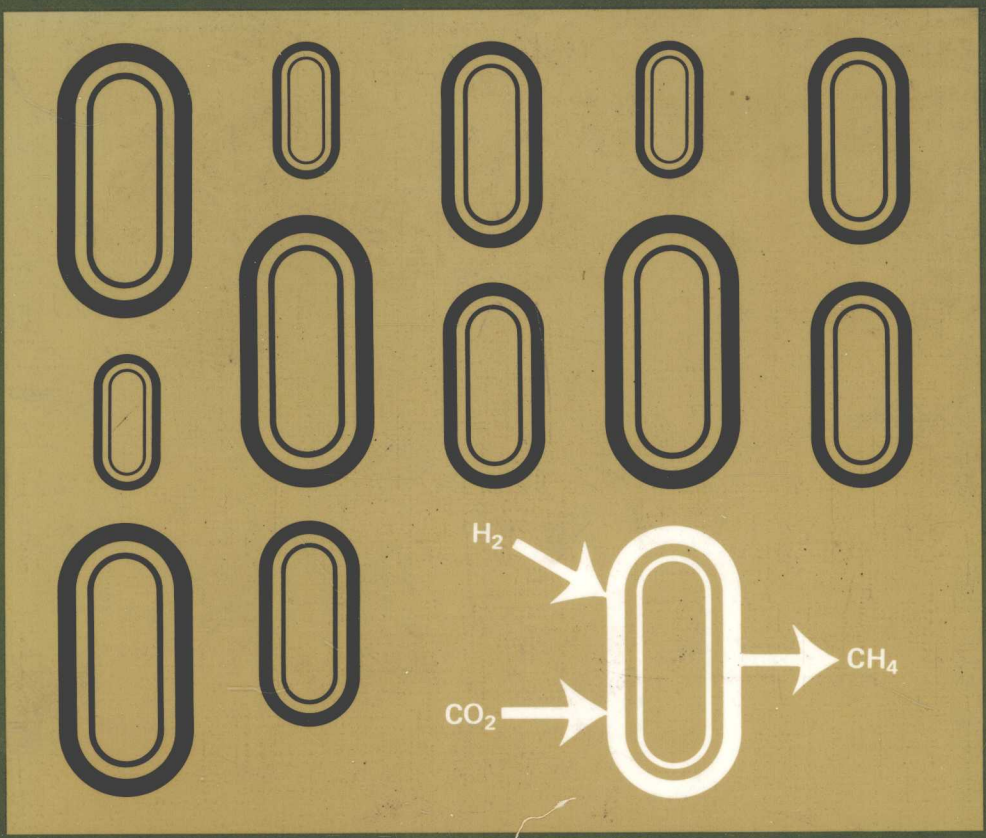

GERHARD GOTTSCHALK

Bacterial Metabolism

Second Edition

Springer-Verlag New York Berlin Heidelberg Tokyo

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With 204 Figures

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To Ellen

Preface to Second Edition

Progress in certain areas of bacterial metabolism has been rapid since the first edition of this book was published. Consequently, large parts of it had to be rewritten or extensively revised for a second edition. Some new material has also been included, sections on chemotaxis, bioluminescence, and catabolic plasmids. The use of NAD, NADP, $NADH_2$, and $NADPH_2$ as abbreviations throughout the first edition of the book has been criticized by some reviewers. The author has taken this to heart; the abbreviations for these coenzymes have been changed to NAD^+, $NADH + H^+$, etc.

Many thanks are due to J. R. Andreesen, B. Bowien, B. Friedrich (Göttingen), L. Ettlinger (Zürich), H.-J. Knackmuss (Wuppertal), H. Mayer (Freiburg), K.-H. Schleifer (München), R. Thauer (Marburg), A. Trebst (Bochum), and W. Zumft (Karlsruhe) who read certain sections of the book and made valuable suggestions; to Claudia Bechtel, Helga Grupe, and Ute Meyer for typing the manuscript and preparing the figures; to Garabed Antranikion, Michael Blaut, Armin Quentmeier, and Bernhard Möller for proofreading; and finally to the publishers for their patience and the pleasant cooperation.

It is the hope of the author that the second edition of *Bacterial Metabolism* will be as well-received as the first edition.

Göttingen, 1985 GERHARD GOTTSCHALK

Preface to First Edition

This book has been written for students who are taking a course in bacterial metabolism. I hope, however, that scholars will also find it useful either as a help in teaching bacterial metabolism or as a review on the special aspects of metabolism in bacteria.

