Burrows

TEXTBOOK OF MICROBIOLOGY

TWENTY-SECOND EDITION

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Chiyoda-Ku, Tokyo 102, Japan

Library of Congress Cataloging in Publication Data

Burrows, William, 1908–

Burrows Textbook of microbiology.

1. Medical microbiology. I Freeman, Bob A. II Title
III. Title: Textbook of microbiology

QR46.B84 1985 616'.01 83–18902

ISBN 0–7216–3868–6

Listed here is the latest translated edition of this book together with the language of the translation and the publisher

Spanish (*20th Edition*)—NEISA, Mexico 4, D F , Mexico

Spanish (*21st Edition*)—Nueva Editorial Interamericana, S A de C V , Mexico City, Mexico

Burrows Textbook of Microbiology ISBN 0-7216-3868-6

Last digit is the print number 9 8 7 6 5 4 3 2 1

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Preface

The successive editions of a textbook must reflect the metamorphosis of the science it represents, with changes in emphasis, expansion into new areas of knowledge, and deletion of the outmoded—all dictated by the maturation of the discipline. Yet, it also necessarily reflects the interests, as well as the prejudices, of its author. This textbook began in 1908 as **General Bacteriology**, authored by Edwin O. Jordan. The first edition of 545 pages set forth the essential knowledge of microorganisms of that era. In 1938, William Burrows assumed authorship, and through nine editions, each bearing his unmistakable imprimatur, the textbook has evolved into its present place in the literature of medical microbiology. The present edition seeks to retain the philosophy, established by William Burrows, that it must represent more than a compendium of facts; it must offer to the student an understanding of the science, based on historical evolution and experimental findings.

This edition has been completely revised and rewritten to improve readability, delete outmoded information, incorporate new material, and delineate new directions in medical microbiology; new sections will be found throughout the book. Rigorous attention has been given to the concepts of microbiology that promote an understanding of infectious diseases. Thus, the book is especially suitable for students of medicine and pathogenic microbiology.

This book could not have been completed without the aid and cooperation of many colleagues; the author is especially grateful to those who contributed chapters in their respective specialties. Dr. M. J. Wolin has been joined by his colleague, Dr. T. L. Miller, in his extensive revision of the chapters on microbial metabolism and the effects of physical and chemical agents on microorganisms. Drs. D. J. Kopecko and L. S. Baron have continued their development of the concepts of gene expression and evolution in bacteria in the chapter on microbial genetics. Dr. Preston H. Dorsett has effectively revised the chapter on basic aspects of animal virology, and Dr. J. W. Rippon continues his association with this book by the addition of new material to the chapter on medical mycology. The author is indebted to two new contributors, Drs. Gary R. Pearson and Donald G. Dusanic. Dr. Pearson has reorganized and rewritten the section on immunology, including new developments and perspectives in this exciting discipline; Dr. Dusanic ably succeeds Dr. R. M. Lewert as author of the chapter on medical parasitology. The writer also extends his thanks to Dr. William M. Todd for his critical review of the manuscript and for his many helpful suggestions.

The author is indebted to the many colleagues who contributed illustrative material and to the editors and staff of the W. B. Saunders Company for their invaluable support and cooperation. Finally, special appreciation is expressed to Rosemary Freeman, who has undertaken bibliographic tasks for the book and has aided in countless other ways toward its completion.

BOB A. FREEMAN

Contents

CHAPTER 1

Historical Perspectives in Microbiology

MI-CRO-BI-OL-O-GY (mī'krō-bī-ŏl'ə-jē) *n.* The science that deals with microscopic living organisms and, especially, their effects on other forms of life. (From Greek *mikros,* small + *bios,* life.)

The term microbiology is commonly used in a more narrow sense than its etymology suggests. Although it may be used to describe the broader subject of small or minute living organisms, it generally refers to a study of those microscopic life forms that are directly or closely related to human activity and welfare.

