Nineteenth Edition

Zinsser Microbiology

Joklik, Willett, Amos, Wilfert

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Preface

With each passing year the term *microbiology* becomes a less satisfactory umbrella for the many disciplines that it attempts to cover. Bacteriology, immunology, virology, mycology, and parasitology have each long since become separate and independent disciplines. They are treated together in a single text simply because they deal with the agents that cause infectious diseases and with the mechanisms that hosts elaborate for defense against them.

In spite of the undeniable triumphs of antimicrobial chemotherapy, which has revolutionized the practice of medicine and very likely represents the greatest single triumph of biomedical science, "microbes" are by no means "conquered"; they continue to cause infections that demand a large amount of the physician's time. In fact, knowledge concerning new infectious agents, unsuspected properties of known agents, additional mechanisms for the genesis and persistence of infections, and the behavior of infectious agents at the molecular, cellular, and organismal levels is accumulating at an ever increasing pace. As a result, the scope and complexity of the material presented to students is expanding rapidly, and the compilation of a comprehensive textbook of manageable size is becoming ever more difficult.

This new edition of *Zinsser Microbiology,* the nineteenth, is designed for medical students experiencing their first exposure to medical microbiology. To that end, we not only describe the pathogenic infectious agents and the diseases that they cause, but also discuss the basic principles of bacterial physiology and genetics, of molecular and cellular immunology, and of molecular virology, our purpose being to provide a firm basis for growth with the field throughout the student's professional career. The book is also designed as a reference source for instructors; to that end each chapter is supplemented with a selection of both reviews and important original papers that provide a rapid entrée to any specialized topic.

The nineteenth edition represents a very extensive revision of the eighteenth edition. Many portions of the text have been completely rewritten and the remainder have been thoroughly updated. In the Basic Bacteriology section very important chapters on the molecular basis of genetics and genetic variation and exchange have been completely rewritten by a new contributor, Dr. Ken Kreuzer; he has taken the place of Dr. Richard Burns who died in 1984. The Clinical Virology section also has several new contributors who provide completely new chapters on arboviruses, rhabdoviruses, arenaviruses, and the human immunodeficiency viruses including the virus that causes the acquired immune deficiency syndrome (AIDS); and the chapter on recent and current developments in molecular pathogenesis has also been completely rewritten. In the Basic Virology section there is much new material, particularly in the chapters on the molecular aspects of virus multiplication cycles and on tumor viruses; these are areas in which a wealth of very important new information is coming to hand, information embodying new principles that are modifying drastically our views of the nature of genetic material and of the mechanisms that regulate its expression. Clearly these are areas of vital importance to medical practitioners. The same applies to the Immunology section where new chapters on the cellular basis of the immune system, immunopathology, and on the immune responses to infection have been provided. This section provides a comprehensive account of both basic and clinical immunology, organized so as to highlight topics currently deemed of maximum relevance to medical students. Finally, all chapters in the Medical Bacteriology, Medical Mycology, and Parasitology sections have been thoroughly updated, with new material added on recently recognized diseases such as Legionnaire's disease, the toxic shock syndrome, and Lyme disease. Increased emphasis has also been placed on the various organisms commonly associated with opportunistic infections which develop in immunocompromised patients or in patients with prosthetic device implants. In these sections, which like all other sections have been carefully edited by a single editor so as to ensure uniformity of format, emphasis is again placed on correlating the basic and clinical aspects of each infectious agent so that the student may acquire an appreciation of how fundamental research unravels the complexities of host–parasite relationships. Each chapter consists of (1) an introduction to the important biologic properties of the organism, (2) a description of the clinical infection in humans, including a discussion of the mechanisms of pathogenicity, (3) a section on laboratory diagnosis that provides information on modern culture and immunologic procedures, and (4) a discussion of the currently recommended treatment.

With regard to the bibliography, we have again elected not to reference specific statements in the text but to append to each chapter a list of recent reviews and key original papers. The former will quickly guide the reader to any specific aspect of microbiology and immunology that he or she wishes to pursue; the latter provide the detailed considerations and circumstances that have gone into the genesis of the most important discoveries. Many of the papers that are cited already are, or no doubt will soon become, "classics."

