Topley and Wilson's

Principles of bacteriology, virology and immunity

Seventh edition in four volumes

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Volume 1

General microbiology and immunity

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and
Heather M. Dick

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General Editors' Preface to 7th edition

After the publication of the 6th edition in 1975 we had to decide whether it would be desirable to embark on a further edition and, if so, what form it should take. Except for the single-volume edition of 1936, the book had always appeared in two volumes. We hesitated to alter this arrangement but reflection made us realize that a change would be necessary.

If due attention was to be paid to the increase in knowledge that had occurred during the previous ten years two volumes would no longer be sufficient. Not only had the whole subject of microbiology expanded greatly, but some portions of it had assumed a disciplinary status of their own. Remembering always that our primary concern was with the causation and prevention of microbial disease, we had to select that part of the newer knowledge that was of sufficient relevance to be incorporated in the next edition without substantial enlargement of the book as a whole.

One of the subjects that demanded consideration was virology, which would have to be dealt with more fully than in the 6th edition. Another was immunology. Important as this subject is, much of it is not directly concerned with immunity to infectious disease. Moreover, numerous books, reviews and reports were readily available for the student to consult. What was required by the microbiologist and allied workers was a knowledge of serology, and by the medical and veterinary student a knowledge of the mechanisms by which the body defends itself against attack by bacteria and viruses. We resolved, therefore, to provide a plain straightforward account of these two aspects of immunity similar to but less detailed than that in the 6th edition.

The book we now present consists of four volumes. The first serves as a general introduction to bacteriology including an account of the morphology, physiology, and variability of bacteria, disinfection, antibiotic agents, bacterial genetics and bacteriophages, together with immunity to infections, ecology, the bacteriology of air, water, and milk, and the normal flora of the body. Volume 2 deals entirely with systematic bacteriology, volume 3 with bacterial disease, and volume 4 with virology.

To this last volume we would draw special attention. It contains 27 chapters describing the viruses in detail and the diseases in man and animals to which they give rise, and is a compendium of information suitable alike for the general reader and the specialist virologist.

The first two editions of this book were written by Topley and Wilson, and the third and fourth by Wilson and Miles. For the next two editions a few outside contributors were brought in to bridge the gap that neither of us could fill. For the present edition we enlisted a total of over fifty contributors. With their help every chapter in the book has been either rewritten or extensively revised. This has led to certain innovations. The author's name is given at the head of each chapter; and each chapter is prefaced by a detailed contents list so as to afford the reader a conspectus of the subject matter. This, in turn, has led to a shortening of the index, which is now used principally to show where subjects not obviously related to any particular chapter may be found. A separate but consequently shorter index is provided for each of the first three volumes and a cumulative index for all four volumes at the end of volume 4. Each volume will be on sale separately. As a result of these changes we shall no longer be able to ensure the uniformity of style and presentation for which we have always striven, or to take responsibility for the truth of every factual statement.

We are fortunate in having Dr Parker, who has been associated with the 5th and 6th editions of the book, as the third general editor of all four parts of this edition and as editor of volume 2. Dr Geoffrey Smith with his extensive knowledge of animal disease has greatly assisted us both as a contributor and as editor of volume 3. Dr Fred Brown, of the Animal Virus Research Institute, has organized the production of volume 4, and Professor Heather Dick the immunity section of volume 1.

Two small technical matters may be mentioned. Firstly, in volume 2 we have retained many of the original photomicrographs and added others at similar magnifications because they portray what the student sees when he looks down an ordinary light microscope in the course of identifying bacteria. Electronmicrographs have been used mainly to illustrate general statements about the structure of the organisms under consideration. Secondly, all temperatures are given in degrees Celsius unless otherwise stated.

Apart from those to whom we have just expressed our thanks, and the authors and revisers of individual chapters, we are grateful to the numerous workers who have generously supplied us with illustrations; to Dr N. S. Galbraith and Mrs Hepner at Colindale for furnishing us with recent epidemiological information; to Dr Dorothy Jones at Leicester for advice on the *Corynebacterium* chapter and Dr Elizabeth Sharpe at Reading for information about *Lactobacillus*; to Dr R. Redfern at Tolworth for his opinion on the value of different rodent baits; to Mr C. J. Webb of the Visual Aids Department of the London School of Hygiene and Tropical Medicine for the reproduction of various photographs and diagrams; and finally to the Library staff at the London School and Miss Betty Whyte, until recently chief librarian of the Central Public Health Laboratory at Colindale, for the continuous and unstinted help they have given us in putting their bibliographical experience at our disposal.