The concept of this book results from my experience in teaching bacterial metabolism. In the first chapters the principal reactions of the energy and biosynthetic metabolism have been discussed using *Escherichia coli* as a model organism. Then the diversity of aerobic metabolism has been outlined. Following a brief description of the regulation of the level and the activity of enzymes in bacteria the characteristic features of fermentative, chemolithotrophic and phototrophic metabolism have been discussed. Finally, the last chapter has been devoted to nitrogen fixation. Throughout the text I have tried not only to describe metabolic pathways and enzyme reactions but also to elucidate the physiology of the microorganisms which carry out all these metabolic reactions.

Two comments regarding the formulas used in this book are necessary. Organic acids are usually called after the names of their salts which are shorter (formate for formic acid, pyruvate for pyruvic acid). However, in schemes and figures the formulas of the free acids are given. Furthermore, it should be pointed out that $NADH_2$ and $NADPH_2$ and not NADH and NADPH are used as abbreviations for reduced nicotinamide-adenine dinucleotide and reduced nicotinamide-adenine dinucleotide phosphate, respectively. This has been done as these compounds are two electron carriers and redox reactions involving these carriers are thus easier to formulate.

I am particularly indebted to Joan Macy, Lynne Quandt, Jan Andreesen and Peter Hillmer for reading the manuscript, for their criticisms and their suggestions, and I thank Ute Gnass for typing the manuscript and for her invaluable help with the indexing and with the preparation of the figures. Finally, I am grateful to the publishers for their patience, willing help, and cooperation.

Göttingen, 1978 GERHARD GOTTSCHALK

Contents

Chapter 1
Nutrition of Bacteria

Bacteria, like all other living organisms, require certain nutrients for growth. These nutrients must contain those chemical elements that are constituents of the cellular materials and that are necessary for the activity of enzyme and transport systems. In addition, the nutrients must provide the organisms with materials for the production of biologically utilizable energy.

I. Major and Minor Bioelements

Only a small number of the elements of the periodic system are required by organisms in relatively high concentrations ($> 10^{-4}\ M$). These 12 major bioelements and some of their functions are presented in Table 1.1. Carbon, oxygen, hydrogen, and nitrogen are the main constituents of the organic compounds occurring in organisms. Sulfur is required for the synthesis of the amino acids cysteine and methionine and of a number of coenzymes. Phosphorus is present in nucleic acids, phospholipids, teichoic acids, and in nucleotides such as ATP, GTP, NAD^+, and FAD. Potassium ions are the principal inorganic cations in the cell.

The next three major bioelements in Table 1.1 are metal ions, which are required as cofactors for enzyme activity and as components of metal complexes. Most of the biologically active phosphate esters are, for instance, present in the cell as magnesium complexes. The phospholipoproteins of bacterial cell walls and membranes are also chelated with magnesium ions. Exoenzymes such as amylases and proteases are calcium proteins, and calcium dipicolinate is an important component of endospores. Ferrous and ferric ions are present in redox carriers such as

Table 1.1. The 12 major bioelements, their sources, and some of their functions in microorganisms

element	source	function in metabolism
C	organic compounds, CO_2	main constituents of cellular material
O	O_2, H_2O, organic compounds, CO_2	
H	H_2, H_2O, organic compounds	
N	NH_4^+, NO_3^-, N_2, organic compounds	
S	SO_4^{2-}, HS^-, S^0, $S_2O_3^{2-}$, organic sulfur compounds	constituent of cysteine, methionine, thiamine pyrophosphate, coenzyme A, biotin, and α-lipoic acid
P	HPO_4^{2-}	constituent of nucleic acids, phospholipids, and nucleotides
K	K^+	principal inorganic cation in the cell, cofactor of some enzymes, e.g., pyruvate kinase
Mg	Mg^{2+}	cofactor of many enzymes (e.g., kinases); present in cell walls, membranes, ribosomes, and phosphate esters
Ca	Ca^{2+}	present in exoenzymes (amylases, proteases) and cell walls; Ca-dipicolinate is an important component of endospores
Fe	Fe^{2+}, Fe^{3+}	present in cytochromes, ferredoxins, and other iron-sulfur proteins; cofactor of enzymes (some dehydratases)
Na	Na^+	involved in various transport processes
Cl	Cl^-	important inorganic anion in the cell

cytochromes and iron-sulfur proteins. Sodium ions, in relatively high concentrations, are required by halophilic microorganisms, by methane-producing bacteria, most rumen bacteria, and many others. Na^+ seems to be involved in various transport processes. Chloride is an important inorganic anion in the cell.