We have tried not to increase the size of the book—no easy task in view of the enormous amount of new information that has accumulated since publication of the last edition in 1984. Obviously, this has entailed the omission of a certain amount of older material; however, we are confident that there are no major gaps and that in our presentation of the newest advances we have not sacrificed careful and logical explanations of fundamental principles.

The list of individuals who have helped to produce this volume extends far beyond the circle of our colleagues who contributed textual material and to whom we are profoundly indebted. We would especially like to thank our many colleagues who permitted us to use illustrative material and who

almost invariably supplied us with original photographs, and the many publishers who allowed us to reproduce previously published material. We would also like to thank the artists who did a superb job in drawing the innumerable charts and diagrams, and the many secretaries who cheerfully massaged the text on their word processors again and again. Finally, we wish to express our appreciation to the staff of Appleton & Lange for their efficient cooperation in producing this new edition.

Wolfgang K. Joklik
Hilda P. Willett
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Catherine M. Wilfert

Preface to the First Edition

The volume here presented is primarily a treatise on the fundamental laws and technic of bacteriology, as illustrated by their application to the study of pathogenic bacteria.

So ubiquitous are the bacteria and so manifold their activities that bacteriology, although one of the youngest of sciences, has already been divided into special fields—medical, sanitary, agricultural, and industrial—having little in common, except problems of general bacterial physiology and certain fundamental technical procedures.

From no other point of approach, however, is such a breadth of conception attainable, as through the study of bacteria in their relation to disease processes in man and animals. Through such a study one must become familiar not only with the growth characteristics and products of the bacteria apart from the animal body, thus gaining a knowledge of methods and procedures common to the study of pathogenic and nonpathogenic organisms, but also with those complicated reactions taking place between the bacteria and their products on the one hand and the cells and fluids of the animal body on the other—reactions which often manifest themselves as symptoms and lesions of disease or by visible changes in the test tube.

Through a study and comprehension of the processes underlying these reactions, our knowledge of cell physiology has been broadened, and facts of inestimable value have been discovered, which have thrown light upon some of the most obscure problems of infection and immunity and have led to hitherto unsuspected methods of treatment and diagnosis. Thus, through medical bacteriology—that highly specialized offshoot of general biology and pathology—have been given back to the parent sciences and to medicine in general methods and knowledge of the widest application.

It has been our endeavor, therefore, to present this phase of our subject in as broad and critical a manner as possible in the sections dealing with infection and immunity and with methods of biological diagnosis and treatment of disease, so that the student and practitioner of medicine, by becoming familiar with underlying laws and principles, may not only be in a position to realize the meaning and scope of some of these newer discoveries and methods, but may be in a better position to decide for themselves their proper application and limitation.

We have not hesitated, whenever necessary for a proper understanding of processes of bacterial nutrition or physiology, or for breadth of view in considering problems of the relation of bacteria to our food supply and environment, to make free use of illustrations from the more special fields of agricultural and sanitary bacteriology, and some special methods of the bacteriology of sanitation are given in the last division of the book, dealing with the bacteria in relation to our food and environment.

In conclusion it may be said that the scope and arrangement of subjects treated in this book are the direct outcome of many years of experience in the instruction of students in medical and advanced university courses in bacteriology, and that it is our hope that this volume may not only meet the needs of such students but may prove of value to the practitioner of medicine for whom it has also been written.

It is a pleasure to acknowledge the courtesy of those who furnished us with illustrations for use in the text, and our indebtedness to Dr. Gardner Hopkins and Professor Francis Carter Wood for a number of the photomicrographs taken especially for this work.

P. H. Hiss, Jr.
H. Zinsser
1910

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SECTION I

BACTERIAL PHYSIOLOGY

1 The Historical Development of Medical Microbiology

The history of the many concepts now embodied in the doctrines of microbiology is an account of attempts to solve the problems of the origin of life, the putrefaction of dead organic materials, and the nature of communicable changes in the bodies of living humans and animals. The visible aspects of these phenomena were as apparent and interesting to ancient observers as they are to modern biologists. In the past, notions of ultimate causes were derived from available factual knowledge colored by the theologic and philosophic tenets of the time. The early history of what has become the science of microbiology is to be found, therefore, in the writings of priests and philosophers.

