MEDICAL MICROBIOLOGY

Edited by

Felix Milgrom, M.D.

and

Thomas D. Flanagan, Ph.D.

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Felix Milgrom, M.D.

Distinguished Professor of Microbiology and Chairman
Department of Microbiology
Schools of Medicine and Dentistry
State University of New York at Buffalo

and

Thomas D. Flanagan, Ph.D.

Professor of Microbiology
Schools of Medicine and Dentistry
State University of New York at Buffalo

With 27 Contributors

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CONTRIBUTORS

C. John Abeyounis, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Boris Albini, M.D. Associate Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Daniel Amsterdam, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, and Associate Professor of Medicine, School of Medicine, State University of New York at Buffalo; Director, Division of Clinical Microbiology and Immunology, Erie County Laboratory, Buffalo

Giuseppe A. Andres, M.D. Professor of Microbiology and Pathology, Schools of Medicine and Dentistry, and Professor of Medicine, School of Medicine, State University of New York at Buffalo; Director, Renal Immunopathology Laboratory, The Buffalo General Hospital; Consultant in Clinical Immunology, Children's Hospital, Buffalo

Ernst H. Beutner, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, and Research Professor of Dermatology, School of Medicine, State University of New York at Buffalo

Arlene R. Collins, Ph.D. Associate Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Roger K. Cunningham, Ph.D. Associate Professor of Microbiology, Schools of Medicine and Dentistry, and Associate Director, The Ernest Witebsky Center for Immunology, State University of New York at Buffalo

Thomas D. Flanagan, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Eugene A. Gorzynski, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Chief, Microbiology Section, Veterans Administration Medical Center, Buffalo

Diane M. Jacobs, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Kyoichi Kano, M.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Consultant in Clinical Immunology, Erie County Medical Center, Buffalo

Joseph H. Kite, Jr., Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Reginald M. Lambert, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Director, American Red Cross Blood Services, Buffalo Region

Kenneth F. Manly, Ph.D. Associate Research Professor of Microbiology, Roswell Park Graduate Division, State University of New York at Buffalo; Cancer Research Scientist V, Roswell Park Memorial Institute, Buffalo

Joseph M. Merrick, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry. State University of New York at Buffalo

Felix Milgrom, M.D. Distinguished Professor of Microbiology and Chairman, Department of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Richard L. Miner, M.D. Clinical Associate Professor of Medicine, School of Medicine, State University of New York at Buffalo

James F. Mohn, M.D. Professor of Microbiology, Schools of Medicine and Dentistry, and Director, The Ernest Witebsky Center for Immunology, State University of New York at Buffalo; Immunohematologist and Director, Blood Bank, The Buffalo General Hospital

David T. Mount, M.S. Clinical Assistant Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Robert A. Nelson, Jr., M.D. Research Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Scientific Director of Research and Development, ImmunoGenetics, Inc., North Berwick, New Hampshire

Erwin Neter, M.D. Professor Emeritus of Microbiology, Schools of Medicine and Dentistry, and Professor Emeritus of Pediatrics, School of Medicine, State University of New York at Buffalo; Former Director, Bacteriology and Serology, Children's Hospital, Buffalo; Consultant to Clinical Staff, Roswell Park Memorial Institute, Buffalo

Russell J. Nisengard, D.D.S., Ph.D. Professor of Periodontics and Endodontics, School of Dentistry, and Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Bernice K. Noble, Ph.D. Assistant Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Cornelius J. O'Connell, M.D. Clinical Associate Professor of Medicine, School of Medicine, and Clinical Associate Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Consulting Physician, The Buffalo General Hospital, St. Joseph's Intercommunity Hospital, and Roswell Park Memorial Institute, Buffalo; Attending Physician, Sisters of Charity Hospital and Erie County Medical Center, Buffalo

Carel J. van Oss, Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, and Adjunct Professor of Chemical Engineering, Faculty of Engineering and Applied Sciences, State University of New York at Buffalo

