclusions. This is chiefly due to the fact that the interpretation of the results obtained by *Gaffron* with the manometric technique is extremely difficult. Thus it is e. g. striking that

in those experiments in which thiosulfate is used alone or in combination with other substances the quantity of CO_2 assimilated is always quite considerably below the amount which had been found to be assimilated when the method of chemically analyzing full-grown cultures was used (2). Now, the last mentioned method yields results which are in agreement with the theoretically required values calculated on the basis of the formulation of photosynthesis as photochemical CO_2-reduction with specific H-donors. Hence it seemed likely that these discrepancies are in some way due to the differences in the methods used. Elsewhere, I have pointed out (12) that rather serious errors have previously been made in the interpretation of results obtained with the manometric technique in studies on bacterial photosynthesis. It lies at hand to suppose that the low values for CO_2 absorption determined by *Gaffron* are due to the fact that these values were obtained at a stage where the thiosulphate had not yet been completely oxidized, although the "end-point" seemed to have been reached because manometrically the uptake of CO_2 could no longer be measured.

One serious objection to *Gaffron*'s theory is, moreover, the fact that in growth experiments I had never detected any difference between the rate of development of cultures in an organic medium with or without added sulfur compounds. Certainly, those media to which no sulfur-compounds had been added may not be considered entirely sulfur-free, because the chemicals used, although of the best grade obtainable, were not specially purified or tested for the absence of sulfates. Yet, the same would be true of the suspensions used by *Gaffron* for demonstrating the effect of sulfate addition on the metabolism of the *Thiorhodaceae*. Thus *Gaffron*'s observations, of fundamental importance for his concept, would tend to indicate that the postulated process of sulfate reduction could proceed at a measurable rate only then when appreciable amounts of sulfate were added. In that case one should have to expect that, up to a certain point, the sulfate concentration determines the rate of the photosynthetic activity, i. e. the rate of development, because photosynthesis would depend upon the presence of H_2S, furnished by the dark reaction. That this apparently was not the case, even after frequently repeated transfers, indicated that possibly *Gaffron*'s interpretation of the phenomena observed by him might not be correct. Therefore an attempt was made to gather sufficient experimental material to allow of an unambiguous interpretation of the results and of a clear concept of the metabolism of the *Thiorhodaceae* in the light. In the first place a quantitative study was made of the extent and importance of the H_2S formation by *Thiorhodaceae* in the dark (section 2).

This was followed by an investigation of the assimilation of carbon dioxide in the presence of thiosulfate, which showed clearly that the manometric technique can be used for the study of this process only if the various reactions involved are sufficiently considered (section 3).

With the experiences thus gathered the problem of carbon dioxide assimilation in the presence of organic substances was attacked. Firstly,

the influence of inorganic sulfur compounds on this process was investigated. The results obtained led to an extension of these experiments, covering the effect of sulfur-free salts (sections 4 and 5).

In this way a more complete understanding of various factors influencing the photosynthetic process of *Thiorhodaceae* was gained. This permitted of studying the assimilation in the presence of organic substances under conditions which might be deemed the most favorable for this purpose (section 6). Also the problem of photosynthesis in the presence of molecular hydrogen could be studied in a similar manner (section 7).

The results obtained have led to the conclusion that the photosynthetic metabolism of the *Thiorhodaceae* and of the *Athiorhodaceae* reveals a striking resemblance. The observed differences in the outcome of experiments with representatives of the two groups appear to be due to a marked difference in acid production, a process independent of the photosynthetic reactions, and especially pronounced in the case of *Thiorhodaceae*.

During the preparation of the manuscript a publication by *Czurda* appeared (16) in which it is claimed that the production of oxygen by illuminated *purple bacteria* cultures has been demonstrated. In view of the far-reaching importance of this statement it will be discussed, together with some new experimental material, in section 8.

The photosynthetic processes of the *purple bacteria* lead to a conversion of CO_2 into organic materials. So far the only endproducts of this conversion have been found in the form of bacterial cells. In view of the fact that the chemical composition of these bacteria had never been determined, although inferences in this respect had been drawn from the quantitative relationships found to exist between the amounts of carbon dioxide reduced simultaneously with the oxidation of known amounts of substrate, some data were collected bearing on this question. They are reported in section 9.

2. On the significance of H_2S formation by Thiorhodaceae.

First of all the problem of H_2S formation by *Thiorhodaceae* in the dark had to be studied, especially in connection with the statement made by *Gaffron* that this process takes place with great velocity and assumes unexpectedly large dimensions. Because *a priori Roelofsen*'s experiments made it seem probable that such a process would not be accompanied by growth of the organisms [cf. also my own experiments on the possibilities of culturing *Thiorhodaceae* in the dark (2)] and because *Gaffron*'s results were obtained with dense suspensions of the organisms, experiments on a large scale were carried out with suspensions obtained by centrifuging large volumes of liquid cultures grown under different

conditions. Furthermore, I considered it advisable to conduct these experiments in such a manner that the H_2S could be isolated or determined as a chemically well-defined substance rather than to titrate the suspensions afterwards with iodine, because it is certainly possible that such suspensions may contain substances other than H_2S which also reduce iodine.

Therefore, the suspensions were acidified, and the H_2S driven off by a current of O_2-free nitrogen, which was subsequently led through a weakly acid solution of $CdSO_4$, or, better, according to the method described by *ter Meulen* (13), through a solution of $ZnSO_4$ in Na-acetate acidified with a drop of acetic acid. Since only H_2S will be retained in these absorbtion vessels, their contents could immediately be titrated with iodine afterwards. The accuracy of this method is demonstrated by the following figures obtained with Na_2S-solutions:

Direct titration of 1 ccm dilute Na_2S solution: 0.440 ccm iodine 0.0754 N.
Titration of ZnS suspension obtained as above
with 1 ccm of the same Na_2S solution: 0.434 ccm iodine.

Exp. 1. 1000 ccm of a full-grown culture of *Chromatium spec. (strain D Roelofsen's)* in standard inorganic medium[1] + 0.2 % Na-malate + 0.5 % $NaHCO_3$, centrifuged, and residue made up to 20 ccm in H_2O + 0.1 % $NaHCO_3$, previously freed from oxygen by passing a rapid current of O_2-free N_2 + 5 % CO_2 through the liquid. On account of the culture method (absence of sulfur compounds in the medium) the bacteria did not contain stored sulfur.

2 ccm of the suspension were tested by the manometric method for assimilation of CO_2 with $Na_2S_2O_3$; in two successive 10 minute periods the amount of CO_2 absorbed after addition of the $Na_2S_2O_3$ were found to be 60 and 70 cmm; the bacteria were, therefore, quite active.

The suspension was divided over 3 small flasks, each connected with a small separatory funnel, a gas-inlet tube and an absorption vessel containing a slightly acid $CdSO_4$ solution.

Flask No. 1: 800 mg bacteria (wet weight, sharply centrifuged) in 20 ccm standard inorganic medium + 0.2 % Na-malate and 0.5 % $NaHCO_3$.

No. 2: As No. 1 + 0.2 % K_2SO_4.
No. 3: As No. 1 + 0.2 % $Na_2S_2O_3$.

After 18 hours at 35° C in the dark a current of O_2-free N_2 was passed through these cultures to which, through the separatory funnel, 5 ccm of N. H_2SO_4 were added. Titration after 3 hours of the precipitated CdS, with iodine yielded the following amounts of H_2S:

No. 1 0.11 mg, No. 2 0.12 mg, No. 3 0.10 mg.

The filtrate of the absorption liquid of No. 3 had a strong odor of SO_2, as was also presented by the culture itself, due to the decomposition of the $Na_2S_2O_3$ by the acid.

Exp. 2. 1000 ccm of a heavy culture of strain D in standard inorganic medium + 0.3 % Na-malate and 0.5 % $NaHCO_3$ to which 18 hours before 0.1 % Na_2S had been added, centrifuged. Bacteria photosynthetically quite

[1] Standard inorganic medium: H_2O, 0.1 % NH_4Cl, 0.1 % KH_2PO_4, 0.02 % $MgCl_2$.

active, containing appreciable quantities of sulfur inside the cells, suspended as before.

No. 1 600 mg bacteria in 20 ccm standard inorganic medium.
„ 2 As No. 1, but heated for 10 min. at 70° C.
„ 3 „ „ 1, + 0.2 % Na-malate.
„ 4 „ „ 1, + 0.2 % Na-malate + 0.2 % K_2SO_4.
„ 5 „ „ 1, + 0.2 % Na-malate + 0.2 % $Na_2S_2O_3$.

After 24 hours at 35° C in the dark H_2S determinations as in Exp. 1 yielded: No. 1 0.570 mg H_2S, No. 2 0.107 mg H_2S, No. 3 0.667 mg H_2S, No. 4 0.657 mg H_2S, No. 5 0.710 mg H_2S.

As before, the culture with $Na_2S_2O_3$ must still have contained considerable amounts of this substance as evidenced by the strong odor of SO_2 in culture and filtrate of the absorption liquid.

Exp. 3. 1000 ccm of a heavy culture of *Strain D* in standard inorganic medium + 0.3 % Na-malate and 0.5 % $NaHCO_3$, to which 8 hours previously 0.15 % Na_2S had been added, centrifuged. Bacteria photosynthetically quite active; cells completely filled with sulfur globules, suspended as before.

No. 1 800 mg bacteria in standard inorganic medium + 0.48 % $NaHCO_3$, used immediately for H_2S determination.
„ 2 As No. 1, but incubated 24 hours.
„ 3 „ „ 2, + 0.25 % Na-malate.
„ 4 „ „ 2, with a constant current of O_2-free H_2, washed through a solution of 8 parts of m/10 Na_2CO_3 and 2 parts of m/10 $NaHCO_3$, passing through.

After 24 hours at 35° C in the dark: No. 1 0.00 mg H_2S, No. 2 0.88 mg H_2S, No. 3 1.22 mg H_2S, No. 4 3.00 mg H_2S.

Exp. 4. As Exp. 3.

No. 1 1000 mg bacteria in standard inorganic medium + 0.4 % $NaHCO_3$ in current of H_2.
No. 2 As No. 1, but with 3 ccm of toluene added.
After 24 hours at 37° C in the dark:
No. 1 4.66 mg H_2S,
No. 2 1.31 mg H_2S.

