The Bacteria

Edited by

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Volume III: BIOSYNTHESIS

The Bacteria

A TREATISE ON STRUCTURE AND FUNCTION

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PREFACE

Our basic concepts concerning the mechanisms of energy-yielding metabolism were developed largely through studies on mammalian tissues and on yeast. The subsequent exploration of energy-yielding metabolism of bacteria did not really lead to the discovery of new principles, but served chiefly to reveal the diversity of the biochemical pathways which cells can use to satisfy their energetic needs. With respect to the biosynthetic aspects of metabolism, the development of knowledge has followed a different path. When the techniques and materials required for the analysis of biosynthetic pathways became available, the advantages of performing biochemical studies with unicellular organisms were fully appreciated, and bacteria and other microorganisms thus became from the start the experimental objects of choice. Thus, in large part, our present understanding of biosynthesis has grown out of studies on the bacteria; studies on biosynthetic pathways in higher organisms have been in the main secondary. The general biochemical picture which emerges is more uniform than that which emerged from the exploration of energy-yielding metabolism. Certain small portions of biosynthesis can be pointed out as more or less specific to bacteria, for example, the synthesis of the unique structural heteropolymers of the cell wall; but in its totality, the process of biosynthesis in the bacterial cell does not seem to differ markedly from that in other types of cells.

The editors wish to thank the contributors to the present volume for their cooperation and patience, particularly in view of the delay in the appearance of this volume. We also wish to thank the publishers and the members of their staff for their encouragement and expert help in the preparation of this, as of the previous volumes of "The Bacteria."

While this volume was in the final stages of its preparation, we learned to our profound regret of the untimely death of Professor S. Hestrin, whose great contributions to knowledge of bacterial carbohydrate metabolism are amply evidenced in the chapter which he prepared for the present volume shortly before his death.

I. C. GUNSALUS
R. Y. STANIER

June 1962

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CHAPTER 1

Photosynthesis and Lithotrophic Carbon Dioxide Fixation

S. R. ELSDEN

I. Introduction*

Biochemical reaction sequences that result in the fixation of carbon dioxide are conveniently separated into two types, which will be referred to as heterotrophic and autotrophic, respectively. The former, which occur in all cells and which will be discussed by H. G. Wood in Chapter 2 of this volume, involve the addition of carbon dioxide to organic acceptors but do not result in the total synthesis of the acceptor from carbon dioxide. In general the heterotrophic reactions are steps in the synthesis of specific compounds. The autotrophic reaction involves the addition of carbon dioxide to a specific acceptor and the over-all process is so constituted that the acceptor is regenerated and is ultimately synthesized entirely from carbon dioxide; the process is in fact cyclic. Organisms capable of synthesizing all their organic matter from carbon dioxide possess both the heterotrophic and the autotrophic enzyme systems, whereas those that grow only upon organic compounds contain, with certain exceptions which will be discussed later, only the heterotrophic systems.

This chapter is concerned with the nature of the autotrophic reaction which enables bacteria to use carbon dioxide as sole source of carbon for growth. The organisms that fix carbon dioxide in this way fall into two distinct groups depending on the nature of their primary energy source. The first group, for which the name chemolithotrophic bacteria has been suggested[1] obtain their energy by the oxidation of inorganic compounds. The second group, the photolithotrophic bacteria, use light as their energy

* The following abbreviations are used: DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate.

source; the photometabolic process of these latter organisms has already been discussed by D. M. Geller in Volume II (Chapter 10) of this treatise.

We owe the discovery of the chemolithotrophic bacteria to Winogradsky, who summarized the results of his studies of the colorless sulfur bacteria[2] as follows:

"En résumé, les Sulfobactéries présentent un type physiologique nouveau et inattendu, dont l'énergétique est différente du type dominant. Leur processus vital se joue d'après un schéma beaucoup plus simple, empruntant toute l'énergie nécessaire à une réaction chimique inorganique, l'oxidation du soufre."

A year later, in 1888, Winogradsky[3] showed that the iron bacteria obtained their energy for growth by the oxidation of ferrous salts to ferric oxide. The iron bacteria, like the sulfur bacteria, grew in the complete absence of organic carbon and Winogradsky concluded that both types of organism were able to use carbon dioxide as their source of carbon.

