INNER STRUCTURES OF BACTERIA

Edited by

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SERIES EDITOR'S FOREWORD

In the 30-odd years since the electron microscope came into general use, there has been a profound change in our concepts of both the inner and outer parts of the bacterial cell. An entirely new understanding of the organization of the bacterial cell resulted from improvements in the electron microscope itself and especially from the introduction of thin sections and positive and negative staining techniques and the introduction of the entirely different freeze-etch technique, which occurred in the late 1960s and early 1970s. In addition, the development of physical fractionation techniques followed by gentler methods permitted a new approach, making it possible to relate structure to function.

Woutera van Iterson. In many papers on cytology it is the selection of the typical photograph that constitutes the prime evidence for the structure reported. To reproduce lengthy papers containing many photographs of the relevant structures would require many volumes to cover the important observed structures. Further, not all bacteria are alike and the structures found in one kind may be completely absent in another. Consequently, the selection of data (consisting almost entirely of electron micrographs) is critical and requires vast experience in the artifacts that can be produced and in the kind of information that can be derived from such micrographs. If, however, one selects only the outstanding micrographs of structures, then increased discussion is also required.

Dr. van Iterson has excerpted many more papers than is usual in a Benchmark volume and because of the nature of the subject she has included a review of an enormous amount of related literature. Indeed, she has produced a monographic discussion of each of the observed structures and has reviewed the literature associated with each throughout the volumes, covering not only cellular cytology but biochemistry and physiology as well. These volumes represent a masterpiece treatment by a recognized world authority of the subject and will stand as benchmarks for years to come.

WAYNE W. UMBREIT

PREFACE

Bacteria have a solid or semisolid, intricate structure within which their numerous physiological functions are integrated. Structure analysis, with the electron microscope in particular, attempts to examine this structure to supply a basis for integrating biochemical data so that function-structure relationships in microorganisms become more understandable.

In this volume, *Inner Structures of Bacteria,* and its companion volume, *Outer Structures of Bacteria,* I have attempted to review the development of ideas during the 35 years that the electron microscope has been applied to structure analysis so intensely that it created the base for modern bacterial cell biology. However, while the cell's main constituent is water, in the electron microscope the specimen is placed in high vacuum. Membrane-integrated and soluble enzymes, ribosomes, and all elementary biological structures function—and virtually exist—only in water. Fixation, dehydration, infiltration, and curing of resin are parts of embedding techniques applied prior to thin sectioning of cells for examination with the electron microscope. Removal of water affects the cell structures.

The criticism of some 35 years ago that the electron microscopist is a student of artifacts is to some extent still recognized by scientists today. With the present electron microscopical point resolution of ±0.3 nm, it still is rarely possible to reveal details smaller than 5–10 nm in biological material. The critical investigator knows that what he or she sees in an electron micrograph is not a picture of a life situation, but only an approximation, the best one can get of the true condition. This approximation should be supplemented and checked with data from other disciplines, keeping in mind *their* limitations.

Today's critical attitude toward electron microscope procedures is a challenge for many investigators to reconsider the physical base for improving preservation of fine detail, looking for new ways to overcome the actual present limitations (Sjöstrand, 1978, 1979; Kellenberger, 1979; Kellenberger et al., 1980). Therefore, this moment could well be the time of choice to review our present picture of the bacterial cell structure as deduced from direct and indirect data.

It has been said that fixation with osmium "fossilizes" cell structures, and because proteins would disintegrate into small fragments, leaving only a "skeleton" (Kellenberger, pers. commun.), we are not looking at life, but at a fossil! But has paleontology not offered us a fascinating picture of the living

forms from the past? Then let us look courageously and critically at these new experimentally produced fossils from the laboratory.

New data are appearing with overwhelming rapidity. The newest are not necessarily the truest. Couldn't it be that they contain the same percentage of error found in the older data? In the addenda found in these two volumes on bacterial anatomy, brief mention is made of some of the additional viewpoints that have appeared after completion of the original manuscripts.

Acknowledgements

I feel very grateful to a number of scientists in the Netherlands and to other scientists abroad, for having read through some of the chapters of the present two volumes on the fine structure of bacteria. I hesitate to mention them by name because of all the alterations and rewritings I did afterwards.