The following conclusions can be drawn from these experiments:

1. Photosynthetically quite active bacteria do not produce demonstrable quantities of H_2S from organic matter and sulfate or thiosulfate in the dark.

2. Cells containing appreciable to large amounts of stored sulfur produce small amounts of H_2S in the dark, and this increases with the original sulfur content.

3. Certainly not all of the H_2S thus formed is a result of "normal" metabolism, as the results of the experiments with heated and toluene-treated bacteria show.

4. The largest amounts of H_2S are produced in the presence of molecular H_2.

What does this H_2S formation signify?

Since it is dependent upon the presence of free sulfur, and since H_2S is not produced from sulfate or thiosulfate, the process responsible

cannot be termed sulfate reduction. The well-known cases of formation of H_2S from added sulfur under the influence of anaerobically metabolizing cells [phytochemical reduction, *Neuberg* and coworkers (14)] seem to supply an analogy. However, it is also known that such phytochemical reductions must be considered as side paths of a normal metabolism and that, quantitatively, they are rather unimportant.

Now a simple comparison shows conclusively, that the quantities of H_2S actually found are but a fraction of those which might have been expected if the conversions had been quantitative. From chemical determinations it appears (see section 9) that such cells as used in experiments 3 and 4 contain as much as 35 % of their dry weight in the form of free sulfur. Consequently 1000 mg of wet, sharply centrifuged bacteria (dry weight ± 20 %) contain 70 mg of sulfur, from which about 75 mg of H_2S might have been formed. The largest amount found in these experiments (4.66 mg) thus appears to fit in with the idea that

the H_2S production by Thiorhodaceae containing considerable amounts of free sulfur would be a phytochemical reduction, and thus cannot be regarded as a normal physiological process.

The experiments reportedby *Gaffron* in 1935 in support of his theory indicated, however, the production of large amounts of H_2S. Because this substance could readily be detected by its odor and with the aid of lead-acetate paper *Gaffron* assumed that the estimated reduction of iodine by his suspensions would be due entirely to H_2S. The above mentioned results made it seem probable, however, that the suspension, after having been kept in the dark, would contain reducing substances other than H_2S. This actually appeared to be the case. If such suspensions are titrated with iodine values are obtained which correspond quite well with those found by *Gaffron* [cf. e. g. (11), table VI, VII, VIII, IX, X, XVI, XVII, XVIII, XIX, XX]. But practically the same values are obtained if these suspensions are titrated after first driving off the H_2S with a current of nitrogen, following acidification. Since suspensions titrated immediately after a period of illumination possess only a feeble reducing power it follows that the metabolism of the *Thiorhodaceae* in the dark gives rise to the production of reducing substances, but for the greater part these are not H_2S.

With the demonstration that in the dark *Thiorhodaceae* do not produce H_2S from sulfates, and that the H_2S production from stored sulfur is quantitatively very insignificant indeed one of the main supports for *Gaffron*'s theory of the metabolism of *Thiorhodaceae* is eliminated.

3. On carbon dioxide assimilation by Thiorhodaceae in the presence of thiosulfate and measuring this process manometrically.

I have remarked before [see also (12)] that the interpretation of *purple bacteria* photosynthesis on the basis of data obtained by mano-

metric measurements is not always simple, and that e. g. the experiments reported by *Gaffron* do not make it clear why the amount of CO_2 assimilated in the presence of thiosulfate is so much below that found as a result of chemical analysis of full grown cultures. It appeared entirely possible that the values reported by *Gaffron* do not at all represent the final stages, but that in this case the manometric method does not directly permit the exact determination of an end-point. If thiosulfate is utilized by *Thiorhodaceae* in a way similar to that demonstrated by *Starkey* (16) for the oxidation of thiosulfate by *Thiobacillus spec.*, i. e. *via* the formation of polythionates, it is obvious that then the respective steps in the photosynthetic process, during which ultimately approximately 2 mols of CO_2 are reduced while 1 mol of $Na_2S_2O_3$ is converted into sulfate (2), can be represented by a series of reactions which at first lead to the accumulation of alkaline products and the formation of sulfur-rich compounds which must be oxidized to the final stage, so that during the later phases free sulfuric acid must be formed. This implies that during the first stages much more CO_2 will disappear from the gaseous phase than that which is actually assimilated by the bacteria because the accumulation of alkali in the medium causes an additional CO_2 absorption. The resulting increase in bicarbonate of the suspension has been observed by *Gaffron* in 1934 (11), and has been correctly interpreted as an indication that the oxidation of thiosulfate proceeds *via* polythionate. But it also implies that during the later stages the formation of H_2SO_4 will cause the decomposition of bicarbonate present in the medium, a purely chemical reaction which may altogether obscure the assimilation of CO_2 by the bacteria. Hence, by manometrically studying this process one would obtain a curve for "CO_2 uptake" by bacteria plus medium according to A in fig. 1. This curve might lead one to believe that at time t_1 the photosynthetic reaction has been completed. If one would follow quantitatively the bicarbonate content of the medium (curve B) one might, however, find that the observed uptake of CO_2 is largely due to the increase in bicarbonate. Correcting for the amount of CO_2 taken up or given off by the medium as a result of secondary, and purely chemical reactions by means of curve B one would, therefore, obtain the "true" photosynthesis curve which in this hypothetical case is a straight line (C) showing the occurrence of photosynthesis at a normal rate long after the pressure changes have become positive.

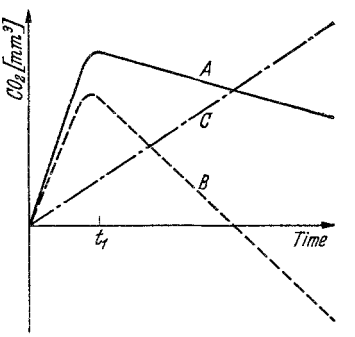

Fig. 1. Diagram of relations between amounts of CO_2 in gas phase (A), liquid phase (B), and assimilated (C) by an organism which produces acid during its photosynthetic activity.

A number of experiments pertaining to this problem have been carried out, and they have borne out the correctness of these theoretical considerations. The curves representing the pressure changes which were observed when suspensions of *Chromatium spec.* (*strain D*) were illuminated in an atmosphere of oxygen-free N_2 + 5 % CO_2 and in the presence of known quantities of $Na_2S_2O_3$ are all fundamentally similar and illustrated by fig. 2.

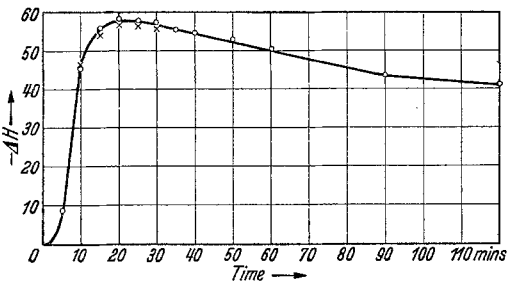

Fig. 2. Pressure changes due to the activity of 2 ccm of an illuminated suspension of *Chrom. spec.* (*strain D*) in the presence of 0.05 ccm m/10 $Na_2S_2O_3$. One experiment terminated after 30 minutes.

Determinations of total CO_2 at different moments showed that the uptake of CO_2, i. e. photosynthesis, proceeds long after the pressure changes would indicate the cessation of CO_2 assimilation; as demonstrated by Table I.

Table I. Assimilation of *Chromatium spec. (strain D)* in the presence of $Na_2S_2O_3$.

2 ccm suspension, addition of 0.05 ccm m/10 $Na_2S_2O_3$ at 12.00. Quantity of carbon dioxide in cmm.

Time	CO_2 in liquid phase	Changes in CO_2 gas phase	Assimilation of CO_2
12.00	102.2 (Initial)	—	—
12.30	162.7	— 124.1	63.6
2.00	18.8	— 96.4	179.8

After $^1/_2$ hour, when negative pressure changes were no longer observed, 124.1 cmm of CO_2 had been absorbed, but the increase of the CO_2 content of the liquid phase amounted to 60.5 cmm. Consequently the quantity of CO_2 assimilated was 63.6 cmm per 1/200 millimol of $Na_2S_2O_3$, i. e. only 28.4 % of the amount required by the theoretical equation

$$Na_2S_2O_3 + 3\,H_2O + 2\,CO_2 \rightarrow 2\,(CH_2O) + Na_2SO_4 + H_2SO_4.$$

It may be recalled that the results of chemical analyses of cultures of *Thiorhodaceae* grown with thiosulfate had supported this equation (2).

After 2 hours, however, the liquid phase contained 83.4 cmm of CO_2 less than at the beginning of the experiment. The quantity disappeared from the gas phase since the start was 96.4 cmm, hence the total quantity of assimilated carbon dioxide was 179.8 cmm, i. e. over 80 % of the amount required by the equation.

It thus appears that the end point of the conversion of the $Na_2S_2O_3$ cannot be determined at all from the continuous observation of the pressure changes. What seems to be an end-point is in reality an intermediate stage in the conversion from where onward the reactions taking place lead to the formation of acid products in contrast to what holds for the initial changes.

This experiment, one out of a large number of similar ones, clearly demonstrates the difficulties connected with an interpretation of data obtained with the manometric technique in studies on metabolizing *Thiorhodaceae*. While in this particular case a known substrate had been added in definite quantity, thus permitting a calculation and comparison of the results with those obtained by purely chemical methods, other experiments have shown convincingly that grave complications arise if bacteria containing stored sulfur are used for manometric studies. Again, a single instance may be discussed here in some more detail. A suspension in oxygen-free tapwater with a trace of $NaHCO_3$ of *Chromatium spec. (strain D)*, grown in a medium containing sulfide, and consisting of cells containing a moderate amount of stored sulfur, was illuminated for 40 minutes in an atmosphere of nitrogen with 5 % CO_2. The manometric readings indicated that no assimilation took place, only slight positive pressures were measured. A determination of CO_2 in the liquid phase revealed that at the end of this period 156.7 cmm of CO_2 remained in solution; the initial CO_2-content of the liquid was 251 cmm. Consequently 94.3 cmm of CO_2 had disappeared. The increase in pressure during the experiment could account for at most 17.9 cmm of CO_2, so that 76.4 cmm of this gas must have been assimilated. Considering the fact that the first phase of the previously discussed experiment appears as a negative pressure of 60 mm of *Brodie* solution, the slight positive pressure observed in the last one (only 7.5 mm in 40 minutes!) could easily be interpreted as a sign that practically no metabolism had taken place, whereas in reality the assimilation of CO_2 was of the same order of magnitude in both experiments.

