microbiology series

volume 3

Microorganisms and Minerals

Microorganisms and Minerals

edited by

EUGENE D. WEINBERG

Department of Microbiology
and Program in Medical Sciences
Indiana University
Bloomington, Indiana

MARCEL DEKKER, INC. New York • Basel

7

Library of Congress Cataloging in Publication Data
Main entry under title:

Microorganisms and minerals.

(Microbiology series ; v. 3)
Includes bibliographical references and indexes.
1. Micro-organisms--Physiology. 2. Mineral metabolism. I. Weinberg, Eugene D.
QR92.M5M53 576'.11'9214 76-53191
ISBN 0-8247-6581-8

MARCEL DEKKER, INC.

270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE

Textbooks of microbiology generally devote few paragraphs to the critical roles of minerals in the growth, differentiation, and death of microbial cells. Moreover, many microbiologists assume that either intact hosts or tissue extracts automatically provide microorganisms with correct qualitative and quantitative balances of minerals in available forms. We hope that the present book will persuade the reader that individual minerals not only have unique functions but must be acquired, stored, and cycled by microbial cells with care and precision. Indeed, all persons who wish successfully to detect, propagate, exploit, or destroy microorganisms should keep aware of the levels and balances of minerals and of organic compounds that bind or transport minerals in the systems with which they are working.

The reader is referred to the Introduction on pp. 1–4 for a general overview of the chapters in this volume.

Eugene D. Weinberg
Bloomington, Indiana

In memory of

HENRY A. SCHROEDER

Dr. Schroeder was for many years Professor of Physiology at Dartmouth Medical School and had built and operated a Trace Element Laboratory at Brattleboro, Vermont. His numerous and systematic publications on each of several dozen elements plus his leadership in trace element research and applications were major factors in the worldwide establishment of trace element biomedical research programs and awareness of health applications since 1955. Although not a microbiologist, Dr. Schroeder was quite cognizant of microbial roles in mineral cycling, and he was always enthusiastic about the use of microorganisms as models in cell biology.

CONTRIBUTORS

Jean E. L. Arceneaux, Department of Microbiology, University of Mississippi Medical Center, Jackson, Mississippi

B. Rowe Byers, Department of Microbiology, University of Mississippi Medical Center, Jackson, Mississippi

Mark L. Failla,* Department of Microbiology, Indiana University, Bloomington, Indiana

William O. Foye, Department of Chemistry, Massachusetts College of Pharmacy, Boston, Massachusetts

Paula Jasper, † Department of Biology, Washington University, St. Louis, Missouri

Ivan Kochan, Department of Microbiology, Miami University, Oxford, Ohio, and Department of Microbiology and Immunology, Wright State University School of Medicine, Dayton, Ohio

Walter A. Konetzka, Department of Microbiology, Indiana University, Bloomington, Indiana

Simon Silver, Department of Biology, Washington University, St. Louis, Missouri

Michael R. Tansey, Department of Plant Sciences, Indiana University, Bloomington, Indiana

Eugene D. Weinberg, Department of Microbiology and Program in Medical Sciences, Indiana University, Bloomington, Indiana

---

Present Address

*Department of Nutrition, Cook College, Rutgers University, New Brunswick, New Jersey

†Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri

ABBREVIATIONS

AAS	atomic absorption spectroscopy
5′-AMP	adenosine 5′-monophosphate
ATP	adenosine triphosphate
CCCP	<u>m</u>-chlorophenyl carbonylcyanide hydrazone
CD	circular dichroism (spectroscopy)
cyclic AMP	adenosine 3′:5′-cyclic monophosphate
cyt	cytochromes
DNP	2,4-dinitrophenol
DTNB	5,5′-dithiobis(2-nitrobenzoic acid)
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
EGTA	ethyleneglycol-bis-(β-aminoethyl ether)-N′ tetraacetic acid
FAD	flavin adenine dinucleotide
FCCP	p-trifluoromethoxy carbonylcyanide phenylhydrazone
HEPES	N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid
K_i	inhibition constant
K_m	concentration for half-saturation of rate
mRNA	messenger ribonucleic acid

NAD	nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NEM	N-ethylmaleimide
ORD	optical rotary dispersion
pCMB	p-chloromercuribenzoate
PHA	phytohemagglutinin
P_i	inorganic phosphate
PIPES	piperazine-N,N′-bis(2-ethanesulfonic acid)
Q_{10}	temperature coefficient
SDS	sodium dodecylsulfate
V_{max}	maximum velocity of uptake

MICROBIOLOGY SERIES

Series Editor
ALLEN I. LASKIN
EXXON Research and Engineering Company
Linden, New Jersey

