General Microbiology

FOURTH EDITION

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PREFACE TO THE FOURTH EDITION

The fourth edition of this book is dedicated to the memory of our friend and colleague Michael Doudoroff, one of the original authors of *The Microbial World.* His entire career was spent at the University of California in Berkeley; he was a leading figure in the group which made Berkeley a great center of research in microbial biochemistry and physiology during the decades following the second World War. He entered the Department of Bacteriology in 1940, and organized single-handed the program of instruction in general microbiology, for which he remained solely responsible until two of us (Roger Y. Stanier and Edward A. Adelberg) joined the Department some years later. *The Microbial World,* first published in 1957, grew out of the introductory courses which we then taught together at Berkeley. The design of these courses was almost entirely the creation of Michael Doudoroff, who was thus the real founder of *The Microbial World.*

When the first edition was written, microbiology still remained—as it had been for many decades—a discipline almost completely isolated from the rest of biology. Our goal in 1957 was to present microbiology within the framework of the concepts of general biology, since it was already evident that the period of isolation was coming to an end. By then, microbiology had begun to provide the experimental material for the discovery of the molecular basis of biological functions, even though the molecular biological revolution was only beginning to get under way. Now, almost 20 years later, the intellectual climate of biology in general and microbiology in particular has changed out of all recognition. This has forced us, in preparing successive editions of *The Microbial World,* to reappraise the scope and level of the material to be included, and to modify profoundly the structure of the book. The present revision is less drastic than the preceding ones. We have retained the organization and scope of the third edition, although few chapters have escaped a complete rewriting. As always, we shall welcome comments and criticisms from readers.

We should like to express our thanks to the many individuals who helped us in the preparation of this edition. Once again, we are grateful to the many colleagues who responded to our requests for new illustrative material. Dr. Mark Wheelis read the entire manuscript and made many constructive suggestions with respect to style and content. Mrs. Marjorie Ingraham gave us invaluable help both with the typing of the manuscript and with the reading of proofs.

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1

THE BEGINNINGS OF MICROBIOLOGY

Microbiology is the study of organisms that are too small to be clearly perceived by the unaided human eye, called *microorganisms.* If an object has a diameter of less than 0.1 mm, the eye cannot perceive it at all, and very little detail can be perceived in an object with a diameter of 1 mm. Roughly speaking, therefore, organisms with a diameter of 1 mm or less are microorganisms and fall into the broad domain of microbiology. Microorganisms have a wide taxonomic distribution; they include some metazoan animals, protozoa, many algae and fungi, bacteria, and viruses. The existence of this microbial world was unknown until the invention of microscopes, optical instruments that serve to magnify objects so small that they cannot be clearly seen by the unaided human eye. Microscopes, invented at the beginning of the seventeenth century, opened the biological realm of the very small to systematic scientific exploration.

Early microscopes were of two kinds. The first were *simple microscopes* with a single lens of very short focal length, consequently capable of a high magnification; such instruments do not differ in optical principle from ordinary magnifying glasses able to increase an image severalfold, which had been known since antiquity. The second were *compound microscopes* with a double lens system consisting of an ocular and objective. The compound microscope has the greater intrinsic power of magnification and eventually displaced completely the simple instrument; all our contemporary microscopes are of the compound type. However, nearly all the great original microscopic discoveries were made with simple microscopes.

THE DISCOVERY OF THE MICROBIAL WORLD

The discoverer of the microbial world was a Dutch merchant, Antony van Leeuwenhoek (Figure 1.1). His scientific activities were fitted into a life well filled with business affairs and civic duties. In this, he was no exception for his time; the great discoveries of this period in all fields of science were made by amateurs who earned their living in other ways, or who were freed from the necessity of earning a living because of their personal wealth. However, Leeuwenhoek differed from his scientific contemporaries in one respect: he had little formal education and never attended a university. This was probably no disadvantage scientifically, since the scientific training then available would have provided little basis for his life's work; more serious handicaps, insofar as the communication of his discoveries went, were his lack of connections in the learned world and his ignorance of any language except Dutch. Nevertheless, through a fortunate chance, his work became widely known in his own lifetime, and its importance was immediately recognized. About the time that Leeuwenhoek began his observations, the Royal Society had been established in England for the communication and publication of scientific work. The Society invited Leeuwenhoek to communicate his observations to its members and a few years later (1680) elected him as a Fellow. For almost 50 years, until his death in 1723, Leeuwenhoek transmitted his discoveries to the Royal Society in the form of a long series of letters written in Dutch. These letters were largely translated and published in English in the *Proceedings of the Royal Society,* so becoming quickly and widely disseminated.

Leeuwenhoek's microscopes (Figure 1.2) bore little resemblance to the instruments with which we are familiar. The almost spherical lens (a) was mounted between two small metal plates. The specimen was placed on the point of a blunt pin (b) attached to the back plate and was brought into focus by manipulating two screws (c) and (d), which varied the position of the pin relative to the lens. During this operation the observer held the instrument with its other face very close to his eye and squinted through the lens. No change of magnification was possible, the magnifying power of each microscope being an intrinsic property of its lens. Despite the simplicity of their construction, Leeuwenhoek's microscopes were able to give clear images at magnifications which ranged, depending on the focal length of the lens, from about 50 to nearly 300 diameters. The highest magnification that he could obtain was consequently somewhat less than one-third of the highest magnification that is obtainable with a modern compound light microscope. Leeuwenhoek constructed hundreds of such instruments, a few of which survive today.

FIGURE 1.1

Antony van Leeuwenhoek (1632–1723). In this portrait, he is holding one of his microscopes. Courtesy of the Rijksmuseum, Amsterdam.