With a view to complicate the interpretation of the experiments on the utilization of organic substances and molecular hydrogen as little as possible the following experiments have, therefore, been carried out with cells free from stored sulfur [cf. also *Gaffron* (11)].

4. On carbon dioxide assimilation by Thiorhodaceae in the presence of organic substances and inorganic sulfur compounds.

Failing to corroborate *Gaffron*'s experimental results concerning the effect of sulfates on the carbon dioxide assimilation in the presence of organic substances *Roelofsen*, working with pure cultures of *Thiorhodaceae*, rejected *Gaffron*'s explanation and ascribed the latter author's finding to the presence of *sulfate reducing bacteria* in his cultures. *Gaffron* has rightly remarked that this explanation is untenable; in order to produce the observed results in these experiments of short duration the number of such *sulfate reducing bacteria* would have to be so large that they could not possibly have been overlooked by microscopical examination.

It is most remarkable that *Roelofsen* (9, 10), although stating: "These experiments were repeated by me in exactly the same way", nevertheless used experimental conditions widely different from those described by *Gaffron* (8). Thus, while the latter suspended the centrifuged *Thiorhodaceae* in m/200 $NaHCO_3$, *Roelofsen*'s suspensions were made up in H_2O with 2 % NaCl and m/28 (0.3 %) $NaHCO_3$. Since, later, *Gaffron* was able to reproduce his previous results, the idea presented itself that the discrepancies between the facts observed by the two authors might have been caused by the different experimental conditions.

Because of the significance which attaches to the positive outcome of these experiments as reported by *Gaffron* it seemed of importance to establish this beyond doubt.

Cultures of *Thiorhodaceae* particularly suited for such experiments, i. e. cultures in which the individual cells do not contain stored sulfur can be obtained by using media containing sulfide or thiosulfate. In that case one has to wait till the sulfur containing substrate has been completely oxidized. Since the storage and further oxidation of sulfur by the individual cells does not take place in exactly the same manner or at exactly the same rate, some cells will be sulfur-free long before the others. By prolonging the incubation period until the last visible traces of sulfur have disappeared, the activity of the culture is often impaired. Furthermore, it is difficult to judge, on the basis of a microscopical examination, whether the cells are entirely sulfur-free. In a number of manometric experiments such microscopically adequate cultures have been found to assimilate quite appreciable quantities of carbon dioxide in the absence of any added substrate with the simultaneous production of acid, and it seems necessary, in these cases, to assume that the assimilation has been made possible on account of the presence of some sulfur in the cells.

A far more certain way to obtain cultures of sulfur-free cells is the method of culturing the organisms in media with organic substrates.

Particularly with sodium malate development is quite rapid and abundant. A prerequisite for such cultures is, of course, an inoculum from a pure culture. For most of the following experiments such malate cultures have been used. The results obtained with this material have all been checked however, with cultures grown in thiosulfate or sulfide media, and these have led, without exception, to a complete confirmation.

Muller (4) has shown that several organic substances enable the *Thiorhodaceae* to develop anaerobically in the light, and the only organic products of metabolism were found to be "bacterial cells". Yet, not all these substances are equally well suited for manometric measurements of

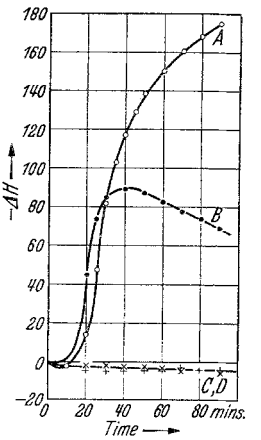

Fig. 3. Pressure changes due to the activity of 2 ccm of an illuminated suspension of *Chrom. spec. (strain D)*.
A: with the addition of 0.1 ccm m/10 $Na_2S_2O_3$ and 0.1 ccm m/10 Na-propionate.
B: with the additon of 0.1 ccm m/10 $Na_2S_2O_3$ only.
C: with the addition of 0.1 ccm m/10 Na-propionate only.
D: no additions.

a "carbon dioxide assimilation", because in the majority of cases the photosynthetic activity of the bacteria also results in the production of carbon dioxide. Only when organic compounds are used of which the percentage composition indicates that they are more reduced than the bacteria themselves the conversion of the organic substance into bacterial cell material is accompanied by the actual disappearance of carbon dioxide. Thus, the saturated fatty acids with more than 2 carbon atoms promised to be the most satisfactory substrates. Now, *Muller* has also demonstrated by culture experiments that, whereas all strains used by him are able to develop in propionate with the uptake of carbon dioxide, only a limited number of them can attack butyrate. In order to avoid negative results by using fatty acids higher than acetate in combination with bacteria which cannot attack them, sodium propionate was used at the start.

A typical experiment is represented in fig. 3, where *Chromatium spec. (strain D)* was illuminated without substrate, in the presence of $Na_2S_2O_3$, $Na_2S_2O_3$ + Na-propionate, and propionate alone.

From this figure it appears that, judging merely by pressure differences, propionate *alone* is not attacked at all, whereas an assimilation with this compound is quite obvious in the presence of thiosulfate. This is borne out by determination of total carbon dioxide. The changes found were as follows:

CO_2 assimilation by control 0,
„ „ with propionate 0,
„ „ „ thiosulfate 197.4 cmm,
„ „ „ thiosulfate + propionate 266.8 cmm.

If, in the last culture, the assimilation with thiosulfate would account for 197.4 cmm, then the assimilation with propionate would have

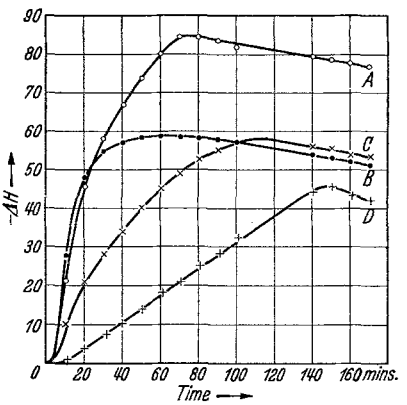

Fig. 4. As Fig. 3.
A: with the additionof 0.05 ccm m/10 $Na_2S_2O_3$ and 0.05 ccm m/10 Na-propionate.
B: with the addition of 0.05 ccm m/10 $Na_2S_2O_3$ only.
C: with the addition of 0.02 ccm m/10 $Na_2S_2O_3$ and 0.05 ccm m/10 Na-propionate.
D: with the addition of 0.01 ccm m/10 $Na_2S_2O_3$ and 0.05 ccm m/10 Na-propionate.

amounted to 69.4 cmm, i. e. approximately 0.25 molecules of CO_2 per molecule of propionate. One has to evaluate this result as a qualitative one, since obviously the experiment was not completed.

The remarkable influence of thiosulfate on the assimilation with propionate led to inquire how this influence would depend upon the concentration of the sulfur compound. Fig. 4 depicts an experiment of this kind.

The corresponding data for total CO_2 are given in the following Table II:

Table II.

	cmm CO_2 in liquid phase	Increase CO_2 in liquid phase	Changes in CO_2 in gas phase	Total CO_2 assimilated
With 0.05 ccm $Na_2S_2O_3$ alone	244.0	101.5	— 124.2	22.7
„ 0.05 „ $Na_2S_2O_3$ + 0.05 ccm Prop.	284.0	141.8	— 209.1	67.3
„ 0.02 „ $Na_2S_2O_3$ + 0.05 „ „	207.0	64.5	— 127.7	63.2
„ 0.01 „ $Na_2S_2O_3$ + 0.05 „ „	206.0	63.5	— 98.9	35.4
Control	142.5		—	—

There can be no doubt that the assimilation with propionate appears the more rapid the higher the initial thiosulfate concentration. A quantitative evaluation of the data is, however, made difficult by the fact that the total quantity of CO_2 assimilated with 0.05 ccm m/10 $Na_2S_2O_3$ is only 22.7 cmm, or 0.1 mol per 0.5 mol of $Na_2S_2O_3$. By assuming that the same amount has been assimilated per 0.05 ccm of $Na_2S_2O_3$ also in the presence of propionate — an assumption which needs not to be correct! — one finds that the decomposition of 0.05 ccm m/10 propionate has caused the uptake of 44.6, 54.1 and 30.9 cmm of CO_2, while the theoretically required amount for a conversion into carbohydrate is 45 cmm.

These experiments prove that *Thiorhodaceae* which, under the given experimental conditions, cannot assimilate CO_2 in the presence of propionate, can be induced to carry out this process if simultaneously thiosulfate is present.

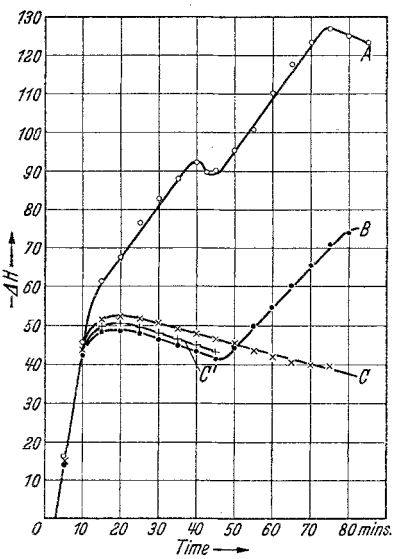

Fig. 5. As Fig. 3.
A: with 0.05 ccm m/10 $Na_2S_2O_3$ and 0.05 ccm m/10 Na-propionate; after 45 minutes again 0.05 ccm m/10 Na-propionate added.
B: with 0.05 ccm m/10 $Na_2S_2O_3$ alone; after 45 minutes 0.05 ccm m/10 Na-propionate added.
C, C': with 0.05 ccm m/10 $Na_2S_2O_3$ alone; C' discontinued after 45 minutes.