In the early history of the science, it was traditional to consider microorganisms as belonging to either the plant or the animal kingdom. In modern concepts of biology, however, the bacteria and blue-green algae are regarded as belonging to a third kingdom, the Procaryotae. The microscopic members of the animal and plant kingdoms—protozoa, fungi, and most algae—share with higher life forms a unit structure, the eucaryotic cell, which is of great organizational complexity. The cellular unit of procaryotes, in contrast, is much more simple in organization and structure (Chapter 2). The viruses represent a special case in that they are noncellular and are distinguished by their obligate parasitic relationship to the cells of their hosts; their evolutionary position is uncertain (Chapter 38).

Although the concept and demonstration of living microorganisms is a relatively recent development in biology, their activities have been familiar to man from prehistoric times. Decomposition of organic matter and especially spoilage of foods, acetic and lactic acid fermentations and alcoholic fermentation, degradation of proteins with the production of new and desirable flavors in certain foods, and the occurrence of infectious disease are obvious examples of familiar phenomena now known to be of microbial origin.

The existence of these biological entities is inferred from the consequences of their activity; their living nature is evident when the observed effects are reproduced in series, *e.g.*, by transfer of a small portion of a fermenting mixture to a fresh, unfermented substrate. Such inferences may be found in statements made in both ancient and later writings. Thus, Lucretius wrote of "the seeds of disease" in his *De Rerum Natura,* Fracastorius of Verona in 1546 suggested a *contagium vivum* as a cause of disease, and Benjamin Marten in 1720 speculated that minute animals were responsible for consumptive disease.

These suggestions were perhaps too abstract for general acceptance, and the beginning of the science of microbiology awaited a concrete demonstration of the existence of these agents; this was accomplished by optical systems so precise as to permit direct visualization of microscopic, living forms.

MICROSCOPY

The name of Antony van Leeuwenhoek (1632–1723) is inseparably associated with the

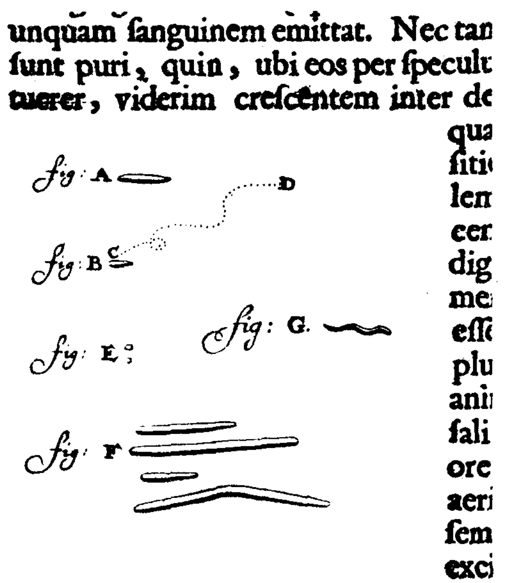

Figure 1–1. The first pictorial representation of bacteria. (Reproduced from *Arcana Naturae Delecta Ab Antonio van Leeuwenhoek*. Delphis Batavorum apud Henricum Crooneveld. 1695.)

early development of microscopy. He held a political sinecure in Delft and devoted the greater part of his time to the hobby of lens grinding. He not only made the best lenses available at that time, but used them to examine a wide variety of materials that interested him. It was characteristic that he first observed bacteria in an attempt to discern in visual terms the nature of the taste of pepper. There is no doubt that he actually observed these microorganisms, for he made recognizable drawings of them that were reproduced in reports of his observations to the Royal Society.[2]

A few of the hundreds of microscopes he constructed are still extant, and it is evident that the maximum magnification he reached was approximately 300 diameters. This is not sufficient to allow observation by transmitted light of objects as small as bacteria. While van Leeuwenhoek was never willing to reveal his method of illumination, it seems probable that he used reflected light as it is used in the modern darkfield microscope.[5]

The direct demonstration of living organisms of such small dimensions was a notable achievement, but any relation of such forms to natural phenomena such as fermentation or infectious disease either escaped van Leeuwenhoek or was of no interest to him. Their possible relation to gross phenomena was apparent to at least some of the scientific men of his day. For instance, in a comment on a report of an epidemic disease of cattle published five years later, his contemporary, Slare, wrote:[11]

> I wish Mr. Leeuwenhoek had been present at some of the dissections of these infected Animals, I am perswaded he would have discovered some strange Insect or other in them.

