CHEMICAL MICROBIOLOGY

AN INTRODUCTION TO MICROBIAL PHYSIOLOGY

Third Edition

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Chem Microbiology

An Intro to Microbial Physiology

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（内部交流）

Third Edition

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Toronto: 2265 Midland Avenue,
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USA

Butterworths (Publishers) Inc
161 Ash Street,
Reading, Mass. 01867

First Edition 1965
Reprinted 1967
Second Edition 1968
Reprinted 1970
Third Edition 1976

ISBN 0 408 70683 X

Printed in England by
Page Bros. Ltd.
Norwich

PREFACE TO THE THIRD EDITION

Biologists have been fascinated by the activities of micro-organisms ever since they became aware of the existence of microscopically small creatures. To begin with, their interests centred mainly on those micro-organisms that cause diseases in Man and animals, and it was some time before they came to appreciate the vitally important roles that micro-organisms play in the natural cycling of elements. Studies on the chemical activities of micro-organisms, on which all other activities of these organisms are based, had to await the birth of the new science of biochemistry during the latter half of the nineteenth century. Since then, interest in the chemical activities of micro-organisms has grown at a tremendous pace, and chemical microbiology is now recognised as a subject in its own right.

In the preface to the first edition of this book, which was published in 1965, I admitted to audacity in having attempted to describe all aspects of the chemical activities of microbes in a relatively small volume. The reception given to the first edition, and also the second edition published in 1968, somewhat allayed my apprehensions. In preparing this, the third edition, I have retained the same basic format, and updated all chapters so that, not surprisingly, the volume is somewhat larger. I have also removed all references from chapters, preferring to include important review articles and papers in a list of suggested reading at the end of each chapter. This change, I believe, makes for a less interrupted text.

As with previous editions, many friends and colleagues have helped with advice, unpublished articles, and micrographs, and to all of them I am deeply grateful. My thanks are especially due to Dr. Ronald Archibald of the University of Newcastle-upon-Tyne, with whom I spent some weeks when we were Visiting Professors at the Biological Laboratories of the Gulbenkian Science Foundation, near Lisbon in Portugal. During these weeks, extensive revisions were made to the second edition of this text, and I greatly valued Dr. Archibald's advice. Several colleagues have kindly read chapters, and offered their criticisms, none more assiduously than Dr. L. Julia Douglas of the Microbiology Department in the University of Glasgow. It would not have been possible to prepare this third edition without the unfailing help and encouragement of my wife Jane. My son Simon gave timely and valuable help in preparing the index.

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ABBREVIATIONS AND SYMBOLS

NUCLEIC ACIDS AND NUCLEOTIDES

AMP, CMP, GMP, IMP, TMP and UMP	5'-Phosphates of adenosine, cytidine, guanosine, inosine, thymidine and uridine
ADP, CDP, GDP, IDP, TDP and UDP	5'-Pyrophosphates of adenosine, cytidine, guanosine, inosine, thymidine and uridine
ATP, CTP, GTP, ITP, TTP and UTP	5'-Triphosphates of adenosine, cytidine, guanosine, inosine, thymidine and uridine Deoxyribonucleotides are distinguished by the prefix d; e.g. dATP for the 5'-triphosphate of deoxyadenosine
DNA	Deoxyribonucleic acid
mDNA	Mitochondrial deoxyribonucleic acid
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
rRNA	Ribosomal ribonucleic acid
tRNA	Transfer ribonucleic acid

AMINO ACIDS

Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
DAP	Diaminopimelic acid
Glu	Glutamic acid

Gly	Glycine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Val	Valine

SUGARS

Ara	Arabinose
Abe	Abequose
Col	Colitose
Gal	Galactose
Glc	Glucose
GlcNAc	N-Acetylglucosamine
Man	Mannose
MurNAc	N-Acetylmuramic acid
PRPP	Phosphoribosyl pyrophosphate
Rha	Rhamnose

VITAMINS AND COENZYMES

FAD, $FADH_2$	Flavin adenine dinucleotide, oxidised and reduced forms
NAD, $NADH_2$	Nicotinamide adenine dinucleotide, oxidised and reduced forms
NADP, $NADPH_2$	Nicotinamide adenine dinucleotide phosphate, oxidised and reduced forms
CoA.SH	Coenzyme A
TPP	Thiamine pyrophosphate

OTHER ABBREVIATIONS

Pi	Inorganic phosphate
PPi	Inorganic pyrophosphate

ENZYMES

Most of the enzymes mentioned in this book are referred to by the trivial names recommended by the Commission on Enzymes of the International Union of Biochemistry

TEMPERATURES

All temperatures recorded in this book are in degrees Centigrade

CONTENTS

1

INTRODUCTION

The aim of the microbial physiologist in studying the chemical activities of microbes is to explain in molecular terms the living processes of these organisms. Information on the chemical activities of micro-organisms has been steadily accumulating ever since the late seventeenth century when Antonie van Leeuwenhoek first observed micro-organisms. The early microbiologists had no option but to study mixed populations of micro-organisms. Following the work of Lord Joseph Lister and Robert Koch, it became possible to study organisms in pure culture, but although this greatly simplified the task of observing and classifying organisms, it meant that they were studied under conditions that were vastly different from those that obtain in natural environments.

