The Bacteria

Edited by

I. C. GUNSALUS
ROGER Y. STANIER

Volume I: STRUCTURE

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

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CONTRIBUTORS TO VOLUME I

THOMAS F. ANDERSON, *Biology Division, University of Pennsylvania, and Institute for Cancer Research, Philadelphia, Pennsylvania*

E. KLIENEBERGER-NOBEL, *Lister Institute of Preventive Medicine, London, England*

E. S. LENNOX, *Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois, Urbana, Illinois*

S. E. LURIA, *Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts*

ALLEN G. MARR, *Department of Bacteriology, University of California, Davis, California*

KENNETH MCQUILLEN,* *Department of Biochemistry, University of Cambridge, Cambridge, England*

R. G. E. MURRAY, *Department of Bacteriology and Immunology, University of Western Ontario, London, Ontario, Canada*

C. F. ROBINOW, *Department of Bacteriology and Immunology, University of Western Ontario, London, Ontario, Canada*

M. R. J. SALTON, *Department of Bacteriology, University of Manchester, Manchester, England*

CLAES WEIBULL, *Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden*

**Also Research Associate of the Carnegie Institution of Washington, Washington, D.C.*

PREFACE

Bacteriology developed in the late nineteenth and early twentieth centuries very largely as an applied science, with momentous consequences for human health and welfare. Its outlook was largely empirical, and its growth was influenced only to a minor extent by general biological theory. During the past twenty-five years, a radical change has occurred. Although the applied aspects of this science have continued to grow, there has emerged a vigorous and widespread interest in the bacteria as biological entities. This originated historically from the realization of the fundamental unity of biochemical processes in living organisms, coupled with the recognition that bacteria provide ideal experimental systems for the investigation of many biochemical problems. Somewhat later, the experimental analysis of bacterial variation by scientists aware of the concepts of classical genetics led to the emergence of bacterial genetics as an important branch of genetic science. Here again, it was quickly realized that the biological properties of bacteria make them particularly suitable for the study of certain genetic problems; in addition the unique mechanisms of gene transfer in bacteria enriched genetic theory with new insights. Lastly, the study of bacterial cell structure has made great strides, aided particularly by the development of electron microscopy and of modern cytochemical techniques.

Heretofore, there has been no comprehensive source of information about modern knowledge of the general biological properties of bacteria. The present treatise will, it is hoped, help to fill this gap in the bacteriological literature. When the editors first discussed plans for the treatise some five years ago, they recognized the desirability of covering also the special properties of the various bacterial groups. However, it soon became apparent that a treatise which dealt with both the general and special aspects of bacteriology would prove an enormous undertaking, and it was accordingly decided to confine the work to general aspects. These will be treated in five volumes, concerned, respectively, with: structure; energy-yielding metabolism; biosynthesis; growth and general physiology; and heredity.

An undertaking of this sort, which involves the collaboration of many different specialists, inevitably lacks the unity and cohesiveness of a work produced by one or two authors. This is unfortunate, but the tremendous expansion of science in our time allows comprehensive coverage of a broad field only at this price. The editors have refrained from attempts to impose a single format, or to delineate in detail the area assigned to individual authors, beyond attempting to eliminate obvious large overlaps. The willingness with which the authors have responded to our appeals for contributions and have accepted suggestions for revisions has made the task of the editors a pleasant one. We are also greatly appreciative of the understanding, enthusiasm and practical assistance extended by the publishers and their staff. We trust that this undertaking will prove to be of value to the community of microbiologists.