Murray W. Stinson, Ph.D. Associate Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Harshad R. Thacore, Ph.D. Associate Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

Konrad Wicher, D.M.Sc. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo; Director, Clinical Microbiology and Immunology Laboratories, Division of Laboratory and Research, New York State Department of Health, Albany

Marek Zaleski, M.D., Ph.D. Professor of Microbiology, Schools of Medicine and Dentistry, State University of New York at Buffalo

PREFACE

This book has been written for the use of medical students and medical practitioners. It may also be of interest to students and professionals in other health-related fields. It provides basic information about microbiology as it pertains to the practice of medicine. Therefore the emphasis has been placed on pathogenic microorganisms. All major pathogens are discussed from the point of view of their disease-producing mechanisms. Specific treatment and prevention of infectious diseases are also discussed at some length. The last chapter is meant as a recapitulation of the most important microbiological procedures as they are used and requested by the physician. Pathological and clinical aspects of diseases are not treated in any detail, since a textbook of microbiology should not intrude into the fields of pathology and infectious diseases.

The text could not remain indifferent to the unprecedented progress that microbiology has experienced in the last quarter of a century. Significantly, a great part of the progress in molecular biology has been made in studies of microbial physiology and genetics. These areas of general microbiology are discussed in this textbook; however, they are not given all the attention that they deserve, and the information provided is limited to that most useful for students of medicine.

The field of immunology, which originally emerged from medical microbiology, has become a part of practically each branch of medicine and biology. Here again, our purpose was to present briefly the vistas of immunology and to concentrate on those aspects of this science that have immediate bearing on recovery from and resistance to infectious diseases.

For many years the authors of this book have taught medical microbiology as a team. They are aware of the fact that with dozens of excellent textbooks of microbiology available on the market, it takes courage to produce one more. They believe, however, that through many years of teaching experience they have developed the skills of communicating with students of medicine and other health-related professions. They also trust that they have learned to select the microbiological material that is essential for students.

F.M.
T.D.F.
State University of New York at Buffalo

CONTENTS

1 The History of Microbiology

James F. Mohn

The farther backward you can look, the farther forward you are likely to see. (Sir Winston Churchill)

In the study of any branch of science, an acquaintance with the historical development of knowledge is an important element in a clear understanding of our present conceptions. To the student of bacteriology such a basis is essential. (Sir Graham S. Wilson and Sir Ashley A. Miles, Topley and Wilson's Principles of Bacteriology and Immunity)

The history of many concepts, such as the origin of life, putrefaction of dead organic materials, and the nature of communicable changes in the bodies of living men and animals, is incorporated in bacteriologic doctrines. At the time of Moses, the Jews believed that leprosy was contagious and could be spread through contact with the patient or his clothes or by living in the house of a former leper. These beliefs led to the Mosaic regulations that are set forth in the Old Testament (Leviticus 13 and 14).

Hippocrates (ca. 460 to 377 B.C.), a Greek physician now known as the Father of Medicine, elaborated a theory of disease that had two components: an intrinsic factor, a pathologic "constitution" of the individual, and an extrinsic factor, air infected with "miasms." He considered miasms to be a modification of air itself to such a degree that it became deleterious. This did not involve any idea of independent, active agents nor did he consider the possibility of contagion. His doctrine of miasms grew and thrived during the Middle Ages and has survived in modern times in certain medical terms, such as malaria (Italian, *mala aria* — bad air) and influenza (Latin, *influere* — to flow in).

Girolamo Fracastoro (ca. 1478 to 1553, Italy), commonly known by the latinized form of his name, Hieronymus Fracastorius, was a scholar, poet, and thinker, who was born in Verona and attended the University of Padua like other Veronese youths. His famous poem, *Syphilis sive morbus Gallicus* (Syphilis or the French Disease), written in Latin as was the custom of the times, was published in Verona in 1530. The disease is named after the youth Syphilis, the main character in his poem.