Figure 5 presents the results of a series of measurements relating to the question whether the influence of thiosulfate is a direct or an indirect one. It was hoped that by determining the effect of the addition of propionate to suspensions of *Chromatium spec. (strain D)* in the presence of thiosulfate after different time intervals an answer to this question could be given. The curves indicate that it makes little or no difference whether thiosulfate and propionate are added at the same moment, or whether the propionate is added 1 hour later. In this experiment the following data for changes in dissolved and gaseous CO_2 were collected (Table III):

By assuming that in all cases the assimilation with thiosulfate has proceeded at the same rate the quantity of CO_2 assimilated with the propionate then is found to be:

For 0.05 ccm m/10 Na-propionate . . . . . 34.7 cmm,
For 2 . 0.05 ccm m/10 Na-propionate . . . . . 70.6 cmm, i. e. about 63 %

Table III.

	CO_2 in liquid phase cmm	Total change in gaseous phase cmm	CO_2 assim. cmm
Initial	140	—	
After 1 hour with 0.05 ccm m/10 $Na_2S_2O_3$	221.6	— 114.8	33.2
After 2 hours			
a) with 0.05 ccm m/10 $Na_2S_2O_3$ only	179.6	— 86.7	47.1
b) with 0.05 ccm m/10 prop. added at start + 0.05 ccm added after 1 hour	313.2	— 291.9	118.7
c) with 0.05 ccm prop. added after 1 hour	256.7	— 198.5	78.8

of the theoretically required amount of $^1/_2$ molecule of CO_2 per molecule of propionate.

It is clear that these results do not lend support to the assumption of a direct influence, which might have been inferred from those illustrated in fig. 4. The fact that the rate of assimilation with propionate is practically independent of the moment at which it is added would lead one to suppose that the influence is mainly indirect.

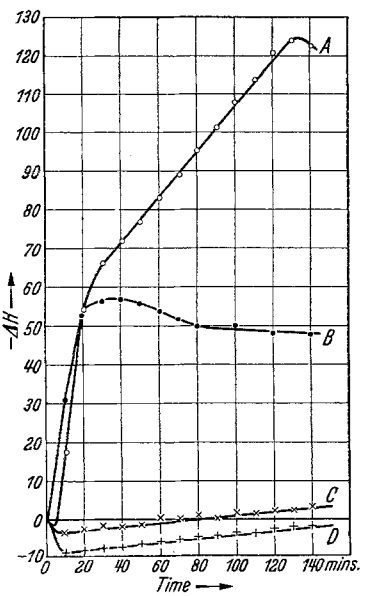

Fig. 6. As Fig. 3.
A: with 0.05 ccm m/10 $Na_2S_2O_3$ and 0.1 ccm m/10 Na-propionate.
B: with 0.05 ccm m/10 $Na_2S_2O_3$ alone.
C: with 0.1 ccm m/10 Na-propionate.
D: with 0.1 ccm m/10 Na-propionate and 0.1 ccm m/10 K_2SO_4.

A comparison of the influence of thiosulfate and of sulfate on the propionate assimilation is represented in fig. 6. The effect of the simultaneous presence of thiosulfate and propionate agrees with the previously reported observations; an influence of sulfate could not be observed.

This seems in contradiction with the results reported by *Gaffron*. It must be remarked, however, that in the before-mentioned experiments the conditions used by *Gaffron* were not always exactly duplicated. Thus, the bicarbonate concentration of the suspension liquid was sometimes less than m/200 in order to facilitate the determination of dissolved carbon dioxide. By varying the initial bicarbonate content of the liquid phase it soon appeared that this had a

tremendous influence upon the results, and it became possible to demonstrate that under quite special conditions also sulfate exerts a pronounced effect on the propionate assimilation. The following composite figure 7 shows this graphically:

Thus it becomes apparent that there are conspicuous differences in the behaviour of the bacteria towards sulfate owing to what might at first sight seem unimportant details in the experimental conditions. The phenomenon observed and described by *Gaffron* is perfectly reproduceable provided the initial bicarbonate concentration of the suspension

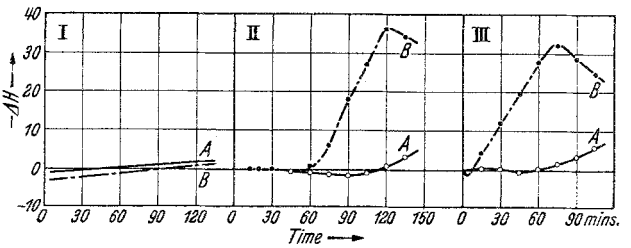

Fig. 7. Pressure changes due to the activity of 2 ccm of *Chrom. spec. (strain D)* in the presence of 0.05 ccm m/10 Na-propionate without (A) and with (B) 0.1 ccm m/10 K_2SO_4, in media with different $NaHCO_3$-content.
I. Original CO_2-content of suspension 90.7 cmm. II. Original CO_2-content of suspension 155 cmm. III. Original CO_2-content of suspension 238.6 cmm.

is adjusted in such a manner that the CO_2-content of 2.2 ccm of suspension amounts to 200—250 cmm, i. e. 1/200 Mol $NaHCO_3$. With concentrations appreciably below this, sulfate has no effect.

The experiments, discussed in this section, and demonstrating that under special conditions propionate leads to a marked carbon dioxide assimilation only in the presence of thiosulfate or sulfate tend to give considerable weight to the theory put forward by *Gaffron*, viz. that the assimilation of organic substances takes place *via* an interaction of these substances with inorganic sulfur compounds.

In repeating these experiments with the substitution of butyrate for propionate the results have been entirely negative. Neither in the presence of sulfate nor of thiosulfate could any uptake of carbon dioxide due to the butyrate be ascertained. Hence it is probable that the culture used (*Roelofsen*'s *strain D*) is characterized by its inability to utilize butyrate. Culture experiments, in which butyrate media of different composition were applied, have supported this view. Whenever development in such media was observed, due to the addition of small quantities of sulfide or thiosulfate, it could also be proved that this development could be ascribed to the conversion of these added substances only; the butyrate could always be recovered. Now, the important problem was to deter-

mine the way in which organic compounds are utilized by *Thiorhodaceae*, and not so much to study which organic substrates can be used, or to detect differences in this respect which might exist between different strains. Hence, for the present no more attention has been paid to the possible assimilation of carbon dioxide by *Thiorhodaceae* in the presence of butyrate.

5. On the influence of sulfur-free salts on carbon dioxide assimilation by Thiorhodaceae in the presence of organic substances.

The experiments discussed in section 2 have shown that *Thiorhodaceae* are unable to reduce sulfates in the dark. Yet, sulfate has a marked effect on the carbon dioxide assimilation with propionate. How can this influence be explained? In order to answer this question it was necessary to establish first of all whether the observed effect is specific for sulfur containing compounds. Because the "sulfate-effect" could be observed only in suspensions with a limited range of bicarbonate concentrations these further experiments were carried out under very similar conditions.

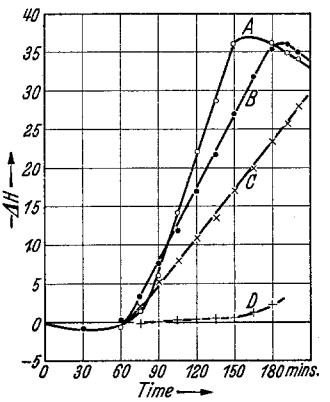

Fig. 8. Pressure changes due to the activity of 2 ccm suspension of *Chrom. spec.* (*strain D*) in the presence of 0.05 ccm m/10 Na-propionate. Original CO_2-content of suspension 155 cmm. After 60 minutes added:
A: 0.05 ccm m/16 $NaHCO_3$.
B: 0.05 ccm m/10 K_2SO_4.
C: 0.05 ccm m/10 KCl.
D: 0.05 ccm H_2O.

Fig. 8 presents the results of a series of measurements on the influence of K_2SO_4, KCl, and $NaHCO_3$ on the assimilation by *Chrom. spec.* in the presence of propionate.

With the additions of 0.05 ccm m/10 Na-propionate only an assimilation cannot be observed during the first $2^1/_2$ hours. The simultaneous addition of 0.05 ccm m/10 K_2SO_4 caused the assimilation to proceed at once. But the same effect was obtained by adding 0.05 ccm m/10 KCl, or 0.05 ccm m/16 $NaHCO_3$. The small differences observed in the rate of CO_2-uptake may be ascribed to the fact that the concentrations which were compared differed in ionic strength. Thus it appears that the influence of sulfate is far from specific and has no connection at all with its sulfur content. The most striking effect is caused by the bicarbonate, even though its concentration is appreciably less than that of the sulfate. That NaCl has the same influence was ascertained in further experiments, and is to be expected.

It must be emphatically stated that the phenomena reported here have been observed only in those cases where the determination of

carbon dioxide dissolved in the 2.1 ccm of suspension + substrate yielded figures between 200 and 250 cmm. If the original carbon dioxide content was near the upper limit yet another observation was made. For then an assimilation became apparent even without the addition of salts, starting, however, at a slow rate, and after a long period during which apparently nothing (i. e. manometrically measurable!) happened, generally about 1 hour. If the bicarbonate concentration of the suspension was such that the initial quantity of dissolved carbon dioxide amounted to 160 cmm this "induction period" was found to be very much prolonged (3 hours!), so that in a normal run, lasting only from 1 to 2 hours, it could not be detected. Quite instructive is the following example for a demonstration of this behavior (Table IV).

Table IV.

2 ccm of *Chromatium spec. (strain D)* suspended in dilute $NaHCO_3$ in each vessel.