I. C. GUNSALUS
R. Y. STANIER

December, 1959

The Bacteria

A TREATISE ON STRUCTURE AND FUNCTION

VOLUME II: METABOLISM

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CHAPTER 1

The Bacterial Protoplasm: Composition and Organization

S. E. LURIA

I. Living Matter, Cell Theory, and the Unity of Biochemistry

A. LIFE AND ORGANIZATION

When we examine living beings, the feature that immediately strikes us is organization. The organization of the world of life expresses itself at various levels. Some are taxonomic levels, which reflect the historical aspects of life and define the common descent and degree of relationship among living beings. Other levels of organization are detected within the individual units of living matter, which are in fact called *organisms*. The organized features of living beings reach all the way from the macroscopic to the molecular level, and one of the major tasks of biology is to understand and describe this organization, its interdependent levels, its stability, and its variability. In fact, life can be defined as the set of processes by which the organization of organic matter is maintained and reproduced, that is, imposed upon other matter which becomes assimilated into living organisms. We may define as *living matter* or *protoplasm* that portion of matter endowed at any one time with this self-maintaining organization.

The progressive evolution and diversification of living forms result from the flexibility of the organization of living matter, which, by reproducing its own pattern with accurate but not always absolute fidelity, makes it possible for new forms of life to arise, to be tried out in the ever-changing material environment, and to persist or disappear, depending on their fitness to carry out the processes of maintenance and reproduction.

B. Cell Theory and the Unity of Biochemistry

The unique features and historical continuity of the organization of living matter or protoplasm are embodied in two basic generalizations: the cell theory and the theory of the unity of biochemistry.

The *cell theory*[1] recognizes that all living matter consists of units, called *cells*, which have a common basic pattern of organization and which represent the simplest elements that can carry out all processes of life. The *theory of the unity of biochemistry*[2] recognizes that these life processes involve the same basic chemical reactions in all cells, because the common pattern of organization is embodied in a chemically common material substrate.

The cell theory was founded on a morphological basis. The microscope showed that all tissues and organs of animals and plants consisted of cells and of intercellular materials. The essential steps in the formulation of the cell theory were, on the one hand, the realization that in any organism only the cells are living, that is, are capable of assimilation and reproduction, while the extracellular materials, no matter how specific or complex, are nonliving, incapable of assimilation, metabolically inert products of cell activity; and, on the other hand, the recognition that all cells have a common basic structure, and that the almost infinite variety of cells represent differentiations and modifications of the common pattern—modulations and variations on a basic theme.

1. Cell Theory and Cellular Structure

The common pattern of organization of all cells is embodied in the idealized cytological picture of a *generalized cell*. The generalized cell has a nucleus, which, within a nuclear membrane, contains one or more nucleoli and a characteristic number of chromosomes. These are identified by cytogenetic experiments as the seat of the discrete genetic determinants that obey Mendelian heredity rules in sexual reproduction. Outside the nucleus is the cytoplasm, consisting of a basic substance or *hyaloplasm*, in which are immersed a variety of organelles and granules, including functional structures—*mitochondria, centrosomes, plastids, kinetosomes*—and reserve materials—starch granules, fat globules, and vacuoles.

The electron microscope recognizes further levels of morphological organization, which reflect more and more closely the patterns of molecular organization. For example, in the hyaloplasm we recognize the *ergastoplasm* or *endoplasmic reticulum*[3] with its constituent granules or *microsomes*; in the mitochondria, elaborate systems of lamellae. On the outer edge the cell is bounded by a *cytoplasmic membrane*, which contains lipids, acts as a semipermeable barrier, and is the site of active transport mechanisms, retaining the larger cellular constituents and regulating the exchanges of smaller molecules between the cell and its environment.

The actual cell types may deviate morphologically from the generalized cell in a number of ways. They may acquire an outer rigid *cell wall*, as many plant cells do. They may develop complex surface structures, such as cilia, flagella, and even "mouth parts," as in certain protozoa, probably by differentiated functions of kinetosomes.[4] They may acquire contractile functions by synthesizing fibers of contractile proteins. They may lose irreversibly their reproductive ability, as in differentiated nerve cells, or even lose their nuclei, as in mammalian erythrocytes. Supracellular or apparently acellular complexes may arise by failure of cellular division to accompany nuclear division, as in fungi with nonseptate hyphae, or by actual fusion of preexistent cells.