In 1546 he published a book in Venice on contagion and contagious diseases that is his chief work, entitled *De Sympathia et*

Antipathia Rerum. De Contagione (Liber Primus), De Contagiosis Morbis (Liber Secundus), De Curatione (Liber Tertius). This is a rare, small quarto book written in Latin in a condensed style. His work on contagion is actually written in three books bound together. The first and most important contains his theory of contagion, in which he expounds on a concept of infectious, communicable diseases, each caused by a living agent, *contagium vivum*; this theory was influenced by his knowledge of the natural history of syphilis. He was the first to indicate that "infection itself is composed of minute and insensible particles and proceeds from them," and he described the transmission of disease by direct contact, by intermediary, inanimate fomites, and through the air (*ad distans*). He noted that "the infection is the same for him who has received or has given the infection; also we speak of infection when the same virus has touched one or the other." In essence this was a true germ theory of disease, but the essentially true statements were unconvincing because they were not based upon the demonstration of the existence of his hypothetical, invisible organisms. Further progress was dependent upon invention of the microscope and the resolution of the problem of spontaneous generation. An account of different contagious diseases is given in the second book, which clearly describes the history of many contagions and makes valuable observations. As examples, the following can be cited: variola (smallpox) and morbilli (measles) affect children by preference and everyone is attacked once, but "it is rare for people who have had these diseases to have them again"; in regard to phthisis (tuberculosis), he believed in the infectivity of consumption, thought the "virus" was very tenacious, persisting in clothing for as long as 2 years, and considered the "germs" infective only for the lungs; rabies he stated was propagated only by the bite of a rabid dog and had an incubation period of about 30 days; his views of syphilis were lucid and accurate, and he gave the first description of gummata, a lesion of tertiary syphilis, his term being "gumositates." The third book deals with his recipes for the cure of various contagious diseases. This all resulted from his practical and broad study of epidemics of syphilis, plague, typhus, and foot-and-mouth disease occurring in northern Italy in his time coupled with deep contemplation and reasoning on what he had observed and how he could interpret it.

Antoni van Leeuwenhoek (1632 to 1723, Holland) has been called the Father of Bacteriology and Protozoology because of his discovery of bacteria and protozoa. He was born in the town of Delft, where he spent his entire life at various occupations beginning as a draper and haberdasher (ca. 1654) and later receiving a series of minor municipal appointments — Chamberlain of the Council-Chamber of the Worshipful Sheriffs of Delft, surveyor, because he was qualified in mathematics, especially geometry, and wine-gauger of Delft. Strangely and with no known explanation, he was an amateur micrographer without any scientific training. He spent his spare time making lenses and mounting them to form a microscope of simple pattern. Nevertheless, he acquired much skill in their manufacture; he taught himself how to grind, polish, and mount lenses of considerable magnifying power, although his method of grinding lenses is unknown. During his life, he made a total of about 550 of these instruments. At the time of his death he left 247 completely finished microscopes and another 172 mounted lenses. The microscopes consisted of a simple, single biconvex lens of short focal length clamped between two metal plates in a fixed position, with the object under examination being moved into focus by means of screws. Of the nine still in existence, the magnification of the best is only 200 times. He left no description of the apparatus used for making his observations on protozoa and bacteria and of his particular manner of observing very small creatures and kept for himself his best microscopes, which probably had a magnification power of 300. Thus, he never divulged what his method was for using his homemade microscope that enabled him to outstrip all other microscopists for at least a century. His micrometry was astonishingly good, although the method of obtaining the illumination he required is unknown. His biographer, Dobell, deduced from his letters that he probably discovered a simple method of dark-field illumination.

