Bacterial Physiology and Metabolism

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PREFACE

This book was written with two purposes in mind: it was intended for use as a textbook for a graduate course in Microbial Biochemistry and as a reference source for workers in fields allied to Microbiology. The emphasis is placed on research done with bacteria with very little space devoted to principles of biochemistry which the student has covered in earlier courses. By eliminating material already covered in books probably owned by the user, the size and cost of this text becomes more reasonable.

A few words about the philosophy used in writing this book are in order. The first section, *Bacterial Physiology*, deals with non-metabolic aspects of Microbial Biochemistry (nutrition, growth and chemistry) which are important to understanding the features that set bacteria apart from other living organisms. The second section, *Energy Metabolism*, deals with ways bacteria attack foods that they find in nature, reduce them to a manageable size, ingest and metabolize them to make chemical energy. The third section, *Biosynthetic Metabolism*, deals with the formation of bacterial protoplasm beginning with simple materials such as inorganic carbon and nitrogen and leading to the formation of the complex molecules of the bacterial cell. Abbreviations used in the text are those recommended in the *Instructions to Authors* of the *Journal of Biological Chemistry*. Each enzyme is listed in the text and index by a common name followed by the name given to it by the Enzyme Commission of the International Union of Biochemistry. (The common name used is not necessarily the same one recommended by the Enzyme Commission.)

Many thanks are due to the large number of authors and publishers who graciously permitted portions of their work to be reproduced here. I feel that these figures add a flavor that would be difficult to obtain otherwise and the figures have been reproduced as they were originally published even though there may be some differences in terminology from the rules outlined in the previous paragraph. Thanks are also due to my Chairman who not only permitted but encouraged me to work on this book and provided the sort of atmosphere that allowed the writing to be done. I would also like to thank the publishers, both in London where the book was published and in New York for their expert technological assistance. Finally, but most important, I would like to thank my wife who helped with typing, proof reading and indexing and provided the expert and dedicated assistance that she did when it was needed most.

November 1968 JOHN R. SOKATCH

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Part One

BACTERIAL PHYSIOLOGY

I. NUTRITION

I. Requirements for Growth

Bacteria as well as all other living organisms require certain essential nutrients in the medium or diet in order to be able to grow. Growth is understood to mean balanced growth, that is, a uniform increase in protoplasm, as opposed to an increase in one or a few components. Essential nutrients fall into two classes, those required to supply energy for growth and those required to supply the chemical elements needed for biosynthesis. Of the various forms of energy available, bacteria can use chemical and light energy for growth. Most true bacteria use organic compounds for chemical energy, but many soil bacteria are able to produce useable energy by the oxidation of inorganic chemicals. Quantitatively, the most important elements required for biosynthesis are those found in protein, namely C, H, O, N and S. These elements may suffice in their inorganic forms or may be required in the form of organic growth factors. Many other elements are required for growth, such as Mg, K, PO_4, Fe, Cu, Co, Mn and Zn, and these are also used as inorganic salts.

II. Nutritional Classification of Bacteria

In contrast to the relatively uniform nutritional requirements of plants and animals, bacteria exhibit characteristic differences in their requirements for energy and carbon sources. A study of the distribution of nutritional types in nature suggests that this might be response to environment; for example, all autotrophic bacteria are soil and water species. A system of classification of nutritional types of organisms which was proposed by a group of prominent microbiologists (Lwoff *et al.*, 1946) forms the basis of the following discussion. Organisms were classified on the basis of energy requirement and on the basis of carbon source for biosyntheses.

Developments in microbiology since 1946 have resulted in changes in the original system of classification. For example, the terms photolithotroph and photoorganotroph are not used here as the organisms formerly grouped in these categories differ in their method of assimilation of carbon (a biosynthetic process) during photosynthesis, but not in the process of converting light energy into chemical energy. If any distinction is to be made among the phototrophs, it should be between those that cleave water during photosynthesis (green plants) and those that do not (bacteria).

Similarly, the requirement for growth factors is no longer considered distinctive enough to merit a separate category, since such a requirement could occur as the result of a single genetic change. This means that the original classification on the basis of minimum nutritional requirements becomes a classification on the basis of carbon source. The organisms formerly classified as mesotrophs are now grouped together with the heterotrophs under this heading. (See review by Guirard and Snell, 1962, for nutritional requirements of bacteria.)

A. Classification on the Basis of Energy Source

1. *Utilization of light energy*

Organisms which use light energy are phototrophs. This type of energy metabolism occurs only among green plants and certain pigmented bacteria. Both plants and bacteria convert light energy into chemical energy in the form of ATP (Arnon *et al.*, 1954; Frenkel, 1954). Most probably this occurs by a process similar to oxidative phosphorylation. Excited electrons, generated during the photochemical reaction pass along the cytochrome chain with the concomitant formation of ATP (Stanier, 1961; Fig. 13.4).

