The Bacteria

Edited by

I. C. GUNSALUS
ROGER Y. STANIER

Volume II: METABOLISM

The Bacteria

A TREATISE ON STRUCTURE AND FUNCTION

edited by

I. C. Gunsalus
Department of Chemistry
University of Illinois
Urbana, Illinois

Roger Y. Stanier
Department of Bacteriology
University of California
Berkeley, California

VOLUME II: METABOLISM

1961

ACADEMIC PRESS • NEW YORK AND LONDON

ACADEMIC PRESS INC.

111 FIFTH AVENUE

NEW YORK 3, N. Y.

United Kingdom Edition
Published by

ACADEMIC PRESS INC. (LONDON) LTD.

BERKELEY SQUARE HOUSE, LONDON W. 1

First Printing, 1961

Second Printing, 1963

Library of Congress Catalog Card Number 59-13831

PRINTED IN THE UNITED STATES OF AMERICA

CONTRIBUTORS TO VOLUME II

H. A. BARKER, *Department of Biochemistry, University of California, Berkeley, California*

M. I. DOLIN, *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*

DAVID M. GELLER, *Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri*

I. C. GUNSALUS, *Department of Chemistry, University of Illinois, Urbana, Illinois*

MARTIN D. KAMEN, *Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts*

L. O. KRAMPITZ, *Department of Microbiology, School of Medicine, Western Reserve University, Cleveland, Ohio*

W. D. MCELROY, *Department of Biology, Johns Hopkins University, Baltimore, Maryland*

JACK W. NEWTON, *Pioneering Laboratory for Microbial Chemistry, Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois*

H. J. ROGERS, *National Institute for Medical Research, Mill Hill, London, England*

C. W. SHUSTER, *Department of Bacteriology, University of California, Berkeley, California*

LUCILE SMITH, *Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire*

W. A. WOOD, *Department of Agricultural Chemistry, Michigan State University, East Lansing, Michigan*

PREFACE

The gross metabolism of microorganisms in energy liberating reactions and pathways has for the most part followed upon the analogous studies of mammalian tissues and of yeast cells. Such studies have been made possible by the abundance of the latter materials and the inclination and affiliation of early enzymologists. In many instances the record of compounds and pathways among the vast array of microorganisms is far from complete. Nevertheless the presently available data show with abundant clarity the occurrence of fundamental differences in catalysts, pathways, and energy coupling steps among the bacteria and between these organisms and mammalian and yeast cells. If the present volume makes evident the gaps and incongruities in knowledge which helps to foster clarification of the actual properties of the individual types of bacteria, the impetus for the present "source book" aspect of this volume will fulfill the objective of the authors and editors.

The variation in pathways among the bacteria and the quantitative preponderance of these pathways under different conditions has in many cases served as a refined tool for the recognition and clarification of processes relatively minor in other cells. One may hope that a view of current understanding, usually of a role in energy supply pathways frequently resulting from enrichment methods of isolation, can be useful to mature investigators and to the growing body of students whose education and research is fostered by the ready availability of such information.

An informed concept of energy liberating patterns is important to a critical appraisal of the biosynthetic pathways and growth phenomena in bacteria now assembled in volumes 3 and 4 of this series. This is true in the sense of reaction types, the raw materials furnishing the monomers for cell structure, and as a basis for an understanding of the limiting factors in various aspects of growth. The rapid rate at which information accumulates in this area should not outdate present viewpoints if the generally held concept of the state of completeness of the data and the validity of the ideas of basic principles considered to prevail in metabolism as related to cell behavior are at all accurate. Thus with the main emphasis shifted to investigations of other aspects of cellular behavior, one may expect for energy metabolism that gaps in knowledge will eliminate gradually, and some hypotheses altered, but the bases for reasoning will, in the main, be sustained.

The editors are appreciative of this opportunity to thank the authors of this volume for their cooperation and patience in the removal of partial

overlap among the chapters and with the difference in rate in which some of the chapters became available. Again we have refrained from suggesting style or choice of material to the authors on the thesis that the selection of material and the freshness of viewpoint of each author is of far greater value than any loss from lack of uniformity. We also wish to express at this time our appreciation to the publishers and to the members of their staff for constant help and encouragement in the many tasks accompanying the assemblage and preparation of this volume.