INFECTION AND CONTAGION

Among ancient peoples, epidemic and even endemic diseases were believed to be supernatural in origin, sent by the gods as punishment for the sins of humankind. The treatment and, more important, the prevention of these diseases were sought by sacrifices and lustrations to appease the anger of the gods. Since humans are willful and wanton by nature, there was never any difficulty in finding a particular set of sins to justify a specific epidemic.

The concept of contagion and the practice of hygiene were not, however, entirely unknown to our ancestors. The Old Testament is often quoted as indicating the belief that leprosy was contagious and could be transmitted by contact. The principle of contagion by invisible creatures was later recorded by Varro in the second century BC, and this concept was familiar to Greek, Roman, and Arabic writers. Roger Bacon, in the thirteenth century, more than a millennium later, postulated that invisible living creatures produced disease. In 1546, the Venetian, Fracastorius, wrote from a knowledge of syphilis that communicable disease was transmitted by living germs, *seminaria morbi,* through direct contact or by intermediary inanimate fomites and through air *ad distans.* Fracastorius expressed the opinion that the seeds of disease, passing from one infected individual to another, caused the same disease in the recipient as in the donor. This clear expression of the germ theory of disease was three centuries ahead of its time.

FIRST OBSERVATIONS OF BACTERIA

Direct observation of microorganisms had to await the development of the microscope. The human eye cannot see objects smaller than 30 μm (0.001 inches) in diameter, and although knowledge of magnifying lenses reaches back to the time of Archimedes, the science of optics was not initiated until the thirteenth century by Roger Bacon. The telescope was invented by Galileo in 1608, and the invention of the microscope occurred later in the same century. The first person known to have made glass lenses powerful enough to observe and describe bacteria was the amateur lens grinder, Anton van Leeuwenhoek (1632–1723), of Delft, Holland. In letters to the experimentalist group, The Royal Society of London, Leeuwenhoek described many *animalcules,* including the three major morphologic forms of bacteria (rod, sphere, and spiral), various free-living and parasitic protozoa from human and animal feces, filamentous fungi, and globular bodies we now know as yeasts (Figs. 1–1 and 1–2). His observational reports were enthusiastic and accurate and created some interest at the time, but unfortunately Leeuwenhoek treated these investigations as a hobby and left no students to continue his work. However, in 1678, Robert Hooke, who developed the compound microscope, confirmed Leeuwenhoek's discoveries. Microorganisms were then occasionally studied by those primarily interested in classifying the various life forms observable with the microscope. These observations lay dormant and were not exploited by those interested in disease. The following 125 to 150 years witnessed the gradual development of knowledge and acceptance of the experimental method, which slowly spread throughout the expanding learning centers of the world. Im-

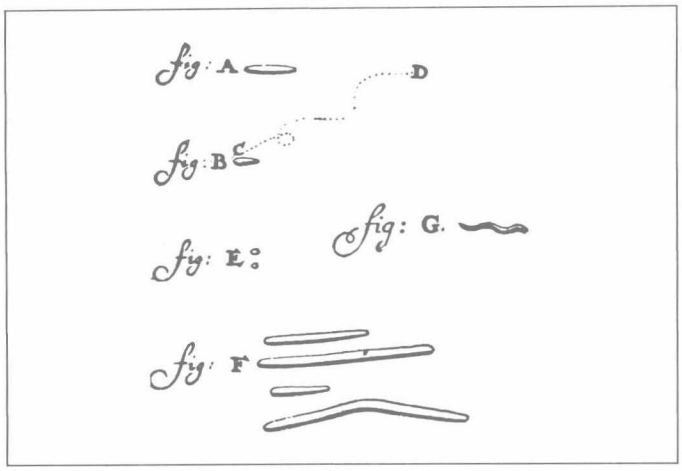

Fig. 1–1. In letters to the Royal Society, Leeuwenhoek described the sizes, shapes, and even the motility of bacteria. These are his drawings of bacteria from the human mouth. **A.** A motile *Bacillus*. **B** to **D.** *Selenomonas sputigena*. **E.** Micrococci. **F.** *Leptothrix buccans*. **G.** Probably *Spirochaeta buccalis*. *(From Dobell: Anton van Leeuwenhoek and His "Little Animals." Harcourt, Brace and Co, 1932.)*