2. Unity of Biochemistry and Cellular Function

In the same way as the cell theory recognizes a general cell pattern and interprets the multitude of actual cell types as modifications of the general pattern, so does the theory of the unity of biochemistry recognize a common chemical substrate of all protoplasm and a common set of chemical reactions providing the energy and the building blocks for the construction of protoplasm. The functionally diverse types of cells represent modifications of the basic chemical pattern, specifically adapted to certain functions and environments.

The unity of protoplasmic chemistry reveals itself in two ways. The first and most basic aspect is the composition of the macromolecules—proteins and nucleic acids—that carry out the specific chemical activities of protoplasm, *enzyme action* and *genetic action*; that is, chemical catalysis and control of the specific patternization of new macromolecules—the so-called "template action." All proteins and all nucleic acids consist of a limited number of common ingredients, in the same way as all words of a language consist of a limited number of letters and syllables: the proteins, of amino acids; the nucleic acids, of nucleotides. As the meaning of language results from combinations and permutations of letters and syllables and from the arrangements of words into sentences, thus all functional specificity or "information" in cells results from the combinations and permutations of about 20 amino acids in proteins or 4 nucleotides in nucleic acids, respectively, and from the folding and juxtaposition of the macromolecules. Folding and mutual relations of the macromolecular chains are probably themselves fully determined by the inner sequence of digits[4a].

Such a system of chemical alphabets makes available innumerable functional specializations embodied in a common substrate; but the actual chemical processes by which proteins and nucleic acids carry out their catalytic or template functions are still largely not understood.

The second way in which the unity of biochemistry expresses itself is that a given chemical result is accomplished in different cells and organisms, not by a great variety of mechanisms, but in only a few alternative ways, sometimes in a unique way. This limited number of alternative pathways reflects the opportunism of evolution, which tends to select and preserve any successful device among the chemically, thermodynamically and ecologically possible ones. Clear examples are observed in energy-yielding and biosynthetic pathways. There are four known prototypes of energy-yielding mechanisms:[5] anaerobic fermentation, pentose phosphate cycle, tricarboxylic acid cycle* and photosynthesis. One or more of these are found with minor variations in all cells and organisms, with similar or identical substrates, key intermediates, and cofactors. Even the enzymes have enough common features to bespeak a common molecular structure. Likewise, each of the necessary chemical ingredients of protoplasm, or *essential metabolites*, is synthesized in different organisms by one of a few alternative pathways. Even the processes involved in all forms of motion of living organisms seem to share a common basic mechanism: stimulation of contractile proteins with ATPase activity by the common substrate adenosine triphosphate (ATP).[6]

All quantitative and qualitative differences among cells and organisms can be traced biochemically to variations of the basic pattern, which fall into three main categories: (1) failures to synthesize some essential metabolite, which thereby becomes a *required nutrilite* (or "growth factor," or "vitamin") and must be supplied from the outside, either by other cells or from the external milieu; (2) specific stimulation or inhibition of chemical processes by endogenous or exogenous substances that regulate enzyme function, like hormones, or enzyme synthesis, like "inducers"[7] or "repressors,"[7a] or agents which alter cell permeability; (3) production of specific functional differentiations, such as contractile proteins for motion,

* The glyoxylate bypass is here considered as an essential variant of the tricarboxylic acid cycle permitting net synthesis of four-carbon compounds for cellular biosynthesis during growth on two-carbon compounds exclusively.[5a]

or rigid materials for structural support, or permeability barriers for selective retention and exclusion of enzymes, substrates, and products. For example, mechanically rigid structures may be achieved either by excretion of intercellular substances, such as collagen and its derivatives in connective and bone tissues of animals, or by production of rigid cell walls, as in plants. On the other hand, a rigid cell wall may be used to provide rigid support for a delicate membrane subject to high osmotic pressures, as in molds and bacteria.