GSW
AAM

Volume Editors' Preface

The decision to publish the present edition in four volumes instead of two, for reasons explained in the General Editors' Preface, has necessitated alterations in the content and sequence of the various chapters. A glance at the contents will show that the present volume covers a wide field corresponding fairly closely to that in Part I of the 6th edition. Most of the chapters have been written afresh by authors who have provided a comprehensive view of their subject embodying the advances made during the past ten years. It will be noticed, however, that the Growth and Death chapter of the 6th edition has been incorporated in the chapter on General Metabolism. A further innovation has been the transference of the five chapters on Ecology, Normal Flora and the Bacteriology of Air, Water and Milk from the end of Volume 2 of the 6th edition, where they were inaptly placed, to form part of the section on General Microbiology to which they really belong. For logical reasons they have been amalgamated so as to form only two chapters. As is made clear in Chapter 8, the subject of Bacteriocines has been tentatively added to that on the Normal Flora instead of ranking with the bacteriophages, as it did formerly.

The greatest change, however, has been the transference of the subject of Immunity from Volume 2 of the 6th edition to the present volume. Rather than attempting to give the complete experimental evidence supporting modern theories of immunity, we have concentrated on those aspects that pertain most closely to infectious disease. In a way, we have returned to the viewpoint of earlier microbiologists, but with the incorporation of modern experimental findings, often derived from work apparently far removed from the study of infection. Much of the detailed description of principles and techniques included in earlier editions has been omitted; this body of knowledge may still be consulted with profit, but has been sacrificed in the present edition to make way for a more wide-ranging discussion. Some of the more recent work on immunoglobulins, on lymphocyte functions, and on the role of the major histocompatibility complex (MHC) in Chapters 10 and 14 may seem strange to those who are not familiar with this rapidly advancing field of knowledge, but of their relevance to infection there can be no doubt. The generation of antibody diversity, the 'fine-tuning' of the controlling influences, and the disastrous consequences of disordered immunological responses (Chapters 10, 15, 16) can be explained only at the cellular level. Details of antigenic structure and the exploitation of this knowledge have also been brought up to date (Chapter 13). The role of antibody in infection (Chapter 12) and the importance of lymphocyte and antibody mediated tissue damage are examined in broad outline (Chapters 11 and 16). The underlying principles of the various problems of infection in patients with defective immunological responses are discussed in Chapter 17. Some readers may feel that we have omitted useful information, both old and new; but we would point to the existence of numerous specialized texts dealing with individual aspects of immunology and to the more important reviews and papers to which references have been made.

We have tried in this volume to provide a firm foundation for the general microbiologist who intends to work on bacterial chemistry, molecular biology, genetic engineering, or the prevention and control of infectious disease, as well as for those engaged in other branches of biology or in clinical medicine who need it for purposes of consultation or reference.

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1983

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1

History

Ashley Miles

Introductory

Bacteriology and virology

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. From Pasteur onwards, for 60 years or more, the great majority of investigators were more interested in what bacteria do than in what they are, and much more interested in the ways in which they interfere with man's health or pursuits than in the ways in which they function as autonomous living beings. The relations of bacteria to disease, to agriculture, and to various commercial processes, presented problems which pressed for solution; and, as a result, we have seen the development of an applied science of bacteriology, or rather its application along many divergent lines, without the provision of any general basis of purely scientific knowledge.

In much the same way, the sister science of immunology grew from attempts to solve problems of immunity in man and animals. Nevertheless the last half of the nineteenth century and the first few decades of the twentieth saw, *pari passu* with the technical mastery of microbial technology, the emergence of the science of microbiology.

It is customary, in summarizing the history of bacteriology, at least in relation to medicine, to refer to the conception advanced by Fracastorius of Verona (1546), of a *contagium vivum* as the cause of infective disease, and to the views advanced by von Plenciz (1762) on the specificity of disease, based on a belief in its microbial origin. A concrete science is, however, seldom advanced by arguments, however ingenious, which are propounded without appeal to experiment, or to wide and detailed observation; and such views have acquired their main significance from knowledge gathered by later generations, rather than from their inherent fertility. The construction and use of the compound microscope was an essential prerequisite to the study of microbial forms. To van Leeuwenhoek (1683) must be ascribed the credit of placing the science of microbiology on the firm basis of direct observation (Dobell 1932). This Dutch maker of lenses devised an apparatus and technique (Cohen 1937) which enabled him to observe and describe various microbial forms with an accuracy and care that still serve as a model for all workers in this field. He observed, drew, and measured large numbers of minute living organisms, including bacterial and protozoal forms. This striking advance was not followed by further rapid progress in our knowledge of bacteria and their activities. Such progress was, however, impossible without further developments in technique. The world of minute living things, opened to morphological study by van Leeuwenhoek, was seen to be peopled by a multitude of dissimilar forms, whose interrelationships it was impossible to determine without preliminary isolation; and, so far as bacteria were concerned, this isolation was not accomplished until the problem of artificial cultivation was solved, almost two hundred years later.