Volume 1 Bacterial Membranes and Walls
edited by Loretta Leive

Volume 2 Eucaryotic Microbes as Model Developmental Systems
edited by Danton H. O'Day and Paul A. Horgen

Volume 3 Microorganisms and Minerals
edited by Eugene D. Weinberg

Other Volumes in Preparation

CONTENTS

Preface		v
List of Contributors		vi
INTRODUCTION, Eugene D. Weinberg		1
Introductory Note to Chapters 1, 2, and 3, Simon Silver		5
Chapter 1		
MAGNESIUM TRANSPORT IN MICROORGANISMS Paula Jasper and Simon Silver		7
I	Introduction	8
II	Magnesium in the Cell	8
III	Magnesium Transport in Intact Bacterial Cells	12
IV	Transport of Magnesium by Other Microbes	30
V	Nonmicrobial Systems	35
VI	Summary	39
	References	40
Chapter 2		
CALCIUM TRANSPORT IN MICROORGANISMS Simon Silver		49
I	Introduction	50
II	Calcium Transport in Intact Bacterial Cells	52
III	Studies with Subcellular Membranes	53
IV	Calcium Accumulation During Sporulation	60
V	Calcium-Binding Factors	65

VI Eucaryotic Microbes 65
VII Bacterial Functions of Calcium 69
VIII Nonmicrobial Model Systems 76
IX Summary 92
References 93

Chapter 3

MANGANESE TRANSPORT IN MICROORGANISMS 105
Simon Silver and Paula Jasper

I Introduction 106
II Manganese Requirements and Functions 106
III Manganese Transport Systems 122
IV Microbes Other than Bacteria 134
V Nonmicrobial Systems 136
VI Summary 139
References 140

Chapter 4

ZINC: FUNCTIONS AND TRANSPORT IN MICROORGANISMS 151
Mark L. Failla

I Introduction 152
II Molecular and Cellular Functions of Zinc 153
III Zinc Transport 181
IV Zinc Storage 197
V Conclusion 198
Recent Developments 199
References 200

Chapter 5

MICROBIAL TRANSPORT AND UTILIZATION OF IRON 215
B. Rowe Byers and Jean E. L. Arceneaux

I Introduction 216
II Microbial Iron Transport Cofactors (Siderochromes) 217
III The Transport Mechanisms 223
IV Metabolic Functions of Iron 237
Recent Developments 239
References 244

Chapter 6

ROLE OF SIDEROPHORES IN NUTRITIONAL IMMUNITY AND BACTERIAL PARASITISM 251

Ivan Kochan

I Introduction 252
II Iron Starvation of Microbes in Tissues and Fluids of Normal Animals 253
III Iron Starvation of Microbes in Tissues and Fluids of Immune Animals 266
IV Competition for Iron Between Iron-Binding Compounds of Animal and Microbial Origin 271
V Relationship Between Siderophores and Microbial Virulence 279
VI Role of Iron in Nutritional Immunity and Microbial Parasitism 280
VII Recent Developments and Concluding Comments 281
References 285

Chapter 7

MINERAL ELEMENT CONTROL OF MICROBIAL SECONDARY METABOLISM 289

Eugene D. Weinberg

I Introduction 290
II Proposed Functions of Secondary Metabolism 292
III Environmental Control of Secondary Metabolism 296
IV Conclusions 310
Recent Developments 311
References 311

Chapter 8

MICROBIOLOGY OF METAL TRANSFORMATIONS 317

Walter A. Konetzka

I Introduction 317
II Mercury 318
III Selenium and Tellurium 323
IV Iron 328
V Manganese 332
VI Arsenic 334
VII Concluding Remarks 335
References 337

Chapter 9

MICROBIAL FACILITATION OF PLANT MINERAL NUTRITION 343
Michael R. Tansey

I Introduction 343
II Mycorrhizae 344
III Lichens 356
IV The Phyllosphere 365
V Summary and Conclusions 374
Recent Developments 375
References 376

Chapter 10

ANTIMICROBIAL ACTIVITIES OF MINERAL ELEMENTS 387
William O. Foye

I Antimicrobial Activities of Copper, Zinc, Mercury, and Silver Ions 387
II Metal Ion Activation by Chelation 397
III References 415

AUTHOR INDEX 421
SUBJECT INDEX 463

INTRODUCTION

Eugene D. Weinberg

> What we learn from Nature's smallest living things often emerge as principles of general biology. [From Ref. 1.]

> It is clear that the initial inorganic matrix in the earth's crust has left its stamp, because metals are still involved in every aspect of biosynthesis, degradation, and macromolecular assembly. [From Ref. 2.]