Added at 11.00	Vessel 1 0.05 ccm m/10 Na prop. + 0.05 ccm H_2O cmm	Vessel 2 0.05 ccm m/10 Na prop. + 0.05 ccm m/10 K_2SO_4 cmm	Vessel 3 0.05 ccm m/10 Na prop. + 0.05 ccm m/10 KCl cmm	Vessel 4 0.05 ccm m/10 Na prop. + 0.05 ccm 0.5 % $NaHCO_3$ cmm
Initial CO_2-content . . .	155	155	155	222
Manometric changes from				
11.00—12.00	—	—	—	—
12.00— 1.00	—	— 40	— 27	— 52
1.00— 2.00	— 7.4	— 30.8	— 43.2	— 18
Total change	— 7,4	— 70.8	— 70.2	— 70.0
Final CO_2 content . . .	147	153	154	218

It will be seen that the addition of the bicarbonate, raising the initial carbon dioxide content to 222 cmm, was insufficient to eliminate

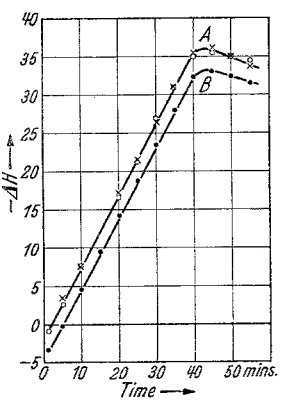

Fig. 9. Pressure changes due to the activity of 2 ccm suspension of *Chrom. spec. (strain D)*; original CO_2-content of suspension 308 cmm.
A: with 0.05 ccm m/10 Na-propionate and 0.05 ccm m/10 NaCl (×××) with 0.05 ccm m/10 Na-propionate and 0·05 ccm m/10 K_2SO_4 (ooo) with 0.05 ccm m/10 Na-propionate and 0.05 ccm m/10 KCl (. . .)
B: with 0.05 ccm m/10 Na-propionate and 0.05 ccm H_2O.

the "induction period" altogether; it merely effected, like the other salts, a decrease of the latter from 3 hours to 1 hour.

If the bicarbonate content is raised still higher (above 300 cmm of CO_2 in solution) the "induction period" of course should no longer be observable. In that case one can no longer detect any influence as a result of the addition of salts. The following figure 9 shows this.

It now becomes clear that *Roelofsen* could not possibly have observed the phenomenon which *Gaffron* had discovered, viz. the effect of sulfate on the assimilation with organic compounds. The high concentrations of bicarbonate and sodium chloride which *Roelofsen* used as a suspension

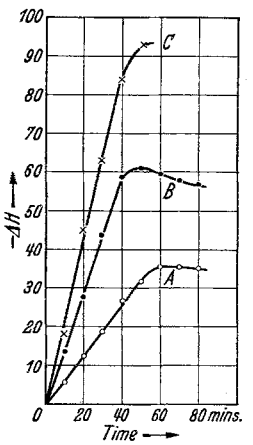

Fig. 10. 2 ccm *Chrom. spec. (strain D)* suspended in dilute $NaHCO_3$-solution. Original CO_2-content of suspension 378 cmm.
A: with 0.05 ccm m/10 Na-propionate.
B: with 0.05 ccm m/10 $Na_2S_2O_3$.
C: with 0.05 ccm m/10 Na-propionate and 0.05 ccm m/10 $Na_2S_2O_3$.

liquid are far beyond the very limited range over which this effect is demonstrable.

Inasmuch as the influence of sulfate is exerted also by sulfur-free inorganic salts it is obvious that *Gaffron*'s explanation cannot be correct. This is also in line with the experimental results reported in section 2. Furthermore, it is clear that the effect of thiosulfate (section 4) is not necessarily due to an interaction of the sulfur compound and the organic substance. In section 3 it has been shown that — in accordance with *Gaffron*'s previous observations — the assimilation in the presence of thiosulfate results in a rapid accumulation of bicarbonate in the medium. Consequently the observed effect of thiosulfate on the assimilation with propionate might have been caused solely by the increase in bicarbonate. That this explanation actually holds good appears most clearly from experiments on carbon dioxide assimilation in the presence of both thiosulfate and propionate in media which contain sufficient bicarbonate to eliminate the "induction period". In such cases one observes that the "propionate assimilation" does not succeed the first stage of the "thio-

sulfate assimilation", but the two processes take place simultaneously, and manometer-readings for the experiment with thiosulfate and propionate during any time interval can be calculated quite accurately from the sum of those observed in the two separate controls (see fig. 10), in contrast to what holds for the experiments reported in the previous section.

The collectivity of data thus far presented shows conclusively that a direct carbon dioxide assimilation in the presence of organic substances by *Thiorhodaceae* can take place, provided care is taken to supply the necessary environmental conditions, i. e. an initial bicarbonate concentration of approximately 0.075 mol.

But why are these precautions necessary in experiments with *Thiorhodaceae*, while they can be disregarded in similar ones with *Athiorhodaceae*? The fact that in media with less than a certain minimum concentration of bicarbonate (0.035 mol) an assimilation cannot be induced even by the addition of sulfates or chlorides, indicates that perhaps the reaction of the medium may be of great importance. In general the *Thiorhodaceae* seem to be decidedly more acid-sensitive than the *Athiorhodaceae*, and for one of the representatives of this latter group *Gaffron* (5) has already demonstrated its sensitivity towards free fatty acid. This has been confirmed for other strains; at p_H 5.8 the assimilation with butyrate is already greatly inhibited at concentrations of m/250 and above of the fatty acid. It is conceivable that the sensitivity of the *Thiorhodaceae* is markedly greater yet, and that an assimilation in media with m/200 fatty acid becomes possible only when the p_H rises well above 7, as is certainly the case if *Thiorhodaceae* are grown in the presence of sulfide. [For the influence of p_H and sulfide concentration on *Thiorhodaceae* cf. (2), p. 34—44. *Roelofsen*, too, has stressed the desirability of maintaining an alkaline reaction in studies on the metabolism of *Thiorhodaceae*.] It must be remembered that in all the experiments reported here the liquid phase was in equilibrium with an atmosphere of nitrogen containing 5 % carbon dioxide, and that the p_H of such a suspension rises above 7 only if the bicarbonate concentration is greater than 0.05 mol.

It has also been shown (2) that the tolerance of *Thiorhodaceae* for sulfide is appreciably increased by the addition of NaCl. Thus the "sulfate effect", also observed with other inorganic salts, might be due to the fact that the inhibitory influence of the fatty acid is decreased by salt additions.

A closer study of all the data at hand reveals, moreover, that this supposed difference in acid-sensitivity between *Thio-* and *Athiorhodaceae* may be greatly enhanced by another circumstance. If representatives of

the latter group of *purple bacteria* are illuminated in the presence of salts of fatty acids one observes that the bicarbonate (CO_2) content of the liquid phase increases by very nearly one molecule per molecule of fatty acid assimilated [*Gaffron* (5, 6)]. This is almost never the case if *Thiorhodaceae* are treated similarly. Frequently the changes in bicarbonate content are negligeable (cf. e. g. Table IV). One would, therefore, be led to assume that the conversions of the fatty acids by *Thiorhodaceae* result in the production of acids. Yet, the studies of *Muller* (4) have proved that here, too, the organic substrate is converted practically completely into bacterial cell material, so that the assumption of an incomplete conversion with the production of some acid must be deemed highly improbable. The only possibility of reconciling these conflicting observations would seem the acceptance of a metabolic process in which acid is produced by the *Thiorhodaceae* but which has no direct connection with the assimilatory activity proper. Now, *Gaffron* (8) has shown conclusively that, in the dark, these organisms produce considerable amounts of acid, accompanied by varying quantities of CO_2, although this author has wrongly stated that these products would be formed in the course of the supposed process of sulfate reduction. *Roelofsen* has confirmed the occurrence of a dark reaction in which some unknown acid and carbon dioxide originate, and believes that this is the normal physiological process by which the *Thiorhodaceae* meet their energetic requirements in the dark. If this process would continue during periods of illumination, for which *Gaffron*'s recent experiments (11) as well as many of the herein reported experiments, also those with completely sulfur-free bacteria, furnish proof, then it might well account for the observed facts. For it is clear that such a reaction would render the immediate environment, or the inside, of the cells rapidly acid, unless the medium is quite strongly buffered. Particularly the fact that the occurrence of a similar process in the *Athiorhodaceae* has not been detected lends support to this idea. In that case the effect of the addition of neutral salts might be the result either of an increased tolerance for acid, as mentioned above, or of an influence on the acid production. Up till the present time too little is known with certainty about these two possibilities.

6. On photosynthesis of Thiorhodaceae in the presence of various organic substances, and the complications caused by simultaneously occurring processes.

It has been shown that the *Thiorhodaceae* are capable of assimilating carbon dioxide in the light in the presence of organic substances, also if sulfur-containing compounds are absent, but that this process takes place at a measurable rate only then, when the medium contains sufficient bicarbonate. Thus one may conclude that the fundamental diffe-

rence in the photosynthetic abilities of the two groups of *purple bacteria* as claimed by *Gaffron* does not exist.

Consequently it seemed of importance to establish whether the quantitative relationships between organic substrate and carbon dioxide assimilation, or production, as found by *Muller* in growth-experiments with *Thiorhodaceae*, and by *Gaffron* in manometric experiments with *Athiorhodaceae*, could now be determined also for *Thiorhodaceae* with the aid of the manometric technique.

In the previous sections numerous experiments have already been discussed in which it was shown that a carbon dioxide uptake can be demonstrated due to the addition of propionate. However, there are only a few amongst these which permit of a calculation of the quantitative relationships mentioned above.

It will be obvious that these can be determined theoretically, apart from any preconceived idea concerning the mechanism, if it is kept in mind that the result of the activity of the bacteria is a conversion of organic substance ($+ CO_2$) into bacterial cells ($+ CO_2$), the relative proportions of carbon, hydrogen, and oxygen of the organic compound and of the bacteria respectively determining whether CO_2 will be used up or produced in this reaction. The experimental examination of these

Table V.

Assimilation of *Chromatium spec. (strain D)* in the presence of propionate. Each vessel contains 2 ccm of bacteria in m/200 $NaHCO_3$ solution; CO_2 produced or absorbed in cmm.