I. C. GUNSALUS
R. Y. STANIER

February 1961

CONTENTS OF VOLUME II

CHAPTER 1

Energy-Yielding Metabolism in Bacteria

I. C. GUNSALUS AND C. W. SHUSTER

I. Metabolism and the Cell*

Fermentation, oxidation, and photosynthesis (light-driven reactions) compose the quantitatively major portion of cellular metabolism. They are also the principal sources of cellular energy supply. The presence in cells of large amounts of catalysts and intermediates of these pathways has simplified both the recognition of the major energy-mobilizing reactions and formulation of the main pathways of carbon and energy flow. Participation of the microbe as experimental material in this advance has permitted a par-

* Abbreviations used in this chapter: DPN, DPNH—Diphosphopyridine nucleotide, reduced; TPN, TPNH—Triphosphopyridine nucleotide, reduced; ADP, ATP—Adenosine diphosphate, Adenosine triphosphate; IDP, ITP—Inosine diphosphate, Inosine triphosphate; DPT—Diphosphothiamine; CoA—Coenzyme A; PEP—Phosphoenolpyruvate; KDPG—2-Keto-3-deoxy-6-phosphogluconate; FP, $FP_{red.}$—Flavoprotein, reduced; fH_4—Tetrahydrofolic acid; N^{10}-formyl-fH_4—N^{10}-Formyltetrahydrofolic acid; N^5,N^{10}-methenyl-fH_4—N^5,N^{10}-Methenyl tetrahydrofolic acid; N^5,N^{10}-methylene-fH_4—N^5,N^{10}-Methylene tetrahydrofolic acid; RNA—Ribonucleic acid; DNA—Deoxyribonucleic acid; Pi—Inorganic (ortho) phosphate; HMG-CoA—Hydroxymethylglutaryl CoA; Glucose-U-C^{14}—Uniformly labeled glucose-C^{14}.

tial visualization of the energy-yielding and material flow machinery serving each of a wide variety of microorganisms. While not complete, this knowledge is sufficiently advanced to provide a chemical basis for comprehending the properties and behavior of specific microbial species. The unity of material and processes[1] in living cells has been most useful in guiding initial studies of little known organisms. Also, the elucidation of new reactions and pathways becomes easier as the recorded cases of systems nature has found workable (thermodynamically probable), both for making energy available biologically and for making essential metabolites, are extended.

Fortunately, the principles and reaction types found in elucidating the energy-furnishing pathways have proved useful in guiding the study of biosynthetic reactions and whole cell investigations of active transport, adaptation, growth and its control, and to a more limited extent, in understanding the chemical changes which accompany modification of genetic characteristics by mutation, transformation, etc. The plan of Volumes II, III, and IV of the present sequence follows the chronology of knowledge accumulation and the context of its application to biological problems. This places the quantitatively major energy transformations in Volume II, the chemistry of the biogenesis of cellular components in Volume III, and the biology and chemistry of growth and general physiology—the coupling of the energy metabolism and biosynthetic reactions and adding of the restrictions of biological behavior—in Volume IV. Volume V, dealing with heredity, will employ the principles and data of Volumes II to IV to an extent dictated by the moderate penetration of molecular understanding into the information and code systems of biology.

The function of the present chapter is to consider problems of energy metabolism which apply to all cells and to ask how far we have progressed, and can progress, in relating this information to the problems of the individual cell—bacteria being, in the main, unicellular organisms. The viewpoint is one of optimism that energy (equilibria), specificity and molecular interactions can tell more of cellular behavior and its control than is now understood. The principal questions concern the quantitative relationships of biologically available energy released by glycolysis, oxidation, and light to chemical bond transformation to whole cell requirements. These questions are actually asked of the data presented in subsequent chapters of this volume.

Undoubtedly the present chapter will raise more questions than it will answer, for the properties which suit the microbe to the solving of pertinent biological problems ask questions in many areas and call for an excellence in many disciplines, not all of which have become the common property of all investigators of the many microorganisms in nature. Bacteria show an increasing ability to attract investigators from a broad area of phys-

ical and biological sciences; the knowledge and skills thus acquired greatly enrich the science of microbiology and the life of the microbiologist, student and investigator. It is with the objective of contributing to the ease of communication and more effective cooperation of multiple attacks on biological and chemical questions with which the microbe can deal that this attempt at an orderly relating of development and status is made.

A. Progress and Problems

Excellent reviews, both critical and authoritative, concerning recent progress in understanding the energy metabolism of bacteria are available; reference to these is made both in this chapter and in the following chapters. In addition, the following chapters present the data in the perspective of their growth and relationship to the microbic processes in recapitulation and evaluation as a basis for further study. Some subjects are, for the moment, relatively complete; a few are changing rapidly; and others, e.g., oxidative energy coupling, barely opened at the chemical level. The specialist can expect to find little beyond a current summary in the area of his immediate interest. It is for the microbiologist with pressing preoccupation in other areas of the subject, the nonmicrobiologist seeking a convenient tool to explore and/or analyze a biological or chemical question, the students, young and old, that the statement of progress and problems is intended.

