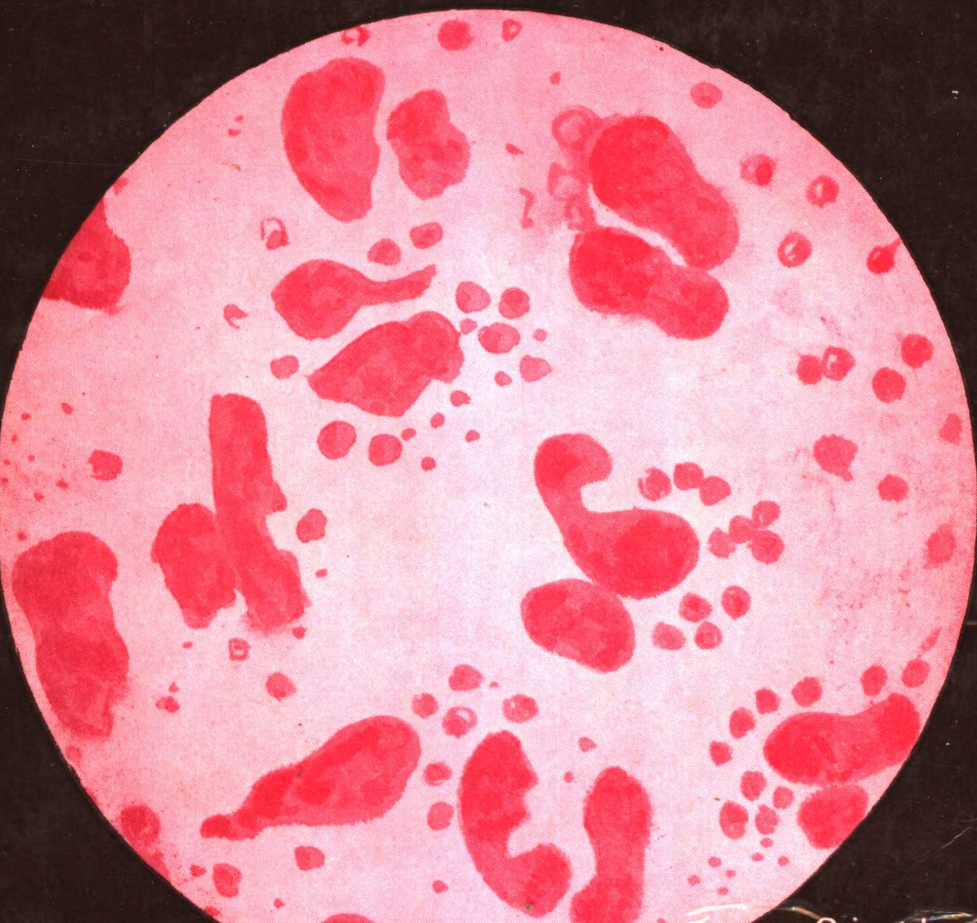
Textbook of Microbiology

R. Ananthanarayan
C. K. Jayaram Paniker



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TEXTBOOK OF MICROBIOLOGY

R. Ananthanarayan
B.A., M.B.B.S (Madras), D.B., Ph.D. (Lond.)
Formerly Professor of Microbiology, Medical Colleges
Calicut, Kottayam, Trivandrum & Gulbaraga
and Adviser to D.H.S., Kerala, on Laboratory Services

C. K. Jayaram Paniker M.D.
Director and Professor of Microbiology,
Medical College, Calicut

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PREFACE

Many of the health problems in developing countries like India are different from those of developed countries. Bacterial diseases still play a considerable role in diseases in our country. Topics such as cholera and enteric diseases are important to us though only of less or academic interest to the developed countries. The increasing importance of the newer knowledge in immunology to health and disease is not adequately stressed in most of the extant textbooks. Virus diseases which are responsible for nearly 60 per cent of human illness require wider coverage. The general approach to the teaching of microbiology in our country has also been rather static. All these factors called for a textbook of Microbiology more suited to countries like India.