C. Biochemical Cytology

The morphological, genetic, and biochemical approaches to cellular organization have converged in recent years to create a "biochemical cytology"[8] which endeavors to give a complete picture of cellular structure and function in terms of the location and mutual relation of functional constituents. Progress has been made, on the one hand, in locating the sites of specific biochemical functions within cells or sections of cells by cytochemical reagents or by microautoradiography using radioactive substrates;[9] and, on the other hand, in fractionating cells, detecting chemical constituents or specific catalysts in certain fractions, and identifying these fractions with cellular layers or organelles. Thus, for example, we recognize the mitochondria as the sites of oxidation, electron transport, and oxidative phosphorylation; the microsomal fraction as the site of cytoplasmic protein synthesis; the chloroplasts as the seat of the primary reactions in photosynthesis; the deoxyribonucleic acid as the basic component of genetic matter, present in constant amount in all cells of a given genetic constitution.

II. The Bacterial Cell

A. Bacteria as "Unicellular" Organisms

Both for technical and for historical reasons, morphology played a relatively less important role in the study of the bacterial cells than in that of other cells. The small size of bacteria long delayed the recognition of inner structures whose specific functions had to be explained. The roots of bacteriology in the study of disease and fermentations caused the attention of bacteriologists to center on chemical function; and the rise of bacteriology as a science coincided with the flourishing of cellular physiology and with the rise of modern biochemistry. Hence, it was possible for some time to ignore the inner organization of bacterial cells or even to deny it and to consider bacteria simply as "bags of enzymes."

The formulation of the theory of the unity of biochemistry led to the recognition of bacterial cells as a superb material for the study of cellular functions. The pure culture methods provided a variety of cell types, with characteristic metabolic patterns, which could be grown and prepared under chemically controlled conditions. In turn, the similarity of metabolic functions in bacteria and in other organisms required a careful comparison between the organization of bacterial cells and other cells. Biochemical cytology revitalized the study of bacterial cytology, bringing to it the tools of enzymology, electron microscopy, chemical fractionation, and tracer methodology.

Thus, there emerged a picture of a *generalized bacterial cell*, comparable to the picture of the generalized cell, as an abstraction embodying the basic feature of organized bacterial protoplasm. In this field, most variations on the common theme represent different organisms rather than different types of cells of the same organism. This is what we mean when we say that bacteria are unicellular organisms. More correctly, we assume that in a pure culture of bacteria we can equate the properties of any one cell with those of all other cells; that is, in a pure culture or *clone* derived from a single ancestor there is no regular morphogenetic differentiation of structure and function, barring genetic mutation, and no irreversible loss of totipotency distinguishable from sterility or death.

Functional coordination within clones derives from metabolic interactions, including competition for substrates, accumulation of by-products, and production of extracellular enzymes, within a population of equivalent individuals. Numerous reports of bacterial "life cycles" have been explained as resulting from an interplay of two types of processes: physiological response of bacterial cells to changes in environment, and genetic variation.[10, 11] Acting together, these two processes lead to changes in growth rates and growth patterns and, secondarily, to a selection of mutant types preadapted or more readily adaptable to the altered environment. Thus, deviations from the prototype or generalized bacterial cell represent either genetic differences or physiological adaptations to a changing environment. These adaptations do not give rise to permanently differentiated cell types with specialized functions within a clone.

Phenomena such as the sporadic appearance and transient persistence of individual motile cells within nonmotile clones[12, 13] indicate, however, that some intraclonal specialization can occur in common bacteria. Under favorable circumstances, such specializations might become stabilized and provide a basis for a true organismic differentiation. Another type of differentiation is observed in cells that initiate the production of an adaptive enzyme.[7, 14] Enzyme adaptation is an "all-or-none" phenomenon and, during the transition from the unadapted to the adapted state, a culture contains a mixture of adapted and nonadapted cells.[15]

Still another intraclonal differentiation in bacteria results from mating phenomena.[16, 17] The only well-analyzed instance, in *Escherichia coli*, re-