proved microscopes became generally available in the 1800s as a result of the rapid technologic advances of the Industrial Revolution. Even then, no notable advance in microbiology was accomplished until after the attention of the scientific world was focused on the role of microbes in the controversies concerning spontaneous generation and the associated phenomenon of fermentation.

SPONTANEOUS GENERATION

The controversy over human ability to create life carried over from Greek mythology. Even Aristotle (384–322 BC) thought animals could originate from the soil. Samson, in the Old Testament, and again Virgil, about 40 BC, described recipes for producing bees from honey, and for centuries it was believed that maggots could be produced by exposing meat to warmth in the air. This was not refuted until Francesco Redi (1626–1697) proved that gauze placed over a jar containing meat prevented maggots forming in the meat. Recipes for producing mice and other similar life forms in litter and refuse were gradually disproved and discarded in similar fashion. However, the question was not settled in all minds. When microbes were discovered, their association with putrefaction and fermentation again raised the question of spontaneous generation. John Needham, in 1749, observed the appearance of microorganisms in putrefying meat and interpreted this as spontaneous generation. Spallanzani, however, boiled beef broth for an hour, sealed the flasks, and observed no formation of microbes. Needham and, in 1859, Pouchet argued that access of air was necessary for the spontaneous generation of microscopic living beings. Disproof came from several lines of evidence. Franz Schulze (1815–1873) passed air through strong acids and then into boiled broth, and Theodor Schwann (1810–1882) passed air through red hot tubes and observed no growth. About 1850, Schroeder and von Dusch filtered air through cotton filters into broth and observed no growth. Pasteur was able to filter microorganisms from the air and concluded that this was the source of contamination, and, finally, in 1859, in public controversy with Pouchet, prepared boiled broth in flasks with long narrow gooseneck tubes that were open to the air. Air could pass but microorganisms settled in the gooseneck, and no growth developed in any of the flasks. Finally, a British physicist, John Tyndall (1820–1893), completed the story by proving that dust carried germs.

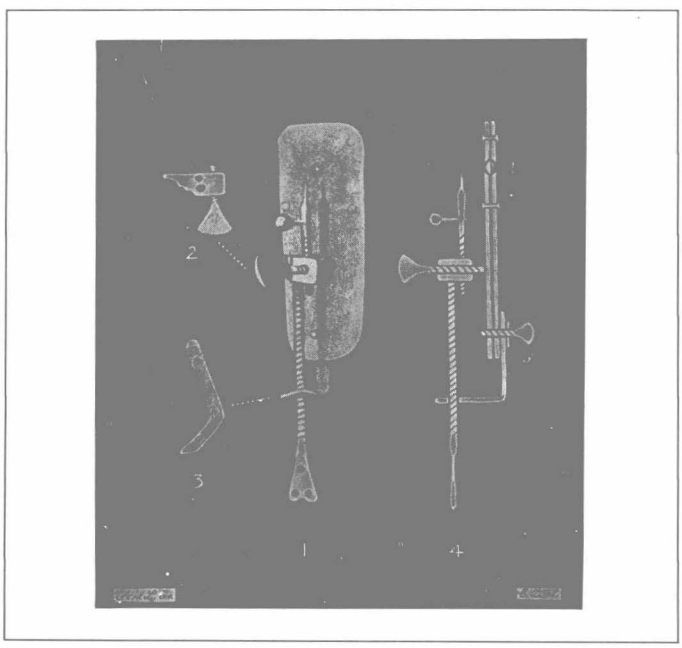

Fig. 1–2. Leeuwenhoek's microscopes consisted of simple biconvex lenses. *(From Dobell: Anton van Leeuwenhoek and His "Little Animals." Harcourt, Brace and Co, 1932.)*