We therefore undertook this endeavour based on our experience of teaching undergraduates and postgraduates for over two decades. We omitted the discipline of parasitology from our book since we already have an excellent textbook on the subject published in India.

This book has taken us over three years to write and over a year in publication. Naturally we would be out of date to a certain and inevitable extent. We do not claim any perfection. On the contrary, we have requested medical students and teachers all over the country to write to us about any shortcomings and give us suggestions as to how to improve the book. We shall spare no pains in seeing that their valuable suggestions are given effect to in our second edition.

Trivandrum

R. ANANTHANARAYAN

C. K. JAYARAM PANIKER

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PART I

1

HISTORICAL INTRODUCTION

Medical microbiology is concerned with the study of the causative agents of infectious diseases of man and his reactions to such infections.

Disease and death have always attracted the attention of the human mind. Ancient man ascribed them to divine wrath and other supernatural forces. Later other concepts such as the influence of environment, of bodily constitution and of faulty diet were proposed. There have been from very early times occasional suggestions that diseases may result from invasion of the body by external contagion. Varo and Columella in the first century B. C. postulated that diseases were caused by invisible beings (*Animalia minuta*) inhaled or ingested. Fracastorius of Verona (1546) proposed a *contagium vivum* as the possible cause of infectious disease and von Plenciz (1762) suggested that each disease was caused by a separate agent. Kircher (1659) reported finding minute worms in the blood of plague victims, but with the equipment available to him it is more likely that what he observed were only blood cells.

As microbes are invisible to the unaided eye, definitive knowledge about them had to await the development of microscopes. The credit for having first observed and described bacteria belongs to Antony van Leeuwenhoek, a draper from Delft, Holland, whose hobby was grinding lenses and observing diverse materials through them. In 1683, he made accurate descriptions of various types of bacteria and communicated them to the Royal Society of London. The significance of these observations was not then realised and to Leeuwenhoek the world of 'little animalcules' represented only a curiosity of nature. It was only some two centuries later that their importance in medicine and in other areas of biology came to be recognised.

The earliest discovery of a pathogenic micro-organism was probably made by Augustino Bassi (1835), who showed that the muscardine disease of silk worms was caused by a fungus. Davaine and Pollender (1850) observed anthrax bacilli in the blood of animals dying of the disease. In fact, even before the microbial aetiology of infections had been established, this was evident to some observant physicians. Oliver Wendell Holmes in the U.S.A. (1843) and Ignaz Semmelweis in Vienna (1846) had independently concluded that puerperal sepsis was transmitted by the contaminated hands of obstetricians and medical students and demonstrated the efficacy of simple measures such as washing their hands in an antiseptic solution.

The development of bacteriology as a scientific discipline dates from Louis Pasteur (1822-95). Though trained as a chemist, his studies on fermentation led him to take an interest in micro-organisms. He established that fermentation was the result of microbial activity and that different types of fermentations were associated with the activity of different kinds of micro-organisms (1857). The basic principles and techniques of bacteriology were evolved by Pasteur during his enquiry into the origin of microbes. This was then the subject of much controversy. Needham, an Irish priest, had in 1745 published experiments purporting the spontaneous generation (abiogenesis) of micro-organisms in putrescible fluids. This view was opposed by Spallanzani, an Italian abbot (1769). In a series of classic experiments, Pasteur proved conclusively that all forms of life, even microbes, arose only from their like and not *de novo*. In the course of these studies, he introduced techniques of sterilisation and developed the steam steriliser, hot-air oven and autoclave. He also established the differing growth needs of different bacteria. His work attracted such attention and he attained such eminence in the world of science that not only France but all Europe looked to him to solve major problems in various fields. Thus started his studies on pebrine, anthrax, chicken cholera, and hydrophobia. An accidental observation that chicken cholera bacillus cultures left on the bench for several weeks lost their pathogenic property but retained their ability to protect the birds against subsequent infection by them led to the discovery of the process of attenuation and the development of live vaccines. He attenuated cultures of anthrax bacillus by incubation at high temperature (42°–43°C) and proved that inoculation of such cultures in animals induced specific protection against anthrax. The success of such immunisation was dramatically demonstrated by a public experiment on a farm at Pouilly-le-Fort (1881) during which vaccinated sheep, goats and cows were challenged with a virulent anthrax bacillus culture. All the vaccinated animals survived the challenge while an equal number of unvaccinated control animals succumbed to it. It was Pasteur who coined the term *vaccine* for such prophylactic preparations to commemorate the first of such preparations, namely cowpox employed by Jenner for protection against smallpox.