But systematic study was delayed for many years, and it was nearly a century later, in 1786, that a Danish zoologist, O. F. Müller, discovered many of the details of bacterial structure. He left drawings so accurate that the bacteria he showed can be identified today as belonging to one or another of the chief divisions. Somewhat later, in 1838, Ehrenberg published *Infusionstierchen*, in which he put the study of these microorganisms on a systematic basis. He established a number of groups by clearly recognizing fundamental morphological distinctions, such as those differentiating the spirochetes from certain of the protozoa. Some of the names he used, such as "bacterium" and "spirillum," are still current in bacteriological nomenclature though with somewhat changed significance.

The perfection of the modern compound microscope, with achromatic and later apochromatic objective lenses, markedly facilitated the study of the morphology of microorganisms. These permitted observations of certain gross internal structures of the bacterial cell, as well as some of the larger viruses. This made possible accurate differentiation of microorganisms on a morphological basis and provided basic criteria for characterization of the larger forms—fungi, protozoa, and metazoa. Phase microscopy, introduced in the 1940s, further facilitated certain aspects of microscopy, especially the observation of microorganisms in the living state, by accentuation of slight differences in the refractive indices of intracellular elements.

Optical microscopy is nevertheless limited by the resolution that may be obtained with visible light. The lower limit of resolution is about 0.2 μm., with practical magnifications no greater than 1000 to 2000 diameters. It is obvious that these resolution limits are inadequate for details of the intracellular structure and organelles of bacteria. The darkfield microscope, although facilitating the observation of tenuous microorganisms such as spirochetes and structures such as flagella, shows minute objects only as points of light against a dark background without improving resolution.

Table 1–1. **Early Developments in Microscopy**[3]

Date	Name	Contribution
(*ca.*) 1600	Hans and Zaccharias Janssen; Cornelius Drebbel	Developed the earliest compound microscopes. These were crude and imperfect and were little more than magnifying lenses. Bacteria could not be resolved.
1665	Robert Hooke	Designed and built microscopes through which he observed small animals. He published these illustrations in *Micrographia*.
1673	Antony van Leeuwenhoek	First of van Leeuwenhoek's letters to the Royal Society. For a period of 50 years he continued this correspondence, communicating his observations of the microscopic world. In 1683, in his 39th letter, he provided the first unequivocal description of bacteria, found in tooth plaque.
1733	Chester Hall; John Dolland	Credited with independent invention of achromatic lenses, correcting for chromatic aberration.
1830	Joseph J. Lister (Father of Lord Lister)	Contributed major improvements in microscopes, leading to modern microscopy.
1878	Ernst Abbe	Invention of homogeneous immersion lenses.
1911	Oskar Heimstadt	Invention of the fluorescence microscope.
1932	Max Knoll and Ernst Ruska	First description of the electron microscope.
1935	Frits Zernike	First description of the phase-contrast microscope.

Beginning in the 1930s, the electron microscope was developed in practical, usable form. It constituted an important advance in microscopy in that an object or structure casting a "shadow" in the electron beam may be resolved, and working magnifications of 30,000 diameters with excellent resolution were obtained. Thus it became possible to view microorganisms beyond the limits of optical resolution and to visualize structures as small as the individual subunits of the virus coat. The subsequent development of the scanning electron microscope opened new dimensions in the visualization of cell topography; the three-dimensional quality of these high resolution images has yielded new information on cell surfaces and cellular interactions.

SPONTANEOUS GENERATION

For many years it was believed that living organisms could arise *de novo* and fully formed from decomposing organic matter. The development of snakes from horse hairs standing in stagnant water and the appearance of mice in decomposing fodder were, at one time, popular legends. The fallacies in such beliefs were suspected by some, and in the seventeenth century a number of individuals carried out experiments designed to show whether living organisms had their origin only in other living organisms (biogenesis), or appeared spontaneously in decomposing organic matter (abiogenesis).

In the middle of the seventeenth century, the poet-physician Redi performed experiments showing, contrary to popular belief, that maggots were not formed spontaneously in decomposing meat but were fly larvae hatched from eggs deposited in the meat. Spallanzani, an Italian monk, showed further that putrescible meat infusions did not spoil when properly heated and did not contain living organisms. Needham, an Irish priest, took issue with Spallanzani on the basis of similar experiments in which spoilage took place and living organisms appeared in spite of previous heating. A second series of elaborate experiments by Spallanzani corroborated his earlier findings and exposed the fallacies in Needham's experiments. It was evident that microorganisms were carried in air, and this was convincingly demonstrated by many other workers, including Schulze, Schwann, Schröder and von Dusch, and Tyndall.

The whole question seemed settled conclusively in favor of biogenesis when it was raised again in the middle of the nineteenth century by the work of the eminent French chemist

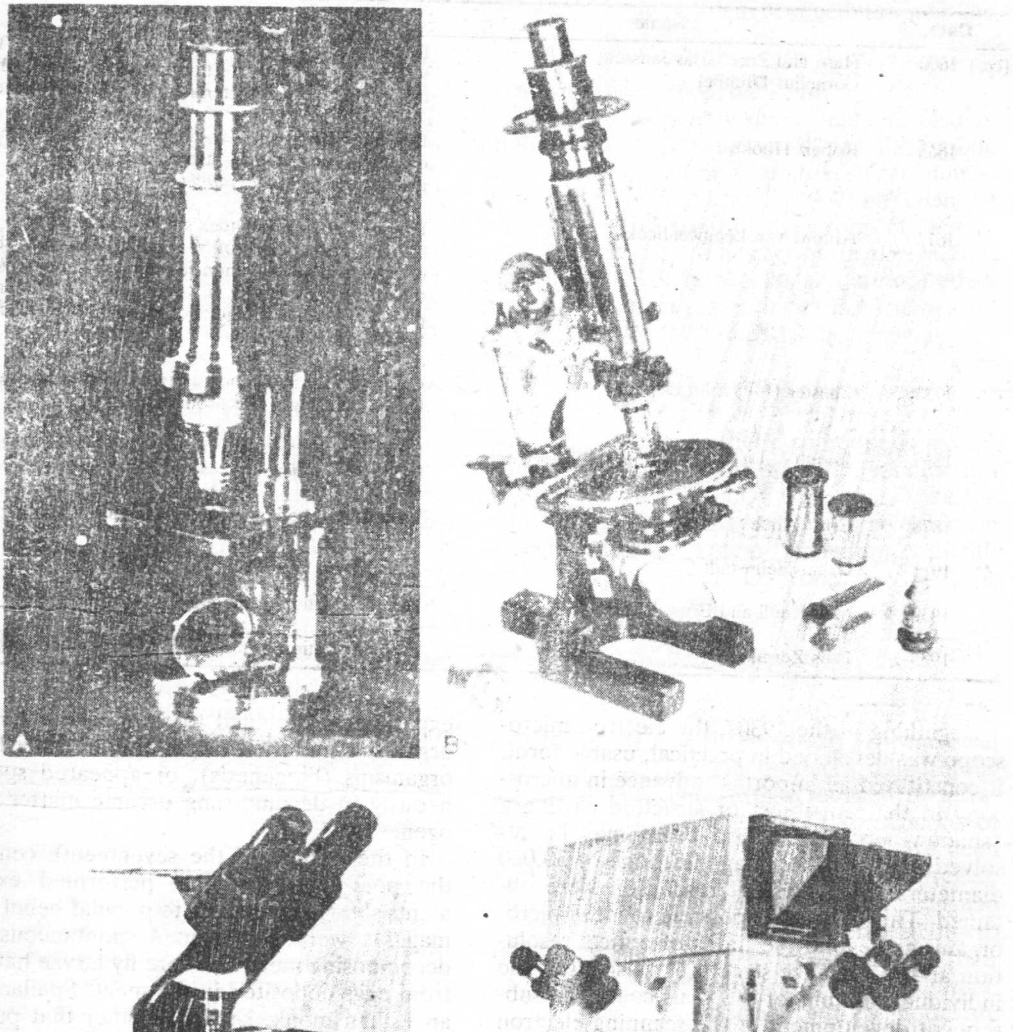

Figure 1–2. Development of light microscopes. *A*, Zeiss microscope, ca. 1860; *B*, Zeiss polarization microscope, ca. 1890; *C*, Zeiss microscope KM; *D*, Zeiss Axiomat NDC microscope. (Courtesy of Carl Zeiss, West Germany.)

Pouchet. He made the same kind of technical errors as some of his predecessors, so that his evidence supported the hypothesis of spontaneous generation of life. It was at this point that Pasteur entered the renewed controversy through his early studies on fermentation. By ingenious and incontrovertible experiments, he demonstrated that microorganisms carried in air were responsible for Pouchet's erroneous results.

The validity of biogenesis has not since been seriously questioned. While biogenesis may seem self-evident today, its establishment was of fundamental importance to the development of modern biological science. Had this not been done, the specific microbic etiology of fermentation, decay, infectious disease, and similar phenomena could not have been established. At the same time, present concepts of evolution, and biochemical evidence in particular, seem to point inevitably toward an original emergence of life from the nonliving.[12]

FERMENTATION AND BIOCHEMICAL PHYSIOLOGY

By the middle of the nineteenth century the general nature of organic material was becoming relatively clear, but the natural decomposition of these substances was not, in that the part played by microorganisms in the processes of putrefaction, decay, and fermentation was not known.

The plant-like nature of yeast had been shown by Schwann in 1837 and Cagniard-Latour in 1838, but the preeminent chemists of the day, Liebig, Berzelius, and Wöhler, regarded the presence of yeast cells in a fermenting mixture as no more than incidental to the decomposition, which was considered by them to have a purely inanimate basis.

Pasteur (1822–1895), originally trained as a chemist, had done his early work on stereoisomerism. The formation of optically active amyl alcohol during the course of the lactic acid fermentation led him to study the processes of fermentation. Having first established the validity of biogenesis, it was not difficult to prove that fermentations resulted from physiological activity of living, growing microorganisms.

Pasteur further established the principle that fermentations are specific, *i.e.*, that different kinds of fermentations, each yielding different end products, result from the activities of specific microorganisms. He applied this principle in work on the "diseases" of beer and wine, showing that these represented secondary fermentations by contaminating microorganisms to produce undesirable end products. It was then logical to control the fermentative process by gently heating the fermentable solution to eliminate undesirable contaminants, followed by inoculation with microorganisms that brought about the desired fermentation. This method of gentle heating, now widely applied to destroy pathogenic microorganisms in milk and other products, is known as pasteurization.

Study of the mechanisms of fermentation was tremendously stimulated by the commercial value of end products such as ethanol, lactic and acetic acids, glycerol, butanol, and acetone. These considerations led to the development of new industries concerned with the large-scale production of organic solvents and subsequently of other microbial products, especially vitamins and antibiotics.

The chemical study of microorganisms grew side by side with the chemical approach to mammalian physiology, eventually fusing into the present-day science of biochemistry. The initial common ground was carbohydrate metabolism and the respiratory processes, astonishingly similar in such widely dissimilar organisms. Thus, the phenomenon of anaerobic respiration, first observed by Pasteur in fermenting mixtures and received with incredulity at the time, is now commonplace in biochemical physiology. More detailed study showed that the catalysis of the processes of respiration is substantially the same also, and characterization of respiratory enzymes, for example, was markedly facilitated by their availability in microorganisms. Many of the vitamins required by mammals, especially those of the B group, are also required by microorganisms, and function in a similar manner.

In other respects, the biochemical potential of microorganisms goes far beyond that of any other living organisms. While some microorganisms simulate mammalian metabolism, others are photosynthetic. These may resemble green plants in the photochemical reduction of carbon dioxide or differ when the photochemical reactions are coupled with the metabolism of inorganic sulfur compounds. Still others are chemoautotrophic, deriving energy for the reduction of carbon dioxide from the oxidation of inorganic substrates such as hydrogen, or inorganic compounds of nitrogen, sulfur, iron, and manganese. The nitrogen-fixing bacteria assimilate atmospheric nitrogen either alone or in symbiosis with leguminous plants. Clearly, the ecological distribution of microorganisms is a function of their physiological properties.[1]

INFECTIOUS DISEASE

Sometimes new vistas are opened prematurely and not appreciated at the time, or too late and are anticlimactic, but occasionally new concepts coincide with an unusually receptive segment of the general stream of thought. Such a monumental coincidence of thought took place with the conceptual transition from the specific microbic etiology of fermentations, advanced by Pasteur, to that of infectious diseases. Inexorably, this transition was to reveal the causes, prevention, control, and possible cure of the rampant infectious diseases, capturing popular imagination as it developed.

The conceptual transition from the specific microbic etiology of fermentations to that of infectious disease was more readily reached than might appear.

The implications of Pasteur's studies on fermentation for infectious disease were almost immediately perceived, notably by the British surgeon Lord Lister. He applied the basic principles to his own work, controlling infection in the operating room by liberal use of phenol. An era of antiseptic surgery, initiated by Lister in 1867, led to a remarkable reduction in intercurrent infection and mortality. These practices were displaced within the next two decades by those of aseptic surgery, largely by von Bergmann in Berlin, following a growing appreciation of infected persons as the primary source of sepsis.

It remained for the German physician Koch (1843–1910) to develop the experimental methods that proved a causal relation between bacteria and infectious disease. From the first applications of microscopy to the study of microorganisms, it was clear that different morphological types existed and that these usually occurred in nature in mixed populations. It was evident also from the early studies on pyemia that morphological criteria did not suffice to differentiate and characterize the bacteria, since morphologically identical forms differed markedly in pathogenic properties. It was essential to separate such microorganisms from one another and to grow each kind in pure culture.

Initially this had been approximately accomplished by diluting mixed bacterial populations in liquid culture mediums; when replicates of high inoculum dilutions showed irregular occurrence of growth, it was assumed that the

Table 1–2. **Significant Developments in the Germ Theory of Disease**

Date	Name	Contribution
Biblical	Leviticus:13, 14	Refers to contagious nature of disease; contains instructions for sanitation and hygiene to inhibit spread of leprosy.
(ca.) 430 B.C.	Thucydides	Infers contagious nature of certain plagues in Athens.
1546	Hieronymus Fracastorius	In a series of books on contagion, wrote of *seminaria* (seeds or germs) of disease. Whether he recognized "seeds" as living is unlikely. Recognized (1) transmission by "fomites" and direct contact, (2) organ specificity of certain infections, (3) age specificity of disease, and (4) resistance to second attacks of the same disease.
1720	Benjamin Marten	In *A New Theory of Consumptions: More Especially of a Phthisis or Consumption of the Lungs* (London), speculated that minute animalcula were responsible for disease. Marten's theories are remarkably similar to modern ideas of infectious diseases.
1872	C. J. Davaine	Experimentally transmitted septicemia in rabbits by serial injections (passage) of "putrid" blood, with observations of increasing virulence.
1878	Robert Koch	In *Etiology of Traumatic Infectious Disease*, Koch undertook to prove experimentally that infectious diseases were due to specific parasitic microorganisms.
1880 – 1883	Alexander Ogston	Building on Koch's studies, Ogston concluded that inflammation and suppuration of acute abscesses are due to micrococci, based on microscopic observations and bacteriological cultures.
1884	Robert Koch	First complete statement of Koch's postulates.

bacteria growing in these tubes were descendants of a single viable cell and thus constituted a pure culture. Probably one of the greatest single contributions to technical bacteriology was the method for isolation of pure cultures developed by Koch. The inoculum was diluted and cultured on a nutrient medium solidified as a gel by the addition of gelatin. Individual viable cells were separated on the solidified medium and their progeny developed as discrete masses of cells of single ancestry—a "clone" in zoological terminology. Modern microbiology was in large part made possible by, and is based upon, this simple technique.

The specific microbial etiology of infectious disease was first clearly established in studies on anthrax, an epidemic and highly fatal disease of cattle and other domestic animals. The microorganism now known as *Bacillus anthracis* had been observed by Pollender in 1849 in the blood and organs of infected animals and by Davaine and Rayer in 1850. The disease was transmitted by Brauell in 1867 by inoculating normal animals with infected blood. It is generally conceded that modern medical microbiology began with Koch's studies on anthrax in 1878, in which he developed conclusive evidence that the anthrax bacillus is causally related to the disease. In this and succeeding works he advanced the conceptual rules, later to bear his name as Koch's postulates, which outline the proof necessary to establish a specific microorganism as the etiological agent of disease.

These advances, coupled with the development of staining methods by Koch, Ehrlich, Weigert, and others, provided tremendous stimulus to the study of infectious diseases, resulting in an immense accumulation of new knowledge within the ensuing two decades. Indeed, most of the principal bacterial pathogens were described and isolated before the turn of the century.

Characterization of the pathogenic bacteria in morphological, physiological, and pathological terms, and studies of their persistence under adverse conditions and behavior in the infected host were obvious and essential corollaries. Out of this kind of information grew an understanding of the basic elements of the spread of infection, providing a firm foundation to the science of epidemiology. In 1854, Snow deduced the presence of the causative agent of Asiatic cholera in the feces of diseased persons and its transmission to others by way of common contaminated water supply in the famous Broad Street Pump epidemic in London. The nature of waterborne enteric disease was to become clear, however, only after the actual isolation and study of enteric pathogens such as the cholera vibrio and the typhoid and dysentery bacilli.

Effective control was an inevitable consequence of the developing understanding of infectious diseases and their dissemination. The application of indicated control measures, such as chlorination of water and pasteurization of milk, coupled with artificial immunization, has been astonishingly successful. The result has been the virtual disappearance of many of the great killing diseases, such as smallpox, typhoid fever, Asiatic cholera, diphtheria, and plague, and tremendous reduction in others, such as tuberculosis. All of this stems in large part from an understanding of the principle of specific microbial etiology of infectious disease.

VIROLOGY

Early in the study of infectious diseases, it became apparent that there were putative agents of disease which could neither be seen with the light microscope nor be cultivated apart from living host cells. Iwanowski in 1892 and Beijerinck in 1899 observed the first of these agents—that causing mosaic disease of the tobacco plant—which Beijerinck described as a *contagium vivum fluidum,* since it was present in bacteria-free filtrates of infected juice. A similar agent causing foot-and-mouth disease of cattle was described in 1897 by Löffler and Frosch, and the causative agent of yellow fever by the American Army Commission under the direction of Reed in 1900. Other such agents producing a transmissible lysis of bacteria were found by Twort in 1916 and d'Herelle in 1917, to give three groups of agents now known as the plant, animal, and bacterial viruses, respectively.

Prior to 1930, the term virus referred to any living agent of disease, including bacteria. During the period from 1930 to 1940, filterable virus was the name used to describe infective agents that would pass through special filters that retained bacteria. Since about 1940, **virus** has been applied only to extremely small, noncellular particles which infect and replicate only within living host cells.

Physical and morphological evidence has shown that these agents range from a diameter of 200 nm., or just at the limits of resolution of the light microscope, to infectious particles as small as 10 nm., visualized only by electron microscopy. Further, chemical analyses re-