In biology, the *concept of unity* and the *principle of variety* in relation to structure and function have provided a viewpoint with which to evaluate, explore, and experiment. Kluyver and van Niel,[2] in 1956, attributed to the microbe a major role in extending our insight into the essence of metabolism "... owing mainly to its impressive metabolic diversity." Thus Kluyver voiced, near the end of his career, his belief in the *principle of variety* as a biological factor among organisms affording a tool to solve problems.

Thirty years earlier, the *concept of unity* arose from Kluyver's recognition as an underlying principle, in the apparent confusion of biological oxidation, of the uniformity among organisms of the mechanisms of hydrogen transport which, by a series of single-step reactions, accomplish biological energy release. Based on a common material substrate, a common reaction sequence was seen to occur in all cells. With this insight, Kluyver had founded comparative biochemistry.[1] These two principles, *unity* and *variety*, underlie the utility of the microbe as a tool for chemical and biological investigation. On their validity rest the general principles elaborated via study of microbial systems. (An excellent account of Kluyver's contributions written by van Niel may be found in the recounting of Kluyver's life.[4])

With respect to comparative biochemistry, it might be appropriate here to urge the student to consider now the variety, perhaps the "uncompara-

tive," of biochemistry—those details of fine structure wherein reside specificity, uniqueness, and the genetic differences which underlie the metabolic differences. Today, comparative biochemistry is as valid as the day Kluyver conceived this generalization; the only change has been the documentation of the hypothesis as a working principle in nature. Knowledge of many an obscure organism became possible because Kluyver had suggested borrowing data from the better documented cases in order to make a start. The need to teach these principles on which to build will continue. The counsel to look for variety is the urge to seek still other hypotheses to guide future investigations and to uncover the next valid and useful generalizations.

Many of the reactions and routes of supply for biologically available energy are known, and an estimate of the magnitude of the remaining problem is possible. The pattern needs to be completed and further analysis made of the mechanisms of action of catalysts as reactants and of "concerted reaction mechanisms," along with other problems. More pressing now, perhaps, are the problems applied to the cell: a reappraisal of the knowledge and its application to metabolism at a cellular level. Among these definable cellular problems are: (1) the availability of substances as substrates based on catalysts for their uptake and turnover at rates compatible with cellular needs, (2) equilibria of sufficient driving force to release free energy for cellular function, (3) coupling mechanisms to convert the available energy to the manifold work functions of the organism, and (4) the control of coupling, rates, and specificity to reproduce the cell and/or perform its work and maintenance functions.

B. Fitness of the Microbe

As an investigative tool, the microbe may well be judged by its contributions made to metabolism; as such, the record is impressive. Yeasts contributed through the battles of Pasteur and Liebig; they have continued to serve modern biochemistry. Highly remembered, as described in the first chapter of Harden, "Alcoholic Fermentation,"[5] are demonstration of cell-free glycolysis in yeast pressed juice (enzyme extracts) (Buchner[6]), discovery of coenzymes, coenzyme I [diphosphopyridine nucleotide (DPN)], yeast carboxylase acting on pyruvate with diphosphothiamine (DPT) as coenzyme,[7] and the identification of the phosphorylated intermediates of glycolysis, hexosediphosphate, and hexosemonophosphate (see Meyerhof[8]).

An equal or even more impressive list derives from the bacteria. *Pseudomonas saccharophila*, via Doudoroff, contributed sucrose phosphorylase,[9] glucosyl transfer, and the formation of multiple disaccharides.[10] Later, 2-keto-3-deoxy-6-phosphogluconic acid and its aldolase,[11] and a direct route (carbon chain intact) from pentose to ketoglutarate were shown.[12,12a]