	1.	2.	3.	4.	5.
Added at 11.00	0.1 ccm 2 n H_2SO_4 + 0.1 ccm H_2O	0.1 ccm H_2O	0.05 ccm H_2O + 0.05 ccm m/10 propionate	0.02 ccm H_2O + 0.08 cmm m/10 propionate	0.1 ccm m/10 propionate
11.00	+ 256	—	—	—	—
12.00		+ 12	— 10	— 6	+ 12
1.00		+ 10	— 58	— 60	— 53
2.00		+ 9	+ 12	— 50	— 51
3.00		+ 8	+ 12	+ 18	— 42
Added:	—	0.05 ccm $NaHCO_3$ (66 cmm CO_2) + 0.05 ccm m/10 propionate	0.05 ccm K_2SO_4 + 0.05 ccm m/10 propionate	0.05 ccm NaCl + 0.05 ccm m/10 propionate	0.05 ccm H_2O + 0.05 ccm m/10 propionate
4.00	—	— 43	— 43	— 39	— 51
5.00	—	— 36	— 28	— 27	— 27
Added:	—	0.1 ccm 2 n H_2SO_4	0.1 ccm 2 n H_2SO_4	0.1 ccm 2 n H_2SO_4	0.1 ccm 2 n H_2SO_4
5.10	—	+ 202	+ 173	+ 199	+ 230

relationships, especially by manometric methods, is, however, seriously complicated by the fact that one must be certain that the conversions studied are the only ones which take place during the period of measurement, or, else, that the simultaneously occurring reactions are quantitatively known. The striking lack in agreement between several such experimentally determined values for one and the same compound made it necessary to study the conditions which could be held responsible for this situation. The following experiment (Table V and figure 11) suggests a simple solution of this problem.

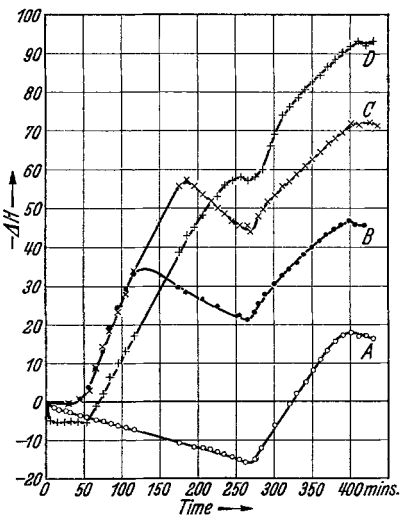

Fig. 11. *Chrom. spec.* (*strain D*) and Na-propionate. Original CO_2-content of suspension 256 cmm.
A: Originally no additions, after 265 minutes 0.05 ccm m/10 Na-propionate and 0.05 ccm m/16 $NaHCO_3$ (= 66 cmm CO_2).
B: Originally 0.05 ccm m/10 Na-propionate; after 265 minutes 0.05 ccm m/10 Na-propionate and 0.05 ccm m/10 K_2SO_4.
C: Originally 0.08 ccm m/10 Na-propionate; after 265 minutes 0.05 ccm m/10 Na-propionate and 0.05 ccm m/10 NaCl.
D: Originally 0.10 ccm m/10 Na-propionate; after 265 minutes 0.05 ccm m/10 Na-propionate and 0.05 ccm H_2O.

A calculation with the aid of these data shows that the assimilation in the various vessels amounted to:

160	cmm of CO_2	for	0.05	ccm	m/10	propionate	=	320	cmm	per	0.01	millimol
198	„	„	„	„	0.1	„	m/10	„	=	198	„	„	0.01	„
221	„	„	„	„	0.13	„	m/10	„	=	170	„	„	0.01	„
238	„	„	„	„	0.15	„	m/10	„	=	159	„	„	0.01	„

If these figures are compared with more reliable ones, obtained in other experiments (80—90 cmm per 0.01 millimol of Na-propionate) they all appear too high, and they deviate the more the smaller the quantity of propionate has been. This undoubtedly must mean that besides the assimilation with propionate some other assimilation process has also occurred, and the influence of the latter on the final figures must have increased with a decrease in the absolute figure for the assimilation with propionate.

The difference in CO_2 uptake between Nos. 2 and 3 (38 cmm) might be ascribed to the assimilation of CO_2 with 0.05 ccm m/10 propionate.

Then, the "unknown" assimilation (probably comparable with the reaction designated as "auto-assimilation" by *Roelofsen*) in these two vessels would appear to have been 126 cmm. Applying this correction to Nos. 4 and 5 one obtains values of 95 cmm of CO_2 for 0.13 ccm m/10 propionate, or 36.5 cmm per 0.05 ccm, and 108 cmm of CO_2 for 0.15 ccm m/10 propionate, or 36.0 cmm per 0.05 ccm. This result indicates that the "unknown" assimilation process proceeded at a normal and unaltered rate besides the propionate assimilation. From the behavior of the curves, as well as from a glance at the final bicarbonate values it also appears that this process gives rise to the formation of acid products.

Yet there are also a number of experiments which tend to show that the assimilation with the added substrate, and the "unknown"

Table VI.

Carbon dioxide assimilation of *Chromatium spec. (strain D)* in the presence of sodium propionate.

Each vessel contained 2 ccm suspension in dilute $NaHCO_3$, and, with the exception of the blank, 0.05 ccm m/10 propionate. Other additions mentioned below.

		cmm of CO_2 in liquid phase	changes of CO_2 in gas phase cmm	Assimilation cmm	Assimilation corrected. Propionate assimilation
Exp. A.	At start:	323.6	—	—	—
	At close:				
	1. 0.05 ccm H_2O	313.0	— 27.1	37.7	38.3
	2. 0.05 „ NaCl	353.0	— 55.2	25.8	26.4
	3. 0.05 „ KCl	332.0	— 47.7	39.3	39.9
	4. 0.05 „ K_2SO_4 . . .	333.0	— 47.8	38.4	39.0
	5. Blank (no propionate) .	316.0	+ 8.2	— 0.6	—
Exp. B.	At start:	323	—	—	—
	At close:				
	1. 0.05 ccm H_2O	342	— 72.9	53.9	26.0
	2. 0.05 „ KCl	315	— 51.2	59.2	31.3
	3. 0.05 „ K_2SO_4 . . .	322	— 57.8	58.8	30.9
	4. Blank	277	+ 18.1	27.9	—
Exp. C	At start:	282	—	—	—
	At close:				
	1. 0.05 ccm H_2O	328	— 80.4	34.4	38.0
	2. 0.05 „ H_2O	320.2	— 75.4	37.2	40.8
	3. 0.05 „ prop.	394	— 193.2	81.2	84.8
	4. 0.05 „ prop.	364	— 179.0	87.2	90.8
	5. Blank	267	+ 11.4	— 3.6	—
Exp. D.	At start:	438.6	—	—	—
	At close:				
	1. 0.05 ccm H_2O	454.6	— 95.9	79.9	39.2
	2. Blank	357.0	+ 40.9	40.7	—

assimilation process are not two entirely independent reactions. For the application of the value obtained in a blank experiment, and thus representing the "auto-assimilation", as a correction in order to obtain the amount of carbon dioxide taken up for a given amount of substrate, leads, in some cases, to abnormally low values. An example is offered by the experiments summarized in the Table VI (p. 347).

It will be seen that, although the figures in one and the same experiment are rather consistent[1], they sometimes deviate quite considerably in experiments made at different times with different material. *Gaffron* has made the same observation in his studies on the assimilation of *Athiorhodaceae* (6). The unavoidable conclusion is that dependable quantitative data cannot be obtained until the nature of this mysterious process of "auto-assimilation" is more completely understood. Hence the following results have primarily a qualitative significance, although a judicious comparison permits of drawing approximate quantitative inferences.

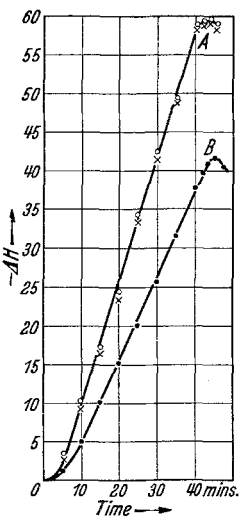

Fig. 12. *Chrom. spec. (strain D)* in the presence of
A: 0.1 ccm m/10 Na-acetate (2 expts.).
B: 0.05 ccm m/10 Na-propionate.

The experiments with propionate have shown that, in general, per molecule of propionate approximately 0.3—0.35 molecules of CO_2 are assimilated. Higher fatty acids appear not to be attacked by the strains used. Acetate, however, gives rise to a rapid uptake of CO_2 from the gas phase. Fig. 12 gives an idea of the observed changes.

Simultaneously there is a considerable increase in dissolved CO_2, as appears from the following data (Table VII):

Table VII.

Assimilation of *Chromatium spec. (strain D)* in the presence of acetate. 2 ccm of suspension in dilute $NaHCO_3$ solution. Changes in cmm of CO_2 resulting from the addition of 0.1 ccm m/10 Na-acetate.

	CO_2 content liquid	Changes in CO_2 gas phase	CO_2-production
Initial	361.8	—	—
At end 1 . . .	506	— 90.4	+ 53.8
2 . . .	508	— 88.7	+ 57.5
Initial	390	—	—
At end 1 . . .	585	— 135	+ 60
2 . . .	582	— 135	+ 57

[1] An exception seems to be Exp. A, 2. It is, of course, possible that the addition of NaCl in this case has also influenced the "auto-assimilation" to an unknown extent.

From one molecule of acetate there would thus result the production of 0.24—0.27 molecules of CO_2, which is in good agreement with the data collected by *Muller* (4) on the basis of chemical analysis of full-grown cultures of *Thiorhodaceae* (average of 0.33 mols of CO_2 per mol of acetate in one set of experiments, and of 0.17 in a second series, in which the recovery of the total carbon disappeared was 75 %), and by *Gaffron* for photosynthesis of *Athiorhodaceae* in the presence of acetate.

Also formate is rapidly attacked, but the pressure changes are small. Yet the end-point can still be determined accurately. Quantitative determinations, uncorrected for auto-assimilation, have yielded values which indicate a production of 0.53—0.55 mols of CO_2 per mol of

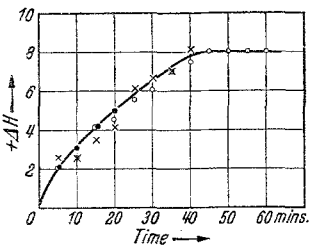

Fig. 13. Gas production by *Chrom. spec.* (*strain D*) in the presence of 0.1 ccm m/10 Na-pyruvate. Accuracy of manometer-readings 0.5 mm! One experiment terminated after 20, the second after 40, the third after 60 minutes.

formate. It is likely, however, that these figures are too low, and that somewhat larger values will be obtained by applying the proper blank corrections.

In addition to the three fatty acids a number of other organic acids were used, chiefly lactic, pyruvic, and malic acid. The results are comparable with those obtained by *Muller* and show in all cases the production of smaller or larger quantities of CO_2. As a result of the considerable acid production, simultaneous with, but not due to the decomposition of the added substrate, also here the observed manometric changes are but small, in the case of pyruvate even slightly, with malate distinctly positive.