The scientific practice of microbiology is presently entering its second century. Well before the beginning of this practice, however, mineral elements were employed to influence microbiological processes. Since early medieval times, for example, mercury and its salts have been important therapeutic agents for skin and intestinal infections. The remarkable selective toxicity of silver for microorganisms was utilized by physicians as early as the 1500s, three centuries prior to an understanding of the microbial etiology of infectious disease. A proper balance of inorganic elements in soils and waters has long been recognized to be a critical factor in cultivation of plants from which desirable products of microbial fermentation are to be obtained.

Nevertheless, identification of the precise roles of minerals in the physiology of microbial cells occurred slowly and sporadically during the first century of scientific microbiology. For example, at the start of the century, Raulin, a student of Louis Pasteur, reported that zinc is required for growth of fungi [3]; in the 1940s, industrial scientists became aware of the importance of this metal in mycotic fermentations. Only within the past decade, however, have microbiologists begun to examine intensively the many metabolic aspects of zinc in microbial systems.

Although, as will be evident in the book, knowledge concerning molecular aspects of mineral roles and requirements has been gained relatively recently, a few significant discoveries were made in the early 1930s. At that time, for example, molybdenum was discovered to be needed for microbial nitrogen fixation [4]. It also became apparent in that decade that the quantity of a metal that is needed and/or tolerated for growth of microbial cells can be quite different from that required and/or permitted for the organisms to carry on a specific physiologic process [5-7]. This important principle of quantitation has only recently been extrapolated to cells of plants and animals [8]. Also recognized in the 1930s and 1940s was the attempt by vertebrate hosts to withhold growth-essential iron from invading microorganisms [9,10]. This phenomenon, now termed "nutritional immunity" [11,12], is finally achieving well-merited, widespread attention.

A number of books [e.g., 8,13-21] have appeared within the past decade on the roles of mineral elements in living matter. In each of these, plants and/or animals have been the focus of concern. This is the first book on biological aspects of a spectrum of mineral elements that is devoted primarily to microorganisms.

The subject matter of the book is divided into four topics. The first concerns the manner in which microbial cells acquire and store selected mineral elements. This topic is important for several reasons. Its study contributes knowledge at the cellular level on mechanisms, active compounds, sites, and homeostatic regulation of acquisition of growth-essential nutrilites. Moreover, specific metal-transport and possibly metal-storage compounds that are being and will be isolated have actual or potential utility in prophylaxis and therapy of metabolic and infectious diseases of animals and plants. Additionally, knowledge of the ability of some intestinal microorganisms to facilitate and others to retard assimilation of essential mineral elements by cells of the gut wall is of potential importance in human and animal nutrition.

Such mineral elements as magnesium, calcium, manganese, zinc, iron, silicon, potassium, cobalt, copper, and molybdenum are important in the life of microbial cells. Because of space limitations and because the first five have been most intensively studied, only these five will be discussed here (Chapters 1 to 5). A review of the momentous competition for iron between invading microorganisms and animal hosts is contained in Chapter 6.

The second topic is concerned with the functions of magnesium, calcium, manganese, zinc, and iron in microbial cells. As with cells of plants and animals, the catalytic function of minerals in microorganisms has become well recognized within the past three decades. The structural and stabilizing functions of minerals in microbial cells presently comprise exciting areas of research. The various roles of the five selected elements in microbial systems are discussed in Chapters 1 to 5 and, where pertinent, are compared with plant and animal systems. A possible novel function, thus far studied mainly in microorganisms, is that of the ability of a few mineral elements (e.g., manganese, zinc, iron) to induce and suppress the processes of secondary metabolism and differentiation (Chapter 7). Comparisons of mineral requirements for growth with those needed for specific functions in plant and animal cells are only now beginning to be made.

The third topic, microbial roles in cycling of mineral elements, is divided into two aspects: geochemical (Chapter 8) and phytochemical (Chapter 9). The first deals with microbial conversion of elements between inorganic and organic forms and among their various oxidation states. The second aspect concerns the role of microorganisms in facilitating assimilation of minerals in plants. Each aspect is indispensable to the economy of life on earth.

The fourth topic is that of the antimicrobial action of mineral elements (Chapter 10). Not only are silver, mercury, copper, and zinc still employed to suppress and kill unwanted microorganisms, but metals are now also important activators of various organic antimicrobial agents.

Within the past half century, the doctrine of the biochemical unity of living matter has become well established. Metabolic pathways as well as methods of storage, transfer, and use of energy and of genetic information are found to have strong similarity in the cells of microorganisms, plants, and animals. The qualitative and quantitative equivalencies in requirements for and roles of minerals in these three forms of life are examples of such unity. As we will see, in some cases principles of mineral aspects of biology were learned first with cells of higher organisms and then applied to microbial cells; in others, microorganisms served as model systems in inorganic cell biology.