Table 1.2. Minor bioelements, their sources, and some of their functions in microorganisms

element	source	function in metabolism
Zn	Zn^{2+}	present in alcohol dehydrogenase, alkaline phosphatase, aldolase, RNA and DNA polymerase
Mn	Mn^{2+}	present in bacterial and mitochondrial superoxide dismutase and in photosystem II; cofactor of some enzymes (PEP carboxykinase, re-citrate synthase)
Mo	MoO_4^{2-}	present in nitrate reductase, nitrogenase, xanthine dehydrogenase, and formate dehydrogenase
Se	SeO_3^{2-}	present in glycine reductase and formate dehydrogenase
Co	Co^{2+}	present in coenzyme B_{12}-containing enzymes (glutamate mutase, methylmalonyl-CoA mutase)
Cu	Cu^{2+}	present in cytochrome oxidase, in nitrite reductase of denitrifying bacteria and oxygenases
Ni	Ni^{2+}	present in urease, hydrogenase and in factor F_{430}
W	WO_4^{2-}	present in some formate dehydrogenases

Besides these 12 major bioelements, organisms require a number of others in small amounts (Table 1.2). Zinc and manganese ions are essential for all microorganisms. Zinc is especially important because RNA and DNA polymerase are zinc-metalloproteins. Manganese is present in bacterial and mitochondrial superoxide dismutase. It is a component of photosystem II in plants and cyanobacteria and functions as cofactor in some enzymes. Specific functions can be assigned to the other metals listed in Table 1.2. Molybdoproteins play an important role in nitrogen metabolism and in formate oxidation. Xanthine dehydrogenase also contains molybdenum. Of the selenoproteins listed in Table 1.2, the glycine reductase contains selenium in the form of selenocysteine. Cobalt is required by all organisms that perform B_{12}-dependent reactions. Copper is present in a number of enzymes transferring electrons from substrates to oxygen and in enzymes involved in denitrification. Nickel has recently been found in hydrogenase and as a Ni-tetrapyrrole compound (factor F_{430}) in methanogenic bacteria. Finally, tungsten is needed by microorganisms in some rare cases.

In nature, most of the bioelements occur as salts, and they are taken up by the organisms as cations and anions, respectively. A greater diversity of compounds utilized by microorganisms is observed only with respect to the first five elements shown in Table 1.1: sulfur, nitrogen, oxygen, hydrogen, and carbon.

Sulfur is normally taken up as sulfate, reduced to the level of sulfide, and then used for biosynthetic purposes. Certain groups of bacteria,

however, depend on the availability of reduced sulfur compounds. Methanogenic bacteria grow only in the presence of hydrogen sulfide as sulfur source. Thiobacilli and a number of phototrophic bacteria require sulfide, elemental sulfur, or thiosulfate as electron donor.

Nitrogen is required in large quantities because it amounts to approximately 10% of the dry weight of bacteria. It occurs naturally in the form of ammonia, nitrate, nitrite, nitrogen-containing organic compounds, and molecular nitrogen. The preferred source of nitrogen is ammonia, which can be utilized by practically all microorganisms. Nitrate is also taken up and used by many microorganisms but not by all. Before it can be incorporated into organic substances it has to be reduced to ammonia. Nitrite is the product of the nitrate–nitrite respiration and of the metabolic activities of *Nitrosomonas* and related species. A number of organisms reduce it to ammonia or N_2. Alternatively, nitrite can be oxidized to nitrate by *Nitrobacter* species. Several bacteria are able to fix molecular nitrogen and to reduce it to ammonia. This capacity is found only in certain prokaryotes but not in eukaryotes. Finally, organic compounds serve as nitrogen sources for many microorganisms. Usually these compounds are degraded such that ammonia becomes available for biosyntheses.

Carbon, **hydrogen**, and **oxygen** can be utilized by bacteria in the form of organic and inorganic compounds. Among the inorganic compounds used are CO_2, CO, H_2, H_2S, NH_3, H_2O, O_2, NO_3^-, and SO_4^{2-}. The number of organic compounds utilized by microorganisms is large. On earth, not a single organic compound formed by organisms is accumulated. This implies that all of them are degradable. Microorganisms play an important role in this degradation. Their versatility has led to the formulation of the "doctrine of microbial catabolic infallibility," meaning that every naturally occurring carbon compound is used by some microbe.

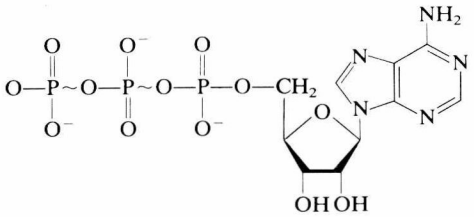
The metabolism of carbon-, hydrogen-, and oxygen-containing compounds is important not only because these elements are the main constituents of the cell. These compounds are important substrates for the energy production in microorganisms.

