MICROBIAL PHYSIOLOGY

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Preface

Many textbooks appear to have been written for the author's peers in the field rather than for students exposed to the subject for the first time. While these volumes serve as excellent reference works for the advanced student or research worker, they often confront the beginning student with unbridged gaps that are difficult to fill, or develop the subject far beyond the scope of the beginning student's comprehension or immediate interest. This textbook on microbial physiology has been directed toward the consideration of each of the major aspects germane to the field, in sufficient depth to provide a basis of comprehension of the nature of microorganisms and their physiological activities. To guide the reader to more detailed reviews and to the original literature, a substantial number of references cite recent as well as historical accounts. Although a substantial background in biology and biochemistry must necessarily be presupposed, it is hoped that the presentation will be understandable and helpful to the advanced undergraduate or beginning graduate microbial physiology student in developing an acquaintance with the field.

Preliminary editions of this material, termed affectionately, "Moat's Notes," have been used by a succession of students while taking the author's courses in microbial physiology and genetics. Since these students found the material useful, it is considered that publishing them in textbook form will happily provide a means of circulation to a wider group. Most importantly, in writing this text I have attempted to keep particularly in mind the students who will ultimately choose to work in areas outside the immediate realm of microbial physiology. A knowledge of this subject should aid them in approaching their particular research problems with a better understanding of the underlying physiological principles involved.

I would like to acknowledge the help and encouragment I received from many of my former colleagues in the Department of Microbiology and Immunology and other members of the faculty of Hahnemann Medical College, particularly Dr. Amedeo Bondi, and Drs. Richard L. Crowell, Richard R. Gutekunst, Burton J. Landau, Jay M. Hammel, Herbert J. Eichel, James K. Alexander, Kathryn E. Fuscaldo, and M. John Boyd. I am also deeply appreciative of the many helpful comments on various portions of the manuscript by Drs. Herman C. Lichstein, Harold L. Sadoff, Ralph A. Slepecky, Charles Panos, Gerald D. Shockman, Michael L. Higgins, Lolita Daneo-Moore, Benedict T. DeCicco, Vincent P. Cirillo, Wayne W. Umbreit, Bernard W. Koft, Adrian M. Srb, Sol H. Goodgal, Joseph S. Gots, Larry Brodsky, and John W. Foster. To many others, particularly the numerous authors who provided original materials, go my thanks and gratitude for their aid in the preparation of this textbook. I also wish to thank William Walsh for making writing facilities available during the early stages of preparation of the manuscript.

A special note of thanks goes to Dr. Arthur J. L. Cooper, who very diligently read through two complete versions of the entire manuscript as well as the galleys. His many invaluable comments and suggestions have been most helpful in the development of this textbook in its final form.

I should also like to thank my daughter, Mary Ellen, and Scottie Marie Ford whose aid in compiling the index is deeply appreciated. The forbearance of my wife, Irene, and other members of my family during the long period required to complete the text has been especially helpful.

Huntington, West Virginia
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ALBERT G. MOAT

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General Introduction and Classification of Microorganisms

GENERAL INTRODUCTION

Physiology is the study of the functions of living organisms and their components. Although frequently interpreted to mean a study of physical functions, a broader definition includes all activities, whether they be physical, chemical, or their combination. Physiological functions depend upon one another and it is the nature of this interdependence that constitutes the substance of the subject.

When applied to microorganisms, physiology generally refers to a study of cell form, structure, growth, and the population dynamics of cells under the influence of changes in their physical and chemical environments. Specific aspects such as cytology, intermediary metabolism, genetics, and molecular biology are often considered as separate areas of study. The recognition of different fields within microbial physiology attests to the great increase in our knowledge and understanding of microbial life and accounts for the recent appearance of a large number of specialized reviews and monographs that treat each of these subjects in detail.

In this text, all major areas comprising microbial physiology are surveyed. As each topic is considered, it will become apparent that a vast array of information exists and that exhaustive coverage of the many facets of a given aspect is impossible. Instead, emphasis is placed upon examples of the most successful approaches to providing an understanding of a given physiological principle and the major findings and conclusions drawn from research on the subject. Where appropriate, areas are indicated in which continued research efforts are required to provide a complete elucidation of a specific problem. As many examples as possible are included from protozoa, algae, fungi, and other higher forms of microbial life, as well as from bacteria. However, a complete discussion of the physiology of all microbial forms is beyond the scope of this text. Specific references to reviews and accounts of research are given so that the reader will have access to further information.