THE GERM THEORY OF DISEASE

EMPIRICAL OBSERVATIONS

A firm basis for the causal nature of infectious disease was established only in the latter half of the nineteenth century. One of the first proofs came from Agostino Bassi, who, in the early 1800s, proved that a fungus, later named *Botrytis bassiana* in his honor, caused a disease in silkworms called *muscardine* in France and *mal segno* in Italy. In the 1840s, the American poet–physician, Oliver Wendell Holmes, wrote "The Contagiousness of Puerperal Fever," in which he suggested that disease was caused by germs carried from one new mother to another. In 1861, Ignaz Semmelweis, who had drastically decreased childbirth deaths by antiseptic techniques and practices, published a seminal work entitled "The Cause, Concept, and Prophylaxis of Childbed Fever." However, the importance of antiseptics in reducing contagious disease was not fully realized until the late 1870s, when Joseph Lister demonstrated the value of spraying operating rooms with aqueous phenol.

LESSONS LEARNED FROM FERMENTATIONS

Further emphasis on microbial activities came from the work of Louis Pasteur between the 1850s and the 1880s. In studies on the diseases of wine, Pasteur demonstrated that alcoholic fermentation of grapes, fruit, and grains was caused by microbes, then called *ferments*. In good wine batches certain types of ferments existed in the vats, whereas in poor or bad fermentations other types of microbes were found, some of which Pasteur found to be capable of growing anaerobically. He suggested eliminating the bad types of ferments from fresh juices by heating them at 63C for half an hour and then cooling and reinoculating them with a culture from the satisfactory vats. Pasteur's success with the problems of the wine industry led the French government to request that he study a disease,

pébrine, which was ruining the silkworm industry in southern France. Pasteur struggled with this problem for several years before he was able to isolate the causative organism.

OBSERVATIONS AND EXPERIMENTS WITH ANIMALS

In 1850, Rayer and Davaine observed rod-shaped microorganisms in the blood of animals that had died of anthrax. Rayer recalled the experiments in 1825 of Barthélemy, who had shown that anthrax was transmissible by inoculation in series in sheep, and by 1863 Davaine had experimentally transmitted anthrax by blood containing these rods, but not by normal blood from which rods were absent. In 1872, Obermeier discovered the relationship of a *Spirillum* to relapsing fever and demonstrated for the first time the presence of a pathogenic microorganism in the blood of a human being.

IMPORTANCE OF PURE CULTURE TECHNIQUES

The etiologic research described above was not based on pure cultures. These were obtained largely by accident, and investigators had no way, except by crude morphologic microscopic examination, of knowing when contaminants were present. This resulted in much equivocal thinking and work that hindered progress.

The first pure or axenic culture technique was developed by Joseph Lister in 1878. He made serial dilutions in liquid media to obtain pure cultures of a bacterium, which he named *Bacterium* (*Lactobacillus*) *lactis*. Meanwhile, Koch, a student of Henle, who insisted on proof that an organism caused disease, was also developing and refining techniques for the isolation of pure cultures. From the work of others, notably Ehrlich, Koch learned methods for staining bacteria on glass with aniline dyes to facilitate microscopic observation. In his early work on anthrax, Koch used sterile aqueous humor of the eyes of animals as a growth medium; later he developed a transparent solid medium by mixing gelatin with Löffler's peptone solution. The gelatin mixture liquefied upon warming, could be heat sterilized and aseptically poured into plates, and solidified upon cooling. Microorganisms streaked upon it developed into macroscopic colonies as the result of the growth of a single invisible cell. However, gelatin does not solidify until it reaches a relatively low temperature (26C), so Koch later switched to agar, the transparent red seaweed extract that solidifies below 43C.

ETIOLOGIC PROOF OF INFECTIOUS AGENTS

Koch was able to isolate the anthrax organism in pure culture by streaking onto his solid media, and he found that even after many transfers, the organism could still cause the same symptoms and disease when inoculated into animals. On the basis of his experiences, Koch formulated criteria that provided proof that a specific bacterium caused a disease. We now call them Koch's postulates:

1. The organism must always be found in the diseased animal but not in healthy ones.
2. The organism must be isolated from diseased animals and grown in pure culture away from the animal.
3. The organism located in pure culture must initiate and reproduce the disease when reinoculated into susceptible animals.
4. The organism should be reisolated from the experimentally infected animals.