Bacterial phototrophs are all soil and water species, most of which are classified in the suborder *Rhodobacteriineae*, with the exception of the genus *Rhodomicrobium*, in the order *Hyphomicrobiales*. The taxonomic distinctions among the *Rhodobacteriineae* are based on the color of the photosynthetic pigments and the preferred type of carbon source. There are three families of *Rhodobacteriineae*, the sulfur purple bacteria, family *Thiorhodaceae*, the non-sulfur purple bacteria, family *Athiorhodaceae*, and the sulfur green bacteria, family *Chlorobacteriaceae*. Sulfur purple bacteria grow well with carbon dioxide as the sole carbon source, in which case they use inorganic sulfur compounds such as hydrogen sulfide and thiosulfate in order to reduce carbon dioxide. They can also assimilate organic carbon, however, in which case no separate reducing agent is required. Non-sulfur purple bacteria grow best with organic carbon, although some species can grow with carbon dioxide as the sole carbon source, using either molecular hydrogen or thiosulfate as the reducing agent. *Rhodomicrobium* is similar to the *Athiorhodaceae* because it requires an organic carbon source and has not yet been grown with carbon dioxide as the sole carbon source. It is separated from the other photosynthetic bacteria on morphological grounds, being a stalked bacterium. Some of the *Athiorhodaceae* are also able to grow aerobically in the dark on organic energy sources. This is true also of some species of algae but not of higher plants. The sulfur green bacteria reduce carbon dioxide with inorganic sulfur compounds. Unlike the other photosynthetic bacteria, *Chlorobacteriaceae* are not able to grow with organic carbon as the sole carbon source, although they can assimilate organic carbon to a certain extent (Sadler and Stanier, 1960).

2. *Utilization of chemical energy*

Organisms which use chemical energy for growth are chemotrophs. Chemotrophs are divided into chemolithotrophs, those which use inorganic energy sources, and chemoorganotrophs, those which use organic energy sources.

Bacteria which use chemolithotrophic energy metabolism are soil and water species in the order *Pseudomonadales*, suborder *Pseudomonadineae*. Some of the non-sulfur purple bacteria are also able to grow in the dark on inorganic energy sources such as hydrogen gas and thiosulfate (van Niel, 1944). This ability does not occur widely outside of bacteria, but strains of *Scenedesmus* and other blue-green algae can be obtained which will grow in the dark with hydrogen as the energy source (Gaffron, 1940).

Chemolithotrophic bacteria whose physiology has been studied with pure cultures can be classified into four groups on the basis of energy source used. It is possible that other physiological groups might be discovered since there are many species of bacteria in the *Pseudomonadineae* which have not been obtained in pure culture. Organisms which oxidize nitrogen compounds are classified in the family *Nitrobacteriaceae*, and these constitute the first physiological group. *Nitrosomonas*, which oxidizes ammonia to nitrite, and *Nitrobacter*, which oxidizes nitrite to nitrate, are the best studied examples of this family. These organisms are strict chemolithotrophs, since they will not grow with organic carbon energy sources. *Nitrosomonas* and *Nitrobacter* are important in maintaining soil fertility because they effect an oxidation of ammonia to nitrate, the preferred nitrogen source for green plants.

Hydrogenomonas (family *Methanomonadaceae*) represents the second group of chemolithotrophs, the hydrogen oxidizers. The ability to oxidize hydrogen occurs frequently among bacteria and occasionally among lower plants such as blue-green algae. *Hydrogenomonas* is also able to grow well with organic energy sources in contrast to *Nitrosomonas* and *Nitrobacter*.

Sulfur oxidizers comprise the third group of chemolithotrophs, most of which are classified in the family *Thiobacteriaceae*. They are able to grow on inorganic sulfur compounds such as hydrogen sulfide, elemental sulfur, thiosulfate and thiocyanate, and produce tetrathionate and sulfate as end products of the oxidation of these compounds. The species of *Thiobacillus* are the best known sulfur oxidizers. At least one species of *Thiobacillus* can grow on organic media (Santer *et al.*, 1959), but this appears to be an exceptional case.

The fourth group, the iron oxidizers, is represented by *Ferrobacillus*. *Ferrobacillus* (family *Siderocapsaceae*) obtains its energy by the oxidation of ferrous iron. This organism grows best in acid media, 3·5 being the optimum pH for growth (Leathen *et al.*, 1956).

Chemoorganotrophs satisfy their energy requirement by the oxidation or fermentation (anaerobic metabolism) of organic compounds. Chemoorganotrophy is the most common type of energy metabolism among bacteria and almost the only kind found in the animal kingdom. Chemoorganotrophy occurs in the plant kingdom among the non-photosynthetic groups such as yeast and fungi. Some species of algae are facultative chemoorganotrophs, being able to grow on organic carbon sources in the dark (Danforth, 1962).

Taxonomically, chemoorganotrophs are almost all those bacteria that have not been mentioned to this point. Rickettsia are able to oxidize a limited number of organic substrates (Moulder, 1962) and should possibly be considered as chemoorganotrophs, although they have not been grown on lifeless media as yet.

The compounds which chemoorganotrophs use for energy range from simple materials such as formate and oxalate to complex hydrocarbons such as camphor. It seems possible that any organic compound which can be oxidized to produce energy is subject to attack by chemoorganotrophs.

3. *Energy supplied by metabolism of the host cell*

Organisms which obtain energy for biosynthetic reactions from the metabolism of host cells are paratrophs. This category includes bacterial, plant and animal viruses. Although rickettsia are known to have some oxidative ability, it is possible that they may obtain part of their growth energy as paratrophs. Many of the reactions involved in supplying energy to paratrophs are those of the host cell and, from that point of view, will be covered in this book. The subject of viral replication as a separate topic will not, however, be treated here.