Winogradsky next turned his attention to the nitrifying bacteria and in his second paper of the series[4] he showed that coincident with the oxidation of ammonia the organic carbon of the medium, determined chemically by a wet combustion process, increased. His conclusions merit repetition in full:

"En résumant, l'agent de la nitrification nous apparaît comme doué de propriétés marquantes, qui en font *un type physiologique nouveau dans la Science*. Ces caractères se résument comme suit:

1° *Développement dans un milieu purement minéral pourvu de substance inorganique oxydable.*

2° *Processus vital étroitement lié à la présence de cette substance, qui est l'ammoniac dans le cas de la nitrification.*

3° *Oxydation de cette substance, comme seule source d'énergie*

4° *Aucun besoin en aliment organique, ni en qualité de materiel plastique, ni comme source d'énergie.*

5° *Incapacité de décomposer les substances organique, dont la présence ne fait qu'entraver le dévelopment.*

6° *Assimilation de l'acide carbonique—seul source de carbone—par chimiosynthèse.*

Table I, taken from his third paper,[5] shows the relationship he found between the amount of ammonia oxidized and the amount of organic carbon formed. Subsequent work showed that the oxidation of ammonia to nitrate proceeded in two stages: first, the oxidation of ammonia to nitrite; second, the oxidation of nitrite to nitrate and culminating with the isolation of the two organisms concerned, *Nitrosomonas* and *Nitrobacter*, respectively.

This work, justly called classic, set the pattern of research for the next fifty years. During this period attention was focused, first, on the isolation of other types of chemolithotrophic organisms, and second, on establishing

the dimensions of the ratio between the amount of carbon dioxide assimilated and the amount of the energy source oxidized. From this ratio the thermodynamic efficiency of the process could be calculated. Table II, taken from Baas-Becking and Parks[6] gives some indication of the results obtained. It will be seen that the thermodynamic efficiency of the process is low. Hofmann and Lees[13] have pointed out that these calculations apply only to the stage of growth the culture had reached at the time the analyses

TABLE I

RELATIONSHIP BETWEEN AMMONIA OXIDIZED AND CARBON DIOXIDE ASSIMILATED[5]

	Culture number			
	11	12	26	30
Ammonia N oxidized (mg.)	722	506.1	928.3	815.4
Carbon assimilated (mg.)	19.7	15.2	26.4	22.4
Ammonia N oxidized (carbon assimilated)	36.6	33.3	35.2	36.4

TABLE II

THERMODYNAMIC EFFICIENCIES[a]

Reaction	Free energy efficiency	Reference
$H_2 + 0.5O_2 = H_2O$	26.4	7
$CH_4 + 2O_2 = CO_2 + 2H_2O$	0.6–29.6	8
$NH_4^+ + 1.5O_2 = NO_2^- + H_2O + 2H^+$	7.9	9
$NO_2 + 0.5O_2 = NO_3^-$	5.9	9
$S + 1.5O_2 + H_2O = H_2SO_4$	8.3	10
$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 2\ N_2$	5.0	11
$8KNO_3 + 5Na_2S_2O_3 + 2NaHCO_3 = 6Na_2SO_4 + 4\ K_2SO_4 + 4N_2 + 2CO_2 + H_2O$	9.0	12

[a] Calculated by Baas-Becking and Parks.[6]

were made and that, if old cultures are examined, then the values obtained may be low. Certainly, in the case of *Nitrosomonas*, which Hofmann and Lees examined, the efficiency of young cultures appears to be of the order of 40% whereas that of old cultures was 7%.

The amount of oxygen consumed may also be taken as an index of the amount of energy made available by an oxidative process; the data for a number of species showing the relationship between carbon dioxide fixed and oxygen consumed are given in Table III. It will be seen that the values obtained for this ratio, using both sulfur and iron-oxidizing bacteria,

and measured in both short-term *in vitro* experiments and in long-term growth experiments agree surprisingly well with one exception, namely, the results of Vogler and Umbreit.[16, 17] Interesting as the calculations of the thermodynamic efficiency may be, such calculations have given no indication whatsoever of the mechanism by which carbon dioxide is fixed.