II. The Two Basic Mechanisms of ATP Synthesis

The principal carrier of biologically utilizable energy is adenosine-5′-triphosphate (ATP), and all energy-requiring processes in living cells are directly or indirectly coupled to the conversion of ATP to adenosine-5′-diphosphate (ADP) and inorganic phosphate (P_i):

$$\mathrm{ATP} + \mathrm{H_2O} \xrightarrow{\text{cellular processes}} \mathrm{ADP} + \mathrm{P_i}$$

ATP contains two phosphate bonds with a high free energy of hydrolysis. These bonds are often symbolized by the squiggle "~":



Because of the high-energy phosphoryl bonds, ATP is an excellent phosphorylating agent, and it is used as such in a large number of reactions by all organisms. At the expense of ATP, intermediates of cell metabolism are activated for further reactions, such as condensations, reductions, and cleavages. Glutamine, for instance, can be synthesized from glutamate and ammonia only if a phosphorylated intermediate is formed. The reaction is, therefore, connected with the formation of ADP and P_i from ATP:

$$\text{glutamate} + NH_3 + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + P_i$$

The high potential of group transfer of the AMP and the ADP group is also taken advantage of in a number of reactions; amino acids are activated by their conversion into the corresponding AMP derivatives with ATP, and AMP is released in the formation of aminoacyl-transfer RNA:

$$\text{amino acid} + \text{ATP} \longrightarrow \text{aminoacyl-AMP} + \text{pyrophosphate}$$
$$\text{aminoacyl-AMP} + \text{tRNA} \longrightarrow \text{aminoacyl-tRNA} + \text{AMP}$$

The enzyme adenylate kinase catalyzes the phosphorylation of AMP to ADP with ATP; pyrophosphate (PP_i) is hydrolyzed to inorganic phosphate by pyrophosphatase so that the end products of this reaction series are also ADP and P_i:

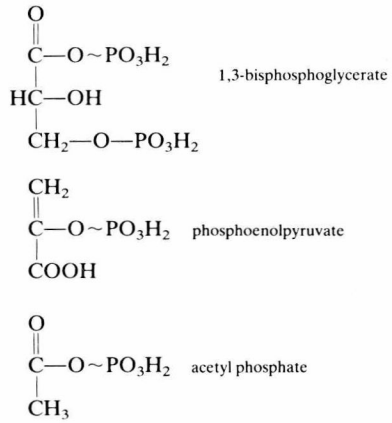
$$\text{AMP} + \text{ATP} \xrightarrow{\text{adenylate kinase}} 2\text{ADP}$$

$$PP_i + H_2O \xrightarrow{\text{pyrophosphatase}} 2P_i$$

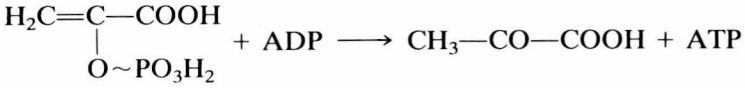
ADP and P_i are thus the principal products of the energy expenditure in metabolism, and the generation of ATP from ADP and P_i is a vital process of all living organisms. There are two basic mechanisms of ATP generation: electron transport phosphorylation and substrate-level phosphorylation.

Electron transport phosphorylation refers to a mechanism in which the flow of electrons from donors with a negative redox potential to acceptors with a more positive redox potential is coupled to the synthesis of ATP from ADP and P_i. Systems in which electron transport phosphorylation occurs are the respiratory chains and the photosynthetic apparatus, they are principally membrane-bound.

Substrate-level phosphorylation is the second mechanism of ATP generation. During the degradation of organic substrates a small number of intermediates is formed containing high-energy phosphoryl bonds. Intermediates of this kind are:



The further metabolism of such organic ~P compounds is coupled to the transfer of the phosphate group to ADP, and this kind of ATP synthesis is called substrate-level phosphorylation:



III. Nutrients as Energy Sources

It has already been mentioned that the function of the nutrients is not only to provide the organisms with the bioelements. Nutrients are also required as energy sources—as fuel for the production of ATP. Various energy sources are available in nature and are taken advantage of by microorganisms, but they cannot be used by every bacterium, and it has become useful to group bacteria on the basis of their characteristic energy source. Organisms using light as energy source are called **phototrophs** (Greek phos = light, trophe = nutrition). If ATP comes from chemical reactions, the organisms that carry out this type of energy metabolism are called **chemotrophs**.

A. Phototrophy

Phototrophs contain a photosynthetic apparatus that enables them to convert light energy into the high-energy phosphate bonds of ATP: