

# General Microbiology

FOURTH EDITION

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## PREFACE TO THE FOURTH EDITION

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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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# CONTENTS

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## PREFACE xvii

## THE BEGINNINGS OF MICROBIOLOGY 1

- The discovery of the microbial world 2
- The controversy over spontaneous generation 4
- The discovery of the role of microorganisms in transformations of organic matter 7
- The discovery of the role of microorganisms in the causation of disease 10
- The development of pure culture methods 13
- Microorganisms as geochemical agents 16
- The growth of microbiology in the twentieth century 18

## THE METHODS OF MICROBIOLOGY 20

- Pure culture technique 20
  - The theory and practice of sterilization 25
  - The principles of microbial nutrition 27
  - The construction of culture media 35
  - Selective media 42
  - Light microscopy 50
  - Electron microscopy 54
- vii

3

**THE NATURE OF THE MICROBIAL WORLD 59**

- The common properties of biological systems 59
- Eucaryotes and procaryotes 64
- Organization and function in the eucaryotic cell 65
- Organization and function in the procaryotic cell 78
- Targets for certain antibiotic agents in procaryotic and eucaryotic cells 84
- The differences between procaryotes and eucaryotes: a summing up 86
- The general properties of viruses 87

4

**THE PROTISTS 89**

- The algae 90
- The protozoa 99
- The fungi 105
- The slime molds 115
- The protists: summing up 117

5

**THE PROCARYOTES: AN INTRODUCTORY SURVEY 119**

- Major taxonomic subdivisions among procaryotes 122
- The mycoplasmas 126
- The Gram-positive bacteria 127
- The Gram-negative bacteria 132
- Constituent groups of Gram-negative bacteria 137
- The procaryotes: a summing up 152

6

**MICROBIAL METABOLISM:  
THE GENERATION OF ATP 154**

- Some thermodynamic considerations 154
- The role of ATP in biosynthesis 155
- The role of pyridine nucleotides in metabolism 157
- Modes of ATP-generating metabolism 159
- The biochemistry of ATP generation by heterotrophs 162

- ix The biochemistry of fermentations 168
- Special pathways for the primary attack on organic compounds by microorganisms 170
- The oxidation of inorganic compounds 172
- Photosynthesis 172
- The mechanism of electron transport 178

7

## **MICROBIAL METABOLISM: BIOSYNTHESIS 187**

- Methods of studying biosynthesis 187
- The assimilation of inorganic carbon, nitrogen, and sulfur 190
- The strategy of biosynthesis 197
- The synthesis of nucleotides 198
- The synthesis of amino acids and other nitrogenous cell constituents 205
- The synthesis of lipid constituents from acetate 216
- The synthesis of porphyrins 224
- Interconnections between catabolic and biosynthetic pathways 225
- The biosynthesis of macromolecules: general principles 228
- The synthesis of DNA 231
- The synthesis of RNA 236
- The synthesis of proteins 237
- The synthesis of polysaccharides 243
- The synthesis of cell wall components 244

8

## **REGULATION 248**

- The biochemical basis of regulation 250
- Regulation of enzyme synthesis 253
- Patterns of regulation 258
- Regulation of DNA synthesis and cell division 268
- Regulation of RNA synthesis 271
- The teleonomic nature of biological systems 273

9

## **MICROBIAL GROWTH 275**

- The definition of growth 275
- The mathematical nature and expression of growth 276
- The measurement of growth 280
- The efficiency of growth: growth yields 283
- Synchronous growth 284
- Effect of nutrient concentration on growth rate 286
- Continuous culture of microorganisms 287
- Maintenance energy 291

**THE EFFECT OF ENVIRONMENT  
ON MICROBIAL GROWTH 293**

- Functions of the cell membrane 293
- Entry of nutrients in the cell 294
- Effects of solutes on growth and metabolism 299
- Effect of temperature on microbial growth 305
- Oxygen relations 309

**THE RELATIONS BETWEEN STRUCTURE  
AND FUNCTION IN PROCARYOTIC CELLS 314**

- Surface structures of the procaryotic cell 314
- The molecular structure of flagella and pili 338
- The chemotactic behavior of motile bacteria 343
- Special procaryotic organelles 346
- The procaryotic cellular reserve materials 351
- The nucleus 356

**THE VIRUSES 364**

- The discovery of filterable viruses 364
- The general properties of viruses 365
- The bacterial viruses 373
- The DNA bacteriophages: the lytic cycle of infection 375
- Lysogeny 381
- The RNA bacteriophages 388
- The animal viruses 389
- The reproduction of animal viruses 391
- The tumor viruses 394

**MUTATION AND GENE-FUNCTION  
AT THE MOLECULAR LEVEL 402**

- The chemical basis of mutation 403
- The effects of mutation on the translation process 415

10

11

12

13



<b>xi</b>	The genetic aspects of regulation	418
	Genetic complementation	423
	Mutations in bacteriophage	425

## **THE EXPRESSION OF MUTATION IN VIRUSES, CELLS, AND CELL POPULATIONS 430**

	The effects of mutation on phenotype	430
	The selection and detection of mutants	434
	The conditional expression of gene mutation	436
	The time course of phenotypic expression of mutation	438
	Population dynamics	441
	Selection and adaptation	448
	The consequences of mutation in cellular organelles	449
	Mutant types of bacteriophages	449

## **GENETIC RECOMBINATION 452**

	Recombination in bacteria	452
	Bacterial transformation	455
	Bacterial conjugation	461
	Properties of plasmids	464
	The conjugation process	470
	F-mediated chromosome transfer in <i>E. coli</i>	473
	The major groups of plasmids	480
	The occurrence of conjugation in different groups of bacteria	486
	Transduction by bacteriophage	488
	Recombination in bacterial viruses	492
	Recombination in eucaryotic protists	493

## **THE CLASSIFICATION OF BACTERIA 502**

	Species: The units of classification	502
	The problems of taxonomic arrangement	505
	New approaches to bacterial taxonomy	509
	The comparison of bacterial genotypes by genetic analysis	518
	The main outlines of bacterial classification	523

**THE PHOTOSYNTHETIC PROCARYOTES 527**

- The functional properties of photosynthetic procaryotes 528
- The blue-green bacteria 540
- The purple bacteria 546
- The green bacteria 555
- The evolution of photosynthesis 559
- Halobacterium and its relations to light 559

**GRAM-NEGATIVE BACTERIA:  
THE CHEMOAUTOTROPHS AND METHYLOTROPHS 564**

- The chemoautotrophs 564
- The methylotrophs 579
- Origins of chemoautotrophs and methylotrophs 587

**GRAM-NEGATIVE BACTERIA:  
AEROBIC CHEMOHETEROTROPHS 589**

**THE ENTERIC GROUP AND RELATED ORGANISMS 612**

- Common properties of the enteric group 613
- Genetic relations among enteric bacteria 618
- Taxonomic subdivision of the enteric group 620
- Coliform bacteria in sanitary analysis 627

**GRAM-NEGATIVE BACTERIA:  
MYXOBACTERIA AND OTHER GLIDING ORGANISMS 630**

- The myxobacteria 630
- The cytophaga group 636

- xiii Filamentous, gliding chemoheterotrophs 638  
The filamentous sulfur-oxidizing bacteria 642

22

**GRAM-POSITIVE BACTERIA:**  
**UNICELLULAR ENDOSPOREFORMERS 644**

- The anaerobic sporeformers: genus *Clostridium* 652  
The anaerobic sporeformers: genus *Desulfotomaculum* 661  
The endospore 662

23

**GRAM-POSITIVE BACTERIA:**  
**THE ACTINOMYCETE LINE 672**

- The lactic acid bacteria 678  
The micrococci 685  
Group II: *Corynebacterium*, *Mycobacterium*, *Nocardia* 686  
Group II: aerobic coryneform bacteria 690  
*Geodermatophilus* and *Dermatophilus* 695  
Group III: the euactinomycetes 695

24

**NONSPORE-FORMING STRICT ANAEROBES 704**

- The methanogenic bacteria 704  
The genus *Desulfovibrio* 707  
Nonspore-forming anaerobes with a fermentative metabolism 711

25

**MICROORGANISMS AS GEOCHEMICAL AGENTS 714**

- The fitness of microorganisms as agents of geochemical change 715  
The cycles of matter 717  
The phosphorus cycle 717  
The cycles of carbon and oxygen 718  
The nitrogen cycle 720  
The sulfur cycle 724  
The cycle of matter in anaerobic environments 726  
The cycle of matter through geological time 727  
The influence of man on the cycle of matter 729

**SYMBIOSIS 733**

- Types of symbioses 733
- The functions of symbiosis 737
- The establishment of symbioses 742
- The evolution of symbioses 744

**SYMBIOTIC ASSOCIATIONS BETWEEN PHOTOSYNTHETIC  
AND NONPHOTOSYNTHETIC PARTNERS 747**

- Symbioses in which the photosynthetic partner is a higher plant 748
- Symbioses in which the photosynthetic partner is a microorganism 757

**SYMBIOTIC ASSOCIATIONS BETWEEN  
TWO NONPHOTOSYNTHETIC PARTNERS 766**

- Symbioses in which both partners are microorganisms 766
- Symbioses between microorganisms and metazoan hosts 774

**MICROBIAL PATHOGENICITY 785**

- Microbial toxins 786
- Infections of the mucosal epithelium 793
- Infections of the subepithelial tissues 794
- Inducible host defenses: the immune response 800

**MICROBIAL DISEASES OF MAN 810**

- Bacterial diseases 811
- Fungal diseases 817
- Protozoan diseases 820
- Viral diseases 826

**THE EXPLOITATION OF MICROORGANISMS BY MAN 831**

- The use of yeast by man 831
- Microbes as sources of protein 836
- The use of acetic acid bacteria by man 837
- The use of lactic acid bacteria by man 838
- The use of butyric acid bacteria by man 841
- The microbial production of chemotherapeutic agents 842
- Microbiological methods for the control of insects 850
- The production of other chemicals by microorganisms 851
- The production of enzymes by microorganisms 851
- The use of microorganisms in bioassays 851

**INDEX 855**

## THE BEGINNINGS OF MICROBIOLOGY

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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.

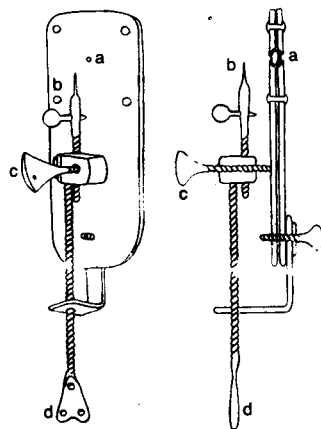
Leeuwenhoek's microscopes (Figure 1.2) bore little resemblance to the instruments with which we are familiar. The almost spherical lens (a) was mounted between two small metal plates. The specimen was placed on the point of a blunt pin (b) attached to the back plate and was brought into focus by manipulating 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.

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 1.1**

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





**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