The first attempt to understand the nature of the processes involved came from the work of Vogler[16] and Umbreit.[17] These authors measured the amount of oxygen consumed and the amount of carbon dioxide assimilated during the oxidation of elementary sulfur by washed suspensions of *Thio-*

TABLE III

RELATIONSHIP BETWEEN OXYGEN CONSUMED AND CARBON DIOXIDE ASSIMILATED[a]

Organism	Substrate	Oxidant	O_2/CO_2	Reference
Thiobacillus denitrificans[b]	$S_2O_3^{--}$	NO_3^-[d]	9	12
Thiobacillus thiooxidans[b]	S	O_2	18	14
Thiobacillus thioparus[b]	$S_2O_3^{--}$	O_2	19	15
Thiobacillus thiooxidans[c]	S	O_2	2.9	16
Thiobacillus thiooxidans[c]	S	O_2	1.5	17
Thiobacillus thiooxidans[c]	$S_2O_3^{--}$	O_2	9–26	18, 23
Thiobacillus thioparus[c]	$S_2O_3^{--}$	O_2	9–26	
Thiobacillus denitrificans[c]	$S_2O_3^{--}$	NO_3^-[d]	4.6–11	18, 23
Iron-oxidizing bacterium[c]	Fe^{++}	O_2	37	19
Iron-oxidizing bacterium[c]	S	O_2	32	19
Hydrogenomonas facilis[c]	H_2	O_2	2.0	20
Hydrogenomonas ruhlandii[c]	H_2	O_2	2.7	21

[a] Data of this table are taken in part from reference 25.

[b] Experiments made with growing cultures.

[c] Experiments made with washed cell suspensions.

[d] Value for oxygen is calculated according to the equation

$$2HNO_3 \rightarrow H_2O + N_2 + 2.5O_2$$

bacillus thiooxidans. Vogler[16] observed that washed suspensions which had been allowed to oxidize sulfur in the absence of carbon dioxide acquired the ability to take up measurable amounts of carbon dioxide when placed in an oxygen-free atmosphere, i.e., under conditions where no further oxidation of sulfur could occur. These experiments were considered to show that the oxidation of sulfur provides the organism with a store of energy which could subsequently be used to fix carbon dioxide. In other words, the fixation of carbon dioxide and the energy supply could be separated in time. It was then shown[17] that if sulfur was oxidized in the absence of carbon dioxide, inorganic phosphate disappeared from the medium and reappeared when carbon dioxide was admitted to the system. It was concluded from these results that during the oxidation of sulfur *Thiobacillus thiooxidans*

synthesized adenosine triphosphate (ATP) and that this compound provided the energy for the fixation of carbon dioxide. The results of these experiments have been subjected to a devastating analysis by Baalsrud and Baalsrud,[23] Baalsrud,[22] Vishniac and Santer.[24] The essential feature of their criticisms was that the amount of carbon dioxide fixed by the cells under anaerobic conditions was very much greater than could have reasonably been accounted for by the inorganic phosphate taken up—the observed ratio of carbon dioxide fixed: inorganic phosphate taken up was 47:1. It is difficult to see how 1 mole of ATP could supply the energy for the fixation of 47 moles of carbon dioxide; indeed, according to the current hypothesis the CO_2:ATP ratio should be 1:3. Attempts to repeat these experiments, while confirming the uptake of inorganic phosphate during the oxidation of sulfur, have failed to confirm the anaerobic fixation of carbon dioxide in the amounts claimed by Umbreit and Vogler.[16, 17] Although, in my opinion, these criticisms were justified, recent work on the mechanism of carbon dioxide fixation has shown that ATP does indeed play an intimate part in the fixation of carbon dioxide. It thus has transpired that although the evidence provided by Vogler and Umbreit was inadequate to substantiate their main conclusion, their concept that the oxidation of sulfur to sulfate is coupled to the synthesis of ATP, which is then used for the fixation of carbon dioxide, is probably correct.