It will be useful and informative to make comparisons among the physiological activities of various microorganisms and higher forms of life in the animal and plant kingdoms. The concept that all living cells have certain common features was first developed by Kluyver and the Dutch School of microbial physiologists and by Marjorie Stephenson and her colleagues in Great Britain during the late 1920s and early 1930s (see Kluyver, 1931; Stephenson, 1949). The concept of **comparative biochemistry** or **comparative physiology** led to investigations that revealed remarkable similarities as well as both obvious and subtle differences among a wide variety of organisms. For example, the biosynthesis of the purines and pyrimidines does not seem to differ in any major way from one group of organisms to another. On the other hand, a major divergence between two groups of organisms is observed in the biosynthesis of the amino acid lysine. Bacteria synthesize lysine by an entirely different pathway from that used by fungi. What has been found to vary widely, even within closely related groups, is the mechanism of regulation of metabolic activities.

Through their seemingly unlimited activities, both favorable and unfavorable, microorganisms influence our lives in a variety of ways. They may give rise to disease in other living forms, including other microorganisms. In the case of diseases that affect human beings or animals, a detailed knowledge of the physiology of the organisms causing them may aid materially our ability to control them. On the other hand, microorganisms may produce metabolic products that are useful to us as foods and beverages or may, of themselves, be edible products (e.g., mushrooms). Microorganisms may affect the flavor and texture of food products or provide a preservative effect through the production of acids or other metabolic products (e.g., in the manufacture of cheeses and other dairy products). Many microorganisms are involved in the various cycles in nature, and by their activities in these cycles are indispensable to the existence of other forms of life. The details of many of these processes are the subject of textbooks on applied aspects of microbiology. In this text, we are concerned with physiological principles that may have broad applicability to many aspects of ecology, public health, or industry.

Knowledge gained from a study of the physiology of microorganisms has recently led to the development of a more unified classification scheme than heretofore available. In any discipline within microbiology, it is essential to be able to distinguish one microorganism from another with considerable precision. To accomplish this, it is necessary to assemble all of the information regarding the physiological characteristics such as cytology, metabolic activities, and patterns of inheritance into an orderly sequence which may be used to identify individual organisms and to categorize related groups of organisms. Because the ability to distinguish one microorganism from another is so important, classification is the major topic of the balance of this chapter.

CLASSIFICATION OF MICROORGANISMS

General Considerations

Microorganisms are self-replicating entities, a characteristic that distinguishes them from viruses, which are wholly dependent upon cellular hosts for replication. Some microorganisms appear to be exceptions to this statement in that they are so dependent on another living host for their survival and multiplication that they cannot be successfully cultivated on artificial media. This dependency may reflect nutritional and physiological requirements so complex that they are difficult to elucidate.

Certain fundamental features universal to all living organisms are considered evidence of common ancestry. The universal nature of the genetic code and the utilization of ATP as a means of energy transfer are major examples. Bacteria, yeasts, molds, algae, and protozoa all contain two major classes of nucleic acid: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The rickettsia and chlamydiae, which are dependent upon another host for their development, also contain both types of nucleic acid. In these dependent organisms, complete enzyme systems are available for the production of energy and the biosynthesis of cellular constituents.

Viruses contain either RNA or DNA, but not both. Viruses are limited to a few specialized enzymes and are otherwise dependent upon a host cell for all their energy requirements and biosynthetic processes. Their replication is, nevertheless, guided by the same genetic code. This feature implies that they must be considered for inclusion in any overall classification scheme.

One of the most clear-cut distinctions that can be made between major groups of organisms is based upon the type of cellular organization. The blue-green algae (Cyanobacteria) and bacteria are **procaryotic.** They possess a single molecule of double-stranded DNA which bears the hereditary traits of the cell. This DNA is not enclosed within a membrane and is not associated with proteins. In those species that have been investigated, the DNA is apparently in the form of a covalently closed circle. No mitotic apparatus is present. The latter appears unnecessary because procaryotic cells apportion copies of their genome to daughter cells with a high degree of fidelity. The exchange of genetic information by a sexual process is either absent or occurs in the form of a unidirectional transfer of the genome from donor to recipient cells. Complex internal organelles such as mitochondria and chloroplasts are absent. Respiration and photosynthesis are apparently compartmentalized by complexing to membranes, but distinct organelles are not observed (Table 1–1). Subcellular structures such as mesosomes may be present. Mesosomes are membranous structures that have been implicated in several unique functions that will be described in later chapters.

Eucaryotes are distinguished by the presence of a discreet nucleus separated from the cytoplasm by a membrane containing pores. Eucaryotic chromosomal DNA is associated with RNA and proteins (Table 1–1). Nuclear division involves **mitosis** which ensures the distribution of the genetic material to daughter nuclei through formation of a complex mitotic spindle. Eucaryotes undergo **sexual reproduction,** a process that permits pooling of genetic material from two parental cells of opposite mating types. There is equal participation of both partners in the fertilization. The diploid state thus formed is reduced to the haploid state by the process of **meiosis.** The processes of mitosis and meiosis may not always be clearly demonstrable cytologically. These activities can, nevertheless, be inferred from analysis of their genetic consequences.