The greatest impact in medicine was made by Pasteur's development of a vaccine for hydrophobia. This was acclaimed throughout the world. The Pasteur Institute, Paris, was built by public contribution and similar institutions were established soon in many other countries for the preparation of vaccines and for investigation of infectious diseases.

An immediate application of Pasteur's work was the introduction of antiseptic techniques in surgery by Lister (1867) effecting a pronounced drop in mortality and morbidity due to surgical sepsis. Lister's antiseptic surgery involving the use of carbolic acid was cumbersome and hazardous, but was a milestone in the evolution of surgical practice from the era of 'laudable pus' to modern aseptic techniques.

While Pasteur in France laid the foundations of microbiology, Robert Koch (1843–1910) in Germany perfected bacteriological techniques during his studies on the culture and characters of anthrax bacillus (1876). He introduced staining techniques and methods of obtaining bacteria in pure culture using solid media. He discovered the bacillus of tuberculosis (1882) and the cholera vibrio (1883).

Pasteur and Koch attracted many gifted disciples who discovered the causative agents of several bacterial infections and enlarged the scope and content of microbiology by their labours. In 1874, Hansen described leprosy bacillus; in 1879, Neisser described the gonococcus; in 1881, Ogston discovered staphylococcus; in 1884, Loeffler isolated diphtheria bacillus; in 1884, Nicolaier observed tetanus bacillus in soil; in 1886, Fraenkel described the pneumococcus; in 1887, Bruce identified the causative agent of Malta fever; in 1905, Schaudinn and Hoffmann discovered the spirochaete of syphilis.

Roux and Yersin (1888) identified a new mechanism of pathogenesis when they discovered the diphtheria toxin. Similar toxins were identified in tetanus and some other bacteria. The toxins were found to be specifically neutralised by their antitoxins. Ehrlich who studied toxins and antitoxins in quantitative terms laid the foundations of biological standardisation.

The causative agents of various infectious diseases were being reported by different investigators in such profusion that it was necessary to introduce criteria for proving claims that a micro-organism isolated from a disease was indeed causally related to it. These criteria, first indicated by Henle, were enunciated by Koch and are known as Koch's postulates. According to these, a micro-organism can be accepted as the causative agent of an infectious disease only if the following conditions are satisfied: 1) The bacterium should be constantly associated with the lesions of the disease. 2) It should be possible to isolate the bacterium in pure culture from the lesions. 3) Inoculation of such pure cultures into suitable laboratory animals should reproduce the lesions of the disease. 4) It should be possible to re-isolate the bacterium in pure culture from the lesions produced in the experimental animals. An additional criterion introduced subsequently requires that specific antibodies to the bacterium should be demonstrable in the serum of patients suffering from the disease. Though it may not always be possible to satisfy all the postulates in every case, they have proved extremely useful in sifting doubtful claims made regarding the causative agents of infectious diseases.