β-Glucose-1-phosphate as a biological intermediate of maltose phosphorolysis and as a step in the formation of α-glucosido-xylose was later added by *Neisseria*.[13] The propionic acid bacteria contributed CO_2 as a heterotrophic metabolite in net fixation (Wood and Werkman[14]), which opened a new era of intermediary metabolism. Recently, their use has shown the second function of B_{12}-coenzyme in carbon chain rearrangement; the movement of the coenzyme A (CoA)-bound carboxyl in the succinyl-methylmalonyl-coenzyme A isomerase;[15] and the role of biotin in transcarboxylation to form propionate in a cyclic nonenergy-requiring system.[16] The clostridia, principally through the efforts of Barker,[17] served to clarify the role of coenzyme A esters in fatty acid oxidation[18] and the function of vitamin B_{12} in coenzyme form[19] as catalyst of carbon chain transfer, from glutamate to β-methyl aspartate.[20] Clostridia also contributed the role of tetrahydrofolic acid (fH_4) in formimino[21] as well as formyl transfer in the generation of phosphate anhydrides.[22] The lactic acid bacteria contributed active acetyl (acetyl phosphate),[23] induced (adaptive) enzyme formation,[24] the existence of lipoic acid,[25] and its role in acyl generation[26] from keto acids, which also opened new approaches to keto acid metabolism.[27] In vitamin B_6 metabolism, these bacteria gave a clue to its active form,[28] coenzyme form,[29] and metabolic role.[30, 31] As auxotrophs resembling mammals in their nutritive requirements, the lactic acid bacteria[32] led to a demonstration of the general synonomy of bacterial growth factors and vitamins (further example of comparative biochemistry) which fostered rapid multiple vitamin assays[33] and the discovery, isolation, and relation to metabolism of a series of vitamin-cofactor prosthetic group substances (see reference 34). *Escherichia coli* contributed extensively to current views of induced enzyme formation,[35] initiated microbial genetics as a study,[36] placed virus studies on a quantitative basis;[37] the related salmonella coupled virus infection and genetic information transfer.[38] Understanding of the role of deoxyribonucleic acid (DNA) in transformation of pneumococci[39] opened the way to new genetic concepts and their chemical implications. Genetic-chemical progress in biological polymer formation has been supported heavily by the microbes: ribonucleic acid (RNA) reactions (RNA-nucleotide diphosphate) by *Azotobacter vinelandii*,[40] DNA in enzyme induction,[41] DNA formation (DNA-nucleoside triphosphate),[42] protein biogenesis by staphylococci[43] and *E. coli*,[44] and amino acid activation by *E. coli*.[45]

This representative but not inclusive list illustrates the extent and scope of indebtedness to microbes for metabolic data and raises the question of the sources of this effectiveness. The answer is not far to seek. It includes (1) speed, (2) variety, (3) adaptability, (4) specificity, and (5) ecological diversity, to list five worthy of brief amplification.

1. Speed and Yield

The high metabolic rate of microbes can be illustrated at many levels; let us take but two examples, growth rate and enzymic activity in extracts. Generation time (time to double protoplasm) approaches 15 minutes in several heterotrophic bacteria, e.g., *E. coli*,[46] *Clostridium welchii*,[47] and *Streptococcus faecalis*.[48] The doubling time for mammalian cells in tissue culture approaches one day (24 hours), thus, a rate advantage of about 100-fold favoring the microbe, i.e., 24 hr. $\times$ 60′/15′ = 96. The cause is not clear, although one could cite correlations of growth rate with size,[49] surface/volume ratio, and ratio of genetic material to cytoplasm.

A comparison of metabolic rates of *whole cells* (dry weight) yields similar figures for both respiration and glycolysis (see Table I). The values correlate inversely with the size relationships[49] as do all the above characteristics (bacteria/muscle cell = 10^2; bacteria/yeast = 10^1). A similar rate advantage is observed with *soluble enzymes* and *enzyme systems*, expressed as activity per unit weight or amount of protein (see Table II). In the latter case, one could attribute the higher specific activity to smaller enzyme (lower molecular weight per active site), higher turnover number (TON) (higher catalytic activity per active site or more active sites per enzyme), fewer enzymes per cell (higher per cent of protein in each, or given enzymes), or less padding with unessential material. The source of higher activity in two cases of energy pathway enzymes is attributable to more enzyme per cell: β-galactosidase,[50] 6 % of soluble cell protein, and formyl kinase[51] crystalline enzyme after 10-fold purification from cell extract.

TABLE I

Relative Size and Metabolic Quotients

Organism or tissue	Cell volume, cm.^{3}	Q_{O_2}[a]	Reference	Q_G[b]	Reference
Rat liver	—	9	228	0.15	228
Rat brain	—	14	228	0.9	228
Saccharomyces cerevisiae	10^{-10}	40–80 (glucose)	228	3.0	229
Escherichia coli	10^{-12}	800 (acetate)	230	19	231
Azotobacter vinelandii	10^{-12}	4200 (acetate)	232	[c]	—
Streptococcus faecalis	10^{-12}	186 (pyruvate)	233	13	225

[a] Q_{O_2} = μl./mg. dry wt./hr.