Of particular interest in this connection is an experiment especially designed to determine whether these minute positive changes observed with pyruvate still permit of an exact determination of the end-point. For this purpose a number of vessels was prepared in exactly the same way, each receiving 2 ccm of a suspension of *Chrom. spec., strain D*, and in the 2 side bulbs, 0.1 ccm 2 n H_2SO_4, and 0.1 ccm m/10 Na-pyruvate respectively. After equilibrium had been reached the pyruvate was added to all, the H_2SO_4 to the first one for the determination of bicarbonate. After 20 minutes the second culture was acidified, after 40 minutes the third, after 60 minutes the last. Pressure changes were determined at 5 minute intervals, and fig. 13 shows the result. It will be seen that, although the manometric changes amount to only one half to

one mm per 5 minutes, yet the changes end abruptly after 45 minutes. A determination of CO_2 production yielded the data presented in Table VIII.

Table VIII.

	Initial CO_2	Gaseous CO_2	Total CO_2 produced
	287.8	—	—
after 20 minutes . . .	338	12.4	62.6
40 „ . . .	402	18.2	132.4
60 „ . . .	413	18.2	143.0

These show convincingly that 45 minutes after the addition of the pyruvate the reaction is completed. A comparison of this result with those published by *Gaffron* for similar studies on *Athiorhodaceae* in the presence of pyruvate (cf., e. g., 6, Fig. 2) shows the effect of the acid production in *Thiorhodaceae* cultures.

The uncorrected values for CO_2 production from these substrates obtained in a large number of experiments were found to be:

Mols of CO_2 per mol of substrate.

Substrate	Values found	*Muller*'s determinations
Lactate	0.14—0.20	0.20 – 0.38
Pyruvate . . .	0.64—0.72	not determined
Malate	1.06—1.18	1.12—1.36

Again, the agreement with *Muller*'s figures is satisfactory.

It should be mentioned here that the previously suggested inhibitory effect of propionate in a medium with acid reaction was not observed with lactate, pyruvate, and malate. The decomposition of lactate and malate proceeds at p_H 6.4, 7.0 and 7.2 at approximately the same rate; the decomposition of pyruvate is distinctly slower at p_H 6.4 than at 7.2, but still it starts immediately upon the addition of the substrate. These observations show that the effect of p_H on assimilation varies with the organic substance, and, for the compounds tested, is pronounced only in the case of propionic acid.

Several experiments with ethyl alcohol as an organic substrate have yielded entirely negative results; an assimilation in the presence of this substance has not been observed.

7. On the assimilation of molecular hydrogen by Thiorhodaceae.

Roelofsen (9, 10) has made the important discovery that, in the light, *Thiorhodaceae* rapidly absorb hydrogen in the presence of CO_2, and has attributed this fact to the ability of the organisms to use molecular

hydrogen as hydrogen donor for a photochemical reduction of carbon dioxide. *Gaffron* (11) has explained this phenomenon as the result of a production of H_2S in the dark, from sulfur-containing compounds and hydrogen, only the sulfide being used in the photosynthetic process. In view of the preceeding experiments on the significance of hydrogen sulfide formation (section 2) and on the existence of a direct assimilation with organic substrates it seemed probable that also the assimilation of molecular hydrogen by *Thiorhodaceae* would proceed directly, as was established by *Gaffron* for *Rhodovibrio*.

Experiments with *Chromatium spec.* (*strains A* and *D*), grown in "sulfur-free" malate media, have shown that this is actually the case. Suspensions in dilute bicarbonate and illuminated in an atmosphere of oxygen-free hydrogen, cause a rapid decrease in pressure, for some time at a perfectly steady rate, which later on decreases, probably due to an increasing alkaline reaction of the medium. Even at p_H 9.5 the reaction rate is, however, hardly affected.

If no carbonate or bicarbonate is added to the suspension, the uptake of hydrogen is slow but measurable, probably owing to a slow production of CO_2 by the organisms. The addition of known amounts of carbonate or of organic substances which are assimilated with the production of CO_2, the elegant procedure followed by *Gaffron* (6) for determining the quantitative relationships between hydrogen- and carbon-dioxide uptake, have shown that per molecule of CO_2 approximately 2.4 molecules of hydrogen are assimilated, and that, under these circumstances, 1 molecule of pyruvate is equivalent to almost 0.5 molecule of carbonate. The experiments with organic compounds and hydrogen, do not, of course, reveal anything about the mechanism of the assimilation and leave open the problem whether the hydrogen uptake is due to a direct photochemical reduction of carbon dioxide, which originates from the simultaneous decomposition of the organic substances, or to a reduction of the latter. *Gaffron* has expressed himself on this question in terms which may be interpreted in favor of the second alternative (8). Elsewhere I have pointed out (12) that the uptake of hydrogen can, for the present, be more easily explained by assuming that the uptake of hydrogen occurs because the organic substrate is a source of CO_2. That acetic acid makes no exception is clearly shown by the experiments on the assimilation with acetate reported in the previous section. The agreement between the data gives there (0.24—0.27 mols of CO_2 produced per mol of acetate added) and those obtained by *Gaffron* on hydrogen assimilation (8, Table XII, 0.47—0.60 mols of H_2 assimilated per mol of acetate) is as perfect as can be expected.

These experiments do not suffice to warrant the conclusion that all *Thiorhodaceae* can assimilate molecular hydrogen, just as *Gaffron*'s

leave this unanswered for the *Athiorhodaceae*. From several preliminary experiments, carried out in the course of the past 5 years, the impression has been gained that there exist just as marked differences between various representatives of *Thio-* and *Athiorhodaceae* in this respect as between strains of *Thiorhodaceae* with regard to their ability to use butyrate.

8. On oxygen production by photosynthesizing purple bacteria.

Quite recently *Czurda* (16) has published experimental results which this author considers as a proof for the production of oxygen during photosynthesis of *purple bacteria*.

The experiment which *Czurda* reports is not new; essentially the same experiment was published by *van Niel* in 1931 (2, p. 102). At that time the oxidation of reduced methylene blue and reduced indigo carmin by illuminated *purple bacteria* was not considered as an indication for the production of O_2 during photosynthesis of the *purple bacteria*, but as a first example of the ability of these organisms to use oxidizable organic substances instead of H_2S for a photochemical reduction of carbon dioxide. This possible interpretation of the experimental results is not considered at all by *Czurda*, who writes: „Schon die ersten Fortzuchtversuche ... haben das unerwartete Ergebnis gehabt, daß auch im CO_2-Assimilationsprozeß dieser Art Sauerstoff entsteht, wie bei der grünen Pflanze" (p. 111).

It is true that the experimental result — the observation that reduced indigo carmin in the presence of illuminated *purple bacteria* turns blue as soon as the excess H_2S is used up — can be interpreted as oxygen production. In view of the great difficulties which this interpretation implies if all the known facts about bacterial photosynthesis are taken into consideration, I have previously adopted the other explanation of the same result.

Because of the importance which attaches particularly to this point, I want to briefly review the main reasons here.

1. With the aid of luminous bacteria, without doubt a still more sensitive reagent for molecular oxygen than reduced indigo carmin, oxygen production by *purple bacteria* could not be demonstrated. This holds also for suspensions in which H_2S was not present [*Molisch* (17)].

2. If one determines, by purely chemical methods, the amounts of carbon dioxide and hydrogen sulfide which disappear during the development of *Thiorhodaceae*, one finds that in no case is there any demonstrable disappearance of CO_2 in excess to that which can be accounted for on the basis of the equation

$$2\,CO_2 + 2\,H_2O + H_2S \rightarrow 2\,(CH_2O) + H_2SO_4.$$

This is quite independent of the time at which the analyses are made; even if the culture is incubated in continuous light for 23 days after the complete oxidation of H_2S to sulfate the amount of carbon dioxide which is used by the bacteria does not increase [(1), p. 166; (2), p. 89].

The production of oxygen would, of course, imply that the photosynthetic process of the *purple bacteria* is altogether independant of the presence of oxidizable inorganic or organic compounds. The mere fact that this is not so, therefore makes the interpretation which *Czurda* now places upon the results doubtful. However, it has become clear that, although the previously given explanation for the oxidation of reduced dyes is in accord with the other facts, the experiment itself is not as conclusive as it might have been. Consequently I have repeated these experiments in a different manner which, *a priori*, would seem to permit of an unambiguous decision which one of the two different explanations is correct.

If the oxidation of reduced indigo carmin is the result of the fact that the organisms can use it as a substitute for H_2S, propionate, etc., then it is obvious that the oxidation could take place only if the bacteria can come in contact with the reduced substance.

If, however, the oxidation results from the liberation of oxygen by the illuminated bacteria, no such contact is necessary. From these considerations the experimental set-up follows logically.

5 ccm of a heavy suspension of a young pure culture of *purple bacteria* in dilute $NaHCO_3$ solution were incubated in an atmosphere of oxygen-free nitrogen. Separated from the suspension, but in contact with the gas phase, was a small quantity (0.3 ccm) of reduced indigo carmin. Two experiments were started simultaneously, and the vessels shaken side by side in a thermostat at 30^0 C. One vessel was illuminated, while the other one was kept dark. The latter control is essential because small amounts of oxygen may remain dissolved in the suspension, even if nitrogen is passed over it for 20 minutes, and, upon shaking, diffuse out, oxidizing the leuco-dye.

It has so far been impossible to carry out these experiments in a system which was completely oxygen-free. Even by using nitrogen which had first been led over heated copper, and subsequently through a solution of reduced indigo carmin, and by flushing the system with this gas for a considerable length of time, the presence of traces of oxygen could still be detected because during the first 15 minutes of incubation the indicator solution changed to a very faint blue. But after this period, during which the color changes of the indicator in the illuminated and in the non-illuminated vessel were exactly the same, no further changes could be observed, not even after 18 hours illumination, as proved by a comparison of the indicator solutions in the illuminated vessel and in the

control. Yet it could be ascertained that the bacteria were quite active afterwards.