Koch's studies thus provided impetus for further work on and a means of proof for the germ theory of disease.

The 20-year period following Koch's work was the Golden Age of Bacteriology. By 1900, almost all major bacterial disease organisms had been described. The list included anthrax (*Bacillus anthracis*), diphtheria (*Corynebacterium diphtheriae*), typhoid fever (*Salmonella typhi*), gonorrhea (*Neisseria gonorrhoeae*), gas gangrene (*Clostridium perfringens*), tetanus or lockjaw (*Clostridium tetani*), dysentery (*Shigella dysenteriae*), syphilis (*Treponema pallidum*), and others.

VIRUSES

Only with advances in technique and improvement in apparatus is it possible to make fundamental advances through new ideas and observations. The development of bacteriologic filters and the discovery of viruses is a case in point.

BACTERIOLOGIC FILTERS

As an alternate to heat sterilization, unsuccessful efforts to remove bacteria from solutions by filtration through paper and similar materials led Chamberland and Pasteur to test and develop unglazed porcelain, which became the first successful bacterial filter (1871–1884). The Berkefeld filter of Kieselguhr (diatomaceous earth) was developed shortly thereafter in 1891. Synthetic polymer filters of cellulose nitrate, cellulose acetate, polyester, and so forth have only come into common use since World War II because of technical advances that allow quality control of pore size. It is interesting that these are essentially space-age products developed in part for the rapid removal of microorganisms from jet and rocket fuels.

DISCOVERY OF VIRUSES

There are three major classes of viruses: animal viruses, plant viruses, and bacterial viruses. Because knowledge concerning each of these classes has accumulated along distinct lines, extensive specialization has developed. Bacterial viruses are, therefore, dealt with only briefly in this book, and plant viruses are not considered at all. Nevertheless discoveries made concerning each of these classes of viruses have profoundly influenced our understanding of the nature of each of the others.

The existence of viruses became evident during the closing years of the nineteenth century, when, as the result of newly acquired expertise in the handling of bacteria, the infectious agents of numerous diseases were being isolated. For some infectious diseases, however, this proved to be an elusive task, until it was realized that the responsible agents were smaller than bacteria. Iwanowski, in 1892, was probably the first to record the transmission of an infection (tobacco mosaic disease) by a suspension filtered through a bacterial-proof filter. In 1898, Loeffler and Frosch conducted similar experiments involving foot-and-mouth disease of cattle. Beijerinck (1898) considered the infectious agents in bacteria-free filtrates to be living but fluid—that is, nonparticulate—and introduced the term *virus* (Latin for 'poison') to describe them. It quickly became clear, however, that viruses were particulate, and the term virus became the operational definition of infectious agents smaller than bacteria and unable to multiply outside living cells. In 1911, Rous discovered a virus that produced malignant tumors in chickens, and during World War I Twort and d'Hérelle independently discovered the viruses that multiply in bacteria, the bacteriophages.

Viruses could not be grown in artificial media, and Koch's criteria could not be specifically applied. Because these pathogens require a living host for propagation, study of viruses progressed slowly. As occurred in bacteriology, each new step had to await the development of appropriate technology. Plant viruses proved easy to obtain in large amounts, which permitted extensive chemical and physical studies. This work led first

to the demonstration that plant viruses consist only of nucleic acid and protein and culminated in the crystallization of tobacco mosaic virus by Stanley in 1935. This feat evoked great astonishment because it cut across preconceived ideas concerning the attributes of living organisms and demonstrated that agents able to reproduce in living cells behave under certain conditions as typical macromolecules.

Work with bacteriophages concentrated on their clinical application. It was hoped that bacteria could be destroyed inside the body by injecting appropriate bacteriophages. Their activity in vivo, however, never matched their activity in vitro, most probably because they are eliminated efficiently from the bloodstream.