II. The Photolithotrophic Bacteria

Pure cultures of photosynthetic bacteria were first isolated and described by van Niel.[26] The organisms, which were strict anaerobes, were of two main types: the green sulfur bacteria or Chlorobacteriaceae, the type species of which is *Chlorobium limicola*; and the purple sulfur bacteria or Thiorhodaceae, of which representatives of two genera *Chromatium* and *Thiocystis* were obtained in pure culture. The isolates of *Chlorobium limicola* converted carbon dioxide to cell material and oxidized hydrogen sulfide to elementary sulfur anaerobically in the light; the sulfur thus formed was deposited outside of the cell. Analysis of the culture fluid at the end of the growth period showed that the chemical changes which occurred fitted equation (1)

$$2H_2S + CO_2 \rightarrow [CH_2O] + 2S \qquad (1)$$

A more detailed study of the green bacteria was published by Larsen[27, 28] some twenty years later. In addition to strains of *Chlorobium limicola*, Larsen, with the aid of the enrichment culture technique, isolated a new species, *Chlorobium thiosulfatophilum*, which was capable of using thiosulfate as well as hydrogen sulfide. In contrast to the organisms isolated by van Niel, Larsen's two species oxidized hydrogen sulfide to a mixture of

sulfur and sulfate. The elementary sulfur, which accumulated in the medium in the early stages of the growth, was subsequently oxidized to sulfate, so that the latter became the major end product. Calculation of the amount of carbon dioxide assimilated from the amounts of sulfur and sulfate formed according to equations (1) and (2) gave a value which agreed with that observed.

$$H_2S + 2CO_2 + 2H_2O \rightarrow 2[CH_2O] + H_2SO_4 \quad (2)$$

Manometric experiments with washed cell suspensions of both species of green sulfur bacteria showed that, in the light, sulfide was oxidized quantitatively to sulfate and the amount of carbon dioxide assimilated was 90% of that predicted by equation (2). Although *Chlorobium thiosulfatophilum* would not grow photosynthetically upon tetrathionate, illuminated washed cell suspensions, in the presence of carbon dioxide, oxidized both this compound and thiosulfate with the assimilation of carbon dioxide according to equations (3) and (4).

$$2CO_2 + Na_2S_2O_3 \rightarrow 2[CH_2O] + Na_2SO_4 + H_2SO_4 \quad (3)$$

$$7CO_2 + Na_2S_4O_6 \rightarrow 7[CH_2O] + Na_2SO_4 + 4H_2SO_4 \quad (4)$$

Washed cell suspensions of both species reduced carbon dioxide with hydrogen in the light according to equation (5).[29, 30]

$$2H_2 + CO_2 \rightarrow [CH_2O] + H_2O \quad (5)$$

In addition, Larsen demonstrated that *Chlorobium thiosulfatophilum* was able to grow upon a mixture of hydrogen and carbon dioxide. The ability of *Chlorobium limicola* to do likewise was not tested.

The Thiorhodaceae like the green sulfur bacteria, metabolize sulfur compounds in the light with the fixation of an amount of carbon dioxide equivalent to the amount of sulfur compound oxidized.[26] During the early stages of growth with hydrogen sulfide droplets of elementary sulfur accumulated within the cells but, when all the sulfide had disappeared from the medium, the intracellular sulfur was oxidized quantitatively to sulfate and the over-all reaction observed was identical with that found in the green bacteria isolated by Larsen. These organisms also resembled the green bacteria in their ability to use thiosulfate; Roelofsen[29] showed that they used hydrogen to reduce carbon dioxide.

In the reactions so far discussed there is an almost stoichiometric relationship between the amount of carbon dioxide assimilated and the amount of the electron donor oxidized. The ability of these photosynthetic bacteria to use radiant energy for growth with carbon dioxide as the carbon source, coupled with the fact that the pigments responsible for the light reaction are related to chlorophyll a, suggests that there is a close relationship be-

tween green plant photosynthesis on the one hand and bacterial photosynthesis on the other. This led van Niel[31-34] to develop his general hypothesis to cover all types of both photosynthesis, both that of bacteria and that of the green plant. According to van Niel, light is used to split water and the over-all chemistry of the process is usually expressed by equation (6)

$$2H_2A + CO_2 \rightarrow [CH_2O] + H_2O + 2A \qquad (6)$$

The general implications of the equation have been discussed by Geller (Volume II, Chapter 10) and will not be enlarged upon further here. What is significant in the present context is the implication that the mechanism of carbon dioxide fixation is the same both in bacteria and in the green plant.