Cytologically distinct organelles such as **mitochondria** or **chloroplasts** are present in eucaryotic cells. Since these organelles have been shown to possess certain attributes of self-duplicating units, it has been considered that they may be derived from procaryotes at some point in evolutionary development. Eucaryotic organisms are aerobic, and their metabolic activities reflect this mode of metabolism. They break down glucose via the Embden–Meyerhof–Parnas pathway and oxidize pyruvate and other metabolites by means of the citric acid (Krebs) cycle. Respiration is mediated by cytochromes, and water is produced by the combination of oxygen with hydride anions or electrons. The terminal oxidative systems are found within mitochondria. Eucaryotes that utilize an anaerobic mode of metabolism are considered to have undergone retrograde evolution.

The organs of locomotion of lower eucaryotic organisms are complex. Flagella or cilia exhibit a characteristic "9 + 2" arrangement of the microtubules (axial filaments). By comparison, the flagella of procaryotes are relatively simple protein filaments.

Table 1–1. Major Distinctions between Procaryotes and Eucaryotes[a]

Procaryotes	Basis of Distinction	Eucaryotes
Microscopic (1–10 μm)	SIZE	Mostly large cells (10–100 μm)
Nucleoid, no membrane separates the DNA from the cytoplasm; no protein in association with DNA	NUCLEAR ORGANIZATION	Nucleus with distinct membrane; chromosomal DNA associated with RNA and proteins
Direct, binary fission	CELL DIVISION	Mitosis
None or rudimentary; transfer of hereditary information is unidirectional—from donor to a recipient cell	SEX	Present in most forms. Equal participation of both partners (equal contribution of hereditary information from both members of a mating pair)
Multicellular forms never develop from diploid zygotes	DIFFERENTIATION	Meiosis yields haploid cell. Two haploid partners fuse to form a diploid zygote
No tissue differentiation		Differentiation into separate tissues is extensive in higher forms
Anaerobic (O_2 is toxic), facultative, and aerobic forms	OXYGEN RELATIONS	Aerobic (exceptions are secondary modifications due to retrograde evolution)
Wide variations in patterns of glycolysis. Mitochondria are absent. Oxidative enzymes are membrane bound	METABOLISM	Similar metabolic patterns Embden-Meyerhof-Parnas pathway of glucose metabolism. Oxidation via Krebs citric acid cycle. Oxidative enzymes (cytochromes) contained in special organelles—mitochondria
Flagella are simple strands of protein (flagellin)	MOTILITY	Flagella or cilia are complex "9 + 2" arrangement of axial filaments
Membrane-bound chromatophores. Anaerobic and aerobic photosynthesis	PHOTOSYNTHESIS	Photosynthetic enzymes contained in chloroplasts. Oxygen-eliminating photosynthesis

[a] Modified from Margulis, 1974.

The surface structures of eucaryotes and procaryotes exhibit several fundamental differences. The cell walls of lower eucaryotes contain chitin, glycan, or other oligosaccharides. Procaryotic cells produce mucopeptide or peptidoglycan structures which contain diaminopimelic acid or lysine as a characteristic constituent. The presence of diaminopimelic acid in the cell walls of many procaryotes reflects the presence of the diaminopimelate pathway for the synthesis of lysine. Eucaryotic cells capable of synthesizing lysine utilize an entirely different pathway that involves the formation of α-aminoadipic acid as an intermediate.

Larger life forms generally fit clearly into either the animal or plant kingdoms and can be classified readily on a phylogenetic basis because there is generally an ample fossil record available. Although bacterium-like structures have been identified from sediments known to be over three billion years old, the microbial fossil record is generally so deficient as to preclude the establishment of any clear-cut phylogenetic basis for classification. The alternative, classification based upon comparison of the empirically determined physiological traits of currently existing forms, often leads to difficulties in establishing true phylogenetic relationships. However, within recent times several methods have been developed which permit the determination of genetic relatedness at the molecular level.

Analysis of the base composition of DNA and comparision of the percentage of guanine and cytosine bases in the DNA of two species affords a measure of their relatedness. Genetic homology of the nucleotide base sequences of the DNAs of different species by means of DNA–DNA hybridization also provides a means of determining the relatedness of organisms at the molecular level. It has also been recognized that many proteins and nucleic acids are "living fossils" in the sense that their structures have been conserved over billions of years of evolutionary development (Schwartz and Dayhoff, 1978). The amino acid and nucleotide sequences of proteins and nucleic acids have evolved from common ancestral sequences by a great number of small changes. Comparison of the number of such changes that have occurred provides a measure of the genetic distance between closely related as well as distantly related organisms.

The DNA base composition can be determined by physicochemical means. The temperature at which the two strands of native DNA separate (melt) is a reflection of the guanine (G) and cytosine (C) content because the hydrogen bonding between G and C base pairs is stronger than the bonding between adenine (A) and thymine (T) base pairs. The G + C content of DNA may also be determined by CsCl density gradient tech-