Early work with animal viruses concentrated on the pathogenesis of viral infections and on epidemiology. Throughout this period, fundamental studies on animal cell–virus interactions were severely hampered by the absence of rapid and efficient techniques for quantitating viruses. The only method then available was the expensive and time-consuming serial end point dilution method, using animals.

Around the year 1940 came several breakthroughs. First, the advent of electron microscopy permitted visualization of viruses for the first time. Second, techniques for purifying certain animal viruses were being perfected, and a group of workers at the Rockefeller Institute headed by Rivers carried out some excellent chemical studies on vaccinia virus. Third, Hirst discovered that influenza virus agglutinates chicken red cells. This phenomenon, hemagglutination, was rapidly developed into an accurate method for quantitating myxoviruses, as a result of which this group of viruses became, in the 1940s, the most intensively investigated group of animal viruses. Finally, this period marked the beginning of the modern era of molecular virology. Until then the interaction of bacteriophages with bacteria had been analyzed principally in terms of populations rather than at the level of single virus particles interacting with single cells. This conceptual block was removed by Ellis and Delbrück's study of the one-step growth cycle, as a result of which the bacteriophage–bacterium system became extraordinarily amenable to experimentation. Indeed, during the past four decades, many of the major advances in molecular biology have resulted from work with bacteriophages.

In animal virology, rapid advances followed the development in the late 1940s of techniques for growing animal cells in vitro. Strains of many types of mammalian cells can now be grown in media of defined composition. As a result, animal cell–virus interactions can now be analyzed using the techniques that have proved so powerful in the case of bacteriophages.

IMMUNITY

Ancient peoples immunized themselves against venomous snakes by introducing small amounts of venom into scratches in the skin. The Chinese used variolization to protect themselves against smallpox more than 2000 years ago. This practice, which involved exposure to dermic lesions from patients who had survived the disease in the hope that it had been caused by a relatively mild virus variant, spread through Asia by trade routes and, in spite of its failure rate of 1 percent or more, was well accepted in the Middle East, and eventually also reached Europe. At the end of the eighteenth century Edward Jenner (1749–1823) noticed that milkmaids who developed cowpox were immune to smallpox and found that he was able to protect susceptible individuals against smallpox by vaccinating them with cowpox. Pasteur developed a chicken cholera vaccine in 1877; he inoculated chickens with old attenuated cultures so that a mild disease rendered the chickens immune to virulent organisms. He called this *vaccination*, after Jenner's procedure (*vacca*, Latin for 'cow'). Shortly afterward, in 1881, applying the same concept, Pasteur prepared temperature-attenuated anthrax grown at 42C to 43C and protected sheep by first injecting them with these bacteria before challenging them with virulent anthrax grown at lower temperatures. Salmon and Smith, between 1884 and 1886, used heat-killed cultures of hog cholera bacillus to develop resistance or immunity in swine against challenge by live virulent organisms. Pasteur developed a rabies vaccine in 1886, again making use of the idea of injecting an attenuated living disease agent. In this case, Pasteur used dried animal spinal cords without, apparently, recognizing the viral form of the disease agent.

Two schools of thought arose in explanation of the increased resistance after vaccination. Metchnikoff developed, in the 1880s, the cellular theory of protection; Bordet and others proposed the humoral, or specific antibody, concept of immunity. There is now evidence that both theories are correct. The last several decades have witnessed the isolation and characterization of the major humoral immune proteins, the immunoglobulins, the generation and function of which are currently being studied intensively. Much research is also being conducted on the cellular interactions in immune reactions that occur not only in infectious diseases caused by bacteria, viruses, fungi, and parasites, but also in rejection reactions of tissue and organ transplants and in cancer.

ANTIMETABOLITES

Many antimetabolites, which were pioneered in concept by Ehrlich in the middle to latter part of the nineteenth century, are now accepted household words, for example, penicillin. The modern era of antibiotics developed only after Domagk reported in 1935 that Prontosil had a dramatic effect on streptococcal infections. It was soon discovered that Prontosil was converted in the body to sulfanilamide, the active chemical agent, which is an analogue of the vitamin *p*-aminobenzoic acid. In the 1940s, in research stimulated by World War II, Florey and Chain and their associates reinvestigated Fleming's penicillin, isolated and characterized it, and demonstrated its practical clinical value. As a result of millions of tests with thousands of organisms, we now have numerous other antibiotics that are active against almost all types of bacteria.