This result throws considerable doubt upon *Czurda*'s interpretation, and may be taken to prove that illuminated *purple bacteria* do not produce molecular oxygen. Hence, at present, there seems to be no need, „die Vorstellungen über den Stoffwechsel der *Thiorhodaceen*, welche *van Niel*, *Muller*, *Roelofsen* auf umfangreiche eingehende Arbeiten und theoretische Überlegungen in so überzeugender Weise zu stützen gesucht haben, neu aufzubauen" (*Czurda*, p. 113).

9. On the chemical composition of the purple bacteria, with remarks on the "photosynthetic quotient" and the mechanism of their assimilation processes.

The quantitative results obtained previously on photosynthesis of *Thiorhodaceae* (2), as well as those published by *Muller* and by *Gaffron* and, finally, the ones discussed in this communication all have shown that the relationship existing between the quantity of carbon dioxide reduced and of substrate oxidized is not as simple as one might expect on the basis of the generalized equation suggested to cover the assimilation processes of *green* and *purple bacteria*

$$CO_2 + 2\,H_2A \rightarrow (CH_2O) + H_2O + 2\,A.$$

From the data furnished by the chemical analysis of full-grown cultures it was concluded that, inasmuch as the (CH_2O)-factor of the equation in that case is represented by the bacterial cells, these cells would be more reduced than carbohydrate. The results obtained with the aid of the manometric technique can still be explained along the same lines if one assumes that the first product of the assimilation does not accumulate, but is rapidly converted into bacterial substances of various composition and belonging to different groups of compounds, or, even, into new bacterial cells. Considering the enormous rate of growth of the organisms, and the high temperature (30—35° C) at which these experiments are carried out, this assumption is not in itself improbable.

Up to the present time, however, no data have been published which support these views by direct chemical analyses. With a view of filling this gap to some extent such chemical analyses of *purple bacteria* have been made[1].

The bacteria used for these analyses, representatives of the groups of *Thio-* and *Athiorhodaceae*, were grown in the standard media, carefully freed

[1] I am very grateful to Prof. Dr. *H. ter Meulen*, Delft, for allowing me to carry out this work in his laboratory, where I could use the elegant and excellent methods developed there for elementary analysis under his experienced supervision. I also want to express my gratitude to Miss *Ir. H. J. Ravenswaay* for much generous assistance.

from precipitates before inoculation, and centrifuged before any precipitate had formed. They were washed thoroughly, and rapidly dried on clean glass plates at temperatures not exceeding 37° C to an air-dry film which was scraped off and kept in vacuum in a desiccator over calcium chloride till the analyses were made. Then they were ground to a fine powder, and dried at 115° C to constant weight, which did not take longer than 1—2 hours. Changes in the appearance of the material were not observed except in the case of "*Streptococcus varians Ewart*", a pure culture of *brown bacteria* resembling the organism described by *Ewart* in 1897 under this name. Here a color change was observed from a reddish brown to a dirty brown-green, apparently caused by the decomposition of some pigment.

The analyses include a determination of carbon, hydrogen, nitrogen, and ash for all strains. The estimations of the ash-content were meant chiefly as a control on the purity of the material and are relatively inaccurate, because an ordinary analytical balance with a sensitivity of 0.1 mg was used,

Table IX. Chemical analyses of some strains of *purple bacteria*.

Organism	mg sample ash-free	mg ash	mg H_2O	mg CO_2	ccm n/10 HCl	% ash	% H	% C	% N
Spirillum rubrum (strain G)	61.0	2.5	40.5	124.5		3.94	7.38	55.7	
	34.5	1.6	23.9	70.4		4.44	7.70	55.6	
	33.7	1.6	22.4	68.7		4.54	7.39	55.6	
	24.1				2.031				11.80
	16.2				1.360				11.82
Spirillum rubrum (strain L)	42.1	2.0	27.2	85.8		4.53	7.17	55.6	
	46.0	1.8	29.7	93.9		3.86	7.18	55.5	
	13.7				1.158				11.81
	14.5				1.222				11.81
Rhodomonas spec.	42.4	3.7	28.3	86.6		8.02	7.42	55.5	
	63.4	4.3	39.9	130.2		6.35	7.00	56.0	
	25.7	2.1	17.6	53.5		8.15	7.60	56.0	
	16.4				1.367				11.68
	22.8				1.808				11.10
	16.0				1.281				11.22
"*Streptococcus varians*"	26.5	1.5	17.3	52.7		5.36	7.25	54.2	
	45.3	2.9	29.7	90.3		6.02	7.28	54.4	
	14.8				1.184				11.20
	14.7				1.188				11.31
Chromatium spec. (D) "Sulfur-free"	31.9	2.4	22.7	64.9		7.00	7.90	55.5	
	35.8	2.3	22.1	72.5		6.04	6.88	55.3	
	28.1	1.5	17.4	57.0		5.50	6.89	55.4	
	26.6				2.064				10.88
	13.6				1.063				10.94

Organism	mg sample	mg $BaSO_4$	ccm n/100 I	% S	Method
Spirillum rubrum (strain G)	79.8		2.81	0.56	Hydrogenation
	76.2		2.31	0.48	
	335	1.3		0.53	As $BaSO_4$ in ash
Chromatium spec. (D) "Sulfur-free"	190	0.7		0.50	As $BaSO_4$ in ash
Chromatium spec. (D) "Sulfur-rich"	12.2		26.54	34.8	Hydrogenation
	10.5		22.60	34.4	

and the total quantity of residue after incineration was of the order of magnitude of 1 mg. Carbon and hydrogen were determined simultaneously, and nitrogen in an additional sample according to the methods worked out by *ter Meulen* c. s. (15).

A determination of the sulfur content of *Chromatium spec. (strain D)*, containing considerable amounts of stored sulfur, was carried out by *ter Meulen*'s hydrogenation method (15). For comparison the sulfur content of a sample of a "sulfur-free" culture of the same organism and of one of the *Athiorhodaceae* was also determined.

The results are collected in Table IX.

A most striking feature is the remarkable consistency of the results; it seems as if the composition of all the strains used is practically identical. This is the more astonishing because the culture media were not the same in all cases; the two strains of *Spirillum rubrum* were grown in 1 % peptone-solutions, *Rhodomonas spec.* and *Streptococcus varians* in malate media with the addition of 1 % yeast autolysate, *Chromatium species* in a malate medium with inorganic salts only.

Of importance is also the fact that a comparison of the composition of the *purple bacteria* with that of a carbohydrate reveals a much lower oxygen content of the former:

	Purple bacteria %	Carbohydrate %		*Purple bacteria* %	Carbohydrate %
C	55.7	40.0	O	15.1	53.3
H	7.4	6.7	N	11.8	—

This proves conclusively the correctness of the previously made assumption that the *purple bacteria* would be more reduced than carbohydrate. It also implies that in the conversion of organic substrates of the empirical compositions of a carbohydrate (e. g. acetic and lactic acids) into bacterial cells some extra hydrogen is necessary, or, expressed differently, some CO_2 will be produced.

Of more interest yet is a comparison of the analytical data with those published by *Gaffron* (5) for a substance isolated by him from *purple bacteria*, and to which a composition $(C_4H_6O_2)_n$ is assigned on the basis of carbon and hydrogen determinations.

	Purple bacteria %	*Gaffron*'s product %
C	55.7	55.8
H	7.4	7.0

This comparison reveals a nearly complete identity of the results as far as carbon and hydrogen content are concerned. Now it must be remembered that *Gaffron* has considered this substance as a direct assimilation product of *purple bacteria* photosynthesis, and this partly because the quantitative relationships found to exist between the amounts of carbon dioxide assimilated per molecule of various fatty acids fitted in with his supposition. The mechanism which *Gaffron* (16) postulates for these conversions is not satisfactory. Brutto equations like

$$2\,CH_3COOH + H_2 \rightarrow C_4H_6O_2$$
$$C_7H_{14}O_2 + CO_2 \rightarrow C_8H_{14}O_4 \rightarrow (C_4H_6O_2)_2$$
$$C_6H_{12}O_2 + CO_2 + CH_2O \rightarrow (C_4H_6O_2)_2 + H_2O$$
$$C_9H_{18}O_2 + 2\,CO_2 + CH_2O \rightarrow (C_4H_6O_2)_3 + H_2O$$

fail to express one of the most important advances made in biochemical research, viz. the conviction that even the most complex biological conversions are the ultimate result of a series of simple step reactions. It is true that the present knowledge of bacterial photosyntheses does not yet permit of its reduction to a series of intelligible steps. But the demonstration that the composition of these cells as far as the relative amounts of carbon and hydrogen are concerned corresponds so closely with *Gaffron*'s product, then serves to show that the considerations applied to furnish evidence for the assumption that this product would be a direct photosynthate can be used with equal justification in defence of the more probable view that the reactions studied till now have dealt chiefly with the conversion of the substrate into entire bacterial cells (12).

10. Summary and conclusions.

The foregoing studies have shown that there is no need to modify the previously expressed concept of the metabolism of *Thiorhodaceae* in the light. It could be demonstrated that oxygen is not evolved during photosynthesis, and that *Thiorhodaceae*, like *Athiorhodaceae*, are capable of utilizing molecular hydrogen and organic substances directly in their assimilation process.

The production of H_2S by *Thiorhodaceae* in the dark is quantitatively insignificant, and, inasmuch as its extent depends upon the

amount of stored sulfur in the cells, it is probably due to a process similar to the "phytochemical reductions".

The manometric study of photochemical carbon dioxide reduction by *Thiorhodaceae* yields results which are in agreement with those obtained by the method of chemical analysis of full-grown cultures, although the mere determination of pressure changes during photosynthesis may be misleading.

In a medium which contains sufficient bicarbonate to maintain a slightly alcaline reaction the fatty acids up to propionic acid are assimilated immediately; if the medium is slightly acid, this process takes place only in the presence of (inorganic) salts. The latter exert a non-specific effect. In a still more acid environment propionate cannot be assimilated.

In the light as well as in the dark the *Thiorhodaceae* produce acid (s). This accounts for the difference in the rate and extent of CO_2 uptake from the gas phase by suspensions of *Thio-* and *Athiorhodaceae* in the presence of salts of organic acids.

Chemical analyses of representatives of *Thio-* and *Athiorhodaceae* prove that the organisms are more reduced than carbohydrate, and that the degree of reduction corresponds with that of a substance which *Gaffron* considers as a direct product of photosynthesis by these organisms.

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