Pasteur's work

The real development of bacteriology as a subject of scientific study dates from the middle of the nineteenth century, and is the direct outcome of the work of Louis Pasteur (1822–95). Isolated observations of microbial parasites, by Brassi, Pollender, Davaine and others, have priority in particular instances, just as Schultze, Schroeder and Dusch and others initiated technical methods which Pasteur applied to his own researches. But it was Pasteur and his pupils who settled the fundamental questions at issue, and developed a technique which made possible the cultivation and study of bacteria.

Trained as a chemist, Pasteur was led to the study of microscopic organisms by his observations on fermentation. His early studies on molecular asymmetry, had led him to believe that the property of optical activity possessed by certain organic compounds was characteristic of substances synthesized by living things. It was known that small amounts of an optically active substance, amyl alcohol, were formed during the fermentation of sugar. Since it was impossible to regard the molecule of amyl alcohol as derived from the molecule of sugar by any simple break-down process, he concluded that the optically active sugar was first broken down to relatively simple substances without optical activity, and that from such inactive substances the optically active amyl alcohol was synthesized. For Pasteur this was evidence of the presence of living things, and he therefore started on his study of fermentation with a strong *a priori* leaning towards the microbial theory of fermentation, and away from the then dominant hypothesis of Liebig. He was prepared to adopt the theories already propounded by Cagniard-Latour in 1836, and by Schwann in 1837, concerning the living nature of the yeast globules, which were always to be found in sugar solutions undergoing alcoholic fermentation, and which had been described by van Leeuwenhoek in 1683.

Since, however, the production of amyl alcohol had been observed in fermenting brews in which lactic acid also appeared, Pasteur first selected lactic fermentation for experimental study. Van Helmholtz (1843) had already indicated that alcoholic fermentation was due to the yeast itself or to some other organized material. He had shown that the substance, responsible for initiating alcoholic fermentation would not pass through membranes that allowed the passsage of organic substances in solution but held back particles in suspension. This experiment, successful with alcoholic fermentation, failed with many other ferments and fermentable liquids. Pasteur's mind was naturally addicted to generalization, and his interest lay in the phenomenon of fermentation as a general type of reaction. It was therefore natural that he should at first neglect the field in which the battle was more evenly balanced between the purely chemical conceptions of Liebig, and the biological theories of Cagniard-Latour, Schwann and Helmholtz, and turn to the field in which Liebig's views had never been successfully attacked. In Pasteur's first memoir published in 1857, he declared the lactic ferment to be a living organism, far smaller than the yeast cell, but which could be seen under the microscope, could be observed to increase in amount when transferred from one sugar solution to another, and had very decided preferences as regards the character of the medium in which it was allowed to develop; so that, for instance, by altering the acidity of the medium one could inhibit or accelerate its growth and activity. In this memoir Pasteur laid the first foundations of our knowledge of the conditions to be fulfilled for the cultivation of bacteria.

Fig. 1.1 Louis Pasteur (1822–1895).

He also showed that the fermentation of various organic fluids was always associated with living cells, and that different types of fermentation were associated with microscopic organisms that could be distinguished from one another by their morphology and their cultural requirements. Thus, at this early stage, the idea of specificity entered into bacteriology.

Putrefaction and fermentation

Spontaneous generation

It was impossible for Pasteur to pursue these studies without facing the problem of the origin of these minute living organisms, which he regarded as the essential agents of all fermentations. At this time (1859) there were two opposed schools of thought with regard to the genesis of microbial forms of life. One school, deriving from the great naturalists of antiquity, believed in the *spontaneous generation* of living things from dead, and especially from decomposing organic matter; but, as Pasteur astutely noted, the species of animals or plants believed to arise by spontaneous generation were diminishing in number, and the average size of those organisms still included in this category was getting smaller and smaller. However the discovery by Leeuwenhoek of the world of microbial organisms gave a powerful stimulus to the somewhat decadent theory. Here, at all events, were living things which obeyed no known law of reproduction, and whose existence seemed to lend support to a belief which had long been accepted by eminent authorities, and which had thereby acquired a natural prestige.

From the start of his inquiry, Pasteur assumed that these microscopic organisms, like other living things, were reproduced in some way from similar pre-existing cells. He had already convinced himself that these organized cells were the active agents of **fermentation.** Clearly then they could not arise *de novo* during the changes for which they were themselves responsible, but must have been introduced from without. Their striking specificity, maintained through repeated transferences from one specimen of fermentable fluid to another of the same kind, was strong evidence in favour of their autonomous reproduction. Here again Pasteur had tentatively adopted the correct solution before starting his experimental inquiry, but the main interest of his part in the controversy lies in the consummate skill with which he developed methods which enabled him to give clear demonstrations where others had left doubt and confusion, and which determined the main rules of a technique which made possible the cultivation and study of bacteria. Needham (1745) described spontaneous generation of microbes in closed flasks of heated putrescible fluid; but Spallanzani (1769) found no such generation after longer heating. Among the non-living substances suggested as the cause of **putrefaction,** oxygen occupied a prominent place at the beginning of the nineteenth century. Apperts' (1810) success with the preservation of foodstuffs, by heating and hermetical closure of the containing vessels, followed by a weighty expression of opinion by Gay-Lussac, had led to a general belief that the exclusion of this gas was the essential factor in ensuring the absence of fermentation. Schultz (1836) renewed the air in a heat-sterilized flask of putrescible fluid with air drawn through solutions of strong potash or concentrated sulphuric acid, and Schwann (1837) with air drawn through a heated tube; and could detect no putrefaction. But other treatments of the air were unsuccessful. A real advance was made by Schroeder and Dusch (1854), who sterilized the air by passing it through cotton wool, thereby avoiding exposure to strong chemicals. But their results were equivocal with some fluids owing, it appears, to their use of too short periods of initial heating.

This, then, was the position when Pasteur began his investigations in 1859. In a series of admirable mémoirs, starting in 1860 and continuing for more than four years, he went over the ground already covered, added new and illuminating experiments of his own devising, and terminated the controversy by clear and decisive demonstrations. He showed that the material removed from air by passage through cotton-wool contained organized particles which were similar in appearance to the spores of moulds. Introduced into flasks of sterilized organic material, they were capable of giving rise to the growth of numerous kinds of living organisms. By other methods, he showed that these germs were numerous in the streets of cities, less numerous in the air of country uplands, rare in the quiet air of closed and uninhabited rooms or cellars, where the dust had deposited and remained undisturbed, and very rare in the pure air of the high Alps, above the level of human habitation. He showed that the failures of Schroeder and Dusch were due to the inadequate sterilization of their material, and that certain animal fluids, such as blood or urine, known to be eminently liable to undergo putrefaction, could be collected in such a way as to remain permanently unaltered (see Vallery-Radot 1919).

In 1876 Bastian published a communication controverting an early statement by Pasteur that urine, sterilized by boiling, remained free from growth on subsequent incubation. Bastian declared that, if the urine were made alkaline at the start, growth often ensued. Pasteur, on repeating the experiment, was forced to admit the truth of Bastian's statement. A careful retracing of all his steps resulted in the demonstration that fluids with an acid reaction, after sterilization at 100°, might remain apparently sterile because certain organisms, which remained alive, were unable to develop, while in an alkaline medium they might grow freely. It was found also that ordinary water frequently contained organisms which were not killed by heating to 100°, and that organisms which had become deposited on the surface of glassware in the dry state might withstand far higher temperatures. We know now that it is especially for those bacteria which form spores that these conditions hold true. As a result of this controversy Pasteur established the practice of heating fluid material to 120° under pressure for the purpose of sterilization, thus introducing the autoclave into the laboratory, and the practice of sterlizing glassware by dry heat at 170°.

Tyndallization An important advance was made by Tyndall who, observing that actively growing bacteria are easily destroyed by boiling, and that a certain amount of time is required for bacteria in culture in the resistant, inactive phase to pass into the growing phase in which they are heat-sensitive, introduced the method of sterilization by repeated heatings, with appropriate intervals for germination between them. This method is still known as *Tyndallization* (see Bulloch 1938).

Lister's work Pasteur's demonstration that both fermentation and putrefaction were initiated by airborne microbes prompted Joseph Lister's (1827–1912) work on wound sepsis. Lister was deeply concerned with the post-operative sepsis that exacted such a terrible toll on the lives of hospital patients. Assuming that putrefying wounds were analogous to putrefying organic matter, he became convinced that the key to the prevention of sepsis lay in denying access to the wound by the microbes of the environment, particularly of the air. This he achieved by the antiseptic dressing, first described in 1867, with strikingly successful results. With his antiseptic technique, the scope of surgery, hitherto limited by the fear of sepsis, was enormously enlarged. That this technique has largely been replaced by the aseptic technique in no way detracts from the merit of Lister's discovery, nor from the debt we owe him for fighting the usual battle against ignorance and prejudice. Although Lister did not directly prove that his success was due to the destruction of potentially infective microbes, in the light of contemporary work by French and German bacteriologists on the relation of microbes to disease, it became evident that his achievement was one of the first great triumphs of applied bacteriology in medicine.

While investigating fermentation Pasteur had used very various kinds of natural organic fluids and solutions, and had succeeded in growing micro-organisms on simple media. As a result he had realized that a medium eminently suitable for the growth of one bacterium or mould may be ill adapted for the growth of another, and that a prime necessity for the successful cultivation of any species of micro-organism is the discovery of a suitable growth medium. He had learned the need for the scrupulous sterilization of everything that came into contact with material to be examined bacteriologically; and had devised the necessary methods of sterilization in the steamer, in the autoclave, in the hot-air oven, or by direct flaming. He had proved the serviceableness of the cotton-wool plug for protecting media in flasks or tubes. He had realized the importance of the constitution of the nutrient material offered to a given bacterium, of the acidity or alkalinity of that medium, and of the oxygen pressure to which it was subjected. Armed with this knowledge, he proceeded to break new ground.

Pasteur was before all else a scientist, intensely curious, and loving knowledge for its own sake, but he was also a convinced utilitarian, and a Frenchman. He desired greatly that his discoveries should benefit mankind in general, France in particular, and, if possible, his neighbours in the first place. Thus we find him investigating the troubles of the local vintners, brewers, and vinegar-makers, and many of his memoirs are devoted to the diseases of wines or of beers, and the methods of preventing them. Here he faced the question whether one species could change into another, in particular whether mycoderma vini could change into the ordinary yeast of wine. Deceived on this point at first, he resorted as usual to rigorous and repeated experiments, and not only demonstrated that this mutation did not occur, but indicated clearly the conditions which led to its apparent occurrence, and the care which must be exercised before accepting any reported variation of this kind.

Microbial diseases It is evident from his memoirs on fermentation and spontaneous generation (see Vallery-Radot, P., 1922–1933) that the possibility of applying this new knowledge to the elucidation of infective disease was already in Pasteur's mind. A request from Dumas to investigate the disease then ruining the silkworm industry in the South of France, turned him permanently towards the study of infective processes. We cannot follow here, even in outline, Pasteur's researches into pébrine, anthrax, chicken cholera, or hydrophobia. Some of them are referred to in later chapters. But certain contributions are noteworthy. It was Pasteur who showed, in the case of anthrax, that a culture of a pathogenic organism could be passed through successive subcultures, in such a way as to dilute beyond possibility of significant action, any other material introduced with it into the primary culture from the blood or tissues, and still produce the disease when inoculated into a susceptible animal; though it is to Koch that priority must be given for much of our knowledge on the nature and mode of action of the anthrax bacillus. It was Pasteur who introduced into bacteriology the conception of virulence and of attenuation, and who demonstrated that an attenuated bacterial culture will act as a vaccine, that is, will confer immunity against subsequent infection with a virulent strain of the same bacterium. For Pasteur, indeed, a vaccine was synonymous with an attenuated culture, as opposed to a virulent culture on the one hand and to a dead culture on the other. It was Pasteur who, in the case of rabies, showed that it was possible to study the virus of an infective disease by animal passage when the organism could not be cultivated, and even to prepare an apparently effective vaccine by using suitably treated animal tissue.

Thus, throughout a long scientific life, Pasteur was largely concerned with the practical application of knowledge gained during his studies on fermentation; and with the problems which occupied the last thirty years of his life, the solution of which made his name a household word. But we shall miss the real significance of his work if we fail to realize that his fertile generalizations were of infinitely more importance for the progress of science than were his successful attacks on these isolated problems.