The first experiments on the chemical activities of micro-organisms were mainly concerned with the ability of growing organisms to change the chemical composition of their environment by removing some compounds and excreting others. Many famous names were associated with this early period in chemical microbiology, including Louis Pasteur, Sergius Winogradsky and Martinus Willem Beijerinck.
A major turning point in the history of the subject came in 1897 with the discovery by the Buchner brothers that fermentation of sucrose could be carried out by cell-free extracts of yeast, an observation which is often said to have given birth to the science of biochemistry. The Buchners' discovery sparked off a series of studies into the metabolic pathways used by micro-organisms beginning with the process of alcoholic fermentation by yeast. It showed, too, the tremendous advantages that can be gained

by using micro-organisms for studying the chemical activities of living cells, and in the years that followed research in many areas of general biochemistry came to depend more and more on the Microbial Kingdom for experimental material. Today, most of the major metabolic pathways used by micro-organisms have been charted at least in outline, and the comprehensive metabolic maps which adorn the walls of biochemical laboratories provide eloquent testimony to the success that the biochemist has achieved in this venture.

During the past decade, microbiologists have become increasingly interested in the physiological aspects of microbial activity, aspects that were temporarily overshadowed by the spectacular success of the enzymologist in charting metabolic pathways. Great strides have been made in studies on regulation of microbial metabolism. This has been accompanied by an expanding interest in the relationship between structure and function in microbial organelles, particularly of the cell wall, plasma membrane, mitochondria and chloroplasts, and therefore in the physiology of the intact micro-organism. Differentiation processes in micro-organisms, though restricted compared with those carried out by higher organisms, are also attracting an ever-increasing number of workers, mainly because of the advantages which they offer as systems for elucidating the basic molecular processes of differentiation.

The chemical activities of microbes are studied by many different groups of biologists and, as a result, the literature on the subject is rather widely scattered. The main task in writing this book has therefore been to condense the vast quantity of published data on the various branches of microbial physiology. Inevitably, there have been occasions when it has been necessary to omit material that would have found a place in a more lengthy treatise, but I hope that these omissions have not been too arbitrary. The book is intended for readers who already have a basic knowledge of microbiology and biochemistry, and who wish to combine and extend this knowledge to make a study of the chemical activities of algae, bacteria, fungi, protozoa and yeasts. The tempo of research on this subject is feverish, and it has to be admitted that many sections of this book will rapidly become out of date. Readers who wish to keep abreast of developments in the subject are recommended to peruse the following review organs which regularly furnish up-to-date and authoritative reviews on a variety of

aspects of the chemical activities of micro-organisms. In this way they will be able to share in the excitement as the molecular secrets of Leeuwenhoek's animalcules continue to be revealed.

Advances in Microbial Physiology, published biannually by Academic Press in London.

Annual Review of Biochemistry, published annually by Annual Reviews Inc., Palo Alto, California, U.S.A.

Annual Review of Microbiology, published annually by Annual Reviews Inc., Palo Alto, California, U.S.A.

Bacteriological Reviews, published quarterly by the American Society for Microbiology, Washington, D.C., U.S.A.

Critical Reviews in Microbiology, published by the Chemical Rubber Company Press Inc., Cleveland, Ohio, U.S.A.

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MOLECULAR ARCHITECTURE

The chemical composition of micro-organisms has been analysed ever since techniques were devised for growing large quantities of microbes in pure culture. Inevitably, the results of these analyses reflected the precision of the analytical methods employed. Today, a large number of extremely sensitive micro-analytical techniques are available for studying the chemical composition of micro-organisms, and these methods permit the detection and separation of molecules that are present in microbes in very small amounts.

However, data on the overall chemical composition of micro-organisms tell us nothing about the ways in which the component molecules are located in the microbial cell. Fortunately, at the same time that progress was being made in the development of analytical methods, important advances were taking place in the subject of microscopy. The limit of resolution of the light microscope (about 0.2 μm) severely restricts its use in studying intracellular structures in microbes. By using the electron microscope, which has a limit of resolution nearer 0.001 μm, it is possible to examine intracellular structures in even the smallest microbe. The link between analyses of the chemical composition of micro-organisms on the one hand, and investigations into their fine structure on the other, came from studies in which intracellular structures and organelles in microbes were separated, and then analysed chemically or examined in the electron microscope.