[b] Q_G = μmoles glucose utilized/mg. dry wt./hr.

[c] Anaerobic, no glycolysis.

TABLE II

RELATIVE ENZYME ACTIVITIES OF BACTERIAL AND TISSUE EXTRACTS

Enzyme or system	Bacteria	Specific activity[a]	Reference	Tissue	Specific activity	Reference	Ratio
Pyruvic oxidation	*Escherichia coli*	0.7	234	Pig heart	0.31	235	2.2
Pyruvic oxidation	*Proteus vulgaris*	2.9	236	Pigeon breast muscle[b]	0.1	237	29
α-Ketoglutarate oxidation	*Escherichia coli*	0.62	234	Pig heart[b]	0.23	238	2.7
					0.003	239	207
Succinic thiokinase	*Escherichia coli*	3.0	240	Spinach	0.013	242	230
	(succinate-adapted)	58	241	Spinach	0.013	242	4460
Amino acid incorporation (leucine)	*Escherichia coli*	0.03	44	Liver	0.0016	243	19
Butyryl-CoA dehydrogenase	*Clostridium kluyveri*	0.4	17	Liver	0.001	244	400

[a] Specific activity = μmoles/mg. protein/hr.

[b] Extract of acetone powder.

2. VARIETY AND SPECIFIC SELECTION

The variety of compounds which serve as carbon and energy sources for some microbes is almost without limit[52] (see Chapter *5*, p. 258). The working hypothesis of the general microbiologist, experimentally applied in the enrichment, or elective culture, method of Beijerinck, has an excellent record of accomplishment. The proposition as usually stated is: any compound which can react with a negative free energy change ($-\Delta F$) is a potential energy source for some organism, or as frequently stated in more restricted form: any organic compound in nature is broken down by some organism with the return of carbon to the atmosphere. Thus organisms can be isolated by selective enrichment on diverse carbon sources (Chapter *5*, p. 260). The metabolic rates on these sources will be high in consequence of their function in the energy release routes. Examples of the use of carefully selected enrichments to solve important metabolic problems, frequently by enhanced enzyme abundance, are well represented among Barker's contributions to microbiology and biochemistry.[17] To list a few: both purines and glycine led to folic acid-mediated energy release systems in *Clostridium acidi-urici*,[21] and *Clostridium cylindrosporum*;[22] glutamic acid fermentation led to the role of B_{12}[19] (see Chapter 3), and

ethanol-acetate to fatty acid oxidation. The oxidation of aromatic compounds provides an excellent example of the use of unique carbon compounds which permit the recognition of induced enzymes independently of enzymes which are always present to transfer essential metabolites; see Stanier,[53] Evans,[54] or the review of Elsden and Peel.[55] Further specific examples of selection for specific activities and variability of pattern can be found in any general microbiology text,[56] publications of the Delft school of microbiology,[57] or survey of microbic activities.[17, 52]

3. Adaptability

With a given strain selected for its metabolic potential, catalytic activity can be increased many fold by added substrates for enzyme induction by physiological conditions of culture.[58-60] Vitamin level,[61] conditions of pH,[62] and aerobiosis,[63] to mention a few of the latter, are also determinative. Examples could be extended; they will not be cited here, but are discussed where pertinent in other parts of this chapter. As pointed out by Monod,[64] in reference to bacterial growth, these are not subjects of study but the tools of the science.

4. Specificity

At the enzyme level, present data do not indicate the superiority of one organism over another in substrate range or specificity of the enzymes formed. In contrast, at the cellular level, the specific activity, and therefore relatively lower level of side reactions, can be greatly altered through both selection (Section I, B, 2), and adaptation (Section I, B, 3). These changes have been most helpful in tracing pathways and in the further purification of enzymes, i.e., the purification is simplified because of high enough protein[50, 51] (or system[65]) concentration for their physical properties to exert an effect. Also, purification can be accomplished with smaller, and thus manageable, amounts of material. Enhanced enzyme stability not directly attributable to this cause has been observed in several instances. The overlap in methodology to gain the advantages indicated under headings 2, 3, and 4 is apparent.

5. Carbon vs. Energy Economy of Cells

The Doudoroff hypothesis[66] concerns the limiting factor in natural ecological conditions for aerobic and for anaerobic organisms, or conditions of growth. In this view, the limitation during anaerobic growth is energy; during aerobic growth, carbon. Complicated as are the metabolic interactions in whole cells, two primary causes would seem to account in large measure for these conditions: (1) in glycolysis, a low energy yield per sub-