How can I thank Dr. H. L. van Vierssen Trip in Ottawa for patiently altering my "Netherlandiisms" and for retyping of the text? Where my ways of expression remain outlandish this must be attributable to my ecological niche on the North Sea shore.

WOUTERA VAN ITERSON

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Part I

BACTERIAL CYTOLOGY: AN INTRODUCTION

Editor's Comments on Papers 1, 2, and 3

1 **VAN ITERSON**
Excerpt from *Much Within Little*

2 **STANIER and VAN NIEL**
Excerpt from *The Concept of a Bacterium*

3 **VAN ITERSON**
Excerpt from *Membranous Structures in Micro-organisms*

During the past thirty years, bacterial cytology has seen a period of rapid development with increasing opportunity to analyze more minute detail. The discipline of bacterial cytology (bacterial anatomy) continues its expansion in the direction of molecular cytology, a subdivision of submicroscopic morphology (see Paper 1).

All physiological processes, studied in biochemistry and its affiliated discipline of molecular biology are, in their final analyses, based on anatomical structure. The processes and reactions studied in living microorganisms may involve components that are integrated in elementary biological structures. When the reactions take place in a water-dissolved state, they may involve endproducts that are determined by the lipoprotein membrane that surrounds the cell or that partitions the cell into membrane-covered parts (organelles). The structures involved in these physiological processes and reactions are so infinitesimally small that it was impossible to think of them realistically prior to visualization in the electron microscope.

It is possible, as has been done in these volumes, to subdivide bacterial morphology into a number of sections, each dealing with a separate structural part, and to present more detailed knowledge in each section. In the end, this should all merge into "The Concept of a Bacterium". This mental integrating activity has graciously been left to the reader.

"The Concept of a Bacterium," a well-known paper by Stanier and van Niel (Paper 2), replaced the old division of the living world into animal and plant kingdoms, to which Haeckel (1866) had added the kingdom of the protists. It is now commonly accepted that the living world may be divided into eukaryotes and prokaryotes, based

on fundamental structural differences between eukaryotic and prokaryotic cell types. The terms eukaryotes (*Eucaryotae*) and prokaryotes (*Procaryotae*) introduced by Dougherty (1957) have proved to represent a sharp demarcation, with few vague intermediate forms.

The only organisms with prokaryotic features are the bacteria and the blue-green algae (*Cyanophyceae*), presently called blue-green bacteria (Cyanobacteria) (see Buchanan and Gibbons, 1974; Stanier et al., 1978). *Inner Structures of Bacteria* and *Outer Structures of Bacteria* deal with bacteria in the original sense; the blue-greens are not discussed in either volume.

The triumph of thirty years of ultrastructural research is our present awareness of a basic unity in cell structure, five decades after Kluyver and Donker (1926) advocated unity in biochemisty. In eukaryotic and prokaryotic cells, analogous functions take place on analogous elementary structures, on fibrillar macromolecules such as the nucleic acids or the flagellar proteins, and on membranes and ribosomes. But the analogies are accompanied by constant fundamental differences in ultrastructural anatomy (see Stanier and van Niel, Paper 2; van Iterson, Paper 3).

SITE OF RESIDENCE OF GENETIC INFORMATION

The activities of the cell are directed by genetic information, which resides in its deoxyribonucleic acid (DNA).

In the eukaryotic cell, the genetic information is primarily contained in a membrane-covered cell nucleus; it also is contained in smaller cell organelles,—the mitochondria and the plastids (chloroplasts). In the nucleus, the genetic information is spread over a large number of highly organized elements called chromosomes, which consist of DNA, histones, and nonhistone proteins. The chromosomes replicate and divide by mitosis, which involves a spindle composed of a number of microtubules. During mitotic division, the nuclear envelope (membrane) disappears in most types of cell, to be formed again after the two daughter sets of chromosomes have parted. The nuclear division is followed by the cell division in a plane coinciding with the spindle's equatorial plane. The DNA in mitochondria and plastids, on the other hand, resembles bacterial DNA in that it is not organized in a composite chromosome. It is more or less a "naked" molecule, usually in a closed loop structure, shorter than bacterial DNA and dividing by an amitotic process.

In the prokaryotic cell (bacteria and blue-greens), the site of genetic information is mainly in a small number of nuclear equivalents—the nucleoids—devoid of a nuclear membrane. The