With the recognition of the metabolic and structural differences, at the molecular level, between pathogenic microorganisms and viruses on the one hand and human or animal cells on the other, the rationale for developing new chemotherapeutic agents is now often based on exploiting these differences.

ROLE OF MICROBIOLOGY IN THE DEVELOPMENT OF MOLECULAR BIOLOGY AND MOLECULAR GENETICS

The enormous advantages of having homogeneous populations of cells available for every conceivable type of investigation were soon realized. Many of the epoch-making advances in cell physiology, biochemistry, and genetics occurred as a result of studies with microorganisms, primarily bacteria and viruses. During the last two or three decades, these advances have led to a precise way of investigating the structure and function of nucleic acids and proteins that has become known as *molecular biology*. For example, the demonstration of the central role of DNA as the repository of genetic information resulted from the studies of Griffith in the 1920s that pneumococci could be transformed from one capsular type to another, followed by the demonstration by Avery and associates during the 1940s that the transforming factor was DNA; proof beyond doubt was provided by the demonstration by Hershey and Chase in

1952 that viral nucleic acid itself contained all the information necessary for virus multiplication. At the same time, Watson and Crick developed the double-helix model of DNA structure, which led them to suggest that one of the complementary DNA strands could serve as the template for the synthesis of the other, thus providing a description of self-perpetuating gene replication and continuity.

Demonstration of the transcription from DNA of information in the form of messenger RNA synthesized in complementary sequence to DNA soon followed, again in a microbial system. Messenger RNA was then found to be translated into polypeptides on ribosomes. By the early 1960s, Nirenberg, Ochoa, and others had worked out the nature of the triplet RNA base sequences corresponding to the codon signals for all amino acids.

More recently, attention has focused on the arrangement of genetic material, including the nature of genes and the mechanisms that control their expression. This wide area of research also originated in microbiology; it is now known as *molecular genetics*. Although much of this research continues to be carried out with microbial systems, cells of higher organisms, particularly cells of vertebrates, are also now being used very extensively. A very important factor in this connection has been the development of the technique of tissue culture, which permits animal cells to be grown, cloned, and passaged in the same manner as microorganisms; in fact, the primary impetus for developing this new technique was the need of virologists to grow and measure viruses. Using this technique, new concepts concerning the regulation of gene expression in cells of higher organisms, and particularly in human cells, are now being developed very rapidly. Among the goals that should be within reach in the foreseeable future are an understanding of the fundamental control mechanisms that operate in both normal and abnormal cell differentiation, including cancer; insight into the mechanisms that control the immune response; and the development of a rational system of antiviral chemotherapy for controlling diseases caused by viruses, just as antibiotics are used to control diseases caused by bacteria.

FURTHER READING

Bulloch W: The History of Bacteriology. London, Oxford University Press, 1938

Dobell C: Anton van Leeuwenhoek and His "Little Animals." New York, Harcourt, Brace, 1932

Dubos RJ: The Professor, the Institute and DNA. New York, Rockefeller University Press, 1976

Dubos RJ: Biochemical Determinants of Microbial Disease. Cambridge, Mass, Harvard University Press, 1954

Dubos RJ: Louis Pasteur, Free Lance of Science. Boston, Little, Brown, 1950

Florey HW: Antibiotics. London, Oxford University Press, 1949

Marquardt M: Paul Ehrlich. New York, Henry Schuman, 1951

Meleney FL: Treatise on Surgical Infections. London, Oxford University Press, 1948

Metchnikoff E: Immunity in Infective Diseases, Binnie FG (trans). Cambridge, Mass, Harvard University Press, 1905

Tyndall J: Essays on the Floating-Matter of the Air in Relation to Putrefaction and Infection. New York, D Appleton and Co, 1882