The discovery of Müller[35] that the Thiorhodaceae will grow anaerobically upon organic compounds if the cultures are illuminated complicated matters. Analysis of the culture medium at the end of growth showed that most of the organic compound supplied was assimilated and depending on the oxidation level of the substrate, carbon dioxide was either produced or assimilated. While there was no doubt that growth under these conditions was photosynthetic in the sense that radiant energy was essential, in the case of those substrates in which there was a net output of carbon dioxide it was not established that fixation of this gas was involved; in contrast to growth in the presence of inorganic hydrogen donors there appeared to be no stoichiometric relationship between the amount of carbon dioxide fixed and the amount of organic substrate assimilated.

The third group of bacteria which grow at the expense of radiant energy is the Athiorhodaceae.[36] These organisms, in the main, use organic compounds as hydrogen donors but many strains will, in addition, use hydrogen to reduce carbon dioxide in the light[30] in a manner similar to the Chlorobacteriacae[27] and the Thiorhodaceae.[29] Gaffron[37] introduced the use of the manometric method to study the photometabolism of organic compounds by members of the Athiorhodaceae, and examined in particular the photometabolism of fatty acids from acetic to nonanoic. He observed that, with acetate, there was a net output of carbon dioxide, whereas with the higher fatty acids carbon dioxide was assimilated. The amount of carbon dioxide fixed was proportional to, but not equivalent to, the chain length. Results similar to those of Gaffron was subsequently obtained by van Niel[32] using *Rhodospirillum rubrum*. Gaffron[37] considered that radiant energy was used for the assimilation of the fatty acids by these organisms and claimed to have isolated the assimilation product formed from acetate. This view was not generally accepted but it has recently received support from the work of Stanier and his colleagues,[38] who have isolated and identified the assimilation product as poly-β-hydroxybutyric acid.

There is one recorded exception to the general rule that during the photo-

metabolism of organic compounds the carbon of the substrate is converted to cell material. Foster,[39] using the enrichment culture technique with isopropanol as the substrate, isolated an organism which in the light oxidized isopropanol to acetone and assimilated carbon dioxide. The acetone so produced accumulated in the medium and did not appear to be further metabolized. Analysis of the culture medium showed that over-all changes which accompanied growth could be approximated to equation (7)

$$2\ \begin{matrix}CH_3\\ \diagdown\\ \quad CHOH\\ \diagup\\ CH_3\end{matrix} + CO_2 \rightarrow 2\ \begin{matrix}CH_3\\ \diagdown\\ \quad C{:}O\\ \diagup\\ CH_3\end{matrix} + [CH_2O] + H_2O \qquad (7)$$

These observations of Foster's were used by van Niel[32-34] to support his view that the photometabolism of organic compounds involved reactions similar to those found in the Chlorobacteriacae and the Thiorhodaceae, namely, that the primary light reaction is the photolysis of water followed by the oxidation of the H-donor and the reduction of carbon dioxide. Unfortunately the organism isolated by Foster was lost. Subsequent attempts by Siegel and Kamen[40] to isolate new strains capable of growing upon an isopropanol in the light in a manner similer to that of Foster's organism failed. They did, however, obtain a pure culture of an organism identified as *Rhodopseudomonas gelatinosa* which would use isopropanol in the light; but during the growth of this organism, the isopropanol was assimilated rather than converted quantitatively to acetone.

The Athiorhodaceae share with the Thiorhodaceae and the Chlorobacteriacae the property of being able to reduce carbon dioxide with hydrogen on illumination and the fact that the chlorophyll of the Athiorhodaceae is identical with that of the Thiorhodaceae suggests that the metabolic processes of all three have much in common. But, at the same time, the facts thus far discussed do not permit us to draw the conclusion that, during the photometabolism of organic compounds, such carbon dioxide as is fixed is assimilated by the autotrophic process.

During the photometabolism of acetate carbon dioxide is produced, approximately 0.2 moles of carbon dioxide per mole of acetate metabolized.[32, 37] Cutinelli and his colleagues[41-43] investigated in detail the metabolism of acetate by *R. rubrum*. They showed that during this process some carbon dioxide was fixed and at least some of the acetate was assimilated without rupture of the carbon chain. To establish this point they used, first, unlabeled carbon dioxide and acetate labeled as follows: $C^{13}H_3C^{14}OOH$, and second, $C^{14}O_2$ and unlabeled acetate. In this way they could follow the fate not only of the individual carbon atoms involved but also of the intact acetate molecule. At the end of the experiment the cells were harvested, the protein extracted, hydrolyzed, and the amino acids separated and degraded.