But within the overall theme of bioinorganic unity lie variations of fascinating diversity. Sufficient examples of uniqueness in the metabolism and roles of minerals exists in specific groups of microorganisms to provide a wealth of productive areas of research. As importantly, the examples of uniqueness described in this book may suggest potentially useful and novel methods for (a) environmental enhancement of desirable microbial groups; (b) suppression of microbial pests; (c) microbial detoxification of poisoned local environments; and (d) microbial accumulation of elemental metals. Thus we hope that the material will be useful not only to bioinorganic scientists but also to environmental, medical, molecular, and physiological microbiologists.

REFERENCES

1. J. W. Foster, First Henrici Memorial Lecture, Department of Microbiology, University of Minnesota, Minneapolis, 1963.
2. J. M. Wood, in Biochemical and Biophysical Perspectives in Marine Biology (D. C. Malins and J. R. Sargent, eds.) vol. 3, Academic Press, New York, 1976, p. 408.
3. J. Raulin, Ann. Sci. Nat. Botan. Biol. Vegetale, 11, 93 (1869).
4. H. Bortels, Arch. Mikrobiol., 1, 333 (1930).
5. A. Locke and E. R. Main, J. Infect. Dis., 48, 419 (1931).
6. E. D. Weinberg, Persp. Biol. Med., 5, 432 (1962).

7. E. D. Weinberg, Advan. Microb. Physiol., 4, 1 (1970).
8. H. A. Schroeder, The Trace Elements and Man, Devin-Adair, Old Greenwich, Connecticut, 1973.
9. A. Locke, E. R. Main, and D. O. Rosbach, J. Clin. Invest., 11, 527 (1932).
10. A. Schade and L. Caroline, Science, 104, 340 (1946).
11. I. Kochan, Curr. Top. Microbiol. Immunol., 60, 1 (1973).
12. E. D. Weinberg, Science, 184, 952 (1974).
13. H. J. M. Bowen, Trace Elements in Biochemistry, Academic Press, New York, 1966.
14. V. Sauchelli, Trace Elements in Agriculture, Van Nostrand Reinhold, New York, 1969.
15. E. J. Underwood, Trace Elements in Human and Animal Nutrition, 3rd ed., Academic Press, New York, 1971.
16. D. R. Williams, The Metals of Life, Van Nostrand Reinhold, New York, 1971.
17. I. J. T. Davies, The Clinical Significance of the Essential Biological Metals, Charles C. Thomas, Springfield, Illinois, 1972.
18. H. G. Gauch, Inorganic Plant Nutrition, Dowden, Hutchinson, & Ross, Stroudsburg, Pennsylvania, 1972.
19. M. N. Hughes, The Inorganic Chemistry of Biological Processes, Wiley, New York, 1972.
20. S. K. Dhar (ed.), Metal Ions in Biological Systems, Plenum Press, New York, 1973.
21. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mertz (eds.), Trace Element Metabolism in Animals — 2, University Park Press, Baltimore, Maryland, 1974.

INTRODUCTORY NOTE TO CHAPTERS 1, 2, AND 3

Our laboratory has been concerned with bacterial divalent cation metabolism for the last 8 years or so. Whereas an enormous body of information about microbial iron metabolism is available because of the importance of iron compounds to microbial metabolism and also because of the clinical use of microbial extracellular iron chelates, the other essential divalent cations of all living cells have experienced an era of benign neglect. We have attempted in the following three chapters to redress the balance with regard to microbial magnesium, calcium, and manganese metabolism. Each of the three chapters represents the first attempt that we are aware of to organize and rationalize what is known about transport of the divalent cation as well as about nutritional requirements and cellular roles. The three cations (and transport systems) are very different. Magnesium is the major intracellular divalent cation, and active transport systems for its accumulation and maintenance have evolved and been retained by all living cells. Calcium is the major extracellular divalent cation, and the different locations and properties of Mg^{2+} and Ca^{2+} are frequently used to regulate metabolic activities. All cells, we believe, actively exclude calcium; Chapter 2 presents the first explicit exposition of this hypothesis. Already much supportive data are available. Manganese differs from Ca^{2+} and Mg^{2+} in that it usually is found as a "trace" element. For most, if not all, living cells, Mn^{2+} is an essential micronutrient; therefore highly specific active transport systems evolved to provide cells with this needed material in the face of a Mn^{2+}-famine environment rich in other divalent cations, especially Mg^{2+} and Ca^{2+}. It has proved easier to demonstrate the universal existence of Mn^{2+} transport systems than it has been to prove universal requirements for Mn^{2+} for cell growth. This, of course, is a tribute to the potency or high efficiency of these cellular systems.

Simon Silver