Although the only language he knew was Dutch, he reported all of his experimental observations to the Royal Society of London in the form of letters that were translated into English and published in Philosophical Transactions. The first of these arrived in April 1673, curiously enough the very month and year he was elected a Fellow of the Royal Society. He discovered protozoa in 1674 with his observations on free-living protozoa seen in freshwater (Letter 6, September 7, 1674); bacteria in 1676 — the first account ever written on them being his celebrated Letter 18 (October 9, 1676) — from his examinations of rainwater, wellwater, seawater, and melted snow and strange and exotic watery extracts of pepper, ginger, clove, and nutmeg; anaerobic bacteria in 1680 (Letter 32, June 14, 1680); intestinal protozoa and bacteria of man in 1681, reported in his letter of great historic interest and importance (Letter 34, November 4, 1681) as "living animalcules in excrements"; and bacteria of the human mouth in 1683 in a letter as famous as any ever written to the Royal Society (Letter 39, September 17, 1683). Among all his observations of bacteria, the most fundamental that is scientifically accurate and the basis of all bacteriologic morphology to this date was his discovery of the three basic forms of bacteria: coccus (pl. cocci) — sphere, like a tennis ball; bacillus (pl. bacilli) — rod, similar to a piece of chalk or a cigar; and spirillum (pl. spirilla) — spiral, a rigid form analogous to a comma, the letter S, or a corkscrew.

In a report published in 1762, Marcus Antonius von Plenciz (1705 to 1786, Austria), connected the speculations of Fracastorius with the observations of van Leeuwenhoek. He presented an advanced view on the specificity of disease based on a belief in specific microbes as agents of infectious diseases. The first infectious disease shown to be caused by a specific microorganism was the disease of silkworms called *mal segno* in Italy and *muscardine* in France. In 1835 in a remarkable series of investigations, Agostino Bassi (1773 to 1856, Italy) proved that a fungus, now named in his honor as *Botrytis bassiana*, was the causative agent of this disease. From his study he prophesied that microscopic organisms would be found as the causes of human disease.

In theoretical discussions of miasmatic and contagious diseases, Jacob Henle (1809 to 1885, Germany) affirmed his belief in the animate nature and specific action of agents of contagion and made the same prediction as Bassi. The early investigators of his time obtained pure cultures of bacteria only by accident and never knew when contaminants were present. This led to much speculation and loose thinking resulting in considerable equivocal work that hindered the development of the discipline. Henle's logical and critical point of view was a firm corrective. In 1840 he published a statement of the conditions that would have to be satisfied to prove the causal relationship between a particular organism and disease, anticipating Koch's posulates by 36 years. They were (1) demonstration of the constant presence of morphologically identical microorganisms in typical lesions, (2) isolation of these microorganisms *in vitro* in pure culture, and (3) reproduction of the same disease in experimental animals by inoculation of the pure culture.

The real development of bacteriology as

a science was the direct outcome of the research of Louis Pasteur (1822 to 1895, France). His work overshadowed all of his predecessors, and it can be unequivocally stated that he not only founded bacteriology but revolutionized medicine as well. Trained as a chemist, he was led to the study of microscopic organisms by his earlier studies on stereoisomerism and by observations on the phenomena of fermentation. In 1857 and 1858 he reported the results of his investigation on the basis of the formation of amyl alcohol during the course of lactic fermentation. It was his conclusion that such substances were synthesized by living organisms that caused the fermentative process. At this time he also turned his attention to alcoholic fermentation. His classic memoir, published in 1860, staunchly upheld the theories of Caignard-Latour (1836, France) and Schwann (1837, Germany) on the living, plantlike nature of "yeast globules." His findings that yeast cells increased in amount when transferred from one sugar to another and had decided preferences as regards the acidity or alkalinity of the media in which they grew disproved the theories of Leibig, Berzelius, and Wöhler that yeast cells were dead. His research was the first foundation of our knowledge of the conditions to be fulfilled for the cultivation of bacteria. In these investigations that spanned some 20 years, he proved that the fermentations of various organic fluids were always accompanied by the presence of living cells. He further showed that organisms that had different morphologic and cultural characteristics occurred in different types of fermentations. Thus the concept of the specificity of microorganisms was originated.

In these and other studies of this period, Pasteur made major advances in the technology required to further the progress of the study of microorganisms, such as introducing the extraordinary sterilizing effect of superheated steam that led to the development of the autoclave, the practice of sterilizing glassware with dry heat at 170°C, the cotton plug, and specific media.