Leeuwenhoek's place in scientific history depends not so much on his skill as a microscope maker, essential though this was, as on the extraordinary range and skill of his microscopic observations. He was endowed with an unusual degree of curiosity and studied almost every conceivable object that could be looked at through a microscope. He made magnificent observations on the microscopic structure of the seeds and embryos of plants and on small invertebrate animals. He discovered the existence of spermatozoa and of red blood cells and was thus

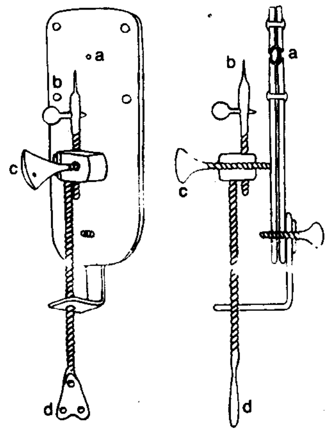

FIGURE 1.2

A drawing to show the construction of one of Leeuwenhoek's microscopes: (a) lens, (b) mounting pin, (c) and (d) focusing screws. After C. E. Dobell, *Antony van Leeuwenhoek and His Little Animals.* New York: Russell and Russell, Inc., 1932.

the founder of animal histology. By discovering and describing capillary circulation he completed the work on the circulation of blood begun by Harvey half a century before. Indeed, it would be easy to fill a page with a mere list of his major discoveries about the structure of higher plants and animals. His greatest claim to fame rests, however, on his discovery of the microbial world: the world of "animalcules," or little animals, as he and his contemporaries called them. A new dimension was thus added to biology. All the main kinds of unicellular microorganisms that we know today—protozoa, algae, yeasts, and bacteria—were first described by Leeuwenhoek, often with such accuracy that it is possible to identify individual species from his accounts of them. In addition to the diversity of this microbial world, Leeuwenhoek emphasized its incredible abundance. For example, in one letter describing for the first time the characteristic bacteria of the human mouth, he wrote:

> I have had several gentlewomen in my house, who were keen on seeing the little eels in vinegar; but some of them were so disgusted at the spectacle, that they vowed they'd never use vinegar again. But what if one should tell such people in future that there are more animals living in the scum on the teeth in a man's mouth, than there are men in a whole kingdom?

Although Leeuwenhoek's contemporaries marveled at his scientific discoveries, the microscopic exploration of the microbial world which he had so brilliantly begun was not appreciably extended for over a century after his death. The principal reasons for this long delay seem to have been technical ones. Simple microscopes of high magnification are both difficult and tiring to use, and the manufacture of the very small lenses is an operation that requires great skill. Consequently, most of Leeuwenhoek's contemporaries and immediate successors used compound microscopes. Despite the intrinsic superiority of compound microscopes, the ones available in the seventeenth and eighteenth centuries suffered from serious optical defects, which made them less effective working instruments than Leeuwenhoek's simple microscopes. Thus, Leeuwenhoek's English contemporary, Robert Hooke, who was a very capable and careful observer, could not repeat with his own compound microscope many of the finer observations reported by Leeuwenhoek.

The major optical improvements which were eventually to lead to compound microscopes of the quality that we use today began about 1820 and extended through the succeeding half century. These improvements were closely followed by resumed exploration of the microbial world and resulted, by the end of the nineteenth century, in a detailed knowledge of its constituent groups. In the meantime, however, the science of microbiology had been developing in other ways, which led to the discovery of the roles the microorganisms play in the transformations of matter and in the causation of disease.

THE CONTROVERSY OVER SPONTANEOUS GENERATION

After Leeuwenhoek had revealed the vast numbers of microscopic creatures present in nature, scientists began to wonder about their origin. From the beginning there were two schools of thought. Some believed that the animalcules were formed spontaneously from nonliving materials, whereas others (Leeuwenhoek included) believed that they were formed from the "seeds" or "germs" of these animalcules, which were always present in the air. The belief in the spontaneous formation of living beings from nonliving matter is known as the doctrine of *spontaneous generation,* or *abiogenesis,* and has had a long existence. In ancient times it was considered self-evident that many plants and animals can be generated spontaneously under special conditions. The doctrine of spontaneous generation was accepted without question until the Renaissance.

As knowledge of living organisms accumulated, it gradually became evident that the spontaneous generation of plants and animals simply does not occur. A decisive step in the abandonment of the doctrine as applied to animals took place as the result of experiments performed about 1665 by an Italian physician, Francesco Redi. He showed that the maggots that develop in putrefying meat are the larval stages of flies and will never appear if the meat is protected by placing it in a vessel closed with fine gauze so that flies are unable to deposit their eggs on it. By such experiments, Redi destroyed the myth that maggots develop spontaneously from meat. Consequently, the doctrine of spontaneous generation was already being weakened by studies on the development of plants and animals at the time when Leeuwenhoek discovered the microbial world. For technical reasons, it is far more difficult to show that microorganisms are not generated spontaneously, and as time went on the proponents of the doctrine came to center their claims more and more on the mysterious appearance of these simplest forms of life in organic infusions. Those who did not believe in the spontaneous generation of microorganisms were in the position, always difficult, of having to prove a negative point; in fact, it was not until the middle of the nineteenth century that the cumulative negative evidence became sufficiently abundant to lead to the general abandonment of this doctrine.

One of the first to provide strong evidence that microorganisms do not arise spontaneously in organic infusions was the Italian naturalist Lazzaro Spallanzani, who conducted a long series of experiments on this problem in the middle of the eighteenth century. He could show repeatedly that heating can prevent the appearance of animalcules in infusions, although the duration of the heating necessary is variable. Spallanzani concluded that animalcules can be carried into