One further point must be noted. Pasteur and his colleagues had shown how to obtain cultures of micro-organisms, and propagate them indefinitely in the laboratory; but the methods which they employed were not well suited to the isolation of pure strains of bacteria from an originally mixed culture, except in those relatively rare instances in which it was possible to use a highly selective medium. Since all media were employed in the fluid state, the only method of purifying a culture was to make successive transfers with very small amounts of material, in the hope that only a few bacteria, all of one kind, would be carried over. Such a technique was very uncertain in its results.

Koch's work

Pasteur, starting as a chemist, founded bacteriology and revolutionized medicine. At about the time when he was propounding his germ theory of disease, a young German physician, some twenty years his junior, was turning from clinical medicine to bacteriology. Robert Koch (1843–1910), at that time a practising physician at Wollstein, attacked the problem of anthrax, and produced, as his first contribution to science, a demonstration of the character and mode of growth of the causative bacillus, which opened a new era in bacteriological technique. This memoir he published in 1876. In the following year he published his methods of preparing, fixing, and staining film-preparations of bacteria, using the aniline dyes introduced into histology by Weigert. In 1878 he published his memoir on traumatic infectious diseases, which remains a classical example of the study of experimental infections in laboratory animals. In 1881 he described his method of preparing cultures on solid media, a technical advance of the first importance, since it made possible the isolation of pure strains of bacteria from single colonies. Solid media prepared from naturally occurring material such as pieces of potato, had previously been used for the isolation of micro-organisms, particularly by mycologists, and the general principles to be observed in the preparation of pure cultures had been clearly enunciated by Brefeld, who had suggested the solidification of a nutrient medium by the addition of gelatin. The media and methods available for the cultivation of fungi were not, however, well suited for bacteria; and it was left for Koch to devise, in the form of his nutrient gelatin, and later, at the suggestion of Frau Hesse, of nutrient agar, a solid, transparent medium, easy to sterilize and handle, and thus admirably adapted for obtaining isolated colonies of bacteria (see Bulloch 1938). In 1882 and 1884 he published his classical papers on the bacillus of tuberculosis. In 1883 he discovered the vibrio of cholera. Already, Koch had enlisted the services of Loeffler and of Gaffky as his assistants. Later came Pfeiffer, Kitasato, Welch and many others, and, with his growing fame, he began to gather round him a group of keen and able young men, who were destined to introduce the methods he devised into the laboratories of many lands. In 1885 he was appointed Professor of Hygiene and Bacteriology in Berlin, and in 1891 he was made Director of the newly founded Institute for Infectious Diseases. His later years were devoted almost entirely to the investigation of bacteriological problems in their relation to the prevention and cure of disease, and many of his contributions to our knowledge will be considered in later chapters. Koch was, above all, an able and careful technician. He was greatly aided by the vigour and initiative of the large German chemical and optical firms, and the advances which he made in staining methods, in the use of the microscope for the observation of bacteriological preparations, and in the technique of cultivating bacteria, revolutionized this branch of science.

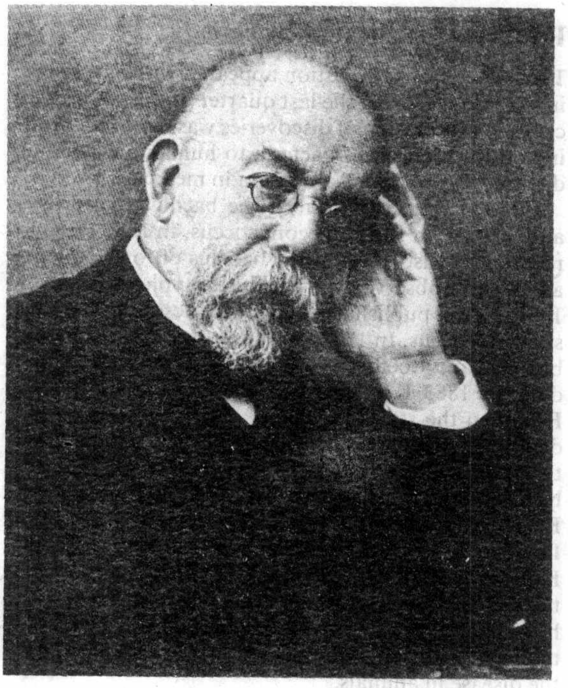

Fig. 1.2 Robert Koch (1843–1910).