In 1861 he turned to the examination of butyric fermentation and made the important discovery that it proceeds in the absence of oxygen. He showed that this fermentation was caused by certain microorganisms that could live without oxygen; he proved this by inhibiting the fermentative process by passing a stream of oxygen through an active butyric-fermenting liquid. This led him to find other organisms that lived in the absence of air, and in 1863 he introduced the terms aerobes (*aérobies*) and anaerobes (*anaérobies*) to indicate those microorganisms that live with and without free oxgyen.

In 1862 Pasteur was called upon by the government to determine the cause of the widespread spoilage of wine that was almost paralyzing this important French industry. His investigations, published in 1866 as his famous *Études sur le Vin*, proved that the damaging effect on the palatability and actual potability of wine was caused by wild fermentation produced by contaminating bacteria, which altered the wine's chemical and physical properties. This led him to introduce in 1863 the process known today in his honor as pasteurization, whereby heat-labile products such as wine and milk are subjected to heat sufficient to kill certain types of microorganisms and to decrease the viable population of many other types but insufficient to damage the material. In a similar vein, in 1865 he began research culminating in 1870 with the institution of procedures that saved the silk industry from being destroyed by a parasitic infection of silkworms, a disease called *pébrine*. This led him to espouse the principle of controlling the spread of an infection by detecting and isolating the infected individuals.

Robert Koch (1843 to 1910, Germany), an investigator previously unknown among bacteriologists, made his first outstanding contribution to the rapidly developing field of microbiology at the time he was a country practitioner and district physician at Wollstein. Under primitive conditions in his own home, with a microscope given by his wife but with all his other apparatus entirely homemade, he undertook an investigation of anthrax, which he had had the opportunity to observe in animals in the course of his medical duties. He showed that the disease was transmissible from mouse to mouse over 20 generations and that the lesions in each animal of the series were identical; he cultured the anthrax bacilli in drops of sterile blood serum or aqueous humor in slide cultures on a primitive warm, microscopic stage. He described the appearance of oval granular bodies in the filaments that he recognized were spores, which had not been seen before; he determined accurately the optimal thermal conditions for spore formation; and he reproduced the disease in mice by injection of his cultures. From these observations Koch concluded that only one kind of bacillus could induce the specific morbid process of anthrax, clearly enunciating the doctrine of a specific agent for a specific disease. The enormously significant report of his findings was published in 1876, and at once he was recognized as a great investigator in the field of bacteriologic research. This study of anthrax clearly established the criteria that had to be met in determining that a given microorganism was the specific etiologic agent of a disease. Thus he confirmed the doctrine promulgated previously by Jacob Henle, one of his teachers at the University of Göttingen. The conditions that he emphatically stated must be met are usually referred to as Koch's postulates but more correctly should preferably be called Henle-Koch postulates.

One of the major hindrances impeding the progress of these early microbiologists was the almost total lack of pure cultures of any human disease-producing bacteria. The notion of the existence of bacterial species as proposed by several of Koch's distinguished predecessors had led to the idea that it might be possible to obtain pure cultures *in vitro* of a particular variety of microorganism. This was supported by Pasteur's observations on specific fermentations. When Koch began his experiments in this area, almost all bacteria were being cultured in liquids that usually allowed the free intermingling and growth of all the different kinds of bacteria present in the original inoculum. Several attempts were made by different researchers to obtain pure cultures from fluid growths by dilution. One of these researchers was Lord Lister (see below), who in 1878 had published the results of his dilution studies in which he employed a specially constructed syringe to deliver a precise volume of bacteria countable under the microscope. It became increasingly apparent, however, that solid media would be required to effect proper separation and isolation of bacteria from mixtures.