2.1 PROKARYOTES AND EUKARYOTES

The detailed information on the subcellular organisation of micro-organisms which emerged from studies with the electron microscope confirmed what had, in fact, been suspected for some time, namely that a major morphological discontinuity exists between bacteria and blue-green algae, and other micro-organisms. That bacteria and blue-green algae are atypical organisms was suspected as long ago as 1875 by the botanist Ferdinand Cohn, and since the availability of fine-structure data, microbiologists have appreciated that bacteria and blue-green algae are fundamentally different from other micro-organisms in that they are always much less differentiated intracellularly. The writings of Roger Y. Stanier and Cornelius van Niel of the University of California have contributed significantly to an understanding of this basic division in the Microbial Kingdom, and it was largely following their suggestion that bacteria and blue-green algae are now known as *prokaryotes*, and other micro-organisms as *eukaryotes*. The basic distinction between prokaryotic and eukaryotic micro-organisms is that the former do not possess a nuclear membrane, a structure which envelopes the genome in all eukaryotic organisms. Other differences, such as the absence of mitochondria, chloroplasts and endoplasmic reticulum from prokaryotes, also exist and these, together with differences in the molecular architecture of other organelles, are discussed in later sections of this chapter.

2.2 METHODS USED IN STUDYING THE MOLECULAR ARCHITECTURE OF MICRO-ORGANISMS

In order to study the molecular architecture of micro-organisms it is necessary to isolate subcellular structures from the organisms in an intact condition and free from contamination with other subcellular components. This is a two-stage process. In the first, micro-organisms are disintegrated to free subcellular structures from one another; in the second, these structures are separated by various types of centrifugation regime. The isolated structures and organelles can then be subjected to chemical analysis or microscopic examination.

2.2.1 DISINTEGRATION OF MICRO-ORGANISMS

Methods for disrupting organisms have undergone extensive development since 1897 when the Buchners first obtained a cell-free yeast juice by grinding yeast with a mixture of kieselguhr and sand. The number of different methods that are used today is legion, and it seems that each laboratory has developed its own techniques for preparing those subcellular structures and organelles in which its workers are principally interested.

Basically, there are two classes of method for disintegrating micro-organisms. With the first, mechanical forces which are often quite large are applied to a suspension of micro-organisms, and this stress causes the disintegration of some and frequently all of the organisms in the suspension. Other, gentler methods do not employ a mechanical force and do not cause as much damage to subcellular structures and organelles.

Mechanical methods

These methods can be subdivided into those which employ a solid shear and those which involve application of a liquid or hydraulic shear. Because of the high tensile strength of their walls, hydrodynamic gradients of up to 10^8 s^{-1} are required to disrupt micro-organisms; it is important to note that subcellular organelles are often damaged by gradients as low as 10^4 s^{-1}.

Some microbial physiologists still prefer to use an up-dated version of the Buchners' original method which creates a *solid shear* in the suspension of organisms. Grinding a paste of microbes with glass powder, washed alumina or sand in a chilled pestle and mortar is a very effective disruption procedure, even with organisms that have very tough walls. Another effective method for disrupting micro-organisms exploits the solid shear created by compression of ice crystals. In these methods, a frozen suspension or paste of organisms is forced through a small orifice into a receiving chamber at very high pressures of about 5.5×10^8 Pa (or 80 000 psi). Two widely used pieces of apparatus which employ this method are the Hughes and 'X' presses.

Liquid-shear methods are of three main types. Ultrasound (10-15 kHz) can be used to create a liquid shear. This causes gaseous cavities to form in the suspension which, in turn, lead to acoustic or micro-

streaming of liquid round each bubble, and it is thought that the acceleration due to this streaming generates a sufficiently large force to disrupt the organisms. Several pieces of equipment are available for ultrasonic disruption of microbes, including those marketed by M.S.E.-Mullard and Bronwill Scientific. Another way of imposing a liquid shear on microbes is to force a chilled suspension through a small orifice, using a pressure of $0.69\text{-}2.07 \times 10^8$ Pa. The French pressure cell and the Chaikoff press employ this method, and both pieces of equipment are very effective in causing disruption of microbes. A third type of method imposes a liquid shear on the microbes by rapidly shaking a chilled suspension with small glass beads (such as Ballotini beads) in what is essentially a vibration mill. Several manufacturers market pieces of equipment for this purpose, notably B. Braun of Melsungen in West Germany, all using high-amplitude shaking. Considerable heat is generated in these machines during the disruption process and it is customary to cool the suspension with liquid carbon dioxide. *Figure 2.1* shows electron micrographs of walls obtained after disrupting bacteria by shaking with glass beads. They show how the rigid wall structure has been ruptured by the liquid shear imposed on the bacteria.

Non-mechanical methods

On the whole, non-mechanical methods are less commonly used than those which involve application of a mechanical force. Drying or desiccating a population of micro-organisms is a long-established way of preserving enzyme activity in cells, but clearly it does not permit isolation of subcellular structures and organelles. The most elegant way of isolating subcellular structures and organelles by a non-mechanical method is first to convert the organisms into protoplasts or sphaeroplasts, and then to lyse or disrupt these osmotically sensitive structures under gentle conditions. As a result, subcellular structures and organelles are released from the protoplast or sphaeroplast, and these can then be isolated and examined.