By the beginning of the twentieth century, many infectious diseases had been proved to have been caused by bacteria. But there remained a large number of diseases such as smallpox, chickenpox, measles, influenza and common cold for which no bacterial cause could be established. During his investigation of rabies in dogs, Pastöur had suspected that the disease could be caused by a microbe too small to be seen even under the microscope. The existence of such ultramicroscopic microbes was proved when Ivanovsky (1892) reproduced mosaic disease in the tobacco plant, by applying to healthy leaves, juice from diseased plants from which all bacteria had been removed by passage through fine filters. Beijerinck (1898) confirmed these findings. Loeffler and Frosch (1898) observed that foot and mouth disease of cattle was caused by a similar filter-passing virus. The first human disease proved to have a virus aetiology was yellow fever. The U.S. Army Commission under Walter Reed, investigating yellow fever in Cuba (1902) established not only that it was caused by a filterable virus but also that it was transmitted through the bite of infected mosquitoes. Landsteiner and Popper (1909) showed that poliomyelitis was caused by a filterable virus and transmitted the disease experimentally to chimpanzees. Investigation of viruses and the diseases caused by them wás rendered difficult as viruses could not be visualised under light microscopes or grown in culture media. Though the larger viruses could be seen after appropriate staining under the light microscope, detailed study of their morphology had to wait till the introduction by Ruska of the electron microscope (1934) and subsequent refinements in electron microscopic techniques. Cultivation of viruses was possible only in animals or in human volunteers till the technique of growing them on chick embryos was developed by Goodpasture in the 1930's. The application of tissue culture in virology expanded the scope of virological techniques considerably.

The possibility that virus infection could lead to malignancy was first put forth by Ellerman and Bang (1908). Peyton Rous (1911) isolated a virus causing sarcoma in fowls. Several viruses have since been isolated which cause natural and experimental tumours in animals and birds. Viruses also cause malignant transformation of infected cells in tissue culture. These indications that human malignancies also may have a viral aetiology have stimulated intensive research in this field. But while these studies have contributed considerable information on the basic aspects of cell-virus interactions, it has so far not been possible to prove conclusively that any human malignancy has a virus aetiology.

Twort (1915) and d'Herelle (1917) independently discovered a lytic phenomenon in bacterial cultures. The agents responsible were termed bacteriophages—viruses that attack bacteria. Early hopes that bacteriophages may have therapeutic applications had to be abandoned, but these viruses have paid unexpected scientific dividends. The essential part of viruses is their core of nucleic acid which acts as the carrier of genetic information in the same manner as in higher organisms. The discipline of molecular biology owes its origin largely to studies on the genetics of bacteriophages and bacteria.

It had been noticed from very early days that persons surviving an attack of smallpox did not develop the disease when exposed to the infection subsequently. This observation had been applied for prevention of the disease by producing a mild form of smallpox intentionally (variolation). This practice prevalent in India, China and other ancient civilisations from time immemorial, was introduced into England by Lady Mary Wortley Montague (1718) who had observed the custom in Turkey. Variolation was effective but hazardous. Jenner observing the immunity to smallpox in milkmaids who were liable to occupational cowpox infection introduced the technique of vaccination using cowpox material (1796). This was the first instance of scientific immunisation and though introduced empirically, has stood the test of time. Jenner's vaccination paved the way for the ultimate eradication of smallpox.

The next major discovery in immunity was Pasteur's development of vaccines for chicken cholera, anthrax and rabies. While the techniques introduced by him were successful, the mechanism of protection afforded by them remained obscure. The explanation of the underlying mechanism came from two sources. Nuttall (1888) observed that defibrinated blood had a bactericidal effect and Buchner (1889) noticed that this effect was abolished by heating the sera for one hour at 55°C. The heat labile bactericidal factor was termed 'alexine'. A specific humoral factor or 'antibody' was described by von Behring and Kitasato (1890) in the serum of animals which had received sublethal doses of tetanus toxin. Pfeiffer (1893) demonstrated bactericidal effect *in vivo* by injecting live cholera vibrios intraperitoneally in guinea pigs previously injected with killed vibrios. The vibrios were shown to undergo lysis. The humoral nature of such lytic activity was proved by Bordet (1895), who defined the two components participating in the reaction, the first being heat stable and found in immune sera (antibody or *substance sensibilicatrice*) and the second being heat labile and identical with Buchner's alexine, subsequently