Koch first employed the cut surface of raw potato, a method that had been used to considerable advantage previously by Joseph Schroeter (1872) in his classic work on pigment-producing bacteria. In 1881 Koch reported on his improved technique that included soaking the raw potato in a solution of corrosive sublimate followed by sterilizing it in steam and finally splitting it into two parts with a sterile knife. The two halves were allowed to separate in a sterile, covered glass vessel, and the cut surfaces were then inoculated with starting material. This was not very satisfactory because most of the disease-producing bacteria simply would not grow on raw potato; Koch therefore abandoned this approach.

Koch had foreseen the fundamental importance of pure bacterial cultures very early, and thus, he concentrated all of his energies on developing a simple and consistently successful procedure. His goal was to obtain a good, supportive medium that was simultaneously sterile, transparent, and solid. He first solidified proven nutrient fluid media by the addition of 2.5 to 5 percent gelatin to a 1 percent meat extract broth, referring to this product as *nutrient gelatin*. He introduced inoculating the surface of this gelatin by means of a sterilized needle or platinum wire, drawing a minimum quantity of inoculum in several crosslines rapidly and lightly over its surface. When different colonies of bacteria did make their appearance, each was transferred to a test tube plugged with cotton that contained sterile nutrient gelatin set in a slanted position. In this way he was able to achieve isolation and pure cultures and solve a problem that 3 years before he thought to be incapable of solution. Bulloch states concerning this achievement, "By this means he opened the door for one of the greatest advances ever made in the history of medicine."

He soon learned, however, that nutrient gelatin had one distinct and insurmountable disadvantage. It melted at 37°C, the temperature felt to be optimal for the cultivation of most disease-producing bacteria.

In 1882 Frau Hesse, wife of an early coworker of Koch, first suggested the use of agar-agar as a solidifying base for culture media. Through some Dutch friends she had obtained samples from Batavia, where this material was well known and widely used for cooking, especially as a substitute for gelatin in the making of jams, fruit preserves, and jelled desserts. Agar, as it is now called, is a mixture of polysaccharides extracted from agar-bearing marine algae. The principal algae from which it is obtained live in the seas of eastern Asia from Sri Lanka (Ceylon) to Japan. Its distinctive virtue that rapidly established its dominance in bacteriologic culture technique is its peculiar property of liquefying if brought to 100°C, but once melted it will resolidify as a relatively stiff, transparent solid mass when it is cooled to 40 to 42°C. Thus any medium in which it serves as a solidifying base will remain solid on 37°C incubation.

In 1882 Koch wrote his classic paper describing the etiologic agent of tuberculosis that he had discovered, and in 1883 he wrote of his identification of the causative agent of Asiatic cholera. Koch was awarded the Nobel prize in 1905.

Sir Joseph Lister (1827 to 1912, England), a famous surgeon and scientist, was an early student of bacteriology and, in 1878, was the first investigator to obtain a pure culture of a bacterium by his dilution technique. In 1865 Lord Lister had his attention drawn to an article concerning Pasteur's report that air was full of living microorganisms that were carried on particles of dust floating in the atmosphere. He immediately related this to the possibility of preventing wound suppuration by applying in the dressing some material capable of destroying the life on these airborne, floating particles. He introduced lint soaked in carbolic acid (phenol) as a dressing for compound fractures. His early clinical studies soon indicated that pure carbolic acid was injurious to tissues. Nevertheless, having proved that this method prevented infection and was beneficial for the treatment of already contaminated wounds, he carried this idea into the operating room by introducing 1:20 diluted carbolic acid for soaking all surgical instruments and 1:40 diluted carbolic acid as a skin preparation prior to any surgical incision. This system of antiseptic surgery was announced in 1868. He augmented it in 1870 by the addition of carbolic acid sprays in the operating theater. His antiseptic treatment revolutionized surgery, and this was acknowledged in 1897 when he was raised to the peerage as Baron Lister.

The greatest scientific worker in medicine of his era, Paul Ehrlich (1854 to 1915, Germany), the founder and leading exponent of the science of hematology, turned his attention to the new field of immunology, becoming principally responsible for the direction that it would take